Journal of Molecular Evolution: A European Meeting

Venue: National Museum of Natural Sciences-CSIC

Madrid (Spain)

25-27th August 2025

Program &

Book of Abstracts

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JOURNAL OF MOLECULAR EVOLUTION

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Program

Venue: Salón de actos

Monday, August 25th

9:00-9:25 Opening (CSIC authorities, David Liberles)

9:25-10:40 Section 1 (3T) New functions I:

- **David Liberles**, Structural and functional characterization of divergence of genes in a duplicated pathway
- **Erich Bornberg-Bauer**, "Chance and necessity in protein sequence space"
- **Beatriz Sabater-Muñoz**, Adaptive experimental evolution and genomic changes of newly duplicated genes in *Saccharomyces cerevisiae*

10:40-11:10 Coffee break

11:10-12:25 Section 2 (3T) New functions II:

- **Gaurav Diwan**, A phylogeny aware analysis of gene function for the biodiversity genomics era
- **Ivan Ayuso-Fernandez**, Redox robustness through hole hopping drives enzyme evolution)
- **David Alvarez-Ponce**, Solving the G-value paradox: Organismal complexity strongly correlates with the number of protein families

13:00-14:15 Lunch

14:15:15:30 Section 3 (3T) Speciation and populations:

- **Silu Wang** "On the mitonuclear energetics behind the tree of life"
- **Davorka Gulisija**, Evolution of phenotypic plasticity owing to migration
- **Rosa Fregel**, Paleogenomic inference of the effects of insularity in Canary populations

15:30-16:00 Coffee break

16:00-17:30 Free time for interactions

17:30-20:30 Visit to museum and welcome cocktail

Tuesday, August 26th

9:00-10:40 Section 4 (4T) Biomedical:

- **Mario Benitez-Prian**, Molecular evolution and *in silico* biomedical potential of antimicrobial peptides in caecilians
- **Kostantinos Voskarides**, The role of cancer genes in adaptation and evolution

- **Anet Filipova,** "Evaluating Aging Markers in Blood: Stable Telomeres and Sex-specific Changes in mtDNA Copy Number"

10:40-11:10 Coffee break

11:10-12:50 Section 5 (3T) Ecology I:

- **Jake Barber**, Mutators increase survivability and stability of pairwise communities
- **Eyal Privman**, Evolution of supergenes: lessons from social chromosomes
- **Zu-Shi Huang**, "Molecular phylogeny and adaptation of the cavefish genus *Sinocyclocheilus*"

13:00-14:15 Lunch

14:15:15:30 Section 6 (3T) Ecology II:

- **Yuan Fu Chan**, Phenotypic and genomic signatures of urban adaptation in *Raphanus raphanistrum*
- **Hector Lorente-Martinez**, Adaptive Evolution of Aquaporins in Amphibious Fishes: Insights into Structural Determinants of Ecological Transitions
- **Rafael Zardoya**, Venomics of cone snails

15:30-18:30 Coffee break and poster section

17:00-18:30 Workshop on Bayesian statistics (**Rosina Savisaar**, Mondego Science). Sala de Juntas

18:30-20:30 Editorial Board meeting (Sala de juntas) and Dinner

Wednesday, August 27th

9:00-11:05 Section 7 (5T) Methods:

- **Sudhir Kumar**, Revising Felsenstein's bootstrap support for phylogenomics
- **Cristina Landa**, The Regularized Maximum Likelihood and Minimum Evolution (REGMLAME) score improves and unifies ML, ME, Bayesian and Least square methods for phylogenetic inference.
- **Praveen Karanth**, Exploring patterns of mito-nuclear discordance in divergence estimates among tetrapods
- **Christina Toft**, Mapping transcriptional and fitness responses to acute and chronic oxidative stress in *Saccharomyces cerevisiae*
- **Miriam Caballero**, Integrating transcriptomics and genome occupancy data to identify epigenetically-regulated genes in plant-virus interactions

11:05-11:35 Coffee break

11:35-13:15 Section 8 (4T) New proteins:

- **Cara Weisman**, Genes from pieces: a new mechanism of gene birth in *Drosophila*
- **Lars Eicholt**, "Exploring structure and sequence space of *de novo* emerged and random proteins"
- **Evandro Ferrada**, On the foldable fraction of the protein sequence space

- **Lev Y. Yampolsky**, T>C Transitions Disproportionally Eliminate Stop Codons in Drosophila de novo ORFs

13:15-14:15 Lunch

14:15:15:30 Free time for interactions

Abstracts

1. **David Liberles**, "Structural and functional characterization of divergence of genes in a duplicated pathway", daliberles@temple.edu, Temple University.

Whole genome duplication duplicates every gene in a pathway. This is one mode through which new pathways can emerge through the loss of cross talk among duplicates. Using the myostatin pathway in Atlantic salmon as a model, where two rounds of whole genome duplication have occurred, the ongoing process of the formation of new pathways is evaluated. Sequences from Atlantic salmon (2WGD events) together with zebrafish (1 WGD event) and mice (0 WGD events) were used to construct gene trees for myostatin and two of its interacting partners (activin type II receptors and follistatin). The phylogenetic analysis establishes sets of 1:2:4 groupings and deviations from that due to gene loss. Further, structural modeling and docking programs are employed to assess changes in protein structure and their potential impact on binding interactions between copies within a species. The goal is to understand how WGD events influence pathway evolution through changes in protein binding interaction functions over the time frame of fish whole genome duplication events.

2. **Erich Bornberg-Bauer** "Chance and necessity in protein sequence space", ebb@bornberglab.org, University of Münster.

A long-standing riddle in molecular evolution concerns the question of how proteins, forming so many shapes and exercising so many fundamentally different functions, can evolve into new proteins, with new structures and functions. Several models try to explain such innovations without depriving organisms of its existing, possibly essential function exerted by the ""old"" protein. The most popular models include neofunctionalization, subfunctionalization (SUBF) by degenerative mutations, and dosage models, all focusing on adaptation after gene duplication. "Escape-from-Adaptive-Conflict" partially resolves this riddle by including adaptive processes before and after gene duplication that lead to multifunctional proteins (promiscuous enzymes), and divergence (SUBF).

We developed a theoretical framework that uses biophysical principles to infer the roles of functional promiscuity, gene dosage, gene duplication, point mutations, and selection pressures.

We find that selection pressures and duplication rates alone can determine which scenario will prevail. Multi-functionality becomes a crucial advantage when gene duplications are rare and an increase in mutational robustness, not necessarily functional optimisation, can be the sole driving force behind SUBF. Overall, this is the first model in which all three processes are unified and demonstrates that, given a certain rate of gene duplications and point mutations, selection pressure determines which processes will prevail. Furthermore, by mapping both RNA and protein-like models on a unified landscape with tunable neighbourhood properties, we demonstrate that the relationship between robustness and evolvability depends critically on the ratio of viable mutations, which are neutral (coding the same phenotype) and innovative (coding for a new phenotype). "

3. **Beatriz Sabater-Munoz**, "Adaptive experimental evolution and Genomic changes of newly duplicated genes in *Saccharomyces cerevisisae*", b.sabater.munoz@csic.es, i2SysBio CSIC

Since S. Ohno's foundational work on duplicated genes' fate (1970), several studies have been exploring how the scale of duplication — whether small-scale (SSDs) or whole-genome (WGDs) duplication — influences the functional evolution of duplicated genes. Mounting evidence suggests that duplicated scale can differentially affect the evolutionary trajectories of duplicated genes. However, the functional disparities between sequence divergence, expression divergence, and essentiality of the duplicates still remain as a general dispute between researchers, involving different evolutionary scenarios driven by the duplication event scale.

It is widely recognized that, *Saccharomyces cerevisiae* originated from an ancestral hybridization event, which involved whole genome duplication, small-scale duplications and genome reduction coetaneous steps. Its current genome retains approximately one-third duplicated genes, with nearly half of them derived from the WGD event, and the other half as SSDs. Despite advances in genome, transcriptome and proteome research, the interplay between gene duplicability, evolvability and evolution of transcriptional regulation—and how these factors contribute to functional divergence— remains an active area of investigation.

From an integrative perspective, we used *S. cerevisiae* strain BY4741, to explore how the interaction partners contribute to the relationship between sequence identity and functional similarity. Our aim was to identify key factors influencing duplicability and the retention of duplicated genes within an established genome structure. We selected three different genes, with differential number of protein-protein interactions, and tested: (i) their duplicability, (ii) their retention and how they affected the mutational landscape, and (iii) their phenotypic response to several adaptive stresses. This approach allowed us to investigate how duplication scale influences innovation, robustness and buffering.

4. **Gaurav Diwan**, " A phylogeny aware analysis of gene function for the biodiversity genomics era", gaurav.diwan@bioquant.uni-heidelberg.de, University of Heidelberg

A fundamental question in biology is: when did a certain function originate? Was it recent or existent millions of years ago in the ancestors or extant species? We are currently in an era where the burgeoning amount of whole genomic data and advancements in annotation methods has given rise to an unprecedented number of complete, well-annotated proteomes from across the tree of life. It is thus possible to leverage the data available in public databases and reconstruct the detailed evolutionary history of genes, pathways and more broadly biological functions. In this study, we used the homolog and ortholog information of ~4.5 million proteins from 508 species across the tree of life and traced the emergence and evolutionary history of every gene. Next, we grouped genes by their function (GO terms/protein domain architecture) or membership to biological pathways (KEGG/Reactome) and traced the emergence and evolutionary histories of these broader functional entities.

We found that the most biological entities originated in Eukaryotes, more specifically in vertebrates, angiosperms, and slime molds. Upon inspection of GO terms and KEGG/Reactome pathways enriched at each node in the tree, we observed characteristic adaptations emerging. We also show parallel evolution of functions across clades with large transitions such as aquatic to terrestrial and unicellular to multicellular. Overall, our work is the first to elucidate the detailed evolutionary histories of biological functions using genomic annotations, which can be explored on a web application. We envision this work will be useful to a wide variety of scientists interested in the molecular history of life on earth.

5. **Iván Ayuso Fernández**, "Redox robustness through hole hopping drives enzyme evolution", ivan.ayuso@cib.csic.es, Margarita Salas Center for Biological Research - CSIC

Enzymes known as lytic polysaccharide monooxygenases (LPMOs) are exceptionally powerful small redox enzymes that master the controlled generation and productive use of potentially damaging hydroxyl radicals in what essentially is a H2O2-driven peroxygenase reaction. We have used ancestral sequence reconstruction and enzyme resurrection to unravel evolutionary steps leading to this unprecedented catalytic power. Real-time monitoring of copper re-oxidation and amino acid radical formation showed evolutionary improvement of both the capacity to avoid futile turnover of H2O2 and of the ability to scavenge damaging radicals resulting from such turnover through a hole hopping pathway. These results show how selective pressure imposed by the need for generating a highly oxidizing intermediate shapes metalloenzymes, involving large parts of the enzyme, well beyond the catalytic center.

6. **David Alvarez-Ponce**, "Solving the G-value paradox: Organismal complexity strongly correlates with the number of protein families and domains", david.alvarez.ponce@gmail.com, University of Nevada

In the pre-genomic era, scientists were puzzled by the observation that haploid genome size (the C-value) did not correlate well with organismal complexity. This phenomenon, called the "C-value paradox", is mostly explained by the fact that protein-coding genes occupy only a small fraction of eukaryotic genomes. When the first genome sequences became available, scientists were even more surprised by the fact that the number of genes (G-value) was also a poor predictor of complexity, which gave rise to the "G-value paradox". The proposed explanations usually invoke mechanisms that increase the information content of each individual gene (protein–protein interactions, intrinsic disorder, post-translational modifications, alternative splicing, etc.). Less attention has been paid to mechanisms that increase the amount of genetic material but do not increase (or not to the same extent) the amount of information encoded in the genome, such as gene duplication and domain shuffling. Proteins belonging to the same family and/or sharing the same domains often carry out similar or even redundant functions. We thus hypothesized that an organism's number of different protein families and domains should be suitable predictors of organismal complexity. In agreement with our hypothesis, we observed that the number of protein families, clans, domains and motifs increases from simple to progressively more complex organisms. In addition, these metrics correlate with the number of cell types better than and independently of the number of protein-coding genes and several previously proposed predictors of organismal complexity. Our observations have the potential to represent a resolution to the G-value paradox.

7. **Silu Wang**, "On the mitonuclear energetics behind the tree of life", silu.wang@yahoo.com, State University of New York

The energy expenditure of mating signals is often divergent between species, mediating hybridization and introgression, and shaping speciation in the face of gene flow. The relative energetics of the mating signals can be underpinned by mitochondrial functional divergence, which contributes to hybrid mitonuclear incompatibility and speciation. This talk explores the connection between mitochondrial activity, mating signal energetics, and their impact on the formation and maintenance of species boundaries. In particular, I illustrate the mating signal energetics in four sensory modalities: visual, acoustic, kinesthetic, and chemosensory.

Integrating mitochondrial functions and mating signal energetics empowers the understanding of diverse forms and functions across the tree of life.

8. **Davorka Gulisija**, “Evolution of phenotypic plasticity owing to migration”, dgulisija@unm.edu, University of New Mexico

Phenotypic plasticity evolves when changing conditions drive populations away from their fitness optimum, enabling them to produce phenotypes better suited to the new environment. However, what maintains plasticity’s wide presence in nature remains unclear since plasticity must remain advantageous to persist. While many populations experience ongoing changing conditions, the maintenance of plasticity requires generational turnover such that parents and offspring regularly encounter different environments. Here, we show that migration between locally adapted populations significantly broadens conditions for the evolution and maintenance of phenotypic plasticity without the need for environmental change. These findings challenge traditional views on the evolution of plasticity by revealing that migration alone can drive its emergence and stable persistence. We support this by investigating a broad range of regimes and the effect in a two-locus, two-deme population genetic model using stochastic simulations and analytical approximation. We demonstrate a wide support for the maintenance of plasticity in finite populations due to migration, even if plasticity is costly, regardless of its genetic basis, provided migration rates are sufficiently large. We provide analytical conditions for adaptive maintenance of plasticity in terms of the strength of selection, migration, and fitness costs and benefits of plasticity.

9. **Rosa Fregel**, "Paleogenomic inference of the effects of insularity and isolation in human populations from the Canary Islands”, rfregel@ull.edu.es, Universidad de La Laguna

Multidisciplinary evidence gathered on the indigenous people of the Canary Islands indicates that their geographical origin was North Africa and that they colonized the islands at the beginning of the Common Era (2nd – 3rd centuries CE). However, aspects like how adaptation to the insular environments affected these populations until the European conquest (15th century CE) have received less attention. Recently, next-generation sequencing techniques have been applied to studying the Canarian indigenous peopling. In this talk, we will review all paleogenomic evidence gathered so far and present new genome-wide data from the islands of El Hierro and Gran Canaria.

Paleogenomic data indicate that insular populations were heterogeneous within the archipelago in terms of both their genetic composition and diversity. Although western and eastern islands share a similar genetic composition, the archipelago is structured, with the islands closer to the continent having a greater contribution from prehistorical populations from Europe. Island sub-populations also show differences in their genome-wide genetic diversity, with the smaller islands or those with fewer natural resources exhibiting the effects of isolation. In particular, El Hierro population was affected by strong genetic drift, leading to low genetic diversity and long runs of homozygosity. By combining genomic and radiocarbon data, we observe that the effects of genetic drift in El Hierro accumulated over time, in such a way that the diversity observed in early individuals steadily declined by the last centuries of the indigenous settlement, reaching the lowest values in the archipelago. On the contrary, the island of Gran Canaria shows one of the highest diversity values and a relatively constant effective population size over time. However, no migrants were detected in our dataset, suggesting that the lower effects of the genetic drift were likely due to a larger population size rather than gene flow.

10, **Mario Benítez Prián**, "Molecular evolution and *in silico* biomedical potential of antimicrobial peptides in caecilians", mario.benitezprian@gmail.com, Universidad Complutense de Madrid

Antimicrobial peptides (AMPs) are key components of vertebrate innate immunity, providing broad-spectrum antimicrobial activity while evading pathogen resistance. Although amphibians are a major source of AMPs, research has primarily focused on frogs, with caecilians (Gymnophiona) remaining largely understudied. This study investigates the diversity and evolutionary dynamics of AMPs in caecilians using genomic and transcriptomic data from eight representative species.

Through bioinformatic analyses, we identified over 400 candidate AMPs, classified into 29 peptide families. Four of these families predominantly exhibit primary antimicrobial activity, while the others may have secondary functions. Phylogenetic analyses revealed gene duplications, convergent evolution, and signatures of positive selection in five AMP families, suggesting evolutionary adaptations linked to antimicrobial function. Artificial intelligence (AI)-based predictions identified several peptides with strong antimicrobial potential and no cytotoxicity, highlighting their promise as therapeutic candidates. Additionally, structural modeling with AlphaFold revealed correlations between peptide 3D conformation and function.

Our findings suggest that caecilians, with their distinct evolutionary history, represent an untapped reservoir of bioactive peptides with pharmaceutical potential. *In silico* approaches provide a cost-effective strategy for identifying novel therapeutic agents, serving as a crucial preliminary step before experimental validation. Furthermore, we are developing a neural network model to detect antimicrobial peptides directly from genomic sequences, enhancing the discovery pipeline for novel AMPs. This research underscores the biomedical potential of caecilian AMPs in combating antibiotic resistance.

11. **Konstantinos Voskarides**, "The role of cancer genes in adaptation and evolution" voskarides.c@unic.ac.cy, University of Nicosia Medical School

Mutations in Tumor Suppressor Genes can cause several types of cancer. TP53 gene is mutated in at least 50% of tumors. However, evidence is increasing that these mutations can be adaptive, in human or animal populations, and at the somatic level as well.

Germline TP53 carcinogenic mutations have been associated with increased longevity in mouse, *Drosophila*, *C. elegans* and humans, and with higher fertilization rates in mice. Additionally, p53 amino-acid residues that cause cancer in humans, are part of the normal p53 protein sequence in some mammals, reducing apoptosis potential of their cells. It is assumed that this is the way that their cells resist under extreme cold and high altitude (hypoxia). Laboratory experiments gave evidence that carcinogenic TP53 mutations could be adaptive for zebrafish larvae, contributing to higher survival rate under extreme starvation conditions.

Similar evidence exists for humans. Extreme starvation exposure has been associated with some cancer types in humans. Additionally, genetic variants in or close to tumor suppressor genes are under selection in people living in extreme cold and high-altitude environments. These human populations exhibit very high incidence of cancer. Another study showed that BRCA1/2 mutations are related with increased fertility in Utah women.

At the somatic level, NOTCH1 and TP53 carcinogenic mutations were found under selection in a large percentage of somatic cells in healthy humans. It seems that carcinogenic mutations may protect our cells under harmful micro-environments. “Mutator” bacteria use the same mechanism to survive under antibiotic stress or other stressful conditions. This selection procedure may also explain why excessive antibiotic use predisposes humans for colorectal cancer.

These data show that evolution, adaptation, and selection can explain multiple phenomena related with cancer.

12. **Anet Filipova**, “Evaluating aging markers in blood: stable telomeres and sex-specific changes in mtDNA copy number”, amf0120@auburn.edu, Auburn University

Head-starting programs are increasingly used in reptile conservation to improve early survival by promoting expedited growth during early vulnerable juvenile stages. However, in species like the gopher tortoise (*Gopherus polyphemus*), endemic to the Southeast USA, the head-starting environment that promotes fast growth is often a dramatic shift from what those animals would be experiencing in the wild in their first year of life in its natural populations. Therefore, there is a significant gap in knowledge regarding how bypassing natural cold dormancy early in life impacts essential biological functions including growth trajectories, metabolic processes, and molecular responses in gopher tortoises that may impact fitness. In this study, we experimentally test the effects of bypassing first-year cold dormancy on growth rate, telomere length, mitochondrial DNA (mtDNA) density, and metabolic markers in head-started tortoises. We used a split-clutch design to assign 60 hatchlings from 14 clutches to two treatment groups: (1) a constant-warmth group raised under standard head-starting conditions without dormancy, and (2) a cold-dormancy group housed under simulated Alabama winter conditions. We took monthly body measurements and collected blood and plasma at four timepoints: before-dormancy (before), during-dormancy (during), 3 weeks post-dormancy (3Wk.Post) and 3 months post-dormancy (3Mo.Post). We estimated growth rate across timepoints, used qPCR to estimate mtDNA density and telomere length, and quantified glucose, total triglycerides and acetyl-CoA in blood plasma. We found that animals that experienced cold-dormancy were 8.47 mm smaller at 3Mo.Post dormancy relative to the constant-warmth group. This was due to the cold-dormancy group having a growth rate 0.54 g/day lower than the constant-warmth group during dormancy, and 0.51 g/day lower in the three weeks after coming out of dormancy (both results were statistically significant). However, 3Mo.Post dormancy there was no statistically significant difference in growth rate between treatment groups. This indicates that although the cold-dormancy animals were smaller, they were able to resume the same growth rate as the constant-warmth group by 3Mo.Post dormancy but not the same body size. Additionally, there was no effect of either growth rate or treatment on telomere length across timepoints. However, while there was no difference in mtDNA density between the two treatment groups during dormancy, we found that the cold-dormancy group showed a significant 27.1% increase in mtDNA density at 3Wk.Post dormancy followed by a significant 17.1% decrease compared to the constant-warmth group 3 Mo.Post dormancy. Furthermore, during cold-dormancy tortoises had reduced plasma glucose compared to the constant-warmth treatment, but there were no differences at post-dormancy timepoints. Finally, our results showed that experiencing cold-dormancy caused a significant decrease in both total triglycerides and acetyl-CoA at 3Wk.Post dormancy compared to the constant-warmth. Total triglycerides at 3Mo.Post were not significant (acetyl-CoA was not measured). Our findings suggest that the period of growth and

metabolic suppression associated with cold dormancy persists for at least 3 weeks after dormancy but is reversible by 3 months after dormancy. While telomere length might be maintained through compensatory cellular mechanisms during early life stage, the metabolic responses could influence energetic priorities and recovery processes associated with arousal from dormancy. These results have important implications for conservation management, suggesting that supporting the growth and metabolic health of reintroduced gopher tortoises may require greater attention to their natural physiological rhythms.

13. **Jake Barber**, "Mutators increase survivability and stability of pairwise communities", jake.barber@upm.es, Universidad Politecnica de Madrid

Mutualisms underpin many major phenomenons in Biology, in both fundamental and applied fields. However, work has previously shown that mutualisms can break down into competing individuals under environmental stress, as the inability of one partner incentivises the other partners to revolve independence. Therefore to predict (and prevent) mutualism breakdown we need to understand the balance between opposing classes of beneficial mutations - those that invest in the mutualism and those that promote independence. Furthermore, it is not known what role mutators - strains with an elevated and biased mutation rate - play in this interaction, and which class of beneficial mutations that mutators would promote. To explore the stability of microbial communities across mutational spectra, we evolved synthetic auxotrophic communities of E. coli with and without mutator alleles, against a background of periodically increase antibiotic concentration. We found that only pairwise communities where both members contained mutator strains were consistently able to survive until the end of the experiment, whilst also remaining mutualist communities. This shows that reversion to autonomy is not the only route to adapting to environmental stress in synthetic communities, and that mutators can prevent mutualism breakdown in stressful environments.

14. **Eyal Privman**, "Evolution of supergenes: lessons from social chromosomes", eyal.privman@gmail.com, University of Haifa, Israel

Supergenes are large genomic regions with suppressed recombination that determine complex polymorphic traits. "Social chromosomes" harbor supergenes that determine colony structure in polymorphic ant species, which form either monogyne (single queen) or polygyne colonies (with multiple queens). Two analogous supergenes evolved independently in two diverged ant lineages – *Solenopsis* fire ants and *Formica* wood ants. In both cases, the monogyne form is associated with one supergene haplotype (M) and the polygyne form is associated with the other (P). We discovered a third such system in the desert ant *Cataglyphis niger*, by reduced-representation genomic sequencing (RAD-seq) of 20 individuals from each of 30 nests. Our analyses identify a large chromosomal region of suppressed recombination between the M and P haplotypes, at least 6Mbp long, which is associated with the social structure. Surprisingly, the *Cataglyphis* social chromosome is homologous to the *Solenopsis* chromosome, even though these lineages diverged more than 90 million years ago. We suggest that this ancient chromosome harbors an ancestral genetic toolkit that was reused for the repeated evolution of sociobiological traits across diverse ant species.

Previous studies in *Solenopsis* and *Formica* suggest that selfishness of the supergene contributes to its maintenance over millions of years. The *Solenopsis* social chromosome is an exceptional example of a "green beard" effect, whereby polygyne workers kill queens unless they harbor the P chromosome. The Formica social chromosome illicit a so-called "maternal

effect killing", whereby offspring of a polygyne queen fail to develop unless they harbor the P chromosome. Our preliminary results suggest similar maternal effect killing in *Cataglyphis*.

The repeated evolution of supergenes (in *Solenopsis* and *Cataglyphis*) on an ancient social chromosome present excellent opportunities for the study of evolution and maintenance of genomic architectures underlying adaptive complex polymorphisms.

15. **Zu-Shi Huang**, "Molecular phylogeny and adaptation of the cavefish genus *Sinocyclocheilus*", huangzs@ioz.ac.cn, Institute of Zoology, Chinese Academy of Sciences

Cave animals possess remarkable phenotypes associated with existence in their dark environments such as eye degeneration. Genetic data provide an important opportunity to understand the phylogeny and adaptive evolution of the cavefish *Sinocyclocheilus* endemic to China that comprises the river-dwelling surface fish and the cave-dwelling cavefish. The genomic data helped to resolve that the more than 100 species of the genus *Sinocyclocheilus* can be divided into four groups, and the species with degenerate eyes can belong to the same group as the species with normal eyes. It suggests that the absence and degeneration of eyes may be the result of convergent evolution, adapting to cave environments through varying degrees of defects in photoreceptor cells. Some genes such as foxg1b, Otx2, Otx5, sox2 and crx were identified as possibly having an important role in the adaptive evolution of cave fishes, showing different genetic mechanisms of eye degeneration. Neural compartmentalization of lipid distribution and lipid metabolism is associated with the evolution of troglomorphic traits in *Sinocyclocheilus*.

16. **Yuan Fu Chan**, "Phenotypic and Genomic Signatures of Urban Adaptation in *Raphanus raphanistrum*", yfchan48@gmail.com, University of Naples Federico II

Urbanisation leads to the transformations of abiotic and biotic environments in ways that mediate evolutionary adaptation in organisms. However, the impact of urbanisation on evolutionary processes in populations remains poorly understood. Here, we investigate how urbanisation shapes the evolutionary response of *Raphanus raphanistrum*. We conducted field surveys across the Campania region in southern Italy to examine the phenotypic divergence between urban and natural populations of *R. raphanistrum*. Seeds collected from these populations were used in a common garden experiment to assess the genetic basis of observed trait differences. We also performed a genome-wide association study (GWAS) to identify genetic variants linked to urban adaptation. Our results show that urban populations had significantly smaller flowers and shorter flowering times compared to natural populations. The common garden experiment suggested that genetically based adaptation underpinned this divergence. Selection analyses indicated that natural selection generally favours shorter flowering times and larger flowers. However, a trade-off between these traits suggests habitat-specific selection: shorter flowering times were favoured in urban environments, while larger flowers were selected in natural ones. The GWAS identifies 32 single-nucleotide polymorphisms (SNP) significantly associated with urbanisation. 21 of which are located within gene regions. Notably, one of these genes, AT3g27390 is linked to the transition to reproductive development. Overall, our findings highlight how natural selection shapes adaptive phenotypic divergence in *R. raphanistrum* and reveal candidate genes underlying this response.

17. **Hector Lorente**, "Adaptive evolution of aquaporins in amphibious fishes: insights into structural determinants of ecological transitions", hlorente@ucm.es, Universidad Complutense de Madrid

Aquaporins (AQPs) are transmembrane protein channels known for their capacity to transport water and a diverse amount of small, uncharged solutes. Each AQP monomer typically comprises six transmembrane alpha helices and two conserved NPAs (Asparagine-Proline-Alanine) motifs at the central part of the pore, integral to the final function of these proteins. It has been hypothesized that the emergence of three novel AQP classes played an important role in the conquer of land by tetrapods. In this context, actinopterygian amphibious fishes serve as a valuable framework for understanding the initial stages of adaptation to terrestrial environments. Our study, explored the genomes of 22 actinopterygian amphibious fishes in order to determine events of contractions or expansions in the AQP repertoire of these animals and to link adaptive changes in these proteins to the evolution of amphibious lifestyles. Our findings revealed potential positive selection events in 19 positions within 16 AQPs across 13 different species of amphibious fishes. Particularly interesting was the result concerning one of the NPAs motifs of AQP11b in (terrestrial) mudskippers and other (fully aquatic) gobioid relatives; the canonical N was substituted with S (serine). The initial structural analysis of this aquaporin revealed an additional alpha-helix, a feature that appears to be common among all teleost AQP11 genes but not in other vertebrates. We are now investigating in detail whether this mutation could have led to an enlargement of the pore channel, indicating a potential modification of function compared to the canonical AQP11 protein. The presence of this mutation suggests a possible role in the transition from marine to freshwater environments in the gobioid ancestor that could have subsequently facilitated the terrestrial transition of mudskippers.

18. **Rafael Zardoya**, “Venomics of cone snails”, rafaz@mncn.csic.es, Museo Nacional de Ciencias Naturales-CSIC

Cone snails (Gastropoda: Conidae) are marine predators, best known for their colorful shells and great species diversity that live in tropical seas worldwide. They produce venoms to capture their preys, which are worms, snails or fish. The venoms are complex cocktails of >200 short peptides named conotoxins that are species-specific. We conduct comparative analyses at the genomic, transcriptomic and proteomic levels to unravel key details about the genetic basis of conotoxin diversity and evolution.

19. **Sudhir Kumar**, "Revising Felsenstein’s bootstrap support for Phylogenomics”, genome.sk@gmail.com, Temple University

Felsenstein’s bootstrap method is widely used for assessing the robustness of evolutionary inferences. Bootstrap support increases rapidly with the addition of genomic loci in clades inferred using concatenated super-alignments, CSA, in phylogenomics. However, it may support incorrect organismal relationships with high confidence, because it fails to incorporate uncertainty contributed by phylogenetic incongruence across sites and loci. We introduce Net Bootstrap Support (NBS) to account for the overconfidence in Felsenstein’s bootstrap support by embracing the phylogenetic variation in CSA analysis. NBS is estimated using the little bootstraps approach in which multiple phylogenomic site subsamples are bootstrapped independently, producing a collection of bootstrap support for each clade in the CSA phylogeny. Analyses of empirical and simulated datasets demonstrate that NBS solves the overconfidence problem of CSA bootstrap analysis. Also, NBS matched or exceeded the performance of partitioned and multi-species coalescence methods, despite not requiring any knowledge of loci boundaries or data partitions. NBS analysis makes CSA phylogenetics very computationally efficient, as it runs hundreds of times faster than whose CSA analysis, while reducing memory needs from gigabytes to megabytes. These efficiencies make phylogenomics

feasible even on personal computers, with NBS analysis obviating the need for the multi-species coalescence and multi-partition phylogenetic analyses. Therefore, NBS is practical and reliable for reconstructing organismal relationships with confidence and efficiency.

20. **Cristina Landa**, "The Regularized Maximum Likelihood and Minimum Evolution (REGMLAME) score improves and unifies ML, ME, Bayesian and Least square methods for phylogenetic inference", ubastolla@cbm.csic.es, Centro de Biología Molecular Severo Ochoa (CSIC-UAM)

The maximum likelihood (ML) principle for phylogenetic inference is probably the most used criterion for phylogenetic inference. However, despite the hope that this principle would infer unbiased trees, early simulations of Felsenstein and co-workers already showed that biases are indeed present. We show that this bias is inherent to the essence of the method, since the likelihood score, for its very mathematical definition, is very strongly correlated with the fitted parameters, i.e. the branch lengths. In the context of regression, in such situations it is known that the regression must be suitably regularized, minimizing a combination of the score plus some function of the fitted parameters (branch lengths). In the context of phylogenetic inference, regularized ML is also equivalent to minimum evolution (ME) regularized with the likelihood score and to Bayesian inference with exponential tree priors. Based on the correspondence between least square fits and ML, distance methods should be regularized in a similar way. We show that the regularization makes tree inference less biased and more robust and propose an algorithm for regularized fits.

21. **Praveen Karanth**, "Exploring patterns of mito-nuclear discordance in divergence estimates among tetrapods", karanth@iisc.ac.in, Indian Institute of Science

Phylogenetic studies across a range of tetrapod groups have historically utilised mitochondrial markers, and in more recent times, concatenated mito-nuclear matrices, or nuclear loci to infer divergence estimates. These studies have often reported discordance between divergence dates estimates across different data types. Here we aimed to quantify the extent of divergence disparity inferred by the aforementioned data types in four tetrapod groups, namely primates (Order Primates), birds (Class Aves), squamates (Order Squamata), and anurans (Order Anura), while controlling for calibration strategies, taxon sampling and other a priori distributions. We also explored substitution saturation for all groups across data types in order to elucidate its role in generating unreliable divergence estimates. Results indicate that mitochondrial estimates consistently underestimate basal divergence times and overestimate recent divergences across nearly all groups apart from anurans, as compared to the nuclear datasets. We also find that divergence times estimated using concatenated matrices skew in favour of the nuclear tree for all groups. While substitution saturation was substantial in all of the mitochondrial marker datasets across groups, interestingly it was also present in the nuclear dataset for anurans, resulting in a reduction in overall mito-nuclear divergence disparity for the crown ages of the group. These results call for a revisit of divergence dates estimated using mitochondrial markers, while advocating for saturation testing for all datasets prior to molecular dating. Lastly, this study highlights the inherent nuclear marker bias of divergence dates estimated using concatenated mito-nuclear alignments.

22. **Christina Toft**, "Mapping transcriptional and fitness responses to acute and chronic oxidative stress in *Saccharomyces cerevisisae*", christina.toft@csic.es, I2SysBio-CSIC

Organisms are constantly exposed to environmental stressors that vary in both intensity and duration, requiring flexible and time-sensitive responses at physiological and molecular levels. Oxidative stress, a common challenge arising from both internal metabolism and external

sources, is one such condition. Understanding how organisms respond to oxidative stress across different timescales is essential for uncovering the evolutionary mechanisms of stress tolerance and adaptation. In this study, we investigate how yeast populations adapt to oxidative stress by comparing physiological performance and gene expression under both acute and chronic exposure.

To assess the impact of stress duration on adaptation, we compared growth and gene expression patterns across multiple time points. We found consistent differences in growth dynamics between stress-exposed and control populations. Populations evolved under chronic oxidative stress retained higher fitness in the stress environment but exhibited reduced performance under standard conditions, indicating a trade-off in adaptation. At the transcriptional level, more significantly differentially expressed genes were observed in the chronic state compared to the acute response. While most expression changes were specific to individual populations and time points, functionally similar genes tended to respond across conditions. Broadly, stress exposure was associated with downregulation of biosynthetic processes and upregulation of genes involved in protein production, a pattern supported by pathway-level analyses.

These findings illustrate how the duration of environmental stress shapes both physiological and molecular adaptation, revealing distinct strategies for coping with acute versus chronic oxidative stress. The observed trade-offs in fitness and functional convergence in gene expression suggest that organisms may prioritize different adaptive mechanisms depending on the timescale of exposure.

23. **Miriam Caballero Cerveró**, "Integrating transcriptomics and genome occupancy data to identify epigenetically-regulated genes in plant-virus interactions", miriam.caballero@csic.es, Universidad de Valencia

Our project aims to identify epigenetically regulated genes in plant-virus interactions using Arabidopsis thaliana infected with Turnip mosaic virus (TuMV). This research explores how epigenetic modifications, such as histone marks, may influence plant responses to viral infections. Introduction: Plant-virus interactions are governed by complex mechanisms, with epigenetic regulation playing a crucial role. This study investigates how modifications to histone marks H3K4 and H3K36 affect gene expression in Arabidopsis during viral infection, using mutants with defects in histone-modifying proteins (sdg8 and atx1). These mutants have shown differences in symptom severity and viral load, suggesting a potential role for epigenetic regulation in antiviral defense. Objectives: The main objectives of this project are: 1) To analyze ChIP-seq data from infected and control epigenetic mutants, 2) To perform transcriptomic profiling to identify differentially expressed genes, 3) To select genes potentially regulated by epigenetic mechanisms, and 4) To verify the involvement of transposable elements in gene regulation. Methodology: The study follows a work plan that includes collecting samples from mutant and wild-type plants, both infected and uninfected. ChIP-seq analysis will be conducted to study histone modifications (H3K4 and H3K36), and RNA-seq will be used to evaluate gene expression. Bioinformatics tools will be applied to integrate these data sets to identify genes whose epigenetic control is associated with viral infection. Additionally, the role of transposable elements in gene regulation will be investigated. Results: The project identified key genes that are epigenetically regulated and play a significant role in the plant response to viral infections. Transposable elements are also anticipated to be involved in the modulation of these genes, providing new insights into plant defense mechanisms. Conclusions: This thesis provides new evidence on how epigenetic marks

influence gene expression during plant-virus interactions and offers a solid foundation for future research aimed at enhancing plant disease resistance through epigenetic manipulation.

24. **Cara Weisman**, “Genes from pieces: a new mechanism of gene birth in *Drosophila*”, cara.weisman@gmail.com, Princeton University

Where do new genes come from? Reconstructing the evolutionary history of a novel *Drosophila* gene has revealed what I propose are “tinkering loci”: genomic regions where genes are copied, broken into pieces, reshuffled, rapidly refined, and often transcribed, creating new chimeric transcripts. I will discuss the structure and function of these loci, describe their patterns across species, and speculate on their implications for the evolution of eukaryotic genomes.

25. **Lars Eicholt**, “Exploring structure and sequence space of *de novo* emerged and random proteins”,lars.eicholt@uni-muenster.de, University of Muenster

De novo proteins are newly emerged proteins from previously non-coding DNA, while random proteins are artificially generated sequences with no evolutionary history. Both types challenge traditional bioinformatics, as their positions in protein sequence space are far removed from conserved protein families. This remoteness renders homology-based structure and function prediction tools largely ineffective due to the absence of detectable sequence similarity and co-evolutionary signals. We systematically analyzed large datasets of de novo, randomized, and conserved proteins using state-of-the-art structure prediction tools such as AlphaFold and ESMFold. We observed substantial discrepancies in predicted structures for de novo and random proteins, along with markedly different behaviors in confidence scores (pLDDT) compared to conserved proteins. These findings underscore the limitations of current prediction models when applied to evolutionarily novel or synthetic sequences, revealing potential biases and blind spots in their training data. To overcome these limitations, we propose a roadmap integrating clustering techniques and molecular dynamics (MD) simulations to refine structure predictions and assess protein stability beyond static models. Our preliminary clustering results suggest that some de novo proteins share biophysical features with structured proteins despite lacking detectable homology. To further explore the position of de novo and randomized proteins within sequence space, we employed alignment-free, k-mer-based distance metrics and statistical approaches to compare the spatial distributions of conserved, de novo, intergenic and random sequences. Overall, our work provides a framework for evaluating novel protein sequences and lays the foundation for improving predictive tools for proteins remote in sequence space It highlights the need for more inclusive models and offers new perspectives on the biophysical and evolutionary landscapes of de novo emerged and randomized proteins.

26. **Evandro Ferrada**, "On the foldable fraction of the protein sequence space”, evandro.ferrada@uv.cl, Universidad de Valparaiso

A fundamental question in molecular evolution relates to the probability of a random sequence to fold onto a stable structure. Answering this question could shed light on the evolution of the first foldable peptides, or the emergence and evolution of de novo protein-coding genes. To explore this question I took a large and homogeneous sample of short peptides, and use AlphaFold to estimate whether they fold. To assess the prediction ability of AlphaFold I use sequences of known structures, as well as some known to be structurally disordered.

27. **Lev Y Yampolsky**, “"T>C transitions disproportionally eliminate stop codons in *Drosophila de novo* ORFs”, yampolsk@etsu.edu, East Tennessee State University

Creation of a *de novo* ORF from non-coding sequence requires elimination of multiple stop codons, a process that eludes both neutral and selective explanation. In particular, in the neutral case, the ubiquitous mutational biases and strand asymmetry should prevent elongation of a de novo ORF by introducing rather than eliminating stops. However, we observed an inverse pattern, namely that stop codons in 103 de novo *Drosophila* genes were disproportionally frequently eliminated by the T->C substitution (relative to all substitutions in the gene region Fisher's Exact Test, P<0.0001). We hypothesize that this bias may be created by the of ADAR adenosine deaminases acting on DNA/RNA hybrids during transcription. Such A>I deamination in the template strand is likely to cause T>C transitions in the coding strand in the next round of replication. Thus, once transcription of an incipient gene starts, a mutational bias introduced by ADAR DNA editing with generate a net loss of stop codons in the coding strand.

POSTERS
(In alphabetical order)

1. **Miguel Arenas**, "Investigating evolutionary patterns in SARS-CoV-2 proteins”, marenas@uvigo.es, Universidade de Vigo

The understanding of molecular evolutionary trajectories of emerging viruses such as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is fundamental for the design of appropriate therapies. In this context, we investigated evolutionary patterns observed in SARS-CoV-2 proteins, including the development of substitution models that can be useful to make phylogenetic inferences. The reduced protein diversity observed in this virus complicated the identification of evolutionary patterns presenting sufficient statistical support, but the evolution of certain structural properties of these proteins could be predicted. This study was supported by the Grant CNS2023-144363 funded by MICIU/AEI/10.13039/501100011033 and by European Union NextGenerationEU/PRTR.

2. **David Ferreiro**, "Evaluation of insertion and deletion rates in the Coronaviridae family”, david.ferreiro.garcia@uvigo.gal, Universidade de Vigo

Insertion and deletion events usually inform about significant evolutionary changes but they are often overlooked in phylogenetic studies. Indels are essential to understand genomic diversity and often influence biological processes including immune escape, drug resistance and adaptive selection. In order to understand the emergence of SARS-CoV-2, we think that indels should be took into account and this makes necessary to investigate the presence of indels in the Coronaviridae family, and making comparisons with those observed in SARS-CoV-2. Thus, following some previous studies, we developed a method to estimate insertion and deletion rates based on the approximate Bayesian computation (ABC). We defined a variety of summary statistics informative about the frequency and distribution of indels along sequences and we performed extensive simulations for training and testing the accuracy of the ABC method. We found that the method was able to accurately differentiate between different indel distribution models and could also accurately estimate insertion and deletion rates, but only when the data has enough diversity. We applied the method to compare the insertion and deletion rates at the whole genome and the S gene in a set of viruses of the Coronaviridae as well as in the SARS-CoV-2 alone. In general, we found a higher deletion rate compared to the insertion rate in all the study data. Indeed, as expected, we found that the insertion and deletion rates were lower in SARS-CoV-2 than in the entire Coronaviridae family. Still, further work that we are not performing is required to understand the evolution of these indels. This

study was supported by the Grant CNS2023-144363 funded by MICIU/AEI/10.13039/501100011033 and by European Union NextGenerationEU/PRTR. LDGV was funded by «Programa de axudas á etapa predoutoral da Xunta de Galicia (Consellería de Cultura, Educación, Formación Profesional e Universidades) cofinanciado pola Unión Europea no marco do Programa FSE+ Galicia 2021-2027.

3. **Joseph Hannon Bozorgmehr**, "The retrogenic origins of many lineage-specific orphans", bozorgmehr@hotmail.co.uk, C3-AI

Pseudogenes have often be thought of as defunct ""junk"", only of interest to those studying rates of neutral evolution. Most are copies of genes that have been disabled and are either not transcribed or functionally inactive. Growing evidence now suggests that some pseudogenes, or at least fragments of them, are transcribed and translated. If their sequences have diverged significantly, owing to drift, they may be ""resurrected"" as a new gene with a modified function. Given the recent interest in the ""de novo birth"" of genes from non-coding DNA, the resurrection of pseudogenes, especially retrogenes, may provide a clearer picture of what is reported in various studies where homology detection is awry.

4. **Paula Iglesias-Rivas**, "Heterogeneous evolution and diversity among genomic regions of emerging viruses", paula.iglesias.rivas@uvigo.es, Universidade de Vigo.

Despite widespread vaccination efforts, the severe acute respiratory syndrome coronavirus 2 (SARS_CoV_2) continues to pose significant questions regarding its future impact on public health. Indeed, other emerging viruses, such as the Crimean-Congo haemorrhagic fever virus (CCHFV), do not present yet a vaccine. In this regard, understanding the virus molecular evolution, particularly in protein-coding regions targeted by treatments, is fundamental. We analyzed a large number of SARS-CoV-2 and CCHFV genomes to assess the diversity, rates of evolution and adaptation of the corresponding protein-coding regions. Overall, we found a wide variation of evolutionary patterns among genomic regions and over time, indicating that forecasting molecular evolution in these viruses, which could provide relevant information for treatments design, is complex and remarks the importance of monitoring virus evolution. This study was supported by the Grant CNS2023-144363 funded by MICIU/AEI/10.13039/501100011033 and by European Union NextGenerationEU/PRTR. LDGV was funded by «Programa de axudas á etapa predoutoral da Xunta de Galicia (Consellería de Cultura, Educación, Formación Profesional e Universidades) cofinanciado pola Unión Europea no marco do Programa FSE+ Galicia 2021-2027».

5. **Iker Irisarri**, "Stitch or cluster? A comparison of alternative phylogenomic dataset assembly strategies for blenny fish", Iker.irisarri@mncn.csic.es, Museo Nacional de Ciencias Naturales-CSIC.

Phylogenomics has revolutionized the way we infer evolutionary relationships. Several bioinformatic pipelines have been developed for assembling phylogenomic datasets, in which orthology inference is a key step. We compared two alternative dataset assembly strategies: sequence clustering (Orthofinder) and a new similarity-based approach that enriches a predefined set of BUSCO genes (Patchwork). With data reuse in mind, we downloaded all publicly available genomic data for the fish family Blenniidae, which is a typical scenario of heterogeneous source data (genome skimming, transcriptomes, genomes) obtained by different sequencing technologies (Illumina short reads, long Nanopore reads, 454 pyrosequencing). These data are characterized by various levels of read length, sequencing depth and per-base accuracies, which allowed us to benchmark the power and limitations of the two assembly pipelines and establish minimum quality standards on various sequencing data types. Our study is the first attempt to test the evolutionary relationships among combtooth blenny fish with phylogenomic data. We also explore two approaches to combine marker-rich phylogenomic data with taxonomically broad legacy multilocus markers.

6. **Irene Martinez-Velasco**, "Genomic approaches to conserve partially clonal systems under semi-intensive management: The case of *Agave karwinskii*", imarvel@ecologia.unam.mx. Universidad Nacional Autónoma de México (UNAM)

In Mesoamerica, the management of wild plants is practiced, and these are cultivated under different production contexts. In economically significant species, such as agaves, direct extraction and agricultural management practices alter populations' genetic composition. Currently, many agaves used in mezcal production are intensively propagated to meet the national and international demand for this beverage. Two agave species facing strong selective processes are *Agave karwinskii* and *Agave cupreata*. Both species are ecologically, economically, and culturally important regional resources. The current demand in mezcal production has positioned them nationally as intensely exploited species. The prevailing reproduction method in most agave species, such as *Agave karwinksii*, involves underground rhizomes, facilitating the establishment of commercial plantations. However, species like *Agave cupreata* do not produce basal shoots or bulbils, depend entirely on seed production, making nursery establishment indispensable for mass plant production. What genomic patterns can we expect in a species with dual reproductive modes and a restricted distribution (*Agave karwinskii*), compared to an agave that reproduces exclusively sexually and has a wide distribution (*Agave cupreata*)?

To address this question, we analyzed the relationships within and between populations under different degrees of agricultural management, from wild and minimally managed to commercially exploited, to understand in detail the distribution of their genomic variation and whether it may be lost due to agricultural management.

Individuals from both species were collected across their natural distribution: 39 sites for *Agave karwinskii* and 26 for *Agave cupreata*. DNA extraction was performed using previously published protocols. The resulting genomic DNA was then converted into double-digest RAD-seq libraries. Raw sequences were processed by removing adapters and low-quality bases. ipyrad was used for assembly and SNP calling, using a transcriptome of Agave tequilana as a reference. Final filtering included read depth, allele frequency, missing data thresholds, Hardy-Weinberg equilibrium, and linkage disequilibrium. Final files were generated identifying the SNPs, which were then used in downstream analyses.

We will present the results of an analysis of 300 individuals of *A. karwinskii* collected in the states of Puebla and Oaxaca and 142 individuals of *A. cupreata* from Guerrero and Michoacán. We used 7,156 and 11,852 SNPs, respectively, to obtain diversity statistics and describe the genomic differentiation patterns in the studied populations.

From a genomic perspective, establishing Evolutionary Significant Units (ESUs) and Management Units (MUs) is essential for the conservation of both species. The results suggest that in the case of *Agave karwinskii*, the populations from Puebla and Oaxaca should be considered separate ESUs. Within each, MUs should account for the two reproductive strategies present (sexual and clonal). For *Agave cupreata*, two MUs are proposed, corresponding to the region of Guerrero and Michoacán.

Although our results indicate overall good genomic health for both species, we identified populations at risk. Current extractive management practices have overlooked the traditional selection method that once contributed to the genomic diversity enrichment. Moreover, the future outlook points toward a steady decline of wild populations. Therefore, urgent measures must be implemented, including the establishment of nurseries, local germplasm banks, and reforestation programs aimed at restoring pollination corridors and connectivity among populations.

7. **Brynleigh Payne**, “Acute heat stress results in sex-specific mortality in birds“, bep0022@auburn.edu, Auburn University.

As temperatures reach unprecedented highs due to global warming, understanding of mechanistic basis for adaptability to heat stress is essential to predict the impact of extreme temperature events on the persistence of animal populations. The overarching goal of this study was to understand the sex-specific effects of acute heat stress on physiological blood measures and cellular/molecular damage. Zebra Finches (*Taeniopygia guttata castanotis*), desert-adapted Australian birds, provide an ecologically relevant study system. Stress resistance has been suggested to be sex-biased with possible links to homogamety/heterogamety of the sex chromosomes and differential hormone expression. As the heterogametic sex, we hypothesized that females (ZW) would be more susceptible to damage from heat stress than males (ZZ).

We conducted an acute heat stress experiment using zebra finches bred at Auburn University, that were all adults under one-year-old. Male and female birds were assigned to two groups: Control, maintained at 22.5°C, and Acute Heat, exposed to 43°C for 5 hours. Baseline blood samples were taken two weeks prior to treatment and blood/tissues were collected immediately after treatment. Fresh whole blood was used for hematocrit, glucose, and ketone measures. DNA from blood cells was used to quantify telomeres and mtDNA copy number by quantitative real-time PCR. Telomeres are known biomarkers of acute and chronic stressors, having demonstrated to be predictors of longevity in the species. Mitochondrial DNA copy number is predictive of metabolic capacity and autophagy in response to mitochondrial damage. Specifically, we predicted that telomere lengths would be shorter and mtDNA copy number lower in females prior to heat exposure if they are more vulnerable to the stressor.

Despite the treatment of 43°C for 5 hours reported to be non-lethal in the literature, in this experiment we observed 12.5% mortality (11 birds) from this acute heat stress treatment. Only female birds died. Upon closer inspection of the data, we observed that all but one of the birds that died were from a “younger” cohort of adults (4-6 months old), relative to a slightly older cohort of adults (9-11 months old). We generated model-predicted probabilities for mortality and found both sex and age-category to be significant predictors (P-value < 0.05), with young females having 22.2% predictive probability of mortality and older females only 4.3%, while males of both age categories have near zero predictive probability of mortality. To test if molecular biomarkers taken at baseline were predictive of mortality due to acute heat, we compared young heat stressed females that did and did not die. Preliminary analysis suggests none of the baseline molecular biomarkers were predictive of mortality.

Our unexpected mortality results indicate that young adult females are the most susceptible group of young adults to heat stress, which could have important consequences on future recruitment into the population under climate change scenarios. This finding emphasizes the importance of studying the effects of stress across sexes and age classes to understand the impact of extreme heat events on population viability. However, this was a lab experiment on captive reared birds. Further research is needed to determine if these results are replicable in lab and field populations. Additional research is underway in our group to examine how males and females differed in cellular damage and repair processes that may contribute to the sex-biased mortality. This research brings new insight to sex-biased response to heat stress in birds.

8. **Elena Pazos-Linares**, "Estimating substitution and recombination rates from protein sequences accounting for structural constraints with approximate Bayesian computation", elena.pazos@uvigo.gal, Universidade de Vigo, Spain.

Mutation and recombination are fundamental evolutionary processes, upon which selection operates, to produce the observed diversity in nucleotide and amino acid sequences. Unlike nucleotide sequences, the estimation of the recombination rate in protein sequences has been little explored. Indeed, commonly used evolutionary analyses of protein sequences ignore evolutionary constraints on the protein structure. Considering these concerns, following our previous works, we present the design of a method to estimate population substitution and recombination rates from protein sequences under structurally constrained substitution models through the approximate Bayesian computation approach. The believe that substitution and recombination events can be characterized by summary statistics based on structural constraints considering the consequences of some of these events on the protein folding stability. We will apply the method to viral proteins and the findings will be compared with traditional estimation methods that ignore selection on the protein structure. This work was supported by the Project PID2023-151032NB-C22 funded by MICIU/AEI/10.13039/501100011033 and by FEDER, UE.

9. **Rosina Savisaar**, "Modelling rates of horizontal transfer as a function of species relatedness", rosina@mondegoscience.com, Mondego Science.

Horizontal transfer of transposable elements (HTT) is an important driver of genome evolution, yet the factors conditioning this phenomenon remain poorly characterized. Here, we screened 247 animal genomes from four phyla (annelids, arthropods, mollusks, chordates) to evaluate the suspected positive effect of phylogenetic relatedness on HTT. Exploratory analysis indeed revealed a tendency for more transfers between species that were more closely related to each-other. We next rigorously quantified the strength of this effect, species by species, so as to evaluate its prevalence across different animal clades. We chose a Bayesian modelling approach, as it provides intuitive estimates of uncertainty. A negative binomial regression model was fit to each species, modelling the number of transfer events to each other species as a function of the divergence time. As the total number of transfers detected was low for some of the species (≤30 events for 67 of the species), there was a risk of over-estimating the effect due to overfitting. To counter this potential issue, a regularising prior was set on the coefficient for divergence time, biasing our models towards an underestimation of the effect of phylogenetic proximity. Despite this conservative approach, 162 of the 219 species involved in transfers (~74%) presented a confidently negative effect of divergence time on transfer rates (the 95% Highest Posterior Density Interval (HPDI) was entirely negative). These species are widely spread across the animal phylogeny and for many, the size of the effect is likely to be considerable. For example, the manatee Trichechus manatus sits at the median across species regarding the strength of the phylogenetic effect and is thus representative of the typical effect size. Strikingly, this species is expected to present 9.93 times as many HTT events at 250 My divergence time than at 700 My divergence (median of the posterior: 9.93; 95% HPDI 1.15-31.87). In conclusion, our models reveal evolutionary relatedness as a major driver of HTT rates across the tree of animals.

10. **Roberto Sevilla-Ortega**, "Study of the evolution of laccases in basidiomycete fungi by ancestral sequence reconstruction and enzyme resurrection", robertsev99@gmail.com, Margarita Salas Center for Biological Research, CSIC.

Laccases are well-known multicopper oxidases that play a crucial role in lignin degradation carried out by basidiomycetes fungi during lignocellulose decay, and have a wide applicability as biocatalysts in different sectors. Recently, we described three new groups of laccase-like enzymes in basidiomycetes species, which are phylogenetically related to but distinct from laccases *sensu stricto*. Here, we reveal the evolutionary trajectory of basidiomycete laccases by

means of ancestral sequence reconstruction, from the last common ancestor of the four groups of laccase-type enzymes (comprising the three mentioned new groups plus the group of laccases *sensu stricto*) to the most recent ancestor of each clade. We show first evidence for crucial changes in key amino acid residues determining enzymatic activity that led to the emergence of today's laccases *sensu stricto*. The ancestral laccase-type enzymes were resurrected (heterologously expressed) and characterized, showing noticeable differences among them at the sequence/structure and biochemical/activity levels. The ancestral laccase showed higher oxidation versatility on typical laccase substrates than the other ancestral laccase-like enzymes.

11. **Cécile Tuchot-Taillefer**, "Clarifying taxonomic boundaries in *Sechium* P. Browne (Cucurbitaceae) and exploring potential center of domestication of chayote through genomics analysis", truchot.cecile@outlook.com, Instituto de Ecología, UNAM.

The genus *Sechium* (Cucurbitaceae), historically considered monospecific with only *S. edule* ssp*. edule* (chayote), has undergone significant taxonomic revision. Numerous Mesoamerican species were initially placed in distinct genera but were later reassigned to *Sechium*. The species count has fluctuated, with recent studies suggesting that *S. chinantlense* and *S. compositum*, once considered separate species, may instead be subspecies of *S. edule*. This challenges conventional classifications and remains debated. Additionally, the domestication history of *S. edule* remains unclear due to the perishable nature of its seeds and the lack of direct archaeological evidence.

To address taxonomic uncertainties, we used SNP (Single Nucleotide Polymorphism) analysis, which is effective for understanding adaptive processes and genetic diversity. SNPs are efficient for resolving recent plant radiation due to their rapid evolutionary rate. We applied multispecies coalescent (MSC) methods for species delimitation. The study included 79 individuals from species across their geographic range in Mexico: four wild taxa (*S. chinantlense, S. compositum, S. hintonii, S. edule ssp. sylvestre*) and one cultivated subspecies (*S. edule ssp. edule*). The selection of samples focused on three objectives: DNA phylogeny, admixture assessment, and population genetics analysis. Due to computational constraints, we reduced the dataset to 40 individuals for phylogenetic analysis. For structure analysis, 76 samples were used, and 69 samples for population genetics. The calibration of the *Sicyos* group, including *Sechium*, was updated with mitochondrial and ribosomal DNA sequences from multiple studies, covering 61 species, to refine divergence time estimates.

Our results confirm that *S. edule ssp. edule, S. chinantlense, S. compositum*, and *S. hintonii* are distinct species, contrary to prior studies suggesting *S. chinantlense* and *S. compositum* were subspecies of *S. edule*. Genetic differentiation, supported by SNP data and FST values, reinforces these species distinctions. Low levels of hybridization were detected in some individuals, but these were minimal. *S. hintonii* was identified as the earliest diverging species in the Sechium group. Divergence times inferred from rcbL, ITS sequences, and SNPs indicate a more recent separation between these taxa than previously estimated. Additionally, we observed that *S. edule ssp. edule, S. chinantlense,* and *S. compositum* are closely related species that diverged recently. Furthermore, S. edule ssp. sylvestre was found to be genetically distinct from cultivated varieties, supporting its classification as a separate entity.

This study enhances our understanding of the evolutionary relationships within *Sechium*, highlighting the distinctiveness of species previously considered subspecies. Our analyses provide clearer insights into phylogeny, suggesting that speciation occurred during the Pleistocene, likely driven by habitat shifts and climatic fluctuations. The observed genetic structuring and range reductions, coupled with the potential threat of climate change, underscore the need for conservation programs like the IUCN Red List. Given ongoing habitat loss and limited distributions, proactive strategies are essential to protect the genetic diversity of this important genus.