

NEOTROPICAL ANOPHELES TRIANNULATUS COMPLEX: PHYLOGEOGRAPHY AND DEMOGRAPHIC HISTORY BASED ON MITOCHONDRIAL AND NUCLEAR MARKERS

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Anopheles triannulatus is a complex of at least three sibling species: *An. triannulatus* s.s., *An. halophylus* and *An. triannulatus* "C". *Anopheles triannulatus* s.l. has been incriminated as a malaria vector in some South American countries, especially when it occurs in high densities. Morphological and behavioral differences, such as larval habitat exploitation, have been described. In addition, molecular analysis using isoenzymes detected a barrier to gene flow among the three species in sympatry. To decipher the evolutionary forces that may have led to the current distribution and genetic history of members of *An. triannulatus* s.l., we analyzed the mitochondrial *COI* and the nuclear *white* gene in mosquitoes from 7 countries and 15 different locations, including samples of sympatric *An. halophylus* and *An. triannulatus* "C" from Salobra, (SW Brazil). The median joining network based on the *COI* gene depicted the haplotypes grouping in 7 different lineages with a high number of mutational steps between them, a) Panamanian, N and NW Colombian and Venezuelan, b) Venezuelan c) N and NE Brazilian, d) Ecuadorian, e) NW Colombian, f) SE Brazilian, g) Bolivian, Argentinian and Central Brazilian (including *An. halophylus* and *An. triannulatus* "C"). In contrast, statistical parsimony analysis of *white* gene showed 4 different clusters, 1) Panamanian, Venezuelan, Colombian and NE Brazilian (except Ceará), 2) Venezuelan, Ecuadorian and SW Brazilian 3) *An. halophylus* and *An. triannulatus* "C" plus SW Brazilian, 4) NE Brazilian (Ceará). Signatures of population expansion were detected with the *COI* gene in NE Brazil, Venezuela (Casigua), Panamá (Gamboá) and NW Colombia. Estimated time of expansion was during the Pleistocene. Results of Bayesian Inference support network lineages, with stronger posterior probabilities in the *white* gene data. Neither marker supports monophyly of any of the three taxa. Both markers detected Casigua (Venezuela), as an area where lineages converge, supporting the idea of a hotspot for diversity perhaps as a consequence of repeated Andean uplifts.

LOW LINKAGE DISEQUILIBRIUM IN ANOPHELES GAMBIAE S.L. POPULATIONS

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In the malaria vector *Anopheles gambiae*, understanding diversity in population biology and genetic components of important phenotypes like resistance to malaria infection is crucial to develop new malaria transmission blocking strategies and requires the study of polymorphism.

Linkage disequilibrium determines the density of Single Nucleotide Polymorphisms (SNPs) to be genotyped to represent the majority of haplotypes present. Here, we aim to determine linkage disequilibrium in *A. gambiae* populations in genes potentially involved in mosquito immune responses against pathogens. We analyzed fragments containing exons and introns of four immune related genes (Gambicin, NOS, REL2 and FBN9) distributed on *A. gambiae* genome in natural populations of seven species of the complex. We used already published and new sequences. Genes were cloned and sequenced for 8 to 16 individuals per population. Detected polymorphisms allowed the measure of linkage disequilibrium decay along the genes. In all tested genes and species, linkage disequilibrium between SNPs was very low: at a distance of less than 200 bp, SNPs were rarely linked to each other. The linkage observed in the *A. gambiae* could be the result of large population sizes and high recombination rates. These results are of great interest in the development of large scale polymorphism studies for population genetics and association studies. It indicates that very fine scale SNP detection will be required to detect association to phenotypes of interest in malaria transmission and to give a general view of genome polymorphism to decipher for example vector immunity, *Anopheles-Plasmodium* interactions or vector behavior.

POPULATION GENETICS OF LUTZOMYIA LONGIFLOSCA (DIPTERA: PSYCHODIDAE) POPULATIONS FROM COLOMBIA USING THE CYTOCHROME OXIDASE 1 GENE

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During the 2003-2004 epidemic of leishmaniasis in central Colombia, an indoor feeding phlebotomine sand fly, *Lutzomyia longiflora*, was found to link the disease to a domestic transmission cycle. Because this sand fly has a distribution beyond the extents of the transmission zone, a molecular genetic assessment was conducted to assess its population structure. Three field populations of *Lu. longiflora* from the endemic regions of Tello (n=29), and Chaparral (n=28) (Tolima Province) and San Antonio (n=10) (Cundinamarca Province) were compared with 620 base pairs of the cytochrome oxidase 1 mitochondrial gene. A high-fidelity Taq polymerase along with both forward and reverse sequencing was used to ensure robustness of the sequencing results. A total of 38 variable sites in the sequence were identified in the 67 specimens. Considerable genetic differentiation was revealed between the San Antonio and Chaparral/Tello samples. Prior morphological analysis identified all samples as *Lu. longiflora*. However, the molecular genetic results suggested the occurrence of a genetically distinct taxon in San Antonio. Of the 620 bp sites, San Antonio differed from Chaparral and Tello at 15 segregating sites for all of its 10 samples, along with three additional variable sites. Chaparral and Tello each had seven segregating sites. A total of 20 haplotypes were identified with a haplotype diversity of 0.911. Analysis in pairwise comparisons resulted in an overall nucleotide diversity (Nei's) of 1.3%. The within population nucleotide diversity was highest for San Antonio (0.8%), but similar for Chaparral (0.4%) and Tello (0.3%). The genetic distance was highest between Tello or Chaparral and San Antonio (0.038 and 0.039 respectively) and lowest between Tello and Chaparral (0.005).