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Polymorphic chromosomal inversions in *Anopheles moucheti*, a major malaria vector in Central Africa

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Abstract

Anopheles moucheti Evans (Diptera, Culicidae) is a major vector of malaria in forested areas of Central Africa. However, few genetic tools are available for this species. The present study represents the first attempt to characterize chromosomes in *An. moucheti* females collected in Cameroon. Ovarian nurse cells contained polytene chromosomes, which were suitable for standard cytogenetic applications. The presence of three polymorphic chromosomal inversions in *An. moucheti* was revealed. Two of these inversions were located on 2R chromosomal arm. The homology between 2R chromosomal arms of *An. moucheti* and *An. gambiae* Giles was established by fluorescent *in situ* hybridization of six *An. gambiae* genic sequences. Mapping of the probes on chromosomes of *An. moucheti* detected substantial gene order reshuffling between the two species. The presence of polytene chromosomes and polymorphic inversions in *An. moucheti* provides a new tool for population genetic, taxonomic, and ecological studies of this neglected malaria vector.

Keywords

Anopheles gambiae; *Anopheles moucheti*; fluorescent *in situ* hybridization; inversion; malaria mosquito; physical mapping; polytene chromosome

Mosquitoes of the subgenus *Cellia*, *Anopheles gambiae*, *An. arabiensis* Patton, *An. funestus* Giles, *An. nili* Theobald, and *An. moucheti*, are the major malaria vectors in Africa. *Anopheles gambiae*, *An. arabiensis*, *An. funestus*, and *An. nili* have wide geographic distributions, spreading across most of West, Central and East Africa. In contrast, *An.*

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moucheti is restricted to the evergreen forest areas of Central Africa, where it breeds in slowly moving streams and rivers (Antonio-Nkondjio *et al.*, 2009; Ayala *et al.*, 2009). In this ecological setting, *An. moucheti* is the most abundant mosquito ensuring malaria transmission throughout the year. For instance, in the village of Simbock situated close to Yaounde, *An. moucheti* accounts for >54% of total anophelines caught, and is responsible for 39.2% of malaria transmission (Antonio-Nkondjio, Awono-Ambene, *et al.*, 2002). Similarly, *An. moucheti* is the most abundant mosquito in the rural village of Olama within the equatorial forest zone of Cameroon (Antonio-Nkondjio *et al.*, 2005). Moreover, a recent study conducted in Gabon has discovered that *An. moucheti* is not only responsible for the natural transmission of *Plasmodium falciparum* to humans, but it is also involved in the transmission of *P. praefalciparum* among great apes and therefore constitutes a main bridge vector candidate for transferring the malaria parasite from apes to humans (Paupy *et al.*, 2013).

Differential ecological adaptations and behaviours of mosquitoes are often associated with dramatic changes in composition and frequency of polymorphic chromosomal inversions. For example, inversions in *An. gambiae* are non-randomly distributed temporally and spatially with respect to degree of aridity increasing the epidemiological significance of the vector (Coluzzi *et al.*, 2002). Despite the importance of *An. moucheti* in malaria transmission, population cytogenetic studies have not been performed on this vector. Here, polytene chromosomes in *An. moucheti* are described and an evaluation of their suitability for the analysis of inversion polymorphism and physical genome mapping is made.

Females of *An. moucheti* were collected by pyrethrum spraying and bednet traps in three localities of Cameroon: Lapse (03°52'N; 11°25'E), Moloundou (02°08'N, 15°23'E), and Olama (03°24'N, 11°18'E), which are situated along different river systems. Specimens were identified in the field as members of the *An. moucheti* complex by using morphological identification keys (Gillies & Coetzee, 1987) and were further characterized by molecular assays as *An. m. moucheti* (hereafter *An. moucheti*) (Kengne *et al.*, 2007). Immediately after collection, semi gravid females were dissected under a binocular and their ovaries were isolated and fixed in Carnoy's solution. Chromosomal preparations were made from Carnoy-fixed ovaries as described elsewhere (Sharakhova *et al.*, 2011). The *in situ* hybridization procedure was conducted as previously described, using probes and primers from the *An. gambiae* genome assembly (Sharakhova *et al.*, 2011).

The cytogenetic study was performed on chromosomal preparations obtained from 40 *An. moucheti* females collected in the three localities. Seventeen out of these females had ovarian nurse cells containing polytene chromosomes of sufficient quality for standard cytogenetic applications. The polytene chromosome complement of *An. moucheti* consisted of one short sex chromosome and four autosomal arms (Fig. 1A). The assignment of chromosomal arms was done based on their relative length and associations. Three polymorphic paracentric chromosomal inversions were found in *An. moucheti*. Two inversions located on chromosome 2 were named 2Ra and 2Rc (Fig. 1B, C). One inversion found on chromosome 3 was named 3Rb (Fig. 1A). Naming of the inversions was made according to the chronological order of their discovery. No inversions were found on chromosome X. Five of seven analyzed females from Lapse had one, two or three

heterozygous inversions. Four of seven females from Olama had at least one heterozygous inversion. Three females from Moloundou were chromosomally monomorphic. To our knowledge, this is the first description of polymorphic chromosomal inversions in *An. moucheti*. Populations of other major malaria vectors, *An. gambiae*, *An. arabiensis*, *An. funestus*, and *An. nili* have significantly reduced inversion polymorphism in forested areas of Central Africa as compared with West Africa (Coluzzi *et al.*, 2002; Cohuet *et al.*, 2005; Sharakhova *et al.*, 2011). The presence of inversions in *An. moucheti* urges further exploration of a role of chromosomal polymorphism in ecological adaptation and behavior of this mosquito.

Polymorphic inversions in *An. gambiae*, *An. funestus*, and *An. nili* have been primarily found on 2R chromosomal arm, which is homologous in these species (Sharakhov *et al.*, 2002; Sharakhova *et al.*, 2011). Similarly, two of three polymorphic inversions were found on 2R in *An. moucheti*. To confirm the 2R arm homology between *An. moucheti* and *An. gambiae*, we successfully mapped six DNA probes designed based on *An. gambiae* genes AGAP001759, AGAP001763, AGAP001983, AGAP001984, AGAP002934, and AGAP002935 to the 2R arm of *An. moucheti*. The physical locations were compared with the positions of the homologous sequences in the *An. gambiae* genome. We observed substantial gene order reshuffling between the two species. For example, genes AGAP001759 and AGAP002935 were located in close proximity to each other in *An. moucheti* (Fig. 2A) but far away from each other in *An. gambiae*. Genes AGAP001759 and AGAP001763 were close neighbours in the *An. gambiae* genome but they mapped far apart on the *An. moucheti* chromosome (Fig. 2B). High rates of gene order reshuffling have been observed among other malaria vectors (Sharakhov *et al.*, 2002; Sharakhova *et al.*, 2011) indicating that paracentric inversions have been the major type of chromosomal rearrangements in the evolution of mosquitoes.

The present study calls for a more detailed characterization of chromosomes in the *An. moucheti* complex in order to facilitate population genetic, taxonomic, and genomic studies of this neglected malaria vector. The taxonomic relationships within the *An. moucheti* complex are unclear. Morphological and behavioural variations observed among natural populations suggest that several taxa may belong to the *An. moucheti* complex: *An. moucheti*, *A. m. nigeriensis*, and *An. m. bervoetsi* (Gillies & Coetzee, 1987). Contrary to these observations, a study of the diversity of isoenzyme markers and the inheritance of morphological characters in F1 progenies obtained from field-collected females has suggested that all three forms belong to a single species, at least in Cameroon (Antonio-Nkondjio, Simard, *et al.*, 2002). Similar results have been obtained using ten microsatellite markers, revealing a low level of genetic differentiation among four *An. moucheti* populations from South Cameroon (Antonio-Nkondjio *et al.*, 2007). However, when mosquitoes were collected from their type localities (in the Democratic Republic of the Congo (DRC) and Nigeria for *An. m. bervoetsi* and *An. m. nigeriensis*, respectively), sequence differences were revealed in the gene encoding for mitochondrial cytochrome oxidase b (CytB) and in the ribosomal internal transcribed spacers (ITS1 and ITS2) between the three morphological forms. These differences were similar in degree to the differences shown previously for members of other anopheline species groups or complexes (Kengne *et*

al., 2007). Microsatellite analysis has further demonstrated significant genetic differentiation between *An. m. bervoetsi* populations from DRC and *An. moucheti* populations from Cameroon, suggesting that they may represent two different species (Antonio-Nkondjio et al., 2008). Cytogenetic studies have been useful for understanding population genetics and taxonomy of malaria mosquitoes (Coluzzi et al., 2002). A recent analysis of polytene chromosomes in another neglected malaria vector, *An. ovengensis*, has revealed high karyotypic divergence within the *An. nili* group (Sharakhova et al., 2013). Future studies will aim at understanding the role of inversion polymorphism in ecological adaptation, population differentiation, and speciation in the *An. moucheti* complex and will open the way for comprehensive comparative cytogenetic analyses across major African vector species.

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Figure 1. Polytene chromosomes in ovarian nurse cells of *An. moucheti* from Cameroon
 A) The complete chromosome set from one nucleus. A heterozygote inversion b/+ is shown on chromosome 3. B) The heterozygote inversion 2Ra/+. C) The heterozygote inversion 2Rc/+. The arm names are shown near the telomeric regions. C—centromeric regions.

Figure 2. Mapping of *An. gambiae* genes to polytene chromosomes of *An. moucheti*
Three DNA probes, AGAP002935 (A), AGAP001759 (A, B), and AGAP001763 (B), hybridized to euchromatic regions on the 2R chromosomal arm of *An. moucheti*. Arrows indicate signals of hybridization.