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

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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).

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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%