Preliminary program

Bat1K satellite meeting at Biodiversity Genomics Meeting 2020

Tuesday 6th Oct @ 19:00-21:00 BST

Chair: Sonja Vernes

19:00 -19:10	**Sonja Vernes**	Sonja.vernes@mpi.nl	Introduction and getting involved in Bat1K
19:10 -19:15	**Martin Pippel**	pippel@mpi-cbg.de	Bat1K genome assembly
19:15 -19:20	**Michael Hiller**	michael.hiller@senckenberg.de	Bat1K genome annotation
19:20 -19:25	**Burton Lim**	burtonl@rom.on.ca	Frozen tissue collection and genomic research on bats at the Royal Ontario Museum
19:25 -19:30	**Huabin Zhao**	huabinzhao@whu.edu.cn	Molecular adaptations of Chinese bats revealed by genome sequencing
19:30 -19:35	**Joana Damas**	joanadamas@gmail.com	Chromosome evolution in chiropterans
19:35 -19:40	**Legana Fingerhut**	legana.fingerhut@my.jcu.edu.au	Using bat genomes to understand their immunity through antimicrobial peptides
19:40 -19:45	**Danielle Adams**	dadams37@umd.edu	Using DNA methylation to predict age and longevity of bats
19:45 -19:50	**Guilherme Oliveira**	guilherme.oliveira@itv.org	The genomes of three Amazon bat species, rare and endangered
19:50 -19:55	**Hannah Kim Frank**	hkfrank@tulane.edu	Exceptional diversity and selection pressure on SARS-CoV and SARS-CoV-2 host receptor in bats compared to other mammals
19:55 -20:00	**Ksenia Lavrichenko**	ksenia.lavrichenko@mpi.nl	Transcriptomic signatures in tissues of vocal learning bats

20:00 -20:05	**Laurel Yohe**	loloyohe@gmail.com	Olfactory receptor gene families, not ecology, explain olfactory diversity in bats
20:05 -20:10	**Juan Manuel Vazquez**	aging@berkeley.edu	Stress Response and a P53 Duplication in the Long-Lived Bat, Myotis Lucifugus
20:10 -20:15	**Alexa Sadier**	asadier@ucla.edu	Understanding the GRN behind bat molar extreme diversification
20:15 -20:20	**Chul Lee**	swear0712@gmail.com	ChrOrthLink: Visualization tool to understand chromosomal evolution across diverse lineages
20:20 -20:25	**Aaron Irving**	aaronirving@intl.zju.edu.cn	Alternative immune activation in bats in response to ligands/pathogens compared to other mammals/humans
20:25 -20:30	**Karen Sears**	ksears@ucla.edu	Investigating relationships between inflammation, aging, and viral susceptibility in wild bats from Cameroon
20:30 -20:35	**Armin Scheben**	scheben@cshl.edu	Unraveling molecular mechanisms of immunity and cancer-resistance using the genomes of two Neotropical bats
20:35 -20:40	**Sébastien Puechmaille**	sebastien.puechmaille@umontpellier.fr	Recovering NUMTs from full bat genomes, a mine of information
20:40	**Breakout room discussions** List of questions to discuss in breakout rooms 1. What opportunities for funding bat genomic work are there (small or large; locally, nationally, internationally)? 2. How can we facilitate collection and transport of samples for genome sequencing? (including permit, CITES, and Nagoya issues) 3. How can we get the community more involved in Bat1k and its activities? 4. What do you want to see from Bat1K in the future? 5. Have you encountered any problems relating to Bat1k? (eg. communication, access to data, interaction)		
Plenary discussion of breakout room topics			

Abstracts

Burton Lim

Frozen tissue collection and genomic research on bats at the Royal Ontario Museum

Newer technologies for genomic sequencing require high molecular weight DNA from fresh or flash-frozen tissues. Although originally collected for allozyme electrophoresis studies and Sanger sequencing, the Royal Ontario Museum has been freezing tissue samples in liquid nitrogen during field collections of small mammals over the past 3 decades. Since 1989, we have collected about 14,700 specimens representing 15 families, 120 genera, and 400 species of bats from 30 countries. We are collaborating on the Bat1K initiative by contributing frozen tissue samples for several families in Phase 1 of the project, a coronavirus rhinolophoid genome study, and a vampire bat genomics project. In addition, we have received funding for sequencing of the blunt-eared bat from Canada's Genomics Enterprise (CGen) and submitted a proposal to Genome Canada for funding research on Canadian bat species.

Huabin Zhao

Molecular adaptations of Chinese bats revealed by genome sequencing

There are more than 150 bat species in China, accounting for over 10% of all bat species in the world. These species are affiliated with eight bat families across the bat phylogeny. We am planning or have finished whole genome sequencing for around 10 representative species of Chinese bats, using Oxford Nanopore Technologies (ONT) sequencing and the high-throughput chromosome conformation capture (Hi-C) analysis. We are planning to investigate lineage-specific molecular adaptations of these bat species using these new genome data and those publicly available genome data. We pay particular attention to immune genes and virus receptor genes, and perform cell-based functional assays to dissect bat-specific immune functions and interactions of viruses and their receptors. Our study facilitates a better understanding of molecular basis of bat adaptations, and adds to an important component of Bat1K project.

Joana Damas

Chromosome evolution in chiropterans

The comparison of species' chromosome organization reveals valuable information for

reconstructing the phylogenetic relationships between species, understanding the evolutionary forces that shape genomes, and shedding light on the link between chromosome rearrangements and the emergence of novel phenotypes during evolution. Highly contiguous chromosome-scale genome assemblies permit accurate identification and classification of chromosomal breakpoints and at high resolution. Herein, we used chromosome-scale assemblies generated by the Vertebrate Genome Project and the Bat 1K Project to investigate chromosome evolution in chiropterans. We defined evolutionary breakpoint regions (EBRs) in bats using human as a reference genome, and the lynx, platypus, and chicken genomes as outgroups. After verifying that the EBRs were in reliable regions of the bat genome assemblies, we investigated the genes that were within and around these evolutionarily dynamic regions. We observed that a chiropteran EBR that corresponds to a locus on human chromosome 6p is associated with pseudogenization and complete gene loss in bats. Species in which one or both ends of the breakpoint was reused in the bat lineage were found to have a larger number of deleted genes and pseudogenes. ZSCAN12 and GPX6 appear to be absent from all analyzed bat species. These genes have important function in immunity and metabolism. These results point to chromosome rearrangements that may have contributed to speciation and traits associated with the bat lineage.

Legana Fingerhut

Using bat genomes to understand their immunity through antimicrobial peptides

Antimicrobial peptides are part of the innate immune system, which help defend the host against pathogens and regulate the microbiome. Bats have a higher tolerance to pathogens which could be reflected in their immune system and antimicrobial peptide repertoire. We are interested in using Bat1K genomes to scan for antimicrobial peptides using the R package, ampir. This could reveal novel antimicrobial peptides and aid in understanding bat immunity. Furthermore, this could be used as a base for future studies investigating the role of the innate immune system on the microbiome of bats which has potentially important implications on bat health.

Danielle Adams

Using DNA methylation to predict age and longevity of bats

Exceptionally long-lived species, including many bats, rarely show overt signs of aging, making it difficult to determine why species differ in aging rates and lifespan. Here, we use DNA methylation (DNAm) profiles from 700 known-age bats, representing 26 species, to identify epigenetic changes associated with age and longevity. We demonstrate that DNAm accurately predicts the chronological age of individuals. Across species, longevity is negatively associated with the rate of DNAm change at age-associated sites. Furthermore, analysis of several bat genomes reveals that hypermethylated age- and longevity-associated sites are disproportionately located in promoter regions of key transcription factors (TF) and enriched for histone and chromatin features associated with transcriptional regulation. Predicted TF binding site motifs and enrichment analyses indicate that age-related methylation change is influenced by developmental processes, while longevity-related DNAm change is associated with innate immunity or tumorigenesis genes, suggesting that bat longevity results from augmented immune response and cancer suppression.

Guilherme Oliveira

The genomes of three Amazon bat species, rare and endangered

Genomic information can provide information that is central to the conservation of bat species. We aim to generate reference genomes and population data for three Amazon endangered bat species in this work. The first three species are *Furipterus horrens* (VU), *Lonchorhina aurita* (VU), and *Natalus macrourus* (VU). We were granted a license to collect one specimen of each species. The animals were collected at the Carajás National Forest in the eastern area of the State of Pará, Brazil. DNA was extracted mainly from the wing tissue punches. Genomic data were obtained using short-read Illumina libraries and PacBio long reads. Samples were also collected from 19 tissues for RNASeq analysis. The genome sizes for the species were estimated to be 2.5 Gb. Short read libraries provided 75X coverage for *Furipterus horrens*, 56X *Lonchorhina aurita* (VU), and 75X for *Natalus macrourus*. We produced additional long read sequences to a 60X coverage for *Furipterus horrens*, 50X *Lonchorhina aurita*, and 60X for *Natalus macrourus*. The initial PacBio and Illumina only assemblies are still fragmented, with N50 values ranging from 113 to 134 kb for the PacBio data. Transcriptome coverage ranged from 27 to 102 million high-quality reads per tissue. The transcriptomes were assembled using Trinity with the number of genes varying

from 320 to 502 thousand, with an average size from 1,09 to 1,2 kb. Integration of short read, further PacBio sequencing, and additional assembly strategies will be used for improved assemblies.

Hannah Kim Frank

Exceptional diversity and selection pressure on SARS-CoV and SARS-CoV-2 host receptor in bats compared to other mammals

Pandemics originating from pathogen transmission between animals and humans highlight thebroader need to understand how natural hosts have evolved in response to emerging human pathogens and which groups may be susceptible to infection. Here, we investigate angiotensin-converting enzyme 2 (ACE2), the host protein bound by SARS-CoV and SARS-CoV-2. We find that the ACE2 gene is under strong selection pressure in bats, the group in which the progenitors of SARS-CoV and SARS-CoV-2 are hypothesized to have evolved, particularly in residues that contact SARS-CoV and SARS-CoV-2. We detect positive selection in non-bat mammals in ACE2 but in a smaller proportion of branches than in bats, without enrichment of selection in residues that contact SARS-CoV or SARS-CoV-2. Additionally, we evaluate similarity between humans and other species in residues that contact SARS-CoV or SARS-CoV-2, revealing potential susceptible species but also highlighting the difficulties of predicting spillover events. This work increases our understanding of the relationship between mammals, particularly bats, and coronaviruses, and provides data that can be used in functional studies of how host proteins are bound by SARS-CoV and SARS-CoV-2 strains.

Ksenia Lavrichenko

Transcriptomic signatures in tissues of vocal learning bats

Vocal learning (VL), e.g. the ability to change or create completely new sounds based on auditory feedback Petkov and Jarvis (1) , is one of the few composite traits of human language that is shared with other animals. An animal clade that is both documented to have strong capacities in VL and amenable to experimental methods is bats (2) . An earlier study from our lab (3) identified a tissue- specific gene network in PAG, a brain region fundamental for mammalian vocalisation in P.discolor, a

bat species with published behavioural evidence for VL (4) . We sequence several tissues of this species, to further characterise transcriptomic profiles in the brain regions, known to play important roles in VL circuits (striatum and cortex) (5) . We aim to ascertain both quantitative (gene expression changes) as well as qualitative (mRNA isoform preference) differences in striatum, cortex and PAG, thus furthering our understanding of these circuits in bats. Furthermore, we plan to compare the transcriptomic profiles in the P.dicolor bat to the data from songbirds, who also possess strong documented capacity of vocal learning (6) . Despite the very different morphology of mammalian and avian brains, the convergence of the transcriptomic profiles was observed for a number of tissues, involved in vocal learning (7) . We expect to be able to refine the resolution of the similarities and differences between those vocal learners in the two kingdoms, thus adding to the knowledge of VL evolution.

Laurel Yohe

Olfactory receptor gene families, not ecology, explain olfactory diversity in bats

Neotropical Leaf-nosed bats (Phyllostomidae) are well
known for their dietary radiation and accompanying morphological adaptations for the consumption of new food resources. The evolution of frugivory, nectarivory, and sanguivory from an insectivorous ancestor suggests novel mechanisms of food detection must have also evolved, and behavioral evidence shows phyllostomids strongly rely on olfaction while echolocation is supplemental. When the profound morphological or genetic changes necessary for dietary diversification emerged is unknown, but enable new diets, these changes must precede dietary diversification. We compared olfactory receptors sequenced from olfactory epithelium transcriptomes in phyllostomids with differing diets to test if adaptive selection or novel morphologies occurred in the olfactory system prior to dietary divergence. We found little difference in plant-visiting bats versus animal feeding bats, but strong differences depending on olfactory receptor subfamily. This suggests some olfactory receptors are evolving faster than others and it is independent of ecology. Rather the mechanisms may lie in where these receptors are located on the chromosomes.

Juan Manuel Vazquez

Stress Response and a P53 Duplication in the Long-Lived Bat, Myotis Lucifugus

Cancer is a disease common to all complex life. Many life history traits, such as size and lifespan, are correlated with cancer risk between individuals of a species; however, this correlation does not hold when comparing between species. This phenomena, known as Peto's Paradox, is resolved as species evolve cancer suppression mechanisms in parallel to increased sizes and lifespans. However, the exact mechanisms involved are largely unknown. Bats represent an ideal clade to study this paradox, as the large number of closely-related species preserves a detailed record of the genetic changes underlying their diversity in body size and lifespan. We show that the long-lived bat, Myotis lucifugus, has 8 copies of TP53, a central regulator of the DNA damage response present in all living organisms. Two of these copies - the canonical locus plus a full-locus duplication - are unique to M. lucifugus, and are actively transcribed. To investigate how these two copies of TP53 influence the stress response of M.lucifugus relative to 4 other closely related bat species (M. evotis, M. thysanodes, M. yumanensis, and E. fuscus), we measured apoptosis, cytotoxicity, and viability in primary fibroblasts in response to chemically induced DNA-damage, unfolded protein response, and oxidative stress. We show that these two copies of TP53 play a role in mediating M. lucifugus's unique response to these stresses relative to the other bat species. These results contribute to our understanding of how pre-existing tumor suppressor mechanisms have been enhanced through gene duplication to resolve Peto's Paradox in large, long-lived organisms.

Alexa Sadier

Understanding the GRN behind bat molar extreme diversification

Among the developmental processes that have been proposed to influence the direction of evolution, the modular organization of developmental gene regulatory networks (GRNs) has shown particular promise. In theory, GRNs have *core modules* comprised of essential, conserved circuits of genes, and *sub-modules* of downstream, secondary circuits of genes that are more susceptible to variation. Such an organisation could explain how some organs, such as teeth, can be both extremely diverse while maintaining their core function.

While this idea has received considerable interest as of late, the field of evo-devo lacks the experimental systems needed to rigorously evaluate this hypothesis. To do so, the bat molar constitutes the perfect system. First, in tandem with the colonization of various ecological niches, bats have evolved all possible mammalians diets such as insects, fruits, nectar, pollen, small vertebrates, and even blood, resulting in an incredible diversity of molar shape. Second, tooth development and its associated GRN

have been well studied and modelled in model and, to a lesser extent, non-model organisms, leading to a good understanding of the conserved mechanisms behind tooth formation.

In this study, we propose to test if the existence of modules within the tooth GRN explains both the conservation and the extraordinary diversity of molar shapes seen in bats. To do so, we will combine genomic, developmental, morphological and computational data, to link shape diversity with GRN module evolution. For both genomic, transcriptomic and developmental experiments, our work will rely heavily on the new bats genomes available.

Chul Lee

ChrOrthLink: Visualization tool to understand chromosomal evolution across diverse lineages

Improvements sequencing and assembly technologies generating high quality reference genome assemblies with chromosome-scale scaffolds provide opportunities to understand chromosomal evolution among species. Genome-wide alignment programs could show relationships among chromosomes of close relative species by detecting conservative synteny blocks, but it is hard to define orthologous regions among distant species across diverse lineages. Here, we developed a program, named as Chromosomal orthologous link (ChrOrthLink) analysis, to visualize links of broadly conserved singleton orthologous genes forming synteny blocks on chromosomes of each species. Based on BUSCO analyses to define orthologous genes in each genome assembly, we checked chromosomal relationships among 15 species including human and the VGP 1st release species in various clades, such as, mammal, bird, reptile, amphibian, teleost fish, and skate. 1,147 complete singleton BUSCO genes conserved in all 15 species visualized chromosomal rearrangements among species, despite those low coverages on whole genomes. We believe this method is universally applicable to trace chromosomal synteny across species in diverse lineages.

Aaron Irving

Alternative immune activation in bats in response to ligands/pathogens compared to other mammals/humans

Bats are unique amongst mammals for their ability to fly, however, their unique adaptations to effectively control immune activation pathways are under-appreciated.

We examine gene and protein expression post-stimulation to compare immune signalling components of bats with other higher-level mammals such as macaque and human to reveal novel insights into immune regulation. Overall there is an overt effort in bats to dampen systemic inflammation while still allowing strong, local responses and systemic triggering of acute phase response proteins, complement etc while still inhibiting large amounts of cytokine release. Several alternative chemokines are differentially expressed in bats. Many of these factors have also undergone evolutionary selection pressure in multiple species of bats. Uncovering the key regulatory factors controlling these specific immune phenotypes may provide insight into new therapeutic options for regulating immune responses to pathogens in humans.

Karen Sears

Investigating relationships between inflammation, aging, and viral susceptibility in wild bats from Cameroon

Bats carry deadly zoonoses but do not get sick, and get older but do not physically age. What causes these apparent paradoxes? Data from genomics, mark-recapture bat populations, and bat cell culture suggest that bats may have evolved a relatively heightened anti-inflammatory response. This change is hypothesized to minimize inflammation-associated cellular senescence and immunopathology, and thereby extend lifespan and limit viral-induced morbidity, respectively. Testing of this intriguing hypothesis requires a thorough understanding of the links between the inflammatory response, aging, and viral susceptibility in diverse organs of many wild bat species. Unfortunately, until now, the field's inability to reliably assess the age of most wild bats severely hampered the generation of these data. Our goal is to establish links between chronological aging, biological aging, inflammation response, and susceptibility to coronaviral (CoV) infection in wild populations of several bat species. This research is now possible because of the recent generation of an epigenetic clock that reliably estimates age in wild bats. As a first step toward our goal, we are currently quantifying chronological age, cellular senescence and pathology, CoV infection status, and inflammation response in organs of wild bats from several species from Cameroon. Preliminary results are consistent with the anti-inflammatory response being heightened in longer than shorter lived bats and in bats versus shorter-lived mammals.

Armin Scheben

Unraveling molecular mechanisms of immunity and cancer-resistance using the genomes of two Neotropical bats

Bats are exceptional among mammals for harbouring diverse pathogens and for their robust immune systems. In addition, bats are unusually long-lived and show low rates of cancer. Contiguous and complete reference genomes are needed to determine the genetic basis of these adaptations and establish bats as models for research into mammalian health. Here we sequenced and analysed the genomes of the Jamaican fruit bat (Artibeus jamaicensis) and the Mesoamerican mustached bat (Pteronotus mesoamericanus). We sequenced these two species using a mix of Illumina and Oxford Nanopore Technologies (ONT), assembling draft genomes with some of the highest contig N50s (28-29Mb) of bat genomes to date. Work is in progress to increase the base-level accuracies of these genomes. We conducted gene annotation and identified a set of 10,928 orthologs from bats and mammalian outgroups including humans, rodents, horses, pigs, and dogs. To detect positively selected genes as well as lineage-specific gene gains and losses, we carried out comprehensive branch-site likelihood ratio tests and gene family size analyses. Our analysis found signatures of rapid evolution in the innate immune response genes of bats. We also found evidence of positive selection of tumor suppressors, which may play a role in the low cancer rates, in the most recent common ancestor of bats. These new genomic resources enable insights into the extraordinary adaptations of bats, with implications for mammalian evolutionary studies and public health.

Sébastien Puechmaille

Recovering NUMTs from full bat genomes, a mine of information

NUMTs, Nuclear copies of MiTochondrial DNA have been transferred from the mitochondria to the nucleus and act as mitochondrial DNA fossils. The study of NUMTs without high quality full genomes is challenging as it is difficult to differentiate an assembly error from a true NUMT. The release of several high quality genomes offers unique opportunities to better understand and use information encoded in NUMTs. For example, their number, length, similarity to the actual mtDNA can be studied to understand the processes behind this transfer. Their sequences can be used to root trees, better understand introgression or correct previous studies that inadvertently amplified NUMTs (instead of the true mtDNA).