control groups over time using weekly assessments of egg, pupal, and adult densities. In addition, subsets of the larval populations from each treatment group will be periodically screened to determine the prevalence of the lethal allele in these populations over time. The implications for potential field use of this strain and genetic control strategy will be discussed.

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GENETIC CONTROL OF *AEDES* MOSQUITOES TO PREVENT DENGUE AND CHIKUNGUNYA

Luke Alphey

Oxitec Limited, Oxford, United Kingdom

In the absence of vaccines, control of dengue and chikungunya can only be achieved by suppression of mosquito populations. However, current control methods are inadequate to reduce the population of *Aedes* mosquitoes below the disease transmission threshold. Recent advances in insect genetic engineering have opened new possibilities for the control of mosquitoes. The genetic approach that is closest to practical application is "population suppression" based on the Sterile Insect Technique (SIT). SIT has been used successfully for the suppression or local elimination of several insect species in agriculture, and mathematical modeling indicates that it would be effective against *Aedes* mosquitoes. Sterile male mosquitoes are released continually over a wide area to mate with the target pest population; no progeny result from these matings and the target population declines. Sterility has conventionally been induced with γ-irradiation, which is too damaging for most mosquitoes, or chemicals which are no longer approved. In the RIDL® method mosquito strains are homozygous for one or more dominant lethal genes. We have successfully constructed RIDL strains of *Aedes aegypti* and *Aedes albopictus*, using the tetracycline-repressible 'tet-off' gene expression system to repress the lethal effect with dietary tetracycline. The first RIDL strains have been successfully tested in confined semi-field conditions for mating competitiveness with wild-type mosquitoes and a range of life history and behavioural traits. An area-wide control program based on mass-release of mosquitoes would preferably not release biting females. Sex-separation methods are therefore required. Effective mechanical separation methods are available, and we are developing genetic sexing methods which will be more accurate and efficient. Our successful development of such systems in *Aedes aegypti* will be discussed.

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THE EFFECT OF GENE DRIVE ON CONTAINMENT OF TRANSGENIC MOSQUITOES

John M. Marshall

University of California at Los Angeles, Los Angeles, CA, United States

Mosquito-borne diseases such as malaria and dengue fever continue to be a major health problem through much of the world. Several new potential approaches to disease control utilize gene drive to spread anti-pathogen genes into the mosquito population. Prior to a release, these projects will require trials in outdoor cages from which transgenic mosquitoes may escape, albeit in small numbers. Most genes introduced in small numbers are very likely to be lost from the environment; however gene drive mechanisms enhance the invasiveness of introduced genes. Consequently, introduced transgenes may be more likely to persist than ordinary genes following an accidental release. Here, we develop stochastic models to analyze the loss probabilities for several gene drive mechanisms, including homing endonuclease genes, transposable elements, Medea elements, the intracellular bacterium Wolbachia, engineered underdominance genes, and meiotic drive. We find that Medea and Wolbachia present the best compromise between invasiveness and containment for the six gene drive systems currently being considered for the control of mosquito-borne disease.

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GENE EXPRESSION PROFILE ANALYSIS OF *ANOPHELES GAMBIAE* AGING AND BLOOD FEEDING: IDENTIFICATION OF CANDIDATE GENES FOR AGE GRADING

Mei-Hui Wang[1], Osvaldo Marinotti[2], Anthony A. James[2], Edward Walker[3], Guiyun Yan[1]

[1]Public Health, University of California at Irvine,, Irvine, CA, United States, [2]Molecular Biology and Biochemistry, University of California at Irvine, Irvine, CA, United States, [3]Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, United States

Information on population age structure of mosquito vectors under natural conditions is not only fundamental to the understanding of vectorial capacity, but it is also crucial to the assessment of the impact of vector control measures on malaria transmission. A recent study demonstrated that transcriptional profiles of the genes strongly associated with age are excellent molecular markers for age grading in *Aedes aegypti*. The objectives of this study are to examine gene expression profile changes associated with ageing and bloodfeeding in *Anopheles gambiae*, and then to identify molecular markers that may be used for *An. gambiae* mosquitoes age grading. We used Affymetrix Anopheles Genome Array GeneChip® and examined the genome-wide gene expression profile changes associated with ageing through comparisons of gene expression patterns for mosquitoes at days 1, 4, 10, 19, and 28 post emergence. The examination of gene expression changes due to blood feeding involves mosquitoes bloodfed at these age points. We compared the global gene expressions in non-blood-fed adult female mosquitoes at these age points, and found 9,116 transcripts differentially expressed at $P < 0.001$. We then excluded genes which expression was affected by blood feeding, and identified a total of 299 candidate genes. The majority of these 299 genes codes for proteins involved in metabolic process and oxidation reduction. The K-means cluster analysis identified 40 clusters. We chose 6 genes as most promising candidate genes for age grading from cluster 28 because they showed a linear reduction in expressions with mosquito ages. Using the *An. gambiae* mosquitoes from laboratory cages, we generated a calibration model and estimated 95% confidence for mosquito age predictions. The calibration mode, based on the canonical redundancy analysis, produced a R^2 value of 0.79 between the calculated redundancy variates and observed age. We are currently validating the expression profile based age grading method using *An. gambiae* mosquitoes reared in semi-natural MalariaSphere in western Kenya.

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POLYMORPHISMS IN *ANOPHELES GAMBIAE* IMMUNE GENES ARE ASSOCIATED TO MALARIA RESISTANCE

Caroline A. Harris[1], Isabelle Morlais[2], François Rousset[3], Luc Abate[1], Didier Fontenille[1], Anna Cohuet[4]

[1]Institut de recherche pour le developpement, Montpellier, France, [2]OCEAC, Youndé, Cameroon, [3]University of Montpellier, Montpellier, France, [4]IRSS, Bobo Dioulasso, Burkina Faso

In nature Anopheles mosquitoes are found to have varying levels of resistance to the malaria parasite, a characteristic known to be under strong genetic control. Understanding the molecular basis of their immunity will give essential insights for novel malaria control strategies. This study aims to uncover single nucleotide polymorphisms (SNPs) associated with mosquito resistance to malaria in the natural vector/parasite couple *Anopheles gambiae/Plasmodium falciparum* through genotype to phenotype associations. *An. gambiae* M form mosquitoes from Cameroon were experimentally infected with a sympatric wild *P. falciparum* isolate. The number of oocysts/midgut at day 8 post blood meal was counted giving each mosquito a quantitative phenotype. These mosquitoes were then genotyped for selected SNPs in known immunity genes and statistical tests applied to determine association (genotype/phenotype). 6 out of 157 SNPs show an association to phenotype, located within or upstream of SP Snake-like, TOLL6, SP PPO activate, CLIPB4,

AgMDL1 and CEC1. These 6 SNPs were then tested for association in 2 subsequent infections (same mosquito colony infected with different local wild parasite isolates) where 2 out of the 6 SNPs showed association in the second infection and none in the third. The SNP located within the gene SP Snake-like shows the same trend in all three infections with the homozygous G allele mosquitoes harboring the lowest number of oocysts. This study reinforces the importance of genetic variability in mosquito immunity. The SP Snake-like G allele confers partial resistance and although not significant in all infections, it is likely to play an important role in the mosquito immune response to the parasite. As associated SNPs do not show association to all parasite isolates it suggests their role in immunity is parasite genotype specific to varying degrees. From these results the effect of genotype appears to have a strong effect on immunity and will have to be seriously considered in the development of novel control strategies.

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DUPLICATION AND CONCERTED EVOLUTION OF VITELLOGENIN GENES IN MOSQUITOES

Song Chen, Jason L. Rasgon

Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States

The mosquito vittellogenin (Vg) genes belong to a small multiple gene family that encodes the major yolk proteins precursors required for egg production. Several Vg genes have been cloned and characterized from several mosquito species, but, their origin and molecular evolution are poorly understood. We isolated four distinct full length Vg genes from the West Nile virus vector *Culex tarsalis* that likely arose from double duplication events. These genes are organized in two pairs (Vg1a, Vg1b; Vg2a, Vg2b). The sequence comparison indicated that Vg1 and Vg2 genes shared 64.3%-65.5% nucleotide identity. Within each pair, the Vg genes shared very high nucleotide identity (98.1% and 97.0%, respectively). For comparative purposes, the Vg genes were identified from the publically-available *Cx. pipiens*, *Aedes aegypti* and *Anopheles gambiae* genome sequences. Vg gene organization in *Cx. pipiens* was very similar to *Cx. tarsalis*, and indicated that the double duplication event was ancestral to the separation of these two species. In contrast, *Ae. aegypti* and *An. gambiae* had three Vg genes that evolved from independent duplication events in each genus. Signatures of concerted evolution were detected in *Culex* and *Anopheles* Vg gene sequences, but not in *Aedes*. In conclusion, these analyses indicate that the evolution of Vg genes is dominated by independent duplication events in 3 different mosquito genera, and that concerted evolution may contribute to sequence homogenization in some genera.

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RESPONSE OF MOSQUITO PROTEIN INTERACTION NETWORK TO THE DENGUE INFECTION

Zhiyong Xi, Xiang Guo, Yao Xu, Guowu Bian, Andrew David Pike, Yan Xie

Michigan State University, East Lansing, MI, United States

Two fifths of the world's population is at risk from dengue. The absence of effective drugs and vaccines leaves vector control as the primary intervention tool. Understanding dengue virus (DENV) and host interactions is essential for the development of novel control strategies. The availability of genome sequences for both humans and the mosquito vector greatly facilitates genome-wide studies of DENV-host interactions. We developed the first draft of mosquito protein interaction network using a computational approach. The high-confidence network includes 4,214 *Aedes aegypti* proteins with 10,209 interactions, among which 3,500 proteins are connected into an interconnected scale-free network. We demonstrated the application of this network for the further annotation of mosquito proteins and dissection of pathway crosstalk. More importantly, protein interaction network makes the foundation for systems biology study of DENV-mosquito interactions. Using three datasets based on physical interaction assay, genome-wide RNA interference (RNAi) screens and microarray assay, we identified 705 putative DENV-associated mosquito proteins. The integrated analysis of these proteins and mosquito network revealed three main modules with function in replication/transcription/translation, immunity and transport that were targeted by DENV. Putative DENV-associated proteins were further selected for validation by RNAi-mediated gene silencing, and the results showed the dengue viral titer in mosquito midgut was significantly reduced for 57% of these genes. Our results indicate presence of common host requirements of dengue viruses in mosquitoes and humans. We discuss the significance of our findings to pharmacological intervention and genetic modification of mosquitoes for blocking dengue transmission.

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ETIOLOGY OF FEBRILE ILLNESS AMONG HOSPITALIZED HIV-INFECTED AND HIV-UNINFECTED ADULTS AND ADOLESCENTS IN NORTHERN TANZANIA

John A. Crump[1], Habib O. Ramadhani[2], Anne B. Morrissey[1], Venance P. Maro[2], Wilbrod Saganda[3], Mtumwa S. Mwako[3], Andrea V. Shaw[1], John A. Bartlett[1], Hugh Reyburn[4]

[1]Duke University Medical Center, Durham, NC, United States, [2]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania, [3]Mawenzi Regional Hospital, Moshi, United Republic of Tanzania, [4]London School of Hygiene and Tropical Medicine, London, United Kingdom

Few studies describe patterns of HIV co-infections in African hospitals in the ART era. We prospectively studied febrile admissions to two hospitals using modern laboratory methods. We enrolled consecutive admitted patients aged ≥13 years with oral temperature ≥38.0ºC during one year in Moshi, Tanzania. A standardized clinical history and physical examination was done and hospital outcome recorded. HIV antibody testing, aerobic and mycobacterial blood cultures, and malaria film were done. HIV-infected patients also received serum cryptococcal antigen testing and CD4-positive T-lymphocyte count (CD4 count). Of 411 patients enrolled, the median (range) age was 37 (14-97) years, 222 (54.0%) were female, and 159 (38.9%) were HIV-infected. Of HIV-infected patients the median (range) CD4 count was 110 (1-1,140) cells/mm^3, 17 (10.7%) had positive serum cryptococcal antigen tests, and 55 (34.6%) were receiving ART and trimethoprim-sulfamethoxazole prophylaxis. Of 374 (90.1%) with blood cultures, 72 (19.3%) grew a pathogen. Of blood cultures with pathogens, 26 (36.1%) grew *Salmonella* Typhi; 10 (13.8%) *Mycobacterium tuberculosis* complex; 10 (13.8%) *Escherichia coli*; 8 (11.1%) *Streptococcus pneumoniae*; 6 (8.3%) *Cryptococcus neoformans*; and 12 (16.7) grew other pathogens. *Plasmodium falciparum* was identified on blood film of 7 (1.7%). HIV infection was associated with *M. tuberculosis* (odds ratio [OR] undefined, p <0.001) and *C. neoformans* (OR undefined, p=0.002) bloodstream infection (BSI), but not with *E. coli, S. pneumoniae,* or *P. falciparum* BSI. HIV infection appeared to be protective against *S.* Typhi BSI (OR 0.12, p=0.001). Forty-three (10.5%) of patients died in hospital. In conclusion, *M. tuberculosis* and *C. neoformans* are leading causes of blood stream infection in Tanzania in the ART era and are closely associated with HIV infection; we demonstrate a protective effect of HIV against *S.* Typhi BSI in this setting. HIV co-infections continue to account for a large proportion of febrile admissions in Tanzania.

Harris C. A., Morlais Isabelle, Rousset F., Abate L., Fontenille Didier, Cohuet Anna. (2009)

Polymorphisms in *anopheles gambiae* immune genes are associated to malaria resistance

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