and 50% of *An. gambiae*. Microinjection of mosquito eggs at the time of pole cell formation and cellularization of the blastoderm with. 01-1.0 nl of concentrated virus yielded the following percent infected adult G_0 mosquitoes as determined by PCR amplification of retroviral sequences: *Aedes aegypti*: 4/149 + (2.7%); *Ae. triseriatus*: 14/69 + (20.3%). These results demonstrate stable *in vivo* gene expression in adult mosquitoes mediated by a retroviral vector and suggest the feasibility of germline transformation with these agents.

428 THE IMMUNE FACTOR Gambif1, FROM THE HUMAN MALARIA VECTOR *ANOPHELES GAMBIAE*IS A NEW REL-FAMILY MEMBER. Barillas-Mury CV*, Charlesworth A, Gross I, Richman A, Hoffmann JA, and Kafatos FC. European Molecular Biology Laboratory, Heidelberg, Germany; European Institute of Oncology, Milan Italy; and Institut de Biologie Moleculaire et Cellulaire, Strasbourg, France.

For malaria transmission to take place, the *Plasmodium* parasite must develop in the mosquito without eliciting an effective immune response. A new rel-family member, Gambiae Immune Factor 1 (Gambif1), has been cloned from *An. gambiae*, a vector of human malaria. It has the highest homology to the *Drosophila* transcription factors Dorsal and Dif, which are involved in the transcriptional activation of antibacterial peptides. In response to bacterial challenge, Gambif1 protein is translocated to the nuclei in fat body cells, and DNA binding activity to the *An. gambiae* Defensin and the Drosophila Diptericin and Cecropin kB-like sites, is also induced in larval nuclear extracts. Gambif1 has the ability to bind to kB-like sites *in vitro* and can activate transcription in co-transfection assays in mbn-2cells, by interacting with the *Drosophila* Diptericin regulatory elements. In this assay, Gambif1 is not functionally equivalent to Dorsal. These molecular markers open the possibility to study the functional status of the mosquito immune system following *Plasmodium* infection, and the role of the defense reactions in determining their susceptibility/refractoriness to malaria infection, and thus, their vectorial capacity.

429 MOLECULAR CHARACTERIZATION OF OLFACTORY SPECIFIC cDNAS FROM THE MALARIA VECTOR *ANOPHELES GAMBIAE S.S.* Zwiebel LJ* and Kafatos FC. European Molecular Biology Laboratory, Meyerhofstrasse 1, Heidelberg, Germany.

The ability to sense and discriminate a large collection of both chemical and visual cues is critical for several critical behaviors in arthropods. In particular, olfaction plays a major role in host seeking and selection by blood sucking female mosquitoes. As these behaviors make up a critical component of vectorial capacity an understanding of these chemical cues and of chemosensory mechanisms in general will be important in the design and development of several biological control strategies including odor baited traps. In this light, we have initiated a molecular characterization of olfaction within members of the *Anopheles gambiae* species complex which includes the principal African malaria vector, *An. gambiae s.s.* This study has been carried out using PCR) based methods for generating chemosensory specific cDNA pools derived from hand dissected antennae and maxillary palps. These pools have been screened by differential display as well as used as substrates for further enrichment for olfactory-specific cDNAs by subtractive hybridization. To date we have isolated an olfactory tissue specific PCR clone (MosOlf1) that on analysis of the basis of deduced amino acid sequence correspond to a superfamily of lipophilic ligand-binding proteins known as Odorant Binding Proteins (OBPs). Members of this class of proteins have been isolated in several arthropods species as either general odorant-binding proteins (GOBPS) or pheromone-binding proteins (PBPs). Northern blot analysis indicates that the MosOlf 1 clone is specifically expressed in the sensory appendages of female *An. gambiae s.s.* and may therefore play a role in host seeking behaviors. Furthermore, experiments have been undertaken to isolate olfactory cDNAs that are specific for anthropophilic host preference by subtractive hybridization against the zoophilic sibling species, *An. quadriannulatusi.*

430 GEOGRAPHIC STRUCTURE OF *ANOPHELES GAMBIAE* (SAVANNA FORM) IN AFRICA BASED ON MICROSATELLITE, ALLOZYME, AND MITOCHONDRIAL LOCI. Lehmann T*, Hawley WA, Besansky NJ, Fontenille D, Simard F, Fahey TG, Kamau L, and Collins FH. Division of Parasitic Diseases, Centers for Disease Control, Atlanta GA; Department of Biology, Emory University, Atlanta GA; Kenya Medical Research Institute, Clinical Research Centre, Nairobi, Kenya; Laboratire ORSTOM de Zoologie Medicale, Institut Pasteur, Dakar, Senegal.

The genetic structure of *Anopheles gambiae* populations representing extreme geographic scales was studied based on several genetic markers. These scales included houses within a village, villages up to 50 km apart, and countries 6000 km away. The following questions were answered: (1) Are mosquitoes in a house more related genetically to each other than mosquitoes in different houses? (2) What degree of genetic differentiation occurs on these geographic scales? and (3) How consistent are the results obtained by separate genetic markers? No differentiation was detected among houses by FST, RST, and the band sharing index tests applied to the 5 microsatellite loci. Likewise, indices of kinship based on mtDNA haplotypes in houses were even lower than in the pooled sample. Thus, the hypothesis that mosquitoes in a house are more related genetically was rejected. No subdivision of the

gene pool among 4 villages in western Kenya was detected by FST or RST based on the 5 microsatellite loci. Likewise, estimates of haplotype divergence of mtDNA between these villages were not higher than the within population estimates. Significant divergence between populations from Kenya and Senegambia was detected by 3 of the 5 microsatellite loci (average Wright's FST was 0.016 and average Slatkin's RST was 0.036) and by 2 of 6 allozyme loci (average FST was 0.036, calculated based on Miles, 1978). These values are surprisingly low and correspond to an effective migration index (Nm) larger than 3, suggesting gene flow across the continent is only weakly restricted. The concordance between results based on microsatellite loci and mtDNA at the microgeographic levels, and between the allozyme's FST and the microsatellite's RST at the macrogeographical level attested for this description of the population structure.

431 PREVALENCE OF CIRCUMSPOROZOITE PROTEINS IN POTENTIAL MALARIA VECTORS ALONG THE THAI-MYANMAR BORDER IN NORTHERN KANCHANABURI PROVINCE, THAILAND. Gordon SW*, Mahapibul P, Sattabongkot J, Wongsrichanalai C, Heppner DG, and Linthicum KJ. Department of Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Department of Immunology and Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

Two villages in northwestern Kanchanaburi Province, Thailand, along the Thai-Myanmar border, were selected for evaluation as potential drug/vaccine test sites. In an effort to characterize endemic malaria transmission, epidemiological and entomological studies were initated in May 1995. Monthly thick and thin blood smears were made on all residents to monitor malaria infection rates. Mosquitoes, collected over a one week period each month using cow-baited traps, CDC light traps, and human bait, were tested by ELISA for the presence of *Plasmodium falciparum* and *P. vivax* circumsporozoite antigens. Maps were generated to show house locations, roads, trails, streams, and mosquito collection sites, using global positioning system instruments. Position information was combined with village census data to create a geographic information system (GIS) database. Data generated from the monthly blood film survey and mosquito collections were incorporated into the database to provide investigators with ready access to current information. *Anopheles minimus* was the most abundant vector species encountered in human biting collections, accounting for over 50% of all samples. Other species frequently identified in human biting collections included *An. maculatus, An. aconitus, An. sawadwongporn, An. nivipes,* and *An. philippinensis.Anopheles dirus,* recognized as a major vector in Thailand, was collected infrequently. Three species, *An. aconitus, An. dirus,* and *An. minimus,* tested positive for *P. falciparum* by ELISA. *Plasmodium vivax*var 210 was detected only in *An. minimus* from human biting collections,while *P. vivax* var 247 was found only in *An. barbirostris* from cow-baited traps.

432 CHANGES IN GENES FROM CHROMOSOME 7 OF *PLASMODIUM FALCIPARUM* SHOW HIGH DEGREE LINKAGE TO CHLOROQUINE RESPONSES. Su X*, Kirkman LA, and Wellems TE. Laboratory of Parasitic Diseases, NIAID, NIH; Bethesda, MD.

A chloroquine resistant (CQR) determinant was previously mapped to a 400 kb DNA segment on chromosome 7 of *Plasmodium falciparum* in a genetic cross (Dd2 X Hb3). We report here the further localization of the CQR determinant to a 40 kb DNA segment on chromosome 7 by use of high density microsatellite markers (30 markers) across the 400 kb region and over 1000 progeny from the genetic cross. Five independent cross-overs were identified within the 400 kb DNA segment. The CQR locus was reduced to 40 kb DNA segment by mapping the break points in 2 progeny (C188 and C408). Seven open reading frames (ORFs) have been identified in the region. The DNA sequences of the ORFs from both Dd2 (resistant) and HB3 (sensitive) have been determined and compared. Changes, including point mutations and differences in repetative sequences in 2 of the ORFs (CG1/CG2), show high degree of linkage in chloroquine responses in 25 field isolates (over 90%). Both of the ORFs are transcribed. We are testing these ORFs by linkage analysis and transfection to identify the element responsible for CQR in the *P. falciparum* cross.

433 PREVALENCE OF DHFR AND DHPS MUTATIONS AND *IN VIVO* PYRIMETHAMINE-SULFADOXINE SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* IN MALI. Djimde A*, Doumbo OK, Diourte Y, Sagara I, Coulibaly Y, Dicko A, Plowe CV. Division of Geographic Medicine, University of Maryland School of Medicine, Baltimore, MD; and Malaria Research & Training Center, National School of Medicine & Pharmacy, Bamako, Mali.

Rapid and simple methods are needed to monitor for the emergence of *Plasmodium falciparum* resistance to pyrimethamine-sulfadoxine (PS) in settings such as Mali, where PS is the second line antimalarial drug. Point mutations in *P. falciparum* dihydrofolate reductase (DHFR) confer *in vitro* resistance to pyrimethamine, and mutations in dihydropteroate synthase (DHPS) have been proposed to mediate *in vitro* resistance to the sulfa drugs. Nested PCR can detect these mutations in parasite DNA extracted from finger-stick blood samles dried onto filter

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