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Morphological and genetic variability within *Aedes aegypti* in Niakhar, Senegal

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ABSTRACT

Aedes aegypti (Linné, 1762) is a major vector of arboviruses such as Yellow Fever, Dengue and Chikungunya. In Africa, where the species exhibits major variations in morphology, ecology, behavior and vector competence, two subspecies have been described: a light form, named *Ae. aegypti aegypti* (*Aaa*) with highly domestic and anthropophilic habits and a cosmotropical distribution; and a dark form, referred to as *Ae. aegypti formosus* (*Aaf*), which is endemic to Africa and thrives in sylvan environments. In East Africa, both forms were described to occur in sympatry whereas only *Aaf* was reported from Central/West Africa. However, recent findings suggest *Aaa* was also common in Senegal. Here, we report on a longitudinal survey of morphological and genetic variability of *Ae. aegypti* sampled in the rural environment of Niakhar, Senegal. In agreement with recent findings, most of specimens we analyzed were classified as *Aaa* suggesting typical *Aaf* was scarce in the studied area. Among *Aaa*, significant temporal variations in abdominal pale scales pattern were detected. Depending on the season and the nature of larval breeding places, the specimens (particularly females) tend to segregate in two main morphological groups. Microsatellite-based estimates of genetic differentiation did not provide any clear evidence that the two groups were genetically distinct. Overall, these results improve our understanding of the diversity of *Ae. aegypti* in West Africa, where data are crucially lacking.

1. Introduction

The mosquito *Aedes aegypti* (Linné, 1762) is a major vector of Yellow Fever (YF), Dengue (Gubler, 2002) and Chikungunya viruses (Charrel et al., 2007; Yergolkar et al., 2006) throughout most tropical areas in the world. It is arguably one of most domestic mosquito vectors, feeding predominantly on man, mating and resting indoors and breeding in man-made containers in and around human habitations, especially in urban environments (Morisson et al., 2008).

In Africa however, which is considered the native range of the species (Chistophers, 1960), the biology of *Ae. aegypti* is sometimes less dependent on the presence of man than in other tropical regions. Indeed sylvan populations of *Ae. aegypti* breeding in natural water collections such as rock pools, tree holes and leaf axils have been reported (Mattingly, 1957; Paupy et al., 2008). Morphological and bionomical differences were extensively described in Kenya (East Africa), between a dark, sylvan form and a light, domestic form which appeared genetically differentiated using isoenzymatic markers (Petersen, 1977; Tabachnick and Powell, 1979; Wallis et al., 1983). In East Africa, the dark form, also known as *Ae. aegypti formosus* (*Aaf*) is considered indigenous and ancestral, whereas the presence of the derived light form, also called *Ae. aegypti aegypti* (*Aaa*) is thought to result from secondary introduction. Tabachnick (1991) presented a verbal argument for the role of demographic history and vicariance in generating two subspecies of *Ae. aegypti* in Africa. The author proposed that the transition from a "green Sahara" to the present hyperarid desert, which started in mid-Holocene (between 6000 and 4000 years ago, Kröpelin et al., 2008), promoted the isolation of two genetic pools from the original sylvan form (*Aaf*) on both sides of the desert. This model argues that *Ae. aegypti* developed its domestic habits north of the Sahara desert, once separated from the core of the species range in sub-Saharan Africa, where the species persisted as ancestral. The domestic *Aaa* further spread out of Africa, and was later re-introduced in some costal regions of sub-Saharan Africa (e.g., Kenya). In East Africa, gene flow between *Aaf* and *Aaa* was shown to be restricted to the peridomestic habitat where both species co-occur and hybrids with intermediate phenotypes (e.g., morphology and behavior) can be found (Trpis and Hausermann, 1978).

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The situation in West/Central Africa remains unclear, as very few investigations were conducted in this area. Until recently, the idea of Mattingly (1957), which considered *Aaf* as the only subspecies occurring in West and Central Africa, was largely admitted. However, *Ae. aegypti* was recently reported from both sylvan and anthropogenic environments, including large urban centers in Senegal, Cameroon and Gabon (Huber et al., 2008; Paupy et al., 2008, 2009), which represents much broader ecological preferences than typically admitted for *Aaf*. In addition, McClelland (1974) described significant morphological variability within West/Central African populations of *Ae. aegypti*, with specimens showing a mixture of morphological characters that would not allow unambiguous classification as *Aaf* or *Aaa*. Additional studies further supported the presence of populations ecologically (Diarrassouba and Dossou-Yovo, 1997) and morphologically (Hervy, 1977; Huber et al., 2008) similar to *Aaa*. In a recent study carried out in Senegal, Sylla et al. (2009) unambiguously demonstrated that there was a clear northwest–southeast cline in the abundance of *Aaa vs Aaf*. Based on analyses of SNPs showing very little or no genetic differentiation between both subspecies, the authors also suggested *Aaf* and *Aaa* were monophyletic and that *Aaf* is the ancestor of *Aaa* in West Africa. In addition, indirect evidences for chromosomal polymorphism (inversions) in *Aaf* strain originated in Senegal were recently discovered, which might impact on the distribution of molecular and phenotypic (morphology and behavior) polymorphisms within and among natural populations (Bernhardt et al., 2009).

The morphological characters used for the distinction of *Aaa* and *Aaf* – the pattern of pale scales on abdominal tergites and the color of integument of the body – were defined by Mattingly (1957). This author stated the dark scaled parts of the body were generally blacker in *Aaf*, in which pale scales on the first abdominal tergite are never observed. The type form *Aaa*, was defined as "either distinctly paler and browner (at least in females) than *Aaf* or with pale scaling on the first abdominal tergite or both". Among mosquitoes morphologically identified as *Aaf*, significant variation in the pattern of extension of abdominal pale scales was reported in Africa and particularly in areas were both subspecies were sympatric (McClelland, 1974; Hervy, 1977). This aspect of morphological variations in *Ae. aegypti* and its biological significance remains understudied and poorly understood. Because vector competence and biological traits potentially involved in virus transmission to man vary according to *Ae. aegypti* morphological form and geographical origin (Tabachnick et al., 1985; Failloux et al., 2002; Sylla et al., 2009), unambiguous characterization of the distinct genetic entities that make up *Ae. aegypti* in Africa would provide precious elements in risk assessment for viral diseases such as Dengue and Chikungunya which recently emerged in Africa (Peyrefitte et al., 2007; De Lamballerie et al., 2008; Paupy et al., 2009). As a step forward to this endeavor, we assessed morphological and genetic diversity in samples of *Ae. aegypti* collected in Niakhar district in Senegal where several epidemics of YF and Chikungunya have occurred with *Ae. aegypti* as a main vector (Thonnon et al., 1999). First, we estimated the level of local morphological variation based on patterns of abdominal scaling. Second, we estimated the level of genetic differentiation and gene flow between morphological and/or ecological groups, using microsatellite genotyping.

2. Materials and methods

2.1. Mosquito sampling

Mosquitoes were sampled as larvae in Niakhar district, a rural area of central Senegal (Fig. 1), typical of the Sahelian and sub-Sahelian regions of Africa where the rainy season extends from July to October and mean annual rainfall is around 500 mm. Sampling was conducted in 2005 during the dry (May) and rainy (September) seasons in two neighboring villages 1 km apart, Kothiokh (14°28.502′N; 16°32.424′W) and Godel (14°29.564′N; 16°33.396′W). The rainy season corresponds to the highest season of exposure to the bites of *Ae. aegypti* (Remoué et al., 2007). *Aedes aegypti* larvae were collected in three types of breeding sites: domestic, peridomestic and natural. Domestic breeding sites refer to water storage containers (earthenware jars and metallic drums) regularly and consistently filled with water by inhabitants. Peridomestic (e.g., abandoned drums, tires, tin cans in the backyard of human dwellings) and natural (e.g., tree holes, leaf axils) larval development sites were flooded with rainwater during the rainy season only. All larvae from 20 indoor/outdoor domestic containers were sampled during the dry season. During the rainy season all larvae from 20 indoor/outdoor domestic, 10 peridomestic and 10 natural breeding sites were collected. Larvae were pooled according to the type of development sites they were collected from and reared to adults in the insectaries. Emerging mosquitoes were readily identified to species, sexed and stored individually at −20 °C, pending further morphological and molecular analyses.

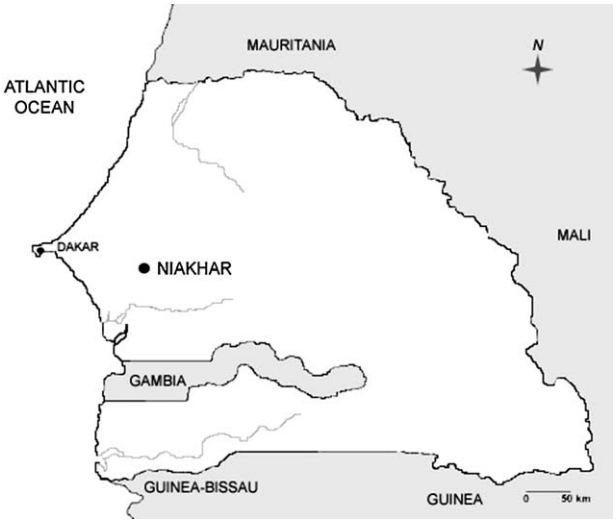

Fig. 1. Map of Senegal showing the localization of the sampling area at Niakhar.

2.2. Morphological analysis

Morphological analyses were performed only on well preserved specimens from both sexes. Specimens were observed and scored according to the method of McClelland (1974). Briefly, the method attributes an individual abdominal pattern value (PV) to each specimen, which calculation considers the number of abdominal tergites exhibiting pale scales spots (excluding apical bands and lateral spots of pale scales). The method differentiates tergites with speckled scales and those that harbor a complete medially pale band of scales. PV ranges from 1 (absence of pale scales on all tergites) to 15 (presence of pale scales on every tergite). Integument coloration, which we considered as subjective and easily biased depending on the investigator, was not considered in our analysis. Influence of season, sex and nature of breeding site on morphology variation was investigated. Comparisons of the distributions of PV across groups were performed using the Wilcoxon test implemented in R software (R Development Core Team, 2005).

Table 1
Characteristics of the microsatellite markers used in the study.

Locus	GeneBank accession no.	Core repeat	Primer designation[a]	Primer sequence (5′ → 3′)	T_a (°C)
34/72	AF338656	GAAAA$(GA)_6$CAGACAGGAAA	34/72-FOR-FAM 34/72-REV	CGT AGT GAT TCT GTG ATA TGG CAT CAG ATT CAG TAA	50
38/38	AF338655	GCT$(GTT)_2$GCTGTT$(GCT)_3(GTT)_3$GCT	38/38-FOR-VIC 38/38-REV	CGG TGG ACG AAT CAT GAT GCC GCC TAG TCC AAT	56
AEDGA	U28803	$(GAA)_3(GAC)_4(GAA)_3$	AEDGA-FOR-VIC AEDGA REV	CCG AAG AAA TTG GGG TGA CC CCT CTC GGT GTT CGC TAA CC	55
AED19	U91680	GGAC$(GGA)_5$	AED19-FOR-FAM AED19-REV	GTA TGA CAA CTC TGG AAT GG TTA TGG AAC TGG TAA GCC C	56
AEDC	T58313	$(GTA)_6(ACG)(GTA)_3$	AEDC-FOR-FAM AEDC-REV	TGC AGG CCC AGA TGC ACA GCC TCC GCT GCC GTT GGC GTG AAC	58
A10	DU169901	$(CT)_3$CGAT$(CT)_{10}$TT(CT)	A10-FOR-PET A10-REV	ATC CCG AAA ACA AAT CGT GA ATC GAA CAT CGC TTC CAA CT 3′	58
M313	DU169909	ATG$_5$(ATA)ATG	M313-FOR-PET M313-REV	CAC CTC GTG ACA TAC AAA CAC C ACG TAC CCA AGC CAC GTA CA	60
H08	DU169903	TCG$_7$	H08-FOR-NED H08-REV	AAA AAC CAC GAT CAC CGA AG ACG CGA TCA CAC ACT GAA AAT G	60

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

2.3. Microsatellite analysis

DNA was extracted from adult mosquitoes of both sexes using DNAZOL (Invitrogen, USA) as described in Huber et al. (2002). DNA pellets were resuspended in 20 μl of sterile water and stored at −20 °C until analysis. Individual genotypes were scored at eight microsatellite markers (Table 1): loci 34/72 and 38/38 (Huber et al., 2001), AEDGA, AED19, AEDC (Ravel et al., 2002) and A10, M313 and H08 (Chambers et al., 2007).

The design, fluorescent labeling and Tm of primers used for microsatellite PCR amplification are given Table 1. PCR amplifications were performed using a 9600 thermocycler (PerkinElmer, USA) in 25-μl reactions containing 4 μl (2 μl for A10, M313 and H08) of a 1/5 DNA dilution, 2.5 μl of 10× reaction buffer (Qiagen, USA), 1.2 mM of $MgCl_2$ (only for 34/72, 38/38, AEDGA, AED19 and AEDC), 125 μM of each dNTP (Eurogentec, Belgium), 10 pmol of each primer and 0.5 U of Taq polymerase (Qiagen, USA). The 5′ end of the forward primer was labeled with a fluorescent dye (Table 1). Cycling temperatures were as follows for loci 34/72, 38/38, AEDGA, AED19, AEDC: 5 cycles of 2 min at 96 °C, 30 s at the annealing temperature (T_a in Table 1), 1.15 min at 72 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at T_a, 1.15 min at 72 °C, and a final extension step at 72 °C for 30 min. For loci A10, M313 and H08, after 5 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at T_a and 30 s at 72 °C were performed, followed by 30 min at 72 °C.

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, USA) 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, USA). Microsatellite alleles were scored using GeneMapper software package (Applied Biosystems, USA).

Genetic diversity by locus and sample and overall was characterized by estimating unbiased expected heterozygosity (H_e, Nei, 1987) and allelic richness (El Mousadik and Petit, 1996) using the software FSTAT2.9.3.2 (Goudet, 1995). Linkage disequilibrium, deviations from Hardy–Weinberg equilibrium (HWE) and genetic differentiation indices were assessed using GENEPOP3.3 software (Raymond and Rousset, 1995). F_{IS} and F_{ST} estimates were calculated according to Weir and Cockerham (1984) and tested for statistical significance with exact tests available in GENEPOP3.3. The overall significance of multiple tests was estimated by Fisher's combined probability tests. Nominal significance levels for multiple testing were corrected using the sequential Bonferroni procedure (Holm, 1979).

A Bayesian approach was used to infer the number of clusters (K) in the dataset without prior on the number of distinct populations in the dataset, as implemented in STRUCTURE 2.2 (Pritchard et al., 2000). A model where the allele 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 30,000 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.

3. Results

Rearing in the insectaries yielded 397 and 351 *Ae. aegypti* adults from larvae collected in the Niakhar area during the dry and rainy seasons, respectively (Table 2).

3.1. Morphological analysis

A total of 247 (156 females and 91 males) and 351 (216 females and 135 males) specimens collected during the dry and rainy season, respectively, were successfully scored. Among them, only

Table 2
Description and yield of *Ae. aegypti* samples collected in Niakhar, Senegal (2005).

Season	Type of larval habitat (N)	Number of emerging adults		
		Males	Females	Total
Dry	Domestic (20)	146	251	397
Rainy	Domestic (20)	50	66	116
	Peridomestic (10)	43	75	118
	Natural (10)	42	75	117
	All (40)	135	216	351

(N): number of breeding sites sampled.

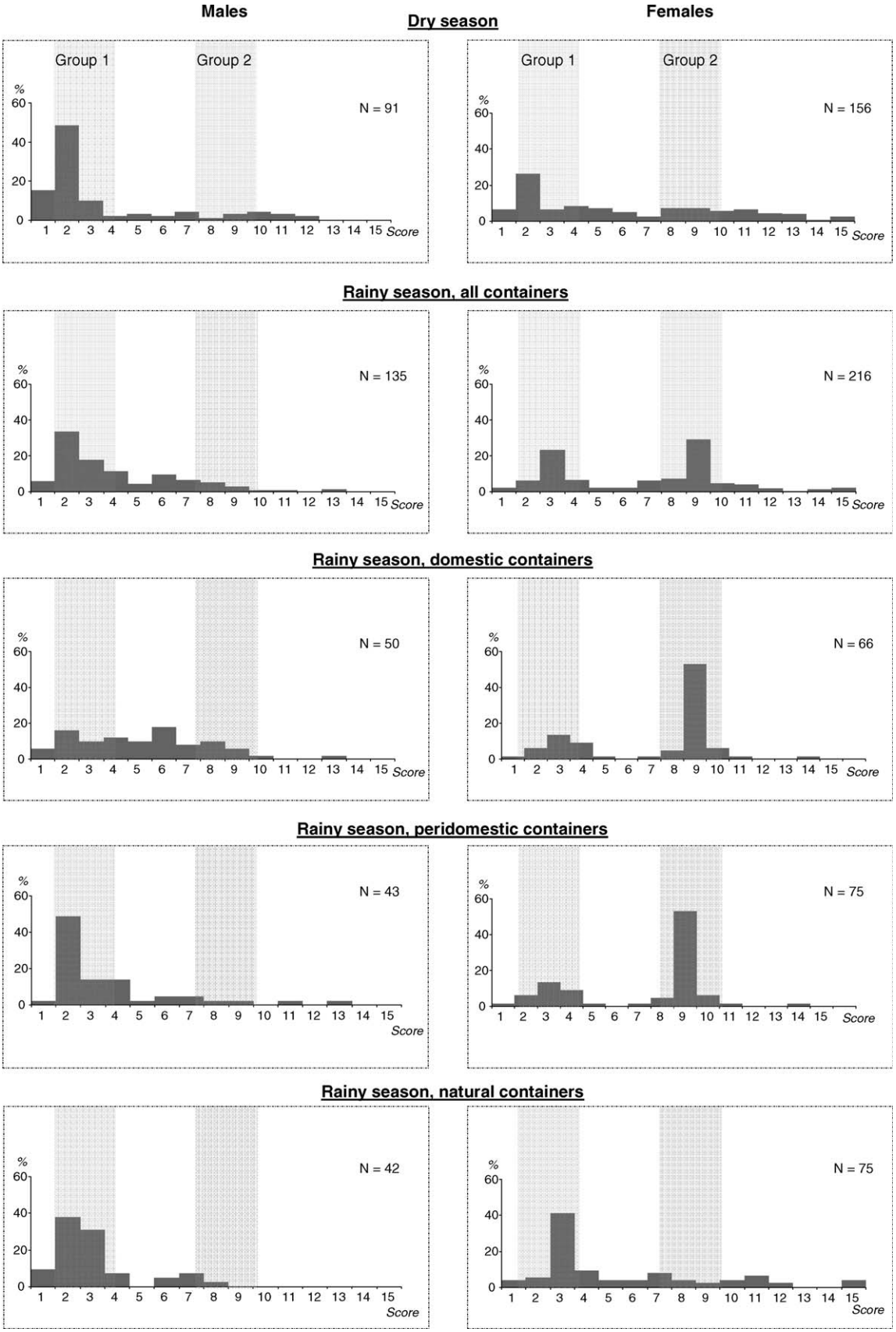

Fig. 2. Distribution of PV across males and females *Ae. aegypti* collected in Niakhar, Senegal (2005). "Group 1" and "Group 2": see Section 3 for definition.

Table 3
Pairwise comparisons of the abdominal pattern values (PVs) distributions in *Ae. aegypti* according to sex, sampling period and nature of the larval habitat.

Comparison			*P*
♂ Dry season (91)	*vs*	♀ Dry season (156)	$<10^{-6}$
♂ Rainy season (135)	*vs*	♀ Rainy season (216)	$<10^{-6}$
♂ Dry season (91)	*vs*	♂ Rainy season (135)	<0.01
♂ Nat (42)	*vs*	♂ Dom (50)	<0.05
♂ Nat (42)	*vs*	♂ Peridom (43)	<0.05
♂ Dom (50)	*vs*	♂ Peridom (43)	<0.05
♀ Dry season (156)	*vs*	♀ Rainy season (216)	<0.01
♀ Nat (75)	*vs*	♀ Dom (66)	<0.05
♀ Nat (75)	*vs*	♀ Peridom (75)	<0.05
♀ Dom (66)	*vs*	♀ Peridom (75)	>0.05

♂: male; ♀: female; in parentheses: sample size; *P*: probability of homogeneity (the Wilcoxon test); Nat, Dom and Peridom refer to the type of larval habitat, respectively, natural, domestic and peridomestic.

24 (dry season) and 13 (rainy season) corresponded to *Aaf* (PV = 1), all other specimens corresponding *Aaa* (PV > 1). Mean PVs were 5.02 ± 3.86 and 6.21 ± 3.60 for mosquitoes from dry season (DS) and rainy season (RS), respectively.

3.1.1. Morphological variation between sexes

Frequency distributions of PV were plotted for both sexes according to the season of collection (Fig. 2). Important differences in the distribution of PV were observed between males and females collected in both seasons. These differences were highly significant (Table 3). Males and females were therefore considered independently in the following analyses.

3.1.2. Morphological variation between seasons

In the dry season collection, 70% of males exhibited a PV between 1 and 3, with a modal value located at PV = 2 (48% of the specimens). All other PV classes grouped together less than 10% of the specimens investigated (Fig. 2). While the modal value is also located at PV = 2 (33%) for males sampled during the rainy season, higher heterogeneity was observed than in the dry season. PV ranging from 1 to 3 encompassed only 57% of the specimens, whereas 11% of the specimens had PV = 4 and 10% had PV = 6. These distributions were significantly different (Table 3).

Females collected during the dry season harbored a modal value at PV = 2 (23% of the females), and all other PV classes grouped together less than 10% of the remaining females. In the rainy season, the distribution of PV in females was bimodal, with two peaks at PV = 3 (23%) and PV = 9 (29%), respectively (Fig. 2). As observed in males, the distributions of PVs were also significantly different between females collected during the dry and the rainy season (Table 3).

3.1.3. Morphological variation according to larval ecology

Fig. 2 shows the distribution of PV in mosquitoes collected during the rainy season, broken down by sex and type of larval breeding site. In both sexes, significant differences were detected in all pairwise comparisons (Table 3), except between females sampled in domestic and peridomestic containers.

Males from peridomestic and natural containers showed a clear unimodal distribution with prevalence of specimens with low PV (PV = 2–4) whereas those from domestic containers showed a relatively flat distribution across the range of PVs. In females from domestic and peridomestic containers, two groups could be distinguished: a main one centered on PV = 9 (group 2) and a smaller one centered on PV = 3 (group 1). Interestingly, females from natural containers were mainly grouped in a single group centered on PV = 3.

3.2. Microsatellite analysis

3.2.1. Genetic variability and Hardy–Weinberg expectations

Genotypes at eight microsatellite loci were determined for 733 *Ae. aegypti* specimens (males and females) collected in Niakhar in 2005 during the dry and rainy seasons (Table 4). All loci were polymorphic showing a number of distinct alleles ranging from 3 (AEDGA) to 9 (A10 and H08). The average allelic richness was assessed after grouping mosquitoes according to the form (*Aaf vs Aaa*), the season and type of larval development site. This grouping was based on studies from Kenya which evidenced that the level of genetic differentiation was linked to differences in larval ecology (Tabachnick and Powell, 1978). Across all loci, the average allelic richness ranged from 3.35 (group *Aaf*) to 3.49 (natural containers in the rainy season) and no statistical difference was detected across samples (Kruskall–Wallis' test, $P > 0.05$). Departure from Hardy–Weinberg proportions associated to significant heterozygote deficits was detected in 16 out of 56 possible tests. One significant deviation was detected in the *Aaf* samples (A10) and five in *Aaa* (A10, AEDGA, AEDC, H08 and M313). Four significant deviations were detected in the dry season (DS) sample (A10, AEDC, H08 and M313), and three in rainy season (RS) sample (A10, AEDC and M313). When RS was subdivided according to larval habitat,

Table 4
Genetic variability and goodness of fit to Hardy–Weinberg expectations in *Ae. aegypti* populations surveyed in Niakhar.

Sample (*N*)		Locus								
		34/72	A10	AEDGA	38/38	AED19	AEDC	H08	M313	ALL
Form										
Aaf (36)	R_s	3.00	4.90	1.69	2.86	3.00	2.55	4.82	4.00	3.35
	F_{IS}	0.86	**0.596**	–	−0.063	−0.030	−0.008	0.264	−0.086	
Aaa (565)	R_s	3.07	5.91	2.15	2.08	2.54	3.36	4.17	4.50	3.47
	F_{IS}	−0.122	**0.526**	**0.041**	−0.093	0.077	**−0.020**	**0.015**	**0.018**	
Season and larval ecology										
Dry season (397)	R_s	2.99	5.48	1.96	1.99	2.25	3.07	4.53	4.74	3.38
	F_{IS}	−0.08	**0.51**	−0.01	0.03	0.09	**0.01**	**0.10**	**0.23**	
Rainy season (351)	R_s	3.12	6.06	2.17	2.19	2.60	3.33	4.03	4.42	3.49
	F_{IS}	−0.15	**0.56**	0.07	−0.12	0.13	**0.01**	0.01	**0.03**	
Domestic (116)	R_s	2.99	5.70	2.24	2.36	2.92	3.55	4.22	4.35	3.54
	F_{IS}	−0.20	**0.64**	0.23	−0.14	0.32	0.01	−0.11	−0.01	
Peridomestic (118)	R_s	2.95	6.29	1.88	2.20	2.62	3.04	3.88	4.49	3.41
	F_{IS}	−0.12	**0.55**	−0.02	−0.10	−0.05	−0.05	0.09	−0.08	
Natural (117)	R_s	3.42	5.96	2.51	2.00	2.20	3.31	4.06	4.49	3.49
	F_{IS}	−0.13	**0.47**	−0.003	−0.123	0.06	0.04	0.06	0.18	

N: sample sizes; R_s: Allele richness (El Mousadik and Petit, 1996); F_{IS}, calculated according to Weir and Cockerham (1984); bolded: significant deficit in heterozygotes ($P < 0.05$) after correction using the Bonferroni procedure (Holm, 1979).

Table 5
F_{ST} estimates computed from seven microsatellite polymorphism for all pairs of samples subdivided according sampling date and ecological characteristics of larval habitat.

Sample	DS	RS	RS-DOM	RS-PERIDOM
DS	–			
RS	0.0007	–		
RS-DOM	0.0010	NC	–	
RS-PERIDOM	0.0025	NC	0.0029	–
RS-NAT	−0.0004	NC	0.0008	0.0013

DS: dry season; RS: rainy season; DOM, PERIDOM and NAT refer to domestic, peridomestic and natural larval development sites, respectively; statistical significance of F_{ST} estimates was assessed using the *G*-test of homogeneity of genotypic frequencies (Goudet et al., 1996). Underlined: $P < 0.0001$. NC: not computed.

heterozygote deficits remained significant only at locus A10 in all samples. Significant linkage disequilibrium was revealed between locus A10 and loci 34/72 and M313 in the DS sample. A single significant association between locus 38/38 and AED19 was detected in the RS pooled samples. Due to the number of significant heterozygote deficits and linkage disequilibrium involving A10, analyses of genetic differentiation and population structure were performed excluding this locus from the dataset.

3.2.2. Genetic differentiation and population structure

Genetic differentiation was first assessed comparing mosquitoes grouped according to the form (*Aaf vs Aaa*) and results suggested that the two forms were not differentiated when the two sampling dates were considered together ($F_{ST} = -0.0036$, $P > 0.05$) or separately (dry season: $F_{ST} = -0.0015$, $P > 0.05$; rainy season: $F_{ST} = -0.0091$, $P > 0.05$). When analyses were ran without morphologic consideration, a very low level of genetic differentiation was detected between mosquitoes collected in the dry and rainy seasons ($F_{ST} = 0.0003$, $P < 10^{-4}$, Table 5). There was no evidence for genetic structuring within the RS sample when mosquitoes were grouped according to the type of container they were colleted from ($F_{ST} < 0.0024$, P>0.05, Table 5). However, significant F_{ST} estimates were detected between the dry season sample (DS) and mosquitoes collected during the rainy season in domestic (RS-DOM) and peridomestic (RS-PERIDOM) containers ($F_{ST} > 0.001$, $P < 10^{-4}$). No differentiation was detected between DS and RS-NAT (Table 5).

Bayesian cluster analysis using STRUCTURE 2.2. was performed to estimate the most likely number of genetic clusters (*K*) in the dataset following the algorithm described by Evanno et al. (2005). Our results revealed the most likely number of cluster (*K*) was equal to 2 and 7 when we successively analyzing the global sample (i.e., males and females from both collection dates pooled), the DS and the RS sample (see Fig. 3) suggesting the number of genetic cluster was higher during the rainy season than during the dry season. Similarly, the number of genetic clusters (*K*) was also found to be higher during the rainy season when analyses were performed considering only those females for which the abdominal morphology was checked. However, no correspondence between these genetic clusters and any grouping of mosquitoes based on either morphology or larval ecology (container type) was

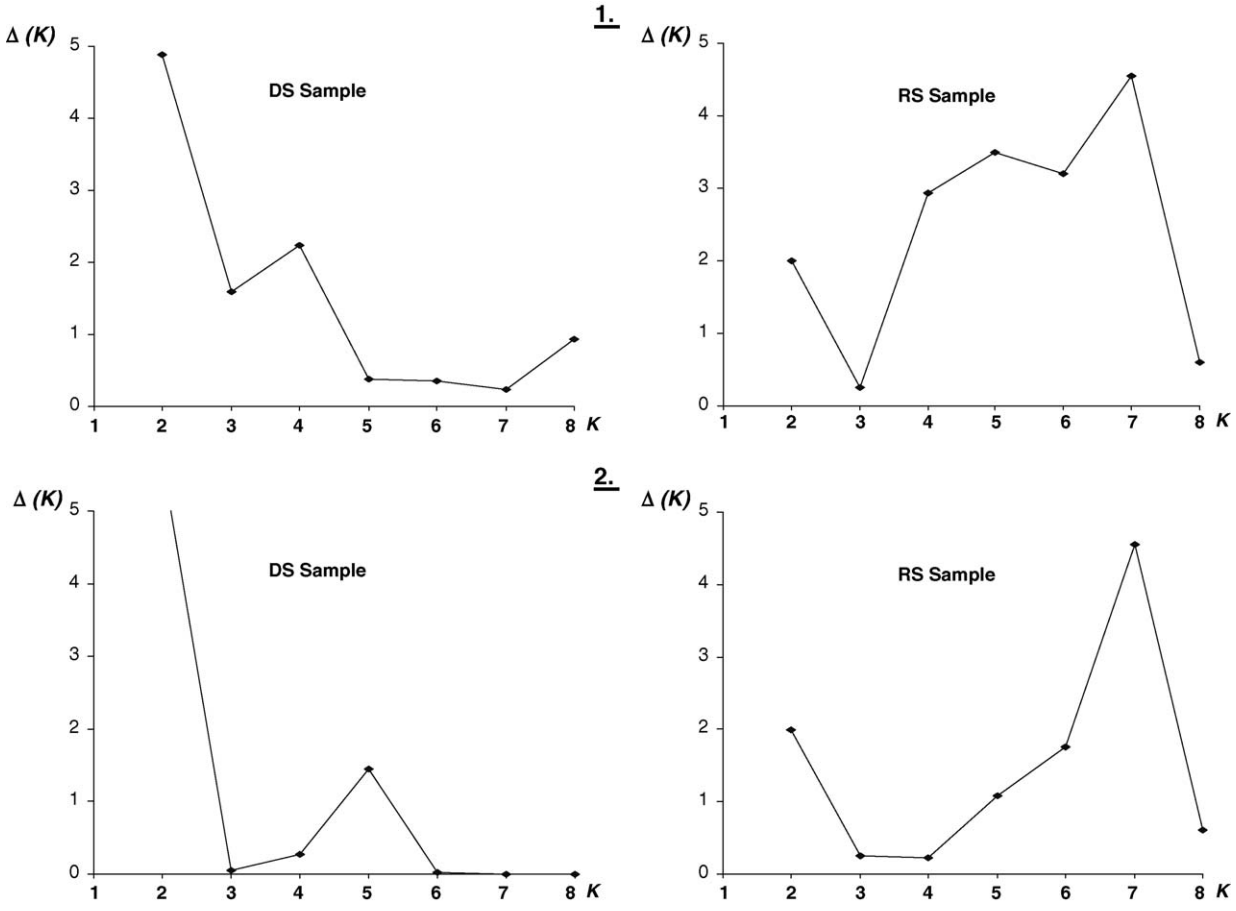

Fig. 3. Bayesian cluster analysis using STRUCTURE (Pritchard et al., 2000) and estimates of the most likely number of genetic cluster (*K*). The estimate ΔK, based on the second order rate of change of the likelihood function with respect to *K* (Evanno et al., 2005), was used to determine the most likely number of clusters (*K*) in the data set. In case 1 (considering the global sample) (*K*) was 2 and 7 for samples collected during the dry season (DS) and the rainy season (RS), respectively. In case 2 (considering females for which a PV was calculated), (*K*) was 2 and 7 for DS and RS, respectively.

detected, all groups appearing as a mixture of the genetic clusters identified (data not shown).

4. Discussion

We estimated the amount of morphological variation among *Ae. aegypti* specimens from the area of Niakhar, Senegal. Relationships between morphological variation and factors such as sex, seasonality and larval ecology were explored. Population structure and the level of genetic differentiation between samples with contrasting larval ecology and/or adult morphology were assessed using a set of eight microsatellite markers.

Morphological analyses based on the pattern of abdominal scaling of *Ae. aegypti* revealed significant variation according to sex. Here, the mean and range of pattern values (PVs) were significantly lower in males than in females. These results are consistent with previous observations (Mattingly, 1957; McClelland, 1974) and suggest that the pattern of abdominal pale scaling is, at least in part, linked to sex. In *Ae. aegypti*, morphological mutant marker loci were reported across all three chromosomes (Munstermann and Craig, 1979; Severson et al., 2002). Some of them, involved in the coloration of the abdomen (e.g., "pale abdomen") were located on the first chromosome, within a linkage group containing one locus involved in sex determination (Anderson et al., 2001).

Mosquitoes from Niakhar mainly exhibited morphological characteristics which are typical of *Ae. aegypti aegypti* (i.e., PV $>$ 1). The specimens that would correspond to *Ae. aegypti formosus* (i.e., blackish appearance of the thoracic and abdominal integument, and absence of pale scales on the first abdominal tergite, PV = 1) occurred at very low frequencies. In addition, our analyses indicated these "*Aaf*-like" mosquitoes were not ecologically or genetically distinct from other morphotypes in our samples. Overall, these results suggest that this level of morphological variation probably reflected intra-population variability rather than phenotypic markers of speciation. Hence, the typical *Aaf*, such as observed in the rural gallery forests around Kedougou in Southern Senegal (Huber et al., 2008), tended to be rare in the anthropogenic area we investigated. Most of the specimens we analyzed would fell under the morphological description of *Aaa*, in agreement with the recent survey by Sylla et al. (2009). It is therefore clear from these results, as well as other reports (e.g., Hervy, 1977) that *Aaa* is common in West Africa. Then, the question of its origins remains to be debated. According to Tabachnick (1991), these West African populations of *Aaa* might have been introduced back to sub-Saharan Africa after lineage splitting through vicariance following the apparition of the Sahara desert. However, the lack or very low levels of genetic differentiation observed using a wide range of molecular markers including isozymes (Huber et al., 2008), SNPs (Sylla et al., 2009) and microsatellites markers (this study) among *Ae. aegypti* populations from Senegal is at odds with this hypothesis. Rather, these results strongly suggest that *Aaa* and *Aaf* are monophyletic. In this context, it is tempting to establish a parallel between the evolution of anthropophily and more general domestic habits such as adaptation to anthropogenic environments (e.g., man-made, permanent breeding sites) in *Ae. aegypti* and in the more widely studied *Anopheles gambiae*, the major malaria vector in Africa (Ayala and Coluzzi, 2005). Indeed, the history of human population settlement and climate changes in Africa may have provided ample opportunities and strong selection pressure for the evolution of anthropophily and domesticity in mosquitoes, especially through the ability of man to provide suitable development sites for the aquatic stages during the phases of "savannization" that occurred across most of the central Africa rain forest, ca. 2500 years ago (Willis et al., 2004). As such, ecologically driven selection might well be the major evolutionary force driving radiation and lineage splitting within *Ae. aegypti* in Africa, rather than allopatric speciation followed by secondary contact. However, it is clear that a much broader assessment of morphological, ecological, behavioral and genetic variations that exist within African *Ae. aegypti* populations is needed for an in-depth understanding of the evolutionary history of the species both within and outside Africa.

In Niakhar, significant morphological variations were evidenced between the dry and the rainy season, particularly in females. Among mosquitoes collected during the dry season, a broad range of PVs was observed, but the females were mainly clustered into a single group centered on PV = 2. Interestingly, females collected during the rainy season segregated into two distinct morphological groups, one centered on PV = 3 (group 1) and one centered on PV = 9 (group 2). Such an observation could indicate the pattern of abdominal pale scales is a polymorphic trait for which the distribution of the different states (i.e., morphotypes) is variable rather than fixed across time and possibly randomly driven, due to stochastic processes in relation to demographic fluctuations. In South Vietnam indeed, it was suggested that seasonal fluctuations in rainfalls (which condition the availability of oviposition sites) affected the population size of *Ae. aegypti* and induced great temporal variation in the allelic distribution of neutral genes (Huber et al., 2002). In Niakhar, the scarcity of suitable larval developmental places during the dry season possibly induced significant demographic crashes enhancing genetic drift that modulated the distribution of characters such as the abdominal morphology we surveyed. The temporal variation in distribution of morphotypes could alternatively be interpreted as the result of the co-occurrence of two morphological forms (or subpopulations) of *Aaa*. Additionally our results suggest the nature of larval breeding site greatly influences the pattern of abdominal scales. The relation between morphology and larval ecology indicated a possible segregation of forms according to the nature of breeding containers. However, microsatellite-based analyses failed to detect any evidence that the two morphological forms are genetically differentiated. Nevertheless, it was possible the set of microsatellite markers we employed was not sufficient to detected genetic differentiation. While such markers are not abundant in *Ae. aegypti* genome, a recent work increased the number of microsatellite suitable for population genetic studies up to 20 (Lovin et al., 2009). The recent study of Bernhardt et al. (2009), suggesting the occurrence of eight large paracentric inversions on three chromosomes of a *Aaf* strain from Senegal, introduced the idea that a chromosomal polymorphism possibly exists in *Ae. aegypti*. These authors also discussed the possibility that genes controlling traits such as morphology, biology and vector competence would be located in inversions and then the traits that they condition would be maintained as correlated characters. To better tackle the heterogeneity of *Ae. aegypti* in Senegal, it would be crucial to study the distribution of such chromosomal inversions within natural populations.

In conclusion, our study demonstrated the scarcity of typical *Ae. aegypti formosus* in Niakhar and evidenced important variations in abdominal morphology within *Ae. aegypti aegypti*, particularly in females. A better understanding of the genetic bases of these morphological and ecological polymorphism and their potential implications in YFV, Dengue and Chikungunya viruses transmission will require the use of additional molecular and chromosomal markers. It is clear however that the genotypic and phenotypic heterogeneity of the system in West Africa is much more complex than previously acknowledged. Disentangling genetic polymorphism and phenotypic plasticity represents a formidable challenge that needs to be addressed to improve our ability to understand, explain and prevent epidemic outbreaks of the arboviruses this vector transmits in Africa.

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