Available online at www.sciencedirect.com
ScienceDirect
Acta Tropica 101 (2007) 61–68
ACTA TROPICA
www.elsevier.com/locate/actatropica

Population genetic structure of the malaria vector *Anopheles moucheti* in south Cameroon forest region

Christophe Antonio-Nkondjio [a,c,*], Cyrille Ndo [a,b], Parfait Awono-Ambene [a], Pierre Ngassam [b], Didier Fontenille [c], Frédéric Simard [a,c]

[a] *Laboratoire de Recherche sur le Paludisme, Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale (OCEAC), P.O. Box 15665, Yaoundé, Cameroon*

[b] *Faculty of Sciences, University of Yaounde I, P.O. Box 337, Yaounde, Cameroon*

[c] *Institut de Recherche pour le Développement (IRD), UR 016, 911, Avenue Agropolis, P.O. Box 64501, 34394 Montpellier Cedex 5, France*

Received 28 April 2006; received in revised form 3 December 2006; accepted 6 December 2006
Available online 21 December 2006

Abstract

We used recently developed microsatellite DNA markers to explore the population genetic structure of the malaria vector, *Anopheles moucheti*. Polymorphism at 10 loci was examined to assess level of genetic differentiation between four *A. moucheti* populations from South Cameroon situated 65–400 km apart. All microsatellite loci were highly polymorphic with a number of distinct alleles per locus ranging from 9 to 17. F_{st} estimates ranging from 0.0094 to 0.0275 ($P < 0.001$) were recorded. These results suggest a very low level of genetic differentiation between *A. moucheti* populations. The recently available microsatellite loci revealed useful markers to assess genetic differentiation between geographical populations of *A. moucheti* in Cameroon.

Keywords: Malaria; *A. moucheti*; Cameroon; Microsatellites DNA; Population genetics

1. Introduction

Malaria remains a major health problem in Africa (Snow et al., 2005). In the equatorial forest region, the disease is transmitted to humans by four major mosquitoes species: *Anopheles gambiae*, *A. funestus*, *A. nili* and *A. moucheti*. Among these four malaria vectors, *A. moucheti* is the only one whose distribution area is restricted to the forest environment. *A. moucheti* occurs in villages situated along slow moving streams or rivers where it is present all year long. It can sustain malaria transmission as high as 100–300 infected bites per human per year (Njan Nloga et al., 1993; Antonio-Nkondjio et al., 2002a). Despite its important role in malaria transmission, this vector remains poorly known and insufficiently studied.

A. moucheti is a group of three morphological forms: the type form *A. moucheti* moucheti, *A. m. nigeriensis*, *A. m. bervoetsi* distinguishable by morphological characters present at the adult and the larval stages (Gillies and De Meillon, 1968). Allozyme markers used to further investigate population genetic structure and the status of these morphological forms, provided no evidence for speciation between morphological forms and geographical populations occuring in different river systems in Cameroon (Antonio-Nkondjio et al., 2002b).

* Corresponding author at: Laboratoire de Recherche sur le Paludisme, Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale (OCEAC), P.O. Box 15665, Yaoundé, Cameroon. Tel.: +237 223 22 32; fax: +237 223 00 61.
E-mail address: antonio_nk@yahoo.fr (C. Antonio-Nkondjio).

0001-706X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2006.12.004

It was not clear wether the lack of genetic differentiation between *A. moucheti* populations in Cameroon was due to high rates of gene flow among these populations, shared ancestral polymorphism from a recent radiation or to the lack of specificity of the markers used.

Following the anti malaria campaign between 1950–1960 in the south Cameroon forest region, vector populations were totally eradicated from this area (Livadas et al., 1958). The stoppage of these control measures in the early 1960s was followed by the progressive reinvasion of vectors populations. It is thought that recolonization took place from forest zoophilic populations which escaped control measures (Livadas et al., 1958).

In the perspective of reducing malaria burden through vector control, a good understanding of vectors populations biology and genetics is vital for a successful management of control measures in the field. Information on the genetic structure of anopheline vectors is necessary for predicting the spread of insecticide resistance genes (Collins et al., 2000) and to reduce malaria transmission through the release of parasite refractory genes into wild malaria mosquitoes (Aultman et al., 2001).

For the major malaria vectors *A. gambiae* and *A. arabiensis* on which various genetic tools have been extensively used, it was reported that large bodies of water or geographical discontinuities can constitute barriers to gene flow (Lehmann et al., 1999, 2003; Simard et al., 1999; Kayondo et al., 2005). In the same way, can the equatorial forest constitute a barrier to dispersal for *A. moucheti*, given that it breeds along streams and rivers? We used, 10 recently described microsatellite loci (Annan et al., 2003) to explore patterns of gene flow between *A. moucheti* natural populations in Cameroon. As microsatellite markers were never used on *A. moucheti*, the purpose of this work was, (i) to assess wether these recently developped markers are suitable tools for population genetic studies in *A. moucheti* and (ii) to examine the genetic structure of *A. moucheti* populations in South Cameroon with particular emphasis on the role of river networks in shaping populations structure.

2. Materials and methods

2.1. Mosquitoes sampling and collection sites

Adult mosquitoes were collected by pyrethrum spraying and bednets traps in four localities of Cameroon: Simbock, Olama, Nyabessan and Mouloundou situated along different river systems (Fig. 1). Simbock (3°51′N, 11°30′E) is located approximately 5 km from Yaounde, along river Mefou. The village of Olama (3°24′N; 11°18′E), is situated 65 km south of Yaounde along river Nyong. Nyabessan (2°80′N; 10°25′E) is situated 220 km south of Yaounde along the Ntem river and Mouloundou (2°08′N; 15°23′E) is situated about 400 km south east of Yaounde, close to Congo popular republic boundary along Dja, Boumba and Ngoko rivers.

All these study sites are located within the Congo-Guinean phytogeographic zone, characterized by a typical equatorial climate with two rainy seasons extending from March to June and from September to November. Mean annual rainfall range 1600–1800 mm. Although most of the equatorial forest region of Cameroon undergoes deforestation, the forest still presents a deep cover in the South, close to the equator (Nyabessan, Mouloundou) while it is highly degraded in the north around Yaounde (Simbock, Olama). The nearest localities were Simbock and Olama situated 65 km apart, while the most distant ones were Simbock and Mouloundou situated about 400 km apart.

Collections were conducted in July 2003 in Nyabessan, August 2003 in Mouloundou, january 2004 in Olama and April 2004 in Simbock. *A. moucheti* specimens were visually sorted from other anophelines according to morphological identification keys (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). All specimens were stored individually and kept at −20 °C until further analysis.

2.2. DNA extraction and genotype scoring

Genomic DNA was extracted from wings or legs of each individual mosquito as described by Cornel and Collins (1996) and resuspended in 100 μl of TE buffer. Ten microsatellite loci over the 24 isolated from *A. moucheti* by Annan et al. (2003) were used for this work. Loci selection was based on high polymorphism and allele sizes ranging from 90 to 180 base pairs. We used loci AM1, AM2, AM5, AM6, AM9, AM10, AM13, AM15, AM16 and AM20. Their cytological locations are not yet known. A standard PCR was ran in a GeneAmp PCR System 2700 thermal cycler in a 12.5 μl reaction mixture containing 50 mM Tris–HCl pH 8.3 (Qiagen France), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate (Eurogentec, Belgium), 5 pmol of each primer, one unit of Taq DNA polymerase (Qiagen, France), and 1 μl of template DNA. The PCR conditions were as follow: denaturation at 94 °C for 5 min, followed by 30 cycles consisting of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and a final elongation step at 72 °C for 10 min. A 10 μl aliquot of PCR product was then mixed with loading buffer containing 30%

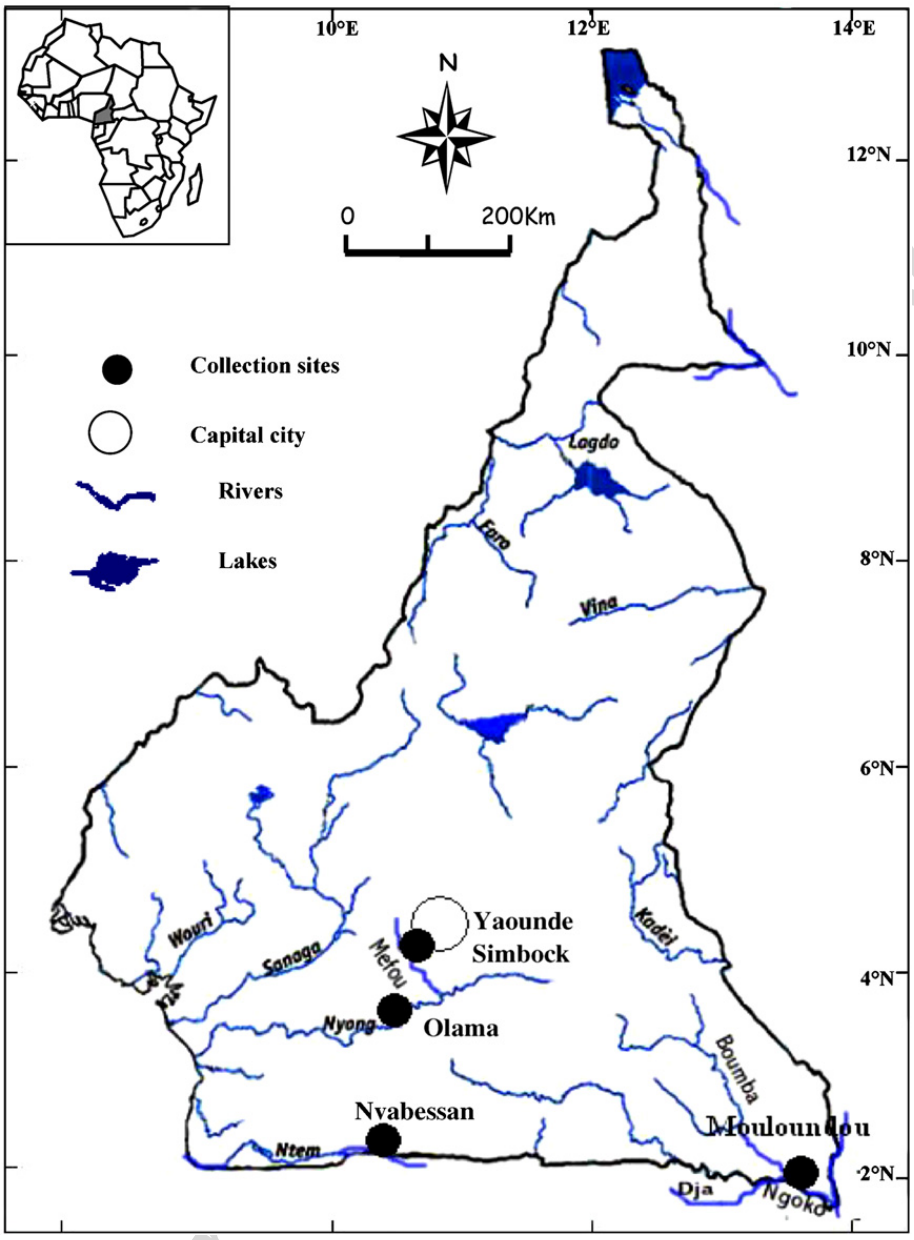

Fig. 1. A map of Cameroon showing the situation of collection sites along rivers networks.

glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanol. This mixture was loaded onto a 10% nondenaturing vertical polyacrylamide gel. The genotypes were determined after electrophoresis and rapid silver staining (Sanguinetti et al., 1994). A set of 47–64 specimens per population was investigated.

2.3. Data analysis

Genetic diversity was measured by the number of alleles and heterozygosity at each locus. Genotype frequencies were tested against Hardy–Weinberg's expectations for each locus in the pooled population and in each single population. Statistical significance was assessed by the exact probability test available in GENEPOP V3.2 computer program (Raymond and Rousset, 1995). Exact tests for linkage disequilibrium between pairs of loci were computed in the pooled population and within each population, using GENEPOP. The Bonferroni correction procedure (Holm, 1979) was applied to evaluate significance when multiple tests were performed.

Differentiation between populations was assessed by F-statistics (Wright, 1978), calculated according to Weir and Cockerham (1984). Significance of F_{is} and F_{st} was assessed using the exact probability test (Raymond and

Rousset, 1995) and the G-based exact test for genotypic differentiation (Goudet et al., 1996), respectively.

3. Results

3.1. Genetic variability and Hardy–Weinberg equilibrium

Genotypes of 223 *A. moucheti* females collected in Simbock, Olama, Nyabessan and Mouloundou were scored at 10 microsatellite loci. All microsatellite loci were highly polymorphic with a number of distinct alleles per locus ranging from 9 to 17 (Table 1). The average number of allele per locus over the 10 markers ranged from 10.6 to 11.2 per population and was not significantly different between populations ($F = 2.85$; d.f. = 3; $P = 0.051$). The average observed heterozygosity and expected heterozygosity across all loci in each population did not differ significantly ($P > 0.6$) (Table 1).

When the pooled samples were analyzed as a single panmictic population, 7 out of 10 loci showed significant deviations from Hardy–Weinberg equilibrium due to significant heterozygote deficiency (Table 1) indicating a Wahlund effect (mixing of different gene pools). At the local population level, 11 out of 40 tests did not conform to Hardy–Weinberg expectations after the multi test analysis was taken into account. All significant tests were associated with heterozygote deficits (positive F_{is}). Deviation from Hardy–Weinberg expectations were found across all populations at locus AM6, across three of the four populations (except Simbock) in locus AM1, in both Nyabessan and Mouloundou for locus AM2 and in Simbock for loci AM5 and AM15. The occurrence of null alleles could explain these deviations as in most cases a few individuals failed to amplify a PCR product on repeated PCR amplifications suggesting that they represented homozygotes for null alleles.

Exact tests for linkage disequilibrium within each population (180 pairwise comparison) were performed to further assess the genetic structure of natural *A. moucheti* populations. They resulted in two significant values (AM1-AM9 in Nyabessan and AM9-AM20 in Mouloundou) after correction by the Bonferroni procedure (nine significant values are expected at the 5% level). This result demonstrated statistical independence of the loci. In the pooled population, 3 of the 45 tests for linkage disequilibrium were significant at the single test level, none of which remained significant when the Bonferroni procedure was applied, suggesting a very low level of linkage disequilibrium among the 10 loci studied. This analysis revealed that each geographical population might be considered as panmictic.

3.2. Genetic differentiation and isolation by distance

The mean F_{st} estimate based on the whole dataset (10 loci and 4 populations) was 0.152 and was highly significant ($P < 0.001$). Jacknifing over loci (Table 2) provided consistent results reflecting homogeneity across loci. Mean pairwise estimates of F_{st} over all loci between different locations ranged from 0.0094 (Olama and Nyabessan) to 0.0275 (Simbock and Mouloundou) and were highly significant ($P < 0.001$). The highest F_{st} estimates were obtained between the most distant populations (Table 3). However, in this case, locus AM9 presented particularly high F_{st} estimates compared to other loci and should be considered as an outlier.

Isolation by distance correlations between pairwise genetic distances measured as $F_{st}/(1 - F_{st})$ and the logarithm of distance between pairs of populations was significant ($R = 0.809$, $P < 0.05$) when considering the whole dataset (Fig. 2). However this correlation was no more significant when locus AM9 was ommitted from the analysis ($t_0 = 1.31$; d.f. = 221; $P > 0.05$).

4. Discussion

This is the first report of the use of microsatellite markers for the study of population genetic structure in *A. moucheti*. Microsatellite loci used for this study amplified well and individual genotypes were easily scored sustaining the reliability of this tool for *A. moucheti* genetic structure studies. All loci were highly polymorphic compared to allozyme markers (Antonio-Nkondjio et al., 2002b). This is consistent with former studies comparing allozymes to microsatellites (Lanzaro et al., 1995; Estoup et al., 1998). However, there was evidence for null alleles occuring, at least, at some loci and that could significantly impact inferences drawn from such loci. Null alleles are commonly reported in studies using microsatellite markers to unravel natural anopheles population structure (Lehmann et al., 1996; Walton et al., 1998; Donnelly and Townson, 2000; Wondji et al., 2002). In this study, the overall lack of linkage disiquilibrium between loci together with the demonstrated homogeneity of differentiation indices obtained across loci, suggest that, null alleles did not significantly bias our conclusions.

Levels of pairwise genetic differentiation estimated by F_{st} (Weir and Cockerham, 1984) were low and statistically significant between all four *A. moucheti* populations. However, inter population differences were not identical across all loci. The exclusion of locus AM9

Table 1
Genetic variability at 10 microsatellite loci in *Anopheles moucheti* from Cameroon

Locus	Populations				
	Simbock ($2n = 118$)	Olama ($2n = 112$)	Nyabessan ($2n = 108$)	Mouloundou ($2n = 108$)	All ($2n = 446$)
AM1					
N_{all}	9	11	7	11	13
H	0.805	0.823	0.805	0.854	0.835
F_{is}	+0.139	**+0.296**	**+0.347**	**+0.458**	**+0.308**
AM2					
N_{all}	11	13	10	12	15
H	0.807	0.847	0.812	0.836	0.832
F_{is}	+0.116	+0.186	**+0.342**	**+0.268**	**+0.230**
AM5					
N_{all}	13	11	14	13	16
H	0.881	0.862	0.898	0.870	0.880
F_{is}	**+0.150**	+0.018	+0.069	+0.114	+0.092
AM6					
N_{all}	12	12	11	10	13
H	0.872	0.826	0.870	0.850	0.873
F_{is}	**+0.285**	**+0.298**	**+0.263**	**+0.217**	**+0.280**
AM9					
N_{all}	11	12	13	12	15
H	0.762	0.800	0.875	0.785	0.841
F_{is}	−0.018	+0.001	+0.042	+0.112	**+0.069**
AM10					
N_{all}	7	6	8	7	9
H	0.704	0.698	0.790	0.764	0.747
F_{is}	−0.065	+0.028	+0.110	+0.032	+0.038
AM13					
N_{all}	8	8	7	9	9
H	0.823	0.824	0.802	0.830	0.824
F_{is}	+0.008	+0.042	+0.177	+0.121	+0.089
AM15					
N_{all}	16	14	16	13	17
H	0.820	0.822	0.869	0.818	0.838
F_{is}	**+0.188**	+0.094	+0.049	+0.178	**+0.130**
AM16					
N_{all}	10	9	10	12	16
H	0.823	0.843	0.822	0.829	0.841
F_{is}	+0.051	+0.101	+0.077	+107	**+0.096**
AM20					
N_{all}	12	13	10	13	14
H	0.844	0.832	0.788	0.848	0.835
F_{is}	+0.218	+0.066	+0.203	+0.173	**+0.172**
Mean across All loci					
N_{all}	10.8	10.9	10.6	11.2	13.7
H	0.813	0.818	0.833	0.830	0.823
F_{is}	+0.112	+0.114	+0.166	+0.180	NC

All: refers to populations pooled. $2n$, number of chromosomes scored; N_{all}, number of alleles per locus; H, expected heterozygosity under Hardy–Weinberg equilibrium (Nei, 1978). F_{is} was calculated according to Weir and Cockerham (1984) and goodness of fit to Hardy–Weinberg equilibrium was estimated by the exact test available in Genepop 3.2 (Raymond and Rousset, 1995). Bolded values: $P < 0.05$ after taking into account multiple tests (Holm, 1979).

Table 2
Jacknifing over loci for the estimation of overall population differentiation (F_{st}) between four *A. moucheti* populations from Cameroon

Locus removed	F_{st}	*P* values
AM1	0.1344	<0.0001
AM2	0.1430	<0.0001
AM5	0.1588	<0.0001
AM6	0.1367	<0.0001
AM9	0.1610	<0.0001
AM10	0.1629	<0.0001
AM13	0.1586	<0.0001
AM16	0.1579	<0.0001
AM20	0.1495	<0.0001
AM15	0.1542	<0.0001
None	0.152	<0.0001

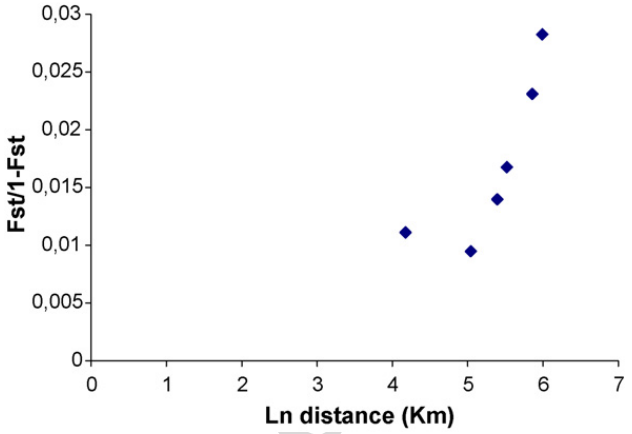

Fig. 2. Correlation between $F_{st}/(1 - F_{st})$ and logarithm of distance (in km) for pairwise comparisons of four *A. moucheti* populations from Cameroon at 10 microsatellites loci.

from the analysis substantially drops level of genetic differentiation between the most remote collection site (Mouloundou) and the other collection sites although it remained significant. The fact that Mouloundou is situated in a forest area with a deep cover compared to the other collection sites, where the forest cover is highly degraded could mean that, loci AM9 is situated close to genes involved in environmental adaptations. It is possible in the light of the data presented above that variation between populations is shaped by differential ecological adaptations and possible barriers to dipersal. However, depending on their position on the chromosome, some loci may be more informative this could be the case of loci AM9 and AM16 which show more variations than the others.

Low F_{st} estimates as obtained in this study, could either result from high levels of contemporary gene flow between natural *A. moucheti* populations in spite of a large geographical distance of 65–400 km between sites, or reflect historical gene flow and the maintenance in the populations of ancestral polymorphism (Krzywinski and Bezansky, 2003). Although the biology and ecology of the species is still poorly known, the hypothesis of high current gene flow across long distances is difficult to reconcile with field observations on mosquito dispersal. In forest environments, adult *A. moucheti* are rarely found beyond 1.5 km from their breeding sites (Languillon et al., 1956; Njan Nloga, 1994; Antonio-Nkondjio et al., 2005). Similar observations were reported for the forest mosquito *A. nili* along the Sanaga river (Le Goff et al., 1997) suggesting that dense forests can act as a barrier to mosquito dispersal. Using direct methods for gene flow estimation such as Mark Release Recapture, it was found that active dispersal of *A. gambiae* is restricted to 2–3 km (Constantini et al., 1996; Touré et al., 1998), but can rise up to 7 km if assisted by winds (Gillies and De Meillon, 1968). For *A. moucheti*, wind assisted migration is possible along the river network as this environment offers no barrier for migration compared to the forest. Passive

Table 3
Pairwise F_{st} estimates between *A. moucheti* populations from Cameroon

Locus	Simbock × Olama	Simbock × Nyabessan	Simbock × Mouloundou	Olama × Nyabessan	Olama × Mouloundou	Nyabessan × Mouloundou
AM1	0.0092*	0.0016	0.0375***	−0.0033	0.0196**	0.0206**
AM2	0.0225**	0.0052	0.0133***	0.0084	0.0037*	−0.0064
AM5	−0.0036	0.0038**	0.0022*	0.0078**	0.0045*	0.0071**
AM6	0.0399***	0.0131**	0.0366***	0.0206***	0.0305***	0.0238**
AM9	0.0054	0.0298*	0.1247***	0.0170	0.0958***	0.0622***
AM10	0.0043*	0.0337**	0.0125*	0.0306***	0.0194*	0.0048
AM13	0.0112	0.0126	0.0118	−0.0016	0.0003	0.0025
AM15	−0.0023	0.0147*	−0.0032	0.0198*	0.0066	0.0063*
AM16	−0.0049	0.0028*	0.0324***	−0.0043	0.0370***	0.0366***
AM20	0.0244**	0.0217**	−0.0005	−0.0012	0.0051	0.0032
Overall	0.0110***	0.0138***	0.0275***	0.0094***	0.0226***	0.0165***

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

dispersal of larvae carried by water current facilitated by the aquatic plant *Pistia strathiotes* (Wanson et al., 1947) may be the principal dispersal mean along the river networks. Indeed high larva densities are often recorded under this plant which is an excellent hiding place and which offers suitable feeding resources. However this migration might be limited to a small geographical scale because of larval predators and rapids.

On the other hand, historical factors such as recent population expansion might explain the low level of genetic differentiation recorded between *A. moucheti* populations, even in the face of restricted gene flow between populations (Donnelly et al., 2001; Krzywinski and Bezansky, 2003). It is likely that, *A. moucheti* has extended its distribution range relatively recently aided by population settlments throughout the forest, and the intensification of fishing activities along river networks. Human activities such as road construction, land cultivation, population settlements along rivers or roads, commercial traffic between sites could also have favoured dispersal of this anthropophilic mosquito. Correlation between gene flow and commercial traffic intensity by planes or boats was indeed reported for *Aedes polynesiensis* between polynesian islands (Failloux et al., 1997), and examples of passive transportation of mosquitoes via trains and boats over large distances have been reported in the litterature (Holstein, 1954; Service, 1997).

Clearly, any conclusion drawn so far on the genetic structure of *A. moucheti* would remain tentative and difficult to justify, and is out of the scope of the present work. We provided evidence that microsatellite loci are suitable tools to study the population genetic structure of *A. moucheti* in the forest of Central Africa but common pittfalls of anophelines population genetics data interpretation remain. Further studies using additional populations collected according, to a hierarchical sampling design along and across hydrographic networks throughout the species distribution area, should allow more refined analysis of the populations structure and the dynamics of gene exchange within and between *A. moucheti* populations. Such knowledge is relevant for a better understanding of malaria epidemiology in Africa, and would be incidental to the development and monitoring, of any control measure to be implemented in areas where *A. moucheti* contributes to the transmission of this life threatening disease.

Acknowledgements

We are indebted to I. Morlais for her comments on earlier versions of the draft, to the reviewers for their helpful comments, to C. Wondji, S. Zebaze-Kemleu J.C. Toto and J.P. Agbor for valuable help in the field and laboratory. This work was supported by grants no. A20727 and A60347 from the UNICEF/UNDP/World Bank/WHO Special programme for Research and Training in Tropical Diseases (TDR) to C.A.N and the French Institut de Recherche pour le Développement (IRD/DSF) through a fellowship to C.A.N.

References

Annan, Z., Kengne, P., Berthomieu, A., Antonio-Nkondjio, C., Rousset, F., Fontenille, D., Weill, M., 2003. Isolation and characterisation of polymorphic microsatellite markers from the mosquito *Anopheles moucheti*, malaria vector in Africa. Mol. Ecol. Notes, 56–57.

Antonio-Nkondjio, C., Awono-Ambene, P., Toto, J.C., Meunier, J.Y., Zebaze-Kemleu, S., Nyambam, R., Wondji, C.S., Tchuinkam, T., Fontenille, D., 2002a. High malaria transmission intensity in a village close to Yaoundé, the capital city of Cameroon. J. Med. Entomol. 39, 330–355.

Antonio-Nkondjio, C., Simard, F., Cohuet, A., Fontenille, D., 2002b. Morphological variability in *Anopheles moucheti* is not indicative of speciation: evidences from sympatric south Cameroon populations. Infect. Genet. Evol. 2, 69–72.

Antonio-Nkondjio, C., Simard, F., Awono-Ambene, P., Ngassam, P., Toto, J.C., Tchuinkam, T., Fontenille, D., 2005. Malaria vectors and urbanisation in the equatorial forest region of south Cameroon. Trans. R. Soc. Trop. Med. Hyg. 99, 347–354.

Aultman, K.S., Beaty, B.J., Walker, E.D., 2001. Genetically manipulated vectors of human disease: a practical overview. Trends Parasitol. 17, 507–509.

Collins, F.H., Kamau, L., Ranson, H.A., Vulule, J.M., 2000. Molecular entomology and prospects for malaria control. Bull. Wld. Health Org. 78, 1412–1423.

Constantini, C., Li, S.G., Della-Tore, A., Sagnon, N.F., Coluzzi, M., Taylor, C.E., 1996. Density, survival and dispersal of *Anopheles gambiae* complex mosquitoes in west Africa Sudan savanna village. Med. Vet. Entomol. 10, 203–219.

Cornel, A., Collins, F.H., 1996. PCR of the ribosomal DNA Intergenic Spacer Regions as a method for identifying mosquitoes in the *Anopheles gambiae* complex. Meth Mol. Biol. 50, 321–332.

Donnelly, M.J., Townson, H., 2000. Evidence for extensive genetic differentiation among populations of the malaria vector, *Anopheles arabiensis*, in East Africa. Insect Mol. Biol. 9, 357–367.

Donnelly, M.J., Licht, M.C., Lehmann, T., 2001. Evidence for a recent population expansion in the malaria vectors *Anopheles arabiensis* and *Anopheles gambiae*. Mol. Biol. Evol. 18, 1353–1364.

Estoup, A., Rousset, F., Michalakis, Y., Cornuet, J.M., Adriamanga, M., Guyomard, R., 1998. Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). Mol. Ecol. 7, 339–353.

Failloux, A., Raymond, M., Ung, A., Chevillon, C., Pasteur, N., 1997. Genetic differenciation associates with commercial trafic in the polynesian mosquito, Aedes polynesiensis Marks 1951. Biol. J. Linn. Soc. 60, 107–118.

Gillies, M.T., De Meillon, B., 1968. The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region), 2nd ed. The South African Institute for Medical Research, Johannesburg.

Gillies, M.T., Coetzee, M., 1987. A supplement to the Anophelinae of Africa south of the Sahara. Publications of the South African Institute for Medical Research, Johannesburg.

Goudet, J., Raymond, M., De Meeüs, T., Rousset, F., 1996. Testing differentiation in diploid populations. Genetics 144, 1933–1940.

Holm, S., 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6, 65–70.

Holstein, M.H., 1954. Biology of *Anopheles gambiae*. Research in French West Africa. WHO, Geneva.

Kayondo, J.K., Mukwaya, G.L., Stump, A., Michel, A.P., Coulibaly, M.B., Besansky, N.J., Collins, F.H., 2005. Genetic structure of *Anopheles gambiae* populations on islands in northwestern Lake Victoria, Uganda. Malaria J. 4, 1–59.

Krzywinski, J., Bezansky, N.J., 2003. Molecular systematics of Anopheles: from subgenera to subpopulations. Annu. Rev. Entomol. 48, 111–139.

Languillon, J., Mouchet, J., Rivola, E., Rageau, J., 1956. Contribution à l'étude de l'épidémiologie du paludisme dans la région forestière du Sud-Cameroun. Méd. Trop. 16, 247–379.

Lanzaro, G.C., Zheng, L., Touré, Y.T., Traoré, S.F., Kafatos, F.C., Vernick, K.D., 1995. Microsatellite DNA and isoenzyme variability in west African populations of *Anopheles gambiae*. Insect Mol. Biol. 4, 105–112.

Le Goff, G., Carnevale, P., Robert, V., 1997. Low dispersion of anopheline malaria vectors in the african equatorial forest. Parasite 2, 187–189.

Lehmann, T., Hawley, W.A., Collins, F.H., 1996. An evaluation of evolutionary constraints on microsatellites loci using null alleles. Genetics 144, 1155–1163.

Lehmann, T., Hawley, W.A., Grebert, H., Danga, M., Atieli, F., Collins, F.H., 1999. The rift valley complex as a barrier to gene flow for *Anopheles gambiae* in Kenya. J. Hered. 90, 613–621.

Lehmann, T., Licht, M., Elissa, N., Maega, B.T.A., Chimumbwa, J.M., Watsenga, F.T., Wondji, C.S., Simard, F., Hawley, W.A., 2003. Population structure of *Anopheles gambiae* in Africa. J. Hered. 94, 133–147.

Livadas, G., Mouchet, J., Gariou, J., Chastang, R., 1958. Peut-on envisager l'éradication du paludisme dans la region forestière du sud Cameroun? Estratto dalla Rivista di Malariologia-Roma 37, 228–256.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.

Njan Nloga, A., Vincent, R., Toto, J.C., Carnevale, P., 1993. *Anopheles moucheti*, vecteur principal du paludisme au sud-Cameroun. Bull. Liais. doc. OCEAC 26, 63–67.

Njan Nloga, A., 1994. Bioécologie et rôle vecteur du paludisme d'*Anopheles moucheti* à Ebogo au Cameroun. Thèse de doctorat Université de Montpellier II Sciences et Techniques du Languedoc, pp. 1–233.

Raymond, M., Rousset, F., 1995. An exact test for population differenciation. Evolution 49, 1280–1283.

Sanguinetti, C.J., Neto, E.D., Simpson, A.J.G., 1994. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques 17, 915–919.

Service, M.W., 1997. Mosquitoe (Diptera: Culicidae) dispersal-the long and short of it. J. Med. Entomol. 34, 579–588.

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

Snow, R.W., Guerra, C.A., Noor, A.M., Myint, H.Y., Hay, S.I., 2005. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature 434, 214–217.

Touré, Y.T., Dolo, G., Petreca, V., Traoré, S.F., Bouare, M., Dao, A., Carnahan, J., Taylor, C.E., 1998. Mark-release-recapture experiments with *Anopheles gambiae s.l.* in Banambani village, Mali, to determine size and structure. Med. Vet. Entomol. 12, 74–83.

Walton, C., Thelwell, T.J., Priestman, A., Butlin, R.K., 1998. The use of microsatellites to study gene flow in natural populations of *Anopheles* malaria vectors in Africa: potential and pitfalls. J. Am. Mosq. Control Assoc. 14, 266–272.

Wanson, M., Wolfs, J., Lebied, B., 1947. Comportement de l'*Anopheles* (Myzomyia) *moucheti* Evans. Recueil Travaux Sci. Méd. Congo Belge 6, 39–62.

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

Wondji, C., Simard, F., Fontenille, D., 2002. Evidence for genetic differentiation between the molecular forms M and S within the forest chromosomal form of *Anopheles gambiae* in an area of Sympatry. Insect Mol. Biol. 11, 11–19.

Wright, S., 1978. Evolution and Genetics of Populations, vol. 4. Variability Within and Among Natural Populations. University of Chicago Press, Chicago.