

POPULATION STRUCTURE OF COLLECTIONS OF THE MOSQUITO *Aedes aegypti* (DIPTERA: CULICIDAE) FROM COSTA RICA

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Aedes aegypti is the main vector of dengue disease, and the most important tropical disease vector in Costa Rica. Population structure of this mosquito in Costa Rica is not well known, and previous studies of *Ae. aegypti* from Central America have not been clear about mosquitoes from. For this reason, a genetic characterization of laboratory collections from different regions of the country could explain the dynamics of populations of this mosquito. For the establishment of the collections, we obtained mosquito larvae and pupae from 19 localities of Costa Rica, in each locality were sampled five different points, and each one of these five sample sites were georeferenced. The mosquito colonies obtained from larvae collected in each locality were maintained in the insectary of the laboratory of Medical Arthropodology of the University of Costa Rica, and generation F_2 was obtained by locality. Genetic characterization of collections was performed with 50 mosquitoes of these F_2 generations. Five different molecular markers were used for the characterization: one mitochondrial marker (the subunit 4 of the NADH gene) and four nuclear markers. Nuclear markers included the microsatellites associated with the locus 19 (a homologous of membrane viteline protein), the locus GA (a subunit of the mRNA of the GABA receptor), and the locus C (mRNA of the ecdysteroid receptor). The fourth nuclear marker was the early trypsin gene, but it was analyzed only for eight localities. Data obtained from the genetic characterization were analyzed using Analysis of Molecular Variance (AMOVA) for comparison of haplotypes or allelic variables within and between mosquito collections and the calculation of F_{ST} value. Hardy Weinberg equation for allelic equilibrium in populations was used to determine the frequency of allelic variants. With these analyses, different haplotypes and allelic variants were obtained from *Ae. aegypti* populations of Costa Rica.

POPULATION STRUCTURE OF THE MALARIA VECTOR *ANOPHELES ALBIMANUS* IN THE ATLANTIC AND PACIFIC REGIONS OF COLOMBIA BASED ON SEQUENCES OF THE MTDNA *COI* GENE

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Anopheles (Nyssorhynchus) albimanus is recognized as an important malaria vector in Colombia and is distributed along the Atlantic and Pacific coasts and on San Andres Island. Prior Colombian studies of *An. albimanus* using enzyme electrophoresis demonstrated low divergence and no differentiation among populations from both coasts; however, populations from the Atlantic coast had higher heterozygosity. Recent studies of this species using microsatellite (MS) loci and a mitochondrial DNA (mtDNA) marker reported differentiation between Central and South America based on geographical barriers. We used sequences of the mitochondrial cytochrome oxidase subunit I (*COI*) gene to investigate the population structure of *An. albimanus* in Colombia. A neighbor-joining tree, analysis of molecular variance, and F_{ST} pairwise comparisons consistently divided the data between the localities from the Atlantic and Pacific coasts; however, populations within each group showed very low divergence. Furthermore, a star-shaped minimum-spanning network and 5/5 significant neutrality tests suggested a past population

expansion or a selective sweep in *An. albimanus* from the Atlantic coast. Although populations from the Pacific coast depicted a star-shaped minimum-spanning network, the neutrality tests were not significant. Our preliminary findings are in contrast with previous studies in detecting higher genetic diversity, and possibly a different demographic history, in Pacific coast *An. albimanus*. Importantly, only *An. albimanus* from the Pacific has been positively incriminated as a malaria vector. Further analysis of mosquitoes from Turbo, located in between these two regions, and sequences of a more conserved mitochondrial DNA marker (*Cyt b*) will extend our preliminary findings and provide more insight into the population genetic structure of *An. albimanus* in Colombia.

DEVELOPMENT OF A HIGH-DENSITY SNP GENOTYPING ARRAY FOR THE VECTOR MOSQUITO *ANOPHELES GAMBIAE*, BY THE AGSNP CONSORTIUM

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Understanding the population structure and genetic mechanisms underlying disease transmission by malaria vectors continues to be hindered by the complex biogeography and reproductive biology of Anopheline mosquitoes, which impede classical genetic approaches to their investigation. The original *Anopheles gambiae* genome sequencing project and the recent completion of two additional genome sequences of the M and S chromosomal forms of *An. gambiae* have provided the genomic information required to develop a pilot single nucleotide polymorphism (SNP) genotyping array for *An. gambiae* (the "AGSNP array") and thereby extend the power of modern genomic approaches to this key malaria vector mosquito. We have initiated formation of a multi-center AGSNP Consortium that will develop, validate, and pursue initial use of high-density pilot AGSNP arrays in Affymetrix format. With the mosquito genome sequences in hand, and using two independent SNP callers, we have identified over 641,000 candidate SNPs, from among a total of more than 5 million primary SNPs, that survive filtering criteria based on distance from insertion/deletion (indel) polymorphisms and neighboring SNPs. These candidate SNPs are currently undergoing evaluation for probe design for a pilot array in the Affymetrix 49 format. We will summarize key questions in vector biology and disease transmission amenable to in-depth investigation using this vector mosquito genotyping tool, describe development of the probe set for the pilot AGSNP array, discuss prospects for pooled probing of Affymetrix-format arrays for analysis of population structure and genome-wide association studies of traits underlying malaria transmission by vector mosquitoes, and present progress on use of the AGSNP array for analysis of population structure in Africa.

GENETIC ASSOCIATION AND LINKAGE DISEQUILIBRIUM IN *ANOPHELES GAMBIAE* IMMUNE GENES

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Understanding the molecular basis of natural mosquito immunity to the malaria parasite will give essential insights for novel malaria control strategies. Genetic variation in the mosquito is known to have drastic effects on immune competence. Understanding this genetic control of refractoriness is fundamental to future control via this route. Association

studies have proved to be useful tools in uncovering specific genetic loci with effect on disease outcome. Linkage disequilibrium (LD) data can help decipher components of molecular pathways and help to piece together the mosquito immunity puzzle. Moreover, as genome wide association studies in *Anopheles gambiae* are rapidly being developed, LD data will be key to determining the necessary resolution of genetic markers in such studies. This study aims firstly, to uncover single nucleotide polymorphisms (SNP's) involved in mosquito susceptibility/refractoriness to malaria in one geographical region. A targeted approach has been applied to select specific SNP's within known mosquito immunity genes for inclusion. *Anopheles gambiae* M form mosquitoes from Cameroon were experimentally infected with a sympatric *Plasmodium falciparum* isolate. The number of oocysts/midgut at day 8 post blood meal were counted to give a quantitative phenotype. Genotyping was then carried out on these mosquitoes for the selected SNP's and statistical tests applied to determine association. Secondly, the LD was measured for immune gene SNP's in *An. gambiae* populations. Data analysis will be fully presented.

1080

INTEGRATE MOSQUITO FORAGING IN ENVIRONMENTAL MANAGEMENT OF AQUATIC HABITATS FOR MALARIA CONTROL

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Malaria campaigns in tropical Africa are concentrated on management of *Anopheles gambiae* s.l., the major vector species, by using insecticide-treated bednets and indoor residual spray. Exploitation of alternative strategies is needed for reducing reliance on chemical insecticides. A new perspective of malaria control is developing based on compromising mosquito foraging for food and oviposition. With limited energy reserve and flight ability, mosquitoes only flourish in environments where foraging movement between hosts and aquatic habitats are facilitated. Reduced availability of oviposition sites by environmental management can prolong the ovipositional cycle and mitigate malaria transmission. In this paper, we develop an agent-based model to characterize foraging behaviors in response to change in the availability of aquatic habitats. In grid-based landscape, we compared intervention scenarios (3 coverages of targeted source reduction, 3 coverages of non-targeted source reduction, 3 coverages of insecticide-treated bednets ITN). Simulations show that interventions targeting ovipositional foraging by selective removal of aquatic habitats proximal to houses are more effective than insecticide-treated bednets in reducing malaria incidence and prevalence. In conclusion, spatial connectivity between human hosts and oviposition sites provides a promising target for malaria control.

1081

IDENTIFYING COVARIATES OF ANOPHELES GAMBIAE S.L. (DIPTERA: CULICIDAE) AQUATIC HABITAT DISTRIBUTION USING A POISSON REGRESSION MODEL, WITH A NON-CONSTANT, GAMMA-DISTRIBUTED MEAN

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We present a geostatistical approach that accounts for spatial correlation in malaria mosquito habitats in two East African urban environments. Multispectral Thermal Imager 5m data encompassing visible bands and the near infra-red bands were selected to synthesize images of *Anopheles gambiae* s.l. Giles aquatic habitats. These bands were used to determine which ecological covariates were associated with urban larval habitat development in Kisumu and Malindi, Kenya. Univariate statistics, correlations and regression analyses were performed on all field and remotely sampled data. Global autocorrelation statistics were generated from georeferenced *Anopheles* aquatic habitats. The results suggest that the geographic distribution of *An. gambiae* s.l. larvae in the study sites exhibit weak positive autocorrelation; similar numbers of log- larval count

habitats tend to cluster in space. A negative binomial regression was used to decompose aquatic habitat data into positive and negative spatial autocorrelation eigenvectors. The principal finding is that synthetic map pattern variables, which were represented as eigenvectors computed for a geographic weights matrix, furnished an alternative way of capturing spatial dependency effects in the mean response term of the regression model. The results suggest the presence of approximately 13% to 32 % redundant information in the larval count samples. Additionally coefficient estimates were used to define expectations for prior distributions for a Bayesian analysis. By specifying the coefficients in a Bayesian framework, we successfully account for depth as being significantly associated with urban *An. gambiae* s.l. aquatic habitats.

1082

ECOLOGICAL BASIS OF SWARMING AND MATING BEHAVIOUR IN NATURAL POPULATIONS OF ANOPHELES GAMBIAE S.S., IN BURKINA FASO

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Recent advances in the molecular genetics of *Anopheles gambiae* have increased the prospects of using genetically modified mosquitoes in malaria control. However the use/release of such genetically modified vectors requires a proper understanding of potential interactions with naturally occurring populations. In many species of insect, larger males are more successful in obtaining mates. Since mating of *Anopheles gambiae* mosquitoes occurs in swarms, we attempted to characterize the swarming behaviour and determined whether size affects mating success of *An. gambiae* males. Swarming behavior of *An. gambiae* s.s. were investigated between July 2006 and December 2007 in Vallée du Kou and Soumouso two villages located in West Burkina Faso. Environmental parameters such as light intensity, temperature and humidity were also monitored. Wing lengths of males collected swarming and mating were measured then compared between both groups. The swarms of *An. gambiae* s.s. were represented by males aggregation in fly. The swarming began generally 1-10 min after sunset and stopped 9-35 min after the onset of darkness. Most of the swarms were observed between 0.6 to 4 m above a variety of markers. The luminosity, temperature and humidity variation had no fundamental impact on the swarming behavior along the months. We measured the wing of 654 swarming males and 152 copulating males. Copulating males were significantly larger than the other males sampled from the swarms ($F = 63.98$; $P = 0.001$). Indicating that the size affect mating success of *An. gambiae* mosquitoes.

1083

HOST-FEEDING PATTERNS OF Aedes Aegypti AND Aedes Albopictus IN NEW ORLEANS, LOUISIANA, 2006

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Field studies targeting *Aedes aegypti* and *Ae. albopictus* in New Orleans, Louisiana from August through October of 2006, yielded 4,027 *Ae. aegypti* and 1,573 *Ae. albopictus* adults. Collections were made with diurnal dry-ice baited light traps and Nasci aspirators in areas moderately to heavily flooded 12-15 months earlier following Hurricane Katrina. The purpose of this study was to evaluate mosquito host-feeding patterns given the drought conditions and varied degree of human repopulation

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