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Population structure of the malaria vector *Anopheles funestus* (Diptera: Culicidae) in Madagascar and Comoros

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Abstract

Microsatellites were used as markers for a study of the population structure of *Anopheles funestus* on Madagascar and Comoros. Mosquitoes were collected in four different localities on Madagascar and one on Comoros. There was a significant genetic differentiation between all samples from Madagascar and that from Comoros ($P < 0.05$). With respect to the Madagascar mosquito samples, it was found that there were no significant genetic differences between samples that were collected at the east coast, and in the highlands, respectively. By contrast, the west coast sample exhibited significant genetic differences (with regard to all Madagascar samples). The results are discussed with respect to population distribution and migration of *A. funestus* from mainland Africa and the islands east of the mainland.
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Keywords: *Anopheles funestus*; Population genetics; Malaria; Microsatellite DNA; Madagascar; Comoros

1. Introduction

Malaria is one of the most important diseases that affects humans, with more than one million deaths and 300 to 500 million cases of acute illness per year (Greenwood and Mutabingwa, 2002; Snow et al., 2005). In sub-Saharan Africa, *A. funestus* Giles is one of the three most important malaria mosquito vectors, in terms of human morbidity and mortality, due to its high susceptibility to malaria parasites and its high degree of anthropophily and endophily (Gillies and De Meillon, 1968). This mosquito is widely distributed throughout tropical Africa, and its activity extends even into the dry season where other malaria mosquito vectors, such as *Anopheles gambiae*, are usually inactive (Fontenille et al., 1997).

Studies on the population structure of malaria vectors have important implications for the prediction and assessment of the effects of many vector control strategies. For example, the suggestion to drive malaria-parasite refractory genes into wild malaria mosquitoes (Crampton et al., 1994; Aultman et al., 2001) or the management of insecticide resistance by studying the spread of resistance genes through target populations (Collins et al., 2000) will be affected by the genetic composition and gene flow of the target vector populations.

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Microsatellites have been extensively applied to detect gene flow or study population genetic structure (Walton et al., 1998). These genetic markers offer many advantages over conventional markers such as polytene banding patterns or allozymes. They seem apparently neutral, are distributed throughout the genome and are codominant. In addition, microsatellites exhibit high levels of length polymorphism and are relatively easy to score (Walton et al., 1998).

During the anti-malarial campaigns of the 1940s and 1950s, extensive household spraying was undertaken to maintain a low incidence of malaria in the Highlands of Madagascar. In the 1970s, household spraying ceased and a slow but progressive resurgence of *A. funestus* took place followed by a reintroduction of malaria. Between 1986 and 1990 malaria incidence was very high and over 100,000 lives were lost. The Ministry of Health began the distribution of chloroquine at the community level, and in 1988 household spraying commenced again (Madagascar Malaria Map, Pasteur Institute of Madagascar, 2002).

The present study was undertaken to examine the current distribution of *A. funestus* on Madagascar and to investigate genetic associations between geographically different populations as well as with that of the Comoros, an island group some 400 km North of Madagascar.

2. Materials and methods

2.1. Collection sites

The study was conducted on the islands of Madagascar and Comoros in the Indian Ocean, off the coast of East Africa. Four of the five different populations of *A. funestus* were distributed within Madagascar (Mahajunga 46°36′E, 15°40′S 25 m alt.; Fenerive-Est 49°24′E, 17°19′S, 0 m alt.; Analamiranga 46°16′E, 19°14′S 870 m alt.; Andranonahoatra 46°25′E, 19°00′S 930 m alt.) and one population was located on the island of Mohéli in the Comoros (43°40′E, 12°19′S, 0 m alt.) (Fig. 1). Two Malagasy localities were on the coast and the other two in the highlands. One village (Andranonahoatra) in the highlands was in an area treated with DDT for malaria control, and the other highland village was in an untreated area.

The study sites were, in general, small hamlets and located in different bioclimatic zones, with considerable differences in annual rainfall and temperatures caused by the specific geography of Madagascar and Comoros and the proximity of the Indian Ocean (Madagascar Malaria Map, 2002).

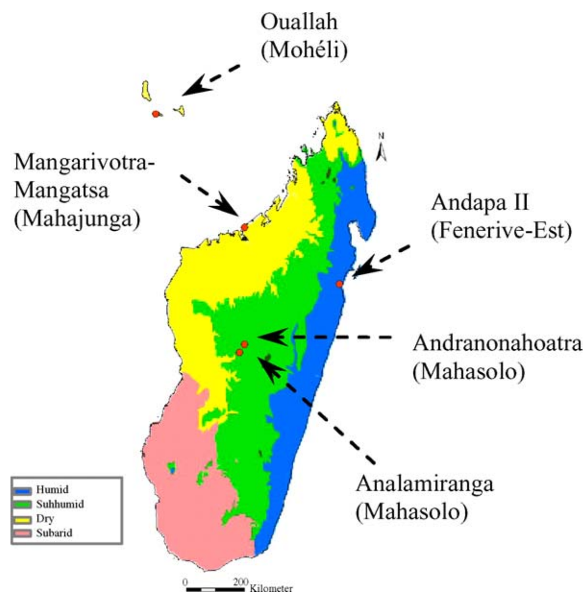

Fig. 1. Collection sites in Madagascar and Comoros. Source: Bioclimate map of Madagascar and Comoros (Koechlin et al., 1974).

2.2. Mosquito collection and identification

Adult mosquitoes were collected with the human bait method, Mbita traps (Laganier et al., 2003) and pyrethrum spray catches. Mosquitoes were collected from January to June 2003.

Morphological identification was performed to separate the *A. funestus* group from the rest of anopheline species (Gillies and Coetzee, 1987). For this purpose, blood meal identification by direct use of enzyme-linked immunosorbent assays (ELISA) was performed according to the protocol described in Beier et al. (1988). This test was performed for those mosquitoes whose 'victim' was unknown (spray catch method). Thus, only mosquitoes feeding on human blood were used. Subsequently, extraction of DNA from single mosquitoes was performed according to a protocol based on Collins et al. (1987).

Species identification within the *A. funestus* group was performed by polymerase chain reaction (PCR), according to a protocol based on Koekemoer et al. (2002). Although in Madagascar, only *A. funestus* sensu stricto has been documented, an exhaustive analysis was performed to confirm that observation and to allow a correct interpretation of the microsatellite data. Five species out of nine, which comprised the whole group, were checked with PCR primers: *A. funestus*, *Anopheles vaneedeni* Gillies and Coetzee, *Anopheles parensis* Gillies, *Anopheles rivulorum* Leeson, and *Anopheles leesoni* Evans (Koekemoer et al., 2002).

2.3. Microsatellite technique

Samples of 47 *A. funestus* sensu stricto females from each population were analysed using 10 microsatellite loci. From Sinkins et al. (2000) were chosen: AF2, AF3, AF19, AF20 and AF5. Subsequently, five additional microsatellites were selected from Cohuet et al. (2002): FunR, FunF, FunG, FunL and FunO. The forward primer of each locus was labelled with a fluorescent dye (FAM, TET, or HEX) compatible with an ABI PRISM sequencer (GENOME Express, France). According to locus colour, a multiple mix was made. The multiple mixes consisted of the following loci: AF2, AF3 and FunO; AF19, AF20, FunR and FunF; AF5, FunG and FunL. Genotyping procedures with fluorescence technology were performed using an ABI 377 automatic DNA sequencer using filter set C (GENOME Express, France). Results were analysed with Genescan 3.1.2. software. All the settings were according to a protocol based upon Cohuet et al. (2002).

2.4. Data analysis

Estimates of linkage disequilibrium were made using GENEPOP v.3.3 (Raymond and Rousset, 1995) and FSTAT v.2.9.3 (Goudet, 1995). Furthermore, tests of deviation from Hardy–Weinberg equilibrium (HWE) were performed using GENEPOP v.3.3 (Raymond and Rousset, 1995).

Population differentiation was analysed using the allelic variation and based upon the null hypothesis that the allelic distribution was identical across populations. Unbiased estimates of the *P*-value of the probability test (Fisher exact test) were produced (Raymond and Rousset, 1995).

Population genetic differentiation was assessed by the fixation index F_{ST} (Wright, 1978). Estimates of F_{ST} were calculated according to Weir and Cockerham (1984), using FSTAT v.2.9.3 (Goudet, 1995) and GENEPOP v.3.3 (Raymond and Rousset, 1995). The FSTAT v.2.9.3 program calculated unbiased estimates of *F*-statistics. In addition, it performed numerical re-sampling by bootstrap and jacknife procedures in order to estimate confidence intervals and the significance of values.

Estimates of expected heterozygosity (*He*) were produced using Nei's unbiased estimator (Nei, 1988) assuming the mutation-drift-equilibrium. *He* is the expected heterozygosity, based on allele frequencies. *Heq* is the expected heterozygosity based on the number of alleles and sample size. Three possible models were assumed to estimate *Heq*: IAM (Infinitive Alleles Model), SMM (Stepwise Mutation Model) and TPM (Two Phase Model).

Wilcoxon signed-ranks tests and Sing-tests were performed to determine if there was a significant number of loci in which $Heq > He$. Tests were performed using BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996).

Isolation by distance was investigated by a simple Mantel test to study the correlation between pairwise genetic differentiation and geographic distance. The significance of the test statistic was assessed by random permutation of one of the matrices using Mantel V2.0 (Liedloff, 1999).

3. Results

3.1. Species identification

A total of 2711 mosquitoes of the *A. funestus* group were captured, of which 260 were identified to species level by PCR. Only *A. funestus* sensu stricto was found. However, 20 of the 260 mosquitoes showed band absence in the agarose gel. To examine whether errors in morphological identification might have erroneously identified *A. gambiae* (which species was also present in the study areas, albeit in much smaller numbers) as *A. funestus*, we conducted an *A. gambiae* specific PCR. Two mosquitoes were identified as *A. gambiae* sensu stricto. The remaining 18 samples showed band absence in the agarose gel and this was ascribed to a failure of the DNA extraction. With exception of the highland village of Andranonahoatra, 47 individuals (out of many more specimens) from each study site were used for genetic analysis. In Andranonahoatra, where the population of *A. funestus* was low at the time of study, only 45 individuals were available. These were all used in the genetic analysis.

3.2. Test for Hardy–Weinberg equilibrium and linkage disequilibrium

Prior to microsatellite data analysis, locus AF3 was rejected due to the lack of scorable genotypes in almost all populations. Within each population, deviations from Hardy–Weinberg expectation were found in four of nine loci, showing a strong heterozygote deficit. This is generally resulting from the presence of *null alleles* (Braginets et al., 2003). Null alleles are alleles that were not amplified because of mutations at the primer binding sites. Thus, to avoid the possibility that null alleles might influence the analysis, the four microsatellites were rejected, pursuing the experiment with the five remaining markers. Linkage disequilibrium was not observed

Table 1
Summary table of mosquito populations used in microsatellite analysis

Population	Microsatellites	No. of females	No. of alleles	Date collected	Collection method	Remarks
Mahajunga	AF 2	44	10	5–6 June 2003	Indoor pyrethrum spray	Hamlet Subhumid forest
	FUN 0	44	4			
	FUN F	42	5			
	AF 5	38	2			
	FUN G	45	5			
Fenerive-Est	AF 2	44	10	4–7 April 2003	Human landing catches Indoor pyrethrum spray	Village Humid forest
	FUN 0	45	4			
	FUN F	42	5			
	AF 5	45	2			
	FUN G	44	5			
Mohéli	AF 2	47	3	24–28 February 2003	Human landing catches	Hamlet Subhumid forest
	FUN 0	47	4			
	FUN F	43	4			
	AF 5	40	2			
	FUN G	39	5			
Analamiranga	AF 2	44	10	9–12 April 2003	Mbita trap Human landing catches Indoor pyrethrum spray	Village Savannah vegetation
	FUN 0	46	5			
	FUN F	42	4			
	AF 5	37	2			
	FUN G	31	6			
Andranonahoatra	AF 2	40	12	15 January–11 May 2003	Mbita trap Human landing catches Indoor pyrethrum spray	Village Savannah vegetation
	FUN 0	37	4			
	FUN F	39	6			
	AF 5	41	2			
	FUN G	33	6			

in any pair of loci. Consequently, the samples were drawn from homogeneous and randomly mating populations, and the microsatellites represent independent markers.

3.3. Genetic diversity and population differentiation

Overall, allele distribution was similar among localities in Madagascar. The number of alleles per locus varied from 2 to 12. However, in Mohéli (Comoros), the number of alleles per locus varied from 2 to 5 (Table 1).

Population differentiation was investigated based on allelic differentiation (allelic distribution across populations). The results showed that both populations from the highland of Madagascar (Analamiranga and Andranonahoatra) were closely related. In addition, these populations were also related to the sample from Fenerive-Est on the East coast (Fisher's exact test, Table 2 and Fig. 1). By contrast, the west coast population (Mahajunga) showed an allelic separation from them. The *A. funestus* population on Mohéli (Comoros) was not related to the samples from Madagascar (Table 2).

3.4. Bottleneck events

Evidence for the absence of recent bottleneck events was provided by the heterozygosity tests (Tables 3 and 4).

Table 2
Allelic differentation

Population pairs		Allelic differentiation χ^2	d.f.	*P*-value
MJG	FEN	Infinity	10	***
MJG	MOH	Infinity	10	***
MJG	AMG	39.404	10	0.00002
MJG	ANH	Infinity	10	***
FEN	MOH	Infinity	10	***
FEN	AMG	17.481	10	0.06437
FEN	ANH	25.127	10	0.00511
MOH	AMG	Infinity	10	***
MOH	ANH	Infinity	10	***
AMG	ANH	10.332	10	0.41189

MJG: Mahajunga; FEN: Fenerive-Est; MOH: Moheli; AMG: Analamiranga; ANH: Andranonahoatra. Alpha corrected by sequential Bonferroni procedure ($P < 0.001$).
*** Highly significant.

+ Model
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Table 3
Base-line data of heterozygosity tests (Cornuet and Luikart, 1996) for *A. funestus* populations in the five study villages, using IAM, TPM and SMM models (see Section 2 for description)

		Mahajunga		Fenerive-Est		Mohéli		Analamiranga		Andranonahoatra	
		He/*Heq*	Prob	*He*/*Heq*	Prob	*He*/*Heq*	Prob	*He*/*Heq*	Prob	*He*/*Heq*	Prob
AF 2	He (Nei's)	**0.808**		**0.771**		**0.652**		**0.780**		**0.734**	
	IAM	0.747	0.273	0.745	0.477	0.328	0.011	0.751	0.458	0.797	0.154
	TPM 70%	0.802	0.457	0.801	0.236	0.399	0.035	0.803	0.265	0.843	0.022
	TPM 80%	0.809	0.399	0.813	0.158	0.405	0.023	0.811	0.220	0.849	0.015
	TPM 90%	0.823	0.317	0.825	0.105	0.425	0.031	0.821	0.160	0.858	0.007
	SMM	0.846	0.113	0.846	0.200	0.457	0.019	0.847	0.038	0.877	0.000
FUN O	He (Nei's)	**0.641**		**0.648**		**0.560**		**0.630**		**0.625**	
	IAM	0.438	0.095	0.440	0.112	0.430	0.273	0.526	0.311	0.447	0.151
	TPM 70%	0.513	0.206	0.510	0.176	0.520	0.455	0.592	0.481	0.531	0.286
	TPM 80%	0.527	0.227	0.530	0.192	0.524	0.478	0.614	0.477	0.542	0.323
	TPM 90%	0.641	0.283	0.558	0.240	0.557	0.436	0.631	0.401	0.563	0.371
	SMM	0.590	0.387	0.594	0.359	0.591	0.299	0.669	0.254	0.598	0.479
FUN F	He (Nei's)	**0.654**		**0.618**		**0.579**		**0.448**		**0.560**	
	IAM	0.579	0.371	0.522	0.345	0.447	0.254	0.445	0.449	0.596	0.333
	TPM 70%	0.666	0.383	0.608	0.454	0.508	0.380	0.516	0.274	0.664	0.153
	TPM 80%	0.682	0.289	0.612	0.460	0.529	0.438	0.528	0.244	0.677	0.115
	TPM 90%	0.695	0.259	0.646	0.310	0.560	0.470	0.552	0.192	0.701	0.058
	SMM	0.733	0.105	0.672	0.212	0.590	0.377	0.586	0.111	0.735	0.021
AF 5	He (Nei's)	**0.489**		**0.457**		**0.491**		**0.373**		**0.270**	
	IAM	0.203	0.077	0.194	0.111	0.190	0.072	0.199	0.224	0.193	0.334
	TPM 70%	0.218	0.083	0.216	0.151	0.220	0.074	0.223	0.271	0.215	0.372
	TPM 80%	0.232	0.090	0.229	0.154	0.226	0.097	0.222	0.261	0.227	0.402
	TPM 90%	0.234	0.097	0.224	0.153	0.230	0.099	0.226	0.276	0.236	0.427
	SMM	0.243	0.114	0.243	0.188	0.233	0.084	0.236	0.303	0.240	0.426
FUN G	He (Nei's)	**0.708**		**0.668**		**0.714**		**0.633**		**0.610**	
	IAM	0.580	0,194	0.521	0.181	0.535	0.092	0.613	0.476	0.606	0.434
	TPM 70%	0.661	0.398	0,594	0.315	0.605	0.175	0.681	0.247	0.682	0.193
	TPM 80%	0.673	0.461	0.617	0.396	0.622	0.214	0.692	0.2070	0.694	0.149
	TPM 90%	0.695	0.474	0.635	0.470	0.650	0.266	0.709	0.148	0.705	0.117
	SMM	0.728	0.308	0.667	0.403	0.674	0.370	0.736	0.069	0.736	0.045

He values are expressed in bold, *Heq* values in regular print.

Deviations ($0.01 < P < 0.05$) from mutation-drift equilibrium, as a result of heterozygote excess, were found in all populations except in Andranonahoatra under the less realistic (IAM) model. These deviations were also observed in the same populations but only with the Wilcoxon signed-ranks test when analysis was performed under TPM 70%. All populations except that of Mohéli did not show any significant deviations from mutation drift equilibrium when the studies were performed under TPM 80%, TPM 90% and the SMM model, in both the Wilcoxon signed-ranks test and the Sign test. Luikart and Cornuet (1998) mentioned that these tests, the Wilcoxon test and the Sign test, under the IAM model could erroneously detect heterozygosity excess in non-bottleneck populations, when microsatellite data were used. Then, those authors suggested using the strict SMM or a TPM model with 5–10% multi step changes with such data.

3.5. Genetic differentiation

Estimates of F_{ST} showed noteworthy patterns of population differentiation. The Comoros population showed significant F_{ST} values (P values ∼0.0001) when compared with the populations from Madagascar (pairwise comparisons, Table 4). Genetic divergence was almost non-existent in highland populations. F_{ST} values supported the data from allelic analysis already mentioned. The west coast population was defined as an isolated subpopulation within Madagascar, showing a significant genetic difference with respect to the other localities on the island. Based on F_{ST} values the population from

Table 4
Estimates of *P*-value for the heterozygosity tests at each population

		IAM	TPM			SMM
			70%	80%	90%	
Mahajunga	Sign test	0.04989	0.26748	0.64005	0.59884	0.36752
	Wilcoxon test	**0.01563**	**0.04688**	0.07813	0.31250	0.59375
Fenerive-Est	Sign test	0.04775	0.27389	0.27002	0.62266	0.63301
	Wilcoxon test	**0.01563**	**0.04688**	0.07813	0.31250	0.59375
Mohéli	Sign test	**0.03701**	0.05228	0.05234	0.05131	0.62858
	Wilcoxon test	**0.01563**	**0.01563**	**0.01563**	**0.01563**	0.07813
Analamiranga	Sign test	0.04809	0.37732	0.39820	0.11495	0.11024
	Wilcoxon test	**0.01563**	0.68750	0.68750	0.95313	0.96875
Andranonahoatra	Sign test	0.59357	0.37853	0.37315	0.37336	0.38800
	Wilcoxon test	0.40625	0.95313	0.95313	0.95313	0.95313

In bold *P*-values <0.05. Alpha corrected by sequential Bonferroni procedure ($P < 0.001$) SMM: stepwise mutation model; IAM: infinitive alleles model; TPM: two-phase mutation model.

Table 5
Estimates of F_{ST} values and their statistical significance (asterisks)

	Mahajunga	Fenerive-Est	Mohéli	Analamiranga
Mahajunga				
Fenerive-Est	0.0162*			
Mohéli	0.1240**	0.1043**		
Analamiranga	0.0306**	0.0053	0.1184**	
Andranonahoatra	0.0498**	0.0100	0.1323**	−0.0017

P-values obtained after 10,000 permutations; Indicative adjusted nominal level (5%) for multiple (10) comparisons is 0.005.
* $P < 0.005$.
** $P < 0.001$.

Fenerive-Est was genetically related to those of Andranonahoatra and Analamiranga, most notably the last one.

A positive and significant ($P < 0.01$) correlation was found between $F_{ST}/(1 - F_{ST})$ and geographic distance (Mantel test) when the five samples were compared. However, pairwise estimates of $F_{ST}/(1 - F_{ST})$ without the Comoros individuals showed a positive correlation but this was not significant (Table 5).

4. Discussion

This study shows significant population structure of *A. funestus* from Comoros and Madagascar, as well as distinct differences between populations on Madagascar. Assuming that *A. funestus* originated on mainland Africa (Sharakhov et al., 2004; Temu et al., 2004), the data suggest that *A. funestus* might have arrived separately on Comoros and Madagascar. A phylogenetic analysis with *A. funestus* samples from East Africa, Comoros and Madagascar would be required to examine with which mainland population the island populations could be related, and also to obtain insight along which route the distribution of these populations might have occurred. Our data furthermore show a weak positive correlation between genetic differentiation with distance on Madagascar, suggesting that the population on the west coast was furthest removed from the highland populations. This would agree with the topographical features of the island, preventing a direct connection between these geographic locations (Fig. 1).

At present, only one study on the population genetics of *A. funestus* on Madagascar has been performed (LeGoff, unpublished data). It means that until now there was no published reference for the genetic distribution of that vector on Madagascar, and no expectations about possible gene flow barriers nor about connections across populations were available. A recent study on genetic differentiation of *A. funestus* from East and Southern Africa (Temu et al., 2004) showed that significant genetic differentiation exists between populations from Uganda and Kenya (west of the Rift valley) on the one hand and those from Malawi, Mozambique and South Africa. The populations from around Lake Victoria were more related than the other populations. The study clearly

demonstrates genetic differentiation with distance, and we might extrapolate that a similar situation applies to the populations from Madagascar.

The ecological relevance of significant genetic differences (F_{ST} values) between *A. funestus* populations of Madagascar provides a new concept of vector population distribution on this island. Our data suggest that genetic exchange between populations has occurred between the east coast of Madagascar and the highlands, but not, or at least less, between the central highlands and the west coast. Geographic barriers may be the reason for this situation, and limited genetic exchange between *A. funestus* populations appears to occur along the coast. Madagascar has dramatically different geographical features, and mountains as well as semi-deserts can provide formidable barriers to gene flow, as evidenced from our results. Perhaps the most interesting, though not surprising, result from this study is the genetic separation of the Comoros population from those on Madagascar. The archipelago of the Comoros is 400 km north of Madagascar, the Indian Ocean providing a strong geographic barrier between the two islands. It would be interesting to study the genetic relationship of *A. funestus* populations from other islands of the Comoros archipelago, as at present the data are limited to those of Mohéli.

With respect to the Madagascar subpopulations from the east coast and the highlands, it appears that *A. funestus* is not affected much by differences in bioclimate and altitude or that it can readily adapt to a wide array of climates. The evidence that the population of Fenerive-Est (East coast) is closely related to that of the highland locations Analamiranga and Andranonahoatra suggests that these populations are in frequent genetic exchange with each other, in spite of a physical distance of 400 km. In the highlands *A. funestus* is strongly seasonal, depending on rain and temperature (Bouma, 2003). On the east coast, however, the population is continuous, and perhaps the exchange between populations occurs more from the east into the highlands than vice versa. As the anti-malaria campaigns of the seventies effectively eradicated *A. funestus* from the highlands (Fontenille and Rakotoarivony, 1988), reinvasion of these areas following termination of indoor residual insecticidal spraying may therefore have occurred from the east coast. Our results suggest that, once eradicated, the vector species that provoked the huge epidemics of malaria in the 1980s arrived from the East coast. The East coast of Madagascar experiences a tropical lowland climate; therefore, there is no limiting effect of temperature or humidity on the vector development and parasite cycle and mosquitoes develop throughout the year. Furthermore, appetitive flights in anopheline species occur usually

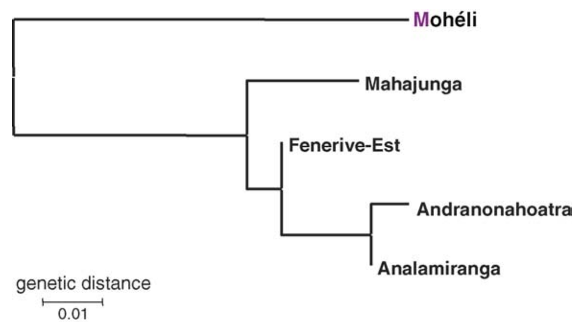

Fig. 2. Unrooted neighbour-joining population tree based on mean F_{ST} values across five loci for the five *A. funestus* populations.

over distances of 1–2 km (Donnelly et al., 1999) and this was not readily reconciled with the high levels of gene flow (low F_{ST} values) observed in this study. Of course, this could be attributable to either constraints on microsatellite loci or a large amount of human-mediated transport (Lehmann et al., 1996). However, the last hypothesis is not likely to be true, as no large human movements have been reported between both locations. It is likely that this species has extended its range relatively recently, aided by new settlements on the plateau and the intensive agriculture, especially rice fields, which are ideal habitats for *A. funestus* larvae.

Analyses of samples from the central area of Madagascar showed that Analamiranga and Andranonahoatra had the highest degree of genetic similarity, as both the F_{ST} values and the other genetic analyses revealed (see Tables 2 and 4). This was not unexpected, as both sites were 25 km apart, situated at similar altitudes and in a similar bio climate. As intensive DDT indoor spraying had occurred in only one of the two sites, we might have expected some genetic differences. Since this was not the case, we propose that reinvasion of Andranonahoatra has occurred from the population present in the unsprayed site of Analamiranga and environs and that frequent exposure to DDT did not affect the genetic composition of the resident *A. funestus* population.

The significance of the genetic difference between *A. funestus* from the west coast and the other populations on Madagascar suggests a clear distinction of two subpopulations on the island: a central-east population and a western population (Fig. 2). Mahajunga remained as an isolated subpopulation, with negligible genetic exchange with the highland populations and limited exchange with the eastern population. There appear to be no relevant geographic barriers between the west coast and central highlands, and rice fields (the ideal habitat for *A. funestus*) are distributed widely across the island. However, Mahajunga is situated in a dry and hot climate where the

temperature during some parts of the year should make the reproduction and spread of the vector more difficult.

This study has several consequences for malaria vector control in Madagascar. First, neighbouring populations can rapidly invade control areas, hindering control methods based on indoor residual spraying. Second, *A. funestus* populations are in genetic exchange over a fairly wide area of the island, highland populations being in contact with coastal populations on the eastern side of the island. This suggests that populations further south may also be in exchange with the eastern population. When bio climates are radically different, genetic exchange seems more limited, and vector control can have a longer lasting effect. As an alternative to insecticidal control, malaria control by the release of genetically modified mosquitoes has been proposed (Collins et al., 2000). Although it is generally accepted that such strategies should be tested on small islands (Pinto et al., 2003), eventually larger areas need to be included, and Madagascar might be the ideal site for this strategy. Given the different environs and subpopulations of vectors as reported in this study, GMM strategies must consider these genetic variations in vector species. The distribution and genetic composition of the vectors seems an essential requirement in this concept of malaria control.

In conclusion, the study demonstrates the existence of genetically different subpopulations of *A. funestus* on Madagascar, with genetic exchange between populations when geographic features allow for such exchanges. Altitude does not seem to be a limiting factor for genetic exchange. The *A. funestus* population on the island of Mohéli, Comoros, is not related to those on Madagascar and may have arrived independently. For an effective control of malaria on Madagascar, the distribution of subpopulations of the main malaria vector should be considered.

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