A/B) bound to ICAM-1 weakly. Most of the residues identified previously as important for ICAM-1 binding are preserved in DBL2β-$C2_{PF11\_0521}$, but less so in the other 15 domains. The binding of ICAM-1 to DBL2β-$C2_{PF11\_0521}$ was almost completely inhibited by pre-incubation of the domain with pooled serum samples obtained from adults living in endemic areas of east Africa, but not by pre-incubation with non-immune serum from the US. The reversal of ICAM-1 binding was much less efficient than blocking, indicating the strong association between DBL domain and ICAM-1 molecule. These data contribute further into understanding of PfEMP1-ICAM-1 interactions, and our high throughput approach will significantly accelerate studies of binding and binding-inhibition between PfEMP1 domains and various cellular ligands. We are presently using this platform to measure anti-adhesion antibody levels in Tanzanian children, and to relate these functional antibodies to malaria outcomes.

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CHARACTERIZATION OF A DOMESTIC TRANSMISSION FOCUS OF AMERICAN CUTANEOUS LEISHMANIASIS IN RURAL COLOMBIA

Clara B. Ocampo-D[1], Cristina Ferro[2], Horacio Cadena[1], Dairo Marin[1], Layder Lozano[1], Cesar Ramirez[1], Leonard Munstermann[3]

[1]CIDEIM, Cali, Valle, Colombia, [2]Instituto Nacional de Salud, Bogota, Colombia, [3]Yale University, New Haven, CT, United States

Between 2003 and 2004, a large outbreak of American cutaneous leishmaniasis occurred in Chaparral County, Tolima, located in a rural mountainous area in Central Colombia. Although no cases were reported here in the past, the initial outbreak documented 2,400 cases. Prevalence among the 69 (157) townships with reported cases in Chaparral varied from 1 to 95%. Thirty percent of the cases were women and children, indicating domestic transmission. The potential risk factors associated with domestic transmission were evaluated in a township with high prevalence (Agua Bonita,74%) and low prevalence (Irco dos Aguas, 1%). Sandfly and wild mammal trapping was undertaken in a concentrated transect design within a 100 m radius from selected houses. For sand fly capture, CDC light traps were placed indoors (n=1) and outdoors (n=16) during three consecutive nights, and for animal capture, Sherman (n=72) and National (n=16) traps were oriented around the house for 4 nights per trapping period. Epidemiological and land use data were recorded at each house to capture environmental parameters associated with leishmania transmission. A great difference between the two townships was seen in sand fly composition and abundance; 1,446 sandflies were capture in Agua Bonita (12 houses) compared with 80 in Irco dos Aguas (10 houses). The most abundant species was *Lutzomyia Longiflocosa*,70% in Aqua Bonita and 49% in Irco dos Aguas. Ten and 7 sand fly species were identified from the two sites, respectively. Eighteen mammals were captured in Agua Bonita and 14 in Irco dos Aguas. Two animals (*Didelphis marsupialis* and *Marmosops impavidus*) tested positive for Leishmania by kDNA PCR at the high endemicity site. No lesions were observed in wild and domestic dogs. Prevalence of infection in the inhabitants of the houses sampled was 45% in Agua Bonitas and 0% in Irco dos Aguas. Although peridomestic land use was similar in the two sites (coffee and scrub brush), adjacent forests occurred only in Agua Bonita. In conclusion, the high risk of domestic leishmania transmission was associated with high densities of a relative uncommon vector species, *L. longiflocosa*, and the proximity of tropical forest.

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MOLECULAR SYSTEMATICS OF THE BARBIROSTRIS SUBGROUP AND HYRCANUS GROUP OF THE GENUS *ANOPHELES* IN SOUTHEAST ASIA

Claudia C. Paredes-Esquivel, Harold Townson

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The *Anopheles barbirostris* subgroup includes six mosquito species that are almost identical in adult morphology: *Anopheles barbirostris*, *An. campestris, An. donaldi, An. hodgkini, An. pollicaris* and *An. franciscoi*. Some of these species are implicated in the transmission of malaria and filariasis in Southeast Asia. Specimens of the Barbirostris Subgroup are also confused in the field with those from the Hyrcanus Group. Such mistakes in identification are an obstacle to the implementation of effective vector control. A phylogenetic analysis of 756 bp of Cytochrome Oxidase I (COI) in the mitochondrial genome revealed five clades within the Barbirostris Subgroup. The same clades were shown using Neighbour Joining and Maximum Parsimony trees, although internal branch points were different. A parsimony-based nested clade analysis also showed five separate networks, congruent with the phylogenetic clades. Analysis of the nuclear rDNA ITS2 region revealed five clades, congruent with those from the COI analysis. In all specimens of the Barbirostris Subgroup, ITS2 was >1.5kb, the largest so far recorded in any insect. The extreme length of the ITS2 was a most interesting finding and resulted from the presence of four or five internal repeats of c.220bp within the ITS2, each repeat comprised of two c.110 bp sub-repeats of variable homology. Within the Barbirostris Subgroup, clade I, II and III were morphologically compatible with *Anopheles barbirostris Van der Wulp,* suggesting that *Anopheles barbirostris Van der Wulp* is a species complex comprising at least 3 species (clades I, II and III). There is limited information on host preferences for clades I and II, but clade III appears to be zoophilic. Clade V was identified as the anthropophilic species *Anopheles campestris.* Clade IV is a zoophilic species with morphological characters intermediate between those of *An. campestris* and *An. barbirostris*, with which it is found in sympatry in Sa Kaeo (Thailand). Clade IV appears to be a new species.

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CHROMOSOMAL INVERSIONS, NATURAL SELECTION AND ADAPTATION IN THE MALARIA VECTOR *ANOPHELES FUNESTUS*

Diego Ayala[1], Michael C. Fontaine[2], Anna Cohuet[1], Carlo Costantini[3], Didier Fontenille[1], Renaud Vitalis[4], Frederic Simard[5]

[1]Institut de Recherche pour le Developpement, UR Caractérisation et contrôle des populations de vecteurs, Montpellier, France, [2]Institute of Integrative and Comparative Biology, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom, [3]Institut de Recherche pour le Developpement, UR Caractérisation et contrôle des populations de vecteurs, Yaoundé, Cameroon, [4]Muséum National d'Histoire Naturelle - Centre National de la Recherche Scientifique UMR [5145] – Université Paris [7], Éco-Anthropologie et Ethnobiologie, Musée de l'Homme, Paris, France, [5]Institut de Recherche pour le Developpement, UR Caractérisation et contrôle des populations de vecteurs, Bobo-Dioulasso, Burkina Faso

Chromosomal polymorphisms, such as inversions, are presumably involved into the rapid adaptation of populations to local environmental conditions. Reduced recombination between alternative arrangements in heterokaryotypes might protect sets of co-adapted genes promoting ecological divergence and assortative mating, eventually leading to reproductive isolation and speciation. The adaptive significance of polymorphic paracentric chromosomal inversions has been evidenced in a number of Diptera such as flies and mosquitoes. Through a comparative analysis of chromosomal inversions and microsatellite markers polymorphisms, we hereby present biological evidence that strengthens this view in the mosquito, *Anopheles funestus* s.s, one of the most powerful and widespread malaria vector in Africa. Specimens were collected across a wide range of geographical, ecological and climatic conditions in Cameroon. We observed a sharp contrast between population structure measured at presumably neutral microsatellite markers and chromosomal inversions. Microsatellite data detected only a weak signal for population structuring among geographical populations (Fst<0.013). By contrast, strong differentiation among ecological zones was revealed by chromosomal inversions (Fst>0.190). Using standardized estimates of Fst, we show that inversions behave at odds with neutral expectations, strongly suggesting a role of environmental selection in shaping their distribution. Using Canonical Correspondance analysis, we demonstrate that heterogeneity in eco-geographical variables (EGVs)

measured at specimens sampling sites explain 89% of chromosomal variance in *An. funestus*. These results are in agreement with a role of chromosomal inversions in ecotypic adaptation in this species. We argue that this widespread mosquito represents an interesting model system for the study of chromosomal speciation mechanisms and should provide ample opportunity for comparative studies on the evolution of reproduction isolation and speciation in major human malaria vectors.

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DOES HEMOLYMPH FLOW DRIVE MALARIA SPOROZOITE MIGRATION THROUGH THE MOSQUITO HEMOCOEL?

Julián F. Hillyer, Jonas G. King, Justin D. Glenn

Vanderbilt University, Nashville, TN, United States

Mosquitoes are obligate vectors of malaria parasites. For successful transmission to the vertebrate host, malaria sporozoites must migrate from the mosquito midgut to the salivary glands. It has long been considered that this migration is parasite-driven by means of active motility. However, our initial experiments showed that after release from oocysts, sporozoites are swept toward the posterior of the insect, enter the dorsal vessel, and traverse the length of the mosquito at speeds much faster than can be accounted for by sporozoite motility alone. Based on these data, we hypothesize that sporozoites passively migrate through the hemocoel to the vicinity of the salivary glands using the mosquito's circulatory system, and once in the anterior ventral thorax, active sporozoite motility allows them to locate and invade the salivary glands. Given the above data, and in prelude to continuing studies on sporozoite migration, we have been conducting a comprehensive assessment of the mosquito circulatory system by tracking the movement of inoculated fluorescent particulates throughout the hemocoel of the malaria vector *Anopheles gambiae*. Here, we will present data that corroborate the general anatomy of the mosquito dorsal vessel, characterize the mechanics of dorsal vessel contraction, unveil novel hemolymph channels, and describe in detail hemolymph flow in mosquitoes. Data on absolute rates of flow speed, acceleration, and direction in different regions of the insect will also be presented, and the general implications of hemolymph flow on *Plasmodium* sporozoite migration will be discussed.

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IDENTIFICATION OF THE BARRIERS PREVENTING SUCCESSFUL DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN *CULEX* MOSQUITOES

Jen Hume, Tovi Lehmann

NIH/ National Institute of Allergy and Infectious Diseases, Rockville, MD, United States

Culex mosquitoes are extremely prevalent across the malarious world serving as important vectors for a number of infectious diseases and are primary vectors of avian Plasmodia, yet they are known to be totally refractory to human Plasmodia species. This is especially fascinating because many species, such as *Culex quinquefasciatus*, are highly anthropophilic and consequently, have repeatedly been exposed to human Plasmodia. However, despite extensive and prolonged exposure, human malaria parasites have never adapted to exploit *Culex* mosquitoes. Thus, there is something unique about the *Culex* physiology that prevents the successful development of human Plasmodia, but to date the exact processes remain unknown. Here, we compare the developmental success of *Plasmodium falciparum* in two African mosquitoes (*Cx. quinquefasciatus* and *An. gambiae*) in key events: fertilization, midgut invasion, oocyst maturation, sporozoite invasion and accumulation in salivary glands. We identify successful fertilization events and subsequent ookinete invasion in *Culex* mosquitoes. However, transformation of ookinetes to oocysts does not occur, suggesting that a developmental barrier exists at the midgut stage preventing successful parasite development. We investigate whether bypassing the midgut allows successful development or whether multiple barriers are responsible for *P. falciparum* refractoriness in *Cx. quinquefasciatus.*

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ENVIRONMENTAL FACTORS INFLUENCE *CULEX PIPIENS QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) SUSCEPTIBILITY TO WEST NILE AND ST. LOUIS ENCEPHALITIS VIRUSES

Stephanie L. Richards, Cynthia C. Lord, Kendra Pesko, Walter J. Tabachnick

University of Florida/Florida Medical Entomology Laboratory, Vero Beach, FL, United States

Three age cohorts from two *Culex pipiens quinquefasciatus* colonies were fed blood meals containing a low or high dose of St. Louis encephalitis virus (SLEV) or West Nile virus (WNV), and each group was held at two different extrinsic incubation temperatures (EITs) for 13 days. The vector competence effects of age, EIT, and dose were complex with interactions between them for WNV and SLEV. The effect of the environment showed differences depending on the virus and colony. Susceptibility of *Cx. p. quinquefasciatus* to both viruses increased with both increasing virus dose and EIT, except for WNV in one of the colonies where infection rates were inversely related to dose. Mosquito age had an effect on vector competence for both viruses, but the effect differed depending on virus, colony, EIT and dose. The relationship between infection and dissemination rates for both viruses changed between colonies, and was dependant on age, EIT and dose where the proportion of disseminated infections relative to infected mosquitoes varied. We observed that the effects of the environment change depending on the virus and the vector strain, here two different laboratory colonies. The complex effects of the environment must be considered in laboratory studies of vector competence that are used to generalize to nature where the extent of the genetic and environmental variation controlling vector competence is largely unknown.

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BLOOD FEEDING IN MOSQUITOES PROMPTS EXPRESSION OF TWO HEAT SHOCK PROTEINS

Joshua Benoit, Giancarlo Lopez-Martinez, David L. Denlinger

The Ohio State University, Columus, OH, United States

Blood feeding in mosquitoes poses two significant stresses: huge changes in size that alters internal pressure, and increases in internal temperature, up to 10°C within 1 minute. In this study, we evaluated expression of two heat shock protein genes (Hsp70 and Hsp90) in three mosquito species (*Culex pipiens*, *Anopheles gambiae*, and *Aedes aegypti*) to see if feeding elicited up-regulation of these genes. High amino acid homology between species was evident for the portions of the genes used for northern blot hybridizations. When exposed to heat shock at temperatures equivalent to the changes that occur during blood feeding (37°C for 5 min), the genes for Hsp70 and Hsp90 where highly up-regulated in all three mosquito species, suggesting that feeding may evoke a stress response. Expression of Hsp70 in response to blood feeding increased within one hour in all three species, and expression persisted above normal background for at least 6h. Blood feeding caused a higher expression of Hsp90 in *Cx. pipiens* and *Ae. aegypti*, but failed to change expression in *An. gambiae*. Our preliminary results suggest that engorging the mosquitoes by injecting blood held at room temperature does not induce Hsp expression, indicating that the high temperature of blood is the critical feature evoking expression. The responses of Hsp70 and Hsp90 to blood feeding are not identical among the three species: responses of *Cx. pipiens* and *Ae. aegypti* are more similar to each other, while the response of *An. gambiae* is more distinct (only Hsp70), suggesting a possible Culicine and Anopheline difference. Our current goal is to understand how heat shock proteins are regulated during blood feeding.

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Chromosomal inversions, natural selection and adaption in the malaria vector *Anopheles funestus*

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