ELSEVIER

Acta Tropica 78 (2001) 241–250

ACTA TROPICA

www.parasitology-online.com

A preliminary study of the population genetics of *Aedes aegypti* (Diptera: Culicidae) from Mexico using microsatellite and AFLP markers

Sophie Ravel [a,*], Nicole Monteny [b], Dolores Velasco Olmos [b], Juan Escalante Verdugo [b], Gérard Cuny [a]

[a] *Laboratoire de Recherche et de Coordination sur les Trypanosomoses, IRD-CIRAD, Programme Santé Animale, TA/30G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France*

[b] *Laboratorio Estatal de Salud Publica, Av. Louis Donaldo COLOSIO Zona Edificios Federales, Col. Las Quintas, CP 83260, Hermosillo-Sonora, Mexico*

Received 4 April 2000; received in revised form 23 November 2000; accepted 6 December 2000

Abstract

Dengue fever recently reemerged in the Americas. Because vaccines are still under development, dengue prevention depends entirely on vector control. Since *Aedes aegypti* (Linnaeus, 1762) is the principal vector of this arbovirus, knowledge of the genetic structure of the insect is therefore required to maintain effective vector control strategies and to estimate levels of gene flow from which movement can be inferred. This preliminary study uses microsatellite and amplified fragment length polymorphism (AFLP) markers, to provide insights into genetic diversity of *A. aegypti* populations from different districts of two towns, located in the north-west of Mexico, Hermosillo and Guaymas. Although the microsatellites used were found to display limited polymorphism, they allowed discrimination between mosquitoes from the northern and the southern districts of Hermosillo. Using AFLP markers, clustering of individuals from the same town and from the same district was observed. Data from microsatellite and AFLP markers analysis both suggest that reinvasion of *A. aegypti* probably occurs from Guaymas to Hermosillo.

Keywords: *Aedes aegypti*; Microsatellites; AFLP; Dengue fever

1. Introduction

Dengue fever is a disease of historical significance which reemerged in the past 20 years with both an expanded geographic distribution of the virus and its mosquito vector, a complex species (Tabachnick and Powell, 1978), *A. aegypti* (Linnaeus, 1762), (Gubler, 1998). Thus, in the Americas, with the exception of Canada, Chile and Bermuda, all countries are infested with dengue vectors (Pinheiro and Corber, 1997). In Mexico,

* Corresponding author. Tel: +33-4-67593919; fax: +33-4-67593920.

E-mail address: ravel@mpl.ird.fr (S. Ravel).

0001-706X/01/$ - see front matter
PII: S0001-706X(01)00083-3

dengue fever is now a major public health problem due to the dramatic increase in the number of severe cases reported in 1995 (Gomez-Dantes et al., 1996). Data for 1997 mentions 50 000 cases of classical dengue and about 1000 cases of dengue hemorrhagic fever (Gorrochotegui-Escalante et al., 1998).

The reinvasion of *A. aegypti* is due to the reduction in surveillance and control programme support, the rapid growth and urbanization of human populations, travelling and transport. Prevention of dengue depends entirely on vector control because no specific treatment and no dengue vaccine are yet available. To maintain effective vector control strategies, knowledge of populations genetic structure and of gene flow between mosquito populations are required. Such information would allow discrimination between resistance and reinvasion processes after insecticide treatments and would also give an insight into vector genetic diversity and vector competence for the virus.

Among the numerous molecular markers available in entomology (Loxdale and Lushai, 1998), microsatellites, because they are highly polymorphic, neutral and of Mendelian inheritance, represent one of the most powerful tools developed for population genetic studies in recent years (Bruford and Wayne, 1993; Jarne and Lagoda, 1996). They have been successfully used for population genetic studies in social insects, like ants (Gertsch et al., 1995; Herbers and Mouser, 1998) and bees (Estoup et al., 1993, 1995, 1996) and in insect vectors of medical importance such as *Anopheles* (Lanzaro et al., 1995a), *Glossina* (Solano et al., 1997) and *Simulium* (Dumas et al., 1998).

A recent DNA fingerprinting technique, called amplified fragment length polymorphism (AFLP), has also been used to analyse genetic diversity in plants (Becker et al., 1995; Mackill et al., 1996) and bacteria (Janssen et al., 1996; Lin et al., 1996; Gibson et al., 1998), and most recently, for insects population genetics (Reineke et al., 1999; Yan et al., 1999).

In the present study, microsatellite and AFLP markers were used to analyse genetic variability in *A. aegypti* populations from Mexico. Mosquitoes were collected from four different areas, two districts of Guaymas, a harbor located on the California bay, and two districts of Hermosillo, 120 km away to the north of Guaymas. The two towns are connected by a main road and by rail. The aim of this work was to ascertain whether reinvasion by *A. aegypti* could occur from Guaymas to Hermosillo in order to explain the dengue cases observed in Hermosillo.

2. Material and methods

2.1. Mosquitoes collection

Two districts from two different towns in Mexico, Hermosillo and Guaymas, were sampled in June, 1998. Mosquito larvae were collected in different points of the same district in which water containers had been previously placed, and reared to the adult stage in the laboratory. Thereafter, 20 adults from each district were used for population genetic analysis (total sample number $n = 80$). Because *A. aegypti* females lay small numbers of eggs at many sites (Reiter, 1996) and consequently several females oviposit in the same container during the sampling period, the likelihood of analysing very closely related individuals, i.e. siblings, is small.

2.2. Detection and analysis of length polymorphism in microsatellite loci

A. aegypti microsatellite repeat sequences were searched for within available data banks and primer pairs designed for the sequences flanking several such regions (see Table 1). One microsatellite was found in a coding region (T58313). Despite the assumed selective pressure on such sequences, significant polymorphism has previously been observed in coding sequences (Michalakis and Veuille, 1996). Thus, the locus T58313 (the ecdysteroid receptor gene) was considered for further analysis.

Legs from each mosquito were taken out, homogenised in 100 μl of a 5% chelex 100 resin (Biorad, CA, USA) solution and incubated for 1 h at 56°C and 30 min at 94°C to obtain DNA. After centrifugation, 5–10 μl of the supernatant

were used for subsequent DNA amplification. Polymerase chain reactions (PCRs) were carried out using a DNA thermalcycler (PE Applied Biosystems, Foster City, CA, USA) in 50 μl final volumes, containing 20 pmoles of each primer pair, 0.2 mM of each deoxyribonucleotide, 1 × incubation buffer with 1.5 mM $MgCl_2$ (Quantum Appligène, Ilkirch, France) and 0.5 units of Taq DNA polymerase (Quantum Appligène, Ilkirch, France). Samples were initially denatured at 92°C for 3 min and then processed through 35 or 40 cycles consisting of a denaturation step at 92°C for 30 s, an annealing step at 53°C (AED19F/AED19R), 55°C (AEDGA1/AEDGA2) or 65°C (AEDC1/AEDC2) for 30 s and an extension step at 72°C for 1 min. The final elongation step was lengthened to 5 min. Amplification products were checked by electrophoresis in 2% agarose gels and visualised by ethidium bromide staining (0.5 μg/ml) under UV light. Allele bands were then resolved in non-denaturing acrylamide gels (10%) and revealed by ethidium bromide staining. Allele sizes were determined by performing PCR in the presence of (α^{35}-S)dATP, followed by denaturing electrophoresis in a 6% acrylamide gel containing 8 M urea. The sequencing of the M13mp18 single stranded DNA, using (α^{35}-S)dATP and the T7 Sequencing Kit (Amersham Pharmacia Biotech, France) was run in the same denaturing acrylamide gel, allowing the allele size to be determined to an accuracy of one base.

For each population, genetic variability parameters (allelic frequencies, observed and expected heterozygosities under Hardy–Weinberg equilibrium) and genotypic differentiation among populations and estimation of F_{is} (inbreeding coefficient) values were assessed using GENEPOP software version 3.1 (Raymond and Rousset, 1995). Differentiation between populations was examined by *F*-statistics, computed according to Weir and Cockerham (1984), using FSTAT version 1.2 (Goudet, 1995).

2.3. AFLP analysis

In this technique, adaptor molecules are first ligated to restriction enzyme fragments and are subsequently used as target sites for primers in a PCR amplification process (Vos et al., 1995). Amplified fragments are then electrophoretically separated to give strain-specific band profiles.

We employed AFLP to analyse 20 individual mosquitoes from Hermosillo and 18 from Guaymas, whose legs have been previously used for the microsatellite analysis.

Table 1
Sequences of microsatellite and AFLP primers used in this study

Primers	Locus	Accession number	Repeat sequence	Primer designation	Primer sequence
Microsatellite primers	19	U91680	(GGA)5	AED19F	5′-TTATGGAACTGGTAAGCCC-3′
				AED19R	5′-GTATGACAACTCTGGAATGG-3′
	GA	U28803	(GAA)3(GAC)4	AEDGA1	5′-CCGAAGAAATTGGGGTGACC-3′
			(GAA)3	AEDGA2	5′-CCTCTCGGTGTTCGCTAACC-3′
	C	T58313	(GTA)6(ACG)	AEDC1	5′-TGCAGGCCCAGATGCACAGCC-3′
			(GTA)3	AEDC2	5′-TCCGCTGCCGTTGGCGTGAAC-3′
AFLP primers				E-ACG	5′-GACTGCGTACCAATTCACG-3′
				M-CAA	5′-GATGAGTCCTGAGTAACAA-3′
				M-CAG	5′-GATGAGTCCTGAGTAACAG-3′
				M-CTT	5′-GATGAGTCCTGAGTAACTT-3′

DNA from each whole individual (minus the legs previously used) was extracted according to Reineke et al. (1998), followed by an additional spermine precipitation. AFLP analysis was performed using AFLP core reagent and starter primer kits from Life Technologies (Gibco BRL, Gathersburg, MD, USA). DNA was digested with two restriction endonucleases, *Eco*RI and *Mse*I, then the genomic DNA fragments were ligated to *Eco*RI and *Mse*I adapters to generate template DNA for amplification. Preamplification was performed with AFLP primers, each having one selective nucleotide (forward primer = E – A and reverse primer = M – C). These PCR products were diluted and used as templates for the selective amplification using two AFLP primers, each containing three selective nucleotides. (The *Eco*RI selective primer was end-labeled with γ-(^{33}P)-ATP using T4 kinase before amplification). Three selective primer combinations were used for each sample (Table 1). Amplification products were then separated on a 6% denaturing polyacrylamide gel (the same AFLP reactions running on the same gel) and scored as discrete character states (present/absent). The data were transformed into band frequencies, and diversity values were calculated using Nei, 1987's unbiased statistic. The band frequencies were also analysed using Phylip analytical software version 3.1 (Felsenstein, 1993). Seqboot was used to generate pseudoreplicates (500) and the output file was processed in the Mix program (which used the Wagner parsimony method) followed by Consense to get a single bootstrapped tree. Since sample H11 presents a banding pattern quite different from the others, with additional bands, it was chosen as outgroup in the analysis.

3. Results

With microsatellite markers, all samples tested from Mexico were found to have the following alleles: one allele at locus GA, two at locus 19 and three at locus C. At locus GA, all mosquitoes were homozygous (fixed) for the 147 bp allele, whatever the district of larval capture. At the two other loci, homozygotes were found to be in the large majority — 80% of individuals. Because of the high ratio of homozygotes observed, we suspected the presence of null alleles. By submitting the samples to amplification with newly designed primers (flanking the original primers) for the three loci, we were able to reject this hypothesis (data not shown).

When samples were separated according to their town of origin, no heterozygote deficiency was observed at any of the two loci for the two Mexican populations (a positive F_{is} value not significant, was only obtained at locus C in the Guaymas population (Table 2)). Mean pairwise F_{st} over all loci between the two populations was near zero so that these two populations could thus not be distinguished. When results were analysed separating samples according to the district of collection, no heterozygote deficiencies were observed in any of the four populations with combined F_{is} values negatives or not significant, over all loci. We noticed for locus 19 that more heterozygotes occurred in the northern district (39%) compared with the southern district of Hermosillo (0%) and the two districts of Guaymas (9 and 20%). When mean pairwise F_{st} over all loci among the four districts were calculated, only the two districts of Hermosillo showed a significant F_{st} value of 0.106, indicating genetic differentiation between mosquitoes from these two districts.

Using AFLP markers, the regional samples displayed different banding patterns (data not shown). Some mosquitoes showed banding pattern with only one or two of the three primer combinations. Therefore, this lack of amplification cannot result from DNA purification, digestion, ligation and preamplification steps, but is more likely to be due to the selective amplification step of the AFLP procedure. Only samples giving banding patterns with each of the three primer sets (29 among 38) were kept for analysis (eight samples from the first district and three from the second district of Guaymas, nine from each district of Hermosillo). Each primer combination revealed between 48 and 144 polymorphic loci (i.e. different bands) in individual populations (Table 3) and between 128 and 182 polymorphic loci across the three populations. Diversity values for primer pairs in individual populations ranged

Table 2
Number of alleles, allele sizes, allelic frequencies, observed and expected heterozygosities, F_{is} value and exact probability for departure from Hardy–Weinberg proportions for the 19 and the C loci in the two populations

Locus	Population	Number of individuals analysed	No. of alleles	Allele sizes (bp)	Allelic frequencies	Observed heterozygosity (%)	Expected heterozygosity (%)	F_{is}	P-value
19	Guaymas	40	2	176 179	0.923 0.076	15.4	14.5	−0.064	1
	Hermosillo	40	2	176 179	0.893 0.106	21.2	19.2	−0.100	1
	Guaymas (1st district)	20	2	176 179	0.954 0.045	9.1	9.1		
	Guaymas (2nd district)	20	2	176 179	0.900 0.100	20	18.6	−0.077	1
	Hermosillo (*N*th district)	20	2	176 179	0.805 0.194	38.9	32.2	−0.214	1
	Hermosillo (*S*th district)	20	1	176	1	0			
C	Guaymas	40	2	222 228	0.112 0.887	16.1	20.3	+0.211	0.315
	Hermosillo	40	3	216 222 228	0.027 0.040 0.932	13.5	13.0	−0.040	1
	Guaymas (1st district)	20	2	222 228	0.100 0.900	20	18.6	−0.077	1
	Guaymas (2nd district)	20	2	222 228	0.125 0.875	12.5	22.6	+0.455	0.190
	Hermosillo (*N*th district)	20	2	222 228	0.078 0.921	15.8	14.9	−0.059	1
	Hermosillo (*S*th district)	20	2	216 228	0.055 0.944	11.1	10.8	−0.030	1

Table 3
Diversity statistics for the three AFLP primer combinations in the different districts of Hermosillo and Guaymas[a]

Population	Sample names	No. of individuals analysed	EACG/MCAA		EACG/MCAG		EACG/MCTT		All loci	
			n	*H*	*n*	*H*	*n*	*H*	*n*	*H*
Guaymas (1st district)	G2,G4,G5,G8,G13 G15,G18,G20	8	124	0.272	113	0.335	121	0.311	358	0.306
Guaymas (2nd district)	G23,G26,G27	3	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Hermosillo (*N*th district)	H2,H3,H4,H5,H6 H11,H15,H17,H19	9	144	0.277	99	0.294	128	0.296	371	0.289
Hermosillo (*S*th district)	H21,H23,H25,H26H28,H33,H34,H35 H38	9	54	0.120	48	0.141	55	0.140	157	0.133

[a] The second district from Guaymas was not taken into account because of the very small sample size (n = number of polymorphic bands; H = genetic diversity).

from 0.120 to 0.335 with average values for all loci ranging from 0.133 to 0.306. The population of Hermosillo south district displayed the smallest number of polymorphic loci and diversity values, whatever the primer combination used.

Fig. 1 shows a consensus tree, built by parsimony methods, based on data for all the 29 samples, from the three primer sets (see legend of this figure for site abbreviations used). Individuals from the same district were generally grouped together resulting in the formation of site-specific subgroups. It is especially evident with mosquitoes from the south district of Hermosillo (ranging from H21 to H38) which clustered on the same branch of the consensus tree with a significant bootstrap value.

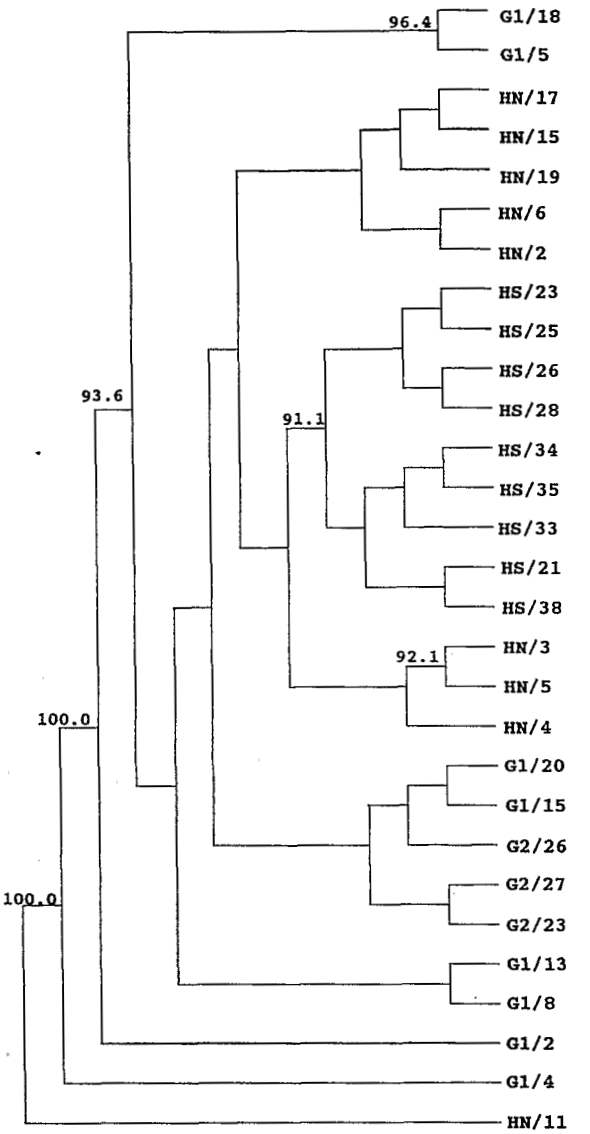

Fig. 1. Consensus parsimony tree built from data obtained with the three primer combinations using Phylip analytical software version 3.1 and parsimony method. Samples HN/2 to HN/19 are from Hermosillo north district, HS/21 to HS/38 are from Hermosillo south district whereas G1/2 to G1/20 are from the first district of Guaymas and G2/23 to G2/27 are from the second district of Guaymas. Bootstrap values for tree nodes for more than 75% are indicated.

4. Discussion

In Hermosillo, after a large epidemic of dengue (DEN-3) in 1996, some further cases were observed within certain defined areas only in 1997 and 1998. Moreover, since the same dengue strain is present continuously in Guaymas, it is possible that reinvasion of *A. aegypti* occurred in Hermosillo, from this site. Especially as the two towns are connected by rail and main road with a high truck traffic between them. Another explanation for the few reported dengue cases in Hermosillo could be the survival of mosquito eggs during the dry season. To check this possibility, mosquitoes from the two towns were collected and genetic variability analysed using both microsatellite and AFLP markers. It has already been shown that understanding the population genetics of the vector may have important implications for the epidemiology of vector-borne diseases (Lanzaro and Warburg, 1995b). For instance, genetic analyses of *Anopheles* (Kamau et al., 1998; Rongnoparut et al., 1999; Simard et al., 1999), *Simulium* (Dumas et al., 1998) and *Glossina* (Solano et al., 1999) on a micro as well as on a macrogeographic scale have gathered important information about population migration and dynamics of transmission of the related diseases.

Compared with other data obtained with microsatellites (Hughes and Queller, 1993; Rongnoparut et al., 1996; Goostrey et al., 1998), the loci used here were only slightly polymorphic in *A. aegypti* mosquitoes from Mexico. However,

high polymorphism have been observed in *A. aegypti* individuals from Cameroon, Central Africa, using the AEDGA1/AEDGA2 and AEDC1/AEDC2 primer pairs (Barbazan et al., 1999) and significant genetic differentiation has been found between mosquitoes from different areas of the Ivory Coast, West Africa (Ravel et al., submitted).

In the present work, mosquitoes from the two Mexican towns could not be distinguished using these three microsatellites. When samples were separated according to their district of collection, genetic differentiation was noticed between mosquitoes from the two districts of Hermosillo. More heterozygotes were observed for locus 19 in the northern district of Hermosillo compared with the three other districts. This could be due to a greater mixing of the population from Hermosillo north district compared with the three other district populations. Together these data may suggest that the vector population from the southern district of Hermosillo might be closer to those of Guaymas, or be introduced from Guaymas.

In order to find support for this possibility, we also tested the recently developed AFLP markers on the same mosquito populations. For each primer pair, the number and the distribution of loci differed significantly from one mosquito to another, indicating genetic diversity among samples. The population of Hermosillo south district displayed a lower genetic diversity as compared with the other populations. The consensus tree built by parsimony method indicated various subgroups, each comprising individuals from the same town and/or from the same district. This is especially clear for the mosquitoes from the south district of Hermosillo which significantly clustered on the same branch.

Taken together, the data sets provided by the two molecular markers both support the view that inadvertent transportation of mosquitoes from Guaymas to the south of Hermosillo occurs, most probably as a result of the high truck traffic between the two towns.

In this preliminary work, the use of microsatellite and AFLP markers has provided an insight into *A. aegypti* genetic diversity and suggests that reinvasion of mosquitoes did indeed take place between Guaymas and Hermosillo. However, a larger study has to be conducted to confirm the reinvasion hypothesis. This will involve larger samples and comparison of *A. aegypti* populations of the same two towns collected one year apart, using new, more polymorphic microsatellite markers. The use of these molecular tools might be extended to other *A. aegypti* populations in order to identify the potential reinvasion period, to study the dynamics of insect vector strains and their viral pathogens, and to adopt effective vector control strategies.

Acknowledgements

This work was conducted as part of a bilateral agreement between the Sonoran Ministry of Health (LESPSON) and the Institut de Recherche pour le Développement (IRD) which both supported the study. We are grateful to Philippe Solano and Christian Barnabé for helpful discussions. The authors also wish to thank two anonymous referees for the significant improvement of the manuscript.

References

Barbazan, P., Dardaine, J., Gonzalez, J.P., Phuangkoson, N., Cuny, G., 1999. Characterization of three microsatellite loci for *Aedes aegypti* (Diptera: Culicidae) and their use for population genetic study. Southeast Asian J. Trop. Med. Public Health 30, 482–483.

Becker, J., Vos, P., Kuiper, M., Salamini, F., Heun, M., 1995. Combined mapping of AFLP and RFLP in barley. Mol. Gen. Genet. 249, 65–73.

Bruford, M.W., Wayne, R.K., 1993. Microsatellites and their application to population genetic studies. Curr. Opin. Genet. Dev. 3, 939–943.

Dumas, V., Herder, S., Bebba, A., Cadoux-Barnabé, C., Bellec, C., Cuny, G., 1998. Polymorphic microsatellites in *Simulium damnosum* s.l. and their use for differentiating two savannah populations: implications for epidemiological studies. Genome 41, 154–161.

Estoup, A., Solignac, M., Harry, M., Cornuet, J-M., 1993. Characterization of (GT)n and (CT)n microsatellites in two insects species: *Apis mellifera* and *Bombus terrestris*. Nucl. Acids Res. 21, 1427–1431.

Estoup, A., Scholl, A., Pouvreau, A., Solignac, M., 1995. Monoandry and polyandry in bumble bees (Hymenoptera;

Bombinae) as evidence by highly variable microsatellites. Mol. Ecol. 4, 89–93.

Estoup, A., Solignac, M., Cornuet, J-M., Goudet, J, Scholl, A., 1996. Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. Mol. Ecol. 5, 19–31.

Felsenstein, J., 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Department of Genetics, University of Washington, Seattle.

Gertsch, P., Pamilo, P., Varvio, S.L., 1995. Microsatellites reveal high genetic diversity within colonies of *Camponotus* ants. Mol. Ecol. 4, 257–260.

Gibson, J.R., Slater, E., Xerry, J., Tompkins, D.S., Owen, R.J., 1998. Use of an amplified-fragment length polymorphism technique to finger print and differentiate isolates of *Helicobacter pylori*. J. Clin. Microbiol. 36, 2580–2585.

Gomez-Dantes, H., Ruiz-Matus, C., Montesano, R., 1996. Dengue hemorrhagic fever in Mexico, 1995. Mosquito Vector Symposium 1995 in J. Am. Mosq. Control Assoc. 12, 469.

Goostrey, A., Carss, D.N., Noble, L.R., Piertney, S.B., 1998. Population introgression and differentiation in the great cormorant *Phalacrocorax carbo* in Europe. Mol. Ecol. 7, 329–338.

Gorrochotegui-Escalante, N., Garcia-Franco, F., Fernandez-Salas, I., Munoz, M.L., 1998. Population genetic structure of *Aedes aegypti*, the vector of dengue in Mexico using RAPD-PCR. Mosquito Vector Symposium in J. Am. Mosq. Control Assoc. 14, 227–228.

Goudet, J., 1995. FSTAT (version 1.2): a computer program to calculate *F*-statistics. J. Hered. 86, 485–486.

Gubler, D.J., 1998. Dengue and dengue haemorrhagic fever. Clin. Microbiol. Rev. 11, 480–496.

Herbers, J-M., Mouser, R.L., 1998. Microsatellite DNA markers reveal details of social structure in forest ants. Mol. Ecol. 7, 299–306.

Hughes, C.R., Queller, D.C., 1993. Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. Mol. Ecol. 2, 131–137.

Janssen, P., Coopman, R., Huy, G., Swings, J., Bleeker, M., Vos, P., Zabeau, M., Kersters, K., 1996. Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxonomy. Microbiology 142, 1881–1893.

Jarne, P., Lagoda, J.L., 1996. Microsatellites, from molecules to populations and back. Trends Ecol. Evol. 11, 424–429.

Kamau, L., Lehmann, T., Hawley, W.A., Orago, A.S.S., Collins, F.H., 1998. Microgeographic genetic differentiation of *Anopheles gambiae* mosquitoes from Asembo bay Western Kenya: a comparison with Kilifi in coastal Kenya. Am. J. Trop. Med. Hyg. 58, 64–69.

Lanzaro, G.C., Zheng, L., Toure, Y.T., 1995a. Microsatellite DNA and isozyme variability in a West African population of *Anopheles gambiae*. Insect. Mol. Biol. 4, 105–112.

Lanzaro, G.C., Warburg, A., 1995b. Genetic variability in phlebotomine sandflies: possible implications for leishmaniasis epidemiology. Parasitol. Today 11, 151–154.

Lin, J.J., Kuo, J., Ma, J., 1996. A PCR-based DNA fingerprinting technique: AFLP for molecular typing of bacteria. Nucl. Acids Res. 24, 3649–3650.

Linnaeus, C. 1762. – Zweyter Theil, enthalt Beschreibungen veschiedener wichtiger Naturalien. Pp 267-606. *In* Hasselquist, F., Reise nach Palastina in den Jahren von 1749 bis 1752. 606 pp. Rostock, Germany

Loxdale, H.D., Lushai, G., 1998. Molecular markers in entomology. Bull. Entomol. Res. 88, 577–600.

Mackill, D.J., Zhang, Z., Redona, E.D., Colowit, P.M., 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. Genome 39, 969–977.

Michalakis, Y., Veuille, M., 1996. Length variation of CAG/CAA trinucleotide repeats in natural populations of *Drosophila melanogaster* and its relation to the recombinant rate. Genetics 143, 1713–1725.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, USA.

Pinheiro, F.P., Corber, S.J., 1997. Global situation of dengue and dengue haemorrhagic fever, and its emergence in the Americas. World Health Stat. Q. 50, 161–169.

Ravel. S., Hervé, J-P., Diarrassouba, S., Kone, A., Cuny, G. Microsatellite markers for population genetic studies in Aedes aegypti from Côte d'Ivoire: evidence for microgeographic genetic differentiation of mosquitoes from Bouaké. Me. Vet. Entomol. Submitted 2001.

Raymond, M., Rousset, F., 1995. Genepop (version 1.2): population genetic software for exact tests and ecumenicism. J. Hered. 86, 248–249.

Reiter, P., 1996. Oviposition et dispersion d'*Aedes aegypti* dans l'environnement urbain. Bull. Soc. Path. Ex. 89, 120–122.

Reineke, A., Karlovsky, P., Zebitz, C.P.W., 1998. Preparation and purification of DNA from insects for AFLP analysis. Insect Mol. Biol. 7, 95–99.

Reineke, A., Karlovsky, P., Zebitz, C.P.W., 1999. Amplified fragment length polymorphism analysis of different geographic populations of the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae). Bull. Entomol. Res. 89, 79–88.

Rongnoparut, P., Yaicharoen, S., Sirichotpakorn, N., Rattanarithikul, R., Lanzaro, G.C., Linthicum, K.J., 1996. Microsatellite polymorphism in *Anopheles maculatus*, a malaria vector in Thailand. Am. J. Trop. Med. Hyg. 55, 589–594.

Rongnoparut, P, Sirichotpakorn, N., Rattanarithikul, R., Yaicharoen, S., Linthicum, K.J., 1999. Estimates of gene flow among *Anopheles maculatus* populations in Thailand using microsatellite analysis. Am. J. Trop. Med. Hyg. 60, 508–515.

Simard, F., Fontenille, D., Lehmann, T., Girod, R., Brutus, L., Gopaul, R., Dournon, C., Collins, F.H., 1999. High amounts of genetic differentiation between populations of the malaria vector *Anopheles arabiensis* from West Africa and eastern outer islands. Am. J. Trop. Med. Hyg. 60, 1000–1009.

Solano, P., Duvallet, G., Dumas, V., Cuisance, D., Cuny, G., 1997. Microsatellite markers for genetic population studies in *Glossina palpalis* (Diptera: Glossinidae). Acta Trop. 65, 175–180.

Solano, P., De La Rocque, S., Cuisance, D., Geoffroy, B., De Meeus, T., Cuny, G., Duvallet, G., 1999. Intraspecific

variability in natural populations of *Glossina palpalis gambiensis* from West Africa, revealed by genetic and morphometric analyses. Med. Vet. Entomol. 13, 401–407.

Tabachnick, W.J., Powell, J.R., 1978. Genetic structure of the East African domestic populations of *Aedes aegypti*. Nature 272, 535–537.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23, 4407–4414.

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

Yan, G., Romero-Severson, J., Walton, M., Chadee, D.D., Severson, D.W., 1999. Population genetics of the yellow fever mosquito in Trinidad: comparison of amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) markers. Mol. Ecol. 8, 951–963.