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SHORT COMMUNICATION

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 the 2R chromosome arm. The homology between the 2R chromosome arms of *An. moucheti* and *Anopheles 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 basis for further population genetic, taxonomic and ecological studies of this neglected malaria vector.

Key words. *Anopheles gambiae*, *Anopheles moucheti*, fluorescent *in situ* hybridization, inversion, malaria mosquito, physical mapping, polytene chromosome.

Mosquitoes of the subgenus *Cellia*, *Anopheles gambiae*, *Anopheles arabiensis* Patton, *Anopheles funestus* Giles, *Anopheles nili* Theobald and *Anopheles moucheti*, are the major malaria vectors in Africa. *Anopheles gambiae*, *An. arabiensis*, *An. funestus* and *An. nili* have wide geographic distributions spread across most of West, Central and East Africa. By contrast, *An. moucheti* is restricted to the evergreen forest areas of Central Africa, where it breeds in slow-moving streams and rivers (Antonio-Nkondjio *et al.*, 2009; Ayala *et al.*, 2009). In this ecological setting, *An. moucheti* is the most abundant mosquito and its presence ensures the transmission of malaria 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 *et al.*, 2002a). 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 discovered that *An. moucheti* is not only responsible for the natural transmission of *Plasmodium falciparum* to humans, but is also involved in the transmission of *Plasmodium praefalciparum* among great apes and therefore constitutes a main bridge vector candidate for the transferral of 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 the composition and frequency of polymorphic chromosomal

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inversions. For example, inversions in *An. gambiae* are non-randomly distributed temporally and spatially with respect to degree of aridity, which increases 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 in this vector. The present paper describes polytene chromosomes in *An. moucheti* and evaluates their suitability for the analysis of inversion polymorphism and physical genome mapping.

Females of *An. moucheti* were collected by pyrethrum spraying and bednet traps in three localities of Cameroon, Lepse (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 using morphological identification keys (Gillies & Coetzee, 1987) and were further characterized by molecular assays as *An. moucheti 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 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 chromosome arms was based on their relative lengths 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). Inversions were named according to the chronological order of their discovery. No inversions were found on chromosome X. Five of seven females from Lepse 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, including *An. gambiae*, *An. arabiensis*, *An. funestus* and *An. nili*, in forested areas of Central Africa have significantly reduced inversion polymorphism in comparison with populations in 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 the role of chromosomal polymorphism in the ecological adaptation and behaviour of this mosquito.

Polymorphic inversions in *An. gambiae*, *An. funestus* and *An. nili* have been primarily found on the 2R chromosome 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 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 from one another in *An. gambiae*. Genes AGAP001759 and AGAP001763 were close neighbours in the *An. gambiae* genome, but were mapped far apart on the *An. moucheti* chromosome (Fig. 2B). High

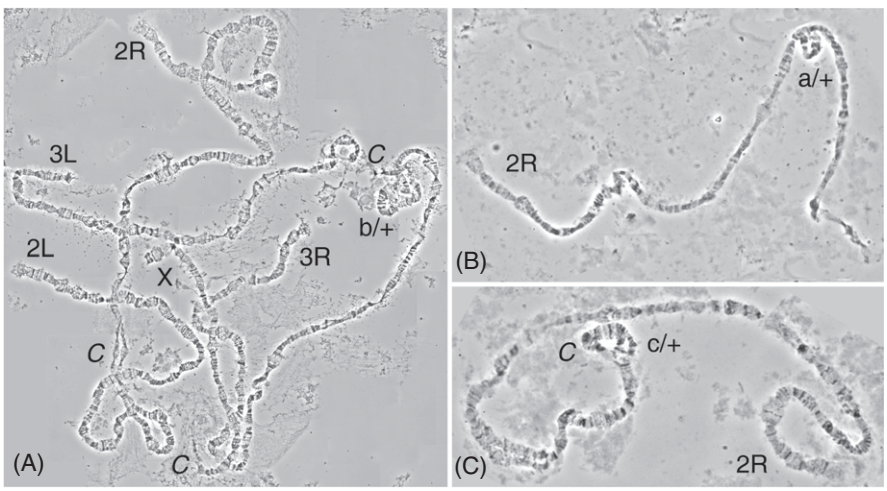

Fig. 1. Polytene chromosomes in ovarian nurse cells of *Anopheles moucheti* from Cameroon. (A) The complete chromosome set from one nucleus. A heterozygote inversion b/+ is shown on chromosome 3. (B) The heterozygote inversion 2Ra/+. (C) The heterozygote inversion 2Rc/+. The arm names are shown near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

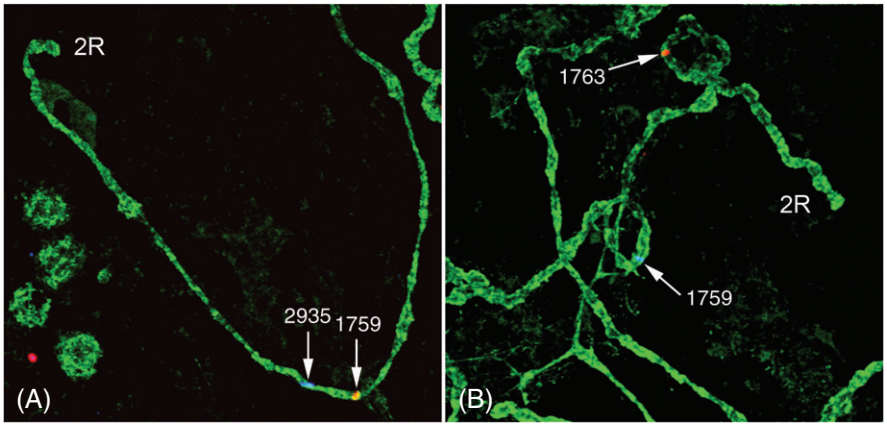

Fig. 2. Mapping of *Anopheles gambiae* genes to polytene chromosomes of *Anopheles moucheti*. Three DNA probes, AGAP002935 (A), AGAP001759 (A, B) and AGAP001763 (B), hybridized to euchromatic regions on the 2R chromosome arm of *An. moucheti*. Arrows indicate signals of hybridization.

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, including *An. moucheti*, *Anopheles m. nigeriensis* and *Anopheles 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 *et al.*, 2002b). Similar results have been obtained using 10 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 Congo (DRC) and Nigeria for *An. m. bervoetsi* and *An. m. nigeriensis*, respectively], sequence differences between the three morphological forms were revealed in the gene encoding for mitochondrial cytochrome oxidase b (CytB) and in the ribosomal internal transcribed spacers (ITS1 and ITS2). These differences were similar in degree to the differences shown previously in 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 the population genetics and taxonomy of malaria mosquitoes (Coluzzi *et al.*, 2002). A recent analysis of polytene chromosomes in another neglected malaria vector, *Anopheles ovengensis*, revealed high karyotypic divergence within the *An. nili* group (Sharakhova *et al.*, 2013). Future studies will aim to elucidate the role of inversion polymorphism in ecological adaptation, population differentiation and speciation in the *An. moucheti* complex and will pave the way for comprehensive comparative cytogenetic analyses across major African vector species.

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