



EVOLUZIONE

**BOOK OF  
ABSTRACTS**

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DIPARTIMENTO DI BIOLOGIA  
UNIVERSITÀ DEGLI STUDI DI PADOVA



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## Opening Lecture

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### **Selectable units of evolution: the importance of developmental symbioses**

*Scott F. Gilbert*

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Evolution involves the selection of heritable variation, and such variations are caused by changes in organismal development. Evolutionary developmental biology, which studies the origins of this variation, has focused on the formation of new structures through changes in gene expression. However, whereas the human genome contains some 22,000 genes, it receives over eight million different genes from its microbial symbionts. Since the expression of microbial genes is critical in producing anatomical, physiological, and behavioral phenotypes, changes in these bacterial genes may be important in generating new phenotypes. Both vertically and horizontally transmitted microbes have been shown to alter development to produce in selectable adaptations. Moreover, recent research has shown that microbial symbionts are necessary for the development of particular organs of several species, for the variation of certain selectable traits within a population, and for the emergence of particular social behaviors. This research also suggests that some major evolutionary transitions have been facilitated by symbiotic microbes. Herbivory, the complex of anatomical, physiological, and behavioral traits allowing animals to eat plants, is one of those transitions. Herbivory will be discussed from the point of view of holobiont evolutionary developmental biology, wherein specific adaptations (such as the rumen), are seen as being induced by interactions between the host and its microbes, and the behavioral and physiological manifestations of herbivorous phenotypes need to be preceded by the successful establishment of communities of symbiotic microbes that can digest plant cell walls and detoxify plant poisons.

**SYMPOSIUM**

**PHYLO3: PHYLOGENETICS, PHYLOGENOMICS & PHYLOGEOGRAPHY**

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**Inferring macroevolutionary processes from phylogenies and fossils**

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Present biodiversity represents a snapshot of a very long and complex evolutionary history, during which species and entire clades have originated, diversified and –to a large extent– gone extinct. Reliable estimates of the processes that have shaped diversity through time and in space are crucial to understanding present biodiversity patterns and the factors that may affect global and local species richness. Here, I present a suite of Bayesian models to infer different macroevolutionary processes including the dynamics of speciation, extinction and dispersal and the evolution of quantitative traits. Both phylogenetic trees of extant taxa and the fossil record provide valuable information about past and present biodiversity, although their integration remains challenging. Using empirical data sets from different plant and animal clades, I will present a unified analytical framework to infer species diversification, historical biogeography, and phenotypic evolution based on neontological and paleontological data. Future developments of an interdisciplinary approach interfacing earth sciences, paleontology, and evolutionary biology will be crucial to further improve our understanding of the processes driving the evolution of taxonomic and phenotypic diversity.

## **Correlating phenology dynamics estimated via Landsat collection with observed avian phylogenetic diversity in the national Park of Peneda-Gerês in northern Portugal**

*Saverio Vicario, Maria P. Adamo, Cristina Tarantino, Palma Blonda*

National Research Council - Institute of Atmospheric Pollution Research

Plant primary productivity is an essential variable both for biodiversity (as driver of ecosystem carrying capacity). In particular in mountain region where local ecosystem food web has as unique source local primary productivity, given the lack of large movement of incoming biomass. Satellite medium resolution data (e.g. MODIS) are typically used to obtain global estimates of green FAPAR (the main component of GPP) at 300-m resolution. This resolution is problematic especially for biodiversity management at local scales, where it is important to appreciate habitat heterogeneity. Higher spatial resolution sensors as Landsat have lower temporal resolution and produce sparse and irregular time series due to atmospheric perturbations making more problematic analysis of time series. We set up a fast and robust approach based on a harmonic model linearly fitted within a hierarchical bayesian framework, to estimate Ecosystem Functional Attributes (EFA) *sensu* Alcaraz et al. 2006, that are 3 features (mean, coefficient of variation, peak's day of the year) of the GPP proxy seasonal dynamics plus the yearly anomalies that are relevant to compare resilience across the area of study. We tested the approach over a large cloudy mountain region – the Peneda-Gerês National Park (Portugal). Estimated EFA were validated with cloud pattern simulation and with coherence with expected patterns due topography (Elevation, Aspect and Slope) and to expected degree of stability though time based of each landcover type. In this area were available 372 observation sites visited twice in 2010 for a total of 4409 individual birds observed. A phylogeny of observed bird was built resampling from the pruned posterior distribution of Birdtree (<https://birdtree.org/>). Preliminary data on the correlation between EFA and the avian biodiversity using permanova and PhyloH on original continuous data and transformed ones (i.e. binned and categorized) are shown.

## BASE: overcoming constraints & limitations of the $\omega$ in a phylogenomic framework

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Detecting molecular signatures of different selection regimes in a phylogenomic framework is a crucial step in order to explore the diverse evolutionary histories of ortholog genes in different clades and genome-wide effects in response to biotic or abiotic events. The standard tool to measure selective pressures in molecular sequences (be they neutral, positive or purifying) along the branches of a phylogeny is the rate of nonsynonymous and synonymous substitution (dN/dS, also known as  $\omega$ ). The most used bioinformatic tool for this purpose is CodeML, a sound tool which lacks in handiness when used on a large number of orthologs clusters and on broad phylogenies, a scenario which is increasingly common nowadays. Its major drawback lies in the involved process to retrieve  $\omega$  values for branches of interest, which leads to serious impediments analyzing ortholog clusters which present missing data (*i.e.* clusters in which sequences are missing for some species). As a matter of fact, its usage is often restricted to the subset of the genes which is shared by all considered species, an approach which limits the scale of the analysis to a fraction of genes which can be as lower as an order of magnitude in respect to clusters which include sequences for a variable number of species. Here we present a front-end named BASE (which stands for Branch And Site Evolution) that, starting from ortholog clusters and a species tree, is able to produce  $\omega$  values for each branch of interest in a phylogeny and for each ortholog group. Although a number of bioinformatic tools have already been developed to fasten and facilitate this kind of analyses, BASE is the only one which allows to retrieve  $\omega$  values for equivalent branches (*i.e.* the branches which includes all the available species of a specific clade in a given cluster). We tested BASE on different published datasets, including scenarios which supported gene specific and genome-wide changes in selection, for different clades.

**“Melons and mangos for my friend and just olives for me, please”: the evolution of food choice in *Bactrocera* fruit flies**

*Federica Valerio*<sup>1</sup>, *Francesca Scolari*<sup>1</sup>, *Ludvik Gomulski*<sup>1</sup>, *Mariangela Bonizzoni*<sup>1</sup>, *Lino Ometto*<sup>1</sup>

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Phytophagous insects rely on chemoreception to localize food sources, mates and oviposition sites. Chemoreception is mediated by multigene families that include odorant binding protein (OBP) genes, chemosensory protein (CSP) genes, olfactory (OR) and gustatory (GR) receptor genes. In turn, the number and pattern of these genes determine the ability to select different host plants. Here, we focused on the evolution of chemosensory gene families in fruit flies of the *Bactrocera* genus, which includes generalists as well as specialist species. In particular, we explored the evolutionary dynamics of these genes in three extremely polyphagous species (*B. dorsalis*, *B. latifrons* and *B. tryoni*), in one oligophagous (*B. cucurbitae*, which feeds mainly on Cucurbitaceae) and one monophagous species (*B. oleae*, which feeds only on olives). We first annotated the repertoire of chemosensory genes in the five available genomes. Then, we analyzed the birth and death processes of these families on a phylogenetic framework. Remarkably, OR and OBP gene families are characterized by high turnover rates in all *Bactrocera* lineages, with the exception of the specialist *B. oleae*, in which only gene losses occurred. Therefore, we hypothesize that the contraction in the *B. oleae* gene families may be functionally tied to its monophagous behavior. In addition, the analysis of the rate of molecular evolution revealed that only in this species OR and OBP genes are evolving under contrasting selective pressures. Thus, the evolution of host choice in this genus is the result of a combination of evolutionary processes occurring at both the gene family and the gene sequence levels. Functional studies of candidate genes can then be used to evaluate their specific roles in chemoreception in these pest species.

**Transcriptome structure and evolution when the size of the genome is 50 gigabases:  
some preliminary data for the Australian lungfish lineage (*Neoceratodus fosteri*)**

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Lungfishes (six species) are the closest living relatives of tetrapods. Both inherited lungs from a Devonian ancestor, and the evolution of this trait can be better understood by comparing their genes and genomes. Genomes of lungfishes, however, are tremendously (and intriguingly) big, ranging from 50 to 130 gigabases in different species. The study of specific genes associated to the unique phenotype, and of the driving factors behind the extreme genome expansion (e.g., transposable elements) occurring only in lungfishes and salamanders among vertebrates, is currently addressed by transcriptomics. The *de novo* assembly of the Australian lungfish (*Neoceratodus fosteri*) transcriptome led to a highly complex and complete transcriptome, with almost 300,000 non-redundant contigs and the identification of all metazoan near-universal single-copy orthologs (BUSCOs). The very low fragmentation rate (5%), together with the presence of >5,000 contigs longer than 5 Kb, indicates that the process was very accurate in reconstructing full-length mRNAs. However, the assembly metrics were lower than those expected for such a complete transcriptome (N50 = 269 bp, mean contig length = 516 bp). This discrepancy can be explained by pervasive transcription of intergenic regions occurring in the giant genome of *N. fosteri*, possibly linked to the high repetitive sequence content (24,08%), with a major contribution from SINEs (7,36%), LINEs (8,98%) and LTRs (3,14%). This was previously noted in the African lungfish *Protopterus annectens*, suggesting a common origin of the giant genome despite > 200 MY of divergence. Conversely, the two transcriptomes differ at functional and possibly adaptive traits, such as the presence of multiple myoglobin genes in *P. annectens*, absent in *N. fosteri*. Our observations suggest that a comparative transcriptomic approach can identify ancestral traits and species-specific genetic adaptations of these endangered living fossils.



**Codon usage indicates that amphibians, reptiles and birds are major hosts for Zika and other arboviruses: implications for epidemiology and surveillance**

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Codon usage studies estimate the efficiency of viral replication in putative hosts and vectors for which there has been a supposed history of adaptation. This can help reconstructing the global epidemiology of arthropod-borne viruses (arboviruses), clarifying aspects regarding their ecology and evolution. Current studies concentrate on primate hosts, promoting a lack of information about alternative reservoirs. We studied if and how the codon usage of a set of 13 arboviruses comprising Zika, chikungunya, dengue, and yellow fever is adapted to a range of 30 different putative animal hosts. Our results indicate that all considered arboviruses are more adapted to amphibians, reptiles and birds than to humans and other mammals. This outcome is consistent over different indexes and correlates with the same analyses in West Nile and Japanese encephalitis viruses, two arboviruses known for their bird-to-bird transmission cycle. This is an unexpected finding indicating that non-mammals are the likely primary hosts of most arboviruses. Thus, we advocate that wild environments may have a stronger influence than previously thought in the reservoiring and spreading of Zika and other arboviruses. This has profound implications for the actual surveillance and management of outbreaks.

**Polymorphism analyses and protein modelling inform on functional specialization of Piwi clade genes in the arboviral vector *Aedes albopictus***

Michele Marconcini<sup>1</sup>, Luis Hernandez<sup>1</sup>, Vincent Houé<sup>2</sup>, Federica Valerio<sup>1</sup>, Umberto Palatini<sup>1</sup>, Elisa Pischedda<sup>1</sup>, Lino Ometto<sup>1</sup>, Federico Forneris<sup>1</sup>, Anna-Bella Failloux<sup>2</sup>, Mariangela Bonizzoni<sup>1</sup>

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Argonaute proteins are key components of the RNA interference (RNAi) mechanisms in eukaryotes, which include the micro-RNA (miRNA), the short-interfering RNA (siRNA) and the PIWI-interacting RNA (piRNA) pathways. Recent studies provided evidence of evolutionary expansion and functional divergence of Argonautes in Dipterans, both in the Ago and Piwi subclades. In mosquito species that transmit epidemic arboviruses such as Dengue, Zika and Chikungunya viruses, the siRNA pathway is canonically considered the main antiviral pathway. However, recent data suggests that the piRNA pathway plays a role in tolerance to infection and that Piwi proteins cross-talk with other proteins of recognized antiviral function. These functions likely stem from specialization of certain Piwi genes, although details are still unknown. Deeper understanding of these evolutionary processes is needed to gain knowledge on the molecular mechanisms that shape vector competence and to develop novel genetic-based vector control strategies. Here, we elucidate the structure, polymorphism and expression of the Piwi clade genes of *Ae. albopictus* in an evolutionary framework using a combination of molecular, population genomics and protein modelling approaches. We show that the genome of *Ae. albopictus* harbors seven Piwi genes, namely Ago3, Piwi1-3, Piwi2, Piwi4, Piwi5, Piwi6 and Piwi7. For the first time in mosquitoes, we show sign of adaptive evolution in Piwi1-3, Piwi4, Piwi5 and Piwi6, including sites in the MID and PAZ domains, two hallmark domains of Piwi proteins. Superposition of molecular predictions of protein structure highlights high levels of conservation in the active site among the Piwi proteins, whereas low conservation is found on the surface of the proteins. Expression profiles during mosquito development and following infection with the Dengue or Chikungunya viruses support functional specialization of Piwi proteins, with a prominent and general antiviral role for Piwi5.

## Phylogeny and divergence of Aedini mosquitoes: evolutionary scenarios and genomic perspectives

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Mosquitoes are the indirect cause of more morbidity and mortality among humans than any other group of organisms. One-third of all mosquitoes species belong to the Aedini, a tribe comprising common vectors of viral zoonoses. In order to improve our understanding of their evolution, we present a detailed multigene estimate of their phylogeny and divergence based on relaxed clocks and fossil calibrations. Our phylogenies using different replacement models and statistical frameworks recover some recognized clades such as *Stegomyia*, *Ochlerotatus*, clades A and B but also indicate unstable supports at many nodes: this is likely due to fast radiation events which should be investigated in future using whole genome data. Our divergence estimates point toward mosquito radiation in the mid-Jurassic and Aedini radiation from the mid-Cretaceous on: this is compatible with a paleo-ecological scenario in which first lineages of mosquitoes co-radiated with reptiles, followed by diversification of Aedini in a more modern ecosystem characterized by mammals, birds, and angiosperms. We observe both topological and chronological incongruence when comparing nuclear and mitochondrial data: this may be explained by either peculiar evolutionary events such as hybridization and/or by wrong taxa assignment in samples collections. Our divergence estimates indicate that emerging model *Aedes albopictus* can be currently compared from a genomic point of view only against distantly related *A. aegypti*: genome data from its sister species *A. flavopictus* would more than half the time from common ancestor, increasing resolution of comparative genome studies. Overall, our results provide an updated view of Aedini molecular phylogeny and suggest paleo-ecological scenarios for their evolution; it also reveals some methodological issues which may be of help in setting the research agenda for future genome studies.

### **Evolutionary study of the crAssphage virus at gene level**

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crAssphage is a bacteriophage virus that thrives in the human gut. It is estimated to be present in half of the human population and it constitutes up to 90% of the viral sequences in human feces. Despite its ubiquitousness, the virus was discovered only recently, due to the lack of reliable genome-centric approaches able to identify viral genomes and the inability to isolate its primary host. Finally, in 2014, CrAssphage genome sequence was determined upon cross-assembly (hence the name) of previously unidentified metagenomic sequences. This study is the first to investigate the evolution of crAssphage at single gene level. Gene duplications in crAss-like genomes were found, albeit most of these have happened in genes whose function is unknown; by investigating the conservation degree of the single genes, a consistent variation across the different functional groups was found. The capsid proteins have been confirmed as the most ubiquitous, despite their overall low quantity of information. By using the mirrortree method, it was possible to find a core of genes whose evolutionary trees strongly correlate with each other, possibly suggesting a strong genetic interaction. Noticeably, these proteins cover a wide array of functions. It is possible to conclude that, despite the variety of crAss-like viruses, a consensus of the evolution of this proposed viral family was produced. Finally, there is the need for a better protein characterization in order to have a complete picture of the evolution of crAss-like phage genomes. The next step in order to understand the evolution of crAss-like viruses will be a better annotation based on protein analysis, e.g. 3D structure analysis.

### Convergent genome reduction patterns in bacterial symbionts of stink bugs

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Nutritional or trophic symbioses are mutualistic associations in which partners exchange nutrients and metabolites. Well characterised systems include bacterial symbionts providing to their animal hosts essential molecules (e.g. amino acids, vitamins) that are absent from their diet. The condition of the phytophagous stink bugs of family Pentatomidae (Hemiptera), typically harbouring gut symbionts necessary for the complete host physiology and development, is considered to be among those cases. Many stink bug symbionts belong to genus *Pantoea* (Erwiniaceae, Enterobacterales), which, in general, encompasses free-living or host-associated forms, including plant and opportunistic human pathogens. In this work, we report the discovery and comparative genome analysis of the novel species “*Candidatus Pantoea persica*”, midgut symbiont of stink bug *Acrosternum arabicum*. After Illumina DNA sequencing from a dissected host midgut, a bioinformatic approach was applied in order to selected only the sequences belonging to the symbiont. The assembled bacterial draft genome (~2.8 Mb) is intermediate in size between freeliving *Pantoea* and other highly-reduced obligate symbionts of stink bugs. A high proportion of total ORFs (approximately one third) was classified as pseudogenes. Interestingly, a similar condition was found in the other previously published *Pantoea* symbiont genomes with intermediate size, contrarily to both free-living and highly reduced forms. In any case, all symbionts retain the predicted capability to perform biosynthesis of essential amino-acids, vitamins and other metabolites, consistently with the hypothesised role of nutritional mutualists. Given that stink bug symbionts are polyphyletic within genus *Pantoea*, the obtained results indicate a recurrent independent establishment of those symbiotic partnerships, possibly driven by analogous host dietary needs leading to preferential recruitment of multiple originally free-living *Pantoea*.

### Towards the understanding of the evolution of intramitochondrial tropism in ticks symbionts

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Despite their wide distribution, *Midichloria* symbionts of ticks are understudied, and their role in the host physiology remains unknown. The most striking feature of some *Midichloria* is their unique capability of residing inside the mitochondria – a characteristic referred to as intramitochondrial tropism (IMT) – in the host oocytes. The exact distribution of IMT in the *Midichloria* genus is unknown: of the previously investigated species, two exhibit IMT, while *Midichloria* in the tick *Ixodes holocyclus* are only located in the cytoplasm. To further investigate IMT and its distribution, we applied a multidisciplinary approach: 999 ticks (47 species) were sampled from three continents and screened for the presence of *Midichloria* bacteria. The tropism of *Midichloria* species was observed in tick ovaries using transmission electron microscopy and *Midichloria* strains were classified as capable of IMT (IMT *Midichloria*) and incapable of such tropism (no-IMT). 15 representative samples of both IMT and no-IMT *Midichloria* were selected for metagenome sequencing, and metagenome-assembled genomes (MAGs) of *Midichloria* endosymbionts were obtained through the bioinformatic pipeline Blobology. MAGs allowed to obtain a robust phylogenetic reconstruction of the genus, and to perform genomic comparisons aimed at the reconstruction of the *Midichloria* core-genome and at the detection of genes involved in intramitochondrial tropism. The analyses on these genes and the reconstruction of the gene content of the *Midichloria* ancestor through birth-death models are being performed, with the goal of understanding the origin and the evolution of this fascinating tropism. Preliminary phylogenetic results suggest that IMT is not restricted to a monophyletic clade, indicating either an homoplastic multiple acquisition of the capability or a single ancestral apomorphic acquisition in the *Midichloria* ancestor, followed by the loss of such capability in at least a member of the *Midichloria* clade.

### **Molecular phylogenetics of early diverging hexapod lineages**

*Antonio Carapelli, Chiara Leo, Francesco Nardi, Francesco Frati*

Department of Life Sciences, University of Siena

An increasing number of molecular data has been recently obtained for basal hexapod lineages. Some are part of genomic projects, aimed at providing essential support for high-ranking relationships and, fewer dedicated to solving within-group phylogenies. Early diverging hexapods are traditionally separated into two major branches, Entognatha and Ectognatha, according to the alternative morphology of mouthparts. Ectognatha contains Pterygota and two groups of primitively apterous taxa (Microcoryphia and Zygentoma) that, altogether, define the “true insects”. Three primitively wingless groups (Collembola, Diplura and Protura) are excluded from the above lineages, thus grouped within Entognatha, and are believed to be among the first arthropod groups to walk on six legs. Mitogenomic data of basal hexapods alone, or in combination with nuclear DNA markers, are used for phylogenetic purposes at different taxonomic levels and with a larger density of compared species with respect to most whole-genome studies. In these analyses, we have tested whether the mitochondrial DNA is useful to establish within and between group relationships among early diverging hexapod taxa; e.g. obtained results have been successfully applied to investigate proturan systematics, at different hierarchical levels, but failed to delimit the closer relatives among arthropods of this bizarre group. Deeper sampling of collembolan mitogenomes has allowed the comparison among all major lineages, with additional insights obtained from gene order data. Similarly, dipluran intra-relationships are plainly assessed. The deeper branching of Insecta supports the monophyly of high-ranking taxa, such as Ectognatha, Monocondylia and Dicondylia. Zygentoma is the only basal hexapod group whose monophyletic status is still questioned according to some analyses. Collectively, mtDNAs are useful markers for hexapod phylogeny and usually successfully applied to establish the relationships among and within groups.

**Multilocus nuclear markers provide new insights into the origin and evolution of the blackbuck,  
*Antilope cervicapra***

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Evolutionary relationships between members of the Antilopina taxon have been much debated in recent years. The ‘true antelope’ clade is currently comprised of 4 genera viz., *Gazella*, *Nanger*, *Eudorcas* and the monotypic genus *Antilope*, that includes *A. cervicapra*. Most studies have focused on the mitochondrial genome or morphological data to study their relationships. However, signals from mitochondrial data can often be misleading when compared with nuclear markers, as has been shown in multiple taxonomic groups. In this study, we revisit the phylogenetic relationships among members of Antilopina, particularly the phylogenetic position of *A. cervicapra*, using 12 nuclear markers and compare it with the mitochondrial tree. Furthermore, we explore the implications of the results of this study on the taxonomy and biogeography of Indian antelopes. The phylogenetic trees built using multiple coalescent and concatenated methods all supported a paraphyletic genus *Gazella*. *Antilope* was nested within *Gazella* as opposed to being sister to it, which was suggested by previous studies and our results based on mitochondrial markers. Our fossil-calibrated larger bovid phylogeny, based on nuclear markers, suggested that the *Antilope* lineage diverged from its sister species more recently in the Pleistocene, rather than in late Miocene as per previous studies. Our biogeographic analyses suggest that the lineage leading to genus *Antilope* dispersed into India from the Saharo-Arabian realm around 2 mya, post the expansion of grasslands. We speculate that the adaptations of this savanna-grassland specialist did not allow them to extend their range beyond the Indian subcontinent. Whereas, the only other true antelope in India, *G. bennetti*, extended its range into India more recently, probably after the establishment of the Thar desert in northwest India.



SYMPOSIUM

UNITS OF DIVERSITY: TOOLS, STRATEGIES AND CASE STUDIES

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**Integrative taxonomy: interface of systematics and evolutionary biology**

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Alpha taxonomy – the science of characterising, classifying, and naming species – is experiencing a renaissance. One reason is the increased availability of techniques from other biological disciplines to taxonomy. Integrative taxonomy (IT) – a multi-source approach that takes advantage of complementarity among disciplines – has especially profited from this. Coined as a term in 2005, IT was reviewed in 2010. Since, case studies on many taxa have been published, diverse in, among others, reproduction strategy, life history, and ecology, and these case studies helped in the fine tuning of procedural details in IT. Here, we highlight IT cornerstones: (a) Data analysis should be made without prior hypotheses, and hypotheses should follow from the data. (b) Using the same individual under every discipline and analysing the data from each discipline separately are paramount to discovering incongruence among disciplines. (c) Resolving such incongruence by invoking evolutionary explanations serves as a strong catalyst to evolutionary biology. (d) When using mitochondrial DNA, awareness of both the chances to evolutionary biology and the pitfalls for taxonomy is required. (e) Validation of a species-delimitation hypothesis using data from an independent discipline is desirable. (f) Morphology is important for linking new insight to existing data. (g) Only through publishing any nomenclatorial consequence, the results of IT gain long-time value. We discuss the prospect of IT by addressing questions such as: Will IT be desirable for each species delimitation? Will genomic data solve all riddles? Will automated species delimitation remove all researcher bias? Is there a citation impediment to taxonomic literature? What will limit advance in IT – funding, technology, education, employability of ITists, collaboration, and/or fieldwork?

### **Integrative analyses on Western Palearctic *Lasiommata* reveal a mosaic of nascent butterfly species**

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Satyrinae butterflies occurring in the Mediterranean have an apparent reduced gene flow over sea straits and for several species recent wide-scale biodiversity surveys indicate the existence of divergent mitochondrial lineages. In this study we apply an integrative approach and examine the phylogeography of the genus *Lasiommata* in the Western Palearctic. Our analyses comprised molecular analyses (mitochondrial and nuclear DNA) and geometric morphometrics (wings and genitalia) for two main species groups, and a comparative GMYC analysis, based on COI, of all the tribes within Satyrinae from this region. Our GMYC approach revealed a particularly fast coalescence rate in the Parargina subtribe and for the *Lasiommata* group identified 12 evolutionary significant units, divided into six clades for the *L. maera* species group, five for the *L. megera* species group and one for *L. petropolitana*, with divergences of about 1%. The patterns of COI were mirrored by ITS2 in *L. maera* but were generally inconsistent in *L. megera*. On the contrary, morphological differences were coherent with the results of COI for *L. megera*, but less clearly so for *L. maera*. Two notable exceptions were *L. paramegaera* and *L. meadewaldoi*, which represent distinct groups for all the analyzed markers that likely advanced faster in the process of speciation because of isolation and reduced effective population size, rendering the rest paraphyletic. Our study illustrates the continuous nature of speciation and the difficulties of delimiting species. Here the recognition of taxa as diverging lineages or distinct, possibly paraphyletic species, mostly depends on the criteria adopted by different species concepts.

**Species delimitation and integrative taxonomy applied to the New Zealand psyllids (Hemiptera: Psylloidea)**

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The psyllid fauna of New Zealand provides a cross section of the superfamily Psylloidea, with species representatives in six of the eight world families, including pests, vectors of plant pathogens and biocontrol agents. Field-collected specimens from almost 600 locations around New Zealand, Australia and United States of America were used to generate an up-to-date list of the New Zealand Psylloidea. When comparing the genetic variation of the cytochrome oxidase subunit I (COI), this highlighted higher-than expected variation, suggesting the presence of cryptic species diversity.

Consequently, a morphological-molecular integrative taxonomy concept applied to species delimitation revealed the presence of more than 20 new taxa divided into multiple species-complexes. In the context of a “unified species concept”, the importance of morphology, ecology and distribution data with genetic information was considered in delimiting species. This work enabled a better understanding of the biodiversity, phylogenetic and evolution of the New Zealand psyllids, resulting in the description of new species. The multi-marker phylogenetic analysis generated was then implemented with data on the bacterial symbionts of each psyllid species, generated using metabarcoding analysis. This enabled to establish a phylogenetic framework resolving evolutionary relationships and highlighted the potential use of symbiotic bacteria as “markers” for the species delimitation of the hosts.

**Systematic revision of the genera *Pleurastrum* and *Chlorococcum* (Chlorophyta) based on molecular and climate data**

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A research work was started to characterize two green coccoid microalgal strains isolated from Terra Nova Bay, Victoria Land (Antarctica). The preliminary analyses carried out on the two isolates suggested their possible attribution to the genus *Pleurastrum* Chodat or the genus *Chlorococcum* Meneghini. The genus *Pleurastrum*, whose type species is *Pleurastrum insigne* Chodat, lives in soil, freshwater habitats, and as photobiont of lichens. It includes species that can show different growth forms depending on environmental conditions, with the coccoid form being the most reported. For its high polymorphism, the taxonomic history of this taxon has been confusing, with authors inquiring its monophyly and several taxonomic revisions during years. The genus *Chlorococcum* (Chlorophyta) Meneghini, with the type species *Chlorococcum infusionum* (Schrank) Meneghini, is a group found ubiquitously; it is reported from soil, freshwater, and marine environments, as well as from extreme habitats (e.g., hot springs and Antarctica). The simple morphology and the lack of unambiguous diagnostic characters make the identification of members of this genus very difficult. In order to better identify the Terra Nova Bay isolates and to throw light on the taxonomy of *Pleurastrum* and *Chlorococcum*, several reference strains of these genera were got from International culture collections and subject to molecular analyses. In particular, phylogenetic reconstructions were carried out based on the 18S rRNA, *rbcL*, *tufA* and ITS2 loci, with a focus on the ITS2 secondary structures. Besides giving a taxonomic position to the two Terra Nova Bay isolates, our results contribute to the circumscription of the genera *Pleurastrum* and *Chlorococcum* and to their revision, with the synonymization of different species. A correlation between lineages and climate zones has also been found and discussed.

**Factors affecting the accuracy of molecular species delimitation: evidences from the species-rich beetle family of Chrysomelidae**

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In the last decade, many molecular species delimitation methods have been proposed; among them nucleotide distance and coalescent tree based methods are the most popular. Using these methods, putative species are inferred from DNA information without a priori knowledge about organisms morphology. Methods accuracy has been already demonstrated, but the impact on the species delimitation of factors intrinsic of the experimental design is not estimated yet. In order to test factors affecting species delimitation accuracy, 7,237 COI sequences of 552 Chrysomelidae species were analysed using four commonly adopted species delimitation methods: two coalescent tree-based, the Generalized Mixed Yule Coalescent (GMYC) and the Multi-rate Poisson tree processes (mPTP); and two based on nucleotide distance, the Automatic Barcoding Gap Discover (ABGD) and the 3% nucleotide distance threshold. Factors possibly affecting species delimitation accuracy (i.e. the number of haplotypes per species and of morphospecies, presence within a dataset of species difficult to be morphologically identified, geographical distance among sampling points, delimitation method used and taxonomic composition of a dataset) were tested fitting a linear mixed effect model. The accuracy was defined as the percentage of matches between morphospecies and molecular identified units. Our results indicate that distance-based methods are more accurate in delimitation than tree-based, while the analysed dataset size and its taxonomic composition are not influencing delimitation performances. High geographic distance among collection points of individuals from the same species is related to better performances of the methods regardless of the increasing of sampled haplotypes per species, which does not influence the methods accuracy. Finally, the presence of species highly difficult to be identified morphologically strongly decreases molecular delimitation accuracy.

### **ExTaxSI: Exploration of Taxonomy Information, a tool to build and visualize amplicon database**

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Sequencing approaches are now a routine in several biology fields and, currently, distinct DNA-based methods were developed to generate data for biodiversity and biomonitoring studies. Especially regarding methods for species identification and traceability, a huge amount of molecular data have populated public repositories, allowing their exploitation to explore information about species, including their diffusion and distribution, and molecular markers used to identify them. In particular, the environmental DNA (eDNA) metabarcoding is one of these powerful tools to explore biodiversity, by now diffused in different fields like ecology, food tracking, microbiome studies, and many others. Being based on HTS sequencing platforms, huge amounts of data are produced and a bioinformatic approach is mandatory. Briefly, pipelines are defined by few steps that are peculiar for each marker gene, but substantially they could be divided in: data cleaning, taxonomy assignment of amplicons and data analysis. We think that the taxonomy assignment is a very pivotal phase, necessary to define correctly species detected. In particular, a lack of reference sequences or an incorrect assembly of the reference database could lead to a not reliable identification and a poor taxonomy annotation, mostly regarding taxa for which the marker used is not so discriminating. To overcome these issues and try to perform a high quality assignment, here we present ExTaxSI, a tool for taxonomy and data information analysis that allows researchers to explore NCBI Nucleotide references and visualize both public and personal annotations. In particular, our tool is modular and it could be used at any stage of taxonomy analysis of DNA sequences, during exploration of database composition, downloading of data, visualization of assignments considering all principals taxonomic ranks and, in addition, to survey the provenance of NCBI records.

### **Mining the microbial word of a drinking water treatment plant: unexplored biodiversity revealed**

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Microorganisms are the most diverse, widespread and abundant life forms on Earth. Nevertheless, there is a great gap in knowledge to the extent that scientists used the expression “*microbial dark matter*” referring to those microorganisms that account for a large portion of the Earth's biodiversity, but their metabolic and ecological properties are unknown. The ability to uncover this neglected biodiversity mainly derives from technical improvements, such as High-Throughput DNA Sequencing techniques (HTS) applied to environmental matrices, going beyond the limit of the culture-based techniques. Our research focused on the water microbiome residing in a drinking water treatment plant (DWTP). Nowadays, it is well known that drinking water, while safe and of high quality, can harbour microorganisms, not pathogenic, unable to grow on culture media, and almost uncharacterized. We investigated through HTS what happens to the microbial communities from the source to the tap, collecting water samples from groundwater throughout the main steps of the potabilization process of a DWTP, in a urbanized area in Northern Italy. Taxonomic analysis of the microbial world inside the DWTP revealed that groundwater samples significantly differ from treated water samples, but on the whole most of the sequences were associated with the phyla Proteobacteria and Patescibacteria, and a high degree of sequences of uncultured bacteria still uncharacterized. We reported for the first time the presence in drinking water of the so-called *microbial dark matter* and of tiny microbes with reduced genomes, defined ultra-small bacteria, across the entire DWTP. This uncultivated majority represents a challenge, since new questions arise about their role in DWTP ecosystem. The analysis of microbial dynamics provides new insights in the drinking water treatment process: a greater capacity of microbial organism identification is essential to address relevant improvement in prevention strategies.

Symposium

MISCELLANEOUS TOPICS

**Dissecting the genetic basis of pharyngeal jaw parallelism in sympatric Midas cichlid fish radiations**

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Cichlid fishes provide textbook examples of explosive phenotypic diversification and sympatric speciation, making them an ideal system for studying the mechanisms underlying rapid evolutionary divergence. Pharyngeal jaws – an additional set of derived jaws used to crush hard food – are considered one of the key reasons why cichlids show unparalleled diversity and propensity to undergo adaptive radiations. Cichlids of the Midas species complex (*Amphilophus* cf. *citrinellus*) from Nicaragua have diversified in their pharyngeal jaw morphology, especially in two young, small and deep crater lakes (Apoyo and Xiloá). Within each of these lakes, the divergence in pharyngeal jaw morphology has accompanied sympatric speciation into open water and bottom-dwelling species, the latter with more enlarged pharyngeal jaws due to more hard prey in their diet. Surprisingly, the same process of sympatric divergence has occurred independently and in parallel across the two lakes. In this study, we combined forward genetic (QTL mapping, GWAS), transcriptomic (RNA-Seq, miRNA-Seq) and morphometric approaches to dissect the genetic basis of the pharyngeal jaw divergence in cichlid species pairs from crater lake Apoyo and Xiloá. This has allowed us to test to which extent the parallel sympatric phenotypic divergence in pharyngeal jaws share the same genetic basis.



### **The interplay between trait, ecology and diversification in *Lupinus***

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Despite the considerable attention that studies in niche evolution and species diversification rates have commonly received, our knowledge on the influence of functional traits on diversification in relation to niche evolution still remains fragmentary. Life history strategy is a key feature associated with climatic niche evolution in plants: longer generation times are closely related with evolution of niche breadth, particularly across elevational gradients. Moreover, shifts from annual to perennial strategies have been commonly documented in oceanic islands and sky islands, where striking niche evolution linked with radiations is perhaps most evident. Here we investigate trait and niche evolution in *Lupinus* (Leguminosae), an amphiatlantic genus comprising ca. 275 species. The primary species diversification rate shift in *Lupinus* is linked with the evolution of perennial life history as a putative key adaptation enabling colonization of montane habitats across the Western New World. Here we use comparative phylogenetics analyses in addition to theory-based model selection and randomization tests to assess: i) the number of shifts that occurred in the evolutionary trajectory of the climatic niche in *Lupinus*; ii) whether annuals and perennials occupy distinct climatic niches; and iii) the association between rates of climatic niche evolution in relation to the evolution of life history strategies and species diversification rates. We assembled a checklist for the genus, coded life history for each species and retrieved 27,217 species occurrence records for *Lupinus* worldwide to quantify the climatic niche of species and perform niche comparisons across clades and life history trait states.

**Symposium**

**EVOLUTION IN MARINE ENVIRONMENTS**

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**Life in cold extremes: adaptations of Antarctic marine species**

*Lloyd Peck*

British Antarctic Survey, United Kingdom

The Southern Ocean is the coldest ocean on Earth. Its temperatures are amongst the most stable of the global marine environment with most places having annual temperature ranges of less than 3°C, and some places less than 1°C. Total darkness in winter and 24 h sunlight in summer results in very short summer phytoplankton blooms that provide the resources for nearly the whole marine ecosystem. This talk will focus on the adaptations of the unique fauna living on the seabed around Antarctica. These include: why some species are giants; how some fish can survive without haemoglobin or myoglobin; the growth and development rates that are slower than they should be even at polar temperatures; and why it seems that proteins are much more difficult to make at temperatures around 0°C than at warmer temperatures. It will also deal with how these adaptations affect abilities to respond to environmental change

**Can prolonged parental exposure to seawater acidification result in different responses of *Paracentrotus lividus* offspring?**

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In marine invertebrates, early life stages are the most vulnerable and sensitive to environmental stressors and they represent a possible bottleneck for the natural populations. However, the environment experienced by the parental generation may influence the offspring phenotype and then their performances. Growing concern about the capability of calcifying invertebrates to cope with ocean acidification and other anthropogenic stressors in the next future led the scientific community to focus on transgenerational studies. It is expected that a long enough exposure can acclimate adult specimens and offspring to the experimental condition as a chain effect. In the present study, adult sea urchins *Paracentrotus lividus* were exposed to two pH values (natural pH and -0.4 pH units). After two and six months, a F1 generation was obtained from nine families for each experimental condition. Each family was composed by a single female and a pool of three males to highlight potential maternal effects. Larvae were exposed to both 8.1 and 7.7 pH for 48 hours after fertilization. Moreover, larvae were exposed to a mixture of environmental contaminants which are currently in the spotlight, the herbicide glyphosate and its main degradation product, aminomethylphosphonic acid. Larval development and growth were assessed 24 and 48 hours postfertilization. The results obtained showed that embryos were affected by the pH value and/or the contaminant presence more intensely whenever their parents were held at low pH for two months. The combination of pH reduction and presence of contaminants exacerbated the development delay. After six months of parental exposure under reduced pH, preliminary observations did not highlight improvements in larval performances. Further analyses will confirm whether or not this parental acclimation in *P. lividus* was long enough to prepare the F1 generation to face better seawater acidification.

## **Together or alone? Protocooperative predation may be an evolutionary driver promoting gregarism in cnidarian polyps**

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Small gregarious colonies of the coral *Astroides calycularis* (Dendrophylliidae) can catch preys several times bigger than the size of their individual polyps. This behavior was recently defined as protocooperation: organisms mutually benefit from each other, but association is not obligated as in mutualism. Protocooperation was hypothesized to be an evolutionary driver favoring gregarism in this dendrophylliid, and possibly in other cnidarians. To further investigate this behavior in such simple animals, we fed polyps of the scyphozoan *Aurelia* sp., whose individuals tend to aggregate in the wild, with specimens of the annelid *Syllis prolifera*; then we compared predation rates of the groups and the solitary polyps. Polyps (1.5 to 2.5 mm long) were reared in plastic bowls, where they established and reproduced forming groups; others were taken isolated. Worm preys (7.5 to 12.5 mm long) were given to both the groups and the individuals. We considered successful those catches in which worms were effectively killed and ingested. After 15 trials, the success rates of the groups and the individual polyps were 100% and 27% respectively. The worms had nearly no chance to escape when captured by two or more polyps; on the contrary, it was rather simple to escape from isolated ones, sometimes recurring to autotomy. Polyps appeared to invest more time in capturing worms when blocked by conspecifics, while they tended to let the prey escape when alone. Although somehow resembling intraspecific competition rather than cooperation, the final result is advantageous for almost every polyp taking part in the hunt. There could be an evolutionary shift between these two behaviors that drives *Aurelia* polyps in gathering together, enhancing their chance to access food resources well beyond their gape, as in *A. calycularis*. New species will be studied, and research will clarify mechanisms of communication among polyps and their food preferences.

**Stem cell based survival strategies in the marine environment: alternative developmental and regenerative pathways in star ascidians**

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Tunicates are the unique chordate species utilizing a stem cells-based asexual reproduction. Among tunicates, the star ascidian *Botryllus schlosseri* produces through embryogenesis a swimming tadpole larva, which metamorphoses into a sessile oozoid. The latter begins a cyclical pallear budding, forming a colony of genetically identical blastozooids joined by extracorporeal blood vessel in the tunic. The colony weekly renews its entire body by a synchronized process of resorption of adult zooids, which are replaced in filtering activity by their buds. Pallear budding, that allows a rapid expansion of a colony in the marine environment, is not the only stem cell-mediated developmental strategy exhibited by the species. The regeneration of a colony can also come from bud fragments isolated by their parents. New buds are emitted by bud fragments that, after healing of the cut surfaces, are progressively resorbed. Strategies for survival are further extreme when the vascular budding is experimentally forced. After the extirpation of all individuals from a colony, circulating stem cells clot close to a vessel epithelium initiating a budding process. The new zooid differentiates through the so-called whole-body regeneration, restoring a new colony. Stem cells involved in budding and regeneration can be considered units of selection. When the vasculature of two colonies come into contact, a genetically controlled recognition process occurs. If compatible, colonies fuse to form a chimaera. Here, circulating stem cells compete each other for the formation of somatic and germline organs in new buds. Stem cells from a looser partner in the chimera can be totally resorbed by those of the winner partner. Moreover, the winner partner for the somatic stem cells can be a germline looser (or *vice-versa*). Here we present these stem cell-mediated developmental strategies, comparing tunicate stem cell features to those of vertebrates.

### First evidence of poecilogony in the Neogastropoda

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Poecilogony is the intraspecific variation in developmental mode, with larvae of different types produced by the same individual, population or species. It is very rare among marine invertebrates, and in gastropods has long been described only in some opisthobranchs. The mechanisms underlying larval evolutionary transitions, such as loss of planktotrophy that occurred repeatedly in many caenogastropod lineages, are still largely unknown. We have studied the inter- v. intraspecific variation in larval development in the NE Atlantic neogastropod genus *Raphitoma*, starting with an integrative taxonomy approach: 17 morpho-species were tested against a COI molecular-distance based method (ABGD), and the retained species hypotheses were eventually tested for reciprocal monophyly on a multilocus dataset. We performed an ancestral state reconstruction on an ultrametric tree of the 10 eventually retained species, time-calibrated by fossils. All interspecific changes were planktotrophy>lecithotrophy, and all have occurred after 2.5 mya. This is suggestive of a major role played by Pleistocene Mediterranean oceanographic conditions - enhanced oligotrophy, unpredictable availability of water column resources - likely to favour loss of planktotrophy. Within this group of species, that has diversified after the Miocene, we identified one pair of sibling species differing in their larval development, evidence of a speciation event associated to the loss of planktotrophy. However, we also identified two poecilogonous species, each characterized by individuals with both larval developmental types. This is the first documentation of poecilogony in the Neogastropoda, and one of the very few in the Caenogastropoda. Although sibling species with different developmental strategies may offer good models to study some evolutionary aspects, poecilogonous taxa are optimally suited for identifying mechanisms of evolutionary transitions.

### Venom diversity and evolution of the most divergent cone snail genus *Profundiconus*

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Many marine organisms subdue the prey and deter predators through the use of venoms, complex mixtures of bioactive compounds that affect relevant physiological functions. Recent studies of venomous animals highlighted interesting evolutionary patterns, such as recurrent convergence in venom components and prey-predator coevolution, but also represented a starting point for the identification of new compounds of pharmaceutical interest. Among them, marine snails of the genus *Conus* have represented an interesting target for venom research, due to their ability to paralyze worms, molluscs and even fishes. *Profundiconus* is the most divergent cone snail genus and its unique phylogenetic position, sister to the rest of the family Conidae, makes it a key taxon for examining venom evolution and diversity. Venom gland and foot transcriptomes of *Profundiconus cf. vaubani* and *Profundiconus neocaledonicus* were de novo assembled, annotated, and analysed for differential expression. We observed a remarkable level of venom diversification between these two closely related species, with only a small fraction of the putative venom components shared between both species. The majority of transcripts corresponded to conotoxins, profunditoxins, turriptides, insulin and prohormone-4. However, there were also a significant percentage of other putative venom components such as chymotrypsin and L-rhamnose-binding lectin. The large majority of conotoxins appeared to belong to new gene superfamilies, three of which are highly differentiated from previously reported venom peptide toxins. The low conotoxin diversity and the type of insulin found suggested that these species, for which no ecological information are available, evolved a worm or molluscan diet, associated to a narrow dietary breadth. Our results indicate that *Profundiconus* venom is highly divergent from that of other cone snails and may have represented a first step towards the adaptation to venom production in Conidae.

## Comparative transcriptomics sheds new light on peculiar cold adaptation strategies in the gills of Cryonotothenioidea

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Cryonotothenioidea represent one of the most striking cases of evolutionary adaptations to an extreme environment known in vertebrates. The icefish *Chionodraco hamatus*, the main target species of this study, lacks hemoglobin and displays a unique cardio-circulatory system and a series of unique morphological and physiological peculiarities that enabled this species to survive and thrive in the sub-zero temperatures of coastal Antarctic waters. However, our knowledge of the genetic and molecular bases of these adaptations is still limited. Here we provide the first large scale comparative analysis between the transcriptional profiles of *C. hamatus*, five red-blooded Cryonotothenioidea, the sub-Antarctic notothenioid fish *Eleginops maclovinus* and seven temperate teleost species. This analysis targeted the gills, the main surface of contact with the external environment, and a tissue of primary importance in fish for gaseous exchange and its role in the fish immunity. We identified a set of genes consistently up-regulated in Cryonotothenioidea, which were surprisingly shared by red- and white-blooded species and revealed several previously unreported alterations which may have contributed to the evolutionary success of this fish lineage in Antarctica. In particular, we detected an increased activity of carbonic anhydrases, paired with an upregulation of the molecular machinery involved in the Golgi to endoplasmic reticulum retrograde transport, consistent with high secretory activity, and molecular signatures of cobalamin deficiency. We discuss the possible biological implications of this condition with respect to hematological alterations and the heavy parasitic loads typically observed in all Cryonotothenioidea.



## Molecular phylogeny of Caecidae micro-snails (Truncatelloidea, Caenogastropoda) and the evolution of a tubular shell

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Caecidae is a species-rich family of micro snails widely distributed in shallow tropical and temperate marine waters. The majority of the approximately 260 species exhibit uncoiled tube-like shells originating from a unique shell development during ontogeny: the larval shell is regularly coiled, but the undergoes a straightening process in early in life. In *Caecum*, the adult shell is added stepwise and parts of the posterior shell are shed, leading to additional morphological variety. To date, we lack a phylogenetic tree of the Caecidae to understand the complex evolution of tubular shells and to critically assess the existing classificatory system. Due to the range of intraspecific variability, small size and different ontogenetic stages, the current purely shell-based taxonomy is highly problematic. Here we present the first molecular phylogeny of the family to explore the evolution of tubular shells and to test the monophyly of the established genera. The study is based on a worldwide sampling of more than 50 species comprising *Caecum*, *Meioceras* and *Parastrophia*. Multi-gene (nuclear 28S rRNA, mitochondrial 16S rRNA and COI) phylogenies were generated using maximum-likelihood and Bayesian analyses. Our results indicate the polyphyly of the *Meioceras* and place *Parastrophia* as well as *Meioceras* within *Caecum*, contradicting independent evolutionary origins. Further, our analyses reveal the existence of cryptic species, showing that shell characters traditionally used for classification might often be insufficient to define species boundaries. Molecular clock analysis suggests that the major diversification events within *Caecum* occurred between 45 to 25 million years ago leading to global colonisation from an Indo-Pacific origin. Character tracing estimation indicated that straight, small, transparent and smooth shells form the ancestral state of *Caecum*, suggesting an evolution from a benthic relative via miniaturisation and adaptation to an infaunal habitat.

## **Vertical evolution across deep water boundaries in the genus *Coryphaenoides***

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Species that live in deep water experience environmental variation in both horizontal and vertical dimensions. We have been investigating a range of species in this environment to better understand the evolutionary processes that shape their populations. One aspect of our study focused on multiple species in the genus *Coryphaenoides*, a deep-water demersal lineage (the ‘grenadiers’) distributed in most oceans and living at depths ranging from ~500 to 5000 m. We used genomic analyses to compare species within the genus and individuals along a depth gradient within a focal species, *C. rupestris*. We consider two potential habitat boundaries in the vertical dimension. The first is around 1 km depth, the base of the mesopelagic zone and where the thermocline ends in temperate regions, salinity levels off, and oxygen is at a minimum. We compared 60 *C. rupestris* genomes, one sequenced at 120X read depth and annotated, and the rest sequenced at ~6X. Samples compared were distributed over a transect ranging from 500 m to 1800 m at the same geographic location. This revealed signals for selection correlated with habitat depth, likely associated with ongoing disruptive selection among maturing individuals (based on genotype data from aged individuals, showing greater digression from HWE in older age classes). The second boundary was across 4 km depth, the division between the bathypelagic zone and the abyss. For this we compared genomes from 14 species, 7 bathypelagic and 7 abyssal, including a new reference genome for the abyssal *C. brevibarbis*. Comparative analyses including protein modelling highlight the importance of specific gene systems, which at the boundary to the abyss are especially associated with muscle and membrane structure and function.

### Extraordinarily rapid speciation in a marine fish

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Barriers are rarely absolute in the sea, hence models of speciation that can operate in the presence of gene flow, such as ecological speciation, are likely to be important in explaining marine biodiversity. Yet, evidence for ecological speciation in the sea is scarce, particularly in vertebrates. Here, we document a case of rapid speciation associated with selection in the postglacial Baltic Sea. European flounders (*Platichthys flesus*) in the Baltic exhibit two reproductive behaviors: pelagic and demersal spawning. Demersal spawning enables flounders to thrive in the low salinity of the Northern Baltic, where eggs cannot achieve neutral buoyancy. We show that demersal and pelagic flounders are a species pair arising from a recent event of speciation, and we officially describe the demersal spawning species as the Baltic flounder *Platichthys solemdali*. Despite an extensive overlap in distribution, the two species show strong reproductive isolation. Using a new, chromosome level genome assembly and genome resequencing data from more than 60 individuals we identified several hard and soft selective sweeps surrounding genes involved in gamete physiology and morphology in the genome of *P. solemdali*, suggesting that both novel mutations and standing genetic variation played an important role in the rapid evolution of this species pair. Furthermore, comparing the genome of the Baltic *Platichthys solemdali* with the genome of a demersal-spawning freshwater population of *Platichthys flesus* from northern Norway, we identified a chromosomal rearrangement associated with the transition from pelagic to demersal spawning. We evaluated different possible evolutionary scenarios under the approximate Bayesian computation framework and estimate that the speciation process started in allopatry ~2,400 generations ago, following the colonization of the Baltic by the demersal lineage. This represents the most rapid event of speciation ever reported for any marine vertebrate.

## **Unveiling the evolutionary history of sulfoxide synthase OvoA in metazoans**

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Sulfur-containing small molecules play a key role in maintaining cellular redox homeostasis and enable organisms to survive changing environmental conditions. Among them, sulfur-derived histidine, commonly known as ovothiols, were discovered in the eggs and biological fluids of marine invertebrates, in some human pathogens, bacteria, and microalgae. Although ovothiols have long been considered as cellular protective molecules, new evidence suggest that their biological function might be more complex than originally thought. The increasingly amount of genomic resources and the recent characterization of the enzymes involved in ovothiol biosynthesis, the 5-histidylcysteine sulfoxide synthase (OvoA) and the PLP-dependent lyase (OvoB), have now paved the way to new studies aimed at elucidating the distribution and biological function of ovothiols in new ecological niches. Here we investigate the structural organization, molecular diversity and evolutionary patterns of the genes/enzymes involved in the biosynthesis of ovothiols in animals. In spite of the absence of OvoB genes, we found that a large number of metazoans possess highly conserved OvoA genes, which likely derived from a single ancestral sequence. As the only exception, Hydrozoa displayed large multi-domain proteins, originated by the fusion between OvoA and an OvoB-like lyase domain. The patchy taxonomic distribution of OvoA genes in extant animal phyla underlies a complex evolutionary history, characterized by several independent gene loss events and horizontal gene transfers, which have led to secondary gene acquisitions. These findings prompt questions as to why OvoA genes have been lost, or possibly replaced, along evolution in some large animal groups, like Ecdysozoa and Teleostomi. Comparative transcriptomic analyses suggest that ovothiols might be important players in the regulation of fundamental biological processes in animal life since early developmental stages.

### **SmithRNAs: a new arena for mito-nuclear interaction and coevolution**

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Evidences of mitochondrially driven RNA interference (RNAi) are recently emerging. Recently, we suggested a new class of small non coding RNAs (sncRNAs) of mitochondrial origin called smithRNAs (Small MITochondrial Highly-transcribed RNAs) in the Manila clam, *Ruditapes philippinarum*, a bivalve species with Doubly Uniparental Inheritance (DUI) of mitochondrial DNA. Among other possible functions, those smithRNAs were predicted to regulate nuclear gene expression in *R. philippinarum* gonad formation, a possibility which has never been suggested before. However, all evidences for smithRNAs were in silico predictions, up to now. We are validating our findings *in vivo*, and we have experimental evidences that smithRNAs are really functional sncRNAs and that they can indeed provide even genome-wide functions. Namely, we experimentally tested for significant modifications in methylation/acetylation levels in specimens treated with smithRNA predicted to target nuclear enzymes connected with such regulatory functions. It is conceivable that smithRNAs will be found in other eukaryotes as well, maybe linked to functions other than gonad development: in fact, we present evidences that putative smithRNA genes are present in other metazoans' mtDNAs. SmithRNAs are good candidates to evolve new functions in the compact mtDNA, because hairpin structures in mitochondrial intergenic regions (needed for correct RNA cleavage) could easily be exapted to evolve RNAi. SmithRNAs make mtDNA a much more complex genome than previously thought, and they represent a new mito-nuclear coevolutionary arena. Actually, the possibility that mtDNA may act as a reservoir of RNAi opens a plethora of new ways for it to interact with the nucleus and significantly raises the level of complexity of mito-nuclear coevolution (for instance, smithRNAs may play a role in reproductive isolation between closely related species).

**Symposium**

**CONSERVATION AND EVOLUTION**

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**Tracking traits to predict declines**

*Christopher Clements*

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Predicting the future states of biological systems is critical if we are to efficiently and effectively preserve biodiversity in the face of ongoing environmental change. However, doing so is challenging, as ecological systems are inherently high dimensional, non-linear, and stochastic, and the data available to make such predictions are often spatially and temporally data limited. These challenges mean that process-to-pattern methods, such as mechanistic models, are often impossible to parameterise for populations or species of conservation interest. Alternatively, the collapse of a population may be inferred from signals detected in available data, a so-called pattern-to-process approach. Recent work has shown that shifts in the distribution of fitness related phenotypic traits such as body size may be particularly useful signals of the collapse of populations, as they indicate the loss of resilience in the system and loss of structure in a population. I will discuss recent research in this area, and the potential for such trait-based approaches to help inform conservation decision making, as well as the need to consider the role of evolution in identifying signals of sudden collapse vs. signals of adaptation.

## Experimental evolution of parasite dispersal strategies in spatially dynamic landscapes

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In the light of global alteration of habitats and changing opportunities for dispersal, a spatial perspective of evolutionary epidemiology is crucial to better understand and predict the spread of pests and pathogens, and to design efficient disease management strategies. In fact, the spatial structure of the environment strongly affects epidemiology and host-parasite evolutionary dynamics. Parasite fitness is usually maximal for an optimal balance between virulence (i.e. host exploitation) and transmission. However, in fragmented landscapes, shifts in optimal virulence may occur due to the additional trade-off between local transmission and global dispersal. Further, this can produce specific adaptations enhancing dispersal if parasites disperse with their host. I will report on an ongoing long-term evolutionary experiment investigating spatial selection in interconnected microcosms populations, where a bacterial parasite disperses with its protozoan freshwater host *Paramecium caudatum*. Using two-patch metapopulations system, we imposed directional selection for or against host dispersal. We predict that positive selection on host dispersal selects for parasites that do not interfere with their host's dispersal ability, thereby lowering optimal levels of virulence and decreased investment in horizontal transmission. Observed epidemiological dynamics are consistent with this prediction, and I will present the results from tests of evolutionary change in both host and parasite.

## Individual variation and evolutionary potential of parasite traits in a songbird-tick system

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Host-parasite interactions reciprocally generate adaptations and counter-adaptations affecting the life history, ecology and ultimately the evolution of both host and parasite. Fundamental elements for the study of co-evolutionary processes in the life history of hosts and parasites are the quantification of heritable individual variation, genetic and phenotypic covariance of traits across life stages and generations, and the determination of selection pressures acting on both host and parasite. Nonetheless, very few studies have investigated selective pressures, tradeoffs, and variation in life-history strategies of parasites at the individual level so far. Hard ticks are blood-sucking ectoparasites that have predominantly been studied with respect to their capacity to transmit pathogens of medical importance. We raised two consecutive generations of tree-hole ticks (*Ixodes arboricola*) in the laboratory feeding on wild great tits (*Parus major*) with partially known pedigree. Feeding and life-history traits (e.g. feeding time, engorgement weight, moulting time) were measured at individual level at larva, nymph, and adult stage, and data were collected on the individual's survival and reproductive success (clutch size and larval hatching success). We show preliminary results on trait covariation across life stages and heritability, and relationships with individual fitness. Moreover, we discuss effects of individual host traits (body condition, hematocrit, sex and age) on parasite success, and their importance for the evolutionary mechanisms and trade-offs of parasite life-history traits.



## **Integrating individual genotyping and eDNA metabarcoding diet analysis for the study of an elusive bird, the hazel grouse (*Bonasa bonasia*)**

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In the last two decades, grouse populations have decreased across the entire Alpine region. In Trentino (Italy, South-Eastern Alps), the hazel grouse (*Bonasa bonasia*) is particularly threatened by habitat fragmentation and degradation that might determine isolation of populations, loss of genetic variability, inbreeding and decreased resource availability. However, little is known about the ecology of the species, due to its elusiveness. A solution to overcome this limitation is offered by non-invasive genetic approaches. We applied non-invasive sampling capture-recapture protocols on faeces collected in a 700-ha area, in the Natural Park Paneveggio – Pale di San Martino (Dolomites, Trentino) during two different winter seasons, to infer number of individuals, genetic variability, sex ratio and diet. A 600 bp fragment of the mitochondrial control region and a portion of the nuclear CHD gene were employed for species and sex assignment, respectively; amplification of 11 microsatellites has been used to identify individuals. Alongside, the amplification of a region of the chloroplast trnL gene (194-310 bp) allowed us to infer diet composition in terms of plants. Moreover, we are optimizing amplification protocols of a fragment of the mitochondrial COI gene (157-159 bp), which could allow the characterization of the animal component (i.e. invertebrates). Our results led to the identification of 45 individuals (36 from the first and 25 from the second sampling season), with a balanced distribution of sexes in both seasons, while the comparison of  $H_e$  and  $H_o$  values and the evaluation of  $F_{is}$  highlighted no deviation from HWE. Lastly, the trnL metabarcoding approach underlined the usefulness of noninvasive samples to gain information about individuals' diet and food resource use, allowing to evaluate differences between male and female and possible seasonal changes. List of abbreviations: bp, base pair; CHD gene, Chromo-Helicase-DNA binding protein gene; COI, Cytochrome c oxidase I;  $H_e$ , expected heterozygosity;  $H_o$ , observed heterozygosity;  $F_{is}$ , fixation index; HWE, Hardy-Weinberg Equilibrium.

**A high-resolution, chromosome-assigned Komodo dragon genome reveals adaptations in the cardiovascular, muscular, and chemosensory systems of monitor lizards**

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Monitor lizards are unique among ectothermic reptiles in that they have a high aerobic capacity and distinctive cardiovascular physiology which resembles that of endothermic mammals. We have sequenced the genome of the Komodo dragon (*Varanus komodoensis*), the largest extant monitor lizard, and present a high resolution *de novo* chromosome-assigned genome assembly for *V. komodoensis*, generated with a hybrid approach of long-range sequencing and single molecule physical mapping. Comparing the genome of *V. komodoensis* with those of related species showed evidence of positive selection in pathways related to muscle energy metabolism, cardiovascular homeostasis, and thrombosis. We also found species-specific expansions of a chemoreceptor gene family related to pheromone and kairomone sensing in *V. komodoensis* and several other lizard lineages. Together, these evolutionary signatures of adaptation reveal genetic underpinnings of the unique Komodo sensory, cardiovascular, and muscular systems, and suggest that selective pressure altered thrombosis genes to help Komodo dragons evade the anticoagulant effects of their own saliva. As the only sequenced monitor lizard genome, the Komodo dragon genome is an important resource for understanding the biology of this lineage and of reptiles worldwide.

**Effective reproductive isolation mechanisms (RIMs) between *Conolophus marthae* and *C. subscristatus* in Galápagos: the possible role of chemical signaling for species recognition**

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No current gene flow occurs between the only known population of *Conolophus marthae* and a population of *C. subscristatus* syntopically co-occurring on Wolf volcano (Isabela Island, Galápagos). The above raises the question of the existence of effective reproductive isolation mechanisms (RIMs) between *C. marthae* and *C. subscristatus*. A variety of organisms, including many reptiles, use chemical signaling for species recognition. In these species, chemical cues may be used to avoid interspecific mating, and studies have shown that chemical signals are important in preventing hybridization between congeneric species of snakes and other squamates. Femoral pore secretions have been indicated as important for inter- and intraspecific chemical communication in squamates. We hypothesized that differences in biochemical profiles should be expected between the two species if such secretions play a role as a RIM. To investigate this hypothesis we collected femoral gland exudate from adult individuals of *C. marthae* and *C. subscristatus* from Vulcan Wolf in three different years and both within and outside the reproductive season. Samples were processed using Gas Chromatography coupled with Mass Spectrometry (GC-MS). We identified over one hundred different chemical compounds. Non Metric Multidimensional Scaling (nMDS) was used to graphically represent the level of similarity of sampled individuals based on their chemical profiles. Results from non-parametric statistical tests indicate that the separation between the two species is significant. Differences between species profile of esters are particularly interesting. Such chemical compounds are known to be involved in olfactory communication, suggesting that the chemical profile signatures of the two species may be play a role in preventing hybridization between *C. marthae* and *C. subscristatus*. Further investigation is needed to better resolve temporal patterns and variation of biochemical profiles.

**From conservation genetics to conservation genomics: the case of the endangered land tortoise  
*Testudo hermanni***

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NGS (Next Generation Sequencing) technologies allow the study of a large number of DNA markers in non-model organisms. Genome-wide datasets improve the power of traditional analyses based on few neutral loci (such as microsatellites) to evaluate population structure and estimate demographic dynamics. In addition, they allow the study of functional DNA regions and local adaptation. In conservation biology, genomic analyses will likely facilitate the incorporation of genetic tools and analyses in conservation and management plans of species at risk of extinction. The Hermann's tortoise (*Testudo hermanni*) is an endangered land tortoise distributed in disjoint populations across Mediterranean Europe. Habitat reduction, intensive agricultural practices and forest fires are major causes of decline in different areas. Intense harvesting for the purpose of pet trade and the release of non-native individuals into autochthone populations represent additional threats. Our previous genetic studies based on a small panel of microsatellites and mtDNA markers were able to clearly distinguish two subspecies and to identify some major geographical groups, allowing also the development of a practical genetic toolkit for geographic assignment. More recently, we performed a ddRAD genomic sequencing that produced approximately 3,000 nuclear SNP markers and revealed further substructure in Western populations, especially in Calabria (Southern Italy). This genomic dataset was simplified into a panel of 48 informative SNPs that can be cost-effectively used to detect the geographic origin of confiscated individuals currently kept in captivity, thus helping their correct relocation in reintroduction plans. In parallel, we are generating a draft genome combining longreads from Oxford Nanopore technology and short-reads from Illumina. The draft genome will enable the resequencing of interesting functional loci in individuals from different subspecies, and, possibly, the mapping of the RADseq loci. This genomic resource will thus allow us to better understand the effective risks of translocation and hybridization in this species, and propose management plans based on functional genomic regions.

## Effects of ovarian fluid on sperm traits and its implications for post-mating sperm selection in the zebrafish

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Sexual selection drives the evolution of traits that maximise the fitness of the bearer. It operates through diverse processes before the mating, and, when females mate with multiple males, also after the mating. In particular, in polyandrous mating systems, females maintain the opportunity to bias male fertilization success after mating in a process known as cryptic female choice. This kind of post-mating sperm selection often occurs in the context of inbreeding avoidance to favour the most genetically compatible males. Mechanisms of cryptic female choice have been found to affect fertilization processes both in internal and external fertilizers. Recently, the fluid surrounding the eggs, known also as the ovarian fluid, has been shown to potentially serve as a mechanism of cryptic female choice as it may directly affect sperm performance. Here, we studied the role of ovarian fluid in post-mating sexual selection using the zebrafish, *Danio rerio*. Firstly, we assessed how ovarian fluid affects sperm swimming performance compared with freshwater. We focused on sperm motility, velocity, swimming trajectory, and longevity, all traits associated with competitive fertilization success in externally fertilizing fish. In a second step, we used a North Carolina II design to explore female, male, and female x male effects by testing sperm motility of 2 males in the ovarian fluid of 2 females in a total of 11 blocks. Our results suggest that the ovarian fluid affects sperm performance differently from freshwater. Specifically, sperm velocity, motility, and longevity were higher in the ovarian fluid than in freshwater, whereas sperm linearity and beat cross frequency showed the opposite pattern. Moreover, these effects varied according to male and female identities, supporting the potential for cryptic female choice mediated by ovarian fluid in this species.

### **Sexual selection under varying predation risk**

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In many animal species, male sexual signals show plastic responses to environmental variation, but how this plasticity affects sexual selection dynamics is currently unknown. Predation is a particularly potent environmental factor with respect to its effects on life-history traits of prey species, including development and maintenance of sexually selected male characters. Using chemical and visual cues, we manipulated the risk of predation perceived by guppies (*Poecilia reticulata*) housed in semi-natural mesocosm populations of equal sex ratios. Replicate male groups were allowed to freely interact and mate with different sets of females in two conditions: presence versus absence of imminent predation risk. Male mating success was assessed from behavioural observations and the resulting offspring were collected and processed for molecular paternity analysis. This design allowed us to measure differences in male mating behaviour and paternity share between the two treatments. We describe how a) male sexual behaviour, b) male and female exploratory behaviour and c) pre- and post-mating male sexual traits (colouration and sperm properties, respectively) relate to differences in male reproductive success and female polyandry. We compare our results to previous lab and field studies of sexual selection processes under the risk of predation and discuss the potential long-term implications of predation pressure for fitness and ensuing population structure.

## Evolutionary implications of translocation for whitefish in Scotland

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Understanding how colonisation of novel environments associated with population bottlenecks affect founder populations is a major question in evolutionary and conservation biology. Translocations have become an important tool for species conservation, but small population sizes and new ecological pressures might lead to the phenotypic and genotypic divergence of translocated populations, with unknown long term impacts. In Scotland, European whitefish *Coregonus lavaretus* is natively present in two lakes, both of which are of conservation concern due to habitat degradation and invasive species. Six refuge populations were established over the last 30 years as a conservation measure. These refuge lakes differ substantially from the source lakes in biotic and abiotic characteristics. In this study we aimed to identify the phenotypic, epigenetic, and genetic effects of rapid environment change and translocation to new habitats. We used a combination of ecological, morphological, genomic, and DNA methylation analyses.

Morphological analyses showed a significant divergence in body shape and functional traits between source and refuge populations, with refuge populations converging in morphospace despite their different ages and genetic founders. Analysis of 6114 genome-wide SNPs showed minimal reduction in genetic diversity in the refuge populations, but significant  $F_{st}$  divergence between source and refuge populations in some cases. Analysis of genotype-environment associations identified loci consistently associated with refuge populations that were involved in tissue and neuronal development, and behaviour. 137 loci were differentially methylated between source and refuge populations and these tended to occur in or near genes whose functions involve muscle and cell functioning, and body development. These results suggest that adaptation to new environments can arise very rapidly, after few generations, and that both genetics and epigenetics facilitate this process.

**Symposium**

**SOCIAL BEHAVIOUR: ECOLOGY AND EVOLUTION**

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**Evolutionary consequences of social transmission in avian brood parasitism systems**

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Obligate brood parasites lay all of their eggs in nests of other species, leaving the burden of parental care entirely to the hosts. As a consequence of being parasitized, hosts' reproductive success is often reduced. This strategy has triggered a coevolutionary dynamic involving behavioural, physiological and morphological adaptations and counter-adaptations from the two players, whose conflicting functions are to successfully parasitize a nest, and prevent or reduce the negative effects of parasitism. In parasite systems studied in the New and Old worlds, warbler hosts exhibited different degrees of learning antiparasite defences from conspecifics. By quantifying strength and direction of selection of intensity and plasticity of nest defence, it was possible to reveal role and evolutionary consequences of social transmission in the frontline defence of this arms race. As group mobbing was also part of antiparasite defence, results indicate that an extended phenotype, including conspecific and heterospecific social phenotype, should be taken into account for continued investigations of the selection trajectories in this ideal coevolutionary model.



**The danger within: relationship quality affects vigilance in wild spider monkeys (*Ateles geoffroyi*)**

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Vigilance is known to be an effective anti-predator strategy. It is often studied in relation to group size, as individuals in larger groups are expected to spend less time vigilant compared to individuals in smaller groups (sensu many-eyes hypothesis). In primate species, however, the relationship between vigilance behavior and group size is inconsistent possibly because of the need for individuals to monitor risky group members as well as extra-group threats. We examined whether the quality of social relationship between group members affects vigilance patterns among spider monkeys, a species with a high degree of fission-fusion dynamics in which subgroup composition changes frequently. We used focal animal sampling to collect data on social interactions and individual vigilance of all adults and subadults (N=22) of a group of well-habituated Geoffroy's spider monkeys living in the protected area of Otoch Ma'ax Yetel Kooch, Yucatan, Mexico. Through a principal component analysis of seven indexes of social interactions, we obtained three components of relationship quality, reflecting the levels of compatibility, value and security. We tested whether individual's vigilance was affected by these three components at three levels. First, we considered the average levels of compatibility, value and security that the focal animal shared with its subgroup members. Second, we considered the lowest levels of compatibility, value and security that the focal animal shared with its subgroup members. Third, we considered the average levels of compatibility, value and security that the focal animal shared with its neighbors (i.e. individuals within 5 m). We did not find evidence for an effect of compatibility and value. However, individuals were more vigilant when they shared lower levels of security with specific members present in the subgroup and with the average neighbor.

### **A long-term study on social behaviour of a wild large herbivore**

*Stefano Grignolio<sup>1</sup>, Alice Brambilla<sup>2</sup>, Marco Apollonio<sup>1</sup>, Francesca Brivio<sup>1</sup>*

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University of Zurich

In polygynous mammals, social dominance is a fundamental aspect of male evolutionary ecology because it determines access to resources as well as to oestrous females. The achievement and the defence of a favourable hierarchical rank requests a remarkable energy investment which can be excessive in demanding environmental conditions. As it is generally difficult to monitor wild marked individuals for a long period, little is known about the determinants of male dominance and the evolution of hierarchy throughout individual life history. We studied the time devoted to interactions and social structure of 79 marked Alpine ibex (*Capra ibex*) males in the Gran Paradiso National Park – Italy – from 2011 to 2016. Differently to other large and polygynous herbivores in continental and Alpine environments, in ibex a clear temporal separation occurred between the period with high-intense interactions to define the social dominance (late spring – early summer) and the mating season (December). Age clearly affects male social rank, but ibex showed a great age-independent phenotypic heterogeneity: rapidly growing males reached high rank at a younger age than slow-growing males. Our results suggested that high-quality males more likely reached high social ranks during their life history than low-quality males. We showed that they are not able to continuously maintain their social rank year by year: generally, they have to alternate years with a more consistent investment to increase their rank with years with lower hierarchical positions. This long-term study on social dominance of a wild large herbivore highlight the complex trade-offs involved in the social dominance process and the pivotal role of behavioural plasticity in individual life history.

**Submissive behaviour is mediated by sex, social status, relative body size, and shelter availability in a social fish**

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Acting submissively may inhibit aggression and facilitate the termination of contests without further escalation. The need to minimise conflict is vital in highly social species where within group interactions are frequent and aggression can dampen group productivity. Within social groups, individual group members may modulate their use of submissive signals depending on their phenotype, the value of the contested resource, their relationship to the receiver of the signal, and on the characteristics of the local environment. We predicted that submissive behaviour would be more common when signallers had limited ability to flee from conflict, when signallers were of a low rank within the group, when signallers and receivers differed substantially in body size (and thus in fighting ability), and when signallers and receivers were of opposite sex and therefore not directly in competition over reproductive opportunities. We tested these predictions using social network analyses on detailed behavioural observations from 27 social groups of the cooperatively breeding cichlid fish *Neolamprologus pulcher*. Congruent with our prediction, submissive behaviour was more common when there were fewer shelters available, suggesting that constraints on fleeing behaviour may increase the use of submission. Also fitting with predictions, submissive behaviour was more common with increasing body size asymmetry between the competitors, among lower ranked fish, and in interactions between opposite sex dyads, which supports the idea that signalling submission is adaptive in contests over low-value resources. Our findings suggest that subordinate *N. pulcher* are primarily concerned with being tolerated within the social group and may use submissive behaviour to avoid escalated conflict. Our findings offer a window into the factors that influence signals of submission in a highly social vertebrate.

### **Social dominance and fertility: indirect genetic effects and antagonistic relationships**

*Cristina Sartori<sup>1</sup>, Nadia Guzzo<sup>2</sup>, Enrico Mancin<sup>1</sup>, Roberto Mantovani<sup>1</sup>*

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The causal effect of a genotype belonging to an individual on the phenotype of another one, also known as indirect genetic effect (IGE) has been widely used in recent studies to analyze the genetic components of social dominance. This trait is generally modelled as response of a dyadic interaction between a winner (dominant) and a loser (subordinate) expecting a genetic correlation of -1 between these two roles. Assuming that different genetic correlations with other traits are expected for the direct and indirect components of a trait, this study aimed to investigate the genetic correlations between social dominance and fertility, known as antagonistic traits. Phenotypic information was obtained in the local Aosta Chestnut and Aosta Black Pied cattle, famous for their attitude to compete, exhibited in traditional tournaments. Here, pairs of cows assess social dominance in bloodless interactions routinely recorded by the breeders' organization. The outcome of 12.3k interactions performed by 4.3k cows in 12 years has been joined to the individual data for fertility, measured using lactation and pedigree information. Bivariate animal model analysis has been carried out via Gibbs sampling using the IGE approach. Different models accounting for variance components were tested and compared. Individual breeding values were used to trace the evolutionary trajectories of traits. An antagonistic genetic correlation of -0.39 was found between the direct component of social dominance and fertility, whereas the indirect component was positively correlated (0.21). The expected genetic correlation close to -1 between the direct and indirect components was confirmed, as well as the divergent genetic trend, leading to a null trend for the whole social dominance. A null trend for fertility was also found. The study provided an empirical evidence in cattle of evolutionary constraints among competition and fitness-related traits as fertility.

**A special case in ant-plant relationships: mutualism or partner manipulation mediated by extrafloral nectar?**

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Interactions involving ants and plants constitute textbook examples in animal-plant biology. Many plants bear extrafloral nectaries (EFNs) that seem to be specially designed to attract ants whose feeding ecology and behaviour make them very efficient plant defenders. Most data available on EFNs-based ant-plant interactions refer to tropical regions and no detailed study has been conducted so far in Italy. In order to reduce this gap we carried out a series of field and laboratory investigation involving different ant-plant study systems. Although, plant-ant associations based on trophobiosis (food in exchange of protection) are generally considered as mutualisms, with both parties gaining benefits from the association, it has recently emerged that some of these associations have evolved towards other forms of relationships and, in particular, that plants may manipulate their partner ants to make reciprocation more beneficial, thereby stabilizing the mutualism. In this context, our data confirm the importance of the ants for the associated-plant fitness showing their crucial role against herbivores. However, some aspects of the plant biology, such as nectar production, amount and composition, seem designed to affect ant behaviour, improving their attraction and performances. In fact, the ants that fed on nectar patrol restless the host plants and are strongly attracted towards EFNs. They showed a higher level of aggression against other insects and changes in response to social and environmental stimuli. Hence, data obtained from some of our model systems support the hypothesis that plants may engage in a sort of “coercive mutualism”, controlling the behaviour of the ants in order to maximize partner-derived rewards and get a better service as bodyguards without disrupting the association.

## **Interactions between individual variation in behaviour and group performances in House Sparrow**

*Beniamino Tuliozi<sup>1</sup>, Elisa Ligorio<sup>1</sup>, Ettore Camerlenghi<sup>2</sup>, Matteo Griggio<sup>1</sup>*

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Social living strongly influences selection on individuals' traits, which in turn evolve to shape the social environment. Individuals in a group vary, and this variation creates social groups that differ from one another with respect to their collective performance and the distribution of resources and fitness of their members. Using captive house sparrows (*Passer domesticus*) we performed a series of experiments investigating the feedback between individual and group traits in situations particularly crucial to survival and fitness. i) Using a novel experimental design we investigated how two groups with different characteristics would exploit limited resources in a novel environment, i.e. if the amount of resources each individual consumed was going to depend on the groups' attributes. We discovered that belonging to the group of the first individual to exploit the resource was critical in determining the amount of resource consumed. ii) We tested house sparrow dyads during an open-field test and during a simulated predator attack. We discovered that individuals assuming the position of leaders and producers during the open-field test switched to being followers during the attack.

Session

EVOLUTIONARY MEDICINE

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**Mr. Darwin incontra il Dr. House: i contributi della biologia evoluzionistica alla medicina e alla psichiatria**

*Alfonso Troisi*

International Medical School, Università di Roma Tor Vergata

La teoria dell'evoluzione ha modificato in modo sostanziale il concetto di causalità dei fenomeni biologici. Applicando il concetto di causa ultima allo studio delle malattie fisiche e mentali, la medicina e la psichiatria darwiniane suggeriscono delle ipotesi causali che non sono comunemente prese in considerazione dai modelli eziologici correnti. In questa relazione sono prese in esame quattro possibili cause ultime che possono essere all'origine di alcune malattie fisiche e mentali: le eredità filogenetiche, le manifestazioni di malattia come risposte adattative, gli ambienti evolutivamente nuovi e la sopravvivenza di geni di vulnerabilità. La psichiatria darwiniana richiama l'attenzione sulla possibilità che l'origine di alcune malattie mentali, come ad esempio le fobie, possa essere pienamente compresa soltanto alla luce dell'analisi filogenetica. Una seconda categoria esplicativa in termini di cause ultime riconduce l'eziologia di alcune manifestazioni di malattia al concetto di reazione adattativa. In altri termini, la medicina e la psichiatria darwiniane riconoscono la possibilità che alcuni sintomi e alcune sindromi siano in realtà risposte biologicamente vantaggiose e non manifestazioni disfunzionali. Questa ipotesi presuppone il riconoscimento di un dato che è ampiamente estraneo non solo al senso comune ma anche al pensiero dominante in medicina e cioè che la selezione naturale non ha necessariamente favorito il benessere fisico e mentale privilegiando invece l'adattamento biologico misurato come integrazione di sopravvivenza e fitness inclusiva. La discrepanza tra le condizioni attuali di vita e le caratteristiche fisiologiche e psicologiche selezionate per l'adattamento all'ambiente ancestrale può essere la causa ultima di alcune condizioni come l'obesità e l'abuso di droghe la cui prevalenza è aumentata sensibilmente negli ultimi decenni. Infine, la persistenza nel genoma umano di geni che aumentano la vulnerabilità a differenti patologie mediche e psichiatriche può essere dovuta a meccanismi selettivi che hanno favorito l'evoltersi di risposte plastiche durante le fasi precoci dello sviluppo ontogenetico (secondo il modello "for better or for worse").

## **Importance and Meaning of Phenoptosis**

*Giacinto Libertini*

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Phenoptosis is defined as the death of an individual caused by its own actions (direct phenoptosis) or by actions of close relatives (indirect phenoptosis), and is a heterogeneous group of adaptive phenomena determined, regulated or influenced by genes favoured by natural selection. Phenoptotic phenomena: (i) can be direct/indirect, rapid/slow, obligatory/optional; (ii) are largely widespread in the living world (both among prokaryotes and among unicellular and multicellular eukaryotes); and (iii) are the main factors that determine the life tables of most species. Aging, defined as age-related increasing mortality in the wild, can be interpreted as a form of slow phenoptosis and is characteristic of a minority of species. Our species manifests multiple forms of optional, direct or indirect, phenoptosis (elimination of foetuses before birth due to at least three types of reasons; infanticide due to at least eight types of documented reasons; sacrifice or endangerment of one's life in particular circumstances) and a single form of obligatory phenoptosis (aging).

As phenoptosis is certainly damaging for the individual, it cannot be explained in terms of individual selection (the Spencerian and then Darwinian "survival of the fittest") and can only be interpreted in terms of supra-individual selection (kin selection, and various possible forms of group selection). Phenoptotic phenomena both for their number and variety of manifestations and for their enormous impact on fundamental aspects of the characteristics of living beings, cannot be considered as a set of unusual biological curiosities but represent a fundamental key to interpret the living world. In fact, it is possible to say that to understand in evolutionary terms the manifestations of life, it is essential to understand death at the same time.



**Symposium**

**POPULATION GENETICS AND POPULATION GENOMICS**

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**Genetic effects of range expansions**

*Montgomery Slatkin*

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I will first review the population genetics theory of the range expansions. During a range expansion repeated founder events occur as a species expands its geographic range. For many purposes, these founder events are similar to episodes of genetic drift that occur in succeeding generations in a single population. The analogy between these two processes leads to relatively simple analytic theory that reveals the common properties of range expansions. I will discuss ways to detect range expansions using the directionality index, to determine their place of origin and to quantify the average intensity of genetic drift during a range expansion. I will present a new method for estimating demographic parameters of a model of range expansion by modifying existing methods applicable to a time series of samples from a single population.

## Highly-resolved strain-level population dynamics of *Wolbachia* from large-scale metagenomes

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Symbiosis studies typically focus on a given bacterial species associated with a specific host organism, yet little is known about how endosymbionts vary within and between hosts. We address this issue in the bacterial endosymbiont *Wolbachia*, the common intracellular manipulator of invertebrate biology. While it has been already studied from a macroevolutionary point of view (i.e. phylogeny and comparative genomics), little is known about its diversity and evolution within its different hosts. Here, we compiled 10 *Wolbachia* strain datasets from a collection of >1,000 *Wolbachia* genomes sampled from 10 populations of insect and nematode hosts. These large-scale datasets allowed us to explore, at an unprecedented depth, how an intracellular bacterial parasite evolves in different hosts. We first found that *Wolbachia* recombination is highly heterogeneous among hosts, both in terms of frequency and fragment sizes, and that it is not responsible for incongruences between *Wolbachia* and host phylogenies. Nucleotide polymorphism patterns indicate that each strain is characterized by distinct demographic histories and has likely undergone distinct selective pressures in different hosts. Overall, our results suggest that the diversity and the evolutionary history of *Wolbachia* are driven by a combination of horizontal transmission and adaptation to the host.

## **Distinguishing among complex evolutionary models using unphased whole-genome data through Approximate Bayesian Computation**

*Silvia Ghirotto<sup>1</sup>, Maria Teresa Vizzari<sup>1</sup>, Francesca Tassi<sup>2</sup>, Guido Barbujani<sup>2</sup>, Andrea Benazzo<sup>2</sup>*

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Inferring past demographic histories is crucial in population genetics, and the amount of complete genomes now available for many species should in principle facilitate this inference. In practice, however, the available inferential methods suffer from severe limitations. Although hundreds complete genomes can be simultaneously analyzed, complex demographic processes can easily exceed computational constraints, and there are no standard procedures to make sure that the estimates obtained are reliable. Here we present an Approximate Bayesian Computation (ABC) framework, based on the Random Forest algorithm, to infer complex past population processes using complete genomes. Under this framework all possible pairs of populations are compared, and the data are summarized by the full genomic distribution of the four mutually exclusive categories of segregating sites (*FDSS*), a set of statistics fast to compute even from unphased genome data. We constructed an efficient ABC pipeline and tested how accurately it allows one to recognize the true model among models of increasing complexity, using simulated data and taking into account different sampling strategies in terms of number of individuals analyzed, number and size of the genetic loci considered. Once assessed the power of the *FDSS* to be informative about even complex evolutionary histories, we analyzed real datasets. We tested alternative models on the dispersal of anatomically modern humans out of Africa and we explored the evolutionary relationships of the three species of Orangutan inhabiting Borneo and Sumatra. The flexibility of our ABC framework, combined with the power provided by the set of statistics proposed, pave the way for reliable inference of past population processes for any species for which high coverage genomes are available.

**Ancient genomes reveal early farmers selected common beans while preserving diversity**

*Emiliano Trucchi<sup>1</sup>, Andrea Benazzo<sup>1</sup>, Martina Lari<sup>2</sup>, Alice Iob<sup>1</sup>, Stefania Vai<sup>2</sup>, Laura Nanni<sup>3</sup>, Elisa Bellucci<sup>3</sup>, Elena Bitocchi<sup>3</sup>, Xu Chunming<sup>4</sup>, Scott Jackson<sup>4</sup>, Lema S Verónica<sup>5</sup>, Babot Pilar<sup>6</sup>, Oliszewski Nurit<sup>6</sup>, Gil Adolfo<sup>7</sup>, Meme Gustavo<sup>7</sup>, Michieli Teresa<sup>8</sup>, De Lorenzi Monica<sup>9</sup>, Lucio Calcagnile<sup>10</sup>, David Caramelli<sup>2</sup>, Bastiaan Star<sup>11</sup>, Hugo de Boer<sup>11</sup>, Sanne Boessenkool<sup>11</sup>, Roberto Papa<sup>3</sup>, Giorgio Bertorelle<sup>1</sup>*

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While selecting desirable phenotypic traits in the last 13,000 years of crop domestication, farmers and breeders also led the domesticated populations through several demographic bottlenecks. As a consequence, erosion of wild genetic variation is typical of modern cultivars making them highly susceptible to pathogens, pests and environmental change. Understanding when the majority of genetic diversity was lost, and how such loss is related with the intensity of the selection process, is not only relevant from an evolutionary perspective but it will also help planning more sustainable breeding options to revert or mitigate crop's genetic erosion. We directly addressed these questions using whole genomes from ancient and modern South American seeds of the common bean (*Phaseolus vulgaris*), one of the major sources of vegetable proteins worldwide, that was independently domesticated in Mesoamerica and the Andes ca. 8,000 years ago. Fifteen ancient beans were radiocarbon dated between 2,500 and 600 years ago and sequenced at >4X and compared with 14 modern seed genomes. Modern Chilean cultivars appear as the direct descendants of the seeds used in Argentina before the Incaic occupation of the area. Analyzing the temporal dynamic of genetic variation and selection during domestication, we found that most of the genetic changes probably occurred before 2,500 years ago, but genetic erosion is, on the contrary, a more recent process, not detectable in 600 years old seeds. Considering that most desirable phenotypic traits are likely controlled by multiple polymorphic genes, a likely explanation of this decoupling is that early farmers used many phenotypically similar but genomically diverse individuals as breeders. On the other hand, selection strategies in the last centuries were probably less sustainable and produced further improvements focusing on few seeds with extreme values of the traits, at the cost of marked genetic erosion.

### Phenotype prediction from ancient low-coverage genomes

*Eugenia D'Atanasio*<sup>1</sup>, *Christiana L. Scheib*<sup>2</sup>, *Rouyun Hui*<sup>3</sup>, *John E. Robb*<sup>3</sup>, *Craig Cessford*<sup>3, 4</sup>, *Sarah A. Inskip*<sup>3</sup>, *Lehti Saag*<sup>2, 5</sup>, *Kristiina Tambets*<sup>2</sup>, *Mait Metspalu*<sup>2</sup>, *Toomas Kivisild*<sup>1</sup>

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Over the past decade, the technological improvements in the shotgun sequencing allowed the analyses of an increasing number of ancient DNA (aDNA) sequences, which can be highly informative about past human evolution and adaptation. However, because of *post-mortem* degradation, low endogenous DNA content and contamination, the average coverage of ancient sample sequences is often not higher than 1×, making the interpretation of the aDNA data challenging. To overcome these problems it is possible to impute missing genotypes by comparing the aDNA sequences with a modern phased reference panel, but this approach is usually considered reliable for samples with a coverage of at least 1×. We performed several imputation accuracy tests, in order to obtain reliable results for hundreds of ancient samples with an average coverage of about 0.15x. Our sample was composed of two groups of individuals, from Estonia and England respectively, dating back to different cultural and time periods, ranging from Neolithic to Middle Age. After adjusting the imputation pipeline for our low coverage ancient genomes, we performed a local imputation to analyze a set of phenotype informative markers, involved in visible features (such as eye, hair and skin color) and in adaptation to external agents (such as diet and pathogens). We used publicly available modern samples as reference panel, after filtering it to discard the rare variants with a minor allele frequency lower than 0.01. The observed differences in frequencies between times and geographic areas allowed us to shed light on past evolutionary changes linked to cultural shifts or pandemics.

### **Polygenic adaptation to high altitude: insights from Tibetan and Sherpa genomes**

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Tibetan and Sherpa populations from the Himalayas present several physiological adjustments evolved to cope with selective pressures imposed by the high-altitude environment, especially hypobaric hypoxia. Nevertheless, only few selective events at hypoxia related genes were confirmed by multiple genomic studies. Moreover, variants at these loci were found to be associated with downregulation of the erythropoietic cascade, which represents just an indirect aspect of the considered adaptive phenotype. Accordingly, the genetic basis of Tibetan and Sherpa adaptive traits remains to be fully elucidated, in part due to limitations of selection scans implemented so far, which have mostly relied on the detection of hard selective sweeps. In order to overcome this issue, we assembled a dataset of newly generated and available from literature Tibetan and Sherpa whole genome sequences and we used them to calculate different haplotype-based positive selection statistics. Then, we explored the obtained genome-wide distributions of selection scores with a recently developed gene network analysis aimed at explicitly testing for the occurrence of polygenic adaptation in these high-altitude populations. This approach allowed us to detect subtle genomic signatures ascribable to weak positive selection acting on multiple genes involved in the same biological function. In particular, we inferred adaptive evolution at loci individually showing small effect on the considered phenotype, but belonging to highly interconnected functional pathways overall involved in angiogenetic processes. Therefore, these findings pinpointed a series of selective events neglected so far that plausibly contribute to the augmented tissue blood perfusion observed in Tibetans and Sherpa, thus enabling to uncover the genetic determinants of a key biological mechanism underlying their adaptation to high altitude.

## **Unravelling genetic adaptations of Mexican populations to their ancestral environments and the potential dis-adaptive loci influencing their present-day disease risk**

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Mexican populations, particularly those groups still showing substantial Native American (NA) ancestry, may have retained at appreciable frequency in their gene pools adaptive variants/haplotypes targeted by natural selection in response to selective pressures experienced during their pre-Columbian history. Being nowadays exposed to different physical (due to historical migration) and cultural (due to westernization of dietary habits and lifestyles) environments, some of the related traits might have become unfavourable (i.e. dis-adaptive), thus influencing present-day patterns of disease risk of these populations. To test such a hypothesis, we used the Native Mexican Diversity Panel [1], composed of 350 samples from 15 Mexican NA populations characterized for 903,800 SNPs, and the 1000 Genomes Project Phase 3 as a reference panel to create a “high-quality imputed dataset” which included information about 6,374,955 SNVs for 271 Mexican samples. Haplotype-based CHROMOPAINTER/fineSTRUCTURE analyses were then performed to identify genetically homogenous population clusters to be submitted to selection scans. In detail, we observed three main macro clusters: one included NA populations from Northern Mexico (Seri and Tarahumara), another one was made up of people from Central Mexico (Huichol, Nahuatl, Nahuatl Jalisco, Nahuatl Puebla and Purepecha) and the last one grouped populations from Southern Mexico (Tojolobal, Tzotzil, Maya, Lacandon, Triqui, Zapoteca and Mazateca) with the Totonaca from Central Mexico. These clusters were found to be consistent with the putative ancestral geographic location, the related environmental conditions, and the historical processes previously described for pre-Columbian cultures distributed along the Mexican territory. This enabled us to infer adaptive evolution of each cluster assuming that populations belonging to it have presumably experienced similar/same selective pressures, and to evaluate the potential correlation of the identified genomic footprints of natural selection with patterns of chronic complex diseases observed in the examined groups.

**Defining contemporary “populations”, and inferring their ancestral histories, using genome-wide DNA**

*Garrett Hellenthal*

University College London

I describe statistical techniques to cluster individuals into genetically homogeneous "populations" using genome-wide autosomal data, and discuss the extent to which these genetics-based groupings correspond with national identity and other factors. I apply these methods to data sampled from multiple world-wide populations, including over 6000 European individuals from Belgium, Denmark, Finland, France, Germany, Italy, Norway, Poland, Spain and Sweden. I show cases where country borders appear to erect barriers to intermixing, though note other instances where older historical and/or recent cultural factors play a larger role. I further detail methods to infer and date when such groups intermixed in the past 4,500 years. Using DNA data from extant individuals, I illustrate how contemporary samples can be used to unearth details about historical interactions among peoples over different time-scales. These analyses demonstrate the genetic impact of well-attested historical events (e.g. empires, migrations), provide evidence for previously unknown or unclear interactions, and point towards the many genetic links among geographically separated humans.



**Paleolithic interactions: hunter-gatherer genetic history across European geo-temporal transects**

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Little is known about the population history of European hunter-gatherers for more than 35,000 years, spanning from the first arrival of modern humans into Europe to the Neolithic transition. Paleogenetic analyses of Upper Paleolithic and Mesolithic hunter-gatherers were performed to investigate demographic changes through time revealing the presence of population structure and genetic turnovers in Ice Age Europe. By expanding the temporal and geographical distribution of genomic data it was possible to infer past population movements and compare them to climatic and archaeological records. While the formation of some genetic clusters tightly matches to the associated archaeological cultures across Europe, other major genomic shifts seem to be more linked to environmental fluctuations. A larger sample size through time and space is pivotal to further reconstruct the interactions among those genetically distinct hunter-gatherer groups.

# POSTERS

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**Molecular evolution of the genus *Hyaella* (Crustacea: Amphipoda) in the Titicaca-Desaguadero-Poopo-Salar water system, High Andes**

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The Titicaca-Desaguadero-Poopo-Salar de Coipasa System (TDPS) comprehends four major water masses, located at the border between Perú and Bolivia. The geological history of these water systems is complex but relatively recent compared to other ancient lakes, as have a Pliocene-Pleistocene formation. Even though the number of endemic aquatic organisms in Titicaca is low compared to other more ancient water systems, species flocks occur for different taxonomic groups including amphipods. The genus *Hyaella* in the Titicaca has been shown to have a polyphyletic origin, with at least five different clades deduced to be older than the lake itself, due to multiple colonization events at different times. We have reconstructed the evolutionary history of the genus in the TDPS using 36 specimens collected at different localities of Peru, Bolivia, Ecuador and Chile. Multiple phylogenetic markers have been obtained by shotgun sequencing to explore the *Hyaella* evolutionary relationships within a phylogenomic framework: protein-coding mitochondrial genes, 18S and 28S ribosomal sequences. Our results agree with previous studies on the existence of five deep TDPS genetic lineages, some related to South American taxa, but also point to an early split in the phylogeny. A group of three TDPS species clusters with a taxon from Tierra del Fuego (Chile), while the remaining taxa appear to be closer to North-American *Hyaella*, including the Ecuadorian clade. The study – in conjunction with a large mitochondrial COI dataset – evidenced a high level of incongruence between molecular and morphological information, shedding doubts on the taxonomic value of some morphological characters such as body spines on tergites and coxal plates. In addition to systematic analyses, details of the comparative mitochondrial and nuclear ribosomal genomic features such as mitochondrial strand bias, phylogenetic informativeness and non-synonymous/synonymous substitution rates are also presented.

## Sexual selection in outcrossing and selfing populations of *Arabidopsis lyrata*: a phenotypic and transcriptomic analysis

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*Arabidopsis lyrata* is a perfect candidate for studying the influence of sexual selection in angiosperms evolution, given the existence of separate populations reproducing with different mating systems: outcrossing (self-incompatible) or selfing (self-compatible). Given the higher variability and the higher chances of pollen competition in outcrossing populations compared to selfers, the former are expected to experience a stronger level of sexual selection, while in the latter the selective pressure should be relaxed. Performing crosses within and between populations of *A. lyrata* can therefore help to draw interesting results about the importance of sexual selection in shaping angiosperm evolution of reproductive traits. We tested for differences between populations in terms of several traits that can play a role in sexual selection: pollen tube growth rate, pollen germination rate and speed, stigma receptivity, size and amount of pollen produced, number of ovules and flower traits such as pistil length and petal size. We also performed crosses within and between populations to assess fertilization ability and multiple donor crosses to test for competition in terms of paternity success. We also performed differential expression analysis of sexual specific tissues, compared with non-sexual tissues, to identify genes that may be possible candidates for the action of sexual selection and to assess whether those genes are expressed at different levels in outcrossing and selfing populations.

**Characterization of root-nodulating bacteria isolated from *Genista cinerea* (Fabaceae) growing in North African drylands using Bayesian Markov chain Monte Carlo (MCMC) methods and 16S rRNA sequencing**

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This study aims to characterize for the first time endosymbiotic bacteria isolated from root-nodules of a wild legume (*Genista cinerea*: Fabaceae). It examines growth variations of endosymbiont isolates under different environmental conditions and compare them with reference strains (RS), using advanced statistical modelling techniques. A phenotypic characterization was conducted using physiological tests (different pH levels, NaCl concentrations, and tolerance to temperature) and nutritional experiments (assimilation of different carbohydrates and amino acids). Data were analyzed using Markov Chain Monte Carlo sampler for multivariate generalized linear mixed models (MCMCglmm) to detect growth differences between isolates and RS. Similarities between isolates and RS were assessed using agglomerative hierarchical clustering (AHC), whereas multiple factor analysis (MFA) was performed to understand factors influencing each group of isolates/RS. Moreover, a molecular identification using 16S rRNA sequencing was carried out to identify genera of isolated bacteria. Symbiotic and cultural characteristics revealed the existence of a large physiological diversity among tested isolates, which showed a broad capability to assimilate different carbonaceous and nitrogenous substances, with consistent and large tolerances to pH [4–10], temperature [4–55°C], and salinity [NaCl=2–10%]. Although the endosymbiont isolates have broad metabolic diversity, they formed two distinct groups with high level of similarity with RS. Results of AHC and MFA evidenced that bacterial diversity of endosymbiont isolates showed high level of similarity with RS, a proof that they are rhizobial strains. The identified genera based on 16S rRNA sequencing included *Achromobacter*, *Klebsiella*, *Luteibacter*, *Pantoea* and *Pseudomonas*.

## Association of TGF $\beta$ 1 cod 10 (C>T) gene polymorphism with longevity in a North-Italian sample

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Human longevity is considered as a complex trait determined by both genetic and environmental factors. The genetic influence seems to increase with the higher ages, since centenarians have significantly better health compared to old but not centenarian subjects. Cytokines are crucial for the regulation of inflammation development in humans and some studies have shown that variations in cytokine genes might play a role in determining human longevity. For these reasons we decided to examine the possible association of six cytokine gene polymorphisms with longevity in an Italian cohort. A total of 1,019 healthy volunteers aged 10-100 and belonging to the North-Italian population were recruited. We genotyped subjects for TNF- $\alpha$  -308 (G>A), IL10 -1082 (G>A), IL10 -819 (C>T), TGF $\beta$ 1 cod 10 (C>T), TGF $\beta$ 1 cod 25 (G>C), IL6 (G>C) gene polymorphisms. In order to evaluate a possible age-associated selection, the sample was split in five age groups: 1-24, 25-49, 50-69, 70-85 and 86-100. No significant differences in cytokine allele frequencies were found between age groups, with exception of TGF $\beta$ 1 cod 10 (C>T) gene polymorphism, for which we observed a significant decrease of the T-allele in the oldest group compared to the younger ones. TGF- $\beta$  is a potent regulatory cytokine that plays an essential role in inflammation and in maintenance of immune response homeostasis. The mutations that alter the blood level of this cytokine, such as (C>T) mutation, could result in lowest levels of the functional cytokine, with consequent increase in the duration of the inflammation process and, consequently, in the cancer risk. In conclusion in this study we provided evidences for a role of TGF $\beta$ 1 cod 10 (C>T) gene polymorphism in longevity, in a sample of Italian subjects.

**Solving a morphological dilemma with genetic tools: species identification and population genetics of the Antarctic fish *Lepidonotothen* spp. in the Scotia Arc and Bouvet Island**

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*Lepidonotothen* (order Perciformes, suborder Notothenioidei, family Nototheniidae) is a genus of benthic notothenioid fishes which inhabit the Antarctic Peninsula, the Scotia Arc and the islands around the continent with a general circum-Antarctic distribution. Species determination in this group has often been problematic. In particular, the existence of two species, *L. squamifrons* and *L. kempi*, has been questioned. In fact, these two species are very similar as adults but show different growth rates and morphological characteristics at the pelagic larval stage making species identification based on morphology traits in adults more complex. In addition, several studies have failed to find genetic markers best suited to successfully identify individuals. In this study, we have analysed several individuals (adults and larvae) previously identified based on morphological traits and assigned to one of the two alleged species. For each individual, we have obtained the sequence of the mitochondrial NADH dehydrogenase 2 and a multilocus haplotype based on 21 microsatellite markers to test whether the morphological identification was consistent with the genetic variability. Preliminary results show little correlation between larvae morphology and the genetic assignment of the individuals to a species. Moreover, a genetically distinct group of individuals, all originally identified as *L. squamifrons*, was found only around the islands of Shag Rocks. This lends the hypothesis that *L. squamifrons* and *L. kempi* may be two differentiated species but that *L. squamifrons* occurs only around Shag Rocks. Future analyses await to clarify the evolutionary history that shaped the divergence of these two species.

### **In cold (white) blood: speciation, introgression and hybridization in Antarctic icefish**

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Hybridization is gaining interest in scientific studies. Once considered an occasional event across animal species, it is now seen as a widespread phenomenon. Hybridization can have different outcomes: it can promote genetic diversity and help the rescue of inbred populations, but it can also decrease the reproductive success of individuals by the production of unfit hybrid offspring and lead to genetic homogenization disrupting local adaptations. Here, we analyze the extent of hybridization among three species of icefish belonging to the genus *Chionodraco* (order Perciformes, suborder Notothenioidei, family Channichthyidae). Notothenioid fishes dominate the cold waters around the Antarctic continent in terms of species diversity, abundance and biomass. These fishes are extensively studied in evolutionary biology since they represent an important example of adaptive radiation, one of the most relevant in the marine realm. By means of a panel of 18 microsatellite loci and morphological analysis of otolith shape, we investigated the species boundaries, population genetics and phylogeographic patterns of the species *Chionodraco hamatus*, *Chionodraco myersi* and *Chionodraco rastrispinosus*. These species are morphologically very similar and difficult to distinguish. *C. hamatus* and *C. myersi* are sympatric while *C. rastrispinosus* is allopatric compared to the other two species. We discovered that *C. rastrispinosus* has a larger distribution than previously known, coming in contact with the two other species in Weddell Sea and hybridizing with them. In order to understand the possible routes of dispersion, a Lagrangian particle model of the regional oceanic currents was implemented. Understanding the population dynamics of species, especially the factors modulating demography and gene flow among populations, is crucial for future predictions of species evolutionary trajectories and for a clear description of biodiversity.



## **Growth regulation in the larval development of the lepidopteran *Pieris brassicae***

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In the view that developmental processes provide for a more complete explanation of observed evolutionary patterns (evo-devo perspective), the specific features of the developmental system, in particular those related to the regulation and control of developmental dynamics across ontogeny, are considered of high interest to understand evolution and evolvability at organismal level. Using geometric morphometrics and adopting a longitudinal-study design, we investigated some selected features of the processes of growth regulation in the larval development of the lepidopteran *Pieris brassicae*. We found that size is highly correlated across stages and that there is evidence for compensative growth. However, compensation is not implemented through the regulation of developmental timing, but rather through the modulation of size-increase rates. Developmental stability, estimated through measures of fluctuating asymmetries, shows no quantitative variation across stages. These results, when compared with those from similar studies in other arthropod species, contribute evidence of the widespreadness of the evolutionary process known as developmental system drift, where conserved features among taxa have diverged in their regulatory underpinning.

**Let mum take care of it! Maternal buffer effects in response to climate change in the sea urchin  
*Arbacia lixula***

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CO<sub>2</sub> emissions, due to fossil fuels consumption, are recognized as the main cause for Global Climate Changes (GCCs), such as increasing temperature and ocean acidification (OA). Temperature may intensify susceptibility of marine organisms to OA, especially in calcifying organisms. Moreover, early life stages are considered more vulnerable than adults, representing a possible bottle-neck for natural populations. However, negative effects of GCCs might be mitigated by maternal anticipatory effects. To verify this hypothesis, the larvae of the sea urchin *Arbacia lixula* from different families were exposed to two pH (pH 8.1 and 7.7) and three temperature values (16, 22 and 28°C) for 48 hours after fertilization, to highlight different pattern of response due to the transmission of phenotypes from mothers differently resilient to the tested stressors. Adults were collected from a current pH site (pH 8.1, Punta San Pietro-SP) and a volcanic CO<sub>2</sub> vents site off the Ischia island (pH 7.7, Castello Aragonese-CA), considered a natural laboratory to study long-term effects of OA. Alterations in the normal larval development were measured at 24 and 48 hours (% of developmental stages, % of abnormal larvae, larval length). Differences in the response of *A. lixula* larvae from the two sites were found. In particular, those from SP adults were significantly influenced by the interaction among pH, temperature and mother of origin, highlighting a different response to the selected stressors among families. On the opposite, larvae from CA adults showed to be resilient to reduced pH, at 22°C at least, while they were more thermosensitive than SP larvae. Beyond these results, we observed a significant influence of the maternal effect on the response trends among the various families exposed to the experimental conditions, demonstrating a transgenerational response effect with the transmission of more or less favourable phenotypes between parents and their offspring.

## **An eDNA protocol for monitoring and preserving biodiversity from genes to species: a case study of Alpine amphibians from Trentino**

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In the last decade, environmental DNA (eDNA) and metabarcoding have provided new opportunities for the study of biodiversity in space and time. The eDNA metabarcoding approach is increasingly used as a cost effective tool for species detection and community characterization, particularly for elusive species. However, technical challenges and case-specific limitations exist and need to be explored. In this study, we tested if eDNA metabarcoding of water samples can be used for developing an effective protocol for the characterization and monitoring of Alpine amphibian communities. By PCR amplification of a 151 bp COI barcode region using primers specific for amphibians, we aimed at assessing the species composition of several selected wetlands and ponds in an Alpine area, part of the MAB UNESCO 'Alpi Ledrensi and Judicaria' Biosphere Reserve (Trentino, south-eastern Alps). Choosing a widespread and abundant anuran, the common frog (*Rana temporaria*) as a model species, we tested for differences in detection success among three developmental stages and various freshwater environments. Lastly, by selecting a more specific COI fragment, we also aimed at assessing mtDNA genetic diversity in the focal species, which previous studies have found to be high in the study region and correlated with nuclear diversity. Preliminary findings will be presented here, together with the discussion of technical challenges and potential applications. Amphibians are threatened worldwide by climate change and habitat alterations: the development of a standardized, cost-effective protocol for monitoring amphibian biodiversity at species and genetic levels may represent an important resource for conservation managers.

## **Association of APOE, eNOS, and FTO gene polymorphisms with longevity**

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Research on the evolutionary genetic bases of biological diversity has shown that longevity varies among individuals and populations and is influenced by the interaction of both genetic and environmental factors. Moreover, human longevity varies in different geographic areas according to the population-specific gene pool and to the socioeconomic level of the population. Among environmental factors, lifestyle and nutritional status seem to play an important role. High levels of adiposity, typical of individuals with a body mass index (BMI) more than 30 kg/m<sup>2</sup>, were linked to increased incidence of many diseases and, consequently, to higher mortality rates. Many gene polymorphisms were positively or negatively associated with longevity. Most of them were metabolic gene polymorphisms. The main objectives of this study were to investigate the possible association of BMI and some metabolic gene polymorphisms with longevity in a northern Italy cohort. The hypothesis is that some anthropometric parameters, such as BMI, and some genetic variants could be differentially represented in long-lived individuals with respect to younger subjects. We genotyped 1,100 healthy volunteers aged 10-100 for APOE, ACE, eNOS and FTO gene polymorphisms. The sample was split into four age groups: 1-24, 25-49, 50-85 and 86-100. Significant differences were found in BMI values between age groups, with the exception of 1-24 with respect to 86-100. A significant decrease of the APO E4, eNOS 393 and FTO A and allele frequencies were observed in the 86-100 age group with respect to younger groups, whereas for ACE gene, no significant differences were found. In conclusion, this study provides evidence for a role of APOE, eNOS, and FTO gene polymorphisms in longevity. It has been estimated that the number of centenarians worldwide will double each decade until 2100, making population data about gene polymorphisms relevant for further studies about longevity.

### **Mechanical design of sea urchin test: an adaptive strategy for flexural resistance**

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Echinoids are echinoderms populating the seas since the late Ordovician, about 450 million years ago. They underwent a relevant adaptive radiation giving rise to a variety of specialized forms and life styles adapted to different marine habitats: the extant groups of regular and irregular sea urchins are widely distributed from the equator to the poles, from the bright shallow waters to the dark and cold deep-sea. The evolutionary success of echinoids is certainly due to the massive strategic employment of their endoskeleton, with particular reference to the coherent shell-structure of the test adapted to efficiently withstand biotic and abiotic mechanical stresses. The present study explores the constructional features of the test in the common sea urchin *Paracentrotus lividus* (Lamarck, 1816) in the light of the related mechanical behavior under load cases. For this purpose, high resolution SEM images, Micro-CT scan, 3D modelling and digital simulation (FE-Analysis) were used to emphasize and interpret 1) the striking diversity of macro- and microstructural specializations related to the different functional needs; 2) the mechanical versatility of the discontinuous plates-divided echinoid test. Results showed that the modular organization of the test, characterized by a strategic subdivision into independent plates joined together by suitable ligamentous sutures (partially-flexible), favours a significant strain energy reduction avoiding the probability of a local catastrophic failure.

## **Effects of phenotypic robustness on adaptive dynamics and evolvability**

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Theoretical and experimental studies have provided evidence for a positive role of phenotype resistance to genetic mutation, or “phenotype mutational robustness”, in enhancing adaptation and fostering evolvability. With the aim of contributing to an understanding of the origin and evolution of phenotypic robustness in living systems, we adopted a theoretical approach, elaborating on a standard model of evolutionary dynamics, complemented by computer simulations. Results show that, under common selective regimes, a certain level of phenotypic robustness is a necessary condition for adaptation to occur, even in the absence of any drift effects. We tested the predictions of the model by analyzing the evolution of some key genomic traits on a sample of 210 eukaryote taxa through a phylogenetic comparative approach. The match between observed and expected patterns provides support to the idea that phenotypic robustness is major quantitative determinant of biological system’s evolvability and a key feature of the genotype-phenotype map which would deserve to be formally included into a more inclusive explanatory framework of the evolutionary theory.

**Overseas invasion and realized niche evolution of the mourning gecko, *Lepidodactylus lugubris* (Duméril & Bibron, 1836)**

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Zoologisches Forschungsmuseum Alexander Koenig (Bonn)

*Lepidodactylus lugubris* is a parthenogenetic gecko which has been increasingly expanding its range during the last century. This invasive species has been reported from multiple tropical and subtropical countries in five continents, most of which were colonized in recent times. In order to better understand the mechanisms behind this dramatic range expansion, we reconstructed the history of the invasion and investigated possible pathways used by the species to spread. Further, we built models of the realized niche of the species at different points in time during the invasion process. This was achieved through the implementation of modern hypervolumes construction methods, based on the Hutchinson's niche concept. The models were then compared to detect possible realized niche shifts over time. A progressive expansion of the realized niche was identified. As the species spread into new areas, we observed a tendency to colonize regions with warmer temperatures and higher precipitation rates. Finally, we found evidence for cargo shipping being the major pathway through which the species expands its range. Further studies on this topic should aim to investigate the role of biological interactions, and how they shape the distribution of *L. lugubris* on a global scale. A deeper understanding of these kind of processes will help us tackle the issue of invasive species, which has become a major challenge in conservation biology.

## **Biodiversity and microbiological complexity studied and analysed throughout the drinking water distribution system**

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Water is a complex ecosystem in which species interact in multilevel networks and dynamics. A universe of bacteria is present in what we call drinking water. An unexplored world, which still contains many aspects to study: the rules that govern it, the connections among different bacteria and the role that each bacterium plays in this environment. A deep knowledge of drinking water ecosystem and its changes, from source to tap, is essential to predict the potential risks caused by its alterations. Our study focused on what happens to the microbial community from the source (groundwater), through the drinking water treatment plant, to the tap, collecting samples along the drinking water distribution system, in drinking water fountains and drinking water distributors. We had the opportunity to collect samples from different sites during a one-year sampling campaign, combining classical microbiological methods with molecular techniques, physico-chemical measurements and cytofluorimetric analysis. We were able to isolate and identify more than two hundred bacteria at the genus level, often belonging to environmental uncharacterized strains, creating a collection of these peculiar microorganisms and a curated reference database of 16S rRNA sequences. Our results showed that differences exist considering localities and sampling points along the distribution system, depicting drinking water treatment plant and drinking water distributors and drinking water fountains as peculiar ecosystems. Noteworthy, we were able to characterize drinking water distributors and drinking water fountains considering bacterial communities and physico-chemical parameters. Our results gave us the awareness that this is only the thin edge of the wedge, and that the drinking water environment is a complex and structured system formed by multiple organisms. A multilevel and integrated approach should be adopted in the effort towards the comprehension of this intricate and intriguing world.



**Determining species boundaries in the Antarctic springtail species *Friesea antarctica*  
(Hexapoda: Collembola)**

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Collembola (springtails) are among the few invertebrate taxa adapted to the Antarctic terrestrial ecosystem, with their habitat limited to seasonally ice-free coastal areas (about 0.5% of the entire continent). The fragmented land sites available for the development of springtail communities, along with their poor dispersal capability, entail a severe degree of isolation among populations. Springtail species composition is remarkably different between Antarctic Peninsula (AP) and Continental Antarctica (Victoria Land, VL), with *Friesea antarctica* being the only 'pan-Antarctic' species. Population genetics studies, so far performed on *F. antarctica*, have shown up high levels of genetic divergence between specimens from AP and from VL, thus suggesting the presence of cryptic species. To further investigate the genetic structure of *F. antarctica* populations, both mitochondrial (*cox1* and *atp6*) and nuclear (28S and EF-1 $\alpha$ ) markers were used for bioinformatics analyses of species delimitation. Although applied tools rely on different algorithms and biological assumptions, and molecular markers generally subjected to different evolutionary pressures, the results obtained in this study would suggest that at least three different species are hidden within the *F. antarctica* complex. This dissonance with morphological characters (expected also for other Antarctic collembolan species) may be the result of springtail adaptation to this extreme terrestrial ecosystem. Given the selective pressures that springtails have to face with, it is likely that a morphological uniformity has been positively selected and conserved in such a harsh environment, in spite of a well-defined genetic divergence. In this respect, an integrative approach, comprehensive of several data sources and tools, may be an effective way both to improve previous results and to support traditional morphological studies in the assessment of the actual collembolan biodiversity in the Antarctic Continent.

## **Integrating water microbiome analyses in aquaculture: emerging potential almost unexplored**

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Aquaculture is one of the fastest growing food industries and the exponential growth needs a continuous implementation of scientific research, to improve fish growth and health, and minimizing the environmental impact. One of the main challenges is represented by novel feeds: optimize quality with new available sources, essential amino acids and other micronutrients, reducing costs production and environmental impact. Without a deep knowledge of water microbiome, with its strict and constant relations with fish, the solutions to address the most urgent challenges faced by aquaculture can not be solved. We focused on water quality, with a global and multilevel approach, since this is a key element to analyse the impacts of different feed formulations on the environment, and to ensure fish health. The analyses were carried out in an experimental aquaculture plant of *Oncorhynchus mykiss*, where, insect feed formulations were tested. The physico-chemical parameters of water were measured such as pH, nitrate, nitrite, total nitrogen, ammoniacal nitrogen and total phosphorus. The analysis of water microbiome using High-Throughput DNA Sequencing techniques combined with classical microbiological tests were integrated in the analysis. We observed that a peculiar ecosystem is present in the aquaculture treatment plant analysed, characterized by the presence of typical environmental bacteria and a percentage of unassigned sequences worthy of note. We compared biofilm and water samples, finding significant differences considering microbial communities. Results showed that the microbial community did not change according to the different feed formulations tested, but depending on seasonality. These findings highlight how less we know about the complex interactions occurring among diet, microbes and host fish: these interactions involve fish health, fish gut microbiome and water microbiome, water and fish microbiome, indicating the strict relation between fish and water microbiome, almost unexplored.

**Extreme environments and vascular plants: genetic diversity of *Genista tinctoria* L. from the Ansanto Valley (Mefite, southern Italy)**

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*Genista tinctoria* L. (Fabaceae) is a highly polymorphic species, with an Eurasian distribution. Currently, many *Genista* sect. *Genista* taxa that were formerly recognized as species have been considered synonymous with *G. tinctoria*, as happened, for instance, with *G. anxantica* Ten. which grows in Ansanto Valley (Avellino, southern Italy). This locality, called also Mefite, represents an extreme environment due to the presence of a large non-volcanic gas emission rich in carbon dioxide (ca. 98%) and other gases in minimal concentrations (< 2%, nitrogen, methane and hydrogen sulphide). The individuals of *G. tinctoria* living in the Mefite have many different morphological peculiarities compared with the sympatric populations of the same species. In order to obtain useful elements to assess the genetics of this population at the Mefite, nuclear microsatellites (nrSSRs) have been employed in a molecular investigation. At present, the Mefite and two sympatric populations of *G. tinctoria* have been analysed using five microsatellites loci. For each individual, genomic DNA was extracted using a CTAB modified protocol and amplified with the M13-tail method. According to the preliminary results, the genetic diversity (Shannon index) varies from 0.9 in Mefite to 1.26 and 1.34 in the sympatric populations, respectively. AMOVA indicates that 5% of the total genetic variation is to be attributed to differences among populations and 95% to differences among individuals within populations ( $\phi$ -PT= 0.048,  $P < 0.05$ ). Bayesian clustering method implemented in STRUCTURE identified an optimal partition in two genetic pools ( $K = 2$ ) that were well defined between Mefite and sympatric populations. This suggests the occurrence of a possible ecological barrier caused by the extreme conditions present in the Mefite locality. These findings offer a promising background for future studies through an increase of the dataset of specimens/populations near this locality.

## **Mito-nuclear coevolution: a perspective from bivalve oxidative phosphorylation genes**

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In Metazoa, 4 out of 5 complexes involved in oxidative phosphorylation (OXPHOS) are formed by subunits encoded by either mitochondrial (mtDNA) or nuclear (nuDNA) genomes, therefore mito-nuclear coevolution is expected. Previous works have shown co-adaptation of subunits encoded by the two different genomes and observed higher evolutionary rates of the nuclear components. This pattern—named “nuclear compensation hypothesis”—was proposed as being adaptive, resulting in the nuclear subunits compensating for the faster-evolving mitochondrial genes. In this study we analysed the sequence evolution (as dN/dS) of 80 OXPHOS genes in 31 species of bivalve molluscs, a taxon showing extraordinary mtDNA variability and a distinctive mitochondrial biology. Overall, our data showed clear signals of coevolution, since the nuclear subunits of complexes formed by genes encoded by both genomes (Complexes I, III, IV, and V) experienced higher evolutionary rates than those belonging to Complex II (entirely composed by nuDNA-encoded genes). Interestingly, Maximum Likelihood trees obtained with either mtDNA-encoded or nuDNA-encoded OXPHOS genes had concordant topologies, despite previous phylogenomic works on bivalves showed that randomly-chosen nuclear markers yield a different topology in respect to mitochondrial ones. However, we did not find evidence of nuclear compensation when comparing the two genomes: mitochondrial genes showed higher dN/dS, contrarily to what observed in previous works. Moreover, evolutionary patterns of mitochondrial subunits were not coherently associated to their nuclear counterparts, and no site-specific signals of compensatory positive evolution were detected. Our analysis shows peculiar deviations from the coevolutionary patterns reported in other Metazoa, and we propose reconsiderations on the nuclear compensation hypothesis.

## Demographic history and molecular adaptation in western Iberian freshwater fish

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In freshwater fish, the isolation of populations in distinct basins is often associated with divergence and speciation processes. Freshwater fish must also cope with challenges imposed by changes in the environment when living in isolated basins. The *Squalius* freshwater fish species in Iberian Peninsula provide an interesting system to study both speciation and adaptation. In Portugal, this genus is represented by four species (*S. carolitertii*, *S. pyrenaicus*, *S. torgalensis* and *S. aradensis*) which have an allopatric distribution across two contrasting climate types (Atlantic in the north and Mediterranean in the south). Here, we infer the species tree describing their evolutionary relationship using a dataset of 25,353 SNPs generated through Genotyping by Sequencing. We recover two main evolutionary lineages: one comprising *S. torgalensis* and *S. aradensis* and a second lineage with *S. carolitertii* and *S. pyrenaicus*. Within the second lineage, demographic modelling indicates introgression, with the population of *S. pyrenaicus* in Tagus (in northern distribution) receiving a contribution of approximately 80% from *S. carolitertii* and 20% from southern *S. pyrenaicus* (Guadiana drainage), which could be due to secondary contact or hybrid speciation. To assess if adaptive processes contribute to differentiation in these species, we tested for signatures of selection in 16 target genes previously reported from transcriptome studies. We found signatures of positive selection in the southern populations, likely due to the harsher environmental conditions of Mediterranean climate type (e.g. high temperatures and drought in summer). Overall, speciation in these species seems to reflect both the drainages' geomorphological history and adaptive events.

***Paracentrotus lividus* sperm performances at current and predicted seawater pH conditions: a transgenerational approach**

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Sea urchins are calcifying marine invertebrates, thus potentially more susceptible to ocean acidification. As they are broadcast spawners, their reproduction depends on the successful meeting and fertilization of gametes in the water column. In sea urchins, sperm swimming and metabolism are strictly linked to internal pH value. Sperm are activated after release in the water column, and the capacity to regulate and keep the internal pH in an optimal range might be influenced by the external seawater pH. Moreover, sperm developed under different pH conditions might differ in term of quality (carry over effect) and in term of performance, when activated at various pH values (phenotypic plasticity). To test this hypothesis, post-spawning adult male sea urchins *Paracentrotus lividus* were exposed to three pH values (8.0, 7.7, 7.4) for two months. Then, from each experimental condition, four males were used to determine the gonadosomatic index and five males were used to analyse sperm ATP content. Sperm was also activated at 8.0, 7.7 and 7.4 pH and motility, swimming velocity, ATP and oxygen consumption were registered within an hour. Males maintained at low pH showed higher reproductive investment in term of gonadic mass and sperm ATP content. Reduction of sperm velocity in time was consistent with sperm ATP charge at activation. Sperm oxygen consumption rate was not affected by the experimental pH values, but interestingly ATP consumption rate was different in sperm developed under different pH conditions. Only in sperm from males acclimated at low pH, this parameter was significantly affected by the activation pH, increasing at 8.0 pH in sperm developed at 7.7 pH, and decreasing at 7.4 pH in sperm developed at 7.4 pH. Prolonged observations on sperm velocity, viability and metabolism are requested to understand how parental exposure could influence sperm performance and if induced changes in sperm have the potential to reduce fertilization capability.

### **Bat in a maze: an in-situ assay of exploration behaviour**

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Many studies have highlighted the importance of migratory species in connecting different habitats, but also stressed the vulnerability of these species to changing environmental conditions. Insights into the processes mitigating animal migration are thus crucial to understand and protect migratory species and the key ecological roles they play. Migratory strategies do not only vary between species, but also within species. These ‘partial’ migratory species are ideal study models to better understand migration. Partial migration is also known to occur in certain bat species, yet studies have mostly focused on birds. Because bats differ from birds in a number of key life-history traits (e.g. hibernation, lactation), insights from partial migration in bats would give us a more comprehensive understanding of the processes mitigating animal migration and thereby the overall vulnerability of this vital ecological phenomenon. A potentially important factor in partial migration is animal personality, i.e. individual differences in behaviour that are consistent over time and/or context. Nowadays, there is strong scientific evidence for animal personality in many species and for links between personality and important life-history traits. A link between specific personality traits and migration strategies would be especially relevant because the presence of personality differences usually implies that individuals are not completely flexible in their behavioral responses. Migrants might thus differ from their conspecifics in their ability to cope with novel (environmental) stressors and changes. We therefore investigate the potential link between migratory strategy and exploration behavior, an established personality trait in a wide variety of species, in partially migratory noctule bats (*Nyctalus noctula*). Individual noctule bats were recently shown to consistently differ in their migratory strategies, using non-invasive isotopic geolocation. In our current study we will now combine isotopic geolocation data with bat personality assays in the field. Here, we will present our first findings.

### **Within- and between-individual mtDNA heterogeneity in a naturally heteroplasmic species**

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Mitochondrial DNA (mtDNA) has a fundamental role in evolution, energy production, cell biology, and disease. Since each cell of a multicellular eukaryote can contain up to tens of thousands of mitochondria—and each mitochondrion harbors multiple copies of mtDNA—a single individual carries a large and heterogeneous population of mtDNAs, a condition known as heteroplasmy. Heteroplasmy was once believed to be rare and/or linked to disease, but it is now clear that is a common condition. Genetic variability is the engine of evolution and studying mtDNA heterogeneity is fundamental to understand mitochondrial biology. In this work, we investigated the within-individual and between-individual variability of mtDNA in *Ruditapes philippinarum*, a bivalve mollusc showing an unusual mechanism of mitochondrial transmission, the doubly uniparental inheritance (DUI). In the DUI system, two separate lineages of mitochondria exist: one is inherited through females (F-type) and the other through males (M-type). DUI has been reported in ~100 bivalve species and the conspecific mtDNA sequence divergence between the two lineages can reach 50%, corresponding to a divergence time of ~250 Myr. This makes DUI the only known stable exception to the maternal inheritance of mitochondria. Heteroplasmy is common in DUI animals and given the high sequence divergence between the two lineages this system can be an excellent model for studying mitochondrial heteroplasmy and mitonuclear coevolution. We performed a mitochondrial enrichment in different tissues of female and male samples and used high-throughput sequencing to investigate the mtDNA heterogeneity among tissues, between sexes and across individuals.



## Past human migrations account for the origins of the major Eurasian linguistic families

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Demographic events in human history leave traces in languages and genes, hence Darwin's intuition that the best possible description of linguistic relationships among populations would be their phylogenetic tree. Studies based on genetic and linguistic data have investigated the question of the origin of Indo-European (IE) and Uralic (UR) languages. At the basis of these studies lies Cavalli-Sforza's hypothesis a major demographic shift with a massive population turnover across a large geographic area be accompanied by the introduction of a new culture/language. In the present study, we combined linguistic and genomic data to shed light on the origin and spread dynamics of the IE and UR linguistic families in Eurasia. We investigated the congruence between linguistic traits inferred from syntactic comparisons and human genome diversity, finding a general correlation with a few exceptions. Then we used genome-wide data to characterize the genetic background and phylogenetic relationships of modern populations in Eurasia speaking IE and UR. Finally, we compared modern and ancient DNA data to investigate the genetic ancestry of these populations. We found that modern populations speaking UR in Europe are genetically closer to the modern and Bronze-Age populations from the Pontic steppes, than present-day IE speakers are. Our preliminary results suggest that the distribution of grammatical diversity of most languages in Europe is largely related to past human migrations, and to the different impact of their culture and genetic legacy during their expansion.

## **Explaining the Bantu expansion through a new Approximate Bayesian Computation (ABC) framework using linguistic and complete genomes data**

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One of the most significant moments in African history is the expansion of Bantuspeaking populations 5000 year BCE. Bantu languages represent the largest African language family occupying a vast territory and spoken by a high number of people. Multidisciplinary studies associated this expansion with the transition from hunter-gatherer societies to food producers that allowed populations to accumulate stored food and to increase in size, resulting in the expansions of populations. However, the dynamics of this expansion are matter of debate. Two main hypotheses have been proposed: an early-split of Bantu farmers into Western and Eastern, at the north of the rainforest; or a later-split, in which the Eastern group branches off the Western group at south of the rainforest. Recent studies have tried to shed light on the modality of the Bantu expansion combining data from different fields. In this project, we propose a new ABC framework in which genomic and linguistic data would be simultaneously considered in the analysis of demographic models. Linguistic evolutionary models will be integrated in the classical ABC framework. Preliminary analysis were performed on this conceptual model, assessing its validity through a power analysis, and obtaining satisfactory results. In the end, we will test both Early and Late-Split models using for the first time whole-genome data, from Bantu-speaking individuals, together with linguistic data. With these two extended datasets, combined with the preferential power produced by the present ABC method, we expect to reveal details of the past history of Bantu population with an unprecedented definition.

**Understanding the impact of translocations in the Australian lungfish (*Neoceratodus forsteri*) using Random Forest Approximate Bayesian Computation: conservation implications and methodological aspects**

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The Australian lungfish is a primitive and vulnerable representative of the subclass Dipnoi. The contemporary distribution of this species is limited to south-east Queensland, with some populations considered as endemic and others possibly descending from individuals introduced in the nineteenth century. Conflicting results are provided by previous genetic studies based on descriptive approaches, but understanding if all populations are autochthonous or some of them are the result of translocation events has important implications for the development of their conservation strategies. In this work we analyzed the genetic variation at three type of genetic markers (mtDNA genomes, 11 STRs, and 5196 nuclear SNPs) in five lungfish populations and exploited the new Approximate Bayesian Computation algorithm based on machine learning to compare several demographic models. The models differed for both the relationships among populations and the pattern of translocation. In particular, we assumed different contributions of Mary river gene pools into the Brisbane and North Pine rivers, possibly related to translocation events occurred in the late nineteenth century. We ran the coalescent simulations for each marker separately, and we also estimated the posterior probabilities of the models combining the three markers. After evaluating on simulated datasets the power of each marker in recognizing the true demographic history, we estimated the probabilities of different demographic models to explain the observed dataset. Our results show that nuclear SNPs have the highest power to correctly recognize the true model in simulated datasets, but in both simulated and real data, different markers usually provide the same answer. The most supported demographic model implies that native gene pools are still present also in the rivers where past translocations are documented, but alternative scenarios assuming additional translocation events will be further investigated.

## Improving the knowledge of the Antarctic toothfish population genetic structure and life cycle to foster its conservation

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The Antarctic toothfish (*Dissostichus mawsoni*, order Perciformes, suborder Notothenioidei, family Nototheniidae) has been the target of an economically relevant fishery in the Southern Ocean for the last 20 years. It is also a key species in the pelagic habitat, both as prey and predator. Overfishing and climate change are the main threats to this fish and its decline in recent years has affected all other species linked to it by trophic interactions. The conservation status, stock differences and much of its early life history are still poorly known, making predictions on the future persistence of this species particularly difficult. Several members of the international scientific community suggested that a moratorium of the toothfish fishery should be put forth until further scientific data and knowledge are collected. The establishment of Marine Protected Areas (MPAs) in crucial regions for the toothfish life cycle would represent an additional powerful tool for species conservation. The largest MPA in Antarctica was established in 2017 in the Ross Sea, where the toothfish is most abundant, and it will stay in force for the next 35 years. A proposal for a MPA for the Weddell Sea, where research on the toothfish is ongoing, has been rejected during the last Commission for the Conservation of Antarctic Marine Living Resources meeting. The aim of our project is to gain further knowledge on *D. mawsoni* life history and genetics (population genomics, transcriptomics). So far, we have focussed on the western region of the Antarctic continent (Ross Sea, Antarctic Peninsula), where effects of global warming are more evident and where climate changes may limit the habitat availability and modify the oceanographic features, potentially impacting the species demography and population connectivity.

**Analysis of the dental microwear in two dwarf fossil elephant species from Sicily, *Palaeoloxodon falconeri* and *P. mnaidriensis***

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The aim of this study is to investigate life aspects of the two Sicilian elephant insular species *Palaeoloxodon falconeri* and *Palaeoloxodon mnaidriensis*, in particular focusing on the diet through dental microwear analysis. Both species are descendants of the mainland *P. antiquus*, who reached Sicily at least twice, evolving into *P. falconeri* 400 thousands years ago and into *P. mnaidriensis* 200 thousands years ago. These are two of the many dwarf elephant species now extinct that confirm the island rule: in a predator and competitor reduced (or free) environment large herbivorous mammals reduce their size and viceversa small mammals increase their body mass. *P. falconeri* was part of a strongly endemic fauna, as attested by its extremely reduced size (1.10 m at the shoulder, 250 kg) and by the absence of other large mammals on island. On the contrary, *P. mnaidriensis* shared the island with four ruminant species and three large carnivores, allowing only a reduced decrease in size (1.80-2.00 m, 1000 kg). We hypothesize that, while *P. falconeri* could choose the most palatable and quality food, *P. mnaidriensis* had to partition the trophic niche with other herbivores. To test this hypothesis, we use the dental microwear analysis. Microwear of the occlusal enamel surface mirrors browse (soft leaves, fruits and seed) and graze (grass and ground level plants) percentage in the diet. We hope to find a different microwear signature in our two Sicilian elephants.

### **Population genetics of the Antarctic mites *Stereotydeus mollis***

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In Antarctica, plants and invertebrates are restricted to ice-free areas where they survived throughout millions of years in isolated refugia. In such as small restricted areas, population divergence and differentiation can occur, potentially resulting in speciation. Because most of the invertebrate species have limited dispersal abilities, specific habitat requirements and populations are often separated by large geographical barriers, the gene flow between different populations is scarce, resulting in high genetic differentiation between clusters of individuals. With more than 100 described species, mites are the most diverse known invertebrate group of Continental Antarctica. Among them, the freeliving genus *Stereotydeus* Berlese, 1901 (Acari: Prostigmata) is represented by 8 Antarctic species of which 2 occur on Victoria Land. In order to examine the variability and the phylogeographic distribution ranges of *Stereotydeus* spp. across Victoria Land, we sequenced the second part of the mitochondrial DNA cytochrome c oxidase subunit I encoding gene using mite specific primers. To obtain an inclusive dataset, we amplified the *cox1* of 60 individuals from 6 localities of Victoria Land (10 individuals for each population) and compared them with 52 haplotypes of *S. mollis* already present on GeneBank. We identified a very high genetic variability among populations distributed along the coast of Victoria Land with contrasting levels of divergence. In particular, we were able to identify 4 main groups within our samples. The distribution of haplotypes suggests a common evolutionary history for some lineages, whereas the divergent *S. mollis* groups are likely to be the result of inter/intra specific events that may relate to several abiotic/biotic factors, such as isolation, geographical distances and/or variation in life history, and probably on the diverse survival and reproductive strategies adopted by mites in Antarctica.

## Multiple signaling pathways control autophagic cell death in the lepidopteran fat body cell line, IPLB-LdFB

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Autophagy is an ubiquitous and highly conserved cell housekeeping process, but also the cornerstone of type II programmed cell death (PCD-II) observed during the insect metamorphosis. IPLB-LdFB is an insect cell line derived from the larval fat body of the lepidopteran pest *Lymantria dispar* and represents an optimal model for studying the PCD specific features of insects. After a 2h incubation with the ATP synthase inhibitor oligomycin A (10  $\mu$ M), IPLBLdFB cells release in the conditioned medium (CM) pro-autophagic factors (PAFs) able to elicit PCD-II. In this study, we looked for the identity of PAFs and the signaling pathways they activate. Size-exclusion centrifugation of CM, evidenced that only the fraction with components below 3 kDa maintained the pro-autophagic activity. Since proteomic studies on CM did not provide < 3 kDa PAFs candidates, we investigated the effects of 20-OH-ecdysone (*i.e.*, a steroid hormone) and ATP (*i.e.*, a purine and key “danger” signal) as potential non-protein candidates. 20OH-ecdysone (0,1÷10  $\mu$ g/mL) was ineffective on IPLB-LdFB cells, whereas flow cytometry and TUNEL assays showed that ATP (0,1÷5 mM) mostly induced PCD-I, *i.e.*, apoptosis. To identify the signaling pathways elicited by the unidentified PAFs and then use this information to determine their identity, we assessed whether single protein kinase (PK) inhibitors could block the CM effects. Cell viability and morphological and flow cytometry analyses showed that all the inhibitors tested (namely H-89 for PKA, calphostin C for PKC and wortmannin for PI3-kinase) specifically modified cell size and cytoplasm organization, but could not prevent MC lethal effects on IPLB-LdFB cells. This suggests that the PAFs in the MC promote PCD-II through multiple signaling pathways. By depicting a complex scenario, our results prompt further molecular analyses on the composition of MC to identify the PAFs mimicking *in vitro* the PCD-II taking place during insect metamorphosis.

**Molecular and evolutionary analysis of candidate genes for docility in Apennines brown bear  
(*Ursus arctos marsicanus*)**

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The Apennines bear is an iconic and endangered Italian mammal, distributed in a small area in Central Italy. Considered as an independent evolutionary lineage within the nominal species *Ursus arctos*, it shows some specific features including a small body size and a very low diversity at long fractions of the genome. It has also been suggested that the Apennines bear is less aggressive as compared to other brown bear populations, and the almost complete lack of historical records of attack to humans seems to confirm this view. A previous analysis on 5 Apennines bear whole genomes showed that a pool of genes associated to aggressiveness in other species were more different than expected when a few Apennines and non-Apennines bears were compared. Motivated by these results, we investigated in more details some of these genes in a larger sample. We focused on three candidate genes: DCC, a gene implicated in neural crest cell migration, DLL3, possibly involved in neuron differentiation pathways, and PLXNB1, which has an important role in controlling axon guidance. Specific primers were developed to amplify and Sanger sequence some regions of interest at these genes in a sample of 20 Apennines bears. The regions were monomorphic at almost all nucleotide sites, and all Apennines chromosomes had mutations never found in other brown bears. These mutations will be analyzed in published sequences and genomes, and especially in species where the comparison between domestic animals and their wild ancestors is possible. Bioinformatics and population genomics analysis will also help predicting the functional impact of these mutations, and the relative role of genetic drift and selection in driving them to fixation.



## Multi-locus phylogeny of *Bactrocera* fruit flies

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Tephritid fruit flies are among the most important pests of economic importance, as their larvae feed and cause losses to a wide variety of fruit and vegetable crops. Around half of the nearly 1000 species belonging to this family are of the genus *Bactrocera*, which is widely distributed in South East Asia and includes highly invasive pest species found worldwide. In order to facilitate comparative studies between these species that may help elucidate the genetic basis of their invasiveness and food preference, we resolved the phylogenetic relationships between 11 *Bactrocera* species, including the commonly studied *B. dorsalis*, *B. oleae* and *B. tryoni*. We performed Bayesian and maximum likelihood analyses using nucleotide, amino acid and 4-fold degenerate sites alignments of 117 nuclear orthologous genes. In contrast to mitochondrial phylogenies, our analyses retrieved a closer relationship of *B. dorsalis* to *B. latifrons* than to *B. tryoni*. Molecular clock analyses further revealed that the clade to which these three species belong experienced a fast radiation about 3 million years ago. The rapid and successive speciation events were possibly associated to incomplete lineage sorting, which would explain the incongruence between nuclear and mitochondrial phylogenies. Overall, our results highlight the importance of using genome-wide data to resolve complex phylogenies and provide a useful framework for future comparative genomics and comparative biology studies in *Bactrocera*.

**On the importance of sister species in comparative genomics: *Drosophila subpulchrella* genome data helps polarizing and tracing the evolution of pest traits in its sister species *Drosophila suzukii***

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Comparative genomics is a powerful tool to understand adaptations to new ecological niches. The invasive fruit fly *Drosophila suzukii* is an excellent model to tackle this issue because, unlike most of its closely related species, it is characterized by unusual egg laying behavior and preference for temperate climates. The genetic basis of these traits can be investigated by combining genome-wide molecular evolutionary studies with ecological and behavioral observations. To increase the resolution power of these analyses, we produced draft genomes and transcriptomes of *D. subpulchrella* and *D. lucipennis*, two species for which a *D. suzukii* phylogenetic affinity has been proposed. Dated phylogenies indicate that *D. lucipennis* is not a member of the *suzukii* subgroup, while *D. subpulchrella* is the sister species of *D. suzukii*. We observe an almost complete sorting of orthologs, which excludes past genetic admixtures between the two species. The rates of nucleotide substitution indicate that both *D. suzukii* and *D. subpulchrella*, and in particular their ancestor, are characterized by fewer generations per year compared to sister clades, compatible with a long history of adaptation to temperate climates in this subgroup. *D. subpulchrella* allow us to polarize with more precision the outcomes of phylogenomics investigations: we identify a high turnover of odorant receptors in the lineage leading toward *D. suzukii* only, and further propose a new scenario for OR22a/b evolution in the melanogaster group. Overall, our results support a progressive modification scenario in the *suzukii* subgroup toward the peculiar *D. suzukii* biology and ecology. It also raises issues on the invasiveness potential of *D. subpulchrella* and on which factors have prevented this event so far. More generally, our study shows that adding a sister species can highly increase the precision of comparative genomics when studying adaptation and the evolution of novel traits.

**The effect of anthocyanins intake on the evolution of redox genes: a comparative genomics approach using *Drosophila***

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Anthocyanins are red/blue vacuolar pigments present in flowers and fruits. They belong to flavonoids, a class of well-studied anti-oxidant agents involved with cancer and ageing protection in animals. It has been shown that anthocyanins can regulate the expression of many genes involved with cell redox biology. Little is known about the evolution of these redox genes in species characterized by different anti-oxidant rich diet. An interesting model to study the adaptive effect of anti-oxidant intake on redox genes is *Drosophila suzukii*: differently from most other *Drosophilidae*, this species lays eggs in ripening berries which are naturally rich in anthocyanins. We hypothesize that *D. suzukii* peculiar diet may have resulted in adaptive genetic differences in redox genes. We used a phylogenomics approach to study if a panel of 31 redox related genes are structurally different and/or differently expressed in *D. suzukii* and *D. subpulchrella* (another species characterized by fresh fruit diet) compared to other eight species of *Drosophila* (*D. takahashi*, *D. biarmipes*, *D. melanogaster*, *D. simulans*, *D. erecta*, *D. yakuba*, *D. ananassae* and *D. pseudoobscura*). In particular, we studied genes known to be influenced by anti-oxidants, and genes encoding for proteins involved in the reactive oxygen metabolism. Annotations and gene phylogenies indicate that while the coding sequences of all 31 genes are well conserved in all species, some gene is characterized by differences in introns length and/or intron number in *D. suzukii*. We further inspected the level of selection pressure using dN/dS and we identified genes which have a different rate or sign of positive selection in *D. suzukii*. We are performing comparative transcriptomic experiments to quantify if these, and other genes, are differently expressed in *D. suzukii* (and *D. subpulchrella*) and we will test gene expression in larvae stadium of *D. suzukii* and other species in different anthocyanins concentrations.

## **Tunas across space and time: population genomics and fishery genetics in temperate and tropical tunas**

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Sustainability of tuna populations and tuna fisheries is challenged by high and increasing consumption demand and products values. Reliable tuna stock assessments and fishery modelling are the main goals of scientific community and are fundamental expectations of fishery managers and policy makers. Since 2010, multiple studies provided scientific data on the spatio-temporal population structure and its changes by analysing genetic and genomics markers on the temperate Atlantic Bluefin tuna (*Thunnus thynnus*; ABFT) and on the tropical yellowfin tuna (*T. albacares*; YFT). In ABFT, low levels of spatial variation among and within populations spawning in the Mediterranean and Gulf of Mexico are detected using several and different types of genetic and genomic markers. Significant though low divergence between ABFT early stages collected for several years from Gulf of Mexico and four main areas in the Mediterranean are consistent with a two-stocks management. Within the Mediterranean, any significant and temporally-stable population genetic structure is supported. Environmental variables and random genetic drift rather than oceanographic and ecological barriers are driving ABFT stock structure and composition in the pan-Atlantic area. Beside spatial structure, long-term temporal variation in Mediterranean ABFT has been deeply and uniquely investigated, looking for correlation with dramatic changes in ABFT fishery and climate. The large-sized Mediterranean population seems to be resilient to such impacts being only affected by slight and not significant changes of genetic diversity over the last century. YFT was assessed with a genomic survey, unprecedented for spatial coverage and type and number of markers used. Straightforward findings obtained using 2b-RAD markers are the consistency of stock subdivision according to that proposed by RFMOs with the exception of the Atlantic stock, likely including two significantly divergent populations.

## Population genomic analyses of a highly mobile large carnivore, the gray wolf (*Canis lupus*), across Eurasia

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Large carnivores, such as the gray wolf (*Canis lupus*), are wide-ranging and can disperse hundreds of kilometers, yet they at times show population genetic structure consistent with ecological and environmental gradients. Within Eurasia we expect east-west gene flow in such species to be higher than that documented for similar distances north-south, owing to generally more similar environmental conditions across latitudinal than longitudinal gradients. We thus expect limited isolation-by-distance in the east-west direction and will test this prediction by analysing gray wolf profiles from across Eurasia. Our analyses encompass existing and newly genotyped profiles from more than 700 individuals genotyped on the Illumina CanineHD BeadChip with over 170,000 single nucleotide polymorphism (SNP) loci. We include wolves sampled in Europe from Scandinavia to the southern Balkans, Iberia, and Italy, plus Caucasus, central and eastern Russia. After merging data sets, filtering for individual and SNP genotyping quality, minor allele frequency, and loci in Hardy-Weinberg and linkage disequilibrium, we examined population genetic structure by comparing results from principal component analyses and maximum-likelihood methods. The most divergent wolf population to emerge across our study area was Italian. Subsequently, we observed other population clusters in central and eastern Russia, Caucasus, Iberia, northcentral Europe, Scandinavia, the Carpathian Mountains, and the Dinaric-Balkan region. The population clusters extending through northcentral Europe, central and eastern Russia suggest relatively high gene flow across these broad geographic regions. This contrasts with finer-scale genetic structure in other localities, including Italy, the Dinaric-Balkan region and the Carpathian Mountains. Over time, wolf recolonization of historic ranges could increase gene flow between currently divergent population such as Dinaric-Balkan and Italian wolves.

### The genetic history of modern humans in Siberia: a bacterial perspective

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The gastric pathogen *Helicobacter pylori* has been associated with anatomically modern humans for at least 100,000 years and it is currently infecting more than half of the world population. Due to a high mutation and recombination rate, *H. pylori* strains show a clear phylogeographic signal and a pattern of genetic diversity that mirrors the one of their hosts. While *H. pylori* strains from Africa, Europe and Southeast Asia have been thoroughly investigated, sequences from northern Asia have yet to be reported. Here we analyze 400 new *H. pylori* sequences from 16 Siberian populations characterized by different lifestyles and spoken languages. Once this data was considered in a worldwide context we discovered several unrecognized strains: Siberia1, Siberia2, Ket and Altai. We also report a more than 100 new hpAmerind sequences, expanding the known distribution of this strain to Northeast Siberia and Western Eurasia. We explicitly simulated different demographic scenarios, developed to explain the origin of the new strains, employing an Approximate Bayesian computation framework. Our results highlighted a recent origin for all of the newly identified strains, and their current distribution supported the hypothesis of a late recolonization of Siberia by humans following the LGM.

**A genomic perspective on population structure and invasion dynamics of the brown marmorated stink bug *Halyomorpha halys* (Heteroptera, Pentatomidae) in Italy**

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The brown marmorated stink bug (*Halyomorpha halys*) is an East Asian invasive pest which has spread worldwide, most notably to North America and most European countries, with the first record in Italy dating to 2012. In addition of being a serious pest of agricultural crops, it is also present in urban areas, which hampers eradication efforts. The genetic diversity of Italian, Greek and Romanian populations has been investigated so far using mitochondrial DNA (mtDNA) markers (*cox1* and *cox2*), providing the first data on patterns of introduction and dispersal of this species, showing that one haplotype is common and widespread in Italy and also in other countries. The present study aimed to deepen the knowledge of the genetics of *H. halys* individuals characterised by the most widespread mitochondrial haplotype, dissecting local population structure and patterns of introduction/dispersal. We analysed genome-wide RADseq markers (1422 SNPs) from 28 specimens sampled in seven Italian regions and Greece. Even though all individuals represented a single mitochondrial haplotype, we detected four genetic clusters: two included individuals from different geographic regions, a third cluster included only individuals from Emilia-Romagna, while the fourth one included only specimens from Veneto region and was characterized by the highest within-cluster differentiation. These results suggest that analysed populations have originated from multiple invasion events and highlight high mobility of the species, as evidenced by the presence of two geographically mixed clusters, likely enhanced by human activities. The lack of information from other countries, made it impossible to trace back the exact sources of invasion. These genomic analyses proved to be effective in disentangling the population dynamics of the recent invasion events, providing important knowledge to guide proper pest management strategies.

## **Complete human genome data account for multiple dispersals of anatomically modern humans out of Africa**

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There is a wide consensus in considering Africa as the birthplace of anatomically modern humans, but the dispersal pattern and the main routes followed by our ancestors to colonize the world are still matters of debate. It is still an open question, indeed, whether early modern humans left Africa through a single major process, dispersing almost simultaneously over Asia and Europe, or in two main waves, first through the Arab Peninsula into southern Asia and Australo-Melanesia, and later through a northern route crossing the Levant. The development of new methodologies for inferring population history, as well as the availability of worldwide high-coverage whole-genome sequences did not resolve this debate, yielded contrasting results. In this work, we test the two main out-of-Africa hypotheses through an Approximate Bayesian Computation (ABC) approach, based on the recently developed Random Forest algorithm. We first explicitly evaluated the ability of the method to discriminate between the alternative evolutionary models (i.e. single vs multiple exits) of anatomically modern humans out of Africa, using simulated data. Once assessed that the two competing models are distinguishable through ABC, we compare simulated data with real genomic variation, from worldwide modern and archaic populations. This analysis supported a multiple dispersal model for the evolution of anatomically modern humans out of Africa, with a first exit (involving current Australo-Melanesian populations) estimated to have happened about 100,000 years ago, and a second exit (giving rise to current Europeans and Asians) about 50,000 years ago.



### Step by step: the unprecedented evolutionary history of family Rajidae

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Among skates, Rajidae represents one of the most enigmatic family of cartilaginous fish whose bioecological traits contributed to an extraordinary evolutionary success in terms of species richness and endemism. Past and present taxonomic conflicts and species misidentifications are linked to their extraordinary level of morphological stasis. In recent years, these issues have been overcome by widescale molecular taxonomy analyses, but also raised questions about their evolutionary history. Concerted actions as the ELASMOMED and ELASMOATL initiatives encouraged and improved large-scale sampling efforts in the Mediterranean and Eastern Atlantic Ocean over years, building unique voucher repositories of thousands of specimens and enabling the exploration of skates' biogeography. Here, the maximum taxonomic (51 OTUs) and molecular (47 OTUs) diversity of tribes Rajini and Amblyrajini were measured using concatenated mitochondrial genes. We also estimated their evolutionary divergence using the molecular clock approach. Evidences produced so far showed that, despite the ancient origin of Rajidae (97 MYA), the Eastern Atlantic and Mediterranean faunas originated more recently, after the closure of connection between these areas and the Indo-Pacific Ocean (15 MYA). The endemic Mediterranean species originated with the recolonization of the Basin, after the Messinian salinity crisis (7-5 MYA). At least five independent vicariant events contributed from 7.4 to 3.15 MYA to the formation of allopatric or parapatric sister species, each distributed in the N-E Atlantic and S-E Atlantic respectively. On the whole, the Quaternary tectonic movement of continental masses, paleoclimatic events and present oceanographic discontinuities occurring along the western African continental shelf might explain this series of parallel and independent speciation events related to the maintenance of low or null levels of gene flow between closely related sibling and cryptic species.

## Yamanaka factors in the budding ascidian *Botryllus schlosseri*: gene evolution and expression

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The Nobel Prize in Physiology or Medicine 2012 was awarded to J.B. Gurdon and S. Yamanaka for the discovery that mature cells can be reprogrammed to become pluripotent. In particular, the so-called Yamanaka's factors (YF), *i.e.* the four transcription factors Sox2, Klf4, Oct3/4 (Pou5f1), cMyc, are able to induce *in-vitro* the de-differentiation of mouse mature cells into stem cells. *In-vivo*, similar events have been never observed in vertebrates, whose regenerative capabilities are very limited when compared to those exhibited by invertebrates. Tunicates, the sister group of vertebrates, show high regenerative capabilities. In particular, the colonial species *Botryllus schlosseri*, undergoes a whole-body asexual development based on a weekly-synchronized growth-resorption cycle mediated by adult stem cells. However, the production of new stem cells by dedifferentiation of mature cells have never been evidenced. Therefore, we investigated the *B. schlosseri* orthologs of the vertebrates YF as markers for putative dedifferentiation events. YF were searched for, or re-annotated in the *B. schlosseri* reference genome, molecularly characterized, and their evolution in deuterostomes was investigated. The expression pattern of these factors was explored during both asexual development and embryogenesis, in order to compare their role in the two distinct developmental pathways. Our results show that two factors (Sox2 and cMyc) are present in the *B. schlosseri* genome; Pou5f1 is the result of a duplication occurred only in vertebrates, therefore the most closely related homologs Pou2 and Pou3 have been analysed; Klf4 was not recognized. These factors are expressed during the two developmental pathways with specific spatio-temporal patterns and they do not co-localize altogether. Therefore, our data do not support the origin of adult stem cells through dedifferentiation governed by the YF even in these invertebrates with high regenerative capability.

## Investigating geographic and temporal genetic variation in the black grouse (*Lyrurus tetrix*) in the Italian Alps

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The black grouse (*Lyrurus tetrix*) is a Galliform distributed across northern Eurasia, and is a game bird in most EU countries. Although the species is listed as 'Least Concern' by the IUCN, populations at the western and southern edges of its range are considered 'Vulnerable' due to increasing habitat fragmentation and human disturbance. Between 1995 and 2017, in collaboration with several hunting associations, we collected more than 600 black grouse samples across seven regions of the Italian Alps. Ten microsatellite markers (STRs) and 2442 Single Nucleotide Polymorphisms (SNPs) were analysed in large subsets of the collected data, with the aim of identifying environmental, temporal and anthropic factors that affect the distribution and level of genomic variation. The main factor shaping the genetic distances between populations based on STRs is the geographic distance between them (i.e. isolation-by-distance), but even the populations on the two extremes of our sampling area are very similar ( $F_{st}$  between the two regions = 0.053). SNP data supports the STR analysis. However, isolation-by-resistance methods for the larger STR data set show that both higher altitudes and urban areas inhibit movement of grouse between populations. While temporal analysis of STRs for the Trentino-Alto Adige region showed no significant change in the mean number of alleles and allelic size range between the two time frames studied (e.g. mean number of alleles 1995-1999: 8.8, 2009-2010: 8.2), and the expected heterozygosity was high in both time frames (1995-1999: 0.740, 2009-2010: 0.722). While black grouse population size is reportedly decreasing, our results suggest there is no measurable genetic impact from this trend. Hence this dataset provides a basis for future monitoring of genetic diversity in this charismatic alpine species.

### **Digit ratio and asymmetry in length investigated in limbs of calves**

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This study aimed to investigate possible sexual dimorphism in the relative digits lengths, known as digit ratio, in calves. Hence, the diverse length of the bone structures of the third (3D) and of the fourth (4D) digits was analysed as evolutionary adaptation to locomotion on soft grounds. A portable X-ray machine was used to measure the length of the digital bones of the right fore limb for 33 females and 15 males. Different traits were studied via mixed model, including the vestigial structure of the second digit (2D), and 3D and 4D measured from metacarpus to the third phalanx. Some digit ratios between 2D and different digit parts of 3D or 4D (2D:3D and 2D:4D) were also considered. As a result, significant differences of the length of the first and the third phalanges of 3D respect to 4D were found. The 2D was shorter in females than in males ( $P < 0.01$ ), as well as the second phalanges of 3D and 4D. Significant 2D:3D and 2D:4D digit ratios were assessed for some digit parts of 3D and 4D and for the first phalanges of 3D:4D. In all cases, a shorter ratio in females than in males was found, an opposite situation than in most mammal species. Concluding, the study found both an asymmetry between 3D and 4D in calves, maybe meaning a functional adaptation. Furthermore, asymmetric 2D:4D and 3D:4D digit ratios in males and females, but with a different direction than in other mammal species. Actually, ungulates limbs notable differ from the limbs of most Mammals, which have maintained five digits during their evolution.

***Clinopodes carinthiacus* as a case study for an integrative approach to species discovery and delimitation in endogeic invertebrates**

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The extensive geological and climatic changes that took place in the Alps are at the base of the complex evolutionary histories of many organisms inhabiting this region. This is especially the case for soil-dwelling, strictly endogeic, invertebrates, which show very limited dispersal ability. Knowledge on the species boundaries and diversity pattern of many endogeic animals still lags behind. As a consequence, their taxonomy needs a reappraisal in the light of the general lineage concept of species and by embracing a rigorous integrative approach, able to exploit different lines of evidence. We studied fine-scale geographic variation of *Clinopodes carinthiacus*, an endogeic centipede distributed in the forest soils of the South-Eastern Alps and the Dinarides. In a sample of 28 populations across the South-Eastern Prealps (spaced 5-20 km from each other), we explored their differentiation in morphological traits (through geometric morphometrics and other methods) and in the DNA sequences of mitochondrial (COI and 16S) and nuclear (28S) loci. The implementation of an array of alternative protocols for species discovery and delimitation (e.g., model-based cluster analyses for morphological characters, distance-based and tree-based methods for molecular sequences) allowed to uncover a hitherto overlooked diversity within the putative species *C. carinthiacus*, which includes different species, some of them even coexisting in syntopy.

## Diversity and evolution of glycoside hydrolase family 19 revised by sequence space mining

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Glycoside Hydrolase (GH) enzymes breaking down chitin and peptidoglycan polymers are called chitinases and lysozymes, respectively. Many different GH families with little to no sequence or structure similarity have converged to these hydrolytic activities, and both functions may coexist in promiscuous enzymes. We focus on the GH 19 family, from which interesting plant and Actinobacteria chitinases were studied in the past due to their capacity to enhance plant defense against pathogens or abiotic stressors, in particular if a Carbohydrate Binding Module (CBM) domain is present in the same sequence. Other members of GH 19 have been recently characterized as lysozymatic endolysins, in some cases being the catalytic part of a multi-modular enzyme. The variability in the presence or absence of CBMs, loop insertions around the active site, and the existence of catalytically inactive isoforms provided other cues for studying how flexibility and key modifications can be responsible for the observed biochemical functions. We described and analyzed GH 19 sequence diversity by integrating all protein sequences available in public databases and by building networks of pairwise sequence identity at domain level. We further rebuilt the classification of GH 19 by combining sequence networks with structural and functional annotations. Finally, we analyzed the conservation of important features for the most studied group of chitinases, considering their evolutionary patterns with respect to a sequence-based phylogeny.

### **Evaluation of different hybrid *de novo* genome assemblies of *Acinetobacter johnsonii***

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The role in biotransformation of bile acids of a strain of *Acinetobacter johnsonii* isolated and purified from an industrial soil sample was already described. However, the enzymes responsible of these transformations are only partially purified and molecularly characterised. To produce a highly accurate functional characterization of this strain, we present an ‘hybrid’ assembly that combine Oxford Nanopore and Illumina sequencing strategies. The dominant sequencing technology provided by Illumina, which generate short-reads (75-300 kb) with low error rate (>1%) presents a significant limitation: short reads can’t resolve genomic repetitive regions, that are common in bacterial genomes. Recently long read sequencer, like MinION (Oxford Nanopore Technologies), has become widely available. This kind of device allows the resolutions of repetitive regions, however all the platforms for long read sequencing still present difficulties in the basecalling process that result in a high error rate (about 10% for the MinION). The genome of *A.johnsonii* was sequenced on a MinION device using a FLO-MIN106 flowcell. The sequencing process generated 12,5 Gbp of output allocated in 2,737,448 reads, with median read length of 2191 bp and median read quality of 11,3. Then the reads was assembled with Canu (v.1.8) and polished using the MinION reads with Nanopolish (v.0.11.1). Currently the genome is being sequenced with an IlluminaNextSeq 500 sequencer with 2×150-bp read, using NextSeq® 500/550 Mid Output Kit v2. The final assemblies will be used to annotate the resulting genomes and, eventually, phylogenetic trees will be generated.

**Investigation of the phylogenetic history of common bean (*Phaseolus vulgaris*)**

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For an appropriate use and conservation of crop species, knowledge about the origin, evolution and diffusion is a crucial aspect. *Phaseolus vulgaris* has a unique evolutionary history with the occurrence of two geographically independent domestication in Mesoamerica and in the South of Andes. The wild form of *P. vulgaris* originated in Mesoamerica and was subsequently introduced in South America with the development of a gene pool in North Peru-Ecuador and one in South Andes. Recently, the hypothesis of a different evolution history (the *pseudovulgaris* hypothesis) was proposed, suggesting that the introduction in North Peru-Ecuador originated much earlier before the diversification of *P. vulgaris* within the *vulgaris* group. The aim of this work is to clarify the phylogenesis of *P. vulgaris* by the analysis of the chloroplast genome diversity of wild common bean accessions.



## **Plastic responses in a trade-off context: lessons from pre- and post-copulatory traits in guppies**

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Costs related to reproduction play a fundamental role in estimating the overall fitness of individuals. Male reproductive success strongly depends on sexually selected traits, which are typically costly. Therefore, in conditions of high environmental variability, males that are able to modify their investment are expected to be favored. Although the strategic adjustment of sperm production has been demonstrated in several species, few studies have investigated the actual costs and benefits of this strategy. Male guppies (*Poecilia reticulata*) enhance sperm production in conditions of perceived high mating opportunities, and consequently reduce courtship rate. We tested the costs and benefits of this flexible adjustment by comparing mating and insemination success of males that were isolated (low mating opportunity, LMO) or kept in visual and olfactory contact with females (high mating opportunity, HMO). We performed two different experiments: i) we measured mating and insemination success of males allowed to mate with 3 pairs of sexually receptive females; ii) we determined differences in mating success between pairs of males, previously exposed to either HMO or LMO conditions, competing for a single female. LMO males, in general, engaged in more intense courtship behavior compared with HMO males. In the first experiment we observed little sperm depletion in HMO males, compared with LMO males. However, HMO males in the second experiment suffered a reduced mating success and had no measurable insemination advantage with respect to LMO males. We confirmed that reproductive plasticity in guppies is constrained by a trade-off between pre- and post-mating investment. Moreover, we showed that each strategy incurs substantial costs with significant fitness consequences, especially when mating conditions strongly deviate from those anticipated. Our results, therefore, highlight the importance of these factors in the evolution of plastic responses.

## Competition for food resources between closely-related species: a nutritional approach

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For closely-related species inter-specific relationships are a major factor that can restrict the potential distribution of the less competitive species. Pine marten *Martes martes* and stone marten *Martes foina* are the most similar European carnivores and co-exist over a large area. While both species prefer forested habitats, the stone marten often occurs in rural and suburban areas. This association with human-dominated habitats has been explained as the result of competition with the pine marten. Although spatial segregation should entail the use of partially different food resources, the two martens' trophic niches overlap extensively. Importantly, as their faeces cannot be distinguished by eye, the diets of the two martens have mostly been studied separately, preventing from differentiating the effects of local and temporal variation in food availability from those of interspecific competition. We tried to overcome this obstacle by quantifying the macronutrient (i.e. protein, lipids and carbohydrates) intakes of the two martens. We hypothesized that macronutrient gains may enable us to compare their feeding requirements independently from the way their populations respond to local food availabilities. We used an indirect approach, reviewing studies that assessed the relative bulk of each food item in marten diet (i.e. volumes or biomasses) by the analysis of faecal samples. The nutrient profiles of martens tightly clustered (average values: 47% protein-, 39% lipid- and 14% carbohydrate energy). Most interestingly, in allopatry the nutritional niches of the two species did not differ, while they did in sympatry. When sympatric with the pine marten the stone marten ate more carbohydrates and less protein, while, in allopatry, the protein intake of the stone marten was high (45-52%) independently from resource availability. These results suggest that stone marten frugivory may result from interspecific competition.

**“Tell me who you go with and I’ll tell you who you are”: using gene co-expression to study temperature adaptation in *Arabidopsis thaliana***

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As plants are sessile, they must be able to regulate their cellular functions to respond to any environmental change that might threaten their survival and competitive abilities. Temperature, for example, affects plant growth and development and in extreme scenarios, causes death. Moreover, temperature fluctuates on an hourly, daily, seasonal and local basis. Therefore, plants must be adapted to compensate physiologically across a range of temperatures. We used the model species *Arabidopsis thaliana* in order to broaden our view on the mechanisms involved in diverse temperature responses and adaptation. It is spread throughout a wide climate range, from Africa to Sweden, hence showing adaptation to disparate local conditions. However, little is known about the underlying genetics. Here, we focused on genome-wide transcriptome expression profiles of four accessions exposed to two non-extreme temperatures (6°C and 16°C). To analyse their relative differences in gene expression we used whole transcriptome shotgun sequencing (RNA-Seq). We inspected transcriptional coregulation of genes in the two temperatures through weighted gene co-expression network analysis (WGCNA). As expected, the level of coexpression of some clusters are conserved, while others are temperature specific, hence suggesting the activation of different pathways. In order to gain insights into the biological processes related to the identified modules, we then performed Gene Ontology (GO) term enrichment. Networks topology and biological annotations can be further investigated for potential gene roles in local adaptation. Linking the differences between accessions networks with respective accessions ecology, we aim to find specific genes involved in temperature adaptation. In this work we want to show that studying the temperature responses and their natural variation from a transcriptome perspective may deliver new insights into the relevant phenomenon of temperature adaptation.

### **Problem solving abilities in three species of macaws: string-pulling paradigm**

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Recent research has been focusing on cognitive abilities in birds, particularly corvids and parrots revealing that these species show similar cognitive skills to those reported in primates. This study aims to investigate and compare the problem-solving abilities of three macaw species. Subjects of the study were eight zoo macaws (four *Ara chloropterus*, two *A. macao* and two *A. ararauna*) housed in mix-species exhibit at Parco Natura Viva, Italy. Macaws were provided with two string-pulling tasks in two periods: in the first period, the apparatus was a horizontal stick with two parallel strings with a reward at the end of only one of them (Test A). In the second period, the strings were crossed, and macaws had to pull the string contralateral to the reward to reach for it (Test B). Per period, ten 1-hour sessions were carried out and all sessions were video recorded. Frequencies of interaction with the apparatuses were collected, considering the first choices of each macaw within each session and per apparatus as well as all other choices. Results suggested individual variability in the interactions with the apparatuses. Considering all choices, all subjects that interacted with the apparatuses were able to solve both tasks. However, individual level differences in the problem-solving skill were reported, with greater number of non-correct choices vs. correct choices in Test B than in Test A. In conclusion, despite inter-individual and inter-specific differences, the study macaws showed problem-solving and learning abilities and were able to solve both the easy and the complex tasks.

## DNA Metabarcoding as a tool for soil's biodiversity assessment in organic and conventional agroecosystems

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Elucidating how agricultural practices affect soil invertebrate communities is of relevant importance. In the present study, using DNA metabarcoding, soil invertebrate communities of selected farms in the Po Valley (Italy) were assessed in order to address the following questions: i) is the structure of soil invertebrates' communities different between organic and conventional farms? ii) To which extent are field use (crop vs stable meadow) and field margins affecting these communities? Soil samples were collected from organic and conventional farms in different periods of the year, covering the margins and three levels towards the centre of fields of stable meadow and barley of both farms. DNA was extracted from soil samples and a fragment of the *cox1* gene amplified using three different pairs of primers; amplicons were sequenced using Illumina Miseq. Raw sequences were analysed using Qiime and ecological and statistical analyses were performed with R in order to address the above-mentioned questions. The recovered invertebrate's communities were dominated by Arthropoda (~ 80% of the OTUs in each sample), a total of 831 OTUs (Insecta 51%, Arachnida 18% and Mollusca 14%) formed the core diversity present in all the analysed samples.  $\alpha$ -diversity indices were not significantly different between the two farming strategies in terms of OTUs richness, Shannon index and Pielou's evenness.  $\beta$ -diversity analyses showed the field margins invertebrate communities were different from the inner field communities. In conclusion, DNA metabarcoding represented a suitable method for soil invertebrates' biodiversity estimation. The recovered communities are significantly affected by the crop and the localization in the field (margin vs center). In addition, the role of field margins as a diversity reservoir is increased in cultivated fields (barley), while in stable meadows fields, the interactions between the margins and the center of the field are lower.

### Assessment of postzygotic hybridization barriers along *Arabidopsis* lineages

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Reproductive isolation maintains the integrity of a species and results in the evolution of many lineages. Interspecies crosses are vital for evolutionary aspects as well as agricultural improvements. Reproductive isolation in plant species is supposed to be complex interactions of a large number of pre- and postzygotic hybridization barriers. The pace of evolution and the evolutionary forces driving postzygotic hybridization barriers remain largely unclear. The *Arabidopsis* genus, with its well-known phylogenetic structure, ecology, physiology and genomic tools offers to address evolution and role of postzygotic barriers in plant speciation. Hybridization between diploid *A. lyrata* and *A. arenosa* causes blockage in seed development due to affected endosperm growth. Hybrid seed lethality among different *Arabidopsis* lineages will be used for assessment of the evolution of hybridization barriers and their relative importance for early to late divergence along the *Arabidopsis* species continuum. Using QTL approach, the genetic basis of well-characterized postzygotic hybridization barriers between Central European *A. lyrata* and *A. arenosa* will be identified. After identification of these genetic loci, using bulked segregant analysis, evolution of “speciation genes” along *Arabidopsis* lineages will be explored. The outcomes will provide a framework for significance of neutral evolution or natural selection in hybrid seed lethality.

### **Organic rice farming: environmental externalities of diversified agro-environments**

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Organic rice cropping systems (ORCS) are a novelty for Italy and are changing under the pressure of bottom-up innovations, resulting in a wide range of agro-ecological practices. Compared to the past, the ORCS seems to have lower mechanical input and shifts in yield. The study aimed to evaluate the ORCS' environmental externalities, considering these changes and the variability of the current agro-ecosystems. 50 fields in North Italy were monitored, collecting data on agronomic inputs and yields. Intermediate (Q2) and higher (Q3) quartiles of yield were computed. The impacts, in terms of Green-House Gas emissions (GHG, CO<sub>2</sub>eq per ton), was assessed by the Life Cycle Assessment method, considering: productive scenarios described by the mean of Q2 (3.9 t/ha) and Q3 (5.6 t/ha), and two variants (A, B) adopted for the most promising crop management strategy. With the aim to integrate this impacts assessment with that of the environmental benefits (e.g. ecosystems services, biodiversity), a preliminary survey on weed flora' was carried out, observing the species presence and the related risk for the production and ecological value. The ORCS' GHG ranged as follow: 1,712 (A) - 1,256 (B) in Q2, 1,293 - (B) in Q3 (- 53% best vs. worst scenarios). The values' range is lower of that found in literature for organic rice (i.e 3,270- 2,000 CO<sub>2</sub>eq; Hokazono & Hayashi, 2012; He et al., 2018; Bacenetti et al., 2016), proving the importance to update the impacts assessment for evolving and diversified agroecosystems such those of ORCS. 14 species resulted invasive and affecting the yield (*Echinochloa* spp., Cyperaceae' family), 11 species were classified as medium-risky, two as low-risky, and 10 as null-risky, showing only an ecological value (e.g. *M. quadrifolia* and *L. procumbens*, belonging to the EU list of rare species for the habitats degradation, Directive 92/43/EEC). The positive externalities of flora biodiversity should be quantified and included in the environmental assessment.

## **Characterization of the complement system in a colonial tunicate: C3 complement receptors and opsonic role of C3**

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The compound ascidian *Botryllus schlosseri* is a reliable model organism for the study of immunobiology. As an invertebrate, it relies only on innate immunity for its defense. We already demonstrated the presence, in *Botryllus*, of homologues of mammalian C3, Bf, MBL and MASP1, referred to as BsC3, BsBf, BsMBL and BsMASP, respectively. All the complement components identified so far, are expressed by morula cells, the most abundant circulating hemocytes. In mammals, once the complement system is activated, a cascade of reactions occurs resulting in the cleavage of the third complement component (C3) to C3a and C3b, the former exerting a chemotactic activity, the latter acting as opsonin and, ultimately, activating the lytic pathway. The best-known receptor for C3a in mammals is C3aR, whereas CR1 is the receptor able to recognize and bind C3b on the microbial surfaces. Here, we describe, in *B. schlosseri*, new genes showing homology with vertebrate C3aR and CR1, respectively, and studied their transcription in the course of the colonial blastogenetic cycle. In addition, we continued our analysis of the role of C3 in *Botryllus* immunity by studying the modulation of BsC3 transcription during the colonial blastogenetic cycle and the effect of *bsc3* knockdown on immune responses. Results indicate that only morula cells, and no other immunocytes type, are labelled by the antisense probe for BsC3aR, whereas phagocytes and young, undifferentiated cells, known as hemoblasts, are the cells stained by the probe for BsCR1. Both the *bsc3ar* and *bscr1* genes are constitutively transcribed. However, a modulation in the extent of transcription occurs during the colonial blastogenetic cycle as the amount of BsC3aR mRNA abruptly decreased at TO, whereas no differences were observed when EC and MC were compared. This is probably related to the renewing of circulating cells at TO, that are replaced by new, differentiating cells entering the circulation in the same period.





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