



**University of  
Zurich** <sup>UZH</sup>



**URPP**  
Evolution  
in Action

# **International Conference Evolution in Action**

**June 11-15, 2023  
Monte Verità, Switzerland**

## **Program & Abstracts**

## Sunday, June 11

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14:00 – 15:00      *Welcome Drink*

### **Session 1: Pathogen Evolution**

Chairs: Beat Keller, Verena Schünemann

15:00 – 15:40      **Francois Balloux**  
Host Adaptation in Zoonotic Pathogens

15:40 – 16:00      **Shraddha Karve**  
Carbapenem Resistance in the Priority Pathogens: A Study of Clinical Isolates

16:00 – 16:20      **Beat Keller**  
Rapid Adaptation of the Fungal Pathogen Wheat Powdery Mildew to Host and Non-Host Resistance

16:20 – 16:40      **Tom Loosli**  
Predicting Dolutegravir Resistance Levels in Southern Africa: The DTG MARISA Model

16:40 – 17:10      *Coffee Break*

17:10 – 17:50      **Eva Stukenbrock**  
Environmental and Ecological Interactions Drive Rapid Adaptive Evolution in Fungal Genomes

17:50 – 18:10      **Donikë Sejdiu**  
Herbarium DNA Traces *Phytophthora infestans* Strain that Caused Irish Potato Famine into 20th-Century Europe

18:10 – 18:30      **Arpan Kumar Basak**  
Horizontal Gene Transfer from Bacteria to Fungi in the Root Microbiome

19:00 – 20:30      *Dinner*

### **Keynote speech: Anthropological Genetics**

20:30 – 21:30      **Anne Stone**  
Ancient Mycobacteria, Human History and One Health

## Monday, June 12

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9:00 – 9:15            **Congressi Stefano Franscini and Monte Verità**  
*Welcome Address*

### **Session 2: Polyploidy and Epigenetic Variation**

Chairs: Ueli Grossniklaus, Kentaro Shimizu

9:20 – 10:00            **Jeffrey Chen**  
Epigenetic Variation and Evolution of Polyploid Plants and Crops

10:00 – 10:20            **Dusan Denic**  
Inheritance and Stability of DNA Methylation in *Arabidopsis thaliana*

10:20 – 10:40            **Kenji Yip Tong**  
Observing Evolution in Action Following Allopolyploidization

10:40 – 11:10            *Coffee Break*

11:10 – 11:50            **Yoav Soen**  
Adaptation by Natural Improvisation

11:50 – 12:10            **Peter Szoevenyi**  
The Masking Hypothesis in Complex Multicellular Organisms with Biphasic Life Cycles

12:10 – 12:30            **Manuel Poretti**  
Chromosome Rearrangements and Evolutionary Diversification of Duplicated Genomes

12:30 – 14:00            *Lunch*

14:00 – 15:00            *Free time for Discussions*

## Workshop: Machine Learning in Evolution

Chair: Simon Aeschbacher

### Part 1 – Machine Learning in Evolutionary Genetics: Concepts and Challenges

- 15:00 – 15:50      **Sara Mathieson**  
Introduction to Machine Learning and Convolutional Neural Networks
- Franz Baumdicker**  
Principles and Challenges of Simulation-based Machine Learning
- 15:50 – 16:30      *Coffee Break*

### Part 2 – Overcoming Simulation Misspecification: Solutions and Applications

- 16:30 – 17:20      **Franz Baumdicker**  
Stabilising Population Genetic Inference with Neural Networks  
through Self-adjusted Training
- Sara Mathieson**  
Using Generative Adversarial Networks to Infer Natural Selection
- 19:00 – 20:30      *Dinner*

### Poster flash talks

- 20:30 – 21:30      Epifanía Arango-Isaza      Keesha Martin Ming  
                         Subhankar Bera              Moeko Okada  
                         Jonas Bucher                Alex Plüss  
                         Harsh Chauhan              Felix Rico-Resendiz  
                         Thibaut Dugay              Michael Schneider  
                         Jana Flury                    Simon Snoeck  
                         Alon Israeli                  Ewa Szlachcic  
                         Jigisha                        Filip Turza  
                         Oliver Johanndrees        Lucas Waser  
                         Kevin Korfmann             Hannah Waterman  
                         Kimberly Louisor            Shunsuke Yoshioka

## Tuesday, June 13

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### Session 3: Domestication

Chairs: Simon Aeschbacher, Kentaro Shimizu

- 9:00 – 9:40            **Takao Komatsuda**  
Genetic Features in Plant Domestication
- 9:40 – 10:00        **Matthias Heuberger**  
Analysis of 487 Wheat Accessions Reveals a Diverse Introgression  
Landscape and Provides Evidence for Inter-Homoeologue  
Recombination in Bread Wheat
- 10:00 – 10:20       **Naoto-Benjamin Hamaya**  
Evolution of Male Gamete Number in Bread Wheat (*Triticum*  
*aestivum* L.)
- 10:20 – 10:40       **Ryo Ishikawa**  
Genetic Dissection of a Loss of Seed-Shattering Trait Uncovers  
Stepwise Routes in Rice Domestication
- 10:40 – 11:10       *Coffee Break*
- 11:10 – 11:50       **Esther van der Knaap**  
The Hidden Gems in Semi-Domesticated Germplasm: The Surprising  
Levels of Genetic Diversity in Fruit Quality Traits in Tomato
- 11:50 – 12:10       **Thomas Grubinger**  
Herbarium Specimens Shed Light on the Origins and Flavour of Early  
European Tomatoes
- 12:10 – 12:30       **Tom Winkler**  
The Ecological Significance and Genetic Basis of a Repeated Seed Color  
Change During Amaranth Domestication
- 12:30 – 14:00       *Lunch*
- 14:00 – 19:00       **Excursion**  
Brissago Islands with Botanical Garden
- 19:00 – 21:00       **Conference Dinner**  
*Ristorante Isole di Brissago Villa Emden*

## Wednesday, June 14

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### Session 4: Emerging Trends in Evolution

Chairs: Chiara Barbieri, Verena Schünemann

9:00 – 9:40	<b>Tal Dagan</b> Multilevel Drift and Selection in the Evolution of Prokaryotic Plasmids
9:40 – 10:00	<b>Abigail Colby</b> Endocranial Volume is Heritable, but is not Related to Fitness, in a Free-Ranging Primate
10:00 – 10:20	<b>Zoe Bernasconi</b> Experimental Evolution through Mutagenesis: An Efficient Method to Unravel the Molecular Basis of Pathogen's Virulence
10:20 – 10:40	<b>Hiromi Matsumae</b> Genomics and Quantifying Various Traits for Understanding Evolution
10:40 – 11:10	<i>Coffee Break</i>
11:10 – 11:50	<b>Monika Moir</b> Genomic Surveillance Agility: From Tracking SARS-CoV-2 Variants in South Africa to the Climate Amplified Diseases and Epidemics (CLIMADE) Global Consortium
11:50 – 12:10	<b>Shevan Wilkin</b> Proteomics of Ancient Immunology: Refined Methods to Detect Active Leprosy in Medieval Individuals
12:10 – 12:30	<b>Weihong Qi</b> Haplotype-Aware Pan-Genome Graph Based Genome Inference for Detection of Transmission Ratio Distortion Signals in Plants
12:30 – 14:00	<i>Lunch</i>
14:00 – 15:00	<i>Free time for Discussions</i>
15:00 – 18:30	<b>Poster Session incl. Coffee</b>
19:00 – 20:30	<i>Dinner</i>

### Keynote speech: Evolution of Plant Reproductive Traits

20:30 – 21:30	<b>George Coupland</b> Variation in Flowering Behaviour of Annual and Perennial Plants
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## Posters

### Arango-Isaza, Epifanía

The Genetic History of the Southern Andes from Present-day Mapuche Ancestry

### Bera, Subhankar

Brassicaceae Unique Chemical Defense System is Involved in the Adaptation to Mutualistic Endophyte, *Phomopsis columnaris*

### Bucher, Jonas

Development of a Reproducible Pipeline for DNA Methylation Data Analysis using SUSHI and Shiny: Addressing the Genomics Reproducibility Crisis

### Chauhan, Harsh

Identification and Isolation of Wheat Powdery Mildew Resistance Genes from *Triticale*

### Dugay, Thibaut

Evolution of the Atlantic Salmon Population in the Burrishoole River System since the 1970s

### Flury, Jana

Self or not to self: Evolution at the S-locus in *Arabidopsis lyrata* at Distribution Edges

### Israeli, Alon

Origin and Evolution of Land Plant Reproductive System

### Jigisha

Population Genetics and Molecular Epidemiology of Wheat Powdery Mildew in Europe

### Johanndrees, Oliver

Investigating the Functional Diversification of a Conserved Plant Peptide-receptor Kinase Signaling Module

### Korfmann, Kevin

Simultaneous Inference of Past Demography and Selection from the Ancestral Recombination Graph under the Beta Coalescent

### Louisor, Kimberly

Complete Bacterial Genome Extracted from a Eukaryote Genome Assembly

**Ming, Keesha Martin**

Combined Effect of Insular Dwarfism and Domestication in Horses of Greece and Japan

**Okada, Moeko**

Co-evolution of Cultivation Syndrome and the Human Agricultural Systems in Bread Wheat

**Plüss, Alex**

Investigating the Distribution and Stability of Epigenetic Variation in Natural Populations of *Arabidopsis thaliana*

**Rico-Resendiz, Felix**

The Kelch-phosphatase BSLM Coordinates Cell Proliferation and Differentiation in *Marchantia polymorpha*

**Schneider, Michael**

Experimental Evolution in Crop Wild Relatives Introgressed Barley, Wheat and Rapeseed Populations - 20 Generations of Natural Adaptation to Organic and Conventional Agro-Ecosystems Assessed by Pool Genotyping

**Snoeck, Simon**

Investigating the Co-evolutionary History of the Family-specific Plant Receptor Kinase MIK2 and its SCOOP Ligands

**Szlachcic, Ewa**

Systemic Orchestration of Cell Size throughout the Body: Evolutionary and Developmental Responses of *Drosophila melanogaster* to Environments

**Turza, Filip**

Can Life Expectancy Determine Altruistic Behaviour in Ants?

**Waser, Lucas**

The Relative Contribution of Genetic and Epigenetic Variability to Adaptation

**Waterman, Hannah**

The Role of DNA Methylation in Shaping Genome Evolution Following Whole Genome Duplication: Insight from Catostomidae Fishes

**Yoshioka, Shunsuke**

A High-throughput Phenotyping of Early Growth Patterns of a Hexaploid Wheat NAM Population by a UAV



## Thursday, June 15

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### Session 5: Adaptation to Changing Environments

Chairs: Chiara Barbieri, Beat Keller

- 9:00 – 9:40            **Hernán Burbano**  
Plant Biotic Interactions Combining Present-day and Historical Samples
- 9:40 – 10:00        **Léa Frachon**  
Genomic Adaptation of a Generalist Plant Species to Local Pollinator Communities and Abiotic Factors
- 10:00 – 10:20       **Abhishek Meena**  
Can Frequent Heatwaves at Different Life Stages Affect the Reproductive Abilities and Recovery of Male and Female *Drosophila melanogaster*?
- 10:20 – 10:40       **Anne Roulin**  
Investigating the Polygenic Architecture of Flowering Time and its Relationship with Local Environments in the Grass *Brachypodium distachyon*
- 10:40 – 11:10       *Coffee Break*
- 11:10 – 11:50       **Tábita Hünemeier**  
Human Adaptation to Amazon Rainforest
- 11:50 – 12:10       **Ramona-Elena Irimia**  
Invasive Species in Changing Environments - Uncovering Spatiotemporal Dynamics of a Global Plant Invasion through Herbariomics
- 12:10 – 12:30       **Elisabeth Authier**  
Phenotypic Response of Flowering Plants to Limited Access to Natural Pollinator Community



# **Abstracts**

## Host Adaptation in Zoonotic Pathogens

Francois Balloux

Genetics Institute, University College London, UK

Most emerging and re-emerging infectious disease are the result of spillover events from wild or domestic animals. There are hundreds of zoonotic pathogens described in the literature that can infect both humans and other animals, yet there are likely many more pathogens with zoonotic potential left to describe. We recently did a screen of RNA viruses in UK bats, and identified four coronaviruses including a sarbecovirus (same genus as SARS-CoV-2) that is able to infect human cells, albeit not very effectively, as well as two previously undescribed species. The diversity of pathogens in circulation in animal populations raises the question whether we can predict which pathogens may jump into humans in the future. One common trend in zoonotic pathogens is a broad host range. SARS-CoV-2 is a good example in this respect, as it has been shown to be able to infect at least one species from all mammalian orders. Despite being a generalist pathogen, SARS-CoV-2 further adapted to its new hosts after jumping into humans, and later on in mink and deer. I will briefly overview possible surveillance approaches including computational and lab-based tools that may allow classifying pathogens as of low- or high concern in terms of the risk of future epidemics in humans. Finally, I will introduce the concept of 'immunological niche', the idea that a pathogen well pre-adapted to circulation in humans may be of lower concern if the host already benefits from cross-immunisation through exposure to a related pathogen.

Sunday, June 11

Session 1: Pathogen Evolution

[↑ back to program](#)

## **Carbapenem Resistance in the Priority Pathogens: A Study of Clinical Isolates**

Shraddha Karve

Ashoka University, India

Rising antimicrobial resistance is currently posing a threat to healthcare systems worldwide. The problem is severe in the tertiary healthcare environments where the pathogens get constant exposure of antibiotics. At the heart of this problem however is a natural process of bacteria adapting to their environments that are teeming with antibiotics. What molecular mechanisms are leading to resistance remains poorly studied in many parts of the world. Most studies mark the presence/absence of known antibiotic resistance genes (ARGs) rather than studying the whole genome.

Using 5-year retrospective data of ~50000 clinical isolates from India, we studied the resistance in the priority pathogens. We identified prevalent resistance profiles and subjected the representative isolates with these resistance profiles to the whole genome sequencing. Our results demonstrate that studying ARGs in isolation is inadequate. Whole genome sequencing can identify the molecular mechanisms of resistance accurately, uncover the intrinsic or extrinsic nature of resistance and allows the study of other ancillary genetic changes that help resistance evolution.

## Rapid Adaptation of the Fungal Pathogen Wheat Powdery Mildew to Host and Non-Host Resistance

Marion C. Müller<sup>1,2</sup>, Javier Sanchez-Martin<sup>1</sup>, Lukas Kunz<sup>1</sup>, Harsh Chauhan<sup>1</sup>, Fabrizio Menardo<sup>1</sup>, Beat Keller<sup>1</sup>

<sup>1</sup>Department of Plant and Microbial Biology, University of Zurich, Switzerland

<sup>2</sup>Chair of Phytopathology, TUM School of Life Sciences, Technical University of Munich, Freising, Germany

The adaptation of pathogens to new hosts is observed rather frequently and is based on evolutionary events occurring in a short time. Similarly, fungal plant pathogens can adapt to the presence of resistance genes in their host species, sometimes within a few years. We are studying at the molecular level the events that occur in this rapid adaptation. We are studying such processes in the fungal pathogen wheat powdery mildew. It has recently expanded its host range to the novel crop Triticale (an amphiploid of wheat and rye), which in its first years of cultivation had been resistant to powdery mildew. We found that the new pathogen with host range expansion from wheat to Triticale is the result of a hybridization of wheat powdery mildew and rye powdery mildew, followed by backcrossing. The new mildew form has an expanded host range, which is dependent on the specific Triticale host genotype. Furthermore, we have studied the adaptation of wheat mildew to resistance genes introgressed from rye into wheat. We found that standing genetic variation present in wheat mildew explains to a large degree the rapid breakdown of resistance conferred by the rye resistance genes.

## Predicting Dolutegravir Resistance Levels in Southern Africa: The DTG MARISA Model

Tom Loosli<sup>1,2</sup>, Anthony Hauser<sup>1,2</sup>, Johannes Josi<sup>1,2</sup>, Nuri Han<sup>1,2</sup>, Richard Lessells<sup>3, 4</sup>, Huldrych Günthard<sup>1,2</sup>, Matthias Egger<sup>5,6,7</sup>, Roger Kouyos<sup>1,2</sup>

<sup>1</sup> Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Switzerland; <sup>2</sup> Institute of Medical Virology, University of Zurich, Switzerland; <sup>3</sup> KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), University of KwaZulu-Natal, South Africa; <sup>4</sup> Centre for the AIDS Programme of Research in South Africa (CAPRISA), South Africa; <sup>5</sup> Institute of Social and Preventive Medicine (ISPM), University of Bern, Switzerland; <sup>6</sup> Centre for Infectious Disease Epidemiology and Research, University of Cape Town, South Africa; <sup>7</sup> Population Health Sciences, Bristol Medical School, University of Bristol, UK

**Background:** HIV drug resistance is a significant challenge for sustainable antiretroviral therapy. In response to the exponentially rising levels of NNRTI resistance, millions of people have recently been switched to Dolutegravir (DTG)-based ART. Data on DTG resistance are limited, and DTG-resistant HIV has rarely been observed. However, given the frequency of use and the recency of the DTG rollout, rates of DTG resistance may increase. Understanding the dynamics of future DTG resistance for medium- and long-term health policy planning is essential. Here, we develop DTG-MARISA, a compartmental model that considers the cascade of care, gender, disease progression and resistance genotype to predict DTG resistance in South Africa.

**Methods:** MARISA (Modelling Antiretroviral drug Resistance In South Africa) is a deterministic compartmental model consisting of the four layers cascade of care, disease progression, sex, and drug resistance. It has been shown to reproduce the HIV epidemic in South Africa, as well as NNRTI acquired drug resistance (ADR) and transmitted drug resistance (TDR). MARISA only distinguished between susceptible and resistant HIV strains, disregarding the complex genetics of HIV resistance that are crucial for high genetic barrier drugs such as DTG. We expanded the resistance genotype as follows: NRTI and NNRTI resistance are classified as either susceptible or resistant. For DTG resistance, we include the key mutations (E138K, G140SR, Q148HR, N155H, R263K) observed in people experiencing failure on DTG-based ART.

**Results:** Based on DTG-MARISA, we model acquired and transmitted drug resistance among people living with HIV in South Africa up to 2040. We explore different scenarios regarding mutation acquisition rates, the impact of resistance on treatment, and NRTI backbone drug resistance on DTG resistance. We show how these factors will affect the emergence of DTG resistance by 2040, compared to a counterfactual scenario. The model predicts that with the introduction of DTG-based ART, the exponential increase in NNRTI TDR will be halted. However, DTG TDR will increase over time and could reach current levels of NNRTI TDR by 2040.

**Conclusion:** Despite high efficacy of DTG at the individual level, a DTG resistance epidemic is likely in the coming years as more and more people are treated with DTG-based therapies. The risk is particularly high in low- and middle-income countries, where pre-existing resistance is more common, and resources for HIV-1 RNA monitoring, and resistance testing or switching to alternative ART regimens are limited.

Sunday, June 11

Session 1: Pathogen Evolution

[↑ back to program](#)

## Environmental and Ecological Interactions Drive Rapid Adaptive Evolution in Fungal Genomes

Pereira DAS<sup>1</sup>, Dutheil JY<sup>2</sup>, Stephan W<sup>3</sup>, [Stukenbrock EH](#)<sup>1</sup>

<sup>1</sup> Environmental Genomics, Christian-Albrechts University of Kiel and the Max Planck Institute for Evolutionary Biology, Plön, Germany

<sup>2</sup> Molecular Systems Evolution, Max Planck Institute for Evolutionary Biology, Plön, Germany

<sup>3</sup> Museum für Naturkunde, Berlin, Germany

Fungal plant pathogens secrete a diversity of effector proteins during host colonization. These effectors have so far been considered as virulence factors, which are produced to overcome host immune responses. Sequence based studies have demonstrated that genes encoding effector proteins often locate in gene sparse regions of the genome, exhibit exceptional nucleotide variation and in some cases show presence-absence variation. Recent studies demonstrate that some effector proteins have antimicrobial properties and are produced, not to interact with plant targets, but to interfere with the plant-associated microbiota. This observation suggests that environmental and ecological interactions more generally drive rapid evolution of genes encoding secreted proteins in fungi. We use the fungal plant pathogen *Zymoseptoria tritici* as a model to study drivers and mechanisms of adaptive evolution in fungi. To this end, we have quantified and compared the proportion of adaptive substitutions and rates of adaptation in sets of effector and non-effector genes by contrasting patterns of polymorphism and divergence. In *Z. tritici* rates of adaptive evolution are indeed significantly higher in genes encoding secreted proteins, including genes with no effect on virulence, but with antimicrobial properties. We extended our analyses to include additionally twenty-four fungal species comprising other plant pathogen species, as well human pathogens and non-pathogen species. Our first results based on 1795 genomes and 62898 gene alignments suggest that genes encoding secreted proteins in general comprise higher proportions of adaptive amino acid substitutions ( $\alpha$ ) and higher rates of adaptation ( $\omega_A$ ) compared to genes encoding non-secreted proteins in pathogenic as well as non-pathogenic species. Overall  $\omega_A$  and  $\alpha$  correlate positively with effective population sizes, supporting the idea that adaptation is more efficient in large microbial populations, in contrast to what was observed, for instance, in animals. These observations suggest that environmental and ecological interactions, including but not exclusively antagonistic host-pathogen interaction, drive the rapid evolution of genes encoding secreted proteins.

## Herbarium DNA Traces *Phytophthora infestans* Strain that Caused Irish Potato Famine into 20th-Century Europe

Donikë Sejdiu<sup>1,2</sup>, Jurriaan M de Vos<sup>3</sup>, Reinhard Berndt<sup>4</sup>, Simon Aeschbacher<sup>2</sup>, Verena J Schünemann<sup>1</sup>

<sup>1</sup> Institute of Evolutionary Medicine, University of Zurich, Switzerland

<sup>2</sup> Department of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland

<sup>3</sup> Department of Environmental Sciences, University of Basel, Switzerland

<sup>4</sup> Department of Environmental Systems Science, ETH Zurich, Switzerland

Plant pathogens have influenced human societies since the cultivation of crops. A notable example is *Phytophthora infestans*, an oomycete that induces late blight in potato and tomato. Introduced to Europe in the 1840s, *P. infestans* led to severe epidemic outbreaks throughout Europe, including the Irish Potato Famine. Since preventing future *P. infestans* outbreaks requires a better understanding of this fast evolving pathogen, we have extracted DNA from 26 infected European potato leaves dating between 1850 and 1961. We sequenced 19 mitochondrial genomes at 6- to 34-fold mean coverage and combined them with 33 published historical and modern sequences for phylogenetic analyses. We found that HERB-1, the strain previously identified as being responsible for the Irish Potato Famine, still existed in 17 European potato specimens collected during the 19th and 20th century. Our study suggests the persistence of HERB-1 into late 20th century Europe and demonstrates the importance of herbarium genomics in biogeographic analyses of plant-pathosystems.



## Horizontal Gene Transfer from Bacteria to Fungi in the Root Microbiome

Arpan Kumar Basak<sup>1</sup>, Thorsten Thiergart<sup>1</sup>, Fantin Mesny<sup>1,2</sup>, Stephane Hacquard<sup>1,3</sup>

<sup>1</sup> Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany

<sup>2</sup> Institute for Plant Sciences, University of Cologne, Germany

<sup>3</sup> Cluster of Excellence on Plant Sciences (CEPLAS), Max Planck Institute for Plant Breeding Research, Cologne, Germany

Horizontal gene transfer (HGT) is considered to be a major force shaping prokaryotic genome evolution. However, the extent to which trans-kingdom DNA transfer from bacteria to eukaryotes has promoted functional diversification and adaptation to environment in eukaryotic genomes remains elusive.

Given that bacteria and fungi co-occur with their plant hosts since 450 MyA, we hypothesised that the root microbiome represents a hotspot for HGT between bacteria and fungi. We investigated the genomes of 120 fungal isolates representative of the root microbiome and identified >700 orthogroups (OG) that were potentially acquired via trans-kingdom HGT. We found that HGT-derived OGs are highly distributed in Ascomycetes isolates including *Plectosphaerella cucumerina* a robust colonizer of *Arabidopsis* roots in nature.

Here, we hypothesised that *P. cucumerina* genes acquired from bacteria via HGT might act as root colonization determinants. By combining transcriptomics and phylogenomic approach, we identified candidate genes (~5) that are induced in *P. cucumerina* upon root colonisation, and share bacterial ancestral origin. These genes are reproducibly detected in the genomes of other *P. cucumerina* isolates that were isolated from diverse plant host across continents. Functional characterization of these genes is ongoing.

Overall, we speculate that during the course of evolution, genes responsible for fungal root colonisation have evolved from root-associated bacteria by HGT.

## **Ancient Mycobacteria, Human History and One Health**

Anne Stone

School of Human Evolution and Social Change, Arizona State University, USA

Tuberculosis (TB) and Hansen's disease (or leprosy) have affected humans, as well as other animals, for millennia. Ancient DNA analyses allow us to examine the evolutionary history of the *Mycobacteria* causing these diseases as well as understand how human migrations have affected their distributions. Our genomic analyses show that *Mycobacterium leprae* from animals reflect exchanges with nearby humans, while Polynesian strains belong to the deepest lineages likely brought with first human migrations from SE Asia. Our analyses of ancient American TB cases from South America and Mexico indicate that these were caused by *M. tuberculosis* complex strains closely related to those found in Southern Hemisphere pinnipeds. We also find evidence for multiple jumps to humans as well as human-to-human transmission to inland parts of South America and to North America. After contact, colonists introduced European TB strains, and our data show that these reflect the source area of colonists.

Sunday, June 11

Keynote speech: Anthropological Genetics

[↑ back to program](#)

## Epigenetic Variation and Evolution of Polyploid Plants and Crops

Jeffrey Chen

Department of Molecular Biosciences, The University of Texas at Austin, USA

Polyploidy or whole genome duplication (WGD) is a major source for evolutionary innovation and species diversification among animals and plants. Most crop plants including wheat, cotton, and oilseed rape are allopolyploids, and many other crops such as potato and alfalfa are autopolyploids. Interspecific hybridization induces genetic and epigenetic changes and heterosis, which are permanently fixed in allopolyploids. Since 1970, research on polyploid genomes has begun a renaissance after publication of “Evolution by Gene Duplication” by Susumu Ohno, who explained how duplication creates redundancy in vertebrates relative to invertebrates, and beneficial mutations may accumulate to provide evolutionary innovation. However, the role of epigenetic variation (without changes in DNA sequence) in genome duplication and evolution remains elusive. We have employed integrated genomic and epigenomic approaches to investigate gene expression changes that have shaped morphological diversity in plant polyploids. In *Arabidopsis* polyploids, we provided molecular evidence for transcriptional and epigenetic control of genomewide nonadditive gene expression (unequal to the sum of two parental values). This finding offers an alternative explanation for “genome shock” in interspecific hybrids, a term coined by B. McClintock (1984). We found that epigenomic modifications mediate genome stability during polyploid formation and evolution. These findings can help explain genomic and epigenomic bases of the Darwin’s “abominable mystery”- sudden appearance and rapid diversification of flowering plants in the Cretaceous. In polyploid cotton, we decoded the genomes of all five tetraploid species including economically important Upland and Pima cotton, the largest renewable source of textile fiber. We discovered hundreds of epialleles that were induced in the interspecific hybrids approximately one million years ago and were heritable among allotetraploid species during evolution and domestication. These findings and resources provide new breeding and genome-editing tools that can explore genetic and epigenetic variation to improve crop yield, nutritional values, and growth resilience under extreme climate.

Monday, June 12

Session 2: Polyploidy and Epigenetic Variation

[↑ back to program](#)

## **Inheritance and Stability of DNA Methylation in *Arabidopsis thaliana***

Dusan Denic<sup>1</sup>, Marc W. Schmid<sup>2</sup>, Ueli Grossniklaus<sup>1</sup>

<sup>1</sup>Department of Plant and Microbial Biology and Zurich-Basel Plant Science Center, University of Zurich, Switzerland

<sup>2</sup>MWSchmid GmbH, Glarus, Switzerland

The long-term goal of this project is crop improvement through unlocking the potential of epigenetic variation. Unfortunately, very little is known about the stability of epigenetic variation. For instance, to which extent are so-called epialleles heritable and how are they influenced by genetic background? We are addressing these questions in the model plant *Arabidopsis thaliana* with a focus on DNA-methylation, a prominent epigenetic mark that can easily be assessed. For this purpose, we analysed publicly available data on DNA methylation from 87 accessions of *A. thaliana*. Approximately 100 loci with a high anticorrelation of gene expression and DNA-methylation were identified. The accessions clearly fell into two groups, i.e., those with either strongly methylated or unmethylated regions (referred to as bistably methylated loci). Some of these also show a correlation with an associated phenotype. The biggest differences were identified in CG and CHG context (H = A, C, or T). To follow the DNA methylation status of selected loci we use Chop-PCR. We monitored the DNA methylation status of 10-15 loci in a set of reciprocal F1 hybrids, and plan to monitor it over several generations during recombinant inbred line generation and in recurrent backcrosses. Our initial results suggest that DNA methylation is stably inherited in additive manner in F1 hybrids, and we have not identified any *trans*-chromosomal methylation or *trans*-chromosomal demethylation events that were previously described in other publications. We are currently investigating whether this is due to the particular genetic backgrounds used in previous studies or is a characteristic specific to bistably methylated loci.

## Observing Evolution in Action Following Allopolyploidization

Kenji Yip Tong

Department of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland

Polyploids have played a significant role in life's evolutionary history. However, the mechanism surrounding their evolution remains contentious. Arguments on the topic often remain purely speculative and evidence is mostly circumstantial. A reason for this is the difficulty of working with polyploid genomes due to their complexity. There are also challenges associated with observing early polyploid evolution. An innovative experiment devised by Dr. Rie Shimizu-Inatsugi is currently emulating early allopolyploid evolution with respect to harsh environmental conditions. Stressful conditions are thought to play a major role in polyploid establishment. However, its effect on early polyploids has rarely been observed directly. Interesting results were already produced thanks to the experiment and to a novel bioinformatic tool tailor made for polyploid research, the ARPEGGIO program. The talk will introduce the experiment, the tool, some results, and some future directions.

Monday, June 12

Session 2: Polyploidy and Epigenetic Variation

[↑ back to program](#)

## Adaptation by Natural Improvisation

Yoav Soen

Department of Molecular Biosciences, Weizmann Institute of Science, Israel

Traditional view of adaptation focuses on natural selection of adaptive variations without regard to how these variations come about in the first place (no consideration of emergence). And yet, every single animal is constantly undergoing newly-forming variations in its epigenome, microbiome and even in its somatic genome. **Many** of these variations appear in **novel combinations** that are **unique to the individual**. Since every new variation might be harmful, it is not clear how every individual can tolerate a large number of novel variations that are forming during its lifetime. To address this conceptual problem, we introduced a principle of dynamic adaptation within a lifetime. It explains how every individual can acquire new adaptations by undergoing stochastic variations under (existing) constraints that suppress the likelihood of reaching non-viable states.

Experimental work-in-progress in dedicated multicellular and unicellular models provide substantial evidence in support of this emergent adaptation. Our multicellular model is based on rearing fly larvae (*D. melanogaster*) on diets in which the primary carbon source cannot be effectively metabolized by the fly's enzymes nor by its initial gut microbiota. This creates a severe nutritional challenge that must be addressed within a few days of larval development. We show that some of the larvae overcome this challenge by taking advantage of stochastic microbiome variations that are formed de novo within a host generation (and subsequently transmitted to the offspring). These variations are also accompanied by non-genetic changes in the host itself, including variations that are individual-specific. Sufficient validation of this concept will close the unrealized gap in the traditional view of evolution by accounting for emergent adaptation on all time scales and levels of organization.

Monday, June 12

Session 2: Polyploidy and Epigenetic Variation

[↑ back to program](#)

## The Masking Hypothesis in Complex Multicellular Organisms with Biphasic Life Cycles

Peter Szoevenyi

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Ploidal divergence of the diploid and haploid phases will exert a profound effect on the evolutionary trajectories of genes showing shared or phase-specific expression. The masking hypothesis suggests that the number of chromosomal copies present in a cell affects the efficacy of selection. The diploid phase is expected to accumulate a greater number of recessive deleterious mutations than the haploid phase, because such mutations can be masked by dominant alleles in a heterozygous state (i.e, masking hypothesis). Consequently, evolutionary rates in haploids and diploids should differ, provided that the majority of mutations are recessive or partially recessive. Therefore, the presence and relative life span of the diploid phase can drive the accumulation of deleterious mutations that in turn is assumed to govern the evolution of basic life cycles. In general, mutational robustness of the diploid phase is assumed to be particularly important for the prevalence of diploid-dominant life cycles in nature. Yet, whether predictions of the masking hypothesis hold for complex multicellular organisms with biphasic life cycles is not clear.

We investigated this fundamental assumption of life cycle evolution by assessing the evolutionary rate and purging of strongly and slightly deleterious mutations in organisms with biphasic life cycles with various mating systems across a broad range of plant species. We found that the evolutionary rates of haploid- and diploid-specific genes contradict the masking hypothesis. Haploid-specific genes do not evolve more slowly than diploid-specific genes in either organism. Our data suggest that gene expression breadth influences the evolutionary rate of phase-specific genes more strongly than masking. Our observations have implications for the role of haploid life stages in the purging of deleterious mutations, as well as for the evolution of ploidy.

Monday, June 12

Session 2: Polyploidy and Epigenetic Variation

[↑ back to program](#)

## Chromosome Rearrangements and Evolutionary Diversification of Duplicated Genomes

Manuel Poretti<sup>1</sup>, Rimjhim Choudhury<sup>1</sup>, Yile Huang<sup>2</sup>, Hussein Anani<sup>2</sup>, Terezie Mandakova<sup>2</sup>, Martin Lysák<sup>2</sup>, Christian Parisod<sup>1</sup>

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Chromosome rearrangements (CRs) are known to promote the reduction of genome size and chromosome numbers (i.e. diploidization) in plants undergoing recurrent whole-genome duplications. Such phenomenon appears decisive for the evolutionary diversification of plants, however the genomic basis of chromosomal restructuring remains underexplored.

In our study, we combine cutting-edge sequencing technologies (Oxford Nanopore, PacBio Iso-Seq and Hi-C) to build, annotate, and compare chromosome-scale genome assemblies of closely related *Biscutella* species. These plant species diversified independently following a whole genome duplication event and colonized different environmental niches, making the *Biscutella* genus a unique system for understanding the impact of diploidization on plant adaptation in natural populations.

The availability of chromosome-scale genome assemblies is crucial for investigating structural variation. Although the complexity of large and repetitive plant genomes often hinders such analyses, we provide guidelines for the assembly and annotation of high-quality genomes and shed light on the molecular mechanisms underlying CRs (with a special focus on transposable elements). Through comparative genomics, we quantify the impact of different restructuring events on genome divergence and assess molecular processes that alter genome size and content, determining to what extent different types of restructuring events affect the gene space vs the repetitive fraction during diploidization and species diversification.



## **Workshop: Machine Learning in Evolution**

Sara Mathieson<sup>1</sup> and Franz Baumdicker<sup>2</sup>

<sup>1</sup>Department of Computer Science, Haverford College, USA

<sup>2</sup>Mathematical and Computational Population Genetics, Cluster of Excellence CMFI, University of Tübingen, Germany

Machine learning is transforming the way evolutionary biologists extract information from data and make inference. In population genetics, the first machine learning approaches began to emerge in the early 2010s. More recently, deep learning techniques including Convolutional Neural Networks (CNNs) have been used to infer recombination rates, natural selection, and effective population sizes. While machine learning offers promising opportunities, challenges arise when simulations are used to generate training data. Misspecification of such simulations can severely limit the performance of deep learning. After a basic introduction to deep learning, this workshop emphasises key considerations needed to address simulation misspecification, and then features two examples of how the simulation of training data can be optimised in a population genetic context. Franz Baumdicker (University of Tübingen) will present a self-adjusting training approach that adaptively reweights the training data by comparing the performance of a neural network estimators with established estimators from theoretical population genetics. Using this reweighting as a guide for the training procedure, overall performance can be stabilised and improved. Sara Mathieson (Haverford College) will focus on a new generative adversarial network (GAN) method that automatically creates simulated data. This method, called pg-gan, works by training a parametric generator and CNN discriminator in concert, until there is a close match between real and simulated data. Overall pg-gan has proven useful in evaluating and strengthening simulated data, especially for understudied human populations that deviate from broad geographic groups. These two applied studies provide avenues for deep-learning solutions to complex inference problems and a better understanding of the evolutionary past of populations.

### **Part 1 – Machine Learning in Evolutionary Genetics: Concepts and Challenges**

Sara Mathieson: Introduction to Machine Learning and Convolutional Neural Networks

Franz Baumdicker: Principles and Challenges of Simulation-based Machine Learning

### **Part 2 – Overcoming Simulation Misspecification: Solutions and Applications**

Franz Baumdicker: Stabilising Population Genetic Inference with Neural Networks through Self-adjusted Training

Sara Mathieson: Using Generative Adversarial Networks to Infer Natural Selection

Monday, June 12

Workshop: Machine Learning in Evolution

[↑ back to program](#)

## Genetic Features in Plant Domestication

Takao Komatsuda

Shandong Academy of Agricultural Sciences, Crop Research Institute, Jinan, Shandong, China

Plant domestication is the process of accumulation of genetic modifications associated with morphological and physiological changes towards human merit. Barley (*Hordeum vulgare* L.) is one of the earliest domesticated crops in the Fertile Crescent as shown by archaeological studies. Six-rowed spikes and non-brittle rachis are diagnostic morphological features of domesticated barley observed in archaeological sites, whereas reduced seed dormancy is a physiological one absent from sites. The genes responsible for these domestication traits are encoded in several parts of the plant's enormous genome, highlighting a rather prominent event in the domestication and development of this plant as a staple food crop in the early civilization.

## **Analysis of 487 Wheat Accessions Reveals a Diverse Introgression Landscape and Provides Evidence for Inter-Homoeologue Recombination in Bread Wheat**

Matthias Heuberger

Department of Plant and Microbial Biology, University of Zurich, Switzerland

Genetically highly diverse genetic material of wild wheat relatives can enter the gene pool of cultivated hexaploid wheat through chromosomal introgressions, both naturally and by breeding efforts, thereby introducing favorable traits such as pathogen resistance and drought. We analyzed sequence coverage from exome-capture data from 487 wheat accessions to identify introgressions from diverse genetic backgrounds. This resulted in a catalog of over 5,000 introgressions, many of them rare and not described as of yet.

Most interestingly, our analysis also reveals frequent recombination events between the homoeologs of the diploid ancestor chromosomes, which goes against the common notion of bread wheat being a functional diploid, with suppressed recombination between the homoeologous chromosomes. Several such events led to duplications of one homoeolog segment and loss of the other, presumably through unbalanced segregation or lineage sorting.

## Evolution of Male Gamete Number in Bread Wheat (*Triticum aestivum* L.)

Naoto-Benjamin Hamaya<sup>1</sup>, Hiroyuki Kakui<sup>1,2,3</sup>, Jilu Nie<sup>2</sup>, Moeko Okada<sup>1,4</sup>, Thomas Wicker<sup>5</sup>, Beat Keller<sup>5</sup>, Shuhei Nasuda<sup>2</sup>, Kentaro K. Shimizu<sup>1,4</sup>

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Male gamete number has been extensively studied in an evolutionary context in plants and animals. Recently, an adaptive reduction of pollen grains was found in the model plant species *Arabidopsis thaliana*. Our study investigates if a decline in the number of pollen grains is occurring in bread wheat related to breeding and to detect loci regulating pollen number.

We analysed pollen numbers for 25 founder lines of a nested association mapping (NAM) population of Asian bread wheat and found a significant reduction in cultivars (originating from modern plant breeding) compared to landraces (old, local wheat originating from traditional selective breeding). This pattern was also found in 32 lines from other geographical origins. Also, pollen number declined over time based on available year data for 39 of the total 57 lines. These declines might indicate a selection for lower pollen numbers during breeding history. Furthermore, QTL analyses found loci significantly associated with the number of pollen grains in the NAM population.

These loci will be further analysed to identify candidate genes for the regulation of pollen number and tested for selection.

## Genetic Dissection of a Loss of Seed-Shattering Trait Uncovers Stepwise Routes in Rice Domestication

Ryo Ishikawa

Laboratory of Plant Breeding, Kobe University, Japan

Asian rice (*Oryza sativa* L.) was domesticated from wild rice (*O. rufipogon* Griff.) by acquiring non-seed-shattering behaviour, allowing humans to increase grain yield. Previous studies argued that a reduction in seed shattering triggered by the *sh4* mutation led to increased yield; but experiments using wild introgression lines showed that the domesticated *sh4* allele alone was insufficient for shattering loss in *O. rufipogon*. By classical genetic approach, the mutation at *qSH3* was found to be responsible for an initial loss of seed shattering together with *sh4*. However, their combination was not sufficient to fully trigger non-shattering phenotype. Through harvest experiments, seed shattering alone did not significantly impact yield; rather yield increases were observed with closed panicle formation controlled by *SPR3*. Complementary manipulation of panicle shape and seed shattering results in a mechanically stable panicle structure, contributing to rice domestication. Further genetic analyses identifying other loci that contributed to reduced seed-shattering behaviour of current rice cultivars will be presented.

## The Hidden Gems in Semi-Domesticated Germplasm: The Surprising Levels of Genetic Diversity in Fruit Quality Traits in Tomato

Esther van der Knaap<sup>1</sup>, Manoj Sapkota<sup>1</sup>, Lara Pereira<sup>1</sup>, Elizabeth Frick<sup>2</sup>, Denise Tieman<sup>2</sup>

<sup>1</sup> University of Georgia, Athens, USA

<sup>2</sup> University of Florida, Gainesville, USA

Wild relatives and semi-domesticated germplasm of cultivated plants provide a significant reservoir of genetic diversity for key regulators of quality traits. Future crop improvement relies on harnessing this diversity. However, mining semi-domesticated and wild germplasm for beneficial alleles is not straightforward because fruit quality is typically a quantitatively inherited trait. One critical quality trait is the flavor of produce such as tomato is a combination of taste and smell. Taste is determined by the levels and balance of sugars and acids in the fruit. The smell is thought to be contributed by up to ~ 400 different volatiles that are emitted by the fruit, especially when consuming the produce. We have analyzed the genome sequence and the volatile aroma of a diverse tomato population ranging from fully wild *S. pimpinellifolium* to fully cultivated *S. lycopersicum lycopersicum* accessions collectively called the Varitome Collection. GWAS and mapping for one such volatile, methyl salicylate, resulted in the discovery of a new gene that encodes a methyl esterase, responsible for the conversion of methyl salicylate to salicylic acid. The research also showed that the locus encodes up to four methyl esterases and that the genetic variation at the locus is huge, especially in the wild parents *S. pimpinellifolium*. Mainly driven by genome structural variants, the function of the different methyl esterase haplotypes and interaction with other loci in tomato will be discussed.

Tuesday, June 13

Session 3: Domestication

[↑ back to program](#)

## Herbarium Specimens Shed Light on the Origins and Flavour of Early European Tomatoes

Thomas Grubinger<sup>1</sup>, Gülfirde Akgül<sup>2</sup>, Alessia Guggisberg<sup>3</sup>, Reto Nyffeler<sup>4</sup>, Jurriaan M. de Vos<sup>5</sup>, Verena J. Schünemann<sup>2,6</sup>, Simon Aeschbacher<sup>1</sup>

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<sup>6</sup> Department of Evolutionary Anthropology, University of Vienna, Austria

The cultivated tomato (*Solanum lycopersicum* L.) showed large phenotypic variation when brought to Europe in the 16<sup>th</sup> century, but its origins and the flavour of its fruits remain unknown. To fill this gap, we analysed 181 modern and historical tomato genomes, some more than 400 years old. Our phylogenomic analyses showed that early European tomatoes originated from Mexico, and that there were both large-fruited and cherry-sized accessions among them. We found that specimens with a higher percentage of fruit-size increasing alleles tended to have a lower percentage of alleles associated with favourable flavour, consistent with genetic constraints underlying a trade-off between fruit size and flavour. Genetic evidence further suggested that historical cherry-sized tomatoes were sweeter than large-fruited ones, but historical large-fruited tomatoes might have tasted less sour than modern ones. Our results illustrate how herbarium genomics can reveal hidden stages of plant domestication.

## **The Ecological Significance and Genetic Basis of a Repeated Seed Color Change During Amaranth Domestication**

Tom Winkler and Markus Stetter

University of Cologne, Germany

Plant domestication can serve as model for rapid evolution, as human cultivation imposed strong selection on fitness relevant traits. The seed as the reproductive and harvestable organ is of particular importance for grain crops. We study the repeated loss of dark seed pigmentation in grain amaranth. During its thrice repeated domestication, amaranth seed color changed from dark to pale while other domestication traits did not change. We study the ecological significance and the genetic basis of the repeated seed color change in grain amaranth. To assess the ecological function of seed pigments, we measure germination properties in a mapping population and find that pale seeds germinate faster than dark seeds. Differential gene expression analysis in diverse accessions with contrasting seed pigmentation revealed downregulation of almost all flavonoid biosynthesis pathway genes in pale seeds. Genomic and molecular analysis suggests a transposable element insertion into a regulator of this pigment pathway as the causal mutation for the seed color change during amaranth domestication.



## **Multilevel Drift and Selection in the Evolution of Prokaryotic Plasmids**

Tal Dagan

Institute of General Microbiology, Kiel University, Germany

Plasmids are an important source of raw material for microbial genome evolution outside the mainstream of bacterial chromosomes. Nonetheless, many plasmids found in nature are lacking a clear trait that is advantageous to their host. Borrowing terminology from evolutionary biology of cellular living forms, we hypothesize that Darwinian fitness is key for the plasmid evolutionary success. At the same time, due to their interaction with a hosting cell, drift and selection operate on plasmid alleles at two hierarchical levels: the collective of plasmids within the host and the collective of cells within the host population. The effect of these two levels on plasmid evolution remains understudied. Using experimental evolution approach integrated with phylogenomic reconstruction we found that: i) drift and selection at the level of the host population leads to contrasting effects on the plasmid fitness and ii) segregational drift of plasmid alleles during cell division constrains the rate of plasmid evolution. Our findings may apply for the evolution of other extrachromosomal genetic elements having a similar population structure. Focusing on plasmids, our research thus uncovers general principles in the evolution of autonomously replicating genetic elements.

Wednesday, June 14

Session 4: Emerging Trends in Evolution

[↑ back to program](#)

## Endocranial Volume is Heritable, but is not Related to Fitness, in a Free-Ranging Primate

Abigail E. Colby<sup>1,2</sup>, Clare M. Kimock<sup>2,3</sup>, James P. Higham<sup>2,3</sup>

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<sup>3</sup>New York Consortium of Evolutionary Primatology, USA

Large relative brain size is a defining characteristic of the order Primates. Arguably, this can be attributed to selection for behavioral aptitudes linked to a larger brain size. In order for selection of a trait to occur, the trait must vary, that variation must be heritable and enhance fitness. In this study, we use a quantitative genetic approach to investigate the production and maintenance of variation in endocranial volume (ECV) in a population of free-ranging rhesus macaques. We measured the ECV and body mass proxies of 542 rhesus macaques from Cayo Santiago. Using a genetic pedigree, we estimated heritability of absolute and relative ECV, and selection gradients of both traits as well as estimated body mass in the sample. Within this population, both absolute and relative ECV are highly heritable, but we found no evidence of selection on absolute or relative ECV. These findings suggest that ECV is not undergoing selection, or that we did not detect it because selection is neither linear nor quadratic, or that we lacked sufficient sample sizes to detect it.

## Experimental Evolution through Mutagenesis: An Efficient Method to Unravel the Molecular Basis of Pathogen's Virulence

Zoe Bernasconi, Ursin Stirnemann, Javier Sánchez-Martín, Beat Keller

Department of Plant and Microbial Biology, University of Zurich, Switzerland

Wheat powdery mildew (short, *Bgt*) is an ascomycete fungus pathogenic to wheat. Race-specific resistance to *Bgt* is controlled by single resistance (R) proteins that recognize specific avirulence (Avr) proteins of certain *Bgt* isolates. However, mildews can avoid host recognition by mutating their *Avrs*.

Functional characterization studies of *Bgt* are challenging due to the lack of stable transformation methods. Therefore, we developed an ultraviolet-based mutagenesis approach to study pathogen adaptation. Starting from a broadly avirulent isolate, we created 24 gain- of-virulence *Bgt* mutants to one or more *R* genes. This evolutionary experiment allowed us to identify novel *Avr* genes, as well as other genetic components governing virulence on different *R* genes. We further unravel that gain of virulence mechanisms are mainly based on single nucleotide polymorphisms, chromosomal deletions, and insertions of transposable elements affecting gene expression.

This approach elucidates how pathogens evolve and escape from recognition by resistant hosts and can deepen our understanding of *R-Avr* gene biology.

## Genomics and Quantifying Various Traits for Understanding Evolution

Hiroshi Matsumae

School of Medicine, Tokai University, Japan

In contrast to genomics, quantifying traits is still behind. We quantified cultures as extended traits of humans and investigated the diversity of language and music in Northeast Asians at the URPP Evolution in Action. Here I show my recent interests in (1) the quantitative analysis of various cultures, such as language and pottery, and (2) the fungal genomes and traits for models understanding human-biodiversity relationships (including diseases) and evolution. In the latter, we focus on genetic and trait variations in a wood-decay mushroom, the basidiomycete *Schizophyllum commune*, known as a cause of respiratory allergy and showing the highest genetic diversity in eukaryotes. We built high-quality reference genomes of two crossable strains of this fungus. We found that the intra-species diversity in *S. commune* was higher than the inter-species diversity in bacteria. We also explore morphological variations of the species corresponding to genetic variations by image analysis.

Wednesday, June 14

Session 4: Emerging Trends in Evolution

[↑ back to program](#)

## **Genomic Surveillance Agility: From Tracking SARS-CoV-2 Variants in South Africa to the Climate Amplified Diseases and Epidemics (CLIMADE) Global Consortium**

Monika Moir<sup>1</sup>, Houriiyah Tegally<sup>1</sup>, Eduan Wilkinson<sup>1</sup>, San Emmanuel James<sup>2</sup>, Cheryl Baxter<sup>1</sup>, Richard Lessels<sup>2</sup>, Tulio de Oliveira<sup>1,2</sup>

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The emergence and evolution of SARS-CoV-2 has been of significant concern globally. South Africa, in particular, has been a focal point for studying the genetic diversity and evolution of SARS-CoV-2, with the identification of several important variants, notably the highly transmissible B.1.351 (Beta) and B.1.1.529 (Omicron) variants, as well as the more recent BA.4 and BA.5 Omicron sublineages. The first major variant of concern, Beta, quickly became dominant across the country and most of southern Africa. It carried a set of mutations in the spike protein, including E484K and N501Y, associated with a selection advantage causing increased transmissibility and potential evasion of immune responses. Continued genomic surveillance efforts in southern Africa then revealed the emergence of the Omicron variant, which at the time harboured an unprecedented number of mutations in the spike protein. Subsequently, we described two new sublineages, BA.4 and BA.5, responsible for a fifth wave of infections in South Africa. The continued discovery of genetically diverse Omicron lineages points to the hypothesis that a discrete reservoir, such as human chronic infections and/or animal hosts, is potentially contributing to further evolution and dispersal of the virus. A continental analysis demonstrated how the pandemic unfolded through multiple waves of infection that were each driven by distinct genetic lineages in specific spatial patterns. Sustained genomic surveillance efforts in Africa have highlighted the importance of continuous monitoring for the emergence of new variants as the virus continues to evolve, which provided early warning signs of potential changes in transmission dynamics and prompted appropriate public health responses. Continued investment in genomic surveillance can be used as a platform to help address the many emerging and reemerging infectious disease threats in Africa. Leveraging the genomic sequencing capacities built and strengthened during the pandemic, we have assembled the CLIMADE consortium with the mandate to monitor climate-aggravated diseases. CLIMADE aims to surveil genetic diversity of vector and waterborne climate sensitive pathogens, in parallel with ecological and epidemiological mapping of pathogens, to identify and control outbreaks before they become epidemics and epidemics before they become pandemics.

Wednesday, June 14

Session 4: Emerging Trends in Evolution

[↑ back to program](#)

## Proteomics of Ancient Immunology: Refined Methods to Detect Active Leprosy in Medieval Individuals

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Palaeopathology and ancient DNA, often used in tandem, have been successfully used to identify diseases in past individuals. While this provides an effective method to detect pathogen presence, much remains unknown about identifying active infections and the host response to past pathogens. Protein analysis offers new insights into how the immune system of those suffering from infections and diseases responded. However, proteomic analysis of bone and dentine samples are often complicated by an overwhelming presence of collagen, the most abundant protein in both tissues. Tandem mass spectrometry focuses on the most common peptide masses which often leads to collagen “swamping” out lower abundance peptides, leaving these lower-abundance proteins undetected. Here we present a protocol combining a recently developed digestion enzyme to reduce collagen with optimized extraction steps to specifically target immune proteins that would be otherwise hidden. We conducted comparisons of tissue types, denaturation agents, extraction fractions, and digestion enzymes in order to identify the most effective protocol for identifying the non-collagenous portion of the proteome, specifically focused on immune-related protein recovery. We illustrate our optimized methodology through a combined DNA and protein immunological case study of individuals from a Medieval Spanish leprosarium, as well as four individuals interred in a non-leprosy associated context. Through this combined approach, we have identified specific immune-related proteins that may indicate active leprosy infections at the time of death of these individuals. The results of this study have widespread implications for future work, and present numerous possibilities for palaeopathological/biomolecular studies focused on past health and disease.

## Haplotype-Aware Pan-Genome Graph Based Genome Inference for Detection of Transmission Ratio Distortion Signals in Plants

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Transmission ratio distortion (TRD) refers to the unequal transmission of parental chromosomes or genes to offsprings. It can result in differences in the expression of specific genes or traits. Cassava haplotype map suggested fixation of deleterious mutations in the species. 20-30% of transcripts in each cassava tissue type showed allele-specific expression (ASE) differences. However, TRD in cassava remain largely unknown. In the present study, we constructed a haplotype-aware pan-genome graph using haplotype-resolved parental genome cassava TME204WT. Genotyping of TME204 F1 progeny based on the haplotype-aware pan-genome graph allowed efficient detection of loci with TRD. Our results laid the foundation for identifying the TRD genes using pan-genome based genotyping. A larger scale experiment involving more parental haplotypes with larger F1 populations will be valuable to illustrate the genetic and molecular mechanisms underlying TRD in cassava.

## The Genetic History of the Southern Andes from Present-day Mapuche Ancestry

Epifanía Arango-Isaza<sup>1,2</sup>, Marco Rosario Capodiferro<sup>3,4</sup>, María José Aninao<sup>5</sup>, Hiba Babiker<sup>6</sup>, Simon Aeschbacher<sup>1</sup>, Alessandro Achilli<sup>4</sup>, Cosimo Posth<sup>7,8</sup>, Roberto Campbell<sup>9</sup>, Felipe I. Martínez<sup>9,10</sup>, Paul Heggarty<sup>11</sup>, Scott Sadowsky<sup>12</sup>, Kentaro K. Shimizu<sup>1,2</sup>, Chiara Barbieri<sup>1,2,6</sup>

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The southernmost regions of South America harbor some of the earliest evidence of human presence in the Americas. However, connections with the rest of the continent, and the contextualization of present-day indigenous ancestries remain poorly resolved. In this study, we analyze the genetic ancestry of one of the largest indigenous groups in South America: the Mapuche. We generate genome-wide data from 64 participants from three Mapuche populations in Southern Chile: Pehuenche, Lafkenche, and Huilliche. Broadly, we describe three main ancestry blocks with a common origin, which characterize the Southern Cone, the Central Andes, and Amazonia. Within the Southern Cone, ancestors of the Mapuche lineages differentiated from those of the Far South during the Middle Holocene, and did not experience further migration waves from the north. A deep genetic split between the Central and Southern Andes is followed by instances of gene flow, which may have accompanied the southward spread of cultural traits from the Central Andes - from crops to loanwords from Quechua into Mapudungun (the language of the Mapuche). Finally, we report close genetic relatedness between the three populations analyzed, with the Huilliche characterized additionally by intense recent exchanges with the Far South. Our findings add new perspectives on the genetic (pre)history of South America, from first settlement through to the present-day indigenous presence. Follow-up fieldwork took these results back to the indigenous communities to contextualize the genetic narrative alongside indigenous knowledge and perspectives.



## **Brassicaceae Unique Chemical Defense System is Involved in the Adaptation to Mutualistic Endophyte, *Phomopsis columnaris***

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Plants have evolved sophisticated defence systems against herbivores, pathogens and microorganisms. Brassicaceae plants produce glucosinolates as defence chemicals that are activated by enzymes called myrosinases, which are stored in the endoplasmic reticulum body (ER body) and myrosin cells. Endophytes are fungi that live at least temporarily in plant tissues during their life cycle, and they are classified as plant mutualistic fungi, but they can be pathogenic to the host plants. *Phomopsis columnaris* is an endophytic fungus reported to help heavy metal accumulation in Brassicaceae plants, but it is unclear whether the plant defence response is involved in the mutualistic interaction between plants and *P. columnaris*. To understand the effect of *P. columnaris* on plants, we inoculated the fungus on *Arabidopsis thaliana* roots and analyzed the gene expressions involved in glucosinolates biosynthesis, myrosinases and ER-body formation. The results revealed that some of these genes were upregulated in the *Arabidopsis* root in response to *P. columnaris* infection. To dissect the effect of individual defense components, we inoculated *P. columnaris* on the *Arabidopsis* mutants deficient in glucosinolates and myrosinases, and we examined the plant growth and the fungus population increase. We found that *P. columnaris* severely infects and reduces the growth of *myb28,29, cyp79b2b3* quadruple mutant (qKO), which is deficient in glucosinolates. We compare gene expressions for ER-body formation between the wild type and the qKO mutant after the fungal inoculation, but none of the genes were changed between them, indicating that ER-body formation genes do not enhance the expression in the glucosinolate absence. The result suggests no compensative effect between ER body gene expression and glucosinolate accumulation. Our findings show that glucosinolate plays a crucial role in endophytic adaptation, and its defect changes the endophyte to be pathogenic in *Arabidopsis*.

## Development of a Reproducible Pipeline for DNA Methylation Data Analysis using SUSHI and Shiny: Addressing the Genomics Reproducibility Crisis

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We developed a reproducible pipeline for the analysis of DNA methylation data using the SUSHI pipeline framework and a Shiny application for exploratory data analysis. The field of genomics has been facing a reproducibility crisis due to the growing number of available tools and the lack of documentation of processing and analytical procedures. SUSHI is a versatile web-based tool for high-throughput sequencing data analysis, while Shiny facilitates interactive web applications and dashboards using R. In our pipeline, SUSHI is implemented for the analysis of DNA methylation data, and a Shiny application is implemented for exploratory data analysis and for making publication-ready figures. Additionally, the pipeline will be implemented in Galaxy framework, a widely used data analysis platform in genomics. To conclude, our tool enables users to analyze DNA methylation data generated from any sequencing protocol, promotes reproducibility of results, and potentially advances the field of genomics with more reliable scientific discoveries.

## Identification and Isolation of Wheat Powdery Mildew Resistance Genes from *Triticale*

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There is a constant battle occurring in nature between hosts and fungal pathogens. Triticale, the man-made wheat-rye hybrid cereal, has recently become host of (wheat?) powdery mildew. In the current project, efforts are directed towards understanding the molecular basis of such host adaptation event. Utilizing a fungal mapping population derived from virulent and avirulent isolates on triticale, QTL analysis indicated that Triticale might have two major resistance loci providing resistance against the unadapted wheat mildew, most likely coming from the rye sub genome. A two-pronged strategy is being undertaken to genetically and molecularly identify these two resistance loci. First, by using a mapping segregating population of contrasting triticale cultivars for mildew resistance and genotyped with wheat and rye SNP arrays we will define the genetic location of both resistance genes. Second, we will apply PacBio isoform sequencing (Iso-Seq) of the wild-type resistant parental genotype with RNA sequencing of multiple EMS mutants to molecularly identify both resistance genes. The identification of these genetic loci will assist the development of resilient crops to pathogens with multiple hosts.

## Evolution of the Atlantic Salmon Population in the Burrishoole River System since the 1970s

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Human-induced disturbances and environmental change are some of the biggest threats to species survival. This results in strong selective pressures leading to rapid genetic changes in wild populations. Long-term data from wild populations can thus bring crucial information regarding its adaptation to human-induced changes. The Atlantic salmon (*Salmo salar*) population of the Burrishoole catchment (Co. Mayo, Ireland) have been monitored continuously since the 1970s, providing a rare genomic dataset covering six decades during which salmon have experienced intense selective pressures. My PhD examines changes in allele frequencies over time in this wild population, the link between genetic and phenotypic changes, and the association between phenotypic and environmental changes. Preliminary analyses focus on studying genetic structure of the modern population in Burrishoole. Indeed, as salmon return to reproduce in their native rivers, it is interesting to study genetic structure in the current population, to establish if there is a spatial differentiation based on reproduction location.

## Self or not to self: Evolution at the S-locus in *Arabidopsis lyrata* at Distribution Edges

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Sexual reproduction can take two extreme modes: obligate outcrossing and selfing. While outcrossing requires a suitable mate, selfing serves high reproductive assurance. However, selfing will decrease heterozygosity, and new genetic variation is only introduced by mutation. *Arabidopsis lyrata* appears to experience a trade-off that favors selfing at the edges of its range. The S-locus determines self-incompatibility, at which outcrossing populations are heterozygous and many different alleles segregate, whereas in selfing populations mostly only one S-allele is present. We investigated its evolution in *A. lyrata* by Pac-Bio Sequel II sequencing in replicate pairs of nearby selfing and outcrossing populations. We located the S-locus with the style-specific gene *SRK* (S-receptor kinase protein) and reconstructed the genealogy using the conserved flanking gene *UBOX*, which revealed an unexpected geographical pattern, separating western from eastern populations, roughly. The orientation of *SRK* differed between samples, as well as the intron sizes. In the future, we will try to locate the pollen-specific *SCR* (S receptor cysteine rich protein) gene within the S-locus and compare *SRK* gene structure between selfing and outcrossing populations.

## Origin and Evolution of Land Plant Reproductive System

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Land plants constitute a significant part of the world's biomass and the human diet. The transition of plants to a land-based habitat led to profound developmental innovations. A major change was the formation of a sexual-based reproductive system. In early-diverged land-plant lineages, the haploid-gametophytic phase dominates the life cycle. In later lineages the diploid- sporophytic phase took a more prominent role. This switch involved the elaboration of an embryo-based reproductive system. However, there is a significant gap in our understanding of the evolutionary, genetic and developmental mechanisms that enabled the formation of a sexual reproductive system. We hypothesize that embryo evolution entailed two key consecutive events: 1. Cell proliferation and growth following fertilization and prior to meiosis, which generates the sporophyte. 2. Specification of a subset of these cells as sporogenous cells, which will generate the spores. We hypothesize that the first step involves class I KNOTTED1- like homeobox (KNOX1), and the second, class III Homeodomain-Leucine Zipper (C3HDZ). We aim to use early-diverged land-plant species to identify regulators of sporogenous formation by elucidating the role of C3HDZ and its targets and determine their role in sporogenous specification. The proposed research is expected to elucidate the molecular network underlying reproductive development of land plants and its evolution. This may unravel basic principles of developmental innovations that enable the evolution of land plants and the conquest of novel habitats.

## Population Genetics and Molecular Epidemiology of Wheat Powdery Mildew in Europe

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*Blumeria graminis*, or powdery mildew, is a fungal pathogen of cereals responsible for major crop losses around the world. Control measures like fungicides and resistant host varieties are often rendered ineffective as pathogen populations rapidly evolve resistance. A holistic understanding of the evolutionary processes in play in these populations is therefore important to design durable control strategies. This study aims to characterize the population structure, dynamics and molecular epidemiology of *B.g. tritici* (wheat powdery mildew) by making use of WGS data of samples collected from across Europe over successive seasons. The project will involve estimating the genetic diversity within and between populations, studying migration patterns, identifying selection pressures and genomic signatures of selection and local adaptation, characterising the extent of sexual reproduction in populations, and tracking different strains over space and time to determine the spatio-temporal dynamics of the epidemic.

## Investigating the Functional Diversification of a Conserved Plant Peptide-receptor Kinase Signaling Module

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Plant genomes encode hundreds of secreted signaling peptides, but only a few of them are functionally characterized. Recently, a family of stress-responsive secreted signaling peptides, called CTNIPs (or SCREWs), and their corresponding receptor kinase HAESA-LIKE 3 (HSL3) (also named NUT) were identified. Notably, the CTNIP-HSL3 signaling module is conserved across Angiosperms, including most Monocots. While *Arabidopsis thaliana* has a single HSL3 homolog, phylogenetic analyses indicate that Poaceae contain an expanded HSL3 repertoire, but *bona fide* CTNIP sequences could not be identified. A similarly increased number of HSL3 homologs was observed in Fabaceae, which have functional CTNIPs. Using both phylogenetic and molecular approaches, we are investigating how CTNIPs and HSL3 might have diverged during plant evolution, which (CTNIP-like) peptides are perceived by HSL3 homologs in Poaceae, and importantly which functions an expanded HSL3 repertoire could serve in Poaceae and Fabaceae.



## Simultaneous Inference of Past Demography and Selection from the Ancestral Recombination Graph under the Beta Coalescent

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The reproductive mechanism of a species is a key driver of genome evolution. The standard Wright-Fisher model for the reproduction of individuals in a population assumes that each individual produces a number of offspring negligible compared to the total population size. Yet many species of plants, invertebrates, prokaryotes or fish exhibit neutrally skewed offspring distribution or strong selection events yielding few individuals to produce a number of offspring of up to the same magnitude as the population size. As a result, the genealogy of a sample is characterized by multiple individuals (more than two) coalescing simultaneously to the same common ancestor. The current methods developed to detect such multiple merger events do not account for complex demographic scenarios or recombination, and require large sample sizes. We tackle these limitations by developing two novel and different approaches to infer multiple merger events from sequence data or the ancestral recombination graph (ARG): a sequentially Markovian coalescent (SM $\beta$ C) and a graph neural network (GNNcoal). We first give proof of the accuracy of our methods to estimate the multiple merger parameter and past demographic history using simulated data under the  $\beta$ -coalescent model. Secondly, we show that our approaches can also recover the effect of positive selective sweeps along the genome. Finally, we are able to distinguish skewed offspring distribution from selection while simultaneously inferring the past variation of population size. Our findings stress the aptitude of neural networks to leverage information from the ARG for inference but also the urgent need for more accurate ARG inference approaches.

## Complete Bacterial Genome Extracted from a Eukaryote Genome Assembly

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*Wolbachia* is a common bacterial parasite (or endosymbiont) found in ~40% of arthropods. They are known to have a variety of reproductive effects on their hosts, including cytoplasmic incompatibility. We isolated a complete *Wolbachia* genome (wDia) from an Oxford Nanopore-based assembly of its host – an adult male oil beetle (in the blister beetle Family Meloidae; *Meloe dianella*). The aim of this study was to characterize the *Wolbachia* genome. The wDia bacteria was identified as belonging to Supergroup B of Wolbachiae and shows no signs of horizontal gene transfer with its oil beetle host. Future steps are to evaluate potential cytoplasmic incompatibility in the sequenced oil beetle caused by wDia, and to examine infection dynamics between the sexes of *Meloe dianella*.

## Combined Effect of Insular Dwarfism and Domestication in Horses of Greece and Japan

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The horse varieties from Skyros and Rhodes islands are extremely small, reaching shoulder heights of only about one meter. Furthermore, the Japanese archipelago is home to eight small, native horse breeds. We aim at investigating the evolutionary morphology and provide a comprehensive review of historical documentations on these horses of great cultural significance in Greece and Japan. Cranial data from historical literature is integrated with data on newly gathered skulls to create and analyse a measurement dataset featuring various domestic and wild horse breeds and varieties. We use non-invasive imaging to study and measure 3D models of the bony labyrinth, housing the inner ear, and the braincase endocast. When considering the effects of allometry, we show that size explains a large amount of the shape variance in the cranium, the inner ear, and the brain. Changes in morphology result from an interplay of domestication and island-living, which both affect body size.

## Co-evolution of Cultivation Syndrome and the Human Agricultural Systems in Bread Wheat

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Wheat's diffusion from its origin, the trans-Caucasia region, to different parts of the world has resulted in genetic changes and adaptations to various environments such as the cold highlands of the Himalayan region and the warm and humid lowlands of East Asia. Cooking cultures have also impacted wheat traits during this diffusion, such as Bulgur in Turkey, baking in West Asia, and steaming and boiling in East Asia. These cooking preferences are considered to impact genetically controlled wheat grain qualities. Despite the importance of adaptation and culinary practices in shaping its genetic makeup, the molecular basis of how wheat's traits changed during diffusion or through human selection remains unclear. To investigate this, 25 Asian wheat cultivars were resequenced, and population genetics were performed. Our analyses provide interesting results including mutations characteristic of Japanese lines, shedding light on the co-evolution of wheat and human agricultural systems.

## **Investigating the Distribution and Stability of Epigenetic Variation in Natural Populations of *Arabidopsis thaliana***

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Epigenetic marks, such as DNA methylation, are important regulators of gene expression. In plants, epigenetic variation (EV) can be meiotically heritable and, therefore, potentially contribute to adaptation. Research in our laboratory aims at understanding the evolutionary significance of EV in plant adaptation and its potential application to plant breeding. We have previously shown that new phenotypes can be selected from genetically uniform populations in as few as five generations. The selected phenotypes, e.g., delayed flowering time and a more highly branched plant architecture, were subsequently heritable in the absence of selection for at least three generations. Thus, EV is subject to selection and can play a role in adaptation under laboratory conditions. However, evidence for its evolutionary relevance in natural populations remains scarce. With this project, we want to bridge the gap between the greenhouse and outdoors to gain a better understanding of the epigenetic basis for plant adaptation.

To test whether epialleles are subject to selection in a natural environment, we are repeatedly sampling wild populations of *Arabidopsis thaliana* over the course of four years. Although sequencing data for many accessions is available, the sample density at a population level is minimal. Therefore, we are monitoring 50 individuals in 10 populations to gain a better understanding of the distribution and stability of EV in natural populations. Our primary interest lies in a set of ~80 epialleles that we identified from public methylome data. The epialleles are present in either a fully methylated or unmethylated state (bistably methylated loci) and methylation is correlated with expression of the respective gene. To probe the methylation state for so many loci in a large number of individuals, we use Chop-PCR, a methylation- sensitive restriction enzyme-based approach. The initial monitoring of a large number of individuals in natural populations showed that, indeed, many bistably methylated loci segregate in these populations, providing an opportunity for selection. The frequency of these segregating epialleles will be monitored over four years in selected populations to see if it changes beyond the level expected by genetic drift, indicating that they are subject to selection.

## **The Kelch-phosphatase BSLM Coordinates Cell Proliferation and Differentiation in *Marchantia polymorpha***

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Plant unique Kelch-phosphatases have been described as components of the brassinosteroid and stomata fate determination pathways in *Arabidopsis thaliana*. However, its role across land plant evolution remains unclear. In *Marchantia polymorpha* we found two loci linked to the sexual chromosomes (MpBSLM and MpBSLF, respectively). Here, we report a severe growth phenotype composed by undifferentiated cells in the *ge-bslm* mutant. Histological analyses confirm aberrations on the appropriate patterning and distribution of the photosynthetic, storage and basal layers. Also, a multi-omic approach was used to explore the differential transcriptional profiles, protein abundances and phospho-sites. Altogether this data set allow us to identify potential substrates differentially phosphorylated in the absence of BSLM. Further experiments will perform to validate direct targets and to explore its role in cell proliferation and fate specification regulation during thallus development. Our findings point to a basic function for Kelch-phosphatases in plant cell proliferation.

## **Experimental Evolution in Crop Wild Relatives Introgressed Barley, Wheat and Rapeseed Populations - 20 Generations of Natural Adaptation to Organic and Conventional Agro-Ecosystems Assessed by Pool Genotyping**

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High productivity in conventional agriculture plays a vital role in food security but also contributes to eminent threads of our time – like biodiversity loss and contribution to climate change. Approaches like organic farming try to minimize this negative footprint of food production, which currently results in lower productivity.

A long-term experiment under two common farming practices monitors diverging and similar fitness on gene allele levels in heterogeneous populations during 20 generations of natural adaptation. The applied WGS pool-sequencing approach allowed a cost-efficient and highly reliable estimation of allele frequencies on gene level. We can show that changes in the allele frequencies are species depended, occur within a few generations, and lead to different adaptation strategies in both agro-ecosystems, attributable to traits.

The results provide knowledge on breeding approaches for organic farming adapted varieties.

## Investigating the Co-evolutionary History of the Family-specific Plant Receptor Kinase MIK2 and its SCOOP Ligands

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Plant secreted signaling peptides regulate growth, development, and stress responses. Nevertheless, specific steps in the evolution of these peptides and their receptors are not well understood. Recent studies reported the characterization of the Brassicaceae-specific family of SERINE RICH ENDOGENOUS PEPTIDES (SCOOPs). In *Arabidopsis thaliana*, SCOOPs are perceived by the receptor kinase MALE DISCOVERER 1-INTERACTING RECEPTOR-LIKE KINASE 2 (MIK2). *In silico* analysis of >30 plant genomes within and outside the Brassicaceae family reveals a strong conservation of potential MIK2 homologues which are family-specific, suggesting the appearance of a common ancestral receptor  $\pm$  30 mya. We aim to functionally validate MIK2-homologues and ancestral MIK2s and leverage them to define evolutionary events leading to the sensing of sequence-divergent SCOOPs. Analysis of MIK2/SCOOP evolution and function will provide a model for functional diversification of other plant receptors and their ligands.



## **Systemic Orchestration of Cell Size throughout the Body: Evolutionary and Developmental Responses of *Drosophila melanogaster* to Environments**

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Organisms have evolved vast disparities in body size and cellularity. Although the body size of ectotherms in environmental gradients has been well studied, little is known about its cellular basis. To fill this gap, we measured body size and five cell types in two groups of *Drosophila melanogaster* flies that originated either from an evolutionary study, by evolving in three thermal environments, or from an experimental study with flies developing at two temperatures combined with two oxygen levels. In general, environmental conditions affected the phenotypic characteristics of flies. Cell size changed systemically across the body, but only to some extent, indicating a more complex nature of cell size response and some level of cell autonomy. Interestingly, despite this, females were consistently larger than males and had larger cells in their organs. We conclude that cell size should be included in studies of insect responses to climate changes and the origin of life-history traits.

## Can Life Expectancy Determine Altruistic Behaviour in Ants?

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The evolutionary success of ants can be attributed to their altruistic tendencies. These tendencies include rescue behaviour occurring between nestmates when one of them is endangered (e.g., trapped). Despite numerous studies, sources of variation in rescue expression in ants remain unclear. In our study, we tested whether life expectancy can play a role in the context of rescue. We manipulated the life expectancies of *Formica cinerea* workers by injury and found that this leads to costs expressed in lower survival. We performed laboratory observations of rescue behaviour and revealed that injured workers take part in rescue actions more likely toward intact nestmates. Our results suggest that workers' tendency to rescue appears to be fine-tuned to their life expectancies, which makes evolutionary sense. The subject that we studied here opens up further research possibilities on the evolution of altruism among social insects.

## The Relative Contribution of Genetic and Epigenetic Variability to Adaptation

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Trans generational epigenetic inheritance is a much researched topic, yet its importance to adaptation in an evolutionary context remains debated. This PhD project is aimed at getting a better understanding of the relative contributions of epigenetic and genetic variability to adaptation. To disentangle the genetic and epigenetic contribution to adaptation, we plan to generate starting populations of *Funaria hygrometrica*, that are only genetically or epigenetically variable and have these populations go through experimental evolution. To induce hypo methylation, we are going to utilize the methyl transferase blocker Zebularine, whereas the genetic mutations are going to be induced through UV-radiation. In order to balance the respective variabilities to reflect the natural state, preliminary experiments will need to be carried out to determine the dose effect relation of the two treatments. The selection pressure for the experimental evolution, is going to be exerted by adding salt to the growth media inducing stress for the plants. Every generation is going to be made up of mutagenesis or epimutagenesis followed by growth on the stress media. During the experimental evolution, the growth of the individual populations is going to be recorded as a proxy for adaptation. After the experimental evolution experiment is over, the finishing populations will be allowed to grow without stress for two generations before sequencing, to ensure the observed adaptation is inheritable across generations and not just phenotypic plasticity. We plan to use a combination of whole genome bisulfite sequencing and whole genome resequencing, to record the changes in both the genome and epigenome. By combining the sequencing data with the phenotypic data we strive to elucidate the respective roles and potential of both genetic and epigenetic variability for adaptation. Questions of particular interest are; How fast can adaptation based solely on the respective variabilities act and how varying are these changes among different populations?

## The Role of DNA Methylation in Shaping Genome Evolution Following Whole Genome Duplication: Insight from Catostomidae Fishes

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DNA methylation plays many important roles within a genome through interacting with histones and other proteins, and DNA. While DNA methylation state can change over short time periods (i.e., minutes) in response to external stimuli, recent work has shown that DNA methylation patterns in key genomic regions associated with development can be conserved across deep phylogenetic splits spanning millions of years. However, it is not known how these DNA methylation patterns change in response to whole genome duplication events (WGD). Our aim is to understand how (or if) methylation plays a role in the conservation of these key genomic regions in a clade of fishes that have gone through an ancient, ancestral WGD. Here we characterized the DNA methylome of five members of the Catostomidae family (*Xyrauchen texanus*, *Myxocyprinus asiaticus*, *Moxostoma valenciennesi*, *Hypentelium nigricans*, and *Catostomus commersonii*) that shared an ancestral WGD approximately 65 million years ago. We compared DNA CpG methylation patterns in these duplicated fish genomes with two unduplicated diploid outgroups, *Gyrinocheilus aymonieri* and *Danio rerio*, using long-read Oxford Nanopore sequencing of two tissues, gill and muscle. Patterns of methylation are generally consistent within the Catostomidae family across duplicated chromosomes with areas of conserved, tissue-specific methylation. Within these areas of conserved tissue-specific methylation, we found several duplicated, conserved hypomethylated valleys within the Catostomidae genomes and are shared with the diploid outgroups, despite more than 100 million years divergence time. These conserved hypomethylated valleys span key developmental genes, including duplicated HOX clusters. Despite the often-labile nature of DNA methylation, our results show that patterns of DNA methylation can be conserved across more than 100 million years of evolution, even following genome duplication events. This study demonstrates the utility of nanopore base modification calling for studying DNA methylation across complex polyploid genomes, and provides new insight about the role of methylation in genome evolution following whole genome duplication.

## **A High-throughput Phenotyping of Early Growth Patterns of a Hexaploid Wheat NAM Population by a UAV**

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We have developed a nested association mapping (NAM) population of wheat (*Triticum aestivum* L.,  $2n=6x=42$ ) employing 24 diverse Asian landraces and cultivars as founder lines to cover the genetic diversity of Asian bread wheat. This population consisted of nearly 5,000 individuals. 1060 lines of this NAM population in the F<sub>7</sub> generation were grown in the field of Kyoto University during the 2020-2021 season. In this study, we aimed to describe the early growth patterns during the cultivation period. We measured height, canopy size, and normalized difference vegetation index (NDVI) using unmanned aerial vehicles (UAVs) for about one month after transplantation and analyzed the dynamics of early growth. We also examined the correlations between these early growth patterns and yield-related traits. We will further analyze correlation between phenotypes and genotypes to infer the locations of causal genes related to early growth patterns.

## Variation in Flowering Behaviour of Annual and Perennial Plants

George Coupland

Max Planck Institute for Plant Breeding, Cologne, Germany

We study variation in flowering behaviour and reproduction among annual and perennial plants. Annual plants typically colonize disturbed environments, flower rapidly to ensure seed production and are short lived. By contrast, perennials have a longer life span and are typically iteroparous, reproducing multiple times in their lifetime, and invest proportionally more in vegetative growth and less in reproductive structures. Perennials present a more complex flowering behaviour, including a longer juvenile period before flowering, control of the duration of flowering and balancing the allocation of resources between reproduction and growth during and after flowering. The life cycles of annual and perennial species diverge rapidly during evolution, and we use comparative analysis within the Brassicaceae family to study this process. Using a combination of forward and reverse genetics, genomics and comparative analyses in a phylogenetic context we have defined some of the mechanisms by which flowering regulation differs between annuals and perennials, contributing to the divergence of these life histories during evolution. The talk will describe the *Arabidopsis* genus as a model system for studying these processes, some of the mechanisms that confer differences in the seasonal patterns of flowering of annuals and perennials and how these are significant in phenotypic variation among European populations of perennial *Arabidopsis alpina*.

Wednesday, June 14

Keynote speech: Evolution of Plant Reproductive Traits

[↑ back to program](#)

## **Plant Biotic Interactions Combining Present-day and Historical Samples**

Hernán A. Burbano

Centre for Life's Origins and Evolution, Department of Genetic, Evolution and Environment,  
University College London, UK

In this talk, I will delve into the realm of plant-pathogen coevolution by integrating present-day and historical samples to examine the interactions between crop and wild plants and their fungal and bacterial pathogens. Furthermore, I will explore the role of bacteriophages in shaping inter-bacterial competition and influencing the outcomes of bacterial infections in metapopulations of historical and modern plant pathogens. To shed light on the multifaceted nature of these interactions, I will contrast the results of plant-pathogen relationships in cultivated crops with those found in natural ecosystems.

## Genomic Adaptation of a Generalist Plant Species to Local Pollinator Communities and Abiotic Factors

Frachon L.<sup>1</sup>, Arrigo L.<sup>1</sup>, Rusman Q.<sup>1</sup>, Poveda L.<sup>2</sup>, Qi W.<sup>2,3</sup>, Scopece G.<sup>4,5</sup>, Schiestl F.P.<sup>1</sup>

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<sup>5</sup> National Biodiversity Future Center, Palermo, Italy

The combined effect of changes in pollinator communities, and the direct impact of soil and climate variation on plant-pollinator interactions can strongly affect the reproductive success of flowering plants. However, knowledge of the adaptative potential of plants to complex ecological networks and the underlying genetic mechanisms is still limited. Based on a pool-sequencing approach of 21 natural populations of *Brassica incana* in Southern Italy, we combined a genome-environmental association (GEA) analysis with a genome scan for signature of selection to discover genetic variants associated with pollinator communities, edaphic and climatic variation. We identified genomic regions involved in adaptive response of *B. incana* to both single pollinator species and the overall pollinator interactions. Interestingly, we observed a significant number of genetic variants shared between the soil texture (fine silt) and the visits of bumblebees and hoverflies, while few genetic variants involved in both pollinator and climate variation were identified. Our results highlight the adaptive potential of generalist species to complex biotic interactions, and the importance of considering multiple environmental factors to describe their adaptive landscape.



## Can Frequent Heatwaves at Different Life Stages Affect the Reproductive Abilities and Recovery of Male and Female *Drosophila melanogaster*?

Abhishek Meena, Alessio De Nardo, Komal Maggu, Sonja Sbilordo, Vitor Kovalov, Stefan Lüpold

Department of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland

Biodiversity is at risk from extreme and unpredictable climate events. Climate change studies often focus on how organisms survive, but successful reproduction is equally important and often more sensitive to heat stress than survival. Ectotherms are particularly affected by varying ambient temperature. Studies, however, mostly examine heat stress on one life stage or sex, without considering how it affects different life stages or the fitness consequences past a heatwave. To address this knowledge gap, we used *Drosophila melanogaster* to explore how heatwaves across different life stages affect reproduction and recover in males and females. We found that heat stress differentially affects reproductive traits between life stages and sexes. This study shows how frequent heatwaves can impair insect reproduction. Such effects could hasten population declines, especially in more vulnerable species, highlighting the need to include reproduction and recovery for models of species persistence.

## Investigating the Polygenic Architecture of Flowering Time and its Relationship with Local Environments in the Grass *Brachypodium distachyon*

Nikos Minadakis<sup>1</sup>, Lars Kaderli<sup>1</sup>, Robert Horvath<sup>1</sup>, Wenbo Xu<sup>1</sup>, Michael Thieme<sup>1</sup>, Daniel D. Woods<sup>2</sup> and Anne C. Roulin<sup>1</sup>

<sup>1</sup> Department of Plant and Microbial Biology, University of Zurich, Switzerland

<sup>2</sup> Plant Sciences Department, University of California-Davis, USA

Synchronizing the timing of reproduction with the environment is crucial in the wild. Among the multiple mechanisms annual plants evolved to sense their environment, the requirement of cold-mediated vernalization is a major process that prevent individuals from flowering during winter. In many annual plants including crops, both long and short vernalization requirement can be observed within species, resulting in so-called early- and late-flowering genotypes. Here, using the grass model *Brachypodium distachyon*, we explored the link between vernalization requirement, flowering time, environmental variation and diversity at flowering genes by combining measurements under greenhouse and outdoor conditions. These experiments confirmed that *B. distachyon* natural accessions display large differences regarding vernalization requirements and ultimately flowering time. We underline significant albeit quantitative effects of current environmental conditions on flowering time and population genomics in 332 natural accessions revealed that eight well-characterized flowering time genes (FT-genes) contribute significantly to flowering time variation and display signs of polygenic selection. FT-genes, however, do not colocalize with GWAs peaks obtained with outdoor measurements, indicating that FT-gene natural variants may not contribute to flowering time variation in the wild. Altogether, our study fosters our understanding of the polygenic architecture of flowering time in a natural grass system and open new avenue of research to investigate the gene-by- environment interaction at play for this trait.

## **Human Adaptation to Amazon Rainforest**

Tábita Hünemeier

Institute of Evolutionary Biology (IBE/CSIC-UPF), Spain

Department of Genetics and Evolutionary Biology, University of São Paulo, Brazil

The history of human settlement in the Americas dates back approximately 15,000 years, when the first individuals arrived in the New World and migrated southward, adapting to new environments and populating all latitudes of the continent. The diverse biomes of the Americas offer an intriguing model for studying human adaptation. Despite the challenges presented by different biomes, Indigenous American populations quickly spread and thrived throughout the continent. The Amazon biome, characterized by lush rainforests, possesses a high level of plant diversity and lower levels of UV radiation than other regions. However, the region also faces significant challenges, including food source instability and the highest concentration of pathogens globally. Inhabitants of the Amazon biome typically have shorter lifespans and rely on specialized thermoregulation mechanisms to cope with the hot and humid climate. Here we will leverage genomic information to explore the impact of natural selection on the genomes of Indigenous populations from the Amazon region.

Wednesday, June 14

Session 5: Adaptation to Changing Environments

[↑ back to program](#)

## **Invasive Species in Changing Environments - Uncovering Spatiotemporal Dynamics of a Global Plant Invasion through Herbariomics**

Ramona-Elena Irimia<sup>1</sup>, Luzia Hamma<sup>1</sup>, Farah Badreldin<sup>1</sup>, Uta Grünert<sup>1</sup>, Christina Richards<sup>1,2</sup>, Oliver Bossdorf<sup>1</sup>

<sup>1</sup> Plant Evolutionary Ecology, Institute of Evolution & Ecology, University of Tübingen, Germany

<sup>2</sup> Department of Integrative Biology, University of South Florida, Tampa, USA

Herbarium collections provide information about how plant species and communities have changed over time and responded to environmental challenges. Molecular marker techniques applied to herbarium specimens can inform about the evolutionary processes that contribute to range expansion and colonization of novel environments. With a global collection of ca. 16.000 herbarium specimens spanning the last 180 years since its introduction into new ranges in Europe and North America, Japanese knotweeds (*Reynoutria*, Polygonaceae) represent a powerful system to investigate spatiotemporal dynamics and species adaptation to heterogeneous environments. For this study, we genotyped hundreds of Japanese knotweed individuals from different time-periods, covering the species distribution across three continents. We also measured variation in leaf functional traits including stomatal density and specific leaf area and leaf chemical defenses against herbivores. Findings will inform about trait variation through time and its putative association with genetic variation and their role in shaping the invasive success in this species complex.

## Phenotypic Response of Flowering Plants to Limited Access to Natural Pollinator Community

Elisabeth Authier<sup>1</sup>, Simon Aeschbacher<sup>2</sup> and Léa Frachon<sup>1</sup>

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Plant–pollinator interactions are essential for plant reproductive success, and pollinators provide essential ecosystem services. However, the ongoing dramatic decline in pollinators threatens the existence of many wild and cultivated flowering plants. There is thus an urgent need to understand how plants respond to quantitative and qualitative changes in pollinator communities. Here, we used an experimental evolution approach to track phenotypic and genetic changes induced by limited access to the natural pollinator community. We artificially manipulated the temporal access of natural pollinators to the flowering plant *Brassica rapa* in a common garden across six generations. We measured morphological traits and fitness components each generation in three different treatments (hand-pollination, full access to natural pollinators, and limited access to natural pollinators). In the last generation, we observed phenotypic variations of floral display and total height of the plant, two traits related to the attractiveness to pollinators, among the three treatments. Moreover, we observed an overall increase of the number of seeds for the limited access treatment across generations, and significant variations of the herkogamy among treatments in the last generation. Our results suggest rapid phenotypic changes in traits related to pollinator attractiveness and plant reproductive function in response to limited pollinator access.

Many thanks for your support

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