

and a combination of bioinformatic and cytogenetic approaches. The minimum number of inversions between members of the *A. gambiae* complex and the outgroup species *A. funestus* and *A. stephensi* was calculated using the Multiple Genome Rearrangements (MGR) and Sorting Permutation by Reversals and block-INterchanGes (SPRING) programs. The physical mapping of *A. merus* chromosomes identified molecular coordinates of the proximal 2Ro+ inversion breakpoint in *A. gambiae*. DNA probes from 2La+ and 2Ro+ inversion breakpoints of *A. gambiae* were mapped to the *A. stephensi* chromosomes. The results suggest that the *A. gambiae* complex shares the 2La and 2Ro arrangements with the outgroup species. Assuming monophyletic origin of the inversions, this study concludes that physical mapping of ingroup and outgroup species can be used for identifying inversion breakpoints and ancestral autosomal arrangements within species complexes. Molecular characterization of the breakpoints in both ingroup and outgroup species will provide a solid basis for reconstructing the inversion history in the *A. gambiae* complex.

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### STRUCTURAL ORGANIZATION OF THE MALARIA MOSQUITO HETEROCHROMATIN

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The development of new genome-based vector control strategies requires detailed knowledge about the organization and function of the mosquito genome. Heterochromatin is a functionally important and rapidly evolving part of the chromosome. Anopheline mosquitoes represent an ideal system for studying the structure and evolution of the heterochromatin because of the presence of polytene chromosomes in several tissues and great variability of heterochromatin among species. Two different morphological types, condensed/dark granulated ( $\alpha$ -) and diffuse/light granulated ( $\beta$ -) heterochromatin have been identified in polytene chromosomes of ovarian nurse cells in *Anopheles gambiae* and *A. stephensi*. Only diffuse  $\beta$ -type forms large visible attachments of chromosomes to the nuclear envelope. The goal of this study was to characterize the two types of heterochromatin using immunostaining and bioinformatic analysis. Immunostaining of chromosomes using antibodies against *Drosophila* Heterochromatin Protein 1 (HP1) and nuclear envelope protein lamin Dm<sub>0</sub> revealed co-localization of these proteins in most of the heterochromatic and euchromatic sites. The total number of sites was 128/158 in *A. gambiae* and 266/268 for in *A. stephensi* chromosomes for HP1/lamin, respectively. Surprisingly, an alternative pattern of protein localization has been detected between the species: both proteins were concentrated in all pericentromeric areas of *A. gambiae* but in internal chromosome regions of *A. stephensi*. No antibodies have been detected in pericentromeric  $\alpha$ -heterochromatin of 2R, 3R and 3L chromosomal arms in *A. stephensi* and intercalary  $\alpha$ -heterochromatin of *A. gambiae*. Gene density, A+T content, and repetitive element content were analyzed in the assembled part of the *A. gambiae* heterochromatin (13.3 Mb) and euchromatin (219 Mb) using Biomart, ATCONTENT and RepeatMasker programs. All heterochromatic regions had higher A+T content and five times lower gene density than euchromatin. Analysis of transposable elements and tandem repeats revealed the major difference in proportion of DNA transposons between the two types of heterochromatin. These findings suggest that  $\alpha$ - and  $\beta$ -types of heterochromatin may have different function in the mosquito genome.

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### CONTRASTING PATTERNS OF EVOLUTION IN FIVE CHROMOSOMAL INVERSIONS OF ANOPHELES GAMBIAE

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Chromosomal inversion frequencies in the malaria mosquito *Anopheles gambiae* exhibit nonrandom distribution with respect to environmental heterogeneities. Although these associations have been known for decades, there exists scant data on genetic variation in *A. gambiae* inversions making it difficult to infer how they might allow for ecological adaptation. By hybridizing genomic DNA from each of 25 wild-caught mosquitoes to high density oligonucleotide arrays, we obtained genome-wide maps of genetic differentiation between alternate arrangements of five common polymorphic inversions. Due to reduced recombination between alternate arrangements, loci captured by inversions tended to show more divergence than collinear loci. However, the degree of divergence varied considerably from inversion to inversion. Furthermore, genetic differentiation of captured loci was not uniform along the length of an inversion. For two inversions (2La and 2Ru) we identified small genomic clusters that had significantly higher divergence than all other regions in the same inversion. Targeted sequencing from ~30 loci in larger sample sizes and in two outgroups allowed us to determine diversity within rearranged regions, perform tests of neutrality, and date the origin of the inversions. Differing ages and sizes partially explained the contrasting patterns of molecular evolution observed between the five inversions. Sequencing in the highly diverged genomic clusters revealed fixed SNPs between alternate arrangements. Known or theorized recombination rates between inversion breakpoints and clusters show that this persistent association is highly unlikely under a scenario of neutral evolution. Based on this high resolution genetic analysis, we suggest mechanisms that could be maintaining *A. gambiae* inversion polymorphisms in natural populations and extend these results to form a qualitative model of inversion evolution that will be applicable to other organisms.

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### DEMOGRAPHIC HISTORY AND MICRO-GEOGRAPHIC POPULATION GENETICS OF ANOPHELES ALBIMANUS IN CENTRAL AMERICA BASED ON MITOCHONDRIAL DNA CO1 AND CYT B SEQUENCES

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*Anopheles (Nyssorhynchus) albimanus* is a malaria vector in Central America and northern South America. Previous research conducted on *An. albimanus* using microsatellite loci and sequences of the mtDNA *ND5* gene hypothesized that mountain ranges across western Panama and Costa Rica restrict gene flow between Central and South America. We analyzed partial sequences of the mitochondrial DNA *CO1* and *Cyt b* genes of *An. albimanus* from 13 localities in Costa Rica, Panama and northwestern Colombia. A minimum spanning network based on *CO1* gene sequences detected three groupings separated by 6 and 7 mutational steps; however, there was no evidence of geographical structure among haplotypes. High haplotype diversity and low nucleotide diversity, unimodal mismatch distribution, 4 of 6 neutrality tests and a star-like shape of the minimum spanning network demonstrate that *An. albimanus* is not at equilibrium, due to either an expansion or selective sweep. Mountain ranges in western Panama and Costa Rica seem to have restricted dispersal historically between the Pacific and Atlantic coasts; however, more recent mutational events in the network suggest higher levels of relatedness

White B.J., Changde Cheng, Hahn M.W., Kern M., Pombi M., Lobo N.F., Coulibaly M., Simard Frédéric, Besansky N.J. (2008)

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