Gene Flow Between Domestic and Sylvan Populations of *Aedes aegypti* (Diptera: Culicidae) in North Cameroon

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J. Med. Entomol. 45(3): 391–400 (2008)

ABSTRACT Polymorphisms at eight microsatellite loci and a fragment of the mitochondrial DNA (mtDNA)-ND4 gene were surveyed in *Aedes aegypti* (L.) (Diptera: Culicidae) populations collected from six localities in North Cameroon, with emphasis on comparing domestic versus sylvan populations. The microsatellites revealed significant genetic differentiation among sylvan populations, with mean $F_{ST} = 0.066$. Domestic collections were genetically homogeneous (mean $F_{ST} = 0.012$). No pattern of isolation by distance was detected, and one of highest levels of genetic differentiation was estimated between populations sampled a few kilometers apart, each in a distinctly different ecological environment ($F_{ST} = 0.076$). Analyses of mtDNA-ND4 polymorphisms and divergence between the two neighboring populations revealed increased genetic diversity within the domestic population, with molecular signatures suggesting recent demographic expansion, whereas a single haplotype was observed in the sylvan sample. These data suggest reduced gene flow between sylvan and domestic *Ae. aegypti* populations in North Cameroon, reminiscent of the situation for *Ae. aegypti* in Kenya in East Africa.

KEY WORDS *Ae. aegypti*, microsatellite, mitochondrial DNA, population genetics, Cameroon

The mosquito *Aedes aegypti* (L.) (Diptera: Culicidae) is the most important vector of yellow fever virus (family *Flaviviridae*, genus *Flavivirus*, YFV) and dengue virus (family *Flaviviridae*, genus *Flavivirus*, DENV) (Gubler 2002). Despite the availability of a safe and effective vaccine against YFV, yellow fever remains an important public health problem, particularly in Africa (Barrett and Monath 2003). No effective vaccine is available against DENV, and an estimated 100 million new infections occur each year involving >100 countries (Lam 1998). Since its emergence in south Asia in the mid-1950s, dengue hemorrhagic fever is now endemic throughout Asia, the Americas, and the Pacific region, and its incidence is increasing worldwide (Gubler 2002). Furthermore, *Ae. aegypti* was the primary vector of Chikungunya virus in several outbreaks in 2005–2006 that occurred in Kenya, Comoro islands, and India, with >1 million cases (Yergolkar et al. 2006, Charrel et al. 2007).

Bionomical and morphological variations reported throughout the distribution range of *Ae. aegypti* led to the description of three morphological forms or subspecies, named *Ae. aegypti* sens. str., *Ae. aegypti* ssp. *formosus*, and *Ae. aegypti* variety *queenslandensis*, which were differentiated based on color of the tegument and the abdominal scales patterns (Mattingly 1957). The taxonomic status of these forms was subsequently questioned (McClelland 1974), resulting in the description of only two taxa: a cosmotropical "light domestic form," corresponding to *Ae. aegypti* sens. str.; and an outdoor (sylvan) "dark form," corresponding to *Ae. aegypti* spp. *formosus*, confined to sub-Saharan Africa (Tabachnick et al. 1979, Powell et al. 1980). The dark form typically breeds away from human habitations in natural breeding sites (e.g., rock pools, tree holes, and leaf axils), and it rarely feeds on humans (Mattingly 1957, Christophers 1960). The sylvan form is considered ancestral to the domestic form because only *Ae. ae. aegypti* is considered to possess the domestic behavior of strong association with human habitats, breeding indoors in water storage containers and readily biting humans among the 34 other species of the subgenus *Stegomyia* in Africa (Christophers 1960). Adaptation to anthropogenic environments is thought to be incidental to the subsequent radiation of the light form out of Africa, through human-mediated transportation (Powell et al. 1980, Tabachnick 1991, Failloux et al. 2002).

In Kenya in East Africa, both forms seem genetically differentiated, although the level of genetic differentiation demonstrated by isozyme markers revealed substantial gene flow between them, suggesting these

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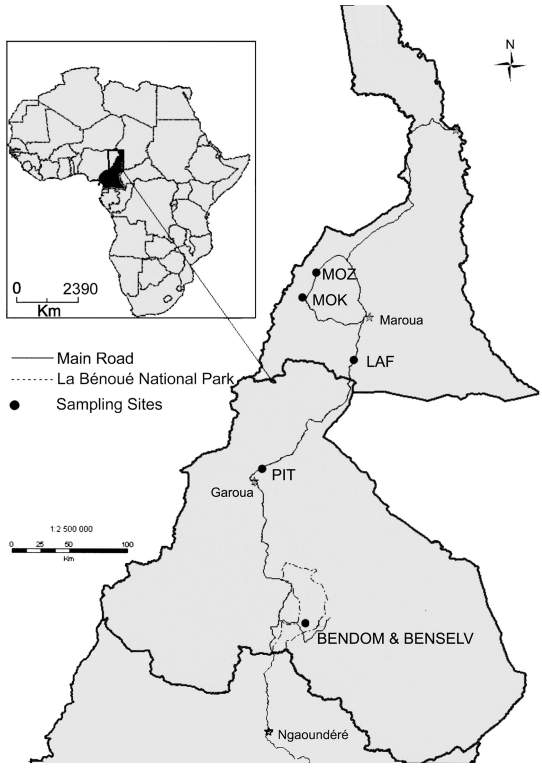

Fig. 1. Location of *Ae. aegypti* samples collected in Cameroon in 2004.

forms might represent distinct adaptive peaks of a single, polytypic species (Tabachnick et al. 1979, Wallis et al. 1983). The dark form shows a greater tendency to colonize anthropogenic environments in Central and West Africa (Mattingly 1957). Genetic analysis using 22 isozyme encoding loci showed that *Ae. aegypti* populations represented by 15 populations in five West African countries were all genetically related to *Ae. ae. formosus*, and genetically more homogeneous, compared with either East African *Ae. ae. aegypti* or East African *Ae. ae. formosus* (Wallis et al. 1983). In contrast to Kenya in East Africa, all *Ae. aegypti* sampled in West Africa were genetically *Ae. ae. formosus* (Tabachnick 1991), including an urban, domestic population responsible for an urban outbreak of yellow fever in Nigeria in 1986 (Miller et al. 1989). This prompted speculation that the absence of *Ae. ae. aegypti* in West Africa was related to a breakdown in barriers to gene flow between the forms that did not occur in East Africa (Tabachnick 1991). A study using microsatellite DNA markers provided information that four *Ae. aegypti* populations from the Republic of Ivory Coast were genetically differentiated from one another (Ravel et al. 2002).

Because vector competence and biological traits potentially involved in virus transmission to humans vary according to *Ae. aegypti* forms and geographical origin (Tabachnick et al. 1985, Failloux et al. 2002), unambiguous characterization of the distinct genetic entities that make up *Ae. aegypti* as well as a clear understanding of the worldwide colonization history of the species would improve comprehension of the epidemiology of the pathogens *Ae. aegypti* transmits. Here, we assessed genetic diversity in samples of *Ae. aegypti* collected from sylvan and domestic environments in North Cameroon. The level of genetic differentiation and gene flow between domestic and sylvan populations were estimated using microsatellite genotyping and mitochondrial DNA (mtDNA) sequencing.

Materials and Methods

Study Sites and Mosquito Sampling. *Ae. aegypti* mosquitoes were sampled as eggs, larvae, and adults in different habitats in North Cameroon during the 2004 early rainy season. Figure 1 shows the geographical localization of sampling sites in Cameroon and Table 1 summarizes information on sample designation, collection method, and habitat characterization. Each sample consisted of larvae, eggs, or both collected from a single breeding site in each location. Based on environment and ecology, six samples were classified as sylvan or domestic. MOK and PIT samples were both collected in earthenware jars (canaris) traditionally located outdoors at the doorstep of human dwellings and in which villagers store water for domestic activities. The BENDOM sample originated from the "Black Buffalo camp" in the Benoué National Park. Mosquitoes were sampled as larvae and emerging adults in an outdoor canari located inside the camp. Moreover females were highly anthropophilic, with a human biting rate (HBR) reaching 11 bites per human per h (unpublished data). In contrast to the BENDOM sample, BENSELV was sampled ≈2 km from the camp, in an undisturbed sylvan environment within the natural fauna reserve, where *Ae. aegypti* larvae were collected from a rock pool. All human landing experiments conducted at this location failed to collect any mosquitoes, whereas eggs of the species were found in

Table 1. Description of *Ae. aegypti* samples collected in Cameroon in 2004

Sample name	Locality	Stage sampled	Ecology
BENSELV	Black Buffalo Camp (Benoué National Park)	Larvae in rock pool	Sylvan
MOZ	Mozogo	Eggs in tree hole	Sylvan
LAF	Laf	Larvae in tree hole	Sylvan
BENDOM	Black Buffalo Camp (Benoué National Park)	Adults and larvae in canari	Domestic
MOK	Mokolo	Larvae in canari	Domestic
PIT	Pitoa	Larvae in canari	Domestic

Table 2. Microsatelitte markers used to characterize *Ae. aegypti*

Locus	GenBank accession no.	Core repeat	Primer designation[a]	Primer sequence (5′→3′)	T_a (°C)[b]
34/72	AF338656	$GAAAA(GA)_6CAGACAGGAAA$	34/72-FOR-FAM	CGT AGT GAT TCT GTG ATA	50
			34/72-REV	TGG CAT CAG ATT CAG TAA	
38/38	AF338655	$GCT(GTT)_2GCTGTT(GCT)_3(GTT)_3GCT$	38/38-FOR-VIC	CGG TGG ACG AAT CAT	56
			38/38-REV	GAT GCC GCC TAG TCC AAT	
AEDGA	U28803	$(GAA)_3(GAC)_4(GAA)_3$	AEDGA-FOR-VIC	CCG AAG AAA TTG GGG TGA CC	55
			AEDGA REV	CCT CTC GGT GTT CGC TAA CC	
AED19	U91680	$GGAC(GGA)_5$	AED19-FOR-FAM	GTA TGA CAA CTC TGG AAT GG	56
			AED19-REV	TTA TGG AAC TGG TAA GCC C	
AEDC	T58313	$(GTA)_6(ACG)(GTA)_3$	AEDC-FOR-FAM	TGC AGG CCC AGA TGC ACA GCC	58
			AEDC-REV	TCC GCT GCC GTT GGC GTG AAC	
A10	DU169901	$(CT)_3CGAT(CT)_{10}TT(CT)$	A10-FOR-PET	ATC CCG AAA ACA AAT CGT GA	58
			A10-REV	ATC GAA CAT CGC TTC CAA CT	
M313	DU169909	$ATG_5(ATA)ATG$	M313-FOR-PET	CAC CTC GTG ACA TAC AAA CAC C	60
			M313-REV	ACG TAC CCA AGC CAC GTA CA	
H08	DU169903	TCG_7	H08-FOR-NED	AAA AAC CAC GAT CAC CGA AG	60
			H08-REV	ACG CGA TCA CAC ACT GAA AAT G	

[a] FAM, VIC, NED, and PET refer to fluorescent phosphoramidite dyes used to end label one of the two microsatellite markers.
[b] T_a, annealing temperature.

ovitraps and rock pools (unpublished data), suggesting zoophilic behavior The LAF sample consisted of *Ae. aegypti* larvae collected from a tree hole in a relic gallery forest, located 2 km outside the village Laf, 50 km south of the town of Maroua. The MOZ sample consisted of desiccated eggs collected with organic matter from a tree hole in a national fauna reserve that is remote from human settlements. All three former samples were considered as domestic (e.g., MOK, PIT, and BENDOM), whereas the latter were regarded as sylvan (e.g., BENSELV, LAF, and MOZ). Eggs and larvae were reared to adults and morphologically identified as *Ae. aegypti.* All mosquitoes were dark to darkish, suggesting they were all *Ae. ae. formosus,* although some specimens presented pale scales spots on first tergites. Specimens were stored individually at −20°C until molecular analysis.

Microsatellite Analysis. DNA was extracted from field-collected mosquitoes by using DNAZOL (Invitrogen, Carlsbad, CA) as described by Huber et al. (2002). DNA pellets were resuspended in water and stored at −20°C until analysis. Genetic variability was assessed at eight microsatellite markers. Based on allele size range and available data on the quality of amplification and ease of genotype scoring, we selected loci *34/72* and *38/38* (Huber et al. 2001), *AEDGA*, *AED19*, and *AEDC* (Ravel et al. 2002), and *A10*, *M313*, and *H08* (Chambers et al. 2007). Characteristics of these loci are given in Table 2.

DNA amplification was performed using a 9600 thermocycler (PerkinElmer Life and Analytical Sciences, Boston, MA) in 25-μl reactions containing 4 μl (2 μl for *A10*, *M313*, and *H08*) of a one-fifth DNA dilution, 2.5 μl of 10× reaction buffer (QIAGEN, Valencia, CA), 1.2 mM $MgCl_2$ (only for *34/72*, *38/38*, *AEDGA*, *AED19*, and *AEDC*), 125 μM of each dNTP (Eurogentec, Seraing, Belgium), 10 pmol of each primer, and 0.5 U of *Taq* polymerase (QIAGEN). The 5′ end of the forward primer was labeled with fluorescent dye (Table 2). Cycling temperatures were as follows for loci *34/72*, *38/38*, *AEDGA*, *AED19*, and *AEDC:* five cycles of 2 min at 96°C, 30 s at TA (annealing temperature), 1.15 min at 72°C, followed by of 35 cycles of 30 s at 95°C, 30 s at TA, 1.15 min at 72°C, and ending with 30 min at 72°C. Cycling temperatures were as follows for loci *A10*, *M313*, and *H08:* 5 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at TA, and 30 s at 72°C followed by 30 min at 72°C.

Polymerase chain reaction (PCR) products were diluted 1/15 in water and pooled with other compatible products according to allele size range and fluorescent dye. Pools were prepared by adding to 1 μl of each diluted amplification product, 0.4 μl of GS 500 Liz internal size standard (Applied Biosystems, Foster City, CA), and HD formamide, for a total volume of 20 μl. The mixture was heated at 94°C for 3 min before migration in an automatic sequencer ABI Prism 3100 (Applied Biosystems). Microsatellite alleles were scored using GeneMapper software package (Applied Biosystems).

Genetic diversity by locus and sample was characterized by estimates of the unbiased expected heterozygosity (H_e, Nei 1987) and allelic richness (R_s, El Mousadik and Petit 1996), available in FSTAT2.9.3.2 (Goudet 1995). Linkage disequilibrium between pairs of loci, deviations from Hardy–Weinberg equilibrium (HWE) and genetic differentiation indices were estimated using GENEPOP3.3 software (Raymond and Rousset 1995). F_{IS} and F_{ST} estimates were calculated using the formula of Weir and Cockerham (1984), and they were tested for statistical significance with exact tests available in GENEPOP3.3. The overall significance of multiple tests was estimated by Fisher combined probability test. Critical significance levels for multiple testing were corrected using the sequential Bonferroni procedure (Holm 1979). Kruskal–Wallis tests were performed for mean comparisons using R software (R Development Core Team 2005).

A Bayesian approach was used to infer the number of clusters (K) in the data set without prior information of the sampling locations, available in STRUCTURE 2.2 (Pritchard et al. 2000). A model where the allele

Table 3. Genetic variability at microsatellite loci from *Ae. aegypti* populations surveyed in North Cameroon

Locus	Estimate[a]	All samples[b] (n = 40)	Sylvan subgroup			Domestic subgroup		
			BENSELV (n = 40)	MOZ (n = 15)	LAF (n = 40)	BENDOM (n = 40)	MOK (n = 13)	PIT (n = 28)
34/72	R_s	3.63	2.65	4.84	3.92	3.32	3.00	3.45
	H_e	**0.52**	**0.30**	0.55	**0.48**	0.55	0.51	0.56
38/38	R_s	2.90	3.07	2.00	2.51	3.06	2.92	2.71
	H_e	**0.31**	**0.49**	0.08	0.35	0.37	0.33	0.42
AEDGA	R_s	2.29	1.85	1.00	1.85	2.52	3.84	1.98
	H_e	**0.08**	-	-	0.12	0.19	0.29	**0.22**
AED19	R_s	3.08	2.97	3.00	2.32	2.30	2.92	2.48
	H_e	0.43	0.52	0.56	0.50	0.47	0.52	0.33
AEDC	R_s	2.42	1.96	3.00	1.31	1.90	2.92	2.69
	H_e	**0.13**	0.19	0.54	0.26	0.07	0.28	0.15
A10	R_s	6.04	5.85	4.92	5.64	6.41	5.77	5.78
	H_e	**0.48**	0.64	0.67	**0.74**	**0.77**	0.69	0.72
M313	R_s	3.70	2.00	3.00	3.69	3.48	4.00	3.87
	H_e	0.50	0.45	0.55	0.63	0.62	0.75	0.66
H08	R_s	5.40	3.21	5.76	5.64	4.85	4.92	5.36
	H_e	**0.57**	0.40	0.81	0.73	**0.74**	0.72	0.69
Mean across all loci	R_s	3.7	2.9	3.4	3.4	3.5	3.8	3.5
	H_e	**0.37**	**0.37**	**0.48**	**0.46**	**0.47**	0.51	**0.46**
	R_s			3.21			3.60	
	H_e			**0.42**			**0.48**	

Values in bold represent significant deficit in heterozygotes ($P < 0.05$ after correction using the Bonferroni procedure (Holm 1979).
[a] R_s, allele richness (El Mousadik and Petit 1996) and H_e, Nei's unbiased estimate of expected heterozygosity (Nei 1978).
[b] n, number of mosquitoes analyzed.

frequencies were correlated within populations was assumed (λ was set at 1, the default value). The software was run with the option of admixture, allowing for some mixed ancestry within individuals, and α was allowed to vary. We did 20 independent runs for each value of K (K = 1–9), with a burn-in period of 100,000 iterations and 100,000 replications. The method of Evanno et al. (2005) was used to determine the most likely number of clusters. This approach uses an ad hoc quantity, ΔK, based on the second-order rate of change of the likelihood function between successive values of K.

ND4 Polymorphism. A 363-bp region of the mitochondrial NADH deshydrogenase subunit 4 (*ND4*) gene was amplified from DNA samples from BENDOM and BENSELV collections. DNA fragments were PCR amplified in 50-μl reactions containing 4 μl of a 1/10 template DNA dilution, 5 μl of 10× reaction buffer, 250 μM of each dNTP (Eurogentec), 20 pmol of each primer, and 0.5 U of *Taq* polymerase (QIAGEN). Primers used were 5′-ATTGCCTAAGGCTCATGTAG-3′ (FOR-ND4) and 5′-TCGGCTTCCTAGTCGTTCAT-3′ (REV-ND4) (Da Costa-da-Silva et al. 2005). After 2-min denaturation at 94°C, 35 cycles of 1-min denaturation at 94°C, 30 s annealing at 56°C, and 1 min extension at 72°C were performed, followed by 7-min final elongation at 72°C. PCR products were purified using Ampure PCR kit (Agencourt, Beverly, MA). Sequencing of amplified fragments was carried out on single strand by using the ABI Prism BigDye terminator version 1.1. (Applied Biosystems). Each 12-μl reaction contained 1.1 μl of Ready reaction mix (Applied Biosystems), 3.3 μl of 5× sequencing buffer, 11 pmol of FOR-ND4 primer, and 2 μl of purified PCR product. After an initial denaturation step at 96°C for 1 min, 25 cycles of 10 s at 96°C, 10 s at 50°C, and 3 min at 60°C were performed, followed by a final elongation step of 3 min at 72°C. Sequence reactions were purified using Seqclean kit (Agencourt) and analyzed on the ABI 330 XL automatic sequencer (Applied Biosystems).

The absence of insertion/deletion polymorphism allowed for unambiguous sequence alignment using Clustal W (Thompson et al. 1994). ND4-mtDNA sequences were numbered with reference to the published *Ae. aegypti* sequence obtained from the Piura strain originated form Peru, South America (GenBank accession no. DQ177154). Basic sequence statistics including the number of distinct haplotypes per samples (K), number of polymorphic sites (S), and nucleotide diversity (π) were computed using DnaSP 4.0 (Rozas et al. 2003). Neutrality tests were performed using the D statistics of Tajima (1989) and Fu and Li (1993) provided by DnaSP 4.0. Haplotype network based on mtDNA polymorphism was designed using TCS 1.21 (Clement et al. 2000).

Results

Microsatellite Analysis. *Genetic Variability.* Genotypes at eight microsatellite loci were determined for 176 *Ae. aegypti* specimens collected in six locations in North Cameroon in 2004 (Table 3). All loci were polymorphic, showing a number of distinct alleles ranging from four (AEDGA) to 11 (H08) (Fig. 2). AEDGA was monomorphic in BENSELV and MOZ samples. Across all loci, the average R_s and expected H_e ranged from 2.9 (BENSELV) to 3.8 (MOK) and from 0.37 (BENSELV) to 0.51 (MOK), respectively. No statistical difference in diversity indices was detected across samples, nor when samples were grouped according to their ecology ($P > 0.05$; Kruskal–Wallis test). Departures from Hardy–Weinberg proportions were associated with significant heterozygote deficits in seven of 45 possible tests, across

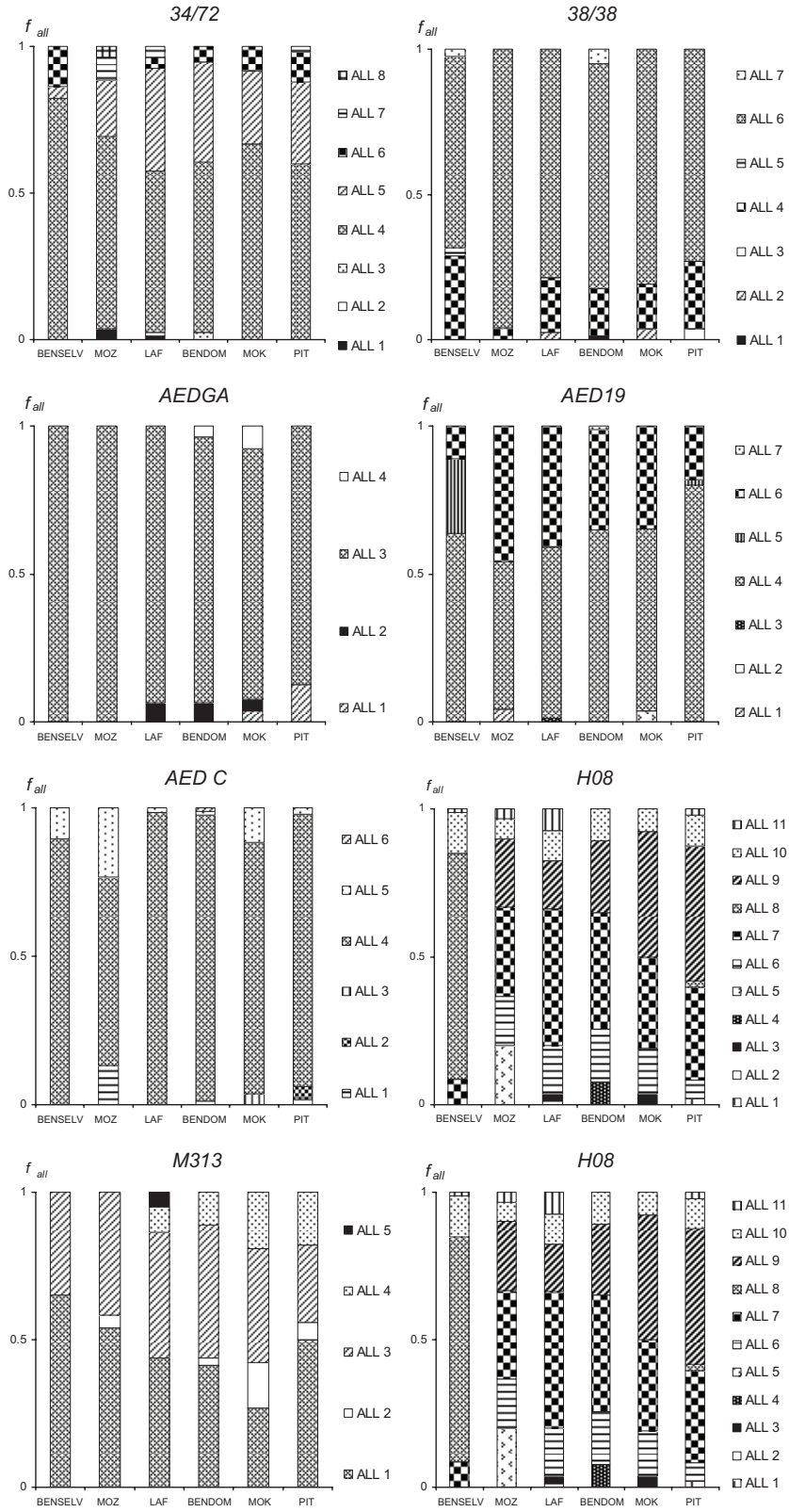

Fig. 2. Allelic distribution at eight microsatellite loci for each *Ae. aegypti* populations from North Cameroon.

Table 4. Pairwise F_{ST} estimates among *Ae. aegypti* populations from North Cameroon

Sample	BENSELV	MOZ	LAF	BENDOM	PIT	MOK
MOZ	0.096††	—				
LAF	0.070††	0.027††	—			
BENDOM	0.076††	0.029†	0.006	—		
PIT	0.075††	0.048	0.025††	0.013	—	
MOK	0.102††	0.025	0.019	0.010	0.013	—

Statistical significance of *F* estimates was assessed using the Fisher exact test of homogeneity of genotyping frequencies (Raymond and Rousset 1995). Underlined, $P < 0.01$; †, $P < 0.001$; and ††, $P < 0.0001$ after correction for multiple tests (Holm 1979).

all loci and collections. Homogeneity of the gene pool was therefore assumed within each sample. Significant linkage disequilibrium was revealed only between locus A10 and loci 34/72, H08, M313, and AEDC in the BENSELV sample.

Genetic Differentiation. Overall genetic differentiation between samples was high and statistically significant ($F_{ST} = 0.042$, $P < 0.0001$). When data were stratified into subgroups (e.g., domestic versus sylvan collections), analyses revealed an absence of genetic differentiation among collections in the domestic subgroup ($F_{ST} = 0.012$, $P > 0.05$), whereas samples from the sylvan subgroup were highly and significantly differentiated from each other ($F_{ST} = 0.066$, $P < 0.0001$). Pairwise estimates of F_{ST} over all loci are presented in Table 4. The BENSELV sample showed the highest degree of genetic differentiation from all other samples ($F_{ST} = 0.070–0.102$, $P < 0.0001$). All three populations from the sylvan subgroup (e.g., BENSELV, LAF, and MOZ) were significantly differentiated from one another with F_{ST} estimates ranging from 0.027 to 0.096 ($P < 0.0001$), whereas no significant genetic differentiation was detected between populations from the domestic subgroup (e.g., BENDOM, MOK, and PIT; $F_{ST} = 0.010–0.012$, $P > 0.05$). No statistically significant correlation was detected between genetic (F_{ST}) and geographic distances between populations, within as well as across groups of populations ($P > 0.23$; Mantel test). This is particularly illustrated by the high and statistically significant F_{ST} estimate observed between the BENDOM and BENSELV samples that were collected a couple of km apart ($F_{ST} = 0.076$, $P < 0.0001$).

Bayesian cluster analysis using STRUCTURE was first performed considering the global data set (all six samples). According to the method described by Evanno et al. (2005) the most likely number of clusters was determined as $K = 3$ (Fig. 3A). STRUCTURE failed to detect a clear clustering because all populations occurred as a mixture of all three genetic clusters

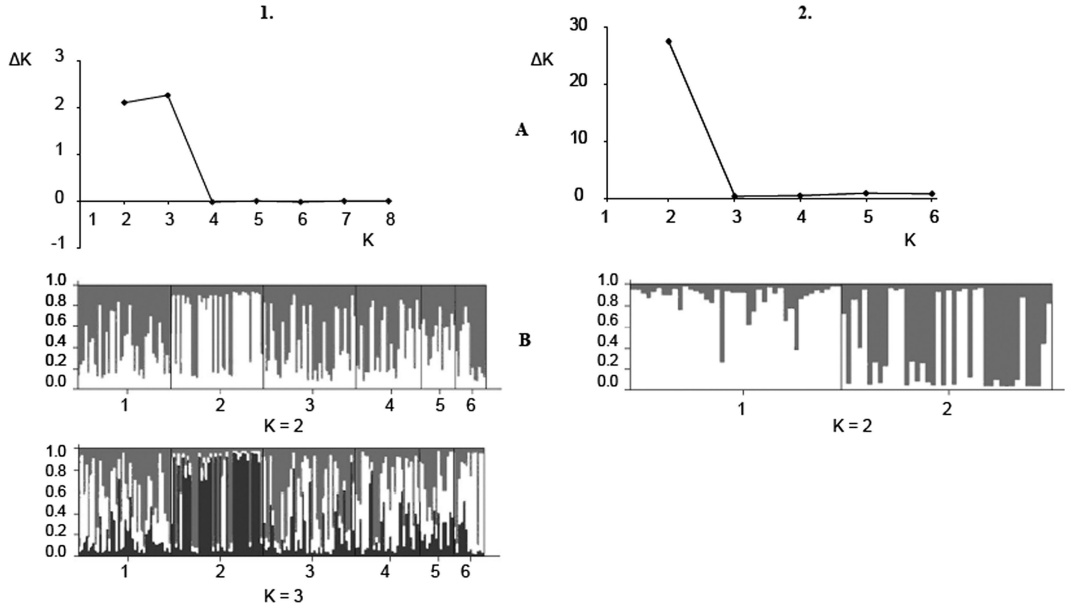

Fig. 3. Bayesian cluster analysis using STRUCTURE (Pritchard et al. 2000). (A) Estimates of Δ*K*, based on the second-order rate of change of the likelihood function with respect to *K* (Evanno et al., 2005), to determine the most likely number of clusters (*K*) in the data set. In case 1 (considering all samples), $K = 2$ or 3. In case 2 (considering BENDOM and BENELV), $K = 2$. (B) Graphical representation of the data set for the most likely *K*, where each color corresponds to a suggested cluster and each individual is represented by a vertical bar. The numbers in the x-axis correspond to a specific sample: 1, BENDOM; 2, BENSELV; 3, LAF; 4, PIT; 5, MOZ; and 6, MOK. The y-axis represents the probability of assignment of an individual to each cluster.

Haplotype	Frequency	*ND4*											
		76	88	100	136	145	154	193	238	271	286	292	316
Piura [DQ177154.1]	-	C	A	G	T	C	G	C	C	G	G	T	T
Haplotype I [EF562501]	27	T	.	.	C	T	A	T	T	A	A	.	C
Haplotype II [EF562502]	2	T	G	.	C	T	A	T	T	A	A	.	C
Haplotype III [EF562503]	1	T	.	.	.	T	A	T	T	A	A	.	C
Haplotype IV [EF562504]	1	.	.	A	.	.	A	.	T	.	A	C	.

Fig. 4. Haplotypes found for the ND4 gene fragment in *Ae. aegypti* populations from The Benoué National Park (North Cameroon). Only polymorphic positions are shown, and these are numbered with reference to the published *Ae. aegypti* (Piura strain) sequence, GenBank accession no. DQ177154.1. Dots represent identity with respect to reference. The frequency indicates the number of times the haplotype was found in the total sample.

identified (Fig. 3B). Nevertheless, the BENSELV sample seemed genetically distinct from the other samples, particularly from BENDOM, supporting the genetic differentiation analysis using F_{ST}. When analysis was performed solely considering BENDOM and BENSELV samples, the most likely number of clusters was $K = 2$. The BENDOM sample mainly contained individuals from a single cluster, whereas BENSELV occurred as a mixture composed of both genetic clusters.

mtDNA Analysis. To more precisely assess the pattern of gene flow between neighboring domestic and sylvan samples in Benoué area, 31 sequences of the mtDNA ND4 gene were retrieved from 17 specimens from the BENDOM sample and 14 from the BENSELV sample. Complete overlap of all fragments spanned 343 nucleotides, among which nine were polymorphic (overall nucleotide diversity, $\pi = 0.00219$), defining four distinct haplotypes (Fig. 4). All four haplotypes were found in the BENDOM ($\pi = 0.00394$) collection whereas only haplotype I was observed in the BENSELV sample.

Significant departure from equilibrium, as determined by the D statistic of Tajima (1989), was found when all 31 individuals were considered as a unique group ($D = -2.12$, $P < 0.05$). A similar trend was observed in the BENDOM population ($D = -1.89$, $P < 0.05$). Significant and negative D value suggests an excess of rare alleles and could reflect demographic instability (population expansion) and/or purifying selection at the sequenced loci. Fu and Li's statistics showed negative and significant values when BENDOM and BENSELV were considered together ($F^* = -2.75$, $P < 0.05$; $D^* = -2.98$, $P < 0.05$). Negative values of F^* (-2.04) and D^* (-2.30) also were observed in the BENDOM sample, although these values were not statistically different from 0 ($P > 0.05$). Negative values of F^* and D^* indicate an excess of recently derived haplotypes and suggest either population expansion or background selection (Fu and Li 1993).

Relationships between haplotypes are represented in Fig. 5. Haplotypes II and III are closely related to the major haplotype I, from which they are separated by a single mutational step. Haplotype IV is clearly isolated from this core group, branching seven mutational steps away from its closest relative (haplotype III). Interestingly, haplotype IV seems more closely

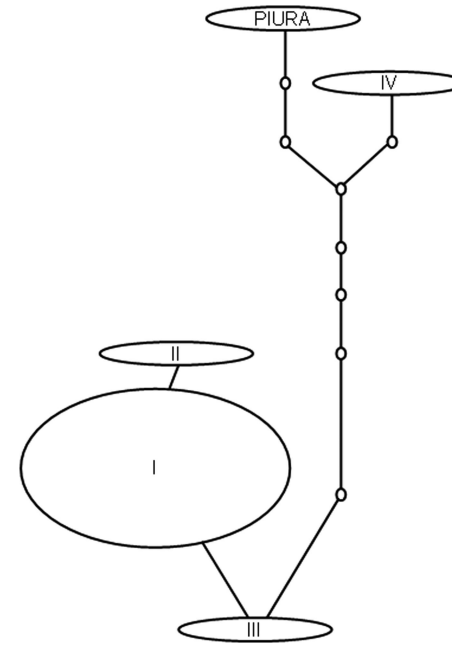

Fig. 5. A 95% parsimony network of the four haplotypes obtained from the mtDNA ND4 sequences as generated by TCS (Clement et al. 2000). Haplotypes are represented as ovals, scaled to reflect their frequencies. Lines connecting haplotypes and small circles (missing or nonsampled haplotypes) represents a single mutation event. PIURA corresponds to the Piura strain (GenBank accession no. DQ177154).

related to the reference haplotype from the Piura strain of *Ae. aegypti*, colonized from South America.

Discussion

Genetic diversity within six *Ae. aegypti* samples from North Cameroon and the level of genetic differentiation between them were assessed using a set of eight microsatellite DNA markers and one mtDNA gene (ND4). The level of polymorphism detected across *Ae. aegypti* populations from Cameroon is comparable with those reported in previous studies that used microsatellite loci 34/72 and 38/38 in Asia (Huber et al. 2002) and loci AEDGA, AED19, and AEDC in West Africa (Ravel et al. 2002). The presence of null alleles at loci AED19 and AEDC reported from West African populations was not detected in the present work. Loci A10, M313, and H08 were never used previously. This study demonstrates their suitability for further use with *Ae. aegypti* natural populations and provides evidence that the present set of microsatellite loci is well adapted and suitable for population genetics studies of *Ae. aegypti* in Africa.

The sampling scheme we used was to compare domestic and sylvan populations of *Ae. aegypti*, in an effort to explore the level of population genetic structuring between ecologically differentiated taxa within the species *Ae. aegypti* in Africa. Microsatellites analyses revealed comparable levels of genetic diversity in both domestic and sylvan populations. However, significant levels of genetic differentiation were detected between sylvan samples, whereas no such trend was observed between domestic samples, suggesting heterogeneous patterns of gene flow among these mosquito populations. Moreover, significant genetic differentiation was also revealed between a domestic and a sylvan population sampled only a few kilometers apart. The domestic sample (BENDOM) was collected from an outdoor domestic container (canari) used for water storage in a remote tourist camp. The sylvan sample (BENSELV) was collected from rock pools in a riverbed, within the surrounding national wildlife reserve where human settlement and land alteration are strictly forbidden. Fixation for the same allele of one of eight microsatellite markers in BENSELV, high level of linkage disequilibrium between loci, and occurrence of a single mtDNA-ND4 haplotype shared by all specimens (whereas four different haplotypes were observed in BENDOM) suggest likely a lower effective population size and increased genetic drift acting on the BENSELV population compared with other collections. If this is the case, then the estimates of population differentiation based on microsatellite allele frequencies comparisons might be biased upward, as was demonstrated, for example, in the malaria mosquito *Anopheles gambiae* (Giles) (Lehmann et al. 1998). However, no significant difference in diversity indices was demonstrated between BENSELV and any other collection, suggesting low, if any, influence of differences in effective populations sizes on our inferences. Additionally, sampling larvae from one or a few larval development sites can bias diversity indices downward, due to possible sampling of siblings from each larval site. Again, homogeneity of estimates of genetic diversity across samples and lack of deviations from HWE in the BENSELV population argues against such bias. The BENSELV sylvan population seems ecologically, as well as genetically, isolated from the other collections studied. Interestingly, however, the results of STRUCTURE suggest that the BENSELV population occurs as a mixture of (at least) two distinct genetic clusters, one cluster of which is almost exclusively constitutive of the BENDOM sample. This result, consistent with the trend for significant heterozygote deficits observed in two of eight loci in the BENSELV sample and significant linkage disequilibrium detected in this sample only, suggests that two (genetically) distinct lineages coexist within BENSELV sample: one lineage representing pure sylvan specimens (ancestral form) and the second lineage (BENDOM-like) representing immigrants from the neighboring domestic population. This suggests, that the widespread domestic population of *Ae. aegypti* found throughout North Cameroon still possesses the ecological plasticity, allowing it to colonize remote sylvan areas. Ecological shift in *Ae. aegypti* behavior (i.e., from domestic to sylvan) was described previously in other parts of the world, for example, on the island of Anguilla where *Ae. ae. aegypti* is able to use rock holes for larval development (Wallis and Tabachnick 1990), and on La Réunion Island where *Ae. aegypti* disappeared from anthropogenic environments and readopted feral ecology after an effective malaria vector control program in 1950s (Hamon 1953, Salvan and Mouchet 1994). Alternatively, we cannot exclude that the contemporary BENDOM population originated from feral, BENSELV-like populations, which still possesses the genetic plasticity allowing adaptation to anthropogenic environments (Trpis and Hausermann 1986). Domestication would have been associated with a series of morphological and behavioral changes, as it has already been described for *Ae. aegypti* (Trpis and Hausermann 1978) and for other insects, particularly in Triatominae species (Schofield et al. 1999).

Reduced genetic exchange between BENDOM and BENSELV populations in the face of small geographic distance between sampling sites, suggest reproductive isolation between sylvan and domestic populations (Coyne and Orr 1998). In the Rabaï district (Kenya), Trpis and Hausermann (1978) proposed the existence of behavioral, seasonal, and habitat isolation between domestic and sylvan *Ae. aegypti* populations. Tabachnick et al. (1979) proposed that assortative mating due to different host preferences helped to maintain genetic differentiation between sylvan and domestic populations of *Ae. aegypti* from Kenya in East Africa. This study demonstrates the same probably applies in North Cameroon settings, although the biological basis for such isolation remains unknown. Meanwhile, the absence of significant genetic structuring among

domestic populations of *Ae. aegypti* in North Cameroon suggests extended gene flow over considerable geographic distance (up to 350 km between BENDOM and MOK; Fig. 1). In anthropogenic environments, *Ae. aegypti* females oviposit in both permanent (water storage) and temporary (rain-dependent) water containers (Christophers 1960). In North Cameroon, as well as in numerous Sahelian areas, water storage in traditional earthenware jars (canaris) is widespread, even when pipe water is available, and constitutes preferential larval breeding sites for *Ae. aegypti* (Rickenbach and Button 1977, Simard et al. 2005). Numerous oviposition sites are therefore available in anthropogenic areas throughout the year, providing ample opportunity for year-round mosquito oviposition. Step-by-step gene flow and population admixture between neighboring anthropogenic areas is also possible in densely populated areas (Paupy et al. 2005), as is the case in North Cameroon. Furthermore, transportation of mosquitoes through human and goods (i.e., canaris and tires) in relation to other agropastoral and/or commercial activities can exacerbate human-mediated dispersal of mosquitoes (Lounibos 2002) and favor population admixture over large geographical distances (Paupy et al. 2005). This latter mechanism probably explains the consistently low F_{ST} estimates between BENDOM and other domestic samples, because, although remote, the Black Buffalo tourist camp is regularly visited and supplied daily. Alternatively, but not mutually exclusive, demographic instability such as large-scale population expansion (after domestication) can explain the lack of genetic structure between domestic samples, in North Cameroon, and probably beyond, as has been postulated for several anopheline diseases vectors (Donnelly et al. 2002). Indeed, signatures for demographic expansion were observed in the BENDOM sample, as demonstrated by negative and statistically significant Tajima' SD and, to a lesser extent, Fu and Li's F* and D* statistics inferred from mtDNA-ND4 polymorphism.

The evidence reported here supports the hypothesis that several distinct ecological lineages of *Ae. aegypti* coexist in Cameroon, as was proposed for Kenya, and they reveal the ecological and genetic diversity of the species on the African continent. Any conclusion on the taxonomic status of the different lineages would be tentative and requires additional field and laboratory studies based on larger sample sizes, higher numbers of genetic markers, and sampling over larger geographic areas. In addition, more work is needed on the biology, ecology, morphology, and cross-population hybridization potential of the *Ae. aegypti* subspecies and forms in different regions, for a better understanding of the natural history and evolutionary mechanisms accompanying the domestication process of *Ae. aegypti* in Africa and its relevance for the transmission and emergence of mosquito-borne diseases such as yellow fever, dengue, and chikungunya.

Acknowledgments

We thank E. W. Chambers and D. W. Severson for kindly providing microsatellite primers and E. Nchoutpouen and J. C. Toto for significant contribution to the fieldwork. We are grateful to the editor and two anonymous reviewers for comments that greatly improved previous versions of this article. We are grateful to F. Lacoste and "La Fondation des Treilles" for support to C.P. This study was funded by the French Institut de Recherche pour le Développement RU016 and Agence Nationale pour la Recherche (ANR-SEST, Epidengue project).

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Received 12 November 2007; accepted 29 January 2008.