PASTEUR MINI-SYMPOSIUM

Evolution & Biodiversity

January 29, 2024, Duclaux Amphitheater

09:30	***Introduction***
09:40	***Keynote lecture*: Laura Eme,** Unité Écologie, Systématique et Évolution, Université Paris-Saclay: ***The origin of eukaryotes***
10:30	**Eduardo Rocha,** Microbial Evolutionary Genomics: ***Bacteria as playgrounds for mobile genetic elements***
10:50	***Short Break***
11:00	**Mart Krupovic,** Archeal Virology: ***Organization and evolution of the viral world***
11:20	**Sebastien Duchene Garzon,** Evolutionary Dynamics of Infectious Diseases: ***Estimating evolutionary rates and dates using ancient pathogen sequence data***
11:40	**Lluis Quintana-Murci,** Human Evolutionary Genetics: ***Evolutionary factors driving immune response variation in humans***
12:00	***Lunch & Poster session***
13:50	**Roberto Toro & Katja Heuer,** Applied and Theoretical Neuroanatomy: ***Role of mechanical morphogenesis on the development and evolution of the mammalian brain***
14:10	**Marc Lecuit,** Biology of Infection: ***An evolutionary perspective on* Listeria *virulence***
14:30	**Aude Bernheim,** Molecular Diversity of Microbes: ***Conservation of anti-viral immunity across domains of life***
14:50	**Javier Pizarro-Cerda,** Yersinia: **Yersinia *stories on pathogen & host evolution***
15:10	***Break***
15:30	**Nicolas Rascovan,** Microbial Paleogenomics: ***Evolutionary and biodiversity insights from ancient DNA data***
15:50	**Thibaut Brunet,** Evolutionary Cell Biology and Evolution of Morphogenesis: ***The evolutionary origin of animal development: insights from our closest unicellular relatives***

INSTITUT PASTEUR

POSTERS

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Dynamics and evolution of body axis elongation in amniotes

P02 - Núria Ros i Rocher (Evolutionary Cell Biology and Evolution of Morphogenesis)
Mixed clonal/aggregative multicellularity is impacted by salinity in a close unicellular relative of animals

P03 - Cyril Anjou (Pathogenesis of Bacterial Anaerobes)
Multiplicity of Thioredoxin systems meets the specific needs of *Clostridia*

P04 - Samuel Garcia Huete (Biology of Spirochetes)
Connecting the unconnected: phylodynamics and evolution of the *Spirochaetes phylum*

P05 - Cyril Savin (Yersinia)
Circulation, evolution and genetic diversity of *Yersinia pseudotuberculosis*

P06 - Nolwenn Dheilly (Pathogen Discovery)
Parasites contribution to viruses evolution

P07 - Juliana Pipoli da Fonseca (Molecular Parasitology and Signaling)
Exploring *Leishmania* genome instability using Nanopore Sequencing

P08 - Dang Liu (Human Evolutionary Genetics)
Dissecting the genetic and evolutionary sources of phenotypic variation in East Polynesians

P09 - Sara Niedbalski (Human Evolutionary Genetics)
The genetic legacy and adaptive dynamics of major lifestyle transition in Siberia

P10 - Arve Lee Willingham Grijalba (Microbial paleogenomics)
Predicting ancient human population movements using phylogenomics of ancient commensal microbes

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Repurposing of the essential metabolic enzyme MoeA

P12 - Gaston Rijo de Leon (Human Evolutionary Genetics)
Unravelling the genetic determinants of human phenotypic variation in East Polynesia: the MATAEA project

P13 - Maite Freire Delgado (Evolutionary Cell Biology and Evolution of Morphogenesis)
Evolutionary History of Amoeboid Cells: Insights from choanoflagellates

P14 - Leo Zeitler (Comparative Functional Genomics)
Decoding the genetics of menstruation in mammals

POSTERS

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Black Death shaped the evolution of immune genes

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The regulatory grammar behind population variation in immune responses to viruses

P17 - Lucas Paoli (Molecular Diversity of Microbes)
Biosynthetic diversity in marine microbiomes: a fragile treasure trove for natural product discovery

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Phylogenetic modeling of gene expression shifts in the mole-rat clade

P19 - Laura Piel (Molecular Parasitology and Signaling)
Beyond genomic adaptation: The role of post-transcriptional regulation in fitness gain of the human pathogen *Leishmania*

P20 - Emelyne Bougit (Yersinia)
Unraveling the depletion mechanism of *Yersinia pestis pla* virulence gene

P21 - Ana Maria Santi (Molecular Parasitology and Signaling)
Translational regulation as a possible driver of fitness gain in the protozoan parasite *Leishmania*

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Type IV-A3 CRISPR-Cas systems drive inter-plasmid conflicts by acquiring spacers in trans

P23 - Alexis Matamoro-Vidal (Cell Death and Epithelial Homeostasis)
Genetic and cellular basis of the repeated evolution of a complex phenotype

P24 - Beatriz Beamud (Synthetic Biology)
Studying the sequence-specificity of bacterial horizontal gene transfer using RandSeq

Dynamics and evolution of body axis elongation in amniotes

P01

Yu Ieda

Gastrulation is a dynamic process, which entails the formation of three embryonic germ layers and concomitantly establishes the primary body axis. Decades of research have focused on the earliest molecular and cellular events underlying gastrulation, yet the subsequent elongation and termination of the primary body axis has received little attention. Using live imaging microscopy and functional perturbations, we investigate these aspects in avian embryos. Furthermore, analyzing reptile embryos, we explore how variations in the process of gastrulation has led to extreme body plan reorganization, as observed in snakes.

Mixed clonal/aggregative multicellularity is impacted by salinity in a close unicellular relative of animals

P02

Núria Ros i Rocher

How animal multicellularity evolved from unicellular ancestors remains an open evolutionary question. Multicellularity in animal cells and in other more distant unicellular species exhibiting facultative multicellularity can be impacted by environmental cues. However, little is known about how signals from the environment regulated unicellular-to-multicellular transitions in early animal ancestors, which in turn could have impacted the mechanisms of integration of such signals along the evolution of multicellularity in animals. In the last decades, considerable advances to reconstruct early animal evolution have come from investigations of the closest living unicellular relatives of animals, notably the choanoflagellates. Here, we are investigating the environmental factors regulating colony formation in the recently discovered choanoflagellate *Choanoeca flexa*. *C. flexa* was originally isolated as multicellular colonies from marine splash pools that naturally undergo cycles of evaporation and refilling. *C. flexa* also exists as swimmer single cells which can adhere to each by cellular aggregation forming multicellular colonies. Here, we found that multicellularity in *C. flexa* is formed through cellular aggregation, which can be also expanded by clonal cell division within colonies. We also found that salinity impacts *C. flexa* multicellularity and we are currently characterizing its aggregative behavior, unique among choanoflagellates.

Multiplicity of Thioredoxin systems meets the specific needs of *Clostridia*

P03

Cyril Anjou

Thioredoxin (Trx) system is a ubiquitous protein repair machinery. In most bacteria, one or several thioredoxins reduce disulfide bonds of proteins and are then recycled by one single pleiotropic thioredoxin-reductase. However, in Clostridia, two to four complete systems (thioredoxin and reductase) are present. Our goal is to understand this atypical composition by studying the three Trx systems of *Clostridioides difficile* and their role in its lifecycle.

By performing phenotypic analysis on simple and multi-mutants, we identified that two redundant systems were involved in the resistance of the vegetative cell to infection-related stresses. However, one of them is also part of the detoxification arsenal of the spore. This spore-associated Trx system is ferredoxin-dependent, allowing activity in absence of an active metabolism, in opposition to the other systems that are classical NAD(P)H-dependent systems.

The third Trx system is part of the reductive Stickland fermentation of glycine. We showed that glycine-reductase and its associated Trx system promote sporulation, probably through consumption of glycine, a known C. difficile co-germinant.

Finally, we found an additional fourth thioredoxin-reductase in several *C. difficile* strains. Phylogenetic analysis demonstrated that this copy was ancestral in *C. difficile* and was lost in some clades. We showed through a trans-complementation approach that this copy is functional and involved in stress response.

Altogether, these results highlight various key roles of Trx systems in *C. difficile* physiology, and provide some clues about the multiplication of these systems by their involvement in crucial Clostridial specific mechanisms, i.e. sporulation and Stickland pathways. More generally, multiplicity of Trx systems meets the constraint of cell differenciation and compartmentation, and this statement can be applied to other organisms, i.e. eukaryotes or Cyanobacteria.

Connecting the unconnected: phylodynamics and evolution of the *Spirochaetes phylum*

P04

Samuel Garcia Huete

Spirochaetes constitute an ancient bacterial phylum that comprises notable pathogens such as *Treponema spp.*, *Leptospira spp.*, or *Borrelia spp.* While *Spirochaetes* cause over 13 million cases of their different diseases annually, comprehensive studies on the complexity and evolution of this bacterial phylum are scarce. Moreover, the recent uncovering of non-spiral *Spirochaetes* challenges established paradigms. To tackle this, we constructed a rooted up-to-date phylogenetic framework for the entire phylum including all cultivable spirochete species sequenced to date. Our results show that the Last Spirochaetal Common Ancestor (LSCA) gave rise to two main clades that separated early in the phylum's evolution. Using phylogenetic profiling, we then revealed that the loss of the spiral shape in the Spirochaetales order correlated with a loss of genes associated with endoflagella and cell shape. Additionally, we demonstrate that the emergence of syphilis-causing species was associated not only with a general metabolic reduction but also with the acquisition of over 50 genes already present in other spirochetes. Our approach sets a precedent for a comprehensive study of Spirochaetes, providing a systematic overview of their evolution and a framework to study relevant evolutionary events.

Circulation, evolution and genetic diversity of *Yersinia pseudotuberculosis*

P05

Cyril Savin

Yersinia pseudotuberculosis (*Ypstb*) is a pathogen of the gastrointestinal tract, closely related to Y. pestis, the plague agent. The population structure of *Ypstb* has been determined using a cgMLST developed in the Yersinia National Reference Laboratory. The population is composed by 155 different genotypes. The epidemiology study of these genotypes revealed that some are restricted to a specific geographical area whereas others are spread worldwide. Two genotypes (1 and 8) are associated with a peculiar form of infection (Far east scarlet-like fever) and two others (5 and 6) are associated with an invasive form of infection, suggesting higher virulence. Phylogenetic analysis showed that *Y. pestis* represents a genotype of *Ypstb*, and that genotype 5 is its closest relative. Spatial and temporal diffusion study of *Ypstb* revealed that this species emerged in Asia before reaching Europe, where it started to spread worldwide. The evolution study of the species revealed that different processes (gene acquisition/loss, SNP, insertion sequences) led to the emergence of the diverse genotypes, leading to differential virulence potential.

Parasites contribution to viruses evolution

P06

Nolwenn Dheilly

Protozoan and helminth parasites are ubiquitous, and responsible for a diverse range of diseases that continue to burden human and animal health worldwide, with major direct and indirect economic consequences. Since the launch of the Parasite Microbiome Project in 2019, and thanks to high throughput sequencing technologies, our knowledge of the taxonomy of viruses of parasites, and role of parasites in virus evolution has been expanding dramatically. Our results reveal (i) the impact of the evolutionary transition towards a parasitic lifestyle on virome composition, (ii) that the intimate relationship between parasites and their hosts provide opportunities for virus host change, and (iii) that most viruses of parasites are vertically transmitted, probably because parasites infrapopulation size - the population of parasites infecting a single host - is often limited, which in itself limits horizontal transmission of viruses. Further studies are now needed to characterize the role of viruses in parasite virulence and host susceptibility, and hence the role of viruses as drivers of parasite evolution.

Exploring *Leishmania* genome instability using Nanopore Sequencing

P07

Juliana Pipoli da Fonseca

Leishmaniasis is a parasitic disease caused by Leishmania spp. with worldwide distribution. A hallmark of *Leishmania* biology is its capacity to adapt to a variety of unpredictable fluctuations inside its human host, notably pharmacological interventions, thus, causing drug resistance. Without transcriptional control, Leishmania responds to environmental change through a mechanism of gene dosage-dependent expression. By intrinsic genome instability, *Leishmania* modulates gene expression and generate genetically highly heterogeneous populations, despite strong selective pressure. Even though chromosome and gene copy number variations fuel the remarkable adaptability of these parasites, little is known on the extent of this heterogeneity in a given parasite population. Here, we use Nanopore sequencing to explore the unique genome of *Leishmania donovani*, map out genetic diversity and assess the existence of asymmetric multi-gene arrays that may drive genomic adaptation by haplotype selection.

Dissecting the genetic and evolutionary sources of phenotypic variation in East Polynesians

P08

Dang Liu

Understanding the links between genetic diversity, local adaptation and phenotypic variation is of upmost importance in evolutionary genetics; yet, most human genomics studies have focused only on European-descent populations. Such an imbalance precludes not only the transferability of genomic findings to other populations, but it provides a partial view of the genetic changes experienced by our species to survive new environments. Polynesia is of particular interest in this context as it has a unique settlement history, made of long-distance voyaging and extensive admixture, and is thought to present among the highest prevalence worldwide of metabolic disorders, raising questions regarding the role of natural selection and genetic drift in driving the genetic architecture of human traits.

Here, we generated whole-genome sequencing data for 1,770 individuals originating from 18 East Polynesian islands and collected a wide range of demographic and phenotypic data relating to metabolism and morphology. Our analyses revealed that East Polynesians from all islands carry genetic ancestries that are maximized in present-day Austronesian speakers from Taiwan and the Philippines, Papuans, western Europeans, and East Asians, as well as native South Americans in the Marquesas Islands only. We observed large variation in East Asian- and European-related ancestries within islands, supporting a common history of recent, extensive admixture.

We confirmed the high prevalence of obesity and type 2 diabetes in the region: > 50% of the population has a BMI > 32, and > 10% has glycated hemoglobin (HbA1c) levels > 6.5%. Applying multiple linear regression models and controlling for sex, age and island of residence, we found that BMI, HbA1c, cholesterol, HDL, and skin pigmentation (melanin index) are associated with genetic ancestry, suggesting that admixture has contributed to phenotypic diversity in Polynesians. For example, we detected a European-specific SLC24A5 variant as a strong determinant of skin pigmentation variation in Polynesians. Furthermore, we identified novel variants associated with variation in metabolic and morphological traits, including variants that are private to Pacific populations. Interestingly, we found no signals of selective sweeps on such variants, suggesting a predominant role of genetic drift in driving phenotypic variation in the region. Together, these results highlight how quantitative genomics in understudied populations can increase our understanding of the genetic and evolutionary sources of human phenotypic variation.

The genetic legacy and adaptive dynamics of major lifestyle transition in Siberia

P09

Sara Niedbalski

Humans have occupied the extreme cold environments of Northern Siberia for 40,000 years, yet the precise genetic interactions and adaptations of peoples from this region are largely uncharacterized. Here, we report new genomic data for 250 Siberians combined with 700 published Eurasian genomes. We investigate population structure as well as how Siberian populations have adapted—through classic sweeps, polygenic adaptation, and post-admixture selection—to extreme environments and lifestyle changes with Russian colonization. We find that contemporary Siberians are best characterized by four genetic sources, a far Eastern Siberian component, isolated within the Chukchi peninsula, two components maximized in overlapping areas of North Central Siberia, the Tunguso-speaking Evenk and Even and the Turkic-speaking Yakut, as well as a European component restricted to Russian communities. We show an admixture event between the Yakut and Even/Evenk ~800-600 years ago, consistent with historical and archaeological hypotheses of a northward expansion of Yakut in the 13th century. Amongst Siberian populations we detect several loci that have been under positive selection, including genes related to metabolic processes, innate immunity, and cardiovascular function, increasing our understanding of human biological adaptations to cold environments. Additionally, we uncover evidence of post-admixture selection in immunity-related loci, suggesting adaptive responses to changing lifestyles and exposure to pathogens introduced by Russian colonizers.

Predicting ancient human population movements using phylogenomics of ancient commensal microbes

P10

Arve Lee Willingham Grijalba

Dental calculus is the calcified biofilm that accumulates on teeth's surface, which can exceptionally preserve the genetic record of an individual's oral microbiome for millennia. Composed of hundreds of species, the human oral microbiome is one of the most stable and conserved microbial communities across worldwide populations. However, little is known on how these human-microbe associations shift over large spatiotemporal scales. To investigate if oral microbial species reflect human population movements, I am analyzing >200 individuals from the Southern Cone of the Americas. This is a region with a rich archaeological record yet underexplored using paleogenomic approaches. Dental calculus will be sequenced at ultra-high depth (>500 million reads) to recover whole genome data from these oral microbes., and phylogenomic analyses will be used to reconstruct the relationship between strains recovered from individuals across multiple spatiotemporal contexts. Thus far, I have analyzed shallow-sequenced data from >50 individuals and created phylogenies for the 6 most abundant oral microbes. Results reveal a strong signal with strains clustering geographically. By analyzing hundreds of microbial species, I expect to bring novel insights into the long-term association of humans and commensal microbes. Thus, contributing a new complementary approach to investigating ancient human demography.

Repurposing of the essential metabolic enzyme MoeA

P11

Anne-Marie Wehenkel

MoeA, also known as gephyrin in higher eukaryotes, is an enzyme that plays a crucial role in the biosynthesis of the molybdenum cofactor (Moco) used by molybdoenzymes involved in redox reactions. Gephyrin has acquired additional functions and acts as a moonlighting protein involved in GABA and GlyR receptor clustering at the synapse. While this feature was thought to be a recent evolutionary trait restricted to eukaryotic Moco biosynthetic enzymes we have recently shown that the clinically relevant phylum of Actinobacteria contains an evolutionary repurposed copy of MoeA (Glp) involved in bacterial cell division. MoeA is present in all domains of life, including Bacteria, Archaea, and Eukaryotes, which motivated our current work to investigate how and how many times MoeA acquired multifunctionality during the evolution of life. We used sequence analyses, phylogenetic inference, and protein structure predictions to study the diversity and evolutionary history of MoeA in all domains of life. Glp-expressing Bacteria such as Corynebacteriales have one or more additional MoeA copies, and structural analysis of their putative active sites suggest that Glp has lost its ancestral role on the biosynthesis of Moco, implying that MoeA multifunctionality was divided into two specialized paralogs. In Archaea, we identified an ancestral duplication of MoeA, and the fusion of one of the paralogs to a periplasmic-binding domain known to be involved in the transport of solute molecules into the cytoplasm, suggesting the acquisition of multifunctionality. On the eukaryotic side, we show that the acquisition of the moonlighting activity of gephyrin was sequential: first, MoeA was obtained from Bacteria by early eukaryotes; secondly, MogA was fused to MoeA in the ancestor of opisthokonts -a clade that includes fungi and animals-, and finally, it acquired the function of anchoring inhibitory neurotransmitters by modifying key exposed residues in the ancestor of animals. Our results support the functional versatility and adaptive nature of the MoeA scaffold, which has been repurposed independently in both eukaryotes and bacteria to carry out analogous functions in network organization at the inner membrane of the cell.

Unravelling the genetic determinants of human phenotypic variation in East Polynesia: the MATAEA project

P12

Gaston Rijo de Leon

Understanding the evolutionary processes that govern phenotypic differences between populations is a central question in evolutionary biology. While the polygenic architecture of most human traits is now understood, the fundamental question of how such traits have evolved remains open, particularly for metabolic diseases, the high prevalence of which poses an evolutionary conundrum. Polynesia, a region spanning >1,000 islands over the Pacific, is of particular interest in this context: it presents the highest prevalence worldwide of metabolic disorders, and has a unique settlement history made of founder events, raising questions regarding the role of natural selection and genetic drift in driving metabolic disease susceptibility.

Here, we generated whole-genome sequencing data for 1,770 individuals originating from 18 East Polynesian islands and collected rich phenotypic data relating to metabolism. We first confirmed the high prevalence of obesity (>50%) and type 2 diabetes (>10%) in the region. We found that metabolic traits are associated with genetic ancestry, suggesting that admixture has contributed to phenotypic diversity in Polynesians. We identified novel variants associated with variation in metabolic and morphological traits, including variants that are private to Pacific populations. Our preliminary analyses show no signals of selective sweeps on such variants, suggesting a predominant role of genetic drift in driving phenotypic variation in the region.

Evolutionary History of Amoeboid Cells: Insights from choanoflagellates

P13

Maite Freire Delgado

In animals, amoeboid cells are crucial cell types underlying fundamental processes like development, immune responses, or cancer metastasis. However, the evolutionary origin of amoeboid cell types remains elusive. In the last decades, considerable advances to reconstruct early animal evolution have come from investigations of the closest living unicellular relatives of animals, notably the choanoflagellates. Choanoflagellates are microeukaryotic organisms that can be found in marine and freshwater environments. In the water column, they are usually thought to swim in the water colum powered by flagellar beating. However, under confinement, these cells can switch to an amoeboid phenotype that starts to crawl powered by bleb protrusions. Given the fact that choanoflagellates are the closest living relatives of animals, the existence of this phenotype might enclose relevant information for the appearance of amoeboid cell types in animals. Here, we are investigating the molecular mechanisms driving the flagellate-to-amoeboid switch in the choanoflagellate species *Salpingoeca rosetta*, which is genetically tractable and experimentally amenable for functional studies. Combining unbiased and hypothesis driven approaches we are functionally testing hypothesis on this question.

Decoding the genetics of menstruation in mammals

P14

Leo Zeitler

In mammals, the uterus undergoes cyclic changes, during which the uterine cell wall and mucosa (endometrium) is built up and-if no fertilization occurs-is broken down. In this case, some species, such as humans and closely related apes, shed the uterine lining, which is known as menstruation. Evolutionary studies revealed that whilst most mammals do not menstruate, the phenotype developed recently and independently in at least four lineages. However, there is still no consensus for what makes menstruation beneficial for those species. We analysed endometrial single-nucleus sequencing data for gene expression (snRNA-seq) and regulation (snATAC-seq) of menstruating and non-menstruating species to identify key differences that give rise to menstruation. The data reveals that the global transcriptome and its regulation of menstruating species substantially changes between different points during the uterine cycle. Our preliminary results shed light on how gene regulation has changed during the adoption of menstruation in mammals.

Black Death shaped the evolution of immune genes

P15

Christian Demeure

Infectious diseases have been among the strongest selective pressure during human evolution. The first outbreak of the Second Pandemic of Plague, the Black Death, killed up to 30-50% of the Afro-Eurasian population. To identify targets of the selection it exerted, we characterized genetic variation around immune-related genes from 321 ancient DNA samples from corpses buried in London and Denmark before, during, and after the outbreak.
We identified 245 variants in immune loci that are highly differentiated, and four of them replicated in both cities represented the strongest candidates for positive selection. One variant controlling the expression of ERAP2 was associated with levels of several cytokines and bacteria killing in macrophages infected with Y. pestis, supporting a role of ERAP2 in immunity against plague. Finally, several of our four variants pointed to alleles today associated with susceptibility to autoimmune diseases, supporting the role played by past pandemics in shaping present-day susceptibility to disease.

The regulatory grammar behind population variation in immune responses to viruses

P16

Yann Aquino

Disentangling the complex predictors of human immune variability is key to understand infectious and inflammatory disease risk disparities among healthy individuals. Towards this goal, single-cell (sc) genomic assays that capture context-specific gene regulation patterns are particularly relevant. We previously used scRNA-seq on immune cells to dissect human population variation in responses to viruses. Building on this dataset, we now report scATAC-seq data on paired basal-state samples from 160 individuals of African or European origin, covering around 200,000 immune chromatin-accessibility landscapes across 21 cell types. Based on this annotation, we mapped over 18,000 variants associated to chromatin accessibility genome-wide at a nominal $p < 1E-5$. These observations will help refine putative causal links between variability in antiviral responses and its genetic predictors, as well as interactions with environmental determinants of the immune response present before infection.

Biosynthetic diversity in marine microbiomes: a fragile treasure trove for natural product discovery

P17

Lucas Paoli

Microbes form complex communities that sustain Earth systems, underpin the health of animals and plants, and fuel biotechnological applications. These communities, or microbiomes, encompass a large discovery potential for bioactive natural products, which are a crucial source of therapeutic developments. However, in environmental microbiomes, the biosynthetic pathways producing these natural products (biosynthetic potential) have not been systematically assessed, owing to the lack of genomic resources. Here, we leveraged sequencing data collected during the Tara Oceans and Tara Pacific expeditions as well as publicly available metagenomes to generate global scale genomic resources for the open ocean and coral reef microbiomes. We found these microbiomes to encode a diverse and novel biosynthetic potential as well as host new candidate superproducer lineages, while highlighting the molecular resources that may be at stake under anthropogenic environmental changes and coral reef decline.

Phylogenetic modeling of gene expression shifts in the mole-rat clade

P18

Maëlle Daunesse

One of the great challenges in evolutionary genomics is understanding the genetic basis of phenotypic adaptations. Despite the morphological and physiological diversity among mammals, their coding genomes exhibit a high degree of conservation. This indicates that changes in gene expression and regulation play a critical role in the evolution of phenotypes. This study aims to use cross-species transcriptomic and epigenomic information to identify and establish a connection between shifts in gene expression and epigenomic regulation, and their potential impact on phenotypic adaptations. To accomplish this, we utilised mole-rats as a model. Naked mole-rats (*Heterocephalus glaber*) and Damara mole-rats (*Fukomys damarensis*) are African rodents that have evolved adaptive phenotypic traits for their underground habitat. These traits include, notably, cancer resistance, tolerance to hypoxia, and exceptionally long lifespans, and have been well-characterised. However, we still have a limited understanding of the gene expression patterns that contribute to these unique characteristics. Here, we profiled the genome-wide gene expression of mole-rats and two rodent outgroups (mouse and guinea pig), in heart and liver. We used a phylogenetic comparative approach to identify genes with a shift of expression in the mole-rat clade, and in naked mole-rat specifically. We found that these genes are associated with functions corresponding to known heart phenotypic adaptations in the naked mole-rat, such as myogenesis or glycolysis. Finally, we demonstrated that genes exhibiting expression shifts also display concordant changes in their regulatory landscape. Altogether, our results provide new insights into the tight relationship between changes in gene expression and regulation, which likely contribute to phenotypic evolution in mammals.

Beyond genomic adaptation: The role of post-transcriptional regulation in fitness gain of the human pathogen *Leishmania*

P19

Laura Piel

Leishmania parasites lack transcriptional control but exploit genome instability for gene dosage-dependent expression changes. Applying experimental evolution and integrative systems analyses on hamster-isolated parasites adapting to in vitro culture, we revealed a fitness trade off with accelerated proliferation correlating with virulence attenuation. We observed the penetrance of a sponteous null mutant for a NIMA-related protein kinase during the first 20 culture passages. Paradoxically, targeting this gene in non-adapted parasites by gene editing caused a lethal phenotype, suggesting the presence of compensatory mechanisms in the spontaneous mutant. Comparative genomics, transcriptomics and proteomics analyses revealed a complex mechanism of compensation involving the post-transcriptional stabilization of non-coding RNAs and differential expression of ribosomal components, suggesting the presence of modified ribosomes that may filter deleterious from beneficial gene dosage effects.

Unraveling the depletion mechanism of *Yersinia pestis pla* virulence gene

P20

Emelyne Bougit

During the 2nd plague pandemic (14th century), a 2-kb deletion was observed in the 9.6-kb plasmid of *Yersinia pestis* pPCP1. This deletion includes toxin-antitoxin genes and pla, an important virulence gene, which may have contributed to end this pandemic.
Here, we aimed at understanding the underlying mechanism of the pla region deletion.
We identified two Xer recombinase Recombination System sequences, xrs1 and xrs2, located at the deleted region extremities. This system is critical for dimer resolution in *Escherichia coli*.
We demonstrated that only xrs2 promotes resolution of vector dimers in *E. coli* or *Y. pestis*. We also showed that SNPs in xrs2, found in *Y. pestis* CO92 reference strain, result in an ineffective dimer resolution. We further studied the importance of Xer system proteins for xrs2 in *E. coli* and found that XerC and PepA, which are crucial for dimer resolution in *E. coli*, are not necessary for dimer resolution mediated by *Y. pestis* xrs2.

Translational regulation as a possible driver of fitness gain in the protozoan parasite *Leishmania*

P21

Ana Maria Santi

Using and Experimental Evolution approach, we previously uncovered genome instability and gene dosage-dependent expression regulation as key drivers of *Leishmania* adaptation and fitness gain. Here we investigate the role of translational regulation as a possible biological filter that can suppress toxic while promoting beneficial gene dosage effects. We mapped and compared the translation initiation sites (TIS) in different *Trypanosomatidae* species uncovering a surprising diversity of Kozak sequences in *Leishmania* with 930 out of the 1024 possible 5-nucleotide combinations identified in the 8419 annotated 5'UTRs. Generating transgenic *L. donovani* that express GFP under the control of different Kozak sequences demonstrated a direct effect of the TIS environment on expression levels. Currently, our focus lies on investigating the presence of regulons governed by the TIS as well as the influence of translational control on Leishmania's adaptation to in vitro culture using RiboProfiling.

Type IV-A3 CRISPR-Cas systems drive inter-plasmid conflicts by acquiring spacers in trans

P22

Fabienne Benz

Plasmid-encoded type IV-A CRISPR-Cas systems lack an acquisition module, feature a DinG helicase instead of a nuclease, and form ribonucleoprotein complexes. Type IV-A3 systems are carried by conjugative plasmids that often harbor antibiotic resistance genes. Their CRISPR array contents suggest a role in inter-plasmid conflicts, but this function remains unexplored. Here, we demonstrate that a plasmid-encoded type IV-A3 system co-opts the type I-E adaptation machinery from its host, *Klebsiella pneumoniae*, to update its CRISPR array. Furthermore, we reveal that robust interference of conjugative plasmids and phages is elicited through CRISPR RNA-dependent transcriptional repression. By silencing plasmid core functions, type IV-A3 impacts the horizontal transfer and stability of targeted plasmids, supporting its role in plasmid competition. Our findings shed light on the mechanisms and ecological function of type IV-A3 systems and demonstrate their practical efficacy for countering antibiotic resistance in clinically relevant strains.

Genetic and cellular basis of the repeated evolution of a complex phenotype

P23

Alexis Matamoro-Vidal

When a complex phenotype repeatedly evolves from different genetic backgrounds, is the goal always achieved by the same genetic and cellular changes or do multiple evolutionary paths lead to the same place? I will present the aims of a project that addresses this question using the wing shape of *Drosophila* as a model system, which has repeatedly and independently evolved towards long or round shapes in several species. First, I will characterise the cellular and molecular modulators of wing shape during morphogenesis in lab strains of the model species *D. melanogaster*. Secondly, I will use strains of *D. melanogaster* originated from three wild populations (Brazil, North America, and Morocco) to artificially evolve the same wing phenotypes and test whether these phenotypes have evolved through the same molecular and cellular changes. In the longer term, I will test whether the mechanisms identified in Aims 1 and 2 are involved in the evolution of wing shape at the interspecific level.

Studying the sequence-specificity of bacterial horizontal gene transfer using RandSeq

P24

Beatriz Beamud

Horizontal gene transfer (HGT), often mediated by mobile genetic elements (MGE) like plasmids, transposons, and bacteriophages, is a main source of bacterial plasticity and adaptation. HGT is not random and bacteria possess several barriers to genetic transfer. Among these, restriction-modification (RM) systems are ubiquitous in bacteria, leading to the degradation of invading DNA which carry specific sequence motifs. Here, we proposed a method to detect targeted DNA based on the delivery of a plasmid library with random sequences (RandSeq). Library members containing a restriction site would be cleaved upon DNA transfer while plasmids without any motif would remain intact. Depleted library members are then identified by high-throughput sequencing of the random nucleotide region before and after DNA transfer. Next, computational methods are used to identify the common motif shared among the depleted members of the library. This method has been validated with *E. coli* strains with known restricted motifs and has revealed unknown specificities. Finally, this approach holds the potential to be extended to non-model bacteria by using a cell-free approach.