EVOLUZIONE 2017

7° Congresso della Società Italiana di Biologia Evoluzionistica

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EVOLUZIONE 2017

7° Congresso della Società Italiana di Biologia Evoluzionistica

Roma 28-31 Agosto 2017

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Opening lecture

Cryptic divergence and sympatric speciation in the main Afrotropical malaria vector species of the *Anopheles gambiae* complex

Alessandra Della Torre

Dipartimento di Sanità Pubblica e Malattie Infettive, Sapienza - Università di Roma

The cryptic genetic heterogeneity within the Afro-tropical *Anopheles gambiae* offer a unique opportunity to provide insights into the mechanisms of speciation, whose implications are not only of interest to evolutionary biologists but also of utmost importance for malaria control. In this lecture I will first summarize the history of the complex, which has been considered as a single variable and opportunistic species until the '60s and is now recognized as a group of at least 8 morphologically indistinguishable, but genetically discontinuous breeding units exhibiting different degrees of reproductive isolation and ecological divergence that change the way in which the mosquito interacts with humans and the environment and, ultimately, the capacity to transmit malaria and efficacy of vector control interventions. Second, I will focus on the two most recently diverged members of the complex – *A. gambiae* and *A. coluzzii* - and show novel genomic and ecological data suggesting that the same process of genetic adaptation to environmental and anthropogenic changes that is believed to have driven historical diversification of the *A. gambiae* complex continues to act within them. I will show how genetic, behavioural, and ecological components interact in a complex way to modulate the strength of reproductive isolation across Africa and then describe the genomic signatures of this dynamic process. Finally, I will show recent data from an area of high hybridization between the two species at the far-west of their range suggesting that extensive introgressive hybridization occurring particularly in the coastal region is leading to a locally selected hybrid form. Overall, the data point to a very dynamic, site-specific and ongoing process of intra-specific sub-structuring within the two major Afrotropical malaria vectors, and highlight the need to monitor these ongoing process and its impact on epidemiologically important attributes, such longevity, fecundity, parasite susceptibility, host seeking behaviour.

SYMPOSIUM

Advanced models and technologies for the study of Evolution

Chair: MARCO PASSAMONTI

The importance of mergers and acquisitions in evolution

James O. McInerney

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Constructing phylogenetic trees is a very interesting and important part of reconstructing evolutionary histories. However, if these histories are replete with merging events caused by recombination, horizontal gene transfer, exon shuffling or any of the myriad ways in which introgression can offer, then the phylogenetic tree is not the correct model. In this talk, I will outline how important merging of evolving molecules has been in the histories of life on the planet. My talk will cover individual genes, populations of organisms, higher level taxa such as the eukaryotes and also methods for investigating mergers and acquisitions.

SmithRNAs: could mitochondria "bend" nuclear regulation?

Andrea Pozzi, Federico Plazzi, Liliana Milani, Fabrizio Ghiselli, Marco Passamonti

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Typically, animal mitochondria have very compact genomes, with few short intergenic regions, and no introns. Hence, it may seem that there is little space for unknown functions in mitochondrial DNA (mtDNA). However, mtDNA can also operate through RNA interference, as small non coding RNAs (sncRNAs) produced by mtDNA have already been proposed for humans. We sequenced sncRNA libraries from isolated mitochondria of *Ruditapes philippinarum* (Mollusca Bivalvia) gonads, a species with doubly uniparental inheritance of mitochondria (DUI), and identified several putative sncRNAs of mitochondrial origin, differentially transcribed in males and females. Some sncRNAs are transcribed by intergenic regions that form stable stem-hairpin structures, which makes them good miRNA-like candidates. We decided to name them small mitochondrial highly-transcribed RNAs (smithRNAs). Many concurrent data support that we have recovered sncRNAs of mitochondrial origin that might be involved in gonad formation and able to affect nuclear gene expression. This possibility has been never suggested before. If mtDNA can affect nuclear gene expression through RNA interference, this opens a plethora of new possibilities for it to interact with the nucleus, and makes metazoan mtDNA a much more complex genome than previously thought.

Comparative transcriptome analysis in species with different mechanism of mitochondrial inheritance.

Mariangela Iannello, Guglielmo Puccio, Federico Plazzi, Fabrizio Ghiselli, Marco Passamonti

Department of Biological, Geological, and Environmental Sciences – University of Bologna, Italy

Mitochondria are a fundamental component of the eukaryotic cell that derived from a free living α-proteobacterium. Although mitochondria retained part of their original genome, most of the genes involved in biogenesis, transmission and homeostasis of these organelles are encoded by nucleus. The genes involved in the main process of energy production of most eukaryotes (oxidative phosphorylation, OXPHOS) are encoded by both nuclear and mitochondrial (mtDNA) genomes, therefore a tight co-regulation between mtDNA and nuclear genome is essential for healthy organisms, and it is the result of a long-lasting mitonuclear co-evolution. While most eukaryotes are characterized by a Strictly Maternal Inheritance (SMI) of mitochondria, some species of bivalve molluscs present the Doubly Uniparental inheritance (DUI), where two distinct mitochondrial lineages are present: F-type, inherited through eggs, and M-type, inherited through sperm. So, in DUI species, the same nuclear background has to coordinate two different mitochondrial mtDNAs, characterized by a high nucleotide divergence (20-40%) and different replication and transcription dynamics. In this work, we used RNA-Seq to compare the transcription of nuclear and mitochondrial genes in males and females of two bivalve species: *Ruditapes decussatus*, a species with SMI, and *Ruditapes philippinarum*, a species with DUI. We focused on genes encoding for subunits of OXPHOS complexes. Our analyses show a divergent pattern of transcription of genes involved in mitochondrial functions among the two species, revealing a different mechanism of regulation between nucleus and mitochondria. We also investigated the relationship between gene expression levels and rate of protein sequence evolution of OXPHOS subunits. Surprisingly, we found different patterns compared to what observed in other animal species.

The Likelihood Decay Index: Branch support for the phylogenomics era

Christopher Creevey[1], Mark Wilkinson[2], James McInerney[3]

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Bootstrap proportion (BP) support remains a commonly used metric of the reliability of genome-scale phylogenetic analyses because sampling error decreases as the length of sequences increases resulting in trend where BP support approaches 100%. However, not all conflicting phylogenetic signal is due to sampling error; processes such as incomplete lineage sorting and horizontal gene transfer can result in valid alternative genetic histories. Despite this, with long-enough alignments, 100% BP can be achieved even if 49% of the data supports an alternative topology. The heterogeneous nature of the underlying support for branches with 100% BP requires a novel approach and a change in our notion of "support". To address this, we suggest a likelihood decay support value. Based on the premise of Bremmer support, it is the difference in likelihoods of the optimal trees that do or do not include a given split. Likelihood decay represents a novel way to assess support which discriminates between different internal branches and is insensitive to alignment length. We demonstrate these properties with simulations and investigate the phylogenetic support in "solved" phylogenomic studies where 100% BP support has been obtained. The likelihood decay index has been implemented in "Machete", freely available at https://github.com/ChrisCreevey/machete.

Large-scale screening of genetic diversity of spatially isolated and taxonomically diverse small mammal communities by intron markers

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As recognized by national laws and international agreements over the last decades, the relevance of preserving genetic diversity as source of evolutionary potential is now widely acknowledged. However, effective monitoring of genetic diversity is challenging. It is important to develop markers that can be compared despite being analyzed in different labs and/or at different times. Boosted by the increasingly powerful NGS approaches and assisted by the availability of open access genomic resources, we optimize a multilocus sequence-based panel of genome-wide markers for research on non-model organism and test it in small mammals. The communities inhabiting two sanctuaries for biodiversity, namely Doñana (Spain) and Kinabalu (Malaysia) national parks, were selected as study system. Specifically, the amplificability and variability of 46 intron loci previously developed on rodents were evaluated for a spectrum of taxonomically diverse species by sequencing amplicon and shotgun libraries. The latter were also used to reconstruct the whole mitogenomes of the sampled taxa. Primer design was optimized through dedicated bioinformatic pipelines and coverage analyses carried out to check for genotype reliability. Even though amplification yield and evenness followed a decreasing pattern sharply reflecting the phylogenetic distance from murids, some loci were amplified also in different families. While genetic variability displayed by some loci point to their utility for addressing question at population genomics level mostly for murids, others will contribute to clarify evolutionary questions at higher taxonomic level for more distantly related groups.

Description of a molecular cytogenetic integrative approach to investigate the evolution of Komodo dragon genome

Alessio Iannucci[1], Martina Johnson Pokorná[2,3], Marie Altmanová[2,3], Michail Rovatsos[2], Lukáš Kratochvíl[2], Ivan Rehák[4], Petr Velenský[4], Jorge Claudio Pereira[5], Malcolm Ferguson-Smith[5], Claudio Ciofi[1]

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The study of reptilian genomes is crucial for accurate investigation of genome evolution across amniotes. To date, the genomes of 13 reptile species have been sequenced and published. Although draft assemblies are often that is required to answer some important biological questions, a greater level of resolution is needed to gain more detailed insights into evolutionary processes. This can be achieved through building high-quality chromosomal maps and anchoring genomes to chromosomes. We report on a preliminary work aimed at identifying the chromosome sequences of the Komodo dragon *Varanus komodoensis*, the world's largest lizard, using an integrated approach based on flow sorting and next generation sequencing (NGS). We isolated *V. komodoensis* chromosomes by flow sorting, assigned each peak to a pair of chromosomes by FISH, and finally amplified chromosome-specific DNA pools for NGS sequencing. Information on chromosomes sequence is crucial for anchoring newly sequenced and assembled Komodo dragon genome scaffolds to chromosomes. Integration of our results to chromosome painting data will allow comparison of genome organization among squamates, facilitate reconstruction of ancestral amniote karyotype and provide a better insight into the evolution of squamate genomes. Finally, production of high-quality genomic information will help the identification of molecular markers to further understand the evolutionary history of wild Komodo dragon populations.

Looking at the present to gain knowledge on the past:

what might be the primeval function of the phytochelatin synthase enzyme?

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The phytochelatin synthase (PCS) enzyme is constitutively expressed in the majority of plants, other eukaryotes and cyanobacteria. In the presence of metal(loid)s (i.e., Cd, Pb, Hg, As; excess Cu and Zn) eukaryotic PCS is activated and produces the so-called phytochelatins, able to bind and compartmentalise these elements inside the vacuole. The constitutive expression of PCS in the plant clade, even in the absence of toxic metal(loid)s, would lead us to postulate other functions of this enzyme, besides toxic metal detoxification; this hypothesis is also supported by the fact that metal hyperaccumulation in plants do not depend on a "super- synthesis" of phytochelatins as far as their metal hypertolerance is concerned. Iron (Fe) has always been widely present in all environments, but at the same time its solubility and bioavailability poses serious problems for the vast majority of organisms. Thus, we hypothesise that PCS in plants and cyanobacteria might possess a primeval and ubiquitarian function geared towards control of physiological requirements of Fe(II) and Fe(III), perhaps in "cross-homeostasis" with Zn. Our work deals with PCS identification and characterisation in green algae and basal land plants, namely the charophytes *Nitella mucronata* and *Chara vulgaris*, the liverworts *Lunularia cruciata* and *Marchantia polymorpha*, the lycophytes *Selaginella kraussiana*, *S. denticulata* and *S. moellendorffii*, and some strains of cyanobacteria (*Geitlerinema* sp., *Gloeobacter* violaceus, *Nostoc* sp.). Functional characterisation of the PCS has been carried out, focusing in particular on the potential post- translational controls of the enzyme activity in the presence/absence of physiological concentrations of Fe and Zn, as well as after exposure to toxic metal(loid)s, such as Cd and As. The results would allow us to achieve comparative characterisations of ancestral PCSs and further clarify their functions in early plants and cyanobacteria.

The impact of orthology selection in assessing the phylogenetic relationships of the living lineages of amphibians

Karen Siu-Ting[1,2,3], Mary O'Connell[3], Maria Torres-Sánchez[4], Diego San Mauro[4], Mark Wilkinson[5], Davide Pisani[6], Christopher Creevey[2]

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Amphibia is a group of vertebrates the living representatives of which (known as Lissamphibia) include the monophyletic crown groups: Anura (frogs), Caudata (salamanders and newts) and Gymnophiona (caecilians). The evolutionary relationships among these three orders is still debated, centring on the Procera hypothesis (Gymnophiona and Caudata together, with the Anura as a sister group to these two) and the Batrachia hypothesis (Caudata and Anura together, with the Gymnophiona as a sister group to these two). While the Batrachia hypothesis is the most supported to date, recent phylogenomic analyses have resulted in conflicting results. We hypothesise this is due to poor ortholog selection. To address this question, we conducted a phylogenomic analysis of the Lissamphibia combining novel transcriptomic data from field specimens and genomic data sourced from databases for 33 species (18 amphibians and 15 vertebrate outgroups). Using a combination of pairwise and phylogeny based methods we identified 2,696 orthologous gene families. These were used to carry out an initial phylogenetic inference using supertree (MRP, ASTRAL) and supermatrix approaches (PhyloBayes, RAxML). To assess the impact of ortholog selection, further phylogenetic reconstructions were carried out following the application of a series of filters to the gene families based on sequence saturation, missing data and monophylies for known uncontested clades in outgroup species. We found that inclusion of all gene families without careful curation supported the Procera hypothesis with high support in the majority of the methods used. However as successive filters are applied to the gene families support for the Batrachia hypothesis increases until all methods retrieve this result with high support. Interestingly, filtering for sequence saturation and missing data had the least impact and methods with complex models (such a Phylobayes) retrieved the Batrachia hypothesis most often. Our results demonstrate careful curation of orthologous genes is a critical factor in phylogenomic studies and in particular for understanding the relationships of the extant lineages of amphibians.

Investigating diet diversity and venom evolution of the family Terebridae (Gastropoda)

Giulia Fassio[1], Emily Lau[2], Juliette Gorson[2,3] Mandë Holford[2,3]

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Terebridae is a family of tropical and subtropical carnivorous marine gastropods that use a venom arsenal for predation and defence. Similar to other venomous animals, it has been hypothesized that variation in venom composition results from adaptive evolution driven by diet prey selection. This study characterizes the vermivorous diet and venom variation of select terebrid species to determine if there is a correlation between terebrid diet and venom composition. The gut content of fourteen terebrid species from Kaveing, Papua New Guinea were selected to represent the major clades of the terebrid family and the variation of foregut feeding anatomy with and without a venom gland. Amplification of the 16S rDNA gene using polychaete specific primers provided gene products that were sequenced with Illumina MiSeq Next Generation Sequencing (NGS). Of the 70 gut samples characterized, three polychaete prey families (Serpulidae, Spionidae and Nereidae) were identified. To determine if terebrid diet variation was tied to venom composition venom gland transcriptomics was conducted on 9 of the same 14 terebrid species which had venom glands. While those species with a venom gland appear to have a more diverse diet, similar prey were found in terebrids with and without a venom gland. This is the first study that molecularly identifies the vermivorous terebrid diet to describe the largely unknown trophic ecology of terebrids.

Paleogenomic evidence for multi-generational mixing between Neolithic farmers and Mesolithic hunter-gatherers in the Lower Danube basin

Gloria Gonzalez-Fortes[1,2,15], Eppie R. Jones[3, 15], Emma Lightfoot[4], Clive Bonsall[5], Catalin Lazar[6], Aurora Grandal-d'Anglade[7], María Dolores Garralda[8], Labib Drak[8], Veronika Siska[3], Angela Simalcsik[9], Adina Boroneanț[10], Juan Ramón Vidal Romaní[7], Marcos Vaqueiro Rodríguez[7], Pablo Arias[11], Ron Pinhasi[12,13], Andrea Manica[3], Michael Hofreiter[2]

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The transition from hunting-gathering to farming involved profound cultural and technological changes. In Western and Central Europe, these changes occurred rapidly and synchronously following the arrival of Early Farmers of Anatolian origin, who largely replaced the local Mesolithic hunter-gatherers. Further east, in the Baltic region, the transition was gradual, with little or no genetic input from incoming farmers. Here we use ancient DNA to investigate the relationship between hunter-gatherers and farmers in the Lower Danube basin, a geographically intermediate area that is characterized by a rapid Neolithic transition but also by the presence of archaeological evidence that points to cultural exchange, and thus possible admixture, between hunter-gatherers and farmers. We recovered four human paleogenomes (1.1x to 4.1x coverage) from Romania spanning a time transect between 8.8 thousand years ago (kya) and 5.4 kya, and supplemented them with two Mesolithic genomes (1.7x and 5.3x) from Spain to provide further context on the genetic background of Mesolithic Europe. Our results show major Western hunter-gatherer (WHG) ancestry in a Romanian Eneolithic sample with a minor, but sizeable, contribution from Anatolian farmers, suggesting multiple admixture events between hunter-gatherers and farmers. Dietary stable isotope analysis of this sample suggests a mixed terrestrial/aquatic diet. Our results provide support for complex interactions among hunter-gatherers and farmers in the Danube basin, demonstrating that, in some regions, demic and cultural diffusion were not mutually exclusive, but merely the ends of a continuum for the process of Neolithization.

The draft genome of *Ruditapes philippinarum* (the Manila clam)

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Bivalve molluscs are a highly successful and ancient Class (20,000+ known species) and an interesting group for both basic and applied biology. They represent a good model for studying adaptation to anoxia/hypoxia, salinity, and temperature, and they are useful bioindicators for monitoring the concentration of pollutants and heavy metals in the water. They also make up an important source of food all over the world, with a production corresponding to ~20% of the global aquaculture yield. A striking feature of bivalves (and the main reason behind this project) is the presence of an unusual mitochondrial inheritance system: the Doubly Uniparental Inheritance (DUI), so far detected in ~100 species. In DUI bivalves, two mitochondrial genomes (mtDNAs) are present: one is transmitted through eggs (F-type), the other through sperm (M-type), and the amino acid p-distance between conspecific M and F genomes ranges from 10% to over 50%. DUI provides a unique point of view for studying fundamental aspects of eukaryote biology. In DUI systems: i) males are naturally heteroplasmic, with two very divergent mtDNAs; ii) it is possible to follow germ line mitochondria during development; iii) mitochondria are under selection for male functions; iv) there are two coexisting mitochondrial genomes in the same nuclear background. All these interesting biological features are in sharp contrast with the lack of genomic resources about bivalves (and molluscs in general). Here we present the draft genome of the DUI species *Ruditapes philippinarum*. A male individual was sequenced with 40x Illumina HiSeq and with 30x PacBio RSII. We have tried to assembly this dataset with all available assembly pipelines. The best results were obtained by Canu assembler with contig N50=76Kb (86% complete, 5% fragmented, and 9% missing metazoan orthologs according to BUSCO). We report a general description of the genome and some highlights of its principal biological features.

Lose it or keep it: (how bivalves can provide) insights into mitochondrial inheritance mechanisms

Elisabetta Punzi, Liliana Milani, Fabrizio Ghiselli, Marco Passamonti

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The strictly maternal inheritance (SMI) of mitochondria is a pattern of mitochondrial transmission observed across the whole animal kingdom. Some interesting exceptions, however, are known in the class Bivalvia, that show an unusual pattern called doubly uniparental inheritance (DUI) whose result is a heteroplasmic pool of mtDNA in males. While widely debated, its molecular bases have not been established yet. The aim of this work is to select classes of proteins known to be involved in the maintenance of SMI and to compare their features in two clam species differing for their mitochondrial inheritance mechanism, SMI *Ruditapes decussatus* and DUI *Ruditapes philippinarum.* It is not clear which is the evolutionary pattern of mitochondrial inheritance in these two related species, if *R. decussatus* lost DUI or if *R. philippinarum* gained it. Either way, both patterns of inheritance probably share a significant portion of the underlying molecular mechanism. The analysis focuses on three main protein groups: nucleases and polymerases, proteins participating in post-translational modifications by small proteins covalent bonding (i.e. ubiquitination, sumoylation, etc.) and proteins involved in autophagy and mitophagy.

These data were then extracted from the analysis of the transcriptome, obtained with an Illumina platform, of male and female ripe gonads of both species. For each protein group of interest, transcription bias (male or female), annotation, and (when necessary) mitochondrial targeting were assessed. We did not find any evidence supporting a role of nucleases/polymerases or autophagic machinery in the enforcement of SMI in *R. decussatus.* Regarding ubiquitination, instead, many proteins with the expected features have been retrieved, providing us with candidates for future targeted research.

Sisters, but not alike: ecological divergence with gene flow in Neotropical nutmeg tree species

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Gene flow is expected to prevent divergence between populations. Divergence with gene flow can nonetheless occur, but the underlying ecological processes and the frequency of such events are yet to be exactly characterised. To contribute to address these issues, we have tested for the presence of gene flow in a pair of phylogenetically related, but ecologically divergent, tree species of the *Virola* genus (Myristicaceae, the nutmeg family). Ten nuclear microsatellites and two chloroplast DNA sequences were used to infer interspecific evolutionary relationships among the bottomland-dwelling *V. surinamensis* versus hilltop-dwelling *V. kwatae*. We assessed the amount of genetic divergence and estimated the extent of interspecific gene flow by a combination of approaches based on conventional *F*-statistics, Bayesian assignment and coalescent ABC modelling. Despite marked ecological divergence, our results indicate that *V. surinamensis* and *V. kwatae* have been connected by gene flow, either continuously or through secondary contact, since their divergence. The existence of a third species, *V. michelii*, ecologically closer to *V. kwatae*, but phylogenetically divergent from the *V. kwatae* / *V. surinamensis* pair, and not exchanging genes with either species on the long term, suggests that evolutionary radiation with habitat divergence in the presence of gene flow has occurred in the *Virola* genus, or that alternatively ecological and genetic divergence is maintained notwithstanding secondary contact.

Advanced models for the study of recent human evolution: application to forensic STRs.

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Human forensic STRs are used for individual identification but have been reported to have little power for inter-population analyses. We genotyped at 16 forensic STRs a large population sample obtained from many locations in the Mediterranean area. In order to reduce the inter-population complexity, yet retaining the signal of spatial trends and genetic structuring, we sequentially applied recent spatial methods. Using spatial PCA (adegenet) on the full dataset, we compiled a list of alleles which display frequency gradients across the area. Based on this list, we generated informative condensed datasets. We then applied the spatially explicit models implemented in GENELAND and EEMS programs to visualize the surfaces of genetic change. These methods provided a concordant view of the population structure. All analyses returned the picture of a background clinal variation, with regional discontinuities captured by each of the condensed datasets. Finally, we applied coalescent simulations to estimate migration rates among the sampled locations, under complex demographic scenarios. These results are promising in a microevolutionary perspective. The methods outlined here may be helpful to orient heuristic searches of specific markers in locations that could confirm/dismiss hypotheses on genetic contributions from the Early Neolithic Levant, the Hellenic world or the Balkans to Southern Italy. On larger scales, it is foreseeable that the same methods will allow the exploitation of an invaluable genotypic resource deposited in forensic STR databases to clarify important aspects in the formation of local gene pools. Grants CUP E81J10001270005 to C.J. and PRIN-MIUR 2012JA4BTY to A.N.

SYMPOSIUM

Biogeography and Systematics

Chair: OMAR ROTA STABELLI

Co-chair: GIULIANA ALLEGRUCCI

Microbial population structure and biogeography from metagenomic data

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Phylogenetic analysis of microbial species from genetic markers or whole genomes permitted to study the population biology and biogeography of several (opportunistic) bacterial pathogens. However, the population structure of most of the key microbial species colonizing our body remains uncharacterized because of issues in obtaining genetic information from organisms that are refractory to cultivation. I will introduce a framework to perform strain-level phylogenetics for hundreds of underinvestigated species that are common in the human microbiome. The framework is based on the extraction of genetic markers from metagenomes and the comparison of such markers across thousands of microbiome samples. Preliminary results highlight that several intestinal bacteria show strong biogeographical patters (e.g. *Faecalibacterium prausnitzii*) and evidence of discrete sub-species structures (e.g. *Eubacterium rectale*). I will show how this framework can be used for a a large set of population genomics analyses for underinvestigated members of the human microbiome.

The genetic diversity of *Helicobacter pylori* in Siberia

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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 biogeographic analysis of loss of planktotrophy in caenogastropod molluscs (Gastropoda, Nassariidae)

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In marine gastropods, the larval development is a key feature for biology and ecology of the species. In planktotrophic development (P), the larvae spend from a few days up to one year in the plankton feeding actively. In non-planktotrophic development (NP), larvae spend very little or no time in the plankton feeding almost exclusively yolk supplies. In the Caenogastropoda, NP development is mostly considered as a derived condition that arises in response to conditions that counterselect P, allowing independence from trophic environmental availability. It is suggested that NP represents an advantage in phytoplankton-poor regions as the Mediterranean Sea, where food availability is strictly limited by seasonality as in Antarctica, or in response to major environmental changes occurred in the past. We have tried to detect and analyze events of loss of P in a group of marine prosobranchs, aiming at identifying patterns of correlation with paleo-environmental changes. We used a robust phylogeny of the family of Nassariidae (Buccinoidea) to identify pairs of sibling species or group of species showing contrasting larval developments, thus representing independent losses of P. Then, we calibrated the phylogeny with known fossils and used a relaxed molecular clock model for dating every single event of loss of P. We found at least 15 P-NP switches in the Nassariidae, 9 involving pairs of P-NP sibling species, 3 resulting in a radiation of NP species and 3 representing single NP lineages species. Most of the P-NP shifts were dated from the Miocene (23-10 Mya) to the Pliocene (4-2 Mya). For most of them a plausible paleoceanographic scenario was reconstructed to explain the environmental conditions that favored the loss of planktotrophy.

Unraveling the evolutionary history of Mediterranean Flabellinidae and Piseinotecidae species (Heterobranchia, Gastropoda)

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Flabellinidae Bergh, 1889 is one of the largest families of aeolid nudibranchs with more than 60 accepted species worldwide. Species ascribing to this family are typically characterized by diagnostic morphological features like the triseriate radula and cerata protruding from a peduncle or directly from the notum. However, recent molecular works, on other groups of aeolids, put in doubts the monophyly of this family since the presence of some Piseinotecidae taxa showing a very low genetic distance with some Flabellinidae species. Furthermore, new cryptic species were recently revealed for the understudied Mediterranean Sea. In this work, we have used genetic data from both mitochondrial (16S and COI) and nuclear (H3) gene fragments as well as morphological data from taxonomically relevant characters, to investigate the systematics of Mediterranean Flabellinidae and Piseinotecidae taxa. Species delimitation analyses and phylogenetic inference were performed to explore the genetic divergence occurring among these two families. Results from Bayesian and Maximum-Likelihood methods indicate that species within the genera *Flabellina*, *Calmella* and *Piseinotecus* do not form monophyletic clades. These results are supported by morphological analyses which allowed the re-evaluation of the triseriate radula condition in some Pisenotecidae and *Calmella* taxa and their inclusion in the genus *Flabellina* as *Flabellina gaditana* comb. nov**.** (synonym of *F. confusa*) *Flabellina gabinierei* comb. nov**.** and *Flabellina cavolini* comb. nov**.** Moreover, species delimitation and barcoding gap analyses allowed uncovering cryptic species within *Flabellina gracilis* (Alder and Hancock, 1844), *F. trophina* (Bergh, 1890), *F. verrucosa* (M. Sars, 1829) and *F. ischitana* Hirano and Thompson, 1990, the latter which is under description. This work corroborates the relevance of an integrative taxonomy from multiple populations and species to study nudibranch evolutionary history and to unravel cryptic diversity.

Population genomics and species-level differentiation in Cerastoderma glaucum (Bivalvia) determined using restriction-site associated DNA-sequencing

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Coastal lagoons form an intriguing example of marine habitat disjunction. Restricted gene flow among isolated populations of lagoon specialist species may induce their genetic divergence, being a first step towards speciation. Patterns of divergence across genome can provide valuable insights into the history of population divergence and dynamics of speciation. In the present study, genetic population structure of the bivalve *Cerastoderma glaucum* has been studied using restriction-site associated DNA-sequencing (RADseq). Populations spanning from the Atlantic and Mediterranean coast, including the Baltic Sea, the North Sea and Black Sea has been compared to determine the degree of genetic differentiation. Detailed population genetic investigation has been performed on genome-wide SNPs derived from RADseq data. Highly significant genetic structuring of populations separated in different basins has been found and the RADseq approach allowed to infer phylogenetic relationships with an unprecedented resolution. Phylogenetic reconstructions point to the existence of three deeply divergent lineages within *C. glaucum* that are separated by an accumulation of many genetic barriers: one of these lineages occupies the Aegean- Black Sea region, another one the Ionian Sea, and the last one is widely distributed from the western Mediterranean to the Baltic Sea. Within the western evolutionary unit significant spatial genetic structuring has been uncovered, reflecting different evolutionary processes.

Genomic insights on the biogeography of the Tiger Mosquito, *Aedes albopictus*, in Italy

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The Asian tiger mosquito, *Aedes* (Stegomyia) *albopictus,* is one of the most invasive animal species, having spread in few decades from its original range in South-East Asia to all continents, thanks to its high ecological plasticity and ability to exploit human mediated transportation. This has a significant public health impact, being the species a competent vector of numerous exotic arboviruses, including Chikungunya, Dengue, and Zika. Notably, in Italy *Ae. albopictus* caused the first outbreak of an exotic arbovirosis, with more than 250 human Chikungunya infections in few weeks. Understanding the biogeography and the dynamics of this extraordinary invasion process is not only of scientific interest but could provide insights on epidemiologically relevant factors, as distinct populations have been shown to be characterized by different susceptibility to arbovirus infections and to insecticides used for control interventions. We took advantage of a reference set of >60,000 SNPs used to genotype across the genome 20 worldwide populations by double digest restriction site-associated DNA sequencing (ddRAD), to focus on the genetic structure, biogeography and invasion dynamics in Italy. We genotyped 9 populations across Italy, 1 from Greece and 1 from Albania and compared the results with those from the 20 genotyped native and invasive populations. Results show that Italy and southeastern Europe were invaded at least twice independently from different source populations, in agreement with worldwide data revealing two genetically differentiated clusters including both native and invasive populations and highlighting a pattern of multiple invasions. High genetic intra-population diversity supports invasion from large propagules followed by genetic drift and admixture among different source populations, which created a complex genomic pattern with generally low levels of differentiation and signs of Isolation By Distance among populations in Italy.

Revealing the genetic diversity of Antarctic springtails

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Eighty million-years of glacial cycles, extreme environmental conditions and isolation of the Continent are the major factors that have shaped the Antarctic terrestrial ecosystem, resulting in low biodiversity (few high-rank taxa and limited species richness). Springtails (and mites) are the largest, year-round, strictly-terrestrial inhabitants of Antarctica. Their evolutionary trajectory arises from the Mesozoic, when the Continent was still placed at lower latitudes and had an ecosystem similar to that of present-day temperate regions. Since the drift-to-south of Antarctica, a limited number of likely pre-adapted arthropod taxa survived the worsening of the climate conditions in Nunataks, from where they dispersed during the present interglacial. Nowadays, the descendants of these lineages populate the coastal sites of the Continent where, during the short summer season, the environmental conditions are compatible with the development of a basic ecosystem. A handful of springtail species live in both maritime and continental Antarctica. Their distribution is sectorial and patchy, and largely dependent on availability of ice-free grounds and bioavailable water. Genetic screening of mitochondrial DNA haplotypes unravels patterns of inter- and intra-specific diversification, and colonization routes and dates. Four Antarctic species (*Cryptopygus antarcticus*, *Cryptopygus terranovus*, *Folsomotoma octooculata* and *Friesea grisea*) are here analyzed using haplotype and complete mtDNA data. Genetic data show high levels of diversification among clusters of haplotypes that usually follow a latitudinal gradient. Molecular markers point for ancient (pre-Last Glacial Maximum) origin of springtail species, although the dates of their colonization routes begun in different geological times. In the lack of clear morphological characters, useful for species diagnosis, complete mtDNAs comparison is also applied to define interspecific borders, among Antarctic springtails.

Ecological genetics and diet composition of Weddell seal (*Leptonichotes weddellii*) from the Ross sea

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In this study, we analyzed the genetic variability and the diet of Weddell seals from Terra Nova and Wood Bay (Ross Sea, Antarctica). Two mitochondrial and one nuclear markers were sequenced to investigate population haplotype diversity and possible recent expansions. Fifteen microsatellites were also used to analyze the population genetic structure and estimate its effective size N_e. For the analysis of seals' diet, $\delta^{13}C$ and $\delta^{15}N$ estimates were obtained from lipid-free muscle fragments of seals and their possible preys. From the genetic analyses, we obtained a N_e of 50,000 Weddel seal females for the Ross Sea and 1,340 individuals for our sampling area. We found high diversity (Hd>0.90) and many exclusive haplotypes (43%-81%), likely due to their surprisingly high site fidelity; nevertheless, low microsatellite differentiation suggests that the colonies are part of the same larger population. Its expansion seems to have started around 58,000 years ago, indicating that seals have been successfully inhabiting the area since then, probably due to the lack of human hunting and predation. The isotopic analyses showed high $\delta^{13}C$ and $\delta^{15}N$ values, likely due to the proximity to a polynya (a key area for biological production) that can enrich the food web. Seal pups showed lower isotopic values, probably due to better diving skills of adult seals, that can reach more efficiently benthonic preys with high $\delta^{13}C$ contents. We also found an increment of 0.18‰ in $\delta^{13}C$ for every 100 Kg of mass, possibly because larger individuals can feed more, due to a greater lung capacity to dive and forage underwater. Their diet mainly consists of *T. newnesi* (43.3%), a fish particularly abundant in areas with polynyas. As a top predator, the role of Weddell seals in the Antarctic food web is surely crucial and their demographic and genetic dynamics should be constantly monitored, to follow the future changes of an important ecosystem such as the Ross Sea.

Peripheral populations of *Salamandra atra* at the southern fringe of the Alps: genetic differentiation and evolutionary history

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Alpine salamanders, *Salamandra atra*, are unusual among amphibians as they are fully terrestrial and adapted to a cool montane climate. The species is widespread throughout the northern and eastern Alps, with largely connected and phenotypically uniform populations. It is also present across the southern Prealps and the Dinarides with rare isolated populations, some of them remarkably distinguished in coloration. Mapping, sampling and investigating these peripheral populations have been insofar hampered by their rarity and the low detectability of individuals. By means of intense field campaigns we have surveyed all the southernmost populations of *Salamandra atra* and, by employing different molecular markers (10 autosomal microsatellite loci, 3 mitochondrial genes and 2 protein-coding nuclear genes), we have estimated the genetic diversity within and between populations. Genetic analyses suggest that most of the prealpine populations are genetically strongly differentiated, even when not differing in coloration, and their genetic diversity is lower in comparison with that of northern populations. The phylogeographic analysis corroborates the hypothesis that extant prealpine and dinaric populations are relic of a broader southern distribution, and most of them did not contribute to the species post-glacial recolonization of the Alps.

The evolutionary history of the insular endemic *Podarcis wagleriana* revealed glacial expansions followed by asymmetric gene flow

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Sea-level changes associated with Pleistocene climatic oscillations largely shaped the current distribution of coastal biodiversity promoting both population movements through formerly separated land masses during glacial marine regression or providing newly exposed habitats suitable for colonization. The Mediterranean basin represents an ideal setting to shed light on whether the increasing of coastal plains during the glacial phase may favored conditions for demographic stability or even expansion as alternative pattern to the Pleistocene 'Expansion-Contraction' (EC) paradigm. We used the Sicilian endemic wall lizard *Podarcis wagleriana* to investigate the extent and the timing of the EC dynamics within a southern Mediterranean insular context. The evolutionary and demographic history has been reconstructed using two mitochondrial and two nuclear gene fragments from 179 individuals across the whole species range in Sicily and neighboring islands. We found three mitochondrial lineages with parapatric distribution, whereas a lack of geographic structure was apparent in nuclear genealogies. The demographic and spatial reconstruction suggest that during the last glacial phase the West and the East lineages expanded from two different ancestral areas up to establish a secondary contact before the last glacial maximum. Such a scenario of glacial expansion is further supported by species distribution modelling, which indicated an increased extent of highly suitable lowland habitat made available by the last glacial marine regression. The pattern of mito-nuclear discordance is explained by (unidirectional) nuclear gene flow between the West and the East lineages following their secondary contact. This study provides additional support for regional variations in the time of demographic changes of temperate species during Pleistocene and also provides future research directions on the role of asymmetric hybridization between lineages as source of mito-nuclear discordance.

Algebra of Biodiversity

Saverio Vicario

Consiglio Nazionale delle Ricerche – Istituto sull'Inquinamento Atmosferico

Biodiversity defined as the measure of the variability of a set of biological communities is an important variable in ecology and land management. The relationship between biodiversity and ecosystem functions and ecosystem services is subject to debate. Even the fact that there was a decrease in diversity through time in last 40 years is contentious. The disagreement it is in part due to the ambiguity to measure such a variable and what mathematical framework should underpin this measure. To the basic concept of taxonomic diversity later were added disparity or specific traits diversity and phylogenetic diversity or expected overall trait diversity. These 3 diversities can be partitioned geographically in within (alpha) and between localities (beta) components. The work of Jost (2006, 2007) grounded the taxonomic estimator within the general framework of Rényi entropies (degree 1 being Shannon and 2 a simple derivation of Gini-Simpson). Chao, Wang and Jost (2010) included also the phylogenetic concept and Marcon, Scotti et al. (2014) produced a coherent partitioning of the alpha and beta components, pointing out that only degree 1 produce independent partitioning. I would like to propose to drop the full family of the Rémyi entropies and concentrate only to degree 1. This allow to use only the well behaved, *sensu* Marcon, beta diversity that overlap with the concept of mutual information. Further, this framework should be used to build interaction network between biodiversity and other predictive variables. We implement this framework and we compare it with a Permanova one using a floristic data set taken from the H2020 Ecopotential project. In prospect to enlarge this framework to discrete or continuous biotic and abiotic variables (i.e precipitations, body size, …), we present the Cumulative Residual Entropy (Rao et al. 2004) and show the equivalence with phylogenetic entropy as akin generalization of discrete Shannon.

Phylogeography, Phylogeny and Systematics of *Hepatozoon* from Galápagos Land Iguanas (*Conolophus* spp.)

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Hepatozoon sensu lato is a hemoparasite that includes hundreds of species. Identification is primarily based on morphological criteria, which may lead to underestimation of taxonomic diversity (Telford, 1984; Harris et al. 2001). We used sequence data from part of the 18S ribosomal DNA gene to investigate the genetic differentiation, phylogeography and phylogeny of *Hepatozoon* infecting land iguanas from Galápagos Islands (*Conolophus* spp.). Our results indicate that the pattern of genetic variation across islands is not consistent with the geological age of islands, with western islands, geologically younger, showing higher haplotype diversity. Such high diversity may correlate with the high density of ticks (*Amblyomma*), which can act as a reservoir for *Hepatozoon* from Galápagos iguanas. Our data demonstrate that *Hepatozoon* from Galápagos are not monophyletic, and at least three separate colonization events occurred, of which, only one shows a clear direct origin from continental South America. In two of three events (the older ones), a radiation throughout the archipelago occurred after colonization. The third colonization seems to be limited to the western islands Fernandina and Isabela. Although a few haplotypes have also been found in the blood of *Amblyrhynchus* (the Galápagos marine iguana) and *Chelonoidis* (the Galápagos giant tortoise) in parallel studies of hemoparasites of Galápagos reptiles (Bataille, 2012; Patino, 2017), most haplotypes seem exclusive to land iguanas and none has been found outside of Galápagos. Similar evidence of specificity was provided by Patino (2017) who found 2 haplotypes exclusive to giant tortoises. Based on intra- and interspecific genetic divergence data, more than one species of *Hepatozoon sensu lato* exist in Galápagos iguanas. In the light of a newly proposed classification of hemogregarine (Karadjian et al., 2015), none of the hemogregarine found in Galápagos reptiles thus far should remain within the genus *Hepatozoon*.

Turning chaos into Order: the phylogeny of Phasmida.

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The Phasmida Order is one of the most species-rich insect taxon, counting more than 3000 species. These phytophagous and mainly nocturnal animals, scattered all throughout the world (except Arctic and Antarctic), are capable of remarkable adaptations: they are one of the clearest example of cryptic mimetism and they also show a wide variety of egg dispersal and reproductive strategies - including parthenogenesis, androgenesis and hybridogenesis. Although major advances regarding biogeographically restricted taxa (i.e. New Zealand), taxonomy and systematics are still debated and a comprehensive phylogenetic analysis is lacking. Many of the subfamilies and higher-order lineages were found not to be monophyletic, taxonomic and phylogenetic uncertainties being hindered by instances of convergent evolution of morphological characters. In 2016, a consortium including internationally recognized taxonomists and evolutionary biologists has been started, and we are now presenting our effort to provide a more complete and solid phylogeny of this taxonomic group. We are using two nuclear genes (28s rRNA and H3 histone genes) and four mitochondrial genes (cytochrome oxidase subunits I and II, 12s and 16s rRNAs) in order to provide a reliable phylogeny at all taxonomic levels. We currently plan to sequence an average of 4000 bp for up to the 70% of the known species of phasmids in the following years, with an integrative taxonomy approach, that includes morphology, reproductive biology and phylogeography. We are also implementing DNA barcoding, with the aim to provide a database resource for quick taxonomic determination. The outstanding biodiversity of phasmids calls for either a validation or a revision of currently recognized taxa and phylogeny. Moreover, this effort will offer the opportunity to investigate about the ecological and historical factors that shaped phasmids current distribution and the evolution of their remarkable adaptations.

Phylogeographic pattern in *Cataglyphis italica* (Emery, 1906) (Hymenoptera: Formicidae): role of the plio-pleistocene marine transgression in the Mediterranean basin

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Mediterranean basin and its biota has been greatly affected by Pleistocene climatic oscillation which induced repeated cycles of marine transgressions. Such scenario had major implications in favoring diversification through vicariant event and allopatric fragmentation. Cataglyphis italica is an endemic species highly thermophilic, associated to the arid environment and widespread in southeast Italy. Its biology is characterized by scavenger behavior, temporary polygyny and lower gyne dispersal than males. This latter feature can lead to an additional level of genetic structuring, as well described in recent studies conducted on C. cursor and C. hispanica. Thus, in this study we wanted to shed light on which processes may have generate the current genetic distribution of C. italica. In doing so, we collected 20 workers in 44 different nests of C. italica from southern Italy. One mitochondrial marker, the cytochrome c oxidase subunit 1 (COI), and two nuclear markers, wingless (Wg) and elongation factor 1-alpha (EF1-α), were amplified and sequenced from one worker for each nest. To evaluate the differentiation degree, the geographic distribution and the genealogical relationships within C. italica we built statistical parsimony network for each marker while the software BEAST was used to have an estimate of the time of their divergence. Our results revealed the presence of five mitochondrial haplogroups strongly differentiated and geographically structured. On the contrary, nuclear markers showed a very low genetic differentiation among populations. We hypothesized that the high level of divergence in the COI and the geographical structure could be caused by the presence of a Plio-Pleistocene paleo-archipelago in southeast Italy togheter with the low dispersal capability of C. italica. Mito-nuclear discordance could be explained by the different inheritance properties and evolution time of the two markers and the differential dispersal of males and gynes.

Phylogeography of *Trichosirocalus horridus* (Panzer, 1801) (Coleoptera: Curculionidae, Ceutorhynchinae) in Europe: a preliminary approach

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Phytophagous weevils of the genus *Trichosirocalus* include 17 species with Palaearctic distribution that mainly feed on Plantaginaceae and Asteraceae. In particular, *T. horridus* shows a distribution from Iberian Peninsula to Caucasus and an association to vegetal species belonging to the genera *Carduus*, *Cirsium* and occasionally *Onopordum* (Asteraceae, Cardueae). Because these thistles are invasive in North America, Australia and New Zealand, *T. horridus* has been used as a biological control agent against them. We investigated the phylogeographical pattern of Italian, French, Spanish, German, Turkish and Georgian populations of this weevil, through a genetic mitochondrial approach. Samples of adult weevils were collected during field trips carried out in Spain, France, Italy, Germany, Georgia and Turkey. We examined 532 bp of the mitochondrial cytochrome c oxidase subunit I (*cox 1*), for 101 individuals. Genetic divergence analysis between geographical groups was performed through MEGA version 7. Bayesian analyses were performed using Beast 1.8.0 version under best-fit models of nucleotide substitution selected by jModelTest. The evaluation of intra and inter-populational genetic relationship was performed by the construction of a haplotype network using PopArt 1.7 software. The inference of demographic history was carried out analysing the shape of the distribution of the number of observed differences between pairs of DNA sequences (mismatch distribution) and using D-Test and Fs-Test, through DnaSP 5.10. Five major haplogroups were detected for these European populations that show little differences compared to Italian populations. These preliminary data show that the genetic pattern follows the geographical distribution of *Trichosirocalus horridus*, probably correlated with different demographic histories of populations.

Species delimitation problems in Miridae (Hemiptera: Heteroptera): the study of a hyperdiverse family through DNA barcoding and Integrative Taxonomy

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Plant bugs or Miridae (Hemiptera: Heteroptera) include over 11,020 described species, and are one of the only two hyperdiverse (>10.000) exopterygotes families. Even though mirids comprise about 25% of all species of heteropterans, recent estimates showed that less than 50% of total species of the family are currently described. On the other hand, many species are cryptic and/or show a high intraspecific variability that hinders the ability to identify different morphs or species. Moreover, the application of molecular techniques to mirid systematics is scarce so far. Our study aimed to verify if the integration of DNA barcoding technique, classical morphological identification and different species delimitation approaches can help in testing taxon relationships within the family. Considering that mirids could have economic impacts as pests of agricultural crops and biological control agents, our analysis was performed on Italian specimens collected in agroecosystems. Out of 53 analyzed specimens, 7 species were identified both by morphological and molecular methods. The comparison of our data with those present in public databases showed contrasting results: while for the genera *Orthops* and *Adelphocoris* there is a clear-cut agreement between morphological and molecular data, for all other genera (*Lygus*, *Polymerus*, *Trigonotylus*, *Deraeocoris*) species delimitation data are incongruent due to erroneous taxonomic assignment, high intraspecific variability and possible cryptic species presence. Our results are the first step for the creation of a Italian mirid database and highlight the need of taxonomic revisions in mirids with the help of integrative taxonomy.

The biogeography of the Antarctic springtail *Cryptopygus terranovus* explained using haplotype screening, Bayesian clustering and comparative mitogenomic analyses

Leo C., Fanciulli P., Frati F., Carapelli A.

Department of Life Science - University of Siena

Since the breakup of Gondwana, the Antarctic terrestrial and marine ecosystems have been characterized by increasing isolation and temperatures. Over a long timescale, those modifications have led to high level of endemism and reduced terrestrial biodiversity (this latter represented by only a few invertebrate species). The two arthropods taxa that live in the terrestrial ecosystem of the continent are mites (Acari) and springtails (Collembola), who have poor dispersal ability and a life cycle restricted to ice-free coastal areas (i.e. to 0.34% of the entire landmass). Springtails are distributed in a limited number of biogeographic sectors, and populations have experienced a severe degree of isolation. In the last decades, this evolutionary scenario has been further confirmed by genetic surveys, highlighting a reduced gene flow and a high genetic divergence among populations, despite a substantial morphological uniformity. Samples of the species Cryptopygus terranovus have been collected from 11 sites along the Victoria Land and screened for the mitochondrial "barcode" gene cox1. Haplotype and Bayesian analyses have confirmed the aforementioned scenario, suggesting that the pattern of intra-specific diversity may follow a latitudinal gradient for northern and central populations of Victoria Land; whereas the southern ones show an even greater subdivision (i.e. multiple lineages living in sympatry). The uniqueness of C. terranovus haplotypes supports the existence of evolutionary lineages isolated from each other, likely due to the presence of insormontable geographical barriers and to the limited dispersal capability of springtails. Furthermore, the high genetic divergence is also confirmed by the comparative mito-genomic analysis, applied to all the mtDNA of springtails, deposited on GenBank. The Antarctic species belonging to the genera *Friesea* and *Cryptopygus* shows high p-distance values, which are comparable to those usually observed at an intra-family level.

Exploring the phylogeny of a megadiverse taxon: the case of *Hycleus* (Coleoptera, Meloidae)

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Hycleus is the most speciose genus of the beetle family Meloidae, with almost 430 species widespread in Africa and Eurasia. It belongs to the tribe Mylabrini, subfamily Meloinae. The systematics of the genus *Hycleus* has never been investigated but, according to the mesosternal morphology, it has been divided by Pardo Alcaide into three main "sections": *Mesogorbatus*, with almost 70 species; *Mesotaeniatus*, with about 50 species and *Mesoscutatus* with almost 300 species. Within these "sections", different lineages have been identified according to other morphological characters. Furthermore, previous and ongoing studies on the phylogeny of this family pointed out that the genera *Paractenodia* and *Ceroctis* are strictly related to the genus *Hycleus* and should probably be included within it. *Paractenodia* includes five species endemic to South Africa and Namibia, and *Ceroctis* 59 species distributed through the tropical Africa and the Saharan-Arabic deserts. In order to understand the relationships among some of the species belonging to these genera, about one hundred species were molecularly tested with mitochondrial (COI, 16S) and nuclear (CAD, ARGK, Wingless) markers. The sequences obtained were analysed under the assumptions of Maximum parsimony, Maximum likelihood and Bayesian inferences and a first set of phylogenetic trees is proposed. Preliminary results seem to corroborate the validity of some lineages already defined according to morphological characters, moreover both nuclear and mitochondrial markers confirm that both *Ceroctis* and *Paractenodia* belong to the genus *Hycleus*, in agreement with previous preliminary molecular results. Further analyses to increment the number of tested species are already in progress.

Genetic structure and gene flow analysis in the Italian wall lizard *Podarcis siculus* revealed a discordant pattern between mitochondrial and microsatellite markers in southern Italy.

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The genetic variability within a species is well known to be driven by a combination of both historical and more recent processes. Recent phylogeographic studies of the Italian wall lizard *Podarcis siculus*, based on mitochondrial and nuclear genes, revealed a complex evolutionary history suggesting an important role of the Pleistocene climatic oscillation in driving allopatric differentiation in scattered refugia areas located throughout the whole Italian Peninsula. Here we used 11 microsatellite loci (i) to investigate the distribution of genetic variability of *P. siculus* and (ii) to assess whether the deeply divergent mtDNA lineages, previously identified, were confirmed or alternatively mixed upon secondary contact and gene flow. We analysed 355 individuals from 113 locality of *P. siculus* form the Italian Peninsula. We explored genetic diversity and gene flow using F-statistics indices while clustering methods have been used to infer the genetic structure. The software STRUCTURE supported the presence of five microsatellite clusters partially confirming the mitochondrial repartition which were instead composed by seven parapatric clades. The conforming pattern among mitochondrial and microsatellite markers for most of the lineages, underlies the active role of the principal geographic barriers in maintaining the current genetic structure. The only discrepancy in the number of clusters has been observed in the Calabrian region, where microsatellite analysis suggested the presence of a single cluster rather than the three identified by mtDNA. This could be explained by events of secondary contact and gene flow, at regional scale, among lineages which shaped the genetic architecture of a species.
Finally, the identified mito-nuclear discordance is a further example of a well-established pattern and additional studies on morphological variation could give insight into mechanisms underlying allopatric differentiation and gene flow upon secondary contact.

Multiple sources of incongruence in *Wolbachia* phylogeny

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Wolbachia are maternally inherited intracellular endosymbionts bacteria, commonly found in the reproductive tissues of arthropods and nematodes. Because of its ability to manipulate host's reproduction and influence the transmissibility of host's pathogens, *Wolbachia* has substantial implications on the host species evolution and is a promising biological tool to control diseases and invasive pests. Various aspects of the biology of *Wolbachia* are still widely unexplored, particular ly its biological interactions, spread and acquisition: this knowledge is an important prerequisite to properly characterize the evolution of this taxon. To further understand the biodiversity and the evolution of *Wolbachia* we compare the phylogenet ic signals of two independent data sets: a gene-rich phylogenomic (GENOME) and a taxon- rich multilocus sequence typing (MLST) alignment. Our MLST data set provides a topology consistent with previous MLST phylogenies, but in conflict with the GENOME data set for the position of wZoo and wCte, infecting respectively termite and cat flea. These incongruences have been explored using various approaches to estimate intragenic recombination, evaluating strain misassignment and multiple *Wolbachia* infections on the same host species, and performing topology comparison tests. Results indicate that these discrepancies are due, at least partially, to undetected co-infectio ns, resulting in different *Wolbachia* strains being sampled in the GENOME and the MLST dataset. Coalescent aware phylogenies further show among genes incongruences for wZoo in the GENOME data set: this is compatible with either horizontal transfer, undetected co-infections, or poor gene sampling for certain *Wolbachia* strains. Overall, our results suggest that discrepancies between our MLST and GENOME dataset are not due to stochastic (poor signal in MLST) or systematic errors (signal misinterpretation), but are caused by the complex epidemiological pattern of *Wolbachia* which may have resulted in different strains being sampled in different specimens and/ or have resulted in chimeric genome assemblies.

SYMPOSIUM

Evo-Devo

Chair: ALESSANDRO MINELLI

Co-chair: GIUSEPPE FUSCO

The Fin-To-Limb Transition

P. Sordino, F. Langellotto, M. Fiorentino, E. De Felice, L. Caputi, V. Nittoli, J. M.P. Joss

Stazione Zoologica Anton Dohrn, Napoli

The major morphological changes that occurred during the fin-to-limb transition are the subdivision of the appendicular skeleton into proximo-distal (PD) domains (upper/forearm-upper/lower leg), the appearance of an autopod, and the reduction of anterior skeletal elements into a single bone (humerus/femur). Understanding the evolutionary history of the genetic developmental systems involved in patterning fish fins and tetrapod limbs, is based upon comparative gene expression analyses and transgenic studies of regulatory elements. In this presentation, I review recent data from genetic and developmental studies in agnathans and gnathostomes. The developing limbs of tetrapods are subdivided into PD and antero-posterior (AP) domains by the activity of genetic modules. Changes in the regulatory networks that integrate fin bud outgrowth and patterning acted as main drivers for the transformation of fins into limbs. A deep ancestry for the AP and PD patterning was posited. Efforts have been devoted to revealing the mechanisms by which the autopod emerged at the distal end of the paired appendages. A limb-specific late-phase pattern of 5'Hoxd expression was interpreted as being consistent with the emergence of the digital arch. Evidence of Meis-Hoxa11 mutual antagonism in Neoceratodus reinforces the view of PD patterning, with humerus and radius/ulna equivalents in sarcopterygian fishes. Similarly, elaboration of the mutual antagonism in Shh-Gli3 signaling, which is absent in multi-basal fins of cartilaginous fishes or in basal actinopterygians, may have shifted the balance between anterior and posterior limb progenitors during fin-to-limb evolution. Together, the emerging scenario is that limb-specific morphologies arose through evolutionary changes of regulatory elements in conserved and flexible patterning mechanisms.

Effects of phenotypic robustness on adaptive evolutionary dynamics

Emanuele Rigato, Giuseppe Fusco

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Though the ubiquity of phenotypic robustness underlying molecular, metabolic and developmental processes is not a topic of major debate, the mechanisms by which robustness might be established during evolution are far from clear and overall little explored. With the aim of contributing to the understanding of the origin and evolution of phenotypic robustness in living systems, we adopted a theoretical approach, elaborating on standard population genetic models of evolutionary dynamics, complemented by computer simulations. Preliminary results show that, under common selective regimes, a high level of phenotypic robustness is a necessary condition (although not sufficient) for adaptation to take place. This appears as a threshold effect, i.e. as a minimum level of phenotypic robustness under which evolution by natural selection cannot occur, even in the case of sizable positive selection coefficients and in absence of any drift effects. This ongoing work represents a first attempt to formally include phenotypic robustness in the more inclusive framework of a theory of adaptation, by providing an explanation for the evolution of this basic feature of living organisms and showing how a key feature of the GP map can directly affect the role of natural selection in evolutionary dynamics.

Do ontogenetic changes trigger the evolution of eco-morphological traits in demersal fishes?

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Shape differences among phylogenetic related species are usually the results of adaptive evolution that allows a better exploitation of ecological niche and reduces potential interspecific competition. While the evolution of new adaptive traits is often studied focusing only on the adult stage, it is well known that the processes of evolution and development are tightly linked: adult phenotypes arise through development and changes in developmental processes provide a source of variation for natural selection. We investigated the origin of morphological novelties in *Diplodus* species using geometric morphometrics. Both juveniles and adults of four species, *D. puntazzo, D. annularis, D. sargus* and *D. vulgaris,* were collected along Giglio Island' coast. Thirty-five landmarks were collected on fish body in lateral view. The aligned configurations were used to investigate shape differences, patterns of integration and modularity and to study the ontogenetic trajectories in the multivariate morphospace. Our results show that juveniles of the four species are already morphologically differentiated at settlement but these differences are not propagated to the adults through a general ontogenetic trajectory. The observed changes of the ontogenetic trajectories are associated to a shift of the integration and modularity of shapes during the transition from settlement to recruitment ontogenetic stages. These evidences suggest that the evolution of novel eco-morphological traits, allowing adult phenotypes to exploit different trophic niches, are triggered by a modification of the post-settlement ontogenesis. The observed changes in the ontogenetic trajectories may reflect a disruption of the general development program shared among closely related species, that in turn allowed species to evolve different adult phenotypes. These findings clearly show the necessity to take ontogeny in full consideration to better understand the origin of phenotypic novelties among closely related species.

Evolutionary compromises in ecological adaptation: urea and ammonia tolerance in *Drosophila suzukii* and *Drosophila melanogaster*

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Drosophila suzukii is an invasive species, and a serious agricultural threat. Unlike other Drosophila, females of *D. suzukii* lay eggs under the skin of fresh fruits, through morphological and behavioral adaptations. Therefore, larvae development and exposure to pathogens result in damage of a wide range of small fruits. The more innocuous *Drosophila melanogaster* lay eggs in fermented fruits and larvae develop in a crowded environment characterized by accumulation of nitrogenous waste such as ammonia and, at lower extent, urea. Behavioral avoidance cannot prevent larvae exposure to environmental toxins, so physiological mechanisms evolved to cope with these compounds. While it is known how *D. melanogaster* respond to high concentrations of urea and ammonia, little is known on the potential effects on *D. suzukii.* We investigated the impact of different concentrations of these compounds on fecundity and larval development in both species. Females and larvae of *D. suzukii* showed a greater sensitivity to high concentration of nitrogenous waste, with a drastic decrease in fecundity and egg viability. To better understand the pathways underlying these differences, we evaluated the effect on enzymes involved in nitrogen metabolism and stress response that are expressed during larval development. Under ammonia and urea exposure, the expression of these enzymes was significantly reduced in *D. suzukii*. Adaptation to a different ecological niche has allowed larvae to develop in a safer and healthier environment. However, metabolic adaptations to different food and environment have probably resulted in less efficient detoxifying and excretory mechanisms. To shed light on those mechanisms in *D. suzukii* is a necessary step to plan effective strategies of sustainable pest control.

SYMPOSIUM

Evolutionary and behavioral ecology

Chair: ANDREA PILASTRO

Co-chair: LISA LOCATELLO

The Polyandry revolution: from societies to genes

Tommaso Pizzari

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Sexual selection is one of the more powerful agents of evolutionary change. Darwin's view of sexual selection was largely restricted to episodes leading up to mating in monandrous mating systems. The realization that females are in fact polyandrous in many organisms has triggered a fundamental revolution of sexual selection theory with wide-ranging ramifications for eco-evolutionary processes at multiple levels. In this talk I will present recent developments from my research group, based on a combination of theoretical approaches and empirical work, which exemplify the ways in which polyandry impacts the operation of sexual selection at the level of (a) social groups, (b) individual strategies, and (c) specific genes.

Life-history strategies and pace-of-life syndrome hypothesis: unmatched predictions and varying correlation structures

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Life-history adaptations to ecological conditions have been widely observed in the animal kingdom, with fast and slow life histories that are respectively favored under unstable and stable environments. The Pace-of-Life Syndrome (POLS) hypothesis expands those predictions by including metabolic costs for body maintenance and behavior into this evolutionary framework. Yet, empirical support for POLS is scarce and the generality of its predictions controversial. In this study, we tested whether first-generation offspring from fish populations adapted to different environments differed on average in life histories, behaviors, and metabolic rates. Secondly, we tested whether correlations among those traits were maintained within each population. We observed that life histories diverged on average between fish populations, with individuals adapted to unstable and human-disturbed conditions that matured earlier, were more fecund, and less incline to develop large propulsion-devoted muscles (i.e., "fast" life history) than fish from stable contexts (i.e., "slow" life history). Yet, contrary to what expected, fish with slow life histories had, on average, higher risk-taking attitudes, activity rates, and mass-specific metabolic costs than fish with a faster life history. Traits correlations also differed between populations, with repeatable amongindividual differences in behavior, metabolic rate, and size correlated only in slow life history fish. Our results suggest predictions from the POLS might not be universal and that ecological adaptations can play a relevant role in driving resources allocation and correlated evolution of phenotypic traits.

Human cytochrome P450 2B6 genetic variability in Botswana: a case of haplotype diversity and convergent adaptation

Giacomo Maria Paganotti

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The present study evaluates the frequency distribution of CYP2B6 alleles, haplotypes and inferred metabolic phenotypes among subjects with different ethnic background in Botswana. A total of 570 individual DNA samples were analysed for *CYP2B6* polymorphisms at position 516G>T (rs3745274), 785A>G (rs2279343) and 983T>C (rs28399499), where most of the genetic ariability shaping the response to drugs and xenobiotics originates. CYP2B6 is involved in the metabolism of several molecules including artemether for antimalarial treatment and efavirenz and nevirapine for HIV treatment. The samples were collected in three districts of Botswana where the human population belongs to two ethnic groups, Bantu-related population in Serowe/Palapye and Chobe (Est and NorthEast) and KhoeSan-related population (also know as Bushmen) in Ghanzi (Central Kalahari). The study is part of a Malaria Indicator Survey aiming to describe parasitological indexes, susceptibility and pharmacogenetics of malaria in the population of Botswana. The results show that haplotype composition is different between the two groups, thus confirming their different evolutionary history and genetic makeup. We created a "metabolic score" taking into account the known metabolic phenotypes associated to the different genotype composition and then applying it on population scale. The three districts show similar metabolic scores distribution. It is important to note that 59.12% of the overall subjects have a extensive metabolic profile, while 36.67% carries a delayed and 4.21% a fast metabolism, respectively. The data presented hint at the possibility of a convergent adaptation of detoxifying metabolic phenotypes despite a different haplotype structure due to the different genetic background of the populations studied. The main implication is that, while there is substantial homogeneity among the country, the response to drugs metabolized via CYP2B6 could be associated to an increased risk of treatment failure and toxicity. These are important facts since Botswana is facing the elimination phase of malaria and a very high HIV prevalence.

Play behaviour in wild juvenile chimpanzee (pan troglodytes)

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Many primates live in social groups in which members coordinate their activities, communicate and interact in affiliative and agonistic ways. In order to integrate social life, play behaviours are considered as an effective and universal learning 'engine' of social rules and facilitate knowledge on other individuals. Play also contributes to social cohesion and development of cognitive skills for social life. The aim of this research project was to test a number of sociobiological assumptions about play behaviour in juvenile chimpanzees. Chimpanzees coordinate their activities by a variety of social interactions and communication, forming strong social bonds. These complex interactions are often expressed in adulthood as dominance, cooperation, coalition and grooming, and could be related to juvenile social play development in term of evolutionary adaptations. In this study, we focused on a community of 50 wild chimpanzees in the Kibale Forest National Park, Uganda. Observations were video recorded using "behavioral sampling" with a focus on play behaviour. Only juveniles (<15 years old) were included as initiators of play behaviour. Time budget of social play was calculated as the proportion of social play duration relative to the total play time. Moreover, the intensity of play was estimated by assigning an intensity value relative to the type of play. Play session intensity was scored by combining intensity values of observed behaviours. We observed that the intensity of play sessions differed relative to gender and age of the player and of the playing partner. Male games were on average more intense. Play also increased in intensity with age, especially when directed to females. Moreover, time allowed to social play activities was found to be longer in males than in females, who spent more time in solitary and mothering games. Finally, we observed the emergence of preferences in the choice of specific playing partners.

The atypical circadian clock of cavefish

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The environment of our planet is dominated by cyclic changes driven by rotation and translation of the Earth. During evolution animals and plants have adopted endogenous timing mechanisms that permit to coordinate physiological and behavioural processes and anticipate cyclic environmental changes. The most common and investigated biological timing system is the circadian clock which allows the anticipation of the day-night cycle and the daily food availability. To shed light on the evolution of the circadian clock in vertebrates we investigate the circadian timekeeping mechanism of cavefish. Cave animals evolved in extreme environments characterized by constant conditions of darkness and temperature, and low nutrient levels. These species share many troglomorphic phenotypes such as anophthalmia, depigmentation, and longevity. Subterranean waters are the home to around 200 species of hypogean fish, more commonly known as cavefish. Among them the cyprinid *Phreatichthys andruzzii* has evolved in the phreatic layer beneath the desert of the central Somalia during the last 5 million years. Previous investigation showed that this species, in addition to the typical extreme troglomorphic phenotype, has a "blind" circadian clock, due to mutations to two nonvisual opsins. Interestingly, the absence of a light-entrained circadian oscillator is compensated by the presence of a food-entrainable circadian oscillator. To better understand the peculiarity of the circadian timekeeping mechanism in *P. andruzzii*, we performed a deep analysis at molecular, cellular and behavioural levels investigating the effect of different feeding and lighting conditions. Our results showed: i) a strong entrainment of locomotor activity to feeding administration provided with different periods (from 24 to 96 hours); ii) characteristic behavioural responses to monochromatic (from red to UV light) light stimuli; and iii) the presence of mutations in clock and light-inducible genes. Our study would reveal the consequences for the circadian clock during evolution in an extreme hypogean environment.

Reproductive strategies and breeding systems in *Reticulitermes* subterranean termites (Isoptera, Rhinotermitidae)

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Termites are eusocial insects that live in colonies characterized by cooperative behavior, where most individuals forego their own reproduction and help to raise the offspring of a few nest members. This may impact on the colony abilities to adapt and exploit the environment. In subterranean termites of the genus *Reticulitermes*, a new colony is settled by a single, heterosexual pair of winged individuals (primary reproductives; kings and queens); as colony maturate and/or upon founders death, neotenics (secondary reproductive; SRs) develop and contribute to offspring production. This leads to inbreeding as SRs are genetically related. In some species, including the Italian *R. lucifugus*, primary queens produce secondary queens by parthenogenesis (P); then, secondary queens will mate with the primary king extending the genetic contribution of the primary queen through time (Asexual Queen Succession, AQS), eventually helping to avoid inbreeding. As a consequence of the increasing genetic contribution of the primary queen, AQS colonies show a female-biased sex ratio of winged reproducers. Here we present population genetics and sex ratio analyses of *R. lucifugus*, and compare results with data on two other species: *R. flavipes* and *R. grassei*. Genetic data indicated the presence of AQS in *R. lucifugus* and its absence in the other two species, accordingly to winged sex ratio observations. Data confirmed that P occurs through a mechanism of terminal fusion. Moreover, winged reproductives resulted all produced by amphigony, confirming that P is only used for secondary queen production. Finally, morphometric and biomass analyses performed on *R. lucifugus* winged reproductives suggested a larger investment in female sex in AQS colonies. The AQS system has been observed to be scattered among termites, occurring through different mechanisms and leading to different outcomes. This variability still need to be explained in the light of social behavior evolution.

How light and genes affect social behaviour in Drosophila

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To which extent is social behaviour affected by other individuals, genetic and environmental variables? These questions can be answered at fine tune by investigating different isogenic lines of fruit flies with automated tracking systems. Studying thousands of dyads of fruit flies, we showed that standing genetic variation is present in Drosophila melanogaster for social interactions, since different strains of the DGRP population significantly differed in distance, orientation and type of interactions. While some variables differed in mean and variability, other differed only in variability, suggesting a dissociation between these components. To test whether social interactions are affected also by environmental factors, such as early exposure to light, we compared individual and social behaviour of flies that had been exposed to complete darkness vs. complete light before eclosion. We found that individual variables such as speed and angular velocity were not affected by light exposure, while social variables, such as inter-fly distance and facing angle between flies, differed between groups. We present here the first evidence that light-exposed flies show stronger social attitudes than flies kept in darkness and a fine analysis of genetically determined individual and social behaviours.

Behavioural plasticity and extreme thermal variation: a story from the world's warmest sea

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Human-induced climate change is one of the most profound threats to global biodiversity. Biodiversity loss associated with increasing variability in temperature is predicted to be particularly extreme within tropical ecosystems, especially coral reefs. Despite this, little is known about how coral reef species will cope with future scenarios of climate change. Plasticity in behaviour will clearly be important, but there is little work focusing on the role of behaviour in structuring tropical species within thermally extreme environments. We focused on examining the foraging activity, vertical movement and sheltering of populations of the Paletail damselfish (*Pomacentrus trichrourus*) within the Persian Gulf – this region has seasonal temperature extremes higher than those by which Indo-Pacific reef fish can tolerate, with summer temperatures comparable to those predicted for the tropical ocean by 2090-2099. We found substantial differences in all three behaviours between winter, spring and summer (21, 27 and 34 °C water, respectively) – particularly at low and high water temperatures fish were significantly less active and bold compared to more benign intermediate temperatures. Phenotypic plasticity is one of the key to understand how Persian Gulf fish populations cope with living in extreme conditions. This may then provide an insight into how marine communities may be impacted, but also cope with increasing changes in global climate.

"Pay to play": costs of phenotypic plasticity of sexual traits in guppies

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Adaptive plasticity in male reproductive strategies (i.e. the ability of males to produce an anticipatory response by adjusting their reproductive effort to perceived mating conditions) is nearly universal, yet its costs have rarely been explored. We investigated long-term costs of plastic responses to varying mating opportunities in the guppy (*Poecilia reticulata*), a polyandrous fish with internal fertilization. Male guppies quickly adjust their sperm investment as well as their sexual behaviour in response to the sex ratio in the population. We examined the effect of expressing iterated plastic responses on male condition. Males were randomly assigned to plastic (fluctuating number of females) or control (constant number of females) treatments. Traits associated with condition, reproductive success (courtship, colour pattern and sperm traits) and lifehistory (life-span and mortality) were compared between groups. We did not find any significant difference between the two groups, suggesting that costs of adjusting the sexual investment are negligible in this species. We can conclude that the selective pressure on the ability to respond plastically to sex ratio variations may have minimized the costs of plastic adjustments.

A morphological approach to study the evolution of explosive defensive systems in Coleoptera

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The ground beetle subfamilies Paussinae and Brachininae have always been of interest to scientists because of their ability to explosively eject a hot (up to 100°C) and irritating quinonoid secretion from pygidial glands. The exceptional nature of this defensive system led many scientists to consider "bombardier beetle" as a monophyletic lineage, emphasizing the general similarities in morphology, function and chemical reactions, despite the different ways of ejecting the spray (directing the abdomen in brachinines; using the elytral flange of Coanda in paussines). However, studies concerning fine morphology and ultrastructure of the components of the pygidial defensive systems (reaction chambers, valves, ducts, reservoirs, and glands that secrete H_2O_2, hydroquinones, catalases and peroxidases) are scarce (brachinines) or still lacking (paussines). In order to render these issues available to a comparative advanced discussion, we performed a morphological and ultrastructural study of representative species belonging to the genera: *Metrius* (Metriini); *Mystropomus* (Mystropomini); *Eustra*, *Goniotropis*, *Pachyteles* (Ozaenini); *Cerapterus*, *Hylopaussus*, *Paussus* (Paussini) for Paussinae; and *Aptinus*, *Brachinus*, *Mastax*, *Pheropsophus* (Brachinini) for Brachininae. Samples were examined through optical microscopy (dissections, histology and fluorescence microscopy), scanning (SEM) and transmission (TEM) electron microscopy, focused ion beam (FIB/SEM) microscopy and synchrotron radiation micro-CT. The relative parts of the defensive systems for each paussine and brachinine species were described, illustrated and comparatively analyzed. We found significant differences between the two systems at both cellular and microstructural levels. Our preliminary results raise some challenges to the acknowledged hypothesis of evolution of this peculiar defensive system that parallel other evidences on the relationship between paussines and brachinines.

The contribution of size and ecotype variation to sexual isolation in *Littorina saxatilis*

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The process of species divergence often involves the evolution of different adaptive changes between populations living in distinct environments. These traits can be directly or indirectly under divergent natural selection and they can promote a genetic and ecological differentiation between populations. Individuals adapted to different habitats will reduce gene exchange and increase reproductive isolation by mating assortatively. The rough periwinkle, *Littorina saxatilis*, occupies distinct microhabitats on the rocky shore habitat where wave action and crab predation constitute influential factors for its fitness. The species forms two ecotypes that can be distinguished by size among other adaptive characteristics and this variation of size follows a clinal pattern along an environmental transect. Besides being subject to divergent natural selection, shell size and shape have been demonstrated to explain a great proportion of the individual sexual isolation and variation in copulation time between the morphs. However, the specific contributions to mate choice between the species ecotypes remain elusive. A preliminary study has been conducted on ecotype populations sampled in four different localities in the west coast of Sweden where 400 reference snails and 600 test snails have been collected at each site, recorded their position in three dimensions and randomly mated under standardised conditions. The initial results have indicated a compelling relationship between size and mating success. Particularly, males that are relatively smaller than females show increased mounting probability and longer copulation time. Assortative mating may result from a joint effect of relative size and other ecotype traits whose intensity may vary along the rocky shore habitat. If these traits are also influencing the mating behaviour of the snail, the information acquired from mating frequency may reflect the spatial variation of the extent of assortative mating between ecotypes of the intertidal snail.

Evolution of DNA UV-damage repair mechanism and photoreception in fish

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Light has dominated animal biology since the origin of life. It serves as the primary source of energy, has an impact on metabolism and coordinates the behaviour of animals. Excess exposure to sunlight also represents a major source of damage for complex biomolecules and thereby underlies pathology. Light is known to have a major effect on many aspects of fish physiology ranging from development and growth to sex determination, behaviour and reproduction. The extreme phenotypes of cavefish which have evolved in the complete absence of light are a testimony to how much light shapes fish evolution. Recent discoveries have revealed the presence of different types of photoreceptors in fish. Using two fish species, the zebrafish and the Somalian cavefish *Phreatichthys andruzzii*, evolved in different photic niches, we investigated the evolution of UV perception and DNA UV-damage repair mechanism. The main elements of these processes are highly conserved: UV opsins are expressed in eye, brain and peripheral tissues of almost all fish and the photolyases, blue-light activated DNA repair enzymes, are essentially preserved throughout the animal kingdom. Analysis of UVc tolerance in zebrafish embryos showed both a significant survivor and a positive effect of blue light treatments with respect to cavefish. Furthermore, 4 hours of blue light exposure are sufficient to increase mRNA levels of the photolyases Cry5 and DASH, but not of XPC and CPD, factors involved in repair of UV-photoproducts. These increases in gene expression are not showed in cavefish embryos exposed to the same lighting condition. To test the effect of UV on adult zebrafish and cavefish behaviour we recorded daily locomotor activity rhythms under a light-dark cycle and phototactic responses using UVa light sources. Taken together our results show that *P. andruzzii* seems to have lost the ability to activate DNA repair events, but it is able to respond to UV light pulse.

Costs and benefits of phenotypic plasticity in sexually selected traits in *Poecilia reticulata*

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In polyandrous species males invest significant resources in producing large and high-quality ejaculates. As sperm are costly, males are expected to modulate their ejaculate investment in order to anticipate future mating conditions (e.g. level of sperm competition or mating opportunities), a capability that have been demonstrated in several species. While this plasticity in male reproductive strategy is likely adaptive, its fitness consequences have rarely been investigated. Male guppies adjust their ejaculate production and sexual behaviour on expected mating opportunities: males maintained in visual contact with females (stimulated) produce more numerous and faster sperm but reduce their courtship rate (and hence their attractiveness) in comparison with males deprived of females. As reproductive success results from the combination of mating and fertilisation success, cost and benefits of male plasticity must be explicitly quantified. Using a repeated-measure design, males, previously stimulated or deprived, were subsequently allowed to mate in sequence with 6 sexually receptive females. Deprived males invested more on first matings, both as number of sperm ejaculated and intensity of sexual behaviour, but depleted more rapidly their sperm reserves and progressively reduced their mating effort as compared to previously stimulated males, which obtained a constant insemination success across the 6 females. Our results suggest that stimulated males are favoured when mating opportunities are high, but pay costs when encountering few females. Although limited by the trade-off between ejaculate and mating effort, ejaculate investment modulation is favoured in the condition to which males had adjusted, highlighting a true case of adaptive phenotypic plasticity.

Feeding entrainment of the circadian clock in zebrafish and cavefish

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Circadian clocks are normally synchronised (entrained) by periodic environmental cues (zeitgeber). Food availability for only a limited time each day is one of the stronger zeitgeber signal for circadian rhythms. This entrainment can be detected by the occurrence of the food-anticipatory activity (FAA), which consists in an increment on the locomotor activity few hours before mealtime. Recent investigations showed that food-entrainable oscillators are present in the circadian system of almost all vertebrates, including fish. We studied the effect of periodic food administration on the locomotor activity rhythms of 3 species of teleosts evolved in different environments: the zebrafish *Danio rerio* and the blind cavefish *Astyanax mexicanus* and *Phreatichthys andruzzii*. Adult fish were fed with different protocols (every 24, 36, 44, 48, 72 and 96 hours) for more than one month under constant darkness, and during this period, locomotor activity was measured. For all species, we observed 1) the FAA, indicative of regulation by a food entrainable oscillator (FEO), 2) a strong entrainment of rhythmic locomotor activity, and 3) a free running activity under fasting. Taking together, our results point to that food is a stronger zeitgeber in cavefish than in zebrafish. To better understand the developed of feeding rhythms we established a protocol to study feeding rhythms in fish larvae using videotracking setup (Daniovision, Noldus). Preliminary results seem to indicate the onset of the feeding rhythms in young larvae. Several lines of evidence point to the existence of a FEO in vertebrates distinct from the light-entrainable oscillator (LEO). Fish could emerge as powerful models for the investigation of food entrainment. A comparative study involving zebrafish that possess both LEO and FEO and cavefish that has only the FEO could provide important insight into the basis of the evolution of the feeding entrainment mechanism in vertebrates.

Male and female sexual fluids and their interactions influence the outcome of sperm competition in the grass goby *Zosterisessor ophiocephalus*.

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Two mechanisms of postcopulatory sexual selection, sperm competition and cryptic female choice, are major determinants of fertilization success in many taxa. Indeed, the gametic interactions between two (or more) males and a given female may lead to adaptations that enhance both a male's ability to compete with a rival and a female's ability to bias the outcome of sperm competition. However, not only the quality of the gametes but also the properties of the ovarian and seminal fluids that are released with the gametes are expected to play a key role in the fertilization process, notably in external fertilizing species in which ejaculates and eggs are simultaneously released in the fertilization environment. We investigated on the influence of male seminal fluid and female ovarian fluid on sperm performance in a species showing guard-sneaker mating tactics, the grass goby *Zosterisessor ophiocephalus*, with the aim of understanding the comprehensive influence of all fertilizing components on the outcome of competition. We experimentally manipulated grass goby ejaculates by separately combining sperm and seminal fluids from territorial and sneaker males and females. Our results evidenced that cross interactions of sperm and male seminal fluids influence the fertilization success of competing ejaculates, with seminal fluid affecting the performances of rivaltactic sperm. A relation between the specific composition of each female's ovarian fluid and its effect on sperm performance also emerged. These findings shed light on the role of different male and female components on the fertilization outcome in the context of sperm competition and cryptic female choice.

Individual recognition in newborn unexperienced tortoises

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Conspecifics' individual recognition is an ability commonly found among social animals: it has been postulate being essential for successful social interactions; in fact, it is supposed to be at the basis of the evolution of cooperation itself. Being able to discriminate individuals may bring several benefits, allowing to remember and use information from previous encounters to moderate future responses towards the same individual. To investigate the relationship between individual recognition and the evolution of the sociality in vertebrates, we investigated how young non-social reptiles interact with familiar and non-familiar conspecifics. Newborn subjects of two species (*Testudo hermanni* and *Testudo graeca*) were reared in pairs with no experience with any other conspecific until the moment of test. After two weeks, subjects were located in a new arena in pairs with either a familiar or and an unfamiliar conspecific. Overall results show that neonates of both species stay significantly closer to unfamiliar conspecifics than to familiar conspecifics. Interestingly, young tortoises have different social reactions compared to chicks, as in this social species familiar pairs tend stay closer to familiar than to unfamiliar individuals. Nevertheless, the differences we observed between the two experimental groups allow us to infer that tortoises are probably able to recognize conspecifics, despite being Chelons non-social vertebrates.

Early social predispositions in chicks: inherited variability for animacy preferences

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Identifying animate objects is crucial for the survival from the early stages of life. This is particularly true for precocial species, such as chicks of the domestic fowl (*Gallus gallus*). In fact, chicks preferentially approach objects that show cues of animacy, the property of being alive. We investigated whether genetic variability is present for this predisposition by testing naïve preferences for animacy in three breeds: Polverara, Robusta and Padovana. These breeds remained genetically isolated for twenty years in the same farm and were raised identically until the moment of test. In Experiment 1, we tested the first approach response to an animate (accelerating/decelerating) vs. an inanimate (uniform motion) object. In Experiment 2, we tested the approach responses to the same stimuli in a running wheel for 30 minutes. Preliminary results suggest that a difference in predispositions for approaching animate stimuli between breeds exists. Interestingly though, between breed predispositions for hen-like stimuli (stuffed hen vs. scrambled hen) dissociate from predispositions for dynamic cues, suggesting that animacy cues activate independent areas of the chick's brain. We continued the study of social behaviour in three breeds in the subsequent phases of life: in the wild, chicks grow up in groups of 6-10 individuals, and it is essential to recognize social companions for many reasons such as thermoregulation and antipredatory responses. For this reason, in Experiment 3, we investigated the presence of differences in early affiliative responses, studying aggregation to familiar and unfamiliar companions in the three breeds. Our data suggest that breeds differ also in this respect, showing the presence of genetic segregation for several social behaviours is not limited to the first hours after birth.

A sexual selection framework in Primates: raising *ossa genitalia* again

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Evolutionary forces drive rapid divergence of morphological structures directly involved in reproduction, such as primate external genitals, affecting both *baculum* (os penis) and *baubellum* (os clitoridis). As mainly embedded within the distal portion of penis and clitoris, they are heterotopic bones (do not belong to skeleton itself). Among many roles of prenatal androgens, they promote ontogeny of both *baculum* and aggressive/competitive behaviours, the latter characterizing aggressive competition regimes in different mating systems. Several functional hypotheses were proposed for *baculum*, however no theoretical framework for *baubellum* evolution exists yet, except as a consequence of a masculine trait selection in females. To study genital bone evolution in a sexual selection framework first mandatory step would be acquiring a conclusive occurrence database to analyse it with proxies of sexual selection pressures in light of primate phylogeny. Recently, a few works aimed at reconstructing the *baculum* (only) evolution across mammals using occurrence data, based on reviews published since the 70's. Exclusively based on primary literature (1909-2000), we show the first state of the art about occurrence of *ossa genitalia*, covering all primate species now resulting after recent substantial primate taxonomy revisions (N=436, IUCN 2016 database). The usually old primary literature on anatomy must be examined directly to prevent absence of data (i.e., omissions) being possibly interpreted as questionable "absences" of bone. *Baculum* scores 55.7% of the species as data deficient while *baubellum* 87.4%. If considering *baubellum* data certa (sum of reported presence and absence) Strepsirrhini scores 22.4% while Haplorrhini 5.5%, as opposed to *baculum* (37.6% vs. 43.7%). On this basis, we secondly test genital bone phylogenetic distribution and species' mating system to explore to which degree sexual selection pressures may explain evolution of genital bones in primates.

SYMPOSIUM

Coevolution and symbiosis

Chair: MAURIZIO CASIRAGHI

Symbionts and genes: the coevolution of Drosophila and it's viruses

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Within populations it is normal to find considerable variation in susceptibility to infection. In *Drosophila* populations one of the major causes of variation is the bacterial symbiont *Wolbachia*, which can make flies resistant to a broad range of viruses. This is a costly way for flies to protect themselves from infection, as strong antiviral protection is associated with very high symbiont densities, which reduces the survival and fecundity of the flies. A second cause of variation in susceptibility is variation in the *Drosophila* genome. Much of this variation is caused by major-effect genes that confer near total resistance to different viruses. These have recently arisen by mutation and are spreading rapidly through populations. These two routes to becoming resistant are not independent. Populations infected by *Wolbachia* suffer less from viral infection, so there is less selection on insect genes that increase resistance. This may lead to an evolutionary 'addiction', as losing the symbiont would leave the insect vulnerable to infection.

Evolutionary investigations on "*Candidatus* Midichloriaceae" endosymbionts of the ciliate *Paramecium*

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Rickettsiales are a diverse group of intracellular bacteria involved in a broad spectrum of relationships with many different hosts. In order to get insights in the role of such bacteria for their hosts, we characterized the first representative of a novel genus within the order, the endosymbiont of a *Paramecium* sp. named "*Candidatus* Fokinia solitaria", by performing ultrastructural and whole genome analyses. "*Candidatus* Fokinia solitaria" cells were observed in the host cortical cytoplasm as tiny rods and no flagella were detected. The whole genome was obtained through a shotgun sequencing approach coupled to a bioinformatic pipeline that allowed selective assembly of the symbiont genome. Subsequent finishing was performed applying bioinformatic and molecular techniques, resulting in a genome assembly composed of three scaffolds, with a genome size of 833,994 bp and a GC content of 35.8%. Separate phylogenies inferred on the 16S rRNA gene and 74 single copy orthologous genes (phylogenomic approach) are consistent and support "*Candidatus* Fokinia" as a novel genus within the "*Candidatus* Midichloriaceae", one of the three families within the order *Rickettsiales*. *"Ca.* Fokinia solitaria" possesses by far the smallest known genome within the family, and one of the smallest among the *Rickettsiales*, suggesting a strong genome reduction. Detection of genome reduction features and analyses on metabolic pathways are ongoing. In-depth genome analysis of "*Ca.* Fokinia solitaria" will be of use in unraveling the knot of relationships between "*Ca.* Midichloriaceae" symbionts and their hosts, thus potentially disclosing the secret of their intracellular evolution and widespread ecological distribution. Moreover, the comparative genome analyses based on the genome of the endosymbiont of *Paramecium biaurelia* "*Candidatus* Fokinia cryptica" (which is currently being assembled) might highlight distinctive patterns of the "*Candidatus* Fokinia" genus.

Patterns of evolution and functional differentiation in the multipartite genome of a bacterial facultative symbiont

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Multipartite genome consisting of two or more large DNA fragments is particularly interesting and is common among relevant bacterial species, including rhizobia. Several aspects of the evolution and biological role of this genome organization remain unclear, such as the possible selective advantage conferred. The N_2-fixing endosymbiont of legumes *Sinorhizobium meliloti*, known for a remarkable genomic polymorphism, contains a tripartite genome consisting of one chromosome, one chromid and one megaplasmid. Previous studies showed the chromid and the megaplasmid to be ancient alien elements introgressed in different times within the paleo *S. meliloti* cells. To investigate the evolutionary forces behind tripartite genome, we performed comparative genomic analyses and reconstruction of the regulatory and metabolic network. Results presented strong evidences that replicons underwent nearly independent evolutionary routes and that secondary replicons evolved to fulfill specialized functions. In particularly, we found that the chromid contributed the ability to colonize the rhizosphere, while the megaplasmid to perform nitrogen-fixing symbiosis. Interestingly, while the megaplasmid was a hot spot for mobile genetic elements and strong genome rearrangements, the chromid showed structural stability but important patterns of positive selection. Finally, to test the functional and regulatory independence of the replicons, we performed experiments of replicon transplantation inserting secondary replicons from wild strains to a recipient laboratory strain. Results are in favour of the independence of secondary replicons in contributing host cell's phenotypes, however, few unexpected phenotypes, possibly due to genetic/metabolic interactions among functions carried on different replicons were detected, suggesting the presence of some level of genomic coadaptation.

Nectar chemistry is not only a plant's affair

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Plant-pollinator interactions are considered essential in the evolutionary process of Angiosperms and several mutual relations developed due to this connection. Entomophilous plant species evolved lure and reward mechanisms that guarantee visits and fidelity of pollinator insects. Pollinators, in their turn, developed specific adaptations to interact with different types of flowers, ensuring plant pollen transfer from one individual to another, favouring allogamy and the fitness of the plant population. Floral nectar is the primary reward directly consumed by floral visitors. It is an aqueous solution dominated by three main simple sugars (sucrose, glucose and fructose); in addition, secondary components as amino acids, organic acids, proteins, lipids, vitamins, minerals and other compounds are presents in minor concentrations. It has been demonstrated that nectar chemical composition affects the attractiveness to pollinators, and it can also modulate their behaviour and fidelity. We investigated floral nectar composition, specifically sugars and amino acids, in a wild population of *Gentiana lutea* L., throughout the entire flower lifespan. Our results indicate that, soon after flower anthesis, nectar becomes contaminated by pollen and yeasts. Yeasts, as well as pollen grains from different plant species, were mainly found in nectar of mature open flowers, that were accessible to insect visits. We can thus hypothesize that yeasts are transferred to floral nectar by floral visitors, together with pollen. Our results demonstrate that pollen contamination enriched the amino acid profile of gentian nectar, both quantitatively as well as qualitatively, while yeasts probably affected nectar sugar ratio. These findings indicate that nectar modulating the pollination process is not the pure secreted solution. By contrast, floral nectar gets altered by numerous factors, in a complex process of ecological relationships.

Follow the flow: hypothesis of symbiotic interactions between ultra-small bacteria and stable community in drinking water treatment plant

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Drinking water shows a wide diversity of microorganisms interacting in a dynamic network. Recent studies revealed that drinking water treatment process can affect the microbiome community structure. Moreover, the presence of unculturable and ultra-small bacteria in groundwater has been demonstrated. We hypothesized that Drinking Water Treatment Plants (DWTPs) can be a source of unexpected biodiversity in terms of environmental microorganisms, still poorly known. Molecular techniques can give a deeper knowledge, going beyond the limit of culture-dependent methods. The term "microbial dark matter" was used to group taxa poorly investigated and not necessarily monophyletic. We focused on such under-investigated microbial dark matter of drinking water treatment plant from groundwater, across carbon filters, to post-chlorination. Our results highlight the presence of under-investigated microorganisms, and among these ultra-small bacteria, across the entire DWTP, even after the potabilization process. Carbon filters probably act as substrate for microorganism growth and contribute to seeding water downstream, since chlorination does not modify the incoming bacterial community. The so called Microbial dark matter is not routinely screened. Nevertheless, the detection of this group of uncultivable bacteria in drinking water and their incredible persistence in DWTP open new scenarios. For instance, according to their extremely small genomes, it is likely that ultra-small bacteria depend on other bacteria to survive. The common theory is that they are extracellular obligate symbionts. We explored what are the real interactions in the microbial network characterising drinking water and if it is possible that ultra-small bacteria occurrences are indirectly linked to the peculiarities of drinking water through the bacteria symbioses.

Allergic and autoimmune diseases in the interpretation of Evolutionary Medicine

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Allergic and autoimmune diseases are widespread and involve significant costs and suffering. Worldwide, respiratory allergic diseases alone affect nearly 700 million subjects. In the USA, the NIH estimates annual direct health care costs for autoimmune diseases to be in the range of $100 billion (in comparison, cancers costs are $57 billion). Moreover, the frequency of these diseases has strongly increased in the last 50 years. According to a fundamental principle of Evolutionary Medicine (EM), the considerable frequency increase of a disease in a limited time is incompatible with a primary etiology by genetic alterations. Conversely, this indicates that the primary causes must be investigated within possible changes in the ecological niche to which the species is adapted. In medicine, a common error is to consider our species such as something that evolved on its own without the coevolution with many other species that which, under natural conditions, are in continuous relationship with it. Defining as "holobiont" our species along with all the species that in the wild have close relationship with it, in the traditional view the holobiont is divided between the single host organism and all the other species that are more or less harmful parasites of it: so, the ideal would be to eliminate all the parasites to solve entirely the possible diseases and problems caused by them. Conversely, in terms of EM, the total or partial elimination of such "parasites" is a great change of our ecological niche, and any change in it must be considered as a possible cause of disease until proven otherwise. Depending on this concept, the serious alterations of the holobiont caused by the massive use of antibiotics, helminthicides, disinfectants, etc., plus the contacts with countless artificially synthesized new substances, are the likely primary causes of allergies and autoimmune diseases that are consequent to alterations in the development and modulation of the immune system.

Water-bears as holobionts: tardigrades select their microbiota

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Symbiotic associations of metazoans with bacteria are ubiquitous and virtually no animal is axenic. The microbiome associated with animals has been characterized for many phyla and has a strong influence on their evolution and fitness (*e.g.* Porifera, Cnidaria, Nematoda, Arthropoda, Chordata, Annelida, Mollusca), but we are far from a complete understanding of the currently recognized diversity of animal hosts. Tardigrada (also known as "water bears") is a phylum of micrometazoans famous for their ability to undergo cryptobiosis (*i.e.,* ametabolic state of life in response to adverse environmental conditions), but very little is known about their associated bacteria. We designed an experiment to determine if tardigrades are colonized by a specific microbial community and to identify their potential symbionts. Using 16S rRNA gene amplicons, the microbiome of six tardigrades species from Europe (Italy, Sweden) and Antarctica, spanning the two classes Heterotardigrada and Eutardigrada was characterized. In tandem, we also characterized the bacteria associated with the substrates (mosses, lichens, freshwater sediments) on which these tardigrades were found, allowing us to rule out environmental contamination as a contributor to the tardigrade microbiome. The tardigrade microbiotas consistently differ from that of their substrates and are species-specific. Putative symbionts from Rickettsiales and Holosporales in different tardigrade species were also identified. Our results confirm that like many other animals, tardigrades have their own microbiota that differs among species and harbor potential endosymbionts, and the potential effects on the evolution of the biology (eg. cryptobiosis and parthenogenesis) need to be further investigated.

Functional morphology and chemical strategy of *Microdon mutabilis* (Diptera, Syrphidae), a nest parasite of *Formica cunicularia*

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Despite ants are very aggressive against the intruders, numerous ant parasites live upon the colony, exploiting the resources of the nest. One of the most striking examples of these parasites are the larvae of *Microdon* hover flies (Diptera; Syrphidae; Microdontinae) which are able to feed undisturbed upon the ant brood. We studied the sophisticated structural and chemical parasitic strategies evolved by *Microdon mutabilis*, an obligate symbiont of *Formica cunicularia* ants. To improve the knowledge about its scarcely known immature stages, we analyzed their functional morphology, integrating scanning electron microscopy (SEM), histology and fluorescence microscopy. We studied also the strategy used by *M. mutabilis* to break the chemical communication and identification system of its host. During its development, *M. mutabilis* faces huge structural changes showing a deep larva polymorphism linked mostly to three main characters: the body shape, that changes from flat to strongly convex; the posterior spiracle, narrow and elongated in the first instar and short and dome-shaped in the third instar; the cephaloskeleton, where a progressive separation of the mandibles is evident. Furthermore for the first time we show the presence of resilin on the external layer of the posterior spiracle. The studies of chemical ecology allowed to define, with gas chromatography- mass spectrometry, the average cuticular profile of the larvae, pupae and workers of *F. cunicularia* and of immature stages of *M. mutabilis*. From their comparative analysis, we can confirm that the majority of cuticular hydrocarbons are in common to both ants and fly larvae, suggesting the presence of a chemical mimicry. The active biosynthesis seems to be the strategy adopted rather than chemical camouflage acquired with the diet or the contact with the nest and/or the ants.

Characterisation of a novel epibiotic bacterium of *Paramecium* showing distinctive features

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Ciliate protists are frequently hosts of a great variety of bacterial symbionts, which may inhabit several intracellular subcompartments or sometimes even be epibiotic, namely attached to the host extracellular surface. A new bacterial epibiont was retrieved in association to the ciliate *Paramecium primaurelia.* The bacteria were found covering at a high density variable portions of the host surface, even enveloping cells entirely. The areas of the host surface bearing the epibionts were deprived of cilia, and some further morphological alterations were found in the underlying alveoli. The infected paramecia were smaller than controls and eventually died, possibly due to impairment in movements and in feeding behaviour. The epibiotic bacteria were infectious towards other *Paramecium* strains, causing the same symptoms observed in natural hosts. The complete genome of this bacterial epibiont was sequenced and annotated (1,205,153 bp; 32.9 GC%; 1,129 CDS). The phylogenies inferred on the 16S rRNA gene and on a set of coding sequences were consistent and indicated a relationship with members of *Rickettsiales* (*Alphaproteobacteria*). All members of this order are obligate intracellular bacteria, living in association with diverse eukaryotic hosts. Some *Rickettsiales* can be human and vertebrate pathogens, but in most other cases host-symbiont interactions are not determined precisely. Comparative genomic analyses with other intracellular and free-living *Alphaproteobacteria* provided evidence for distinctive metabolic capabilities respect to closest relatives. Preliminary results on the involved genes provided no evidence of horizontal gene transfer, suggesting a peculiar genomic evolutionary pathway for the epibiotic bacterium. Genetic evidence for proteins involved in extracellular host adhesion was also found, with a possible crucial role in the epibiotic lifestyle. Further functional and evolutionary genomic analyses are ongoing.

Level of mitochondrial heteroplasmy in a natural and stable heteroplasmic system

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Mitochondrial heteroplasmy is the presence of more than one type of mitochondrial genome (mtDNA) within an individual, and in most of the reported cases it seems to be an unfavourable condition. For example, heteroplasmy in humans increases the risk of common age-related disorders as neurodegenerative diseases. The only evolutionarily stable and natural heteroplasmic system in Metazoa is the Doubly Uniparental inheritance (DUI), typical of some bivalves, in which two mitochondrial lineages are present: one transmitted through eggs (F-type) and the other through sperm (M-type). While females are homoplasmic for the F-type, males have M-type-homoplasmic gametes, but heteroplasmic soma. So far, no study has investigated mitochondrial heteroplasmy at the protein level, and no analysis has been performed to clarify if it is present at tissue, cell, or organelle level. We characterized the expression of three mitochondrially-encoded proteins (ND5, CYTB, COX3) in the DUI species *Ruditapes philippinarum*. Specific antibodies were produced to discriminate, with immunolocalization, between the F and M form of the same protein (variants highly divergent in DUI species) in germ line and somatic tissues of females and males in different developmental stages. Unexpectedly, M-type antibodies labelled mitochondria in female primordial stem cells (PriSCs), undifferentiated germ cells, and early oocytes, while mature eggs and female somatic cells expressed only the F-type. M- and F-type expression in male somatic tissues showed mitochondrial heteroplasmy at the cell level. Interestingly, F-labelled mitochondria were detected in male PriSCs and early germ cells. We hypothesize that PriSCs and undifferentiated germ cells carry both types of mtDNA, but during gamete maturation one of the two genomes disappears, and only the sexspecific mtDNA remains, maintaining the homoplasmy of the germ line. This would imply a selective degradation of mitochondria in adulthood.

Dating the impossible: the origin and divergence of the largest infection on Earth

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Wolbachia are common intracellular bacteria of many arthropod and nematode species; they are responsible for likely the most widespread and evolutionary significant infection on Earth. *Wolbachia* are generally acquired by maternal transmission, but they often move across species boundaries, impairing the use of host divergences as independent calibration to infer their evolutionary history. Here we present a first systematic effort to estimate the origin and the divergence of the *Wolbachia* infection by coupling the molecular clock analysis of a genomic and an MLST data sets with three types of disputed calibration priors: a root prior for splits within Proteobacteria, the recently proposed *Nomada* host-symbiont co-divergence, and an adjusted mutation rate gathered from *Drosophila melanogaster* and *Nasonia* species. We first perform a hierarchical based model comparison on the MLST data set to select the best clock, replacement, and tree priors; we then used the best prior combination to calibrate both the genomic and the MLST data sets using different combinations of calibrations. Although divergence estimates are characterized by a high degree of uncertainty and strongly depend on the use of certain calibrations, most results point towards a pre-Cambrian origin of *Wolbachia* (its split from *Ehrlichia*). Diversification of *Wolbachia* supergroups is more recent, from the late Paleozoic to the Mesozoic: this is compatible with a long-fuse model of evolution, and suggests a co-radiation with holometabolan insects. Our results provide a comprehensive first effort to date *Wolbachia* evolution: results are model, prior, and data set dependent and reveal the difficulties embedded in the molecular dating of such elusive bacteria characterized by the absence of reliable calibration priors and a clear evolutionary history.

SYMPOSIUM

Evolution and conservation

Chair: PAOLO CIUCCI

Co-chair: GABRIELE GENTILE

Integrating evolution into conservation: from genes to ecosystems

David R. Vieites

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Conservation planning should derive into actions that effectively preserve the Planet's biodiversity, but those could change dramatically depending on the criteria used for defining priority areas and species for conservation. In the last 20-25 years, the wealth of genetic and genomic data has percolated the field of conservation biology, incorporating evolutionary patterns and processes into effective conservation planning, from genes to ecosystems. Those span from the detection of loci as potential markers for adaptation to novel climates or environmental gradients, to the integration of genetic diversity as well as spatial phylogenetic richness and endemism as proxies for preserving Earth's evolutionary history, as well as species diversity, at wider scales and across taxa. Here I will provide an overview of the current trends in the integration of evolution into conservation planning and future directions.

Unidirectional hybridization between *Amblyrhynchus cristatus* and *Conolophus subcristatus* in Galápagos: naturally driven or man-mediated?

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Species conservation and evolution can be strongly connected. Human activities may influence evolutionary trajectories of species in a variety of ways and it is crucial to understand the mechanisms affecting populations distribution and the natural microevolutionary processes involved. In this work, we investigated hybridization and introgression and their possible role in shaping the evolution of two populations of *Amblyrhynchus cristatus* (marine iguana) and *Conolophus subcristatus* (Galápagos land iguana), syntopic in Plaza Sur island (Galápagos). Our results highlighted the sex-driven unidirectionality of interspecific hybridization and the presence of private alleles in hybrid individuals. Inter-island migration of marine iguana males can explain the phenomenon. In the light of these results, considering the Reproductive Isolation Mechanisms between the two species, data from McLeod et al. (2015) are also re-discussed, in the hypothesis of human-mediated migration of marine iguanas across the archipelago.

Molecular detection of hybridogenetic pattern in the *Pelophylax esculentus* complex in Italy

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Hybridogenesis is an extremely rare form of sexual parasitism. A case in point is the *Pelophylax esculentus* complex that is composed by three morphologically cryptic species: the parental *P. lessonae* and *P. ridibundus*, and their fertile hybrid *P.* kl. *esculentus*. As the hybridogenetic condition depends on the backcross of the hybrid, which in its gametes is able to only transmit the "*ridibundus*" genome excluding the *P. lessonae*-DNA, this system can only exist in the absence of *P. ridibundus*. Nowadays, translocation of alien waterfrogs of the *P. ridibundus* clade from eastern and western Europe is threatening the persistence of this hybridogenetic system in the Po Plain, leading to the extinction of *P. lessonae*, the reduction of *P. esculentus* hybrids, and the establishment of new hybrid lineages with unknown reproductive outcome. From an evolutionary point of view, this scenario offers the opportunity to investigate the effects of alien genome introgression among species and the mechanisms beneath the hybridogenetic complex. Our aim is to study hybridization and genome exclusion adopting a two-way integrative approach that combines whole genome sequencing, through ddRAD-seq (double digested Restriction-site-Associated DNA sequencing), and GISH (Genomic In Situ Hybridization) techniques. To do so we identified pure parental lineages with disagnostic mtDNA and SSR markers, and crossed them by in vitro fertilizations. Obtained tadpoles have been reared indoor until they reached the gametogenesis stage and then analyzed with the approach up-mentioned. In vitro crossing will allow us to compare gDNA information of hybrid offspring and its direct parents. Also, GISH with parental genomic DNA probes will allow us to confirm and strengthen the results. The results will represent a unique resource to study how hybridogenesis works, and to investigate the effects of alien species introduction, thus providing important information to draw up conservation guidelines.

The dark side of hybridization: quantifying prevalence of anthropogenic introgression for conservation

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Hybridization, the interbreeding of individuals from genetically distinct populations, has been considered until recently a relatively rare event across animal species. However, it is with the development of molecular techniques that hybrids have been increasingly detected, suggesting hybridization may be a more widespread phenomenon than originally thought. Hybrids are not necessarily less fit and, under given circumstances, they may also bear more successful adaptive traits with respect to their parental populations. Hybrids can also re-integrate with parental populations by backcrossing, leading to various levels of introgression. Whereas natural hybridization is considered a positive evolutionary force and a source of evolutionary novelty, conservation-wise hybridization caused by human impacts (anthropogenic hybridization, AH) may threaten the genetic integrity of many wild plant and animal taxa. AH has been recently recognized as an increasing threat for biodiversity worldwide due to human induced changes in species abundance and distribution, removal of reproductive barriers, and introduction of non-native and domestic species. Conservation issues raised by AH may range from wasted reproductive effort in threatened, parental species, when the hybrids are sterile, to various degrees of genetic swamping when introgression takes place. We present a review of AH cases focusing on the hybridization between wild species and their and domesticated counterparts. By summarizing factors and conditions that most facilitate AH, we discuss the most relevant implications from both an evolutionary and conservation-wise points of view. In particular, we emphasize the importance of developing of demographic models to accurately estimate the current and future extent of admixture in parental populations in order to explore and accordingly identify the most appropriate management and conservation recommendations.

Conservation genetics of barbel species and ecological index inside Protected Areas of Emilia Romagna

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Italian freshwater fishes are threatened by climate changes, anthropic activities, habitat loss and fragmentation, increase pollution that decrease the native populations. For this reason, Italian IUCN Red List promoted *Barbus plebejus* from LC to VU and *Barbus caninus* from VU to EN. Recently, many studies have shown an intense introgression of allochthons barbel species in particular *Barbus barbus* in Northern Italy. More than 200 samples from 20 sampling sites inside 15 Protected Areas Areas (SCI Site of Community Interest) in the framework of Nature 2000 Network, were studied using 10 microsatellite molecular markers and cytb mtDNA. Even more, three different ecological parameters were analyzed: ISECI (Ecological State of Fish Community Index), STAR-ICMi (Macroinvertebrates index) and IFF (Functional Fluvial Index) inside protected areas. mtDNA results detected three different barbel species inside protected areas: 72% *Barbus plebejus*, 18% *Barbus caninus* and 10% *Barbus barbus*. Structure analysis revealed a complex scenario where in many different sampling sites the autochnous specie are introgressed with *B. barbus*. Two protected areas did not show any barbel presence. In addition, only in two sites *B. caninus* revealed a structured population and in many rivers hybrids between autochnous species are more relevant in the upper part of rivers. Futhermore ecological index showed a strong environmental variation between lower and upper altitude part of protected areas probably due to human activities more intense in plain environment of Emilia Romagna. In this study for the first time a deep analysis inside SCIs was shown. These results will be managed in the European LIFE Barbie project NAT/IT/001129 for the hatchery reproduction of authoctonous barbel species that will be released in SCIs after an environmental assessment.

Diversification and distribution of Barbel species in central Italy: a combined morphological and genetic approach

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Along Italian peninsula the distribution and the diversification of several freshwater taxa have been largely influenced by the hydrographic and geological history. Central and south Italy are biodiversity spots with a high number of endemisms. The *Barbus* genus, freshwater bottom dwellers Cyprinids, has two native fluvio-lacustrine species, *B. plebejus* and *B. tyberinus*, endemic to Padano-Venetian (PV) and Tuscano-Latium (TL) districts, respectively, that represent an ideal model to study the species diversification and distribution, especially in relation to the Apennine biogeographic barrier. The present study focused on *Barbus* population genetic structure along main central Italy, providing evidence of contact zone between the endemic species and identifying the presence of exotic barbel species (i.e. European barbel *B. barbus*, Spanish barbel *Luciobarbus graellsii*). To test species delimitation a total of 629 specimens have been collected in Central Italy. Genetic and phenotypic investigation was conducted analyzing D-loop mitochondrial marker and evaluating shape through morphometric analyses based on 19 landmarkers. Morphometric and genetic results are congruent in the identification of *B. barbus* species, represented by 20 different haplotypes mainly distributed in the TL. The morphological and genetic results obtained for endemic Italian species were not congruent, revealing from one side a lack of morphological discrimination and in opposite a genetic attribution based on p-distance >2%. Moreover, the genetic results evidenced high variability in both endemic species (32 and 18 haplotypes in *B. plebejus* and *B. tyberinus*, respectively), mainly distributed, but not confined, in the respective native districts, suggesting river capture phenomena that have promoted the Apennine barrier permeability. These results highlighted the need for immediate conservation actions and emphasize the value of an integrated approach in the discrimination of *B. barbus* species.

Genetic structure of *Anadara tuberculosa* from East Pacific as revealed by mtDNA: Implications to conservation

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The pustulose ark, *Anadara tuberculosa* (Bivalvia), is considered as an emblematic species of the East Pacific mangrove ecosystem. In the last decades, several studies showed a worrying wild population decline in South American countries (Colombia, Ecuador and Peru) mainly due to overharvesting, habitat loss or degradation, and a lack of effective stock management plans. To reverse this trend, several conservation and restoration strategies have been promoted such as regulation of fishing activities, mangrove concessions programs granted to local communities, hatchery production of spat and small-scale pilot programs to grow *A. tuberculosa*. In this context, understanding the genetic aspects of geographic variations and populations structure of *A. tuberculosa* appears as a priority to fishery authorities in order to elaborate integrated and collaborative conservation policies for fishery management, aquaculture and stock enhancement programs in a concerted way. In this study, we used mtDNA sequence data (partial COI gene) to investigate haplotype diversity, genetic structure, and demography of *A. tuberculosa*. Samples were collected from two sampling sites north of the equator (Colombia and Ecuador) and three south of the equator (Ecuador and Peru). Results indicate genetic homogeneity of populations distributed north and south of the Equator, respectively. The two groups show similar haplotype diversity (H=0.91) and demography reconstruction supports recent, possibly post-glacial, population expansion. However, statistically significant differentiation emerged between northern and southern populations with pairwise FST values ranging between 0.036 and 0.092. The oceanic current system acting in the area (Panama Current and Humboldt Current) might play a role in limiting the larval dispersal of the species, still poorly understood. Our results would suggest separate and independent management of populations north and south of the equator.

Molecular variation and population structure in critically endangered Turks and Caicos Rock Iguanas: identifying intraspecific conservation units and revising subspecific taxonomy

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Identifying conservation units is a great challenge, especially for species inhabiting naturally fragmented habitats. Critically endangered *Cyclura carinata* iguanas are endemic to the Turks and Caicos Islands (TCI). Once abundant across the entire archipelago they now persist on only 5% of their historic range. Analyzing the geographic distribution of genetic variation is now particularly important for this species due to the onset of translocation programs. In this work, we used 29 microsatellite loci to genotype 280 individuals from 30 sampling locations across the TCI. We further investigate genetic variation, within the mitochondrial ND4 gene, through sequencing, or the use of PCR-RFLPs. A protein profiling analysis of femoral pore secretions was also conducted on a subset of samples. Microsatellites suggest that a recent, common population ancestry, or a simple reduction in gene flow, cannot account for the significant differences detected between a western and an eastern lineage within the TCI (F_{ct} = 0.202, p << 0.01). The same pattern was confirmed by mitochondrial DNA sequence analysis, significantly distinguishing between the western and eastern haplotypes (Φ_{ct} = 0.96, p << 0.01). Protein profiling provides further support, differentiating a western and eastern lineage. Implications of these results are that at least two Evolutionarily Significant Units (ESUs) can be recognized across this taxon's geographic range. Multiple Management Units (MUs), within each ESU, can also be defined. Future management planning for *C. carinata* should consider these evolutionarily independent lineages.

Climate-driven range shifts in fragmented ecosystems

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Range shift is the primary short-term response of species to rapid climate change but it is hampered by natural or anthropogenic habitat fragmentation. Fragmented habitats expose different critical areas of a species niche to heterogeneous environmental changes resulting in uncoupled effects. We present a predictive model of a species discontinuous distribution under climate change scenarios. We used i) accurate past and present demographic parameters inferred from a vast genomic dataset, ii) the result of long-term observations of fitness dynamics, and iii) an ensemble of 15 global coupled ocean-atmosphere general circulation models. Using a charismatic bioindicator of one of the most threatened ecosystems by global warming, the King penguin in the subantarctic ocean, our model predicts the disappearance of about 70% of the 1.6 million breeding pairs, and identifies potential future refugia which can be the focus of proactive conservation strategies. Our multidisciplinary approach opens novel opportunities for predicting the effect of global warming on species relying on spatially and ecologically distinct areas to complete their life-cycle (e.g., migratory animals, marine pelagic organisms, central-place foragers) and, in general, on species constrained in fragmented landscapes due to continuously-growing anthropogenic pressure.

Common patterns in "bio-cultural" diversity and the role of ecological barriers: Putting E.O. Wilson's hypothesis to the test

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Biological diversity (BD) and cultural diversity (CD) are deeply interlinked. A well consolidated literature is today confirming that extinction patterns related to BD closely match with those related to CD. What about the patterns related to the evolution and distribution of bio-cultural diversity? Edward O. Wilson suggested that ecologically rich areas are usually associated to cultural hotspots and that ecological and geographic barriers play a significant role in producing both BD and CD. The paper aims at outlining the core ideas of a research project (RP) which the APE Lab in Padua is developing with the collaboration of geneticists, ecologists and archaeologists. The RP aims at testing Wilson's hypothesis to disentangle the concept of "bio-cultural" diversity. After a preliminary phase of collection and exploration of different cultural datasets to detect ubiquitous elements, comparable in extensive or different contexts, the project aims at defining measures of ecological and cultural "diversity" and "distance", through which the distribution of the traits can be interpreted and processes of cultural transmission and evolution can be inferred. We will test a null model of pure cultural diffusion, where interaction between groups is the only determining factor and geographical proximity can predict biocultural similarity. Where such a model isn't met, we advance some hypotheses to explore the relationships between: (1) geographic or ecological barriers and ecological diversity; (2) genetic distance and number of barriers; (3) cultural diversity and genetic diversity; and (4) cultural diversity and ecological diversity. If our tests provide successful insights, this work will provide a significant contribution to the field of conservation of both BD and CD, and it will raise ethic awareness towards the ecological disruption due to anthropic activity, by showing the great amount of "bio-cultural" loss that is to be expected as an ultimate consequence.

When PINK is a question: A possible evolutionary trade-off in the Galápagos Pink Land Iguana (*Conolophus marthae*)

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The lack of complete skin pigmentation in *Conolophus marthae* contrasts with the habitat of the species, the Wolf volcano (Isabela Island). Indeed, adaptation to such an environment, characterized by an extremely high level of solar radiation (UV Index>16) due to the latitude zero and the high altitude (1700m), would imply the acquisition of a protective mechanism against UV-induced DNA damaging effects, mainly directed to epidermis cells. The Wolf volcano offers an opportunity to comparatively discuss the issue in the light of different degrees of body pigmentation. In fact, the only existing population of *C. marthae* is syntopic with a population of *C. subcristatus*, on the top of the volcano and the two species are evolutionary closely related. Additionally, whereas the skin of *C. marthae* is markedly depigmented, the skin of *C. subcristatus* is pigmented throughout the whole body. These circumstances would also suggest that, besides the possible different effects that UVB radiation could cause in the two species, if the "all-things-being-equal" assumption is sufficiently met, *C. marthae* should show plasma levels of Vitamin D higher than (or equal to) *C. subcristatus* in Wolf volcano. We investigated these predictions and found that, according to the prediction, the number of micronuclei and nuclear anomalies in blood red cells is higher in *C. marthae* than in *C. subcristatus*. Conversely, *C. marthae* showed Vitamin D plasma levels lower (but still normal) than *C. subcristatus*. We considered these results in the light of UVB exposure and found out that *C. subcristatus* is most likely found in open areas where UVB radiation is high, whereas *C. marthae* is most likely found in sites where UVB radiation is much less than the maximum radiation available in the environment. On the whole, these findings suggest a possible evolutionary trade-off in which behavioral strategies interplay with ecologic requirements and provide one more reason for pink iguanas' habitat conservation.

Development of a landscape suitability model to assess the role of anthropogenic disturbance for conservation of tool use behavior in wild capuchin monkeys (*Sapajus libidinosus*): a pilot study

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At the present time, anthropogenic activities are predominant in several environments. Tool use behavior by wild nonhuman primates is noteworthy, as it provides the possibility to clarify lithic technology evolution in human lineage. However, the conservation of this "unique" behavior is still not addressed properly, as well as the effects of human impact on it. In this pilot study, we developed a landscape suitability model in order to define the relation between environmental variables and the nut-cracking behavior recorded in a wild population of capuchin monkeys (*Sapajus libidinosus*) living in a Brazilian semiarid habitat characterized by an increasing anthropogenic pressure. The model included (i) behavioral data collected at the anvil sites where tool use behavior was recorded, (ii) natural variables (i.e.: minimum distance between anvilsites and palm trees, Normalized Difference Vegetation Index-NDVI) and (iii) anthropogenic variables (i.e.: minimum distance between anvils and houses or sandy roads). Our results highlight the importance of NDVI in determining landscape suitability for the occurrence of nut-cracking behavior, as they are positively related. Although anvil sites are found at relatively short distances from anthropogenic elements, these variables are less relevant for the model. Importance of high NDVI value in nut-cracking performance might be related to a permanent and healthy vegetation cover, which provides protection from predators and steady fruits supply. The short distance found between anvil sites and the anthropogenic elements (i.e. houses and roads) supports the hypothesis that capuchin monkeys in the study area developed a remarkable adaptability to moderate anthropogenic disturbance, given the low housing/traffic density. Nevertheless, in order to develop the best strategy to preserve tool use behavior in wild capuchin monkeys, model accuracy improvement and a better understanding of the role of anthropogenic disturbance are needed.

Conservation genetics and genomics of the endangered tortoise *Testudo hermanni*

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The Hermann's tortoise (*Testudo hermanni*) is an endangered land tortoise distributed in disjoint populations across Mediterranean Europe. Previous studies based on few microsatellite and mtDNA markers were able to identify major geographic groups and hybrids. Here we show how these data can be used to classify confiscated individuals of unknown origin in groups with different levels of suitability for reintroduction projects. However, the power with this kind of data is limited and several animals were assigned with low probability. We therefore completed a ddRAD (Double Digest Restriction Associated DNA) genomic sequencing that allowed us to obtain around 3,000 nuclear SNP markers in 82 wild individuals from 39 sampling sites across its natural range. We are using this large number of markers to better understand the genetic structure of this species, and to identify a subset of informative SNPs for the rapid and cheap genotyping (e.g., using TaqMan or KASP assays) of many individuals potentially suitable for reintroductions in specific geographic areas, and excluding hybrids. Preliminary results based on PCA and coancestry matrix showed a clear genetic divergence between distant subpopulations, and highlighted fine-grained sub-structure within a previously unresolved cluster. These preliminary results suggest that ddRAD approach can successfully be used to better understand the geographic structure in this species and to contribute to the conservation and possible reintroduction strategies in areas were the species is now extinct. The interest of several institutions and authorities in Italy indicate the importance to develop genomic tools not only to better understanding the status of the Hermann's tortoise, but also develop costs-effective tools to genotype individuals in captivity before reintroduction. The RAD approach is very promising and we expect that this study represents an important step forward for the conservation and the management of this species.

Genetic characterization of MHC class II exon2 in *Bombina pachypus* populations across the entire range: preliminary results

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The Apennine yellow-bellied, *Bombina pachypus*, is an Italian endemic amphibian, listed as "Endangered" in the Red List of the International Union for Conservation Nature (IUCN). This species represents one of the most threatened anurans in Italy and it is a priority of conservation. In fact, its populations are decreasing due to multiple interrelated factors, not all fully understood. Habitat degradation and fragmentation are probably the most influent impact factors, as well as the putative susceptibility to pathogens. Furthermore, *B. pachypus* typically exhibits high levels of population genetic structure over relatively small geographical distances. In fact, gene flow is limited because of high philopatry and relatively low vagility of this species. Habitat fragmentation, local adaptations and local pathogenic infections may also affect the genetic structure. In this context, the Major Histocompatibility Complex (MHC) represents a suitable system to investigate the potential role of selection in promoting adaptation in a context in which migration and genetic drift may act as not negligible evolutionary forces. MHC is a gene-dense region involved in the adaptive immune response and polymorphism is usually extremely high in MHC loci, often due to balancing selection promoted by pathogen pressure. Information about this system are extremely poor in Italian amphibians. The knowledge of the distribution pattern of adaptive variation, as reflected by MHC allelic diversity, provide a useful tool in the planning appropriate conservation strategies for endangered species. In this poster, we show preliminary data of MHC variation detected across 47 sampling locations, representative of the entire range of *B. pachypus*. We amplified fragments of the most variable part of MHC class II exon2 in 271 individuals. By analyzing results of Amplicon-Sequencing (Next Generation Sequencing), we identified all exon 2 putative variants in each individual.

Productivity as the main driver of bird migratory behaviour at different evolutionary scales

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The evolution of migration in birds is still a subject of debate. Some drivers of migratory behaviour have been identified from studies tracking bird movement, in most cases being productivity the main driver, but it is unclear which variables may be related to the evolution of bird migration at deeper evolutionary scales, and if those are the same that are acting in the present. Here, we used phylogenetic comparative methods to assess whether the evolutionary patterns of migratory distances, as a proxy for migratory behaviour, are correlated with several biometric, climatic and productivity variables in a phylogenetic context, using a species' level phylogeny of Sylvia warblers as a case study. We found that migration likely evolved to profit from productivity peaks in areas outside species' original ranges. We recover the Net Primary Productivity in the breeding range and during the breeding season as the variable with stronger correlation with migratory distances in a phylogenetic context, being always included in the best models considering a combination of potential drivers of bird migration. Several climatic variables show correlation with the evolution of migratory behaviour, but those are also tightly correlated with productivity. Considering biometric variables, we only found differences in wing length between migratory and sedentary lineages. In the case of Sylvia, the ancestor of the genus was recovered as migratory, and there were several events of migratory behaviour loss mainly linked to island colonization. Our results suggest that the same drivers of bird migratory behaviour may have acted along the long evolutionary history of these passerines until today.

Genetic signature in peripheral populations of African greenbul in tropical forest ecosystem

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Studies of population genetic patterns at the limit of species' distribution range are useful to infer processes of species adaptation to the recent environmental changes. To evaluate if the genetic signature at periphery zone of geographic range may be shaped by distinct ecological traits we analyze and compare the population genetic signature of three taxa, selected among forest dependent African greenbuls (Pycnonotidae, Passeriformes): the generalist *Andropadus latirostris* and the specialist *Phyllastrephus cabanisi*. These species share their range limits in an Afromontane forest (Cherangani Hills, Kenya) populated also by *Andropadus nigriceps* defined as mountain specialist greenbul. Through molecular analyses, two main aims were investigated: i) find out a correspondence between genetic and ecological signature; ii) provide a preliminary survey on the genetic effects of recent Cherangani fragmentation, started about 50 years ago. Blood and plucked feathers were sampled from 124 greenbuls: all samples were genotyped at 8 microsatellite loci, while cytb mtDNA gene was sequenced in 50 samples. Haplotype and nucleotide diversity, Minimum Spanning Network and mismatch distribution analysis, showed as the two ecological models are defined by different demographic trends: 1) a stable population in expansion in the forest generalist; 2) the co-occurrence of two distinct lineages in the forest specialist. In mountain specialist *A. nigriceps* a recent population bottleneck has been recorded. Then, significant values of pairwise differentiation between populations from two main forest fragments were found, indicating a congruent pattern of reduced connectivity and limited genetic flow supported also by the low number of first-generation migrants inferred (2.8% and 9.3% migrants between *A. latirostris* and *P. cabanisi* populations respectively). In conclusion, this preliminary study on African greenbuls provides evidence of effects of Cherangani forest fragmentation.

Demographic inference using genomic data of endangered species

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The huge amount of genomic data that is becoming available in public databases opens new opportunities in the population genetics field. Development of new methods and software to analyze these data allows researchers to reconstruct major events in the demographic history of populations. The PSMC (Pairwise Sequentially Markovian Coalescent) method reconstructs the population size history assuming a single non structured population. However, the history of real populations is more complex and there is a growing need to use models that incorporate population structure. Our group has shown that it is possible to use the distribution of coalescence times for alleles taken from a single diploid individual to perform model choice in a likelihood framework. The two models that were used were Wright's n-island model and a simple model of stepwise population size change (SSPSC, for single step population size change). Our group developed a method based on coalescence times to estimate the demographic parameters in order to then identify the best of the two models. The method has now been extended to use the number of heterozygous sites in a large number of independent fragments sampled in the genome of a diploid individual to infer parameters of the demographic history of populations. We applied for the first time the approach of Rodriguez, Mazet and Chikhi to real data by obtaining genomic data of several endangered species. We believe that it will help us to reconstruct the demographic history (bottlenecks, expansions, etc.) of species. This kind of approach is complementary to the PSMC as it allows to detect some forms of population structure that may generate spurious signals of population size change.

Old wild wolves: ancient DNA unveils ancestral variability in the Italian wolf population

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The origin and ancient population dynamics of the grey wolf (Canis lupus) have been the subject of several studies based on modern and ancient samples. Recent works describe the spatial-temporal distribution of the 2 mitochondrial haplogroups (defined HG1 and HG2) associated with 2 different ecomorphs of which one (HG2) was dominant in Europe and America during the last glacial period and related to megafauna. After the Last Glacial Maximum (LGM) climate change the HG1 ecomorph has totally replaced the pre-existing North-American wolf and in Eurasia has become dominant over the HG2. The contemporary Italian wolf population is the only remnant population entirely referable to HG2 but the ancient dynamics that led to this situation are not well defined. In this work a portion of HVR1 region of mitochondrial DNA was analysed in 18 ancient samples from northern Italy, ranging from 24.000 ya to 3.500 ya, and the results were compared with ancient and modern sequences of wolves and dogs. The haplotypes found are related to ancient wolves from northern Europe and Beringia, modern wolf and also primitive and contemporary dogs. These results show that the oldest Italian specimens, in continuity with the present age, belong only to the HG2 wolf population, characterised by a larger variability. Presumably the migratory wave from East of wolf HG1 did not reach Italy in substantial numbers, making the Italian Peninsula the last stronghold of an ancient and once widespread wolf lineage.

SYMPOSIUM

The effects of drift and selection on populations and genomes: expectations and observations

Chair: GIORGIO BERTORELLE

Co-chair: SILVIA GHIROTTO

A genomic history of Aboriginal Australia

Anna-Sapfo Malaspinas et al.

University of Bern

The population history of Aboriginal Australians remains largely uncharacterized. Here we generate high-coverage genomes for 83 Aboriginal Australians (speakers of Pama–Nyungan languages) and 25 Papuans from the New Guinea Highlands. We find that Papuan and Aboriginal Australian ancestors diversified 25–40 thousand years ago (kya), suggesting pre-Holocene population structure in the ancient continent of Sahul (Australia, New Guinea and Tasmania). However, all of the studied Aboriginal Australians descend from a single founding population that differentiated ~10–32 kya. We infer a population expansion in northeast Australia during the Holocene epoch (past 10,000 years) associated with limited gene flow from this region to the rest of Australia, consistent with the spread of the Pama–Nyungan languages. We estimate that Aboriginal Australians and Papuans diverged from Eurasians 51–72 kya, following a single out-of-Africa dispersal, and subsequently admixed with archaic populations. Finally, we report evidence of selection in Aboriginal Australians potentially associated with living in the desert.

Evolutionary analysis provides insight into the origin and adaptation of HCV

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Hepatitis C virus (HCV, family *Flaviviridae*, genus *Hepacivirus*) is a genetically heterogeneous virus, with 7 genotypes divided into several subtypes. A few epidemic HCV subtypes spread recently, whereas highly diverse endemic strains are geographically restricted. Using a selection-informed evolutionary model we show that the common ancestor of extant HCV genotypes existed at least 3000 years ago and that the oldest genotypes are endemic to Asia. Equine hepacivirus, HCV most closely related virus, originated around 1100 CE. These time estimates are not consistent with the possibility that HCV derives from a cross-species transmission from horses and suggest a single zoonotic event with subsequent spread and diversification in human populations. Evolutionary analysis in mammals also support a role for hepaciviruses as drivers of CD81 evolution in Chiroptera and Glires, implicating these mammals as the HCV reservoirs. By investigating the evolution of the 7 major HCV genotypes, we describe a number of biologicallyimportant sites that have been positively selected and indicate that drug resistance-associated variants are significantly enriched at positively selected sites. These results shed light on the origin of HCV and provide a catalog of candidate genetic modulators of HCV phenotypic diversity.

Inference of population parameters from low-depth sequencing data

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Accurately estimating genomic diversity is vital for sensible inferences of evolutionary events, including past population size changes and natural selection. However, many studies sacrifice high sequencing depth in favour of increasing sample size, giving a more complete picture of the overall population diversity but increasing the level of uncertainty in the analysis. We present a novel approach to estimate various measures of linkage disequilibrium (LD) from low-read sequencing data. When both haplotypes and genotypes are unknown, LD indexes can be directly measured from expected genotypes or estimated from genotype likelihoods using an expectation maximization algorithm. This approach is particularly suitable for low-depth data where genotypes cannot be assigned with high confidence. Indeed, we show that error in LD estimation is greatly reduced using our approach compared to classic strategies based on genotype calling for low/medium sequencing depth. We also implemented a fast and accurate algorithm to prune linked loci. Additionally, we modeled the LD decay over physical distances, exemplifying its utility in inferring population histories even when phased genotypes are not directly observable. These new methods were applied to several RNA-seq data sets from non-model organisms, where obtaining unbiased estimates of genetic diversity is challenging due to varying depths across genes. For all tested species, we can obtain sensible LD measures and discuss population sizes from LD decay. We finally discuss how a computationally challenging task such as the estimation of multidimensional site frequency spectra (SFS) is feasible under this framework. We explain the rationale behind detecting positive selection by looking at statistical deviations from the expected multidimensional SFS under genetic drift. We believe that these innovative features will be part of the essential bioinformatic tool-kit for evolutionary inferences from high-throughput sequencing data.

Genomic analysis reveals hidden heterogeneity within human population isolates

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Studying groups subject to barriers to gene flow provides a unique opportunity to understand how inbreeding, genetic drift and selection have shaped the structure of human genetic diversity. Currently, the consequences of genetic isolation may be better studied using genome wide approaches (GWA), such as those based on SNP microarrays which enable the simultaneous analysis of very large numbers of loci distributed across chromosomes. Despite the considerable body of knowledge on human genetic isolates, variation occurring among individuals within isolated populations has not been yet thoroughly investigated. This issue is worth exploring since events of recent admixture and presence of sub-structure could potentially disrupt the genetic homogeneity which is expected when isolation is prolonged and constant over time. In a previous study, we have compared intra and inter-population variation measures combining novel and literature data relative to 87,818 autosomal SNPs in 14 open populations and 10 geographic and/or linguistic European isolates. Here, we expand our dataset to a total of 27 groups and move our focus to the variation among individual genomes within populations. Using four measures of within population diversity (homozygosity, identity-by-state and length and number of runs of homozygosity), we observed a significantly higher level of variation among the German-speakers of the linguistic islands of Sappada, Sauris and Timau and North Sardinians. These same populations show a noticeable among individual variation for ancestry components. Caveats, significance and implications of our results are discussed in relation to both microevolutionary aspects and bio-medical applications.

The determinants of genetic diversity across the whole-genome in *Arabidopsis lyrata*

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Genetic diversity is the basis on which evolution acts and is therefore a key parameter in evolutionary biology, conservation and breeding. So far, empirical studies have been conducted to understand the role of local demographic factors such as census size and mating system on genetic diversity, or they investigated the role of historic, species-scale demographic effects, but rarely have their relative roles been considered. Furthermore, most such studies focused on diversity in a small fraction of the genome. The goal of our study was to overcome these issues and to investigate the role of local demographic factors versus historic, species-scale dynamics in explaining genetic diversity on a whole-genome level, including intergenic, intron and coding DNA sequences. Our study system was the North American Arabidopsis lyrata, one of the closest relatives of the plant model organism Arabidopsis thaliana. The study includes re-sequence data of 1300 individuals from 52 populations, covering the entire range of the species' distribution. Best predictors of genomic diversity were the local mating system (selfing compared to outcrossing) and historic range dynamics since the last glaciation cycle. Historic demographic processes pre-dating the last glaciation cycle and admixture between clusters had a much smaller impact and were more visible in intergenic regions. Census size was only positively linked with nucleotide diversity in parts of the distribution area. The study highlights that for a species with a relatively recent expansion history, this history is one of the most important factors explaining diversity in coding and non-coding DNA, with the highest diversity in the area from which expansion happened.

Distinct selective forces and Neanderthal introgression shape genetic diversity at genes involved in neurodevelopmental disorders

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High cognitive abilities and complex social systems are observed in several animal species, but prosocial behaviors are more pronounced in mammals compared to other vertebrates. In addition to high intelligence, humans evolved specialized social-cognitive skills, which are specifically affected in children with autism spectrum disorder (ASD). Herein we analyzed the evolutionary history of two gene modules (M1 and M2, 68 genes) involved in neurodevelopmental disorders. Results indicate that positive selection acted similarly on the mammalian and sauropsidan branches of the vertebrate phylogeny. Analysis of an extended mammalian phylogeny and of genetic data from Homininae identified several positively selected sites. The strongest signals of selection were detected at *MDM2* and *UIMC1*, two genes involved in cancer and highly expressed in testis. ASD missense mutations and positively selected sites map to the same *MDM2* and *UIMC1* gene regions. In human populations, purifying selection was the major force acting on M1 and M2 genes, which also tend to display low levels of archaic retrogression. However, we identified a Neanderthal/Denisova introgressed haplotype that modulates the brain expression of *CUTA* and *PHF1*, genes involved in neurotransmitter signaling. Finally, we detected modern-human-specific variants in *DYRK1A* and *TCF4* that are located in regions targeted by positive selection in early modern human populations. These variants map to functional elements with a possible regulatory role in brain gene expression. Results herein indicate that genetic diversity at genes involved in neurodevelopmental disorders was shaped by distinct selective forces, including natural selection and introgression from archaic hominins. We discuss the possibility that segregation distortion during spermatogenesis accounts for a subset of ASD mutations.

Molecular evolution of light-dependent DNA repair mechanism in the cavefish *Phreatichthys andruzzii*

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DNA damage arising from endogenous and exogenous sources constantly threaten genomes integrity. To avoid fixation of detrimental mutations in genomes, DNA needs to be repaired. DNA integrity is provided by multiple repair pathways evolved to correct specific types of errors and lesions. The crucial role of these mechanisms in sustaining life is supported by their presence in all living organisms, their redundancy and their extreme phylogenetic conservation. Regressive evolution predicts that evolutionary events may trigger the loss of a specific function. For example, the loss of UV photoprotection in eutherian mammals would be related to the occupation of a prevalently nocturnal niche by the ancestors of this group during the Mesozoic era. Besides mammals and other few exceptions, a wide spectrum of organisms are UV-protected by photolyases, light-dependent enzymes that directly reverse UVB-induced pyrimidine dimers. Here we test the hypothesis that the isolation in perpetual darkness for several million years led to the loss of UV photoprotection in the Somalian cavefish *Phreatichthys andruzzii*, similarly to what has been observed in placental mammals. The results of experiments based on biochemical and biomolecular assays showed that *P. andruzzii* lacks direct photoreactivation. As for the genes coding photolyases proteins, 6-4 and dash photolyases are prematurely truncated, and thus lack nuclear localization and DNA-repair activity. Conversely, cpd photolyase gene is full- length and therefore the protein is translocated into the nucleus, but its photorepair activity measured by in vitro experiments is not efficient in cavefish. By means of interspecific analysis of molecular evolution and structure prediction, we infer the evolutionary pattern of 6-4, dash and cpd photolyases genes involved in direct DNA photoprotection, testing the hypothesis that natural selection is no longer actively maintaining the photoreactivation pathway in this cave-dwelling organism.

Adaptive evolution of Asian populations in response to rice-based diets

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Early adoption of massive rice consumption in some pre-agricultural Asian populations and its subsequent domestication by Neolithic farmers has plausibly introduced a substantial challenge for *H. sapiens* metabolism. In fact, among domesticated cereals, rice shows the highest carbohydrates content and glycaemic index, thus prompting a rapid increase in glycaemia that makes its usual ingestion a potential risk factor for the development of insulin resistance and metabolic diseases. This new selective pressure on insulin-related pathways might have triggered genetic adaptation of longterm rice-feeding populations against the dangerous side effects of their peculiar dietary habits. To test such a hypothesis, we assembled a genome-wide dataset made up of 2,483 subjects belonging to 129 South Asian and East Asian populations representative of human genetic variation observable at geographical areas where wild rice was supposed to have originated and then domesticated. We thus inferred genomic ancestry for each of the examined samples and we applied population structure analyses to detect distinct clusters of genetically homogenous human groups. Then, we searched for genomic signatures ascribable to the action of natural selection in the above-mentioned population clusters and we tested whether known candidate loci involved in modulation of insulin metabolism were enriched in the identified set of potentially adaptive loci. According to this approach, we succeeded in shortlisting some of the adaptive events that have characterized the evolution of Asian populations and that might underlie the maintenance of physiological glycaemia even in the context of rice-based diets.

Complex demography shaped the genomic landscape of Himalayan populations in addition to altitude-related selective pressures

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Although many studies corroborated the identification of candidate genes (e.g. *EPAS1, EGLN1*) plausibly involved in high-altitude adaptation of Himalayan populations, the actual adaptive variants and the role of other loci pointed out in independent cohorts were not elucidated so far. Moreover, most of these studies focused generically on Tibetans, whereas little is known about the genetic structure and demographic history of the several populations inhabiting the Tibetan Plateau. Nepalese populations from the Gaurishankar mountain range represent an intriguing case study to test the interplay between demography and natural selection in having shaped the genomic background of Himalayan populations. In fact, this region hosts populations belonging to three main ethnic groups: people speaking Indo-Aryan languages, who live at low altitudes, and groups speaking Tibeto-Burman languages (i.e. Tamangs and Sherpas), who have spread respectively up to medium and high altitudes and are supposed to have moved from Tibet to Nepal in historical times. To reconstruct the genomic relationships of these populations with a large set of East Asian/South Asian groups, we generated genome-wide SNP data for 75 Indo-Aryan, Tamang and Sherpa individuals. We thus found evidence of extensive admixture in Indo-Aryans, with East-Asian ancestry components presumably introduced by Tibeto-Burman migrants, as well as of Tibetan admixture with low-altitude East Asians contrary to substantial Sherpa isolation. Although we partially disentangled the impact of gene flow and drift on the evolution of such Himalayan groups, we sequenced the whole genome of a Sherpa individual from the studied community to refine demographic inferences about high-altitude Nepalese populations. By merging the obtained data with Tibetan/Sherpa whole genomes available from literature we also aim at discerning the possible role of multiple genes in adaptation to hypobaric hypoxia.

The genetic legacy of ancient migrants to Southern Italy as revealed by high-throughput sequencing of the Y chromosome

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The relative contributions from the East to the genetic pool of Southern Italy by seafarers of the early Neolithic, steppe people of the late Neolithic and Greek founders of *Magna Graecia* are unclear. So far, genetic data did not distinguish their legacy. Yet, in the three cases Southern Italy offered appropriate conditions for successful settlement, demographic growth and hence molecular radiation. The presence of the corresponding signatures in the phylogenetic trees of the lineages conveyed by the immigrants can directly be tested by a phylogeographic approach. The Y chromosome haplogroup J (Hg J) has long been considered the clearest marker of East-to-West migrations that impacted South and South-eastern Europe. We used Next Generation Sequencing (NGS) to obtain an exhaustive list of variants within Hg J. Our final goal was to distinguish different time windows and to determine the source population(s), the relative contribution(s), the routes and the Italian locations where the genetic traces of this processes are prominent today. We identified Hg J carriers among ~1,300 unrelated males from locations in Italy, the Balkans, Greece, Turkey and the Middle-East. We selected 58 Hg J chromosomes (7 sub-Hg J) from the most geographically-informative regions. These were re-sequenced, to a mean 50x depth of 4 Mb in the X-degenerate regions of the male-specific portion of the Y, by using Target Enrichment. Dating was obtained by calibration with the Hg J individual "Kotias" (9.7 kya). We identified 1,230 high quality variant positions (SNPs). The corresponding maximum parsimony tree recapitulates the known Hg J phylogeny, but with a refined branch length for the main subclades. We found several unexpectedly deep lineages. Few lineages coalesce to star-like nodes, dated 4-7 kya. Basal lineages within each sub-Hg J were not preferentially the eastern ones, indicating high levels of gene flow in the past. Grants PRIN-MIUR 2012JA4BTY_004, 003 to FC, AN.

The Kurgan migrations revisited: Genome diversity in the Globular Amphorae culture

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It is unclear whether Indo-European (IE) languages in Europe spread from the Pontic steppes in the late Neolithic (the Kurgan hypothesis), or from Anatolia in the early Neolithic (Anatolian hypothesis), or from some other region. In its classical formulation, the Kurgan hypothesis regards the Globular Amphorae Culture (GAC) as largely descended from Eastern ancestors, most likely representing the Yamnaya Culture. However, nuclear (6 individuals typed for 597,573 SNPs) and mitochondrial diversity (11 complete sequences) from a Late Neolithic burial of the GAC in Kierzkowo, Poland, show limited genetic affinities with Yamna ancient DNA, or with ancient DNA from with those of all other populations of Central Europe related with the Kurgan Hypothesis. Instead, the GAC samples appear genetically related to those of the Early and Middle Neolithic periods. Explicit comparisons of alternative demographic models via Approximate Bayesian Computation confirmed this pattern. We observe genetic traces of a possible migration from the Pontic steppes and the Yamna only in ancient DNA from later Central Europe cultures (Corded Ware and Bell Beaker). Taken together, these results confirm that there were Late Neolithic migration processes from the Pontic steppes into Central Europe. However, they also add nuance to this model in showing that the archaeological and genetic evidence of eastern influence do not always match. Our results suggest that the eastern affinities of the GAC evident in the archaeological record may have been due to cultural influences from the eastern groups with whom they were in contact and not to movement of people.

The effects of habitat loss and fragmentation in two species of small mammals (*Apodemus sylvaticus* and *Myodes glareolus*; Rodentia)

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We compared the effect of habitat fragmentation on the genetic structure of two common rodent species, the wood mouse (*Apodemus sylvaticus*) and the bank vole (*Myodes glareolus*), using microsatellite markers and a suite of methods including Isolation by Resistance (IBR) and the estimation of effective migration surfaces (EEMS). The species are characterized by similar habitat requirements, but have different mobility and ability to overcome environmental barriers. Trapping was performed every two months for two years, in an area of central Italy with <15% of residual forest cover. A total of 193 wood mice and 199 bank voles were genotyped using 7 and 8 microsatellite loci, respectively. We found a different genetic structure in the two species. Almost all pairwise FST values in *A. sylvaticus* were not significant. On the contrary, *M. glareolus* shows a large geographic structure, with approximately half of the FST values being significant and a global FST of 0.08. Both bank vole and wood mouse populations showed significant patterns of isolation by distance (IBD). EEMS for both species highlighted areas of higher/lower gene flow with respect to IBD. We analyzed the potential origin of deviation from IBD considering the most common environmental features coded as resistance distances in IBR models. The IBR model providing the best fit for the wood mouse included woodlands and cultivated fields as a source of moderate increase and decrease of resistance to movements, respectively. In the bank vole, the connectivity was favored by both woodlands and cultivated fields, while urban areas had a high resistance coefficient to the movement of this species. Overall, this study shows that habitat fragmentation can have different impacts in these species, suggesting that the risks related to the loss of connectivity, and the consequent management actions to be implemented, cannot be easily generalized to an environmental condition even when similar species are concerned.

Distinguishing among complex models of modern humans evolution through a new ABC framework

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There is wide consensus in considering Africa the birthplace of anatomically modern humans, the dispersal pattern and the main routes followed by our ancestors to colonize the world are still matters of debate. It is an open question whether early modern humans left Africa through a single, major process, dispersing simultaneously over Asia and Europe, or in two main waves, first through the Arab Peninsula into southern Asia and Oceania, 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, yelded contrasting results. Moreover, it has never been formally explored whether, with the genetic evidence currently available, we would have the power of selecting the correct model of evolution of modern humans. As a consequence of this, it is not easy to say if the lacking reconstruction of our past is eventually due to missing information on genetic data, low inferential power of the statistical methodologies used or a combination of these factors. In this work, we addressed this question through an Approximate Bayesian Computation (ABC) approach, based on the recently developed Random Forest algorithm, and introduced a new set of statistics designed to be informative of admixture and divergence events. We constructed an efficient ABC pipeline and tested how accurately it allows to recognize the true model among models of increasing complexity, using simulated data. We also took into account different sampling strategies, testing several combinations of individuals analyzed per population, number and length of genetic loci considered. This pipeline has then been used to explicitly evaluate the power to discriminate between complex demographic models of single vs multiple exit of anatomically modern humans out of Africa, and finally applied to real, worldwide, modern and archaic genomic data.

Application of the constraint-based methods to analyse human mitonuclear co-evolution networks

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Mitochondrial efficiency requires interactions between nuclear and mitochondrial encoded proteins for assembly and function of the oxidative phosphorylation complexes. Accordingly, a strong selection should act to purge deleterious mutations and fix beneficial mitonuclear combinations. This study was designed to detect co-evolution signatures of mitonuclear interactions and explore how they affect human health and disease. This approach may be limited by the amount of individual and population sampled. To overcome this issue, exome-sequencing data of 2,390 healthy unrelated samples from 1000 Genomes Project were analysed. However, the curse of dimensionality challenged this study as about 70,000 nuclear and 1,700 mitochondrial variants were recognised and the number of multiway mitonuclear interactions was very high. So, a network-based method relying on a simple but robust statistics, Mutual Information (MI), was adopted. Five constraint-based learning algorithms (GS, MMPC, IAMB, Inter-IAMB, SI-HITON-PC) implemented in Bnlearn R package were used. To discern genomics signatures of co-evolution from background noise, *i.e.* demographic processes, 555 nuclear synthenic sites mapping in genes evaluated and then excluded with high certainty from MitoCarta2.0 inventory ("Non-Mito"), were selected. Analysis was performed with and without including the Non-Mito set. Only interactions robust to this inclusion were considered good candidates for co-evolution. Firstly, only 996 sites harbouring non-synonymous mutations and mapping on 214 nuclear genes, encoding proteins physically interacting with mitochondrial-encoded subunits, were investigated. We found 146 candidate nuclear sites directly interacting with mitochondrial ones and recognised by at least one of the five methods. Finally, we plan future investigations of a coalescence-based null model, to be compared to the use of the synthenic sites.

ROUND TABLE

Popularization of Evolution

(Divulgazione dell'Evoluzione)

Chair: TELMO PIEVANI

Bingo! La lotteria dell'Evoluzione!

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Evolution is not an easy topic to deal with. Infact, literature on science teaching methods show how student have persistent misconceptions about evolution. For example, evolutionary changes is often explained as the only result of individual needs referred to random variations and natural selection. To make these topics more affordable for school pupils - such as natural selection, random environmental variables, survival of the fittest and hereditariness - in 2014 we designed a game, named "Bingo! The evolution lottery", that was presented in "Festival della Scienza" in Genova, a very important italian science festival. 951 people got involved in the workshop, especially students aged 6-16 years. Kids were organized in teams, each one equipped with a "population" of 5 proto-insects, that could be randomly assembled in 24 possibile ways, to represent intra-specific variability. The variables are: antennae (long/short); eyes (small/big); legs (long/short); colour (green/red/yellow). Each characteristic gives an advantage to the specimen in certain environments. Then, environmental variables are extracted like bingo numbers (volcano's eruption, drought, predators, sexual mating) that have an impact on each population. The survivors will transmit the fitting characteristics to the offspring. In this way, through the game, each population will change and evolve, in a random way that makes each match different. In the present work, strengths and weaknesses of the educational game project are discussed both in methodological and conceptual frameworks.

404 error: (evolutionary) page not found

Sofia Rizzi[1], Sonia Celestini[1], Giulio Menegus[1], Francesca De Giorgi[1], Francesca Failla[1], Silvia Pernechele[1], Marco Salvatori[1], Andrea Silverj[1], Elisa Barbazza[1], Paola Bisaccia[1], Carlotta Bonaldi[1], Ludovica Dal Borgo[1], Lisa De Biasio[1], Laura Drago[1], Giulia Fabbri[1], Jacopo Fabrello[1], Alessandro Franceschini[1], Sabrina Gallo[1], Sandy Gionfriddo[1], Jacopo Grego[1], Carola Leonardi[1], Giulio Maria Menti[1], Ludovica Molinaro[1], Stefano Monteforte[1], Cristina Ottocento[1], Anna Paiola[1], Paolo Panizzon[1], Beste Basak Savasci[1], Federica Zancanella[1], Giuseppe Fusco[2], Omar Rota-Stabelli[3], Lino Ometto[4]

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A good acquaintance with the fundamental evolutionary patterns and processes is considered essential for any biologist and for laypeople as well. Therefore, correct understanding and communication about evolutionary themes should play a central role in modern scientific education and awareness. Wikipedia is probably the largest reference work on the Internet and, while it is mostly consulted by people who are not expert in evolutionary issues, it is also used as a quick and easily accessible reference tool by students and scientists. Nonetheless, we noticed the absence of accurate and complete information about several evolutionary and phylogenetic topics in the Italian edition (for instance selective sweep, long branch attraction and phenotypic plasticity). We therefore decided to edit (when inadequate) and create (when absent) some of these pages, adding the results of the recent literature and, when needed, relying on Wikipedia pages from other language editions. In addition, the contents of these pages will be integrated into the Italian evolutionary web portal Pikaia, with the aim of providing the basis for a glossary on evolution, open to the contribution of both the student and the research communities.