

Cloning and characterizing the 2Rj inversion breakpoints of the malaria vector *Anopheles gambiae* s.s.

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Malaria remains the biggest challenge of parasitic diseases in Sub-Saharan Africa where 90 % of the world's malaria specific mortality occurs. In West Africa, cytogenetic analysis of the primary African malaria vector *Anopheles gambiae* has revealed at least five partially or completely reproductively isolated chromosomal forms, recognized and defined on the basis of combinations of inversions on the right arm of chromosome 2 (j, b, c, u and d). Three of these forms-- Bamako, Mopti and Savanna-- are sympatric in Mali and exhibit clear differences in ecology. As a tool for recognizing different chromosomal forms, karyotyping has limitations. It is technically demanding, limited to half-gravid adult females, and possibly biased due to unreadable chromosome preparations or to complex karyotypes that are difficult to interpret and therefore underrepresented. Molecular markers diagnostic for the different chromosomal forms have been difficult to find. So far, rDNA-based differences distinguish what are known as M and S molecular forms, which correspond to Mopti and Savanna/Bamako chromosomal forms in Mali and Burkina Faso (but not necessarily elsewhere). It remains impossible to distinguish between Savanna and Bamako using molecular tools. As 2Rj inversion homozygotes are characteristic of the Bamako chromosomal form in Mali (but not necessarily elsewhere), we aimed to clone and sequence the j breakpoints to develop a molecular diagnostic tool for this taxon. A colony of the Bamako chromosomal form (BKO) maintained at the University of Notre Dame was the source of polytene chromosomes with arrangement 2Rjcu/jcu. *In situ* hybridization to BKO chromosomes of BAC clones already known to map to the proximal breakpoint of the uninverted arrangement in the PEST strain identified a single 124 kb BAC clone mapping to both distal and proximal breakpoints. The breakpoint was localized within a 1.3 kb segment, which is apparently single copy in the PEST genome. This fragment was used as a probe to screen a BKO genomic library (pSMART LCKan, Lucigen), from which two positive clones were recovered upon secondary screening. Sequence analysis confirmed that both breakpoints were recovered from the BKO library. The sequence from one breakpoint has been used to develop a PCR assay to detect the j arrangement in inverted and uninverted orientation, which in principle can be used to distinguish the Bamako chromosomal form from the other cytotypes in combination with existing rDNA-based

assays. Once validated using karyotyped specimens, the assay will facilitate studies on the biology, ecology and behavior of this unique form.