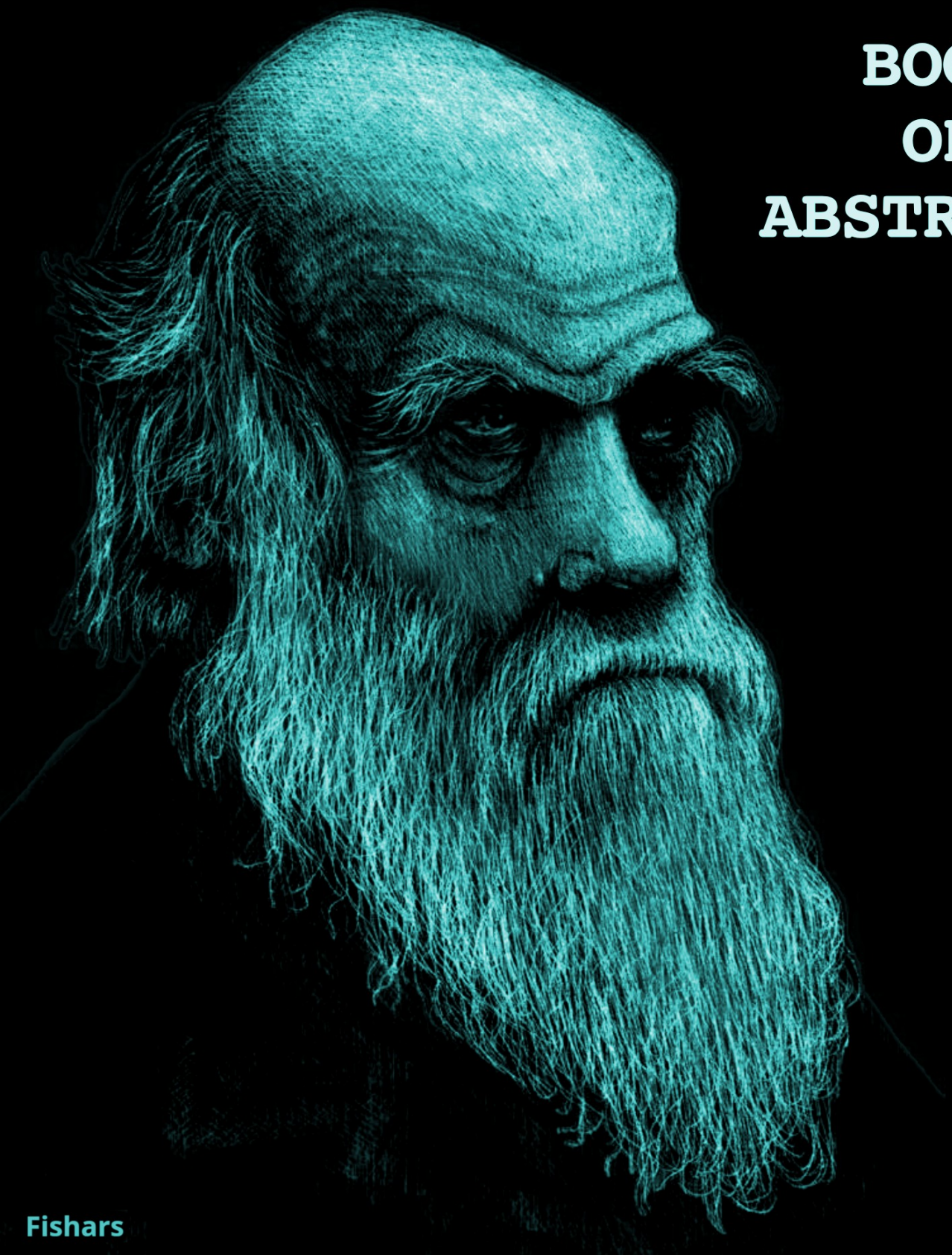
Evoluzione 2022

BOOK OF ABSTRACTS



SOCIETÀ ITALIANA DI BIOLOGIA EVOLUZIONISTICA

Fishars

9° Congresso della Società Italiana di Biologia Evoluzionistica

Book of abstracts

Ancona, 4-7 September 2022

Department of Life and Environmental Sciences and Department of Agricultural Sciences, Food and Environment, Marche Polytechnic University, Via Brecce Bianche, 60131, Ancona

Evoluzione 2022

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SYMPOSIUM

ADVANCES IN PHYLOGEOGRAPHY AND PHYLOGENOMICS

CHAIRS: PAOLO GRATTON, JOAN FERRER OBIOL, ASSUNTA BISCOTTI

Invited speaker DAVIDE PISANI, University of Bristol, UK

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Phylogenomic approaches reconstruct the age and physiology of the last universal common ancestor of life

1_Pisani Davide, 2_Moody Edmund, 3_Donoghue Philip C.J., 4_Williams Tom, 5_Spang Anja, 6_Szöllosi Gergely

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Understanding the early evolution of life is difficult as little information is left in the genomes of living organisms that can easily be tracked back to LUCA (the Last Universal Common ancestor of Life) while fossil evidence for early life is scant. To further complicate the problem, horizontal patterns of descent in prokaryotes make ancestral genome reconstructions challenging and estimates of the genome and physiology of LUCA have, therefore, varied significantly. Some authors see LUCA as “half alive”, an organism that used environmental energy gradients to stay alive, while others suggest LUCA had a genome as complex as that of modern organisms. Here I will synthesise results from our ongoing research on early life, presenting a new timescale of life obtained using novel molecular clock methods that allow us to use the few fossil calibrations available for the deepest history of life more efficiently. In addition, I will present new results illustrating progress in reconstructing the genome of LUCA using new approaches for gene tree species tree reconciliation. Our results indicate that LUCA was most likely a membrane bound cellular organism, with a relatively complex genome and a metabolism that might have been based on the Wood-Ljungdhal pathway. LUCA was alive ~ 4 billion years ago, but the molecular evolutionary processes that led to its existence were already in place well before that time, suggesting a very early emergence of life on Earth.

Talks

A full-plastome analysis to trace the ancient phylogeographic route of the relict tree Platanus orientalis

1_Teresa Rosa Galise, 2_Silvia Fineschi, 3_Antonia Cristaudo, 1_Salvatore Cozzolino, 4_Sandro Strumia, 1_Annalisa Santangelo, 1_Donata Cafasso

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Several tree species now confined within the south-western Eurasian continent represent Tertiary plant communities previously distributed across the Northern Hemisphere. At the end of the Tertiary warm phase (ca. 15 Myr ago), the onset of aridification and cooling drastically changed the vegetation of Europe. As a result, vegetative communities subsequently shifted from medium to high latitude circumboreal distributions to regions southwards, which were typically associated with warm and wet climates (i.e. refugia). Furthermore, Tertiary relict flora members already confined within these refugial areas have shown some resilience against the effects of recent glaciations (see Médail and Diadema 2009). Several studies have demonstrated that older processes of some strictly Mediterranean species than the effect of quaternary glaciations alone can be better explained with tree species' genetic structure and phylogeographic patterns (e.g. Magri et al. 2007, Désamoré et al. 2011, Migliore et al. 2012). Therefore, phylogeographic patterns provide more robust evidence of older migrations than the most recent postglacial expansion.
The oriental plane tree, Platanus orientalis, is one of the largest and longest-lived trees in the eastern Mediterranean and is considered an iconic example of Tertiary relict species. The present study uses whole plastid genome sequencing to better trace the origin and history of P. orientalis across its distribution range from the Iran-Turanian floristic region to the Mediterranean basin. We applied dating methods to estimate divergence time between the P. orientalis haplotypes to determine whether the current distribution of P. orientalis could be better explained by recent (quaternary) or old (tertiary) migrations. We also tested the hypothesis whether the Balkans served as a source of primary colonization for both east Asia and western Mediterranean migrations (and represents the ancestral centre of diversification of the species) or whether the species firstly originated in central Asia and only afterwards migrated west.

Population genetic structure and phylogeography of Papilio machaon (Lepidoptera, Papilionidae) in Europe and North Africa

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In the last two hundred years, researchers described more than 100 aberrations, subspecies, and ecotypes afferent to the P. machaon species. The intraspecific subdivision of P. machaon historically described in Europe has not obtained general consensus due to its high morphological and ecological variability and wide geographical distribution, characterized by extremely different habitats.
The study of the population genetic structure, affected by the paleoclimatic history, can contribute to the subspecies identification, which can be defined on isolated populations in time and space.

Using 179 mitochondrial sequences (COI) from European and N-Africa samples, we analyzed the population genetic structure on three geographical focuses: Europe and North Africa, Europe and Italy. The specimens were divided into groups based on glacial refuges and historically recognized subspecies (P. m. gorganus, P. m. hispanicus, P. m. sphyrus, P. m. emisphyrus) in order to study the variation between and within populations (AMOVA) and to test the historical hypotheses about the intraspecific taxonomy.
We found no genetic support for the traditional intraspecific subdivision in Europe, therefore having to consider all the subspecies proposed as synonyms and the European population almost panmictic.
Instead, we found three distinct main genetic clusters, one in allopatry in the N-Africa region, most likely representing the P. saharae species, and two others living in sympatry in Europe, representing two potential cryptic species based on the degree of genetic differentiation of the two clusters.

Underestimated evolutionary differentiation under the ground: hints from geographic fine-scale variation in soil centipedes

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A comprehensive evaluation of the amount of extant organism diversity, at the species level and below, is paramount for sound ecology and evolution research. However, for the many soil-dwelling animals with poor dispersal ability, the actual degree of evolutionary differentiation among populations is underestimated, based on the taxonomy in use, and the geographical scale of differentiation is still largely overlooked. For instance, even though many centipedes are traditionally thought as widespread species with conservative morphology, our preliminary investigations are disclosing an unexpected amount of genetic and phenotypic geographic differentiation. We report on recent and ongoing studies on different geophilid centipede species within the Italian region, employing different techniques (including molecular methods of species delimitation, and analysis of subtle shape variation by geometric morphometrics), and combining different evidence in an “integrative taxonomy” approach. Our results are consistent with the theoretical expectation that the evolutionary dynamics in organisms with low dispersal ability are prone to produce much higher level of biodiversity than usually recognized.

Congruent evolutionary responses of European steppe biota to late Quaternary climate change

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Quaternary climatic oscillations had a large impact on European biogeography. Alternation of cold and warm stages caused recurrent glaciations, massive vegetation shifts, and large-scale range alterations in many species. The Eurasian steppe biome and its grasslands are a noteworthy example; they underwent climate-driven, large-scale contractions during warm stages and expansions during cold stages. We evaluate the impact of these range alterations on the late Quaternary demography of several phylogenetically distant plant and insect species that are typical of the Eurasian steppes, and for which large SNP datasets were available. We compare pre-defined explicit evolutionary hypotheses by applying an approach using convolutional neural networks for model selection, and approximate Bayesian computation for parameter estimation. We identified congruent demographic responses of cold stage expansion and warm stage contraction across all species, but also species-specific effects. The demographic histories resolved by our models reflect major paleoecological turning points that have impacted the Eurasian steppe biome in the late Quaternary. Our findings highlight that the late Quaternary climate was the driving force underlying patterns of genetic variance on the biome level.

Integrative taxonomy in the 21st century. An example from the intricate plant genus Xanthium L. (Ambrosinae, Asteraceae)

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Natural history collections are extremely important for studies aiming at resolving taxonomies in intricate groups. The use of herbarium material helps both while sampling, especially for widespread taxa, and foremost because the inclusion of name-bearing types facilitates tremendously the nomenclatural work. Herewith, we aim at delimiting evolutionary lineages in the taxonomically intricate genus Xanthium L., employing types and using phylogenomics, morphometrics, and coalescent-based species delimitation approaches.
Xanthium is a peculiar genus of the Asteraceae, characterized by spiny, wind-pollinated, female capitula (burs). Most of the morphological characters used to delimit taxa in the genus are features of burs. Due to their extreme morphological variability, taxonomic treatments have been inconsistent in the past and several names have been given by different authors. A recent study has proven that most of the taxa can be grouped into five species complexes (Tomasello, 2018). However, some of these extremely variable complexes remained widely unresolved.
To address this issue, we examined several herbaria and collected over 270 samples, 39 of which being types (more than 70% of recognisable types), covering a wide range of the morphological variation and geographic distribution of the genus. We retrieved high-resolution specimen images for morphometric analyses of leaf shapes and burs traits, and small leaf fragments for DNA extraction and subsequent phylogenomic analyses. For the latter, we combine standard extraction kits with methods used for ancient DNA and archaeobotanical remains. Thus, we were able to extract DNA and produce sequences from 200 years old samples. We applied target enrichment techniques (Hyb-Seq), and the commercially available “Asteraceae COS baits kit” to retrieve sequence data from hundreds of nuclear loci. Using integrative approaches, we delimit evolutionary lineages and link them to taxonomic names.

Phylogeography of Curruca melanocephala: an enigmatic genetic arrangement along the Mediterranean distribution range

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In the Western Palearctic, the Plio-Pleistocene ice ages have played a prominent role in addressing genetic patterns for a multitude of animal lineages. Several temperate and boreal species in this biogeographic region were profoundly investigated. However, the knowledge regarding the phylogeography of the Mediterranean basin's species still needs extensive investigation to determine broader patterns and processes of diversification. The Sardinian Warbler (Curruca melanocephala) is a largely-sedentary passerine belonging to the Curruca clade, a circum-Mediterranean genus among the Sylviidae family, recently splitted from the Sylvia group. Based on morphological characteristics, four subspecies are currently recognized, with few genetic insights and an open debate on intra-specific classifications. In this study, we analyzed both mitochondrial and nuclear markers of samples collected across the species' distributional range, aiming to deepen the phylogeographical knowledge and subspecies identification. We found some widely distributed haplotypes indicating a panmixia phenomenon among the entire distributional range. More complex scenarios are found when considering the subspecies C. m. leucogastra (Canary Islands) and C. m. valverdei (Western Sahara); for the Canary subspecies, no clear geographic lineages can be confirmed, except for unique haplotypes from Gran Canaria/La Gomera island, while for the Western Sahara subspecies a strongly supported clade was identified, including haplotypes from Morocco and El Hierro island. Furthermore, we found a clear genetic differentiation of C. m. momus from other subspecies, thus validating the Levant subspecies. Our results suggest a higher degree of mobility with respect to what has been attributed to the species, with gene flow between the populations, where recent and spaced expansion are already described. Additional samplings in different areas and advanced analysis could shed light on the genetic scenery of this species.

Mito-nuclear coevolution and phylogenetic artifacts: the case of bivalve mollusks

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Mito-nuclear phylogenetic discordance in Bivalvia is well known. In particular, the Amarsipobranchia clade (Heterodonta+Pteriomorphia), retrieved from mitochondrial markers, contrasts with the Heteroconchia clade (Heterodonta+Palaeoheterodonta), retrieved from nuclear markers. However, oxidative phosphorylation nuclear markers support the Amarsipobranchia hypothesis instead of the Heteroconchia one. Interacting subunits of the mitochondrial complexes

share the same phylogenetic signal irrespective of genomic sources, while other nuclear markers do provide a different signal: this is a clue of coevolution between nuclear and mitochondrial genes. In this work we inferred the phylogeny from mitochondrial and nuclear oxidative phosphorylation markers using different phylogenetic approaches, adding two datasets for comparison: genes of the glycolytic pathway and genes related to the biogenesis of regulative small noncoding RNAs. All trees inferred from mitochondrial and nuclear subunits of the mitochondrial complexes support the monophyly of Amarsipobranchia. Conversely, nuclear genes support the Heteroconchia hypothesis, as expected. However, not every single oxidative phosphorylation marker agrees with the mitochondrial topology: this is clearly visible in nuclear subunits that do not directly interact with the mitochondrial counterparts. Overall, our data support the hypothesis of a coevolution between nuclear and mitochondrial genes for the oxidative phosphorylation. Moreover, we suggest a relationship between the mitochondrial topology and different mitochondrial genome features among clades. We conclude that the Heteroconchia hypothesis is possibly the best evolutionary hypothesis for bivalves to date.

Among genes heterogeneity of the phylogenetic signal in genome data

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Genome-scale inference of phylogeny has reduced the stochasticity associated with single gene phylogenies, but some drawbacks of this approach are not yet fully understood, particularly the among-gene heterogeneity of the phylogenetic signal. I studied this issue in Drosophila using genome-scaled datasets. Although both datasets apparently resolve most of the relationships with high support when analysed at the nucleotide level, there are at least two types of incongruences. First, the phylogenetic signal is not homogenously distributed among nuclear, mitochondrial, and non-coding genes. Second, the phylogenetic signal is not homogenously distributed among ontology classes, whereby nuclear genes involved with the metabolism tend to carry their own signal. Most, but not all of these incongruences, are due to substitutions at synonymous sites which I show being affected by different mutational pressures in different types of data. Counter intuitively, partitioning is not successful in disclosing these incongruences, which are instead revealed by using across-site heterogeneous models or coalescent aware approaches. These results advocate that care should be taken when interpreting high supports from the analysis of genome data even at the intra-genus level. Phylogenetic incongruences may be however extremely instructive in revealing peculiar aspects of species biology such as introgression or incomplete lineage sorting.

Whole mitochondrial genome sequencing to improve population genetic inference and phylogeny of Mediterranean loggerhead turtles

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Female natal phylopatry is a mechanism which shapes population structure in many animal species. However, a breakdown in this behaviour episodically occurs, when founder individuals colonize new sites for reproduction. The Mediterranean population of loggerhead sea turtle (Caretta caretta) is such an example. The life history of this species has been studied for decades. In particular, population genetic surveys conducted mainly by assessing interindividual differences in mitochondrial DNA control region sequences have shown widespread sharing of common haplotypes among rookeries and ocean basins. This has hampered further resolution of matrilines and phylogeographic patterns which can be better addressed by investigating sequence variation of the whole mitochondrial genome. In this study, we sequenced the mitochondrial DNA (mtDNA) of 34 adult loggerhead turtles and 36 unborn embryos or dead hatchlings collected from 28 nests in Tuscany, Latium, Calabria and the Island of Linosa in the Mediterranean Sea. Genomic libraries were prepared following a PCR-free Tagmentation protocol and a low coverage (1X) whole genome sequencing was performed on a Illumina NovaSeq 6000 platform. Using this shotgun sequencing approach, we obtained high coverage (100X) mtDNA sequences. We characterized a total of 150 SNPs which assigned samples carrying the same and most frequent mtDNA control region haplotype (CCA2.1) to 11 different mitogenomic haplotypes. This result will certainly strengthen future mixed-stock analyses, whereby adult and subadult turtles are assigned to their rookeries of origin based on mitogenomic sequence frequency comparisons. Moreover, a more robust phylogeny built on mtDNA sequences will shed light on the Mediterranean origin of Calabrian haplotypes. Our work represents the first mitogenomic data set for Mediterranean C. caretta, includes previously undescribed nesting sites and paves the way for further mitogenomic studies on this species.

Posters

Genomic evidence of postglacial expansion from multiple refugia for alpine plants in the Dolomites

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The climatic oscillations of the Quaternary had a strong influence in biota in Europe and worldwide. Massive glaciers covered a major part of high European mountain ranges, therefore posing a major challenge for mountain species, which were forced to find refugia in peripheral areas or unglaciated spots within the ice core in order to escape extinction. The Dolomites, as several areas along the southern margin of the Alps, experienced strong glaciation but still offered possibilities for cold stage survival, as shown by the high diversity, endemism rates and genetic diversity values of alpine species. It remains unknown though, whether these refugia were restricted to the southern margin of the Dolomites, from where postglacial expansion northwards occurred, or alternatively, whether postglacial expansion started from several microrefugia spread across the current distribution of the species. Here we use demographic modeling based on the site frequency spectrum (SFS) to study the recent evolution of three alpine endemic plants of the Dolomites with known intraspecific genetic structure. The SFSs were calculated for each known genetic group and were used to estimate the effective population size over time. Two and three-dimensional SFS were used to find the best demographic models for the divergence among populations, and to estimate demographic parameters as divergence time, population size and migration. On the one hand, we investigated a major genetic split between eastern and western populations of Campanula morettiana and Primula tirolensis defined by the Piave Valley. The divergence largely predates the last glacial maximum, indicating long term survival of both species on each side of the valley. On the other hand, we explored the demographic dynamics within the western genetic group of the latter two species and Saxifraga facchinii. West of the Piave Valley the distribution is larger and scattered across several massifs, and the observed genetic structure provided no congruent patterns among the study species. Our demographic models indicated idiosyncratic recent demographic histories for each study species, challenging the raise of congruent patterns but rejecting the simple hypothesis of postglacial expansion from a single major southern refugium.

First insights of the invasive beetle Popillia japonica's genome

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Popillia japonica (Coleoptera: Scarabaeidae) is an invasive alien beetle, native to Japan, that in the last century has spread all over the world, from North America to the Azores and Europe, following goods and people movements. Due to its capabilities to attack and destroy a huge variety of plant species, EFSA and the JCR of the European commission have nominated the scarab as a high priority pest. For this reason, we have sampled and sequenced individuals of P.

japonica in order to obtain its first complete reference genome. Structural and functional annotations are going to be completed by means of transcriptome data from samples at different stages (larvae, pupae and adults males and females). The assembled genome is 0.64Gb in length and first encouraging statistical observations describe it with a N50 of about 720,600 nt, composed of 269 scaffold. A first BUSCO survey found 95.2% of completeness, 21% of fragmentation and 2.7% of missing BUSCOs indicating a good genome quality. Furthermore, to unravel the invasion route of the beetle, we are currently resequencing multiple genomes covering the entire distribution of the species. The aim of this part of the project is to analyse mitogenome data and call variants compared to the reference genome studying the data in terms of a) reconstructing the process of invasion from Japan to other regions; b) study of the demography of the species through bottlenecks associated to the invasion; c) study selection that may have taken place at specific genes during the invasion, e.g. life history traits, detoxification, resistance to insecticides.

The evolution of photoreception in Lophotrochozoa

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Opsins are G-coupled receptors playing a key role in metazoan image-forming vision and other photoreceptive capabilities (i.e., body orientation or circadian rhythm entrainment). While many studies enriched our understanding of opsin diversity in several animal clades, we still lack a whole picture of opsin evolution in Lophotrochozoa, one of the largest metazoan clades that includes annelids, molluscs, nemerteans, bryozoans, brachiopods, and platyhelminths. We hereby filled this gap by analyzing 74 lophotrochozoan genomes and proteomes and capitalized on recently developed maximum likelihood estimation methods to reconcile the gene and species trees. We found that the common ancestor of Lophotrochozoa possessed a plethora of opsins that underwent many gene duplications and loss events. While the lineage leading to bryozoans and platyhelminths show dramatic opsin losses, molluscs and annelids maintain opsin richness. We estimate the presence of 4 r-opsins at the base of the clade and several subsequent events of xenopsin duplications, highlighting the importance of this class of molecules. Despite that, many opsins remain functionally uninvestigated and, given the wide plethora of opsins, visual structures and lifecycles, we suggest that Lophotrochozoans (especially molluscs) represent excellent models for studying the evolution of photoreception in animals.

PhyloCloud: an online platform for making sense of phylogenomic data

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Rapid growth of genome data over the last decades generates large amounts of phylogenomic trees and multiple sequence alignments, which may be enormous in size, providing opportunities to the evolutionary history of species. However, the analysis and interpretation of such data still rely on custom bioinformatic and visualisation workflows that are not entirely user-friendly for researchers without prior programming background. Besides, there is increasing demand for fast exploration of large trees and comprehensively managing a large number of phylogenomics data. Here we present PhyloCloud, an online platform aimed provide a one-stop solution of hosting, managing and exploring large phylogenetic tree collections, providing also various options of analysis and operations, such as taxonomic annotation, searching, topology editing, automatic tree rooting, orthology detection, evolutionary events detection, etc. Besides, PhyloCloud provides a handful of phylogenetic tools such as allowing users to reconstruct their own phylogenies using predefined workflows, graphically compare tree topologies, and query taxonomic databases such as NBCI or GTDB. It is worth mentioning that PhyloCloud utilized a novel tree visualisation system empowered by ETE Toolkit v4.0, which can be used to explore very large trees up to one million tree nodes and enhance them with custom annotations and multiple sequence alignments. The platform allows for sharing tree collections and specific tree views via private links, or make them fully public, serving also as a repository of phylogenomic data. PhyloCloud is available at https://phylocloud.cgmlab.org
NCBI: National Center for Biotechnology Information
GTDB: Genome Taxonomy Database
ETE Toolkit: Environment for Tree Exploration Toolkit

Investigation of the evolutionary history of common bean through nuclear and chloroplast genomes

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Knowledge about the origin, evolution and expansion of crop species is crucial for their conservation and exploitation. Phaseolus vulgaris has a unique evolutionary history, with the wild form originated in Mesoamerica and subsequently introduced into South America, leading to the formation of two additional wild gene pools in North Peru and Ecuador and in South Andes. However, the debate on common bean origin is still open. Indeed, recent studies proposed the so-called “Pseudovulgaris” hypothesis on the origin of common bean, that indicates the origin of the North Peru and Ecuador gene pool as occurred much earlier than that of P. vulgaris species and, thus of the diversification of Mesoamerican and Andean gene pools. In this case, the North Peru-Ecuador population represents a different species, named P. pseudovulgaris (P. deboucki) and it shared a common ancestor with the Mesoamerican and Andean groups, that remains to be discovered or has become extinct. Here, by analysing the phylogeny of P. vulgaris we aim to better investigate the P. vulgaris origin and verify the different hypotheses. A wide sample that represents the entire geographical distribution of the wild forms of the species was genetically characterized for chloroplast genome diversity. A concatenated sequence of 3,231 chloroplast informative sites

was used to build a phylogenetic tree. Moreover, 37 de novo chloroplast genomes were assembled and used to provide a temporal frame of the divergence for the analysed genotypes, suggesting that the separation between the Mesoamerican and the North Peru-Ecuador gene pools occurred 0,15 Mya. Nuclear data, from the resequencing of a sample of ten accessions, were used to corroborate results. Overall, analyses of nuclear and plastid data support monophyletic and Mesoamerican origin of common bean.

SYMPOSIUM

BEHAVIOUR AND ECOLOGY: AN EVOLUTIONARY PERSPECTIVE

CHAIRS: LISA LOCATELLO, DONATO GRASSO

Invited speaker GIL GUASTONI ROSENTHAL, Department of Biology, University of Padova, Italy

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Behavioural mechanisms, macroevolution, and global change

As the interface between an animal's internal processes and its environment, behavior constitutes not only a primary target of selection but also a driver of evolutionary change. In particular, individual mating decisions can play a determining role in structuring gene flow among lineages, and this role is highly dependent on natural and anthropogenic changes in the environment. Long-term studies on swordtail fish from Mexico's Sierra Madre Oriental yield insights into how sensory and cognitive mechanisms modulate hybridization and reproductive isolation.

Talks

Molecular mechanisms orchestrating sexual maturation and reproduction with the lunar cycle

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Many species orchestrate behavioral aspects and reproduction with the monthly lunar cycle. These phenomena are particularly widespread in the marine environment, yet examples have been reported in terrestrial species as well, including humans. In spite of their relevance, the underlying molecular mechanisms remain elusive, limiting also our understanding of the potential effects of light pollution on animal physiology. We investigate these aspects in the marine annelid Platynereis dumerilii a genetically-accessible model which synchronizes its reproductive events with the lunar cycle. Specifically, we took advantage of a knockout strain for the light-sensitive cryptochrome (the ortholog of Drosophila cry), which shows altered monthly reproductive rhythms in laboratory conditions. Our molecular analyses revealed that lcry knockouts have altered expression of the homologs of key genes for vertebrate reproduction , such as those encoding gonadotropin-releasing hormone-like peptides and gonadotropins subunits. Based on potential similarities in the role such hormones play in vertebrates and annelids, we predicted lcry knockout worms should delay their sexual maturation and gonadal development as well. Interestingly, we found mutants being characterized by dampened gonadal maturation, achieving sexual maturation with a dramatic delay. Moreover, compared to wild-types, these worms showed a desynchronized onset of the last phase of sexual maturation with respect to the moonlight stimulus. Taken together, our data shed light on the poorly characterized molecular mechanisms underlying lunar-regulated animal reproduction, unveiling surprising similarities in the regulatory networks orchestrating worm and vertebrate sexual maturation/reproductive timing.

When order matters: fertilisation patterns in the polyandrous common cuttlefish

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Besides traditional sexual competition mechanisms, polyandrous species adopt strategies in order to manipulate paternal contribution, including competitors' sperm removal. Males mating order (MO) and females mating history (MH) have a direct influence on such competitive behaviours and eventually on fertilization rate. In cephalopods, both behavioural and genetic approaches were used to investigate sexual competitive mechanisms, but they have seldom been combined. In the present study, we performed controlled mating experiments considering MO and male awareness about female MH coupled with microsatellite analysis to determine: (i) how MO of males and MH of female affect sexual competitive strategies and (ii) how such competitive behaviours affect paternal contribution in the common cuttlefish (Sepia officinalis). We performed 30 controlled mating experiments where each female (N=10) mated with 3 different males (N=30). Males were either aware (AM) or unaware (UM) about female's MH. AMs witnessed the mating of female with the other males. Paternal contribution, assessed by employing 7 microsatellite loci, was highly correlated with MO. The last male had a significantly higher paternal contribution than previous males. Female's MH affected males' performance. Sexual competitive behaviours were observed both in AMs and in UMs, when they mated a female that had previously mated. Thus, female's MH may not be a significant factor in inducing competition. Males could perceive that the female has

already mated with others, without witnessing. Also, some females flushed by using the siphon, maybe for escaping or avoiding spermatophores transfer. So, both sexes seem to play a role in paternal contribution to the offspring.

Deimatic behavior correlates with personality and population history in the Apennine yellow-bellied toad Bombina pachypus

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Understanding the mechanisms underlying the origin and maintenance of inter-individual variation in animal behavior is one of the main challenges of eco-evolutionary research. In this study, we investigated the link between individual personality and anti-predatory behavior by focusing on deimatic display, an anti-predatory strategy consisting in prey suddenly displaying striking behaviors to startle predators. By combining camouflage and aposematic displays in a complex and time-structured behavior, deimatic species provide appealing systems to investigate the processes maintaining alternative behaviours within populations. Yet, the extent of inter-individual variation in deimatic behavior is still almost unexplored. We characterized the extent and pattern of inter-individual variation in the deimatic behaviour named unken-reflex (consisting in prey suddenly arching the body and exposing the aposematically-colored ventral side) and a suite of personality traits along the boldness/shyness axis in three populations of the yellow-bellied toad Bombina pachypus. The unken-reflex behaviour varied among individuals, both within and among populations, and this variation was repeatable across multiple trials. About half of the toads reacted to simulated predator attacks by performing the unken-reflex; the others did perform unken-reflex, but rather moved away. We also found significant association between personality traits and deimatic behavior: shy individuals showed a higher occurrence of the unken-reflex. Finally, we found a geographic structure of phenotypic variation likely reflecting the species' genetic structure: populations from the glacial refugium and genetic diversity hotspot for this species showed higher phenotypic variation than populations that originated via postglacial expansion.

Do cnidarian polyps remember their last meal? The role of learning in the protocooperative capture of large prey

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Small-sized cnidarian polyps can eventually engage in collective catching of large prey through protocooperation. Such collective macrophagous feeding behaviour is rather different from the suspensivorous one characterizing cnidarian polyps, since it requires rapid movements and contractions that are seldom observed when catching small planktonic prey. Little is known on the mechanisms allowing the polyps to switch from one feeding strategy to the other, and if protocooperation is a casual behaviour or a stable strategy characterizing gregarious polyps. Non-associative learning in cnidarians is often associated with feeding stimuli. We hypothesize that polyps learn how to cope with prey based on its dimension. For this reason, we tested the learning ability in groups of Aurelia coerulea polyps (n=5, 5 replicates) by providing three prey types requiring different seizing strategies (the large Syllis prolifera, and the small planktonic Artemia salina and Brachionus plicatilis). After complete digestion, polyps were provided with different homogenates (HOM) of the different preys, observing and categorizing their behaviour each 30 seconds for 10 minutes. The number of tentacles contractions and rapid mouth movements (TC/MM) was used as proxy for feeding excitement. HOMs triggered different behaviours in polyps in relation to their last meal. TC/MM were significantly higher in polyps that received the combination of the large prey and its HOM (S-s treatment). The percentage of polyps playing the feeding behaviour was up to 92%. From these results, we can hypothesize that A. coerulea polyps facing large prey are able to learn and remember how to cope with it. Memory would thereby reinforce the ability of polyps to engage in protocooperation, allowing them to enhance individual predation success and to access to much larger preys.

Investigating physiological differences between native and invasive strains of the arboviral vector Aedes albopictus

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The rapid global invasion of the Asian tiger mosquito, Aedes albopictus, has resulted in public health concern due to its competency for several arboviruses, leading to outbreaks of diseases like Dengue and Chikungunya. Understanding the traits that favour successful expansion and establishment of Ae. albopictus outside of its native range will enable us to develop strategies for mitigating its future spread. To gain insights on traits that can influence the invasion success of this mosquito, we compared physiological and fitness characteristics of one strain from the native home range in China, called Foshan, and one invasive strain from Mexico, Tapachula, where Ae. albopictus was first intercepted in the early 2000.
We observed that invasive mosquitoes are statistically larger than those of the Foshan strain, in both sexes, but there were no differences in lifespan. Invasive mosquitoes also had higher fecundity than Foshan females, but lower fertility, suggesting differences in egg development. To further investigate egg production by the two strains, we performed a proteomic analysis of ovaries after a blood-meal. We identified many proteins responsible for oogenesis, growth and development, transporting mechanism, cellular multiplication and transcription in both strains, with 109 and 86 proteins detected only in Foshan or Tapachula, respectively. Unique proteins found in Tapachula ovaries are mainly related to efficient storage and utilization of energy, development of oocytes and pathways that possibly affect female fecundity and oogenesis. Proteins identified only in Foshan are associated with the process of embryogenesis that follows oogenesis. These results suggest that egg development in Foshan mosquitoes is faster than in Tapachula mosquitoes, whereas Tapachula mosquitoes are better in storing glycogen and lipid reserves, as reflected also in their body size.
Overall, these results show that invasive mosquitoes have a larger size, higher fecundity and higher ovarian protein content, suggesting these traits may be linked to their invasion success.

Whether these phenotypes were selected or emerged during the invasion process is currently under investigation. Knowledge of the molecular phenotypes and physiological responses of different Ae. albopictus strains will enable us to implement novel strategies of vector control such as mass-production of genetically- or biologically manipulated mosquitoes and to design mathematical models of mosquito populations or control programs.

Vampire tales: adaptations to hematophagy in the marine snail Cumia reticulata

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Despite being a quite uncommon trophic habit in molluscs, blood-feeding has convergently evolved in at least three families of Neogastropoda: Cancellariidae, Marginellidae, and Colubrariidae. The buccinoidean family Colubrariidae includes about 30 marine species inhabiting mostly shallow-water hard bottoms in tropical, subtropical and temperate seas. The entirety of the species included in the family are considered hematophagous and feed during the nighttime on benthic fishes, which are contacted by extending an extremely long proboscis to gain access to the blood vessels. In the last years, we have actively investigated the molecular basis of this peculiar feeding habit, in the Mediterranean colubrariid vampire snail Cumia reticulata. Using a transcriptomic approach, we have identified several protein families that play key roles in hematophagous feeding, and we have carried out an in-depth investigation of their molecular diversity and evolution, which is often characterized by recurrent domain and gene duplication events, as reported for other blood-feeding and venomous organisms. We have further focused on the characterization of a novel protein family with a peculiar architecture, comprising exclusively vWA1 domains, for which we were able to experimentally confirm an antiplatelet activity of great adaptive value, but also of remarkable biotechnological interest. Our results shed a new light on the molecular adaptions underlying hematophagy in marine gastropods, which seem to be characterized by a high level of tissue and lineage-specificity, in some cases accompanied by a remarkable intraspecific variability, and by instances of structural and functional convergence with respect to other lineages of blood-feeding Metazoa.

Cognitive plasticity in teleost fish

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The cognitive abilities of an individual are often linked with its success in interacting with the environment and ultimately, with its fitness. Nevertheless, individuals of the same species are often

exposed to spatio-temporal variation in environmental conditions. Considering that the neural tissues are among the most expensive tissues in terms of metabolic requirements, it possible to hypothesise that selection might favour adaptive phenotypic plasticity in cognitive abilities. Here, we analysed cognitive plasticity in a teleost fish, the guppy Poecilia reticulata. In a first study, we focussed on plasticity in response to social environment experienced by the individuals. We subjected new-born guppies to treatments manipulating the dimension and the stability of the social group and when they reached the age of 1 month, we assessed subjects' cognitive control. We found that guppies reared as singletons displayed enhanced ability inhibit an inappropriate foraging behaviour compared to guppies reared in pairs and guppies reared in 6-individual groups. Furthermore, guppies reared in a stable social group, showed a greater level of inhibition compared to individuals reared in an unstable social group, simulated with continuous fission and fusion events. In a second study, we compared guppies experiencing a generally enriched environment provided with vegetation, natural substrate, live prey and social companions, with guppies experiencing a non-enriched environment. Enrichment treatment did not affect our measures of cognitive control (reversal learning task and inhibitory control task). Yet, we found that enrichment enhanced guppies learning ability in a colour discrimination task. In a third study, we manipulated predictability of food resources. Results indicated that guppies exposed to food sources that varied in space and time developed greater inhibitory abilities compared to guppies exposed to predictable food sources; learning was not affected by this treatment. These results highlighted the presence of phenotypic plasticity in the cognitive abilities of the guppy. Interestingly, different cognitive functions were altered according to the environmental factors that we manipulated, suggesting trait-specific plasticity.

Contrasting respiratory responses define different thermal niches in two families of semi-terrestrial crabs

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Multiple independent terrestrialization events contributed to the current vast biodiversity on land, with true crabs being one of the last groups colonizing the land. Among all aspects of adaptation, changes in respiratory system largely determines the species' dependency on aquatic medium and hence, primarily affects the degree of terrestriality, which ultimately determines the extent of the colonization of the terrestrial habitats.
Ocypodidae and Sesarmidae are both semi- terrestrial families in the Brachyura that share the same grade of terrestriality. Three semi terrestrial mangrove species from each of the family were selected for this study. They inhabit the intertidal area with close proximity to each other. Yet, when placed in aquatic and aerial medium respectively, patterns of partial oxygen pressure in their haemolymph and oxygen consumption rate revealed contrasting respiratory performances. Despite the nature of their amphibious lifestyle, these results suggest Ocypodidae are better adapted in extracting oxygen from air and have lowered dependency on gills for respiratory purpose.
According to oxygen- and capacity- limited thermal tolerance (OCLTT) hypothesis, this also implies the benefit of displaying a widened thermal window. This coincides with results from field logged temperature data, showing ocypodids inhabit hotter microhabitats in the mangrove compared to sesarmids. The differing respiratory strategies of the two families lead to ecological consequences that are intrinsically linked to their respective thermal tolerance.
The widened thermal window allows ocypodids to colonize open mudflats in high densities, as their burrows are often found to be located at exposed intertidal regions under direct sunlight, where soil surface temperature can reach up to 40°C. Unlike the ocypodids, sesarmids do not share morphological adaptations such as lung- like organs, instead they recirculate branchial water and

retain gaseous exchange dependency on gills. Hence, their lower thermal tolerance limits their activity close to vegetation or shaded regions.
With the use of comparative physiology analysis, we demonstrated that the respiratory performance of semi terrestrial crabs reflects their true degree of terrestriality, and explains their ecological distribution with their unique thermal niche.

The rise of cryptic female choice: a lesson from two external fertilizers

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When females mate with multiple males within the same reproductive episode, the different ejaculates have to compete for fertilization. Sperm competition has traditionally been viewed as an extension of male competition, and therefore as an intra-sexual process. However, we also know that the arena in which sperm competition occurs permits cryptic female choice (i.e., sperm selection) and that this process is often able to strongly influence the outcome of sperm competition. Despite this, cryptic female choice is likely the least investigated process of post-mating sexual selection.
Female reproductive fluid (FRF) has recently been suggested to be a mediator of cryptic female choice, particularly through its effects on sperm traits. However, the mechanisms by which FRF mediates sperm selection and its overall influence over sperm competition remain obscure.
In my presentation I will describe two examples of how the effects of FRF can overturn the outcome of sperm competition, in two externally fertilizing fish species with different reproductive behaviours.
The first case study is the zebrafish (Danio rerio), a species characterized by a high level of male pre-mating competition and limited female choice. Here we demonstrated that in water the paternity success of competing males is predicted by sperm velocity, but not in presence of FRF, which affects both sperm performance and fertilization success, enabling females to exert a cryptic choice, thus influencing the outcome of sperm competition.
On the other hand, in the grass goby (Zosterisessor ophiocephalus), a species with distinct territorial-sneaker mating tactics and a strong female pre-mating preference towards territorial males, we discovered a differential effect of FRF over sneaker and territorial sperm performance and fertilization success, reinforcing female pre-mating preferences. Such an effect is mediated by the different FRF concentrations experienced by the competing ejaculates, consequence of the males' relative proximity to the female, and allows females to discriminate among male phenotypes and control the sperm competition outcome.
Together, these two examples, depict a powerful mechanism of female post-mating control, mediated by FRF, and “tailored” to the female needs, shedding light on the crucial role of female processes in the sperm competition game.

The role of mimicry in the evolutionary history of Palearctic ants: the case of Colobopsis imitans and Co. truncata revealed through a multidisciplinary approach

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In the W-Palearctic, the genus Colobopsis has long been considered to consist in a single species, Co. truncata. Our field observations combined with scattered literature accounts suggested peculiar differences in chromatic patterns and behavior, possibly used to mimic two different ants as model species. We questioned the significance of these differences by relying on integrative taxonomy: using a multidisciplinary approach combining ecological, ethological, genetic, morphological and biogeographical evidence, we discovered that the W-Palearctic Colobopsis populations actually represented two species. Workers of the newly described Co. imitans are characterized by a chromatic pattern closely resembling the ant Crematogaster scutellaris, on the contrary, workers of Co. truncata resemble those of the ant Dolichoderus quadripunctatus. Both Cr. scutellaris and D. quadripunctatus form populous colonies and possess well-developed defenses. Co. imitans also habitually follows the large foraging trails typical of Cr. scutellaris. The two Colobopsis species show a significant yet subtle morphometric separation and a polyphyletic phylogenetic pattern for the mtCOI gene. These characteristics suggest a recent isolation between the two taxa. Moreover, they are allopatric, following well-known biogeographical boundaries, and their ranges overlap with those of their models, with which they often share the same nesting tree. A strong divergence of appearance and behavior despite a recent isolation suggests that mimicry can play a significant role as a diversification driver in ants. Further investigation is needed to identify key predators responsible for the selective pressures leading to these adaptations.

Kleptopredation: how (and why) do nudibranchs eat plankton?

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Aeolid nudibranchs are often associated with specific species of hydroid. The association has long been assumed to be a simple predator-prey relationship, with the added bonus for nudibranchs capable of assimilating cnidocils from hydranths and appropriating them for their own defense. We have shown that while the aeliod nudibranch Cratena peregrina does prey directly on the hydranths of Eudendrium racemosum, it preferentially consumes polyps that have captured and are handling prey. Stable isotope analyses suggest that hydroid polyps form only a part of the nudibranch's diet by volume. We propose that the nudibranch optimises its use of a finite habitat by maximising energy intake through targeting hydroid prey, and ingestion of the polyps themselves is, to an extent, incidental. Consumption of feeding hydranths provides higher calorific content, satiating the nudibranch with consumption of fewer polyps and thus extending the life of the hydroid colony. Here we explore new data from similar hydroid-nudibranch pairings, report flume experiments showing behavioural reactions to olfactory stimuli, and speculate on the evolutionary origin of kleptopredation.

Posters

Scent pouch microbial communities of wild brown and spotted hyenas from southern Namibia

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Species of the Order Carnivora feature highly developed structures which release volatile odorous compounds implied in social behaviors, including territoriality and recognition of individuals. The “fermentation hypothesis” posits that the released odorous compounds are actually synthesized by symbiotic bacteria colonizing these scent-producing structures. Bacterial communities may confer specific olfactory signatures to social groups. Individuals release specific chemical signals used to discriminate between both single animals and groups. This phenomenon is particularly important in hyenas, which possess specialized scent pouches for odorous secretions production. Indeed, microbiota evidence coherent with fermentation hypothesis has been gathered on wild spotted and striped hyenas in Kenya.
In the present work, we characterized for the first time the microbial communities of the scent pouch of wild brown hyenas of the Namib desert, in collaboration with the Brown Hyena Research Project (B.H.R.P.). This species is one of the most specialized of the Order, featuring a complex scent pouch able to produce two distinct secretions used in different contexts. We sampled the two different secretions from five captured animals and from markings found in the environment in the territories of two clans, and characterized their microbiota by V3-V4 16S rRNA amplicon metagenomics. We also included in the study secretions of two spotted hyenas previously sampled by the B.H.R.P.
The analysis revealed a signal for species-specific bacterial communities in brown and spotted hyenas, including numerous fermentative and anaerobic bacterial taxa. Moreover, in brown hyenas, the two distinct secretions showed different levels of alpha diversity and no effect of clan membership was reported. Our results provide further evidence for the fermentation hypothesis, which has to be considered in the wider context of studies of the same field of research.

Litter caching behaviour in mangrove crabs

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Plant litter is the main food source of many mangrove crabs. Litter caching behaviour has been observed in some of these species. The crabs collect and store litter into their burrows before consumption. It has been proposed that caching litter in burrows reduces competition for food sources among crabs. This study investigates such behaviour in two species, from two families,

inhabiting the supralittoral zone, namely Neosarmatium indicum and Chasmagnathus convexus. Time-lapse cameras were deployed to record the behaviours of the crabs. The occurrences of litter caching behaviours of the two species were compared and time-budget analysis was carried out. Moreover, the 'leaf-aging' assumption suggests that storing litter in burrows enhances the palatability and nutritional value of this food. To test this hypothesis, a field experiment was carried out to assess the effect of litter storing on its chemical characteristics. Mesh bags containing yellow leaves of two mangrove associate species, Hibiscus tiliaceus and Cerbera manghas were placed in the burrows of the above species, artificial burrows and outside the burrows respectively. The changes in carbon: nitrogen ratio, total phenolics content and Klason lignin content were compared among treatments. The results of these observations and experiments improve our understanding to the ecology and behaviours of these important species.

Implementation of physiological performance in Species Distribution Models to assess the vulnerability of tadpoles to climate change.

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Ectotherms are particularly vulnerable to climate change due to their natural dependence on environmental temperatures. Amphibians are acutely threatened because of their biphasic life cycle. In temperate populations of anurans, tadpoles are a crucial life-stage, since they may be more vulnerable to increasing temperatures. Bioclimatic envelope models are useful tolls to understand the potential vulnerability of anurans to climate changes. Nevertheless, most models do not take into account information on the mechanistic links between the species' current distribution ranges and climate. There is a need to combine performance physiology with bioclimatic models to predict ecological and evolutionary responses to global change. We assessed the effects of different temperatures (T0=5 C°, T1=10 C°, T2 =15 C°, T3 =20 C°, T4 =25 C°, T5 =30 C°) on thermal performances curves (TPCs) of swimming speed in tadpoles of common toad (Bufo bufo) and Italian frog (Rana italica). We evaluated the locomotor performance as index of fitness and survival rate of these species. Common toad is characterized by a board geographic range and good adaptations to different niches, while the Italian frog is a geographically restricted specie adapted to specific habitat and environmental conditions. We compared these two amphibians to assess if species with restricted distribution are more susceptible to climate change than species with broader distribution. We used the outcomes from TPCs of the two species to set the fundamental thermal parameters of ecological niche models. The modelled distributions based on our physiological trials allowed us to identify the area that will be most affected by climate change and not suitable for the two species and to better understand the variability of physiological traits, which predict ecological and evolutionary responses to global change. The results can provide useful tools in the management for the conservation of freshwater communities.

Do captive fish need cognitive enrichment? A study with a foraging problem-solving task

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Some animal species might be hardwired to perform cognitive tasks and solve problems during foraging due to their evolutionary history. Providing similar cognitive enrichment in the housing conditions might be a useful approach to increase the welfare of captive animals. In line with this idea, most of the species investigated so far (mammals and birds) displayed a marked preference towards problems and complex foraging situations versus freely available foraging opportunities. We currently do not know whether this holds true for the group of vertebrates with the highest number of individuals breed in captivity, the teleost fish. In our study, we exposed the poeciliid fish guppy to the choice between foraging on freely available food (as in normal housing conditions) and on an enriched foraging context in which they have to solve a problem, i.e., removing a small obstacle that hid the food. If guppies benefit from the foraging enrichment, they were expected to display some interest in solving the foraging problem in spite of the freely available food. Our results indicated that most of the subjects quickly learned to solve the problem. Generally, the guppies preferred to first consume the freely available food. However, in approximately 20% of the trials, the guppies spontaneously tackled the foraging problem before consuming the free food, suggesting at least a modest interest for the cognitive enrichment. Interestingly, male guppies displayed much lower propensity to first solve the foraging problem (males: 11%; females: 24%). Moreover, females usually solved the foraging problem even after consuming the freely available food whereas males did not so. Because males proved to consume two freely available food sources in a control test, we speculate that females (but not males) have been selected to 'go the extra mile' to obtain additional food. Overall, our study indicates that developing cognitive enrichments might improve welfare in captive fish, although this strategy might have a different effectiveness in the two sexes.

Heterozygosity-fitness correlation in a woodland rodent: more heterozygous individuals have higher nutritious quality habitats

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In this communication we use over 100 woodmice (Apodemus sylvaticus) of both sexes to explore the effects of heterozygosity on mouse habitat selection. We show that more heterozygous mice exploit the habitats with highest nutritious quality as measured by the biomass of edible invertebrates. To conduct the study we used 3 years of data from an intensively monitored rodent population at Silwood Park Campus (Imperial College London). We scored heterozygosity at 14 microsatellite loci for over 100 mice to get individual estimates of their genetic quality. We also characterized the diversity and biomass of edible invertebrates across the study site. Finally, we used rodent relocation data using a novel technology of mobile data loggers to determine individual home ranges (HR) and the areas within individual HRs that individuals used more intensively. Our results show that mice with higher individual genetic diversity use areas of the open native woodland in which the abundance of nutritious protein food resources is higher. Whilst more homozygous individuals use areas where the amount of edible invertebrate biomass is lower. This strongly suggests that individual genetic diversity provides an advantage either in terms of increased competitive skills with conspecifics, or in terms of cognitive abilities that allow them to exploit the habitat in a way that maximizes their fitness. This finding has important theoretical implications highlighting a new ecological mechanism (spatially-structured genetic variation)

through which heterozygote advantage can maintain genetic variation in wild populations. nuclear and plastid data support monophyletic and Mesoamerican origin of common bean.

SYMPOSIUM

BIODIVERSITY CONSERVATION USING GENES AND GENOMES

Organized in partnership with the European Reference Genome Atlas (ERGA) consortium

CHAIRS: **GIORGIO BERTORELLE, CLAUDIO CIOFI**

Invited speaker CAMILA MAZZONI, Berlin Center for Genomics in Biodiversity Research, Germany

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Biodiversity conservation and the need of high quality genome

Biodiversity is being lost faster than science is able to catalogue and study the species of our planet. New Genomic technologies can help us describe the genome sequence of virtually any living species, and unveil the most basic and essential information that forms an organism. Reference genomes are highly contiguous, accurate, and annotated sequence assemblies that represent the structure and organisation of the genome of a species. Thanks to the consolidated and standardised efforts of international genome initiatives, reference genomes are becoming a reality for an exponentially growing number of species. This is mostly due to recent technological advances, which enable the assembly of almost any species regardless of genome size and complexity. Conservation genetic studies and applications have increasingly used different genomic techniques as opposed to the traditional low number of markers. These techniques - such as genome reduced representation and SNP chips - can offer statistical power for a number of population and phylogenetic parameters but are mostly “anonymous” and are usually not connected to corresponding regions of the genomes. Even for those species with annotated draft genomes, a lot can be missed in terms of information, such as structural variants, functional variation related to multicopy genes and complex regions of the genome that may be more prone to diverge. In this talk, I will bring examples of the usage of reference quality genomes in species with conservation issues and discuss their need in different situations. Finally, I will introduce a pan-European initiative that aims to promote the sequencing of the entire European biodiversity, the European Reference Genome Atlas (ERGA).

Invited speaker ASTRID VIK STRONEN, University of Ljubljana, Slovenia

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Genomic analyses of gray wolf (Canis lupus) populations in Eurasia

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Background: Wide-ranging species such as the gray wolf (Canis lupus) can disperse several hundred kilometers, although recent studies demonstrate that their population structure at times reflect physical, environmental, or ecological boundaries. For certain populations this structuring represents long-standing isolation and genetic drift, whereas for others there does not appear to be obvious barriers to dispersal. In Eurasia, especially in the western part, north-south gene flow may be more limited than that occurring east-west because of physical features including mountain chains and water. We examined gray wolf genomic profiles from across Eurasia to determine broad-scale population genetic structure. We expected to find stronger north-south than east-west structuring, and a higher degree of isolation and genetic drift in peninsular populations.
Methods: The analyses included over 700 wolves genotyped on the Illumina CanineHD BeadChip with more than 170,000 single nucleotide polymorphism (SNP) loci, encompassing previous and recently genotyped profiles. These comprise samples from central and eastern Russia and the Caucasus, and in Europe we sampled wolves from the Dinaric-Balkans, Italy, and Iberia in the south to Scandinavia in the north. After merging data sets, we filtered for individual and SNP genotyping quality, minor allele frequency, and loci in Hardy-Weinberg and linkage disequilibrium. We examined population genetic structure by comparing results from principal component analyses and maximum-likelihood methods, assessed population differentiation, and evaluated the relationship among populations with TreeMix.
Results: We detected population clusters in central and eastern Russia, Caucasus, Iberia, Italy, the Carpathian Mountains, the Dinaric-Balkan region, northcentral Europe, and Scandinavia. Italy emerged as the most divergent population and, in general, our findings indicate higher east-west than north-south gene flow. Finer-scale genetic structure across relatively short geographic distances was observed in the Carpathian Mountains versus neighbouring regions north and south, between Dinaric and Italian wolves, and between Dinaric and Balkan wolves. Population differentiation results reflected protracted isolation and genetic drift in the Iberian, Italian, and Scandinavian wolves, which contrasted with limited genetic differentiation in other areas, particularly between wolves in central and eastern Russia. The TreeMix results also reflected the isolation and genetic drift observed in peninsular populations, especially for Scandinavia and Italy.

Talks

From conservation genetics to conservation genomics: the case of the endangered land 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. Habitat reduction, intensive agricultural practices and forest fires are major causes of decline in different areas. Intense harvesting for the purpose of pet trade and the release of non-native individuals into local populations represent additional threats. Our previous genetic studies based on STR and ddRADseq markers were able to clearly distinguish two subspecies (the eastern T. h. boettgeri and the western T. h. hermanni) and to identify some major geographical groups, allowing also the development of practical genetic tools for geographic assignment. More recently, we generated a high-quality assembly and annotation of the T. hermanni genome using a combination of PacBio HiFi and Oxford Nanopore Technologies data. The assembled genome size was 2.26 Gb with a contig N50 of 58.76 Mb. The genome included 22,017 protein-coding genes and repetitive elements constituted 45.5% of the assembled genome. The genome assembly and the set of annotated genes yielded 97% and 95% completeness scores, respectively when compared with the BUSCO Sauropsida dataset. In addition, we re-sequenced whole genomes at ~20x coverage of six individuals, three for each subspecies. Preliminary results confirmed a clear distinction among the two subspecies, and the major geographical groups, and a higher diversity in the eastern subspecies. Moreover, we are investigating coding regions across the genome to identify potential loci responsible for adaptive differences among populations/subspecies. In conclusion, these genomic resources will allow us to better understand the effective risks of translocation and hybridization in this species and propose management plans based on functional genomic regions.

News from Mozambican rivers: what we know about freshwater fish biodiversity?

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Most of the biodiversity richness in Mozambique is still unknown. Since 2016 we were sampling and collecting different freshwater fish in six southern rivers of the country. BioForMoz project is involved to describe the biodiversity of different taxa from insects to mammals and fish. With a multiparametric and integrated approach, we will study freshwater biodiversity to improve and fill the gaps in fish distribution and biodiversity. We are presenting the first data on freshwater fish biodiversity studied with DNA barcoding and morphometric characteristic. Molecular barcoding results showed new haplotypes for Tilapia's group, Amphilidae and Cyprinidae families and a new

taxonomy identity for the genus Enteromius. For the first time will be presenting a new genetic identity of different taxa and their communities' relationships.

Freshwater fish conservation are focusing on countries where the missing and scarce datasets are higher than in others. In these countries, due to climate changes and human activities, the most threatened fish species are migrants. In addition to the molecular barcoding approach, we are presenting the setup of the study of the GREB1L: a central regulator of vertebrate development, specifically affecting renal, gonadal, and inner ear organ systems gene. As recently described, GREB1L is linked to the migration phenomenon. Inside BioForMoz we are studying this gene in different eel species (Anguilla sp.). Our results are focusing to understand the evolution of GREB1L gene across different eel species living in Italy and in Mozambique.

Evaluating the past, present and future effects of climate change on a steppe-specialist raptor

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Climate change is rapidly altering local environmental conditions. To mitigate climate change effects, we need to understand how species have coped with past environmental changes, and incorporate knowledge on the neutral and adaptive forces governing present population dynamics. Assessing the potential impacts of climate change is especially important for species which are expected to be most affected by rapid environmental changes, such as habitat specialists and rare or endangered species. We integrated landscape genomics, demographic analyses and ecological modelling to evaluate the effects of environmental change on past, present and future population dynamics of a migratory steppe-specialist raptor, the lesser kestrel (Falco naumanni). Despite low genetic differentiation, we identified two main genetic clusters, corresponding to European and Asian populations. These lineages diverged during the Last Glacial Period (LGP), coinciding with the onset of a five-fold population decline and an overall decrease in suitable breeding areas. Using genotype-environment association (GEA) analyses, we identified 65 variants associated with bioclimatic variables in potential candidate genes for local adaptation. These variants were used to derive metrics of potential maladaptation with and without considering dispersal. Combining this approach with future species distribution models, we show that Asian populations, and in particular populations at the contact zone, are at higher risk of maladaptation, suitable area reduction, increased migratory distance and hence extinction. Integrating assessments of maladaptation with demographic analysis and species distribution models allows us to better understand the responses of species to climate change and to inform conservation efforts.

Genomic variation and accumulation of deleterious mutations in the critically endangered Aeolian wall lizard

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Small populations give a unique opportunity to investigate the relative roles of drift and selection in evolution. In particular, small populations can show an accumulation of deleterious mutations (the genetic load) due to the strong effects of drift. The Aeolian wall lizard Podarcis raffonei is endemic to the Aeolian archipelago, located in the Mediterranean Sea, North of Sicily. The extremely restricted distribution range includes two small islets (La Canna, 1,800 m2, and Strombolicchio, 7,000 m2). Our project aims at investigating the genomic variation pattern and the genetic load in small populations of this species, and to compare them with those observed in the sister species Podarcis waglerianus (the Sicilian wall lizard), where the distribution range and the population size are much larger. A newly assembled genome was produced for the Aeolian wall lizard, and whole genomes were resequenced for ten individuals each from La Canna and Strombolicchio (estimated population size: 50 and 500 individuals respectively) and for ten individuals of the sister species. The Aeolian wall lizard shows a uniquely low level of polymorphism, in particular for the La Canna population that has 300 times less polymorphic sites than the Sicilian wall lizard. Annotation-based estimates of deleterious mutations revealed a high genetic load in the smallest La Canna population. Levels of variation and genetic load estimates are therefore clearly affected by the

population size in this system, suggesting that more conservation actions should be implemented at least in the smallest islets.

Population structure, genomic diversity and demographic history of Komodo dragons inferred from whole-genome sequencing

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Population and conservation genetics studies have greatly benefited from the development of new techniques and bioinformatic tools associated with next-generation sequencing. In particular, analysis of extensive datasets from whole-genome sequencing of even a few individuals allows detection of patterns of fine-scale population structure and detailed reconstruction of demographic dynamics through time. In this study, we investigated the population structure, genomic diversity and demographic history of the Komodo dragon (Varanus komodoensis), the World's largest lizard, by sequencing the whole genomes of 24 individuals from five Indonesian islands comprising the entire range of the species. Three main genomic groups were observed. The populations of the Island of Komodo and the northern coast of Flores, in particular, were identified as two distinct conservation units. Degrees of genomic divergence among island populations were interpreted as a result of changes in sea level affecting connectivity across islands. Demographic inference suggested that Komodo dragons probably experienced a relatively steep population decline over the last million years, reaching a relatively stable Ne during the Saalian glacial cycle (400-150 ka) followed by a rapid Ne decrease. Genomic diversity of Komodo dragons was similar to that found in endangered or already extinct reptile species. Overall, this study provides an example of how whole-genome analysis of a few individuals per population can help define population structure and intraspecific demographic dynamics. This is particularly important when applying population genomics data to conservation of rare or elusive endangered species.

The genome of the subterranean termite: diversity and dynamics of gene families associated to wood feeding and social living

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Termites (Insecta, Blattodea, Termitoidae) are a widespread and diverse group of eusocial insects known for their ability to digest wood matter. Termite genome sequencing evidenced a number of feature associated with social living, mostly gene families expansions. Though, a few considerations have been made to analyze their remarkable ability to digest the wood matter. Here we report the first draft genome of the subterranean termite Reticulitermes lucifugus, an

economically highly impacting species in the Holarctic region. Moreover, this species is among the most studied taxa in respect to its eusocial organization and mating system, displaying the so-called Asexual Queen Succession (AQS) which include the parthenogenetic production of secondary queens. The final assembly resulted about 813 Mb long, covering up to 88% of the expected genome size. coherently with the AQS mating system, the genome was found in completely homozygous. We predicted 16,349 highly supported gene models and a repetitive content of 42%. Transposable elements show similar evolutionary dynamics compared to other termites, showing two main wave of activity localized mainly driven by DNA, LINE and SINE elements. The analysis of gene families expansion and contractions identified multiple instances of gene duplications associated to the R. lucifugus diversification, with significant lineage-specific gene families expansions related to development, stimuli perception and nutrient metabolism pathways. In addition, we further analyzed P450 and odorant receptor genes repertoires, highlighting a huge diversity and dynamic evolutionary history of these proteins also among analyzed termite genomes. This newly assembled genome will provide a valuable resource for further understanding the molecular basis of termites biology as well as their pest control.

COins: a curated reference database of COI sequences for insect species identification through DNA metabarcoding

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Due to the high level of specialization required for their morphological identification, their species richness and ubiquity, Insects represent one of the groups for which DNA-based identification is most commonly adopted. Nowadays, insects biodiversity surveys using DNA metabarcoding are increasingly common; yet, species level identifications are not always easily obtained with such approach. A DNA metabarcoding reference database specifically curated for insect identification can improve this result. Here we present COins, a database of COI-5P sequences of insects that includes over 532,000 representative sequences of more than 106,000 species specifically formatted for the QIIME2 software platform. Through a combination of automated and manually curated steps, we developed this database starting from all COI sequences available in the Barcode of Life Data System for insects, focusing on sequences that comply to several standards, including a species-level identification. COins was then validated on previously published DNA metabarcoding sequences data (54 bulk samples obtained from Malaise traps) and its efficiency compared with other publicly available reference databases for local use (not specific for insects). In our case study, COins allowed an increase of up to 30% of species-level identifications. COins can represent a valuable resource for the insects DNA metabarcoding, especially when species level identifications are required.

Changes in neutral genetic variability of the isolated brown bear (Ursus arctos) population of Trentino (Italy) two decades after its reintroduction

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The current population of brown bear (Ursus arctos) in Trentino is the result of a reintroduction which aimed at rescuing autochthonous individuals from the near extinction. Between 1999 and 2002, 10 bears were trapped in neighbouring Slovenia and released in Trentino. Since then, the population has increased faster than expected, but remained isolated in a small geographical area. The population has been monitored genetically since 2003, and a study by de Barba et al. (2010), using non-invasive samples collected in 2002-2008 and 10 microsatellite (STR) loci, found a significant decrease in expected heterozygosity (He) and allelic richness (A) compared to founders. Here, we studied the genetic variation of the same population for a longer time period analysing the 174 genotypes at 15 STR loci observed until 2019. Changes in diversity indices were estimated according to different subdivisions of the population based on: 1. Year (considering both 15 and 10 STR loci in order to compare the two studies). 2. Generation (founder population F0, first generation F1, etc.) 3. Cohort (individuals born in the same 5-year period, the generation time calculated for this species). 4. Group (30 randomly-chosen individuals from 3-year periods to test if standard population genetic studies would accurately estimate genetic diversity). All the subdivision strategies showed an early increase in diversity indices He and A, followed by a rapid reduction through time and a stabilization of the values in recent years; trend more significant for 15 than 10 STR. Interestingly, levels of inbreeding (Fis) increased, but not as dramatically as expected, given the small number of founders and males which contributed to the gene pool. Simulations indicate that genetic diversity will continue to decrease, although management strategies such as exchanging individuals with or a new reintroduction of few individuals from Slovenia could reverse the trend. These results underline the importance of costantly monitoring small reintroduced populations.

Is the Aeolian wall lizard Podarcis raffonei threatened by hybridisation with Podarcis siculus? Conservation genomic insights from Capo Grosso (Vulcano Island)

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The Critically Endangered Aeolian wall lizard (Podarcis raffonei) is an island endemic of the Aeolian Archipelago in Southern Italy. The species is only recorded in four locations: from three tiny islets; and from the small peninsula of Capo Grosso on the island of Vulcano, the latter representing the largest remaining population in terms of census size. Since the 1980s, it has been hypothesised that the small distribution range of P. raffonei is the result of competition and widespread hybridisation with the introduced Italian wall lizard (P. siculus). However, data supporting the hybridisation hypothesis is scarce, comprising a single study using allozymes and observations of individuals with apparently intermediate colour phenotypes. Using genome-wide SNPs obtained by reduced-representation sequencing, we present the first population genomics survey of P. raffonei and P. siculus from the Capo Grosso peninsula and the island of Vulcano. In 2015 and 2017, 135 individuals were phenotyped and genotyped, including lizards with a typical brown phenotype of P. raffonei and lizards with a green dorsal-colouration phenotype (putative "hybrids") in Capo Grosso, as well as P. siculus from the main island of Vulcano. We ask: 1) Is there evidence of widespread hybridisation with the invasive species P. siculus? 2) Do Capo Grosso lizards with a green dorsal-colouration phenotype represent true genetic hybrids? 3) What is the current status in terms of genetic diversity and effective population size of the Capo Grosso population? 4) Does P. raffonei show demographic perturbations associated with the estimated colonisation time by P. siculus? We interpret our genomic results in the light of conservation and management recommendations for the Critically Endangered Aeolian wall lizard, P. raffonei.

Integration of environmental Niche Modelling and genomic data to investigate the demographic history of Emys orbicularis in relation to habitat availability

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The European pond turtle, Emys orbicularis, is a freshwater turtle with a range extending from northern Africa through most of Europe up to the Aral Sea. Despite its widespread distribution, E. orbicularis is threatened by habitat encroachment, pollution and competition with invasive species such as Trachemys scripta. It is now listed as "Near Threatened" in the IUCN global red list and as "Endangered" in the Italian IUCN red list. A number of population genetic studies using single or multilocus molecular markers have assessed general patterns of the evolutionary history of the species, however, comprehensive analysis of life history traits can make use of a high-quality reference genome of E. orbicularis for genome-wide association and population genomic studies.
We produced a high quality, chromosome level reference genome for the European pond turtle using the online platform Galaxy Europe and genomic data based on SMRT HiFi long reads sequencing, Hi-C chromosome conformation capture technique and Bionano optical mapping according to the Vertebrate Genome Project pipeline 2.0. The assembled genome had a total length of ~2.3 Gbp with a scaffold N50 of 137 Mbp.
In order to understand how environmental processes may have shaped the evolutionary history of the species and how extant populations may respond to climatic change, we used Environmental

Niche Modeling (ENM) and estimated habitat dynamics during the Pleistocene in Europe. The models were produced with the R software "ENMeval" using the geographical coordinates of the sightings of the species from 1960 to 2021, and both recent and Pleistocene bioclimatic data. These models allowed prediction of habitat characteristics from four different periods: early Holocene, last glacial maximum, last interglacial period and Marine Isotope Stage 19 interglacial.
Results from ENM were then compared to effective population size (Ne) fluctuations estimated using a Pairwise Sequentially Markovian Coalescent method (PSMC) from heterozygosity values of the reference genome of E. orbicularis. Preliminary data suggest a reduction in habitat availability during the last glacial maximum. However, the demographic history of the species showed a relatively constant trend until the early Holocene. Here, a significant Ne reduction was recorded despite estimates of good habitat conditions. This pattern suggests possible anthropogenic factors affecting the demography of E. orbicularis.

Exploring fungal biodiversity using a comprehensive python package for automatic (multi-marker) barcode analyses

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Fungi play a pivotal role in all ecosystems: they regulate nutrient cycles, influence soil structure and ecosystem multifunctionality, and undergo symbiotic associations with plants, algae, and animals. Despite their importance in shaping and maintaining ecosystems, it is estimated that we currently know only 3-8% of worldwide fungal biodiversity. Fungi can be detected in environmental DNA samples, but it is difficult to metagenomically extract their typically long polyploid genomes in the absence of reference genomes for mapping reads. For fungi, primary databases are still a hidden source of new information in a classical DNA barcode fashion. However, existing barcoding tools lack a comprehensive pipeline, can be very time-consuming and do not take full advantage of metadata associated with sequences.
To tackle these issues, we developed a comprehensive Python package for the automatic barcode analysis of fungi. The pipeline is composed of different scripts which cover consecutive steps: data retrieved from multiple sources; selection of samples based on length, quality and occurrence; construction of chimeric sequences using sequence identifiers, automatic cleaning of the alignment; concatenation of different markers, barcode gap estimation and visualisation; detection of outliers; inference of phylogenetic tree. The package has been successfully used to define cryptic species and to identify misled samples in various fungal taxa.
Our package is a useful tool for the quick detection of new taxa from often understudied or unbinned nucleotide collections. It is scalable to any type of organism and to many markers for multi-marker barcoding and phylogenomic studies. It may ultimately contribute to improving our understanding of fungi and other organism diversity.

Posters

Long and short term evolutionary dynamics of transposable elements in the genome of the endangered Apennine yellow-bellied toad (Bombina pachypus)

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Transposable elements (TEs) represent a significant fraction of vertebrate genomes, impacting genome architecture and evolution. TEs could also be the driver of genome expansion in some organisms, playing a major role in genome size variation. In particular, a positive correlation was detected between the accumulation of specific TE families and species with very large genomes such as salamanders, which show a much larger amount of long terminal repeat (LTR) retrotransposons due to a slower rate of DNA loss and poor TE silencing mechanisms.
Bombina pachypus is an endangered anuran species endemic to the Italian peninsula, which has experienced a post-glacial range expansion that left a marked signature of southern richness - northern purity on its genetic diversity.
Investigating TEs in B. pachypus large genome (estimated size of 10Gb) will allow us to explore both long and short term TE-host evolutionary dynamics and, in general, to contribute to our understanding of large genomes evolution.
Analysing the activity of TEs in somatic and germline tissues, we aim to identify potentially active TE families, tissue-specific dynamics and different patterns of TE expression.
Furthermore, the investigation of the activity of genes involved in TE silencing mechanisms will help to understand whether there are tissue-specific strategies evolved by the host to repress TE mobilization and/or to control TE expression dynamics.
Finally, we will investigate the short term effects produced by the recent demographic expansion of this species resulting in reduced diversity and predicted lower selection efficacy at the edge of the expansion, by comparing TE dynamics between two populations of Bombina with markedly different effective population sizes, one from the southern refugium and one from the margin of the northern expansion range.

Characterisation of the genetic structure of chestnut trees varieties in the Lario region.

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Edible sweet chestnuts (Castanea sativa Mill.) have been cultivated for centuries representing an important food resource for rural populations in mountain regions of many countries. The tree is the only European species of the genus Castanea and is considered to be native of Asia, then colonising the Balkan region during the middle Eocene then reaching Italy with a rapid expansion to the present areas of cultivation during the Roman period.
The aim of this work is to characterise the genetic structure, genetic variability and demography of chestnut trees and their most important known varieties collected in the Lario region in Northern Italy. Our goal is to study 400 trees using ten SSRs – these preliminary results involve 96 trees from three different “varieties” genotyped by four SSRs.

Because “varieties” are defined by historical/morphological factors it is of interest to verify whether a genetic classification will confirm this. With a degree of surprise we already detected significant differences between the three varieties studied, both at the differentiation level (overall FST = 0.17) and at the population structure level (three genetic clusters of origin detected by STRUCTURE). Following on from this, the results that we intend to obtain are the determination of the degree of distribution of the genetic variability for the populations that are interesting for economic, landscape and cultural aspects; identification of populations with high levels of genetic variability; demographic analysis to identify any demographic variation occurring in the past and possibly related to historical events. Finally, we will propose models of in-situ conservation of these genetic resources. The genetic results, integrated with historical and cultural data, also allow for the correct planning of conservation strategies by identifying mother trees in order to build germplasm collections.

Genome assembly of a butterfly endemic to the pontine islands, Hipparchia sbordonii and estimation of some population diversity statistics

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In conservation biology, an increasing number of projects are taking advantage of the information available in whole genomes of endangered or threatened species in order to understand, by means of various analyses, their population characteristics and health status, their distribution in the habitats, including local adaptation, and how the intervention of biotic and abiotic elements affects their population size. The newly emerging field of conservation genomics needs, however, the data to carry out such analyses, that is the reference genomes of the species to be studied. The aim of our work and study was therefore to produce a genome assembly of an endemic Italian species at risk of extinction, the butterfly Hipparchia sbordoni, and then to analyze a set of population-level genomic data to estimate molecular summary statistics describing population diversity and structure of the species under study. The genome assembly and the study of population characteristics was carried out using state-of-the-art bioinformatics tools according to the gold standard set by international genome assembly consortia.

Integrating is better than separating: integrative approach in the phylogeny and biogeography of an intriguing tardigrade genus

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Background: Specimens of the genus Xerobiotus are relatively rare and little studied. To date, only four species were attributed to this genus and all of them were reported from arid environments:

grasses roots, mosses and lichens on sandy dunes or rocks exposed to sun. Recently, based on molecular phylogenetic data, it was proposed to suppress the genus and transfer its species to the genus Macrobiotus, despite the presence of synapomorphic characters that support its taxonomic validity. This study aims to enrich the knowledge related to biodiversity, biogeography, and phylogeny of the genus Xerobiotus. To better understand the evolutionary causes related to the distribution of this genus we applied an integrative approach integrating morphological, morphometric, molecular, karyological, and reproductive data.
Methods: Samples from several European, Caucasian, and Australian sites were used to collect tardigrade specimens of Xerobiotus and Pseudohexapodibius (the sister taxon) genera. Light and scanning electron microscopy allowed to conduct detailed morphological analyses. Molecular data were obtained sequencing cox1, 18S, 28S and ITS2 genes for population characterization, species delimitation, and phylogenetic analyses. Karyotype and reproductive modes of the populations were also investigated.
Results: In addition to the distinctive morphological structures of the taxon, microscope investigations showed new or still little known characters for Xerobiotus. Several new taxa were identified within Xerobiotus supported by both morphological/karyological and molecular data. Molecular data showed that both parthenogenetic and bisexual taxa have a widespread distribution, a situation not common in tardigrades.
Conclusion: Obtained results, other than to identify new taxa, underline how biogeography patterns of tardigrades are unclear and influenced by their reproductive mode. They also show that integrative approach (morphological, molecular, karyological and reproduction data) is highly useful to identify new taxa and their distribution. The phylogenetic line of Xerobiotus results diversified in several taxa and closely related to Pseudohexapodibius and Macrobiotus species.

Conservation genomics of the Adriatic sturgeon (Acipenser naccarii)

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The Adriatic sturgeon is an important species from an evolutionary and economic point of view. Once distributed in the Adriatic Sea and in several rivers flowing into it, it currently occurs only in a few northern Italian rivers and is considered a critically endangered species. Our project aims at estimating the genomic variation of the small residual population kept in captivity, and to compare it with the estimates based on a closely related species with a much larger population size, the Russian sturgeon (Acipenser gueldenstaedtii). Using kmer-based methods and short reads with approximately coverage of 30x, we first estimated that the haploid genome size of this species is ~ 1.4 Gb. Although the genome is currently considered to be functionally tetraploid, we found that this species has a mosaic of ploidies along the genome. Using the reference genome of a related species, the sterlet sturgeon (Acipenser ruthenus), we sequenced and mapped the genomes of 18 Adriatic sturgeons and 10 Russian sturgeons, and we performed basic population genomics analyses. First results revealed a lower genomic diversity in the Adriatic sturgeon than in the Russian sturgeon. We also performed in silico forward simulations in the software SLiM using genomic, and environmental parameters for tetraploid populations. Simulations of a population size decline and a genetic rescue intervention using a sister species are currently under investigation. The use of population genomics analyses, including estimates of inbreeding levels and genetic load, together with simulations of conservation activities such as translocation and hybridization, will help developing a conservation plan for this species.

Back in time: a glimpse on the demographic history of Galápagos Iguanas using genomic data

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Galápagos iguanas are iconic animals for both evolutionary and conservation biology. The taxon started diversifying about 4.5 million years ago, with four species currently described. One of these species is the marine iguana (Amblyrhynchus cristatus), specialized towards an aquatic lifestyle unique among squamate reptiles. The characteristics and the distribution of the remaining three species (Conolophus subcristatus, C. pallidus and the phylogenetically divergent and critically endangered C. marthae) appear as the result of a complex history of local extinctions and colonizations, with still undetermined roles of selection, genetic drift, and hybridization.
The Iguana Genome Consortium was created in 2020 to shed light on the evolutionary history of these wonderful yet endangered species, through the study of their genomes. At the moment, the completion of the assembly and annotation of the reference genomes of all the four species of Galápagos iguanas is under way. We computed demographic estimates using whole genome sequences and RADseq data aligned to the already completed reference genomes, combined with our field-data based estimates of generation times. In details, we reconstructed the demography for the only known population of C. marthae, for the population of C. subcristatus living in syntopy with C. marthae, for the population of C. pallidus endemic to the Santa Fe island, and for two populations of A. cristatus.
Our results show that all populations have been relatively small ($100 < Ne < 10000$), in the last thousands of years. Specifically, the current effective population size (Ne) of C. marthae is estimated to be of ca. 440 (95% CI: 115-510), similar to mark-recapture estimates of census size (108-174 individuals), and slightly larger than Ne estimates obtained by microsatellite data. For this population, the Stairway Plot 2 shows evidence of a recent decline. All other populations suggest a relatively recent growth at different times in the past (5-50 ka BP), consistent with events of local extinction and colonization.

SYMPOSIUM

DYNAMICS OF GENOMIC DIVERSITY IN DOMESTICATED SPECIES

CHAIRS: **ELENA BITOCCHI, IVAN SCOTTI**

Invited speaker YVES VIGOUROUX, Université de Montpellier, Institut de Recherche pour le Développement, CIRAD, UMR, DIADE, Montpellier, France

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Tracing the origin of African crop domestication

Our understanding of the origin and history of agriculture in sub-Saharan Africa is still spotty. We used genomic datasets to try to decipher and understand the domestication of major African crops: pearl millet, Africa rice, yam, sorghum and fonio. We notably used spatial model to trace the area from which these crops diffused. Our initial result supports the hypothesis that the vicinity of the Niger River was a major cradle of African agriculture. But integration of news species show that several domestications occurred thorough the Sahel zone. Analysis of the timing of plant diffusion suggested desertification of Sahara might have trigger major social change with the adoption of agriculture.

Invited speaker ROBERTO PAPA, Department of Agricultural, Food and Environmental Sciences, Polytechnic University of Marche, Italy

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The complex journey of the common bean

The role of genetic diversity and its characterisation is crucial for future improvements to meet societal demand for healthy and nutritious food for all, especially under a climate crisis. From this perspective, it is extremely important to study the genome structure and evolution of crop species and their wild relatives. Food legumes are crucial for all agriculture-related societal challenges, including climate change mitigation, agrobiodiversity conservation, sustainable agriculture, food security and human health. The transition to plant-based diets, largely based on food legumes, could present major opportunities for adaptation and mitigation, generating significant co-benefits for human health. The common bean (Phaseolus vulgaris L.) is the world's most important food legume for direct human consumption. It has a Mesoamerican origin as most of the other Phaseolus species. Because of migration, P. vulgaris expand its distribution from Mesoamerica to South America in the Andean region. This has led (likely between 100-150 ky BP) to the formation of three gene pools of wild P. vulgaris in different geographical regions: Mesoamerica, Northern and Southern Andes. Domestication took place independently in Mesoamerica and Southern Andes and no sign is available of a domestication in the Northern Andes. The original gene pool structure is still clearly evident in both the wild and the domesticated forms. This evolutionary scenario makes P. vulgaris almost unique among crops, and therefore particularly useful to investigate crop domestication, diversification, and further expansion to the 'Old World' due to the Colombian exchange. Recent results concerning three main episodes of the evolutionary history of P. vulgaris will be presented: 1) speciation and dispersal into South America, 2) the parallel domestication and diversification in Mesoamerica and in the Andes, and 3) the expansion of the common bean outside the centre of domestications in the "Old World".

Talks

The domestication of cat and its dispersal in ancient Europe

1_De Martino Marco, 2_De Cupere Bea, 3_Alhaique Francesca, 4_Bogdanović Sonja, 5_Ceccaroni Emanuela, 6_Cerilli Eugenio, 7_Detry Cleia, 8_Goffette Quentin, 9_Grau Idoia, 10_Gręzak Anna, 11_Küchelmann Hans Christian, 12_Lloveras Lluís, 13_Mazzorin De Grossi Jacopo, 14_Minniti Claudia, 15_Moreno Marta, 16_Nadal Jordi, 17_Onar Vedar, 18_Pereira Vera, 19_Soranna Gabriele, 20_Spassov Nicolai, 21_Wilkens Barbara, 22_Peters Joris, 23_Van Neer Wim, 24_Ottoni Claudio

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Domestication of plants and animals dramatically changed human culture, biology and ecology. Domestication is a complex and multistage process involving natural and artificial selection, intricate patterns of admixture between and within wild and domestic populations and human-mediated translocation, ultimately leading to significant morphological and behavioral changes in the targeted animals.
Among domesticates, the cat is one of the most understudied species. Its domestication process is unconventional: its wild ancestor, Felis silvestris lybica, is an hypercarnivorous and solitary species; moreover, no evident morphological changes differentiate domestic cats from its wild counterpart.
Archeozoological evidence indicates both the Neolithic Levant and the Predynastic Egypt as important centers for cat domestication.
Paleogenetic evidence based on the analysis of few short mitochondrial fragments, demonstrated that cats carrying haplogroup A spread with farmers from Southwest Asia during the Neolithic. On the other hand, haplogroup C, found in Egyptian mummies, became predominant in the Old World since Classical Antiquity spreading with humans across land and sea routes of connectivity. However, evidence on the dispersal of these lineages is lacking with regards to continental Europe and the broader Mediterranean region.
By applying cutting edge methodologies in the ancient DNA field, we aimed at generating complete mitochondrial genomes as well as genome-wide nuclear data from cat remains dated from the Upper Paleolithic to recent times in Europe. We show here the results of shotgun sequencing data from over a hundred of ancient cats. Our data provide a clear temporal and geographic framework of DNA preservation across the Mediterranean region. Moreover, through the reconstruction of complete mitogenomes, we are able to refine the times and modes of cat dispersal from the original domestication centers, with a particular focus on Central and Southern Europe.

The barn swallow genome reveals selection signatures in genes involved in neural development and fear memory formation, suggesting a role for synanthropy

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Insights into the genomic evolution of non-model organisms are often limited by the lack of reference genomes. As part of the Vertebrate Genomes Project's effort to generate complete, accurate and fully annotated genome assemblies, we present here a new chromosome-level, karyotype-validated reference genome for the barn swallow (Hirundo rustica), a charismatic synanthropic migratory bird with six recognized subspecies. We also generated the first pangenome for the species using High-Fidelity long reads, to reduce bias towards a single reference genome. So far, the absence of a high quality reference genome has hindered the understanding of the genetic bases of behavioral phenotypes and evolutionary adaptations in the barn swallow. Our newly generated reference genome allowed us to perform a whole-genome alignment with other bird species and a comprehensive catalog of genetic markers of the species, exploiting all publicly available genomic data from individuals belonging to several populations. We used these genomic resources to identify genetic loci potentially under selective pressures. Conservation analyses performed on the multialignment pointed at genes enriched for DNA-binding, transcriptional regulation and neurodevelopment. The top conserved gene was CAMK2N2, encoding an inhibitor protein of a kinase involved in fear memory formation. Genome-wide linkage disequilibrium scans and extended haplotype statistics analyses on our marker catalog detected signatures of selection at a locus harboring BDNF, a gene involved in neural crest development and neural pathways for learning, memory formation, and stress response. These processes have been previously linked with tameness and domestication in animals, suggesting that the selective pressures acting on these genes might have played a role in the recent evolution of the synanthropic habits of the barn swallow, through the reduction of fear and stress response.

Diversity and adaptation of domesticated chickpea (Cicer arietinum L.) germplasm

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A wide collection of domesticated chickpea genetic resources was genotypically and phenotypically characterized to investigate the level and structure of genetic diversity and to identify the genetic basis of phenotypic variance for adaptation to different agro-environmental conditions.

The set of materials includes 480 chickpea lines developed by Single Seed Descent from genebank accessions and local varieties still cultivated on farm. The accessions are worldwide distributed, with a greater representation from the Mediterranean Basin and Central Asia. Georeferentiation is available for 150 lines.
Whole genome sequencing (WGS) was used to genotype the entire collection, while the phenotypic characterization was based on three years (2019, 2020 and 2021) of field trials carried out in Osimo locality (Central Italy).
By using genomic data, we assessed the genetic diversity and population structure of the entire collection and by coupling these data with environmental variables, available for georeferenced landraces, we applied landscape genomic approaches to investigate the spatial distribution of genetic variation and to detect signatures of selection for adaptation. Results were also coupled with those obtained by the Genome Wide Analysis (GWA) carried out by using genomic and phenotypic data recorded during field trials. This allowed to identify candidate regions potentially involved in adaptation to different agro-environmental conditions.
The results of this work will widen the current knowledge on chickpea evolution and adaptation to different environments following its spread after domestication, especially because it involves a wide sample of materials from Mediterranean Basin, a region usually underrepresented in literature. At the same time, our results will be a very useful tool for chickpea breeding, especially for the development of varieties adapted to the European and Mediterranean environments.

Posters

Reconstructing the phylogeograhy of Linum bienne Mill., the widely distributed ancestor of cultivated flax, using whole plastomes and low-depth nuclear genomes.

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Linum bienne Mill. is the closest wild relative and ancestors of Linum usitatissimum L., also known as fiber flax or linseed. Despite the importance of this crop, which is nowadays grown worldwide for fibre and oil, the phenotypic and genotypic diversity of its wild ancestor have only been described in light of its agronomic importance, ignoring its ecology and evolution. Yet, understanding phenotypic and genotypic variation in light of the ecology and evolution of L. bienne is fundamental for its conservation, to identify adaptations that might be relevant for the improvement of the crop, L. usitatissimum, as well as to understand its domestication history. Moreover, L. bienne sits in a genus that has been historically employed to study the evolution of flower morphology as well polyploidization. Yet, the genus Linum lacks a reference species for which extensive genomic resources have been developed beyond the crop itself. For these reasons, we set out to produce the first phylogeography of L. bienne by: 1) reconstructing and dating a phylogeny for the genus Linum using the recently developed Angiosperm353 kit; 2) reconstructing and dating the phylogeny of L. bienne based on chloroplast and nuclear genomes together with node ages obtained in the previous step; 3) interpreting the phylogeny of L. bienne based on SDM in present and past climate scenarios. A few accessions of L. usitatissimum were also included in this process to understand how it relates to different L. bienne lineages. Preliminary results suggest that geneflow between Linum species and between L. bienne lineages occupying different geographical areas has occurred throughout their evolution. Similarly, L. usitatissimum seems to be the product of a domestication process where wild introgression into the crop's genetic background might have been common despite it being primarily self-pollinating, which confirms previous research.

Setting up an adaptive evolution experiment to unravel co-adaptation mechanisms in a maize/bean intercropping system

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Intercropping (IC), i.e. the simultaneous growth of two or more species in the same field, has been recognized to provide several agroecosystem services, as well as to support yield stability through the exploitation of ecological mechanisms. However, genetic bases of phenotypic traits involved in the process of plant-plant interactions and whole-genome patterns of co-adaptation are still unknown, which demand for an integrated and tailor-made approach when breeding for mixtures.
Within the Horizon 2020 project RADIANT, we aim to combine experimental evolution, whole-genome sequencing of pool of individuals (PoolSeq) and Participatory Plant Breeding (PPB) in maize/common bean and maize/runner bean intercropping systems to examine co-adaptation mechanisms after artificial selection for IC ability.
For this purpose, an Evolve & Resequence study has been set up, involving three Italian farmers in representative environments from North to South Italy. We developed a Maize Composite Cross Population (MCCP) from a collection of Italian flint maize landraces, and a Common Bean Mixture (CBNMIX) from European and American genetic resources. In addition, we also included three

landraces (for flint maize: Spinato di Gandino; Ottofile di Arcevia; for runner bean: Clusven), resulting, in total, in six maize/bean combinations tested over three different agro-environments. Lastly, a dynamic and adaptive workflow for a decentralized approach has been adopted to record farmers comments, set selecting criteria and detect traits of IC ideotype. This includes interviews, questionnaire, meetings and field days.
The integration of results from further population genomics analysis (selective sweeps, causative variants, allelic/genotypic frequencies) with ones from experiments of the H2020 INCREASE project will contribute to the elucidation of genomic patterns of co-adaptation.

SYMPOSIUM

EVOLUTION OF FORMS FROM GENES TO CELLS TO ORGANISMS

CHAIRS: MARIA INA ARNONE, PAOLO COLANGELO

Invited speaker DETLEV ARENDT, European Molecular Biology Laboratory, Germany

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Building bilaterian brains: key innovations in molecular machinery, cell types, and nervous system architecture

We study the evolution of animal form at all scales, with a particular focus on the origin and rise of their most fascinating trait, which is the centralized nervous system. For this, we track the evolution of neurons and other constituent cell types across animal phylogeny, focusing on slow-evolving animals with moderate amounts of secondary loss. We have chosen the nereid Platynereis dumerilii as a powerful model for comparative studies, with morphologically similar organisms already existing as early as the Cambrian. We take advantage of its highly stereotypic development to establish the link between gene expression, cellular morphology, and organ formation for an entire body. To exemplify the evolution of form in the bilaterian brain, I will explain how we trace the assembly of conserved synaptic proteins, transmitters and receptors in a basic set of bilaterian neuron types; and how we trace the assembly of these neuron types into a basic set of neural circuits that make up the bilaterian brain. Enabling this, we have constructed a unique cellular atlas for the nereid, the PlatyBrowser, which allows us to combine genome-wide expression profiling with cellular ultrastructure and connectomics for an entire body. To systematically characterize cellular morphologies we have added AI-based recognition of cellular MorphoFeatures to the atlas that we can now align with cell type-specific gene expression modules. I will explain how we use these unique resources to advance our understanding of bilaterian brain evolution, and to find hotspots of cellular variation at the micro- and macroevolutionary scale that drive the evolution of form.

Talks

Growth regulation in the larva of the lepidopteran Pieris brassicae

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Size and shape are important fitness components along the whole ontogeny in most organisms. Accordingly, the capacity of the organism to regulate size and shape during growth, by buffering against the effects of different sources of developmental disturbances, is considered a key feature of the developmental system. However, observational data on the phenomena of ontogenetic size and shape regulation are relatively scarce. In a recent laboratory experiment, through a morphometric analysis based on geometric morphometrics, we found evidence of size and shape regulation during the larval development of the cabbage butterfly Pieris brassicae. However, the effectiveness of the buffering mechanism under stronger environmental disturbances remained to be ascertained. Here, through a field experiment, by adopting an identical quantification of size and shape variation in the same species, we show that the developmental regulatory mechanisms underlying the capacity of P. brassicae of buffering against the effects of developmental disturbances are also effective in more natural and variable conditions of growth. This study may contribute to a more general understanding of the mechanisms of developmental stability and canalization and their influence on phenotypic evolution.

The genomics of Heliconiini shows strong selective pressures prior Heliconius' adaptive radiation

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Adaptive radiation is the proliferation of species from a single common ancestor that rapidly diversify into ecologically diverse forms. Within the Heliconiini (Nymphalidae), a tribe of neotropical butterflies, the genus Heliconius is known example of sympatric adaptive radiation. Due to its toxicity and Müllerian mimicry, they radiated into the richest species genus of the tribe. This diversity is coincidental with important trait innovations, such as pollen feeding behaviour, a unique feature among Lepidoptera, prolonged reproductive lifespan, and major elaborations of learning and memory centres in the brain. With 63 genome assemblies, we present the most comprehensive genomic resource for the entire tribe of Heliconiini to date. A genome-wise approach was applied to generate a new dated phylogeny, which we use to provide new analyses on incomplete lineage sorting and introgression. We then describe how transposable elements activity affects both genome size and gene structures and estimate how Heliconiini genomes changed throughout the course of their radiation, inferring evolutionary dynamics of gene expansion/contraction. Finally, molecular signals of adaptive evolution reveal how several basal lineages were affected by strong selective pressures prior to the Heliconius' radiation. By doing so we identified candidate genes, pathways, and gene families likely to be responsible for some of the most important innovations we see in these butterflies. Our dataset provides the first tribe-wide quantification of genetic and genomic variation potentially underlying key phenotypical traits, advancing our understanding of the genomic basis of behavioural, physiological and morphological innovation in the context of ecological diversification and speciation.

Reconstruction of Nitric Oxide regulatory networks during chordate embryonic development and novel insights in fish gill development

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A tale from the amphioxus. During animal development the early body patterning is a fundamental process regulated by complex interactions among key signaling pathways that govern simultaneously the correct embryonic grow. We demonstrated that Nitric Oxide (NO) and Retinoic Acid (RA), two potent morphogens that play a pivotal role in vertebrate body patterning, regulate each other during the early development. Although still poorly documented in other systems, we highlighted the functional cooperation between NO and RA during amphioxus embryogenesis (cephalochordate) used as a proxy for vertebrates. To investigate downstream the NO signaling gene network, we applied short term in vivo pharmacological treatment, during early stages of development employing a highly specific inhibitor of NO synthase (Nos), and performed a differential transcriptomic analysis. It comes out clearly the upregulation of RA metabolic genes and on the other hand, we showed that RA regulates Nos genes transcription and therefore that RA probably controls the endogenous NO levels. This discovery of a functional crosstalk between NO and RA during development of the cephalochordate amphioxus opened new questions about the evolutionary conservation of this regulatory loop in all chordates, and in particular in vertebrates. Therefore, we aim to investigate in future more in detail the effect of the NO inhibition by looking at the differential genome-wide chromatin accessibility by ATAC-seq and proteomic analysis.
A tale from fishes. Although our knowledge of the evolution of Nos genes in vertebrates is substantial, the origin of the diversified repertoire of Nos orthologs in fish remains a puzzle. The recent identification of Nos3 in spotted gar opened new perspectives since it was considered lost ray-finned fish lineage. This finding prompted us to explore Nos evolution and expression by surveying vertebrate species representing key evolutionary nodes. Surprisingly, the expression of at least one Nos paralog in developing gills of shark, bichir, sturgeon, and gar but not of lamprey suggests that NO signaling may have a key role in this organ in the last common ancestor of gnathostomes. These results provide a framework for continuing to investigate on Nos subfunctionalization or reciprocal loss-of-function occurred in different lineages, and novel NO functional role(s) during fish evolution.

Neuromodulation by Monoamines is a Bilaterian Innovation

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Monoamines like serotonin, dopamine, and adrenaline/noradrenaline (epinephrine/ norepinephrine) act as neuromodulators that tune the response of the nervous system to the environment with predictable advantages for fitness. For instance, monoamines influence action selection depending on the internal state of the organism, contribute to 'higher' cognitive functions like learning and memory formation and modulate fundamental homeostatic needs such as sleep or feeding. Despite their significance and the extensive research in model organisms, the evolutionary origin of the monoaminergic system is uncertain. Here using a phylogenomic approach, we study the evolution of the majority of genes involved in the production, modulation, and detection of monoamines. Our analyses suggest that most of the genes of the monoaminergic system

originated in the common ancestor of bilaterians. These findings suggest that the monoaminergic synaptic pathway is a bilaterian innovation. We hypothesise that monoaminergic neuromodulation contributed to the diversification and complexification of behaviour and forms found in Bilateria.

The evolution of molecular toolkits for biosilicification in demosponges (Porifera)

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Three out of the four classes of Porifera construct siliceous skeletons but they do it through divergent enzymatic pathways that are still poorly characterised. In demosponges, the biosilicification occurs by polycondensation of silica using silicases around an axial filament formed by a protein called silicatein and also using an axis made of actin. Several other genes have been preliminarily characterised as important for the process of biosilicification in demosponges, including membrane transporters and scaffolding proteins. Most demosponges can produce more than one spicule type, on some occasions with convoluted beautiful ornamentations. Although some demosponges have more than one silicatein gene (with highly divergent evolutionary pathways), it is not clear whether the silicatein diversity is linked to the diversity of spicule shapes. Interestingly, in calcareous sponges, different alpha-carbonic anhydrases present in the group are linked to the production of several spicule types. Here we used complete transcriptomes of 64 sponges (19 newly assembled) to create a sound time-calibrated phylogenomic framework to explore the evolution of biosilicification within demosponges across the phylogeny of Porifera. The enzymes required to produce siliceous spicules including silicases, silicateins, scaffolding proteins, and silicon transporters, were screened within our transcriptomic and other datasets available. Character reconstructions were performed in our datasets to understand the evolution of the spicules. Finally, we used BAMM tools to detect and quantify heterogeneity in evolutionary rates across sponges with diverging silicification levels and molecular machinery. We find a highly complex toolkit of genes involved in biosilicification, that includes a wide diversity of silicateins and silicon transporters aquaporins in those demosponges with high diversity of spicules. We also highlight diversification events at the origin of demosponges that did not co-occur with the origin of spicules. These results allow us to interpret in a more sensible way the evolution of Demospongiae and their fossil record.

Evo-devo approach to study asexual development and whole body regeneration: insights from tunicates

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Asexual propagation and whole-body regeneration are forms of non-embryonic development (NED) widespread across animal phyla and central in life history and evolutionary diversification of metazoans. Among chordates, more than a half Tunicate species can regenerate a functional adult body, after extensive injuries or as part of the asexual life-cycle, through different forms of budding. The tunicate phylogeny suggests that the capacity of undergoing NED is an evolutionarily plastic trait, a view reinforced by the fact that budding mechanisms differ from one species to another, involving non-homologous epithelia and/or mesenchymal cells. Such a scenario provides an opportunity to explore conserved or non-conserved mechanisms underlying the evolution of regenerative capabilities in this group of chordates.
Whereas it is challenging to reconstruct the gains or losses of NED at large phylogenetic scale, comparative studies could benefit from being conducted at more restricted taxonomic scale, in groups for which phylogenetic relationships are well established. The tunicate family of styelidae encompasses strictly sexually reproducing solitary forms as well as colonial species that combine sexual reproduction with different forms of NED.
Our research combines phylogenomic, genomic, cellular and molecular approaches in order to investigate the reproductive plasticity in ascidians styelidae, with the final aims to understand the cellular and molecular mechanisms that allow budding and coloniality in these marine chordates as well as their evolutionary trajectories. Using a phylogenomic approach we provided a robust phylogeny of this family of chordates, which supports two convergent acquisitions of NED. This result prompted us to further mechanistically investigate the presence of conserved developmental modules driving asexual development and whole body regeneration in key ascidian species. Recent findings will be presented.

The genetic basis of a trans-specific alternative life history strategy

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Alternative life-history strategies (ALHS) are genetic polymorphisms generating discrete phenotypes differing in life histories that often arise due to resource allocation tradeoffs. In general, ALHS are evolutionarily young, limited to a single or several closely-related species. Unfortunately, the genetic basis of ALHS in wild populations is poorly understood, at the causal loci have rarely been identified and the role of such loci in generating tradeoffs not understood, both of which limit our ability to understand their origins and evolutionary dynamics. In the butterfly genus Colias, at least one-third of the 70 Colias species have a female limited ALHS called Alba. While many females develop brightly pigmented wings that are yellow or orange, Alba females reallocate nitrogen resources used in pigment synthesis to reproductive development, producing white-winged females that develop faster and are more fecund. Building upon our recent discovery of the Alba locus and the gene generating at least the wing phenotype (BarH1), we have recently used a large-scale comparative genomics approach to develop deeper insights into the evolutionary origins of this ALHS. Unlike to most ALHS, we demonstrate that Alba has an ancient origin more

than 1.5 million generations ago, with additional analyses suggesting it has likely been maintained across species in the genus Colias by both introgression and balancing selection. We also used our comparative genomic data identify the putative cis-regulatory region that all Alba species contain. Importantly, we verified the role of this cis-regulatory region using CRISPR/Cas9 mutagenesis, which generated a phenocopy of pervious mutations in the coding region of BarH1. We hypothesize that this cis-regulatory region of the transcription factor gene BarH1 acts as a modular enhancer for the induction of the ALHS, which has likely facilitated its long evolutionary persistence.

Posters

Ecological, physiological and life-history traits correlate with genome sizes in decapod crustaceans

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Crustaceans are characterized by some of the most variable genome sizes among animals. Significant relationships between genome size and specific eco-physiological and morphological features have been described in many crustacean taxa, such as Amphipoda, Ostracoda, Cladocera and Copepoda. A consistent pattern of genome size variation is yet to be found, however, in one of the most specious order of crustaceans, the Decapoda. We investigated how genome size is related to selected eco-physiological and life-history traits in species belonging to this order. We analyzed the respiratory, excretory and developmental adaptations of 179 decapod species inhabiting shallow marine, deep marine, freshwater, intertidal and terrestrial habitats. Our results show that the genome size is significantly larger in decapods that have a direct development. Moreover, in Anomura, Astacidea and Brachyura we found larger genome sizes in species i) living in freshwater habitats, ii) using gills as a strictly water-breathing organs, iii) presenting a direct development. While specie that i) live in shallow waters, and intertidal and terrestrial habitats, ii) have some degrees of air-breathing adaptations, namely gills that can exchange oxygen with air if wet or branchiostegal lungs and iii) possess an indirect or extended development are characterized by significantly smaller genomes. Our analyses show that developmental complexity affect genome size in decapods, and that multiple eco-physiological and life-history traits correlate with genome size in Anomura, Astacidea and Brachyura.

The evolutionary history of opsin genes in Bactrocera fruit flies

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Fruit flies of the Tephritidae family have a considerable impact over worldwide agriculture. In particular, the Bactrocera genus (which makes up nearly half of Tephritid species) is responsible for extensive damage to fruit cultures. The progressive spread of Bactrocera species in new countries made it necessary not only to develop more efficient control methods but also of prevention by identifying their invasive and adaptative potential before their establishment. Therefore, it is essential to have a thorough understanding of how Bactrocera species select their specific host plants, for instance by studying the evolution of sensory genes - such as those coding for the photoreceptive opsins proteins involved in vision. In this work we annotated opsins coding sequences and transcripts in 11 Bactrocera species and other 17 closely related Diptera. We then analyzed their evolutionary history in a phylogenetic framework to detect the gene family dynamics and sequence divergence pattern. Our results revealed a yet undescribed lineage specific duplication event involving the ancestor of the Rh4 and Rh3 genes. Patterns of molecular evolution further suggest the action of positive selection on some opsin genes, which may be related to the species-specific host preference.

Adaptation of Giant Amphipods and Notothenoids to life in Antarctic Ocean

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Antarctic Ocean has been in extreme, but stable, conditions for at least fifteen million years, allowing many life forms to develop a set of unique adaptations to the several challenges offered by this environment. One of the most important factors is the higher gas solubility in cold antarctic waters, which implies adaptations of the respiratory systems in Antarctic marine organisms. Here we present respiratory adaptations of the giant amphipod Eusirus giganteus and six species of Cryonotothenioids. We assembled the transcriptome of a juvenile of E. giganteus and evidenced an high transcriptional effort in the expression of several hemocyanin isoforms, suggesting that this species acquired gigantism as an adaptation to the different diffusion coefficient of oxygen in sub-zero water, which has to be compensated with more hemocyanin production in the smaller juveniles. By comparing the gills transcriptomic profiles of Chionodraco hamatus and other five red blooded Cryonototenioids with those of the subantarctic Eloginops maclovinus and seven other temperate teleost species we found several transcripts that were consistently up-regulated in Cryonotothenioids compared to the non-antarctic species which shared the same profile in white- and red-blooded species. In particular the analysis evidenced an increased activity of carbonic anhhydrases, together with the up-regulation of genes involved in secretory activity and molecular signatures of cobalamin deficiency, implying a role of the hematological alterations and the heavy parasitic loads typically observed in Cryonototenoids. Finally we investigated the transcriptomic response of Trematomus bernacchii to a temperature increase in line with the expected effects of climate change in this decade, evidencing non-compensatory alterations in gills and brain tissues, with the latter tissue being the most affected, showing alterations in synapse-related pathway, mitochondrial metabolism and protein synthesis and folding.

Evolution of mechanoreceptor genes in Diptera

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True fruit flies (Tephritidae) are among the most important insect pests, as their larvae feed on fresh fruit and cause losses to a wide variety of crops. These insects can locate their host plants using chemoreceptors, with females further using mechanoreceptors to locate, on the fruit peel, the optimal place to insert their ovipositor and lay eggs. Comparative studies between related species can help to clarify the genetic basis of biology and dietary preference. Here, we used available genomic data from six species of the genus Bactrocera, three of the genus Drosophila including two with a similar egg-laying behavior (i.e. D. subpulchrella, D. suzukii), and one outgroup, to identify orthologs of six genes known to be involved in mechanoreception. Specifically, we focused on genes encoding for ion channels belonging to the TRP family (e.g. NompC, TrpA1) and on the gene Cirl (G-protein coupled receptor), which is involved in the perception of tactile stimuli. We then studied the patterns of evolution of these genes in a phylogenetic framework. Our results

revealed clade specific duplications of the BRV gene in Drosophila and possible evets of positive selection that may be associated with the specific egg-laying behavioral preference.

Looking for germline-specific functional signatures in Metazoa: an RNA-Seq approach

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The study of the germline, that is the cell lineage that gives rise to gametes in sexual animals, classically relied on morphological descriptions. Thanks to modern molecular characterizations, the genetic toolkit involved in germline determination and differentiation could be identified for a wide variety of species, making possible to observe that many genes are shared by all investigated phyla. Moreover, the same genes were observed as expressed in totipotent stem cell lineages of some animals with high regenerative potential, refining the knowledge around totipotency itself and its evolution in relation to multicellularity. The present study stems from such conclusions and represents an attempt to outline transcriptional similarities across germline-related lineages of different metazoan species. We sourced online-available RNA-Seq experiments including both germline-related and somatic samples, recovering a data set comprehending 10 species belonging to 8 phyla, and we searched for upregulated germline-related genes and functions. Through phylostratigraphic analyses, we observed that the proportion of germline-related transcripts shared with other phyla was more frequently higher than expected by chance, and that the proportion of metazoan novelties was lower in germline-related samples. Despite germline evolution has been indeed usually associated to cases of novel gene evolution (such as vasa, piwi, nanos), our results suggest how the overall molecular phenotype is characterized by a higher proportion of anciently evolved molecular factors. When looking at the specific nature of germline-related elements, signals related to proper DNA replication resulted the most common across the considered species, while the regulation of post-transcriptional mechanisms, that are regulative pathways usually associated to germ cells, appeared more variable, suggesting a higher level of lineage-specificity and tuning.

SYMPOSIUM

MICROBIAL GENOMICS AND EVOLUTION

CHAIRS: MARCO FONDI, ALESSIO MENGONI

Invited speaker MIREIA VALLES COLOMER, University of Trento, Italy

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Person-to-person transmission of the human microbiome

The human microbiome is an integral component of our body and a co-determinant of many health conditions. However, we have little knowledge on how human body-associated microorganisms are transmitted among individuals, in contrast to the well-studied transmission routes of pathogens, from bacteria to viruses. We assessed person-to-person transmission of the gut and oral microbiomes at large scale capitalising on >9,700 metagenomes and novel computational strain-level profiling tools. We detected extensive microbial strain sharing across individuals with distinct vertical, intra-household, and intrapopulation transmission patterns. Vertical mother-to-infant gut microbiome transmission was found considerable and stable during infancy, remaining detectable in older ages. Transmission of the oral microbiome, in contrast, occurred largely horizontally and was enhanced by the duration of cohabitation. In addition, we showed that microbial strain sharing recapitulated host population structures better than species-level profiles. Finally, distinct bacteria appeared as efficient spreaders across transmission modes, being explained by different predicted bacterial phenotypes linked with out-of-host survival capabilities. Overall, the extent of microbial transmission we identified reinforces the hypothesis that several diseases and conditions that are currently considered non-communicable should be re-evaluated, and that accounting for transmissibility and social network structure will improve the design of future microbiome investigations and modulations.

Talks

It's a long way to the tap: microbiome research at the core of drinking water quality

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Microorganisms are detected in every ecosystem on Earth, as well as in any built environment. However, many of the built environment microbiomes we interact with are still largely unknown. Among these, drinking water treatment plants and distribution systems provide peculiar microbial ecological niches, dismantling the belief of the biological simplicity of drinking water. Being the primary source of human sustenance, unravelling the dynamics of drinking water microbiome will highlight new aspects of the strict interactions between water and humans.
We focused on what happens to the microbiome from the source to the tap, collecting data covering several years and integrating microbiological and molecular techniques.
We were able to isolate and identify >100 bacteria, peculiar to drinking water, and no one of these was pathogenic. Thus, we created a collection of drinking water bacteria, useful for further characterizations. However, most of the bacteria were recalcitrant to grow in lab conditions. DNA-based approaches coupled with machine learning analysis allowed us to describe microbial diversity, but also (and more important) to predict which taxa are most discriminating in response to treatments.
Our results revealed that the microbial structure varies from the source to the tap. Carbon filters harbour a microbial community seeding water downstream, introducing a significant change on groundwater microbiota. Noteworthy, we reported the presence members of the DPANN Archaea superphylum (Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, and Nanohaloarchaeota), especially in groundwater, and the striking dominance of Patescibacteria, uncultivable bacteria with limited metabolic capacities and small genomes, from source to downstream water.
On the whole, microbiome research can support sustainable drinking water management, encouraging collaborations across sectors and involving the society through responsible research and innovation.

Molecular mechanisms for the evolution of gene structure and organization: the histidine case

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The origin and evolution of metabolic pathways represented a crucial event occurred during molecular and cellular evolution, rendering the primordial cells less dependent on the exogenous supply of abiotically formed molecules. The evolution of genes is the result of different molecular mechanisms including point mutations, gene duplication, fusion and elongation, horizontal transfer of external DNA and its homologous recombination in the host genome. One of the most studied metabolic pathways, which shows a plethora of gene structures and organizations, is histidine biosynthesis and analyses of the structure of his genes revealed that these different molecular mechanisms played an important role in shaping this route. The aim of this work was to explore the molecular mechanisms that shaped metabolic pathways during evolution, using the histidine biosynthesis as a model and experimentally simulating these events in Escherichia coli. Through bioinformatic analyses, genome editing techniques and directed evolution experiments, we i) studied the structure and organization of histidine biosynthetic genes in the Bacteroidota-Rhodothermota-Balneolota-Chlorobiota superphylum, highlighting a high variety of genes structures and organizations and allowing to suggest a possible model for the assembly of his genes in operons during bacterial evolution; ii) analyzed the compartmentalization of histidine biosynthetic enzymes in E. coli, demonstrating the in vivo interaction between HisF and HisH enzymes; iii) investigated the evolutionary molecular mechanisms of gene elongation, frameshift mutation and homologous recombination using the hisF gene as a model, simulating its possible early evolution; iv) explored the HisF involvement in different cellular processes in the bacterial world, suggesting its central role in cellular metabolism. Results obtained from the proposed analyses could represent a further step towards the understanding of metabolic pathways evolution.

Oral plaque metagenome: a functional perspective

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Periodontitis is a chronic inflammatory disease, which leads to the progressive destruction of the dental support. A fundamental role is played by the interaction between pathogenic periodontal microbiota and the host immune response, modulated by environmental and genetic factors. Periodontitis can act as a risk factor for systemic disease including cardiovascular disease, rheumatoid arthritis and diabetes.
The objective of the research was to apply whole metagenomic shotgun sequencing to explore functional and taxonomical features in the subgingival microbiome in a pilot study including twelve subjects, characterized by presence/absence of poorly controlled type 2 diabetes and presence/absence of moderate-severe periodontitis.
The analysis of functional activities was performed by DIAMON combined with MEGAN6-KEGG and by HUMAnN 3.0. Statistical analyses were carried out with STAMP and LEfSe.

Functional activity investigations lead to the detection of an enrichment of several features in affected subjects related to the biosynthesis of: 1) peptidoglycan and lipopolysaccharides. These products entering into the bloodstream can trigger a systemic inflammatory response in the host; 2) pyrimidine salvage pathway, that could represents a preferred route for the uptake of nucleobases, important for bacteria not only for nucleic acid biosynthesis but also as carbon and energy sources; 3) fatty acids (FA) and ferroptosis, both involved in cell death: FA can disrupt host defence systems inducting apoptosis in immune cells and ferroptosis induces cell death by iron-dependent reactive oxygen species.

Plant root microbiota can induce dysbiosis in Lepidoptera

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Competition among organisms inhabiting a shared ecological niche is commonly observed. In particular, competition for nutritional resources can result in a very complex network of interactions, especially if we consider eukaryotes as holobionts. Here, in a study-case model system, we demonstrate how the colonization of plant roots by a fungus negatively affects the development and survival of a lepidopteran larva feeding on the colonized plant. The insect does not show any structural damage to the midgut and/or alterations in the digestive capacity, as highlighted by transcriptomics, microscopy, and physiology analyses. Instead, metagenomic and metatranscriptomic approaches demonstrate how the larval midgut microbiota is altered in its composition and activity, impairing its nutritional support to the host. The present study shows how the outcome of a plant-insect interaction can be shaped by a soil fungus manipulating the gut

microbiota of the insect and reducing its fitness. The two do not share the same ecological niche but still rely on the same trophic resources: the plant tissues. Unraveling this complex network of interactions can contribute to our understanding of natural communities evolution.

Population Genomics and Phylogenetics of Phytoplasma Transmissibility by Psyllids and their Coevolving Endosymbionts

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Apple proliferation (AP) is a chronic apple tree disease caused by the bacterium 'Candidatus Phytoplasma mali', which is vectored by the phloem feeding psyllids, Cacopsylla picta and C. melanoneura. Apart from these two psyllids, several related Cacopsylla species also transmit phytoplasma, causing diseases in diverse species of trees and plants. It is hypothesised generally that the extent of phytoplasma transmission is influenced by a complex interaction between the phytoplasma, the psyllid vectors, their primary and secondary endosymbionts, and the targeted plant species. Yet despite the agricultural importance of these diseases, surprisingly little about their genomic structure, diversity and coevolutionary history is known. To address this, our research follows several avenues. We firstly examine the extent of codiversification between phytoplasma, psyllids and their microbial endosymbionts, focusing on 7 species of related Cacopsylla psyllid vectors. For each species, we assemble complete insect mitochondrial, phytoplasma, and primary and secondary endosymbiotic bacterial genomes. After characterising basic genomic structures and defining the array of primary and secondary endosymbionts, a phylogenetic analysis of codiversification is conducted targeting the hypothesis that psyllids, phytoplasma and endosymbionts codiversify, and exploring the possibility of rare horizontal transmissions. Building on this, we explore the potential influences that genetic variation in psyllid populations have on the transmission of phytoplasma, here focusing on AP transmission by C. melanoneura. Specifically, after establishing isolines from male-female crossing of wild caught individuals, and raising their offspring on experimental apple trees infected with 'Ca. P. mali', quantitative PCR was used to determine 'Ca. P. mali' uptake in 10 individuals in each of 124 sibling families. The full nuclear and mitochondrial psyllid and phytoplasma genomes were then assembled and long- and short-read genomic sequencing of the sibling families was conducted, with the resultant dataset allowing us to explore genetic differences potentially associated with disease uptake. Our results provide fresh insight into the coevolutionary genomics of psyllids and their endosymbionts in relation to their role in spreading important phytoplasmic disease.

The origin of autism spectrum disorders in the interpretation of Evolutionary Medicine

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Autism spectrum disorders (ASD or autism) are characterized by stereotypical repetitive behaviors and deficits in intellectual abilities and social interactions. Due to variations in autism definition and diagnosis, its frequency is poorly known, but surely it is high and dramatically increased. In the USA, in 2018, it was calculated that 1 in 59 children were affected by ASD, and was estimated that ASD prevalence more than doubled between 2000-2002 and 2010-2012. Autism has a remarkable and strongly growing economic impact, estimated in the USA, in 2012, to be equal to $ 250 billion with a likely increase to over $ 450 billion by 2025.
Many correlations between ASD and various factors are known, but none of them is considered as a valid explanation for the syndrome. However, considering the principles of Evolutionary Medicine, this explanation should not be sought exclusively in the physiology of the human organism. In fact, the human organism is part of a very complex ecosystem, defined as holobiont, made by the organism itself and also by many other entities (bacteria, fungi, viruses, macroparasites, etc.) and their countless interactions. Moreover, a mother and her child constitute a double holobiont.
It is known that the holobiont is severely altered in several ways and times by many factors. Moreover: (1) alterations of the holobiont cause numberless allergic and autoimmune diseases and other diseases; (2) in mice, the importance of the intestinal microbiome for a correct development of the central nervous system is documented; and (3) there are correlations between autism and (i) gastrointestinal disorders (a sign of altered intestinal microbiome); and (ii) immunological alterations.
It is likely that ASD are caused by alterations of the double holobiont. However, even before such causes will be precisely described, the severity of the consequences of ASD suggests that the holobiontic alterations should be corrected to prevent both autism and other pathologies.

Genetic variation analysis provides insights into diatom population genomics

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The planktonic, domoic acid producing alga Pseudo-nitzschia multistriata belongs to the tremendously species-rich group of diatoms. P. multistriata has a ubiquitous distribution in the world oceans, being generally present with relative low densities. Despite being a DA producing species its presence has so far not been linked to harmful toxic blooms.
In this study, we resequenced the genome of 29 P. multistriata strains from four different geographical locations, including two strains from the Gulf of Naples which were isolated during an event of clonal expansion. Variant analysis showed a high nucleotide diversity and allowed to compare groups of strains, revealing a stronger spatial than temporal influence on genetic diversity. We looked at different features of the genomes, such as highly variable regions, regions characterized by loss of heterozygosity (LOH) and selective sweeps and identified dispensable genes and fast evolving genes. Since some of the used strains seemed non-toxic in the condition of collection, we examined the DA biosynthetic genes, finding no major differences in their predicted protein sequences in the 29 strains, but observing a correlation between their expression levels and toxin levels in selected strains. Finally, we analyzed the mating type determination region of the strains and report the finding of an unexpected allele makeup in an MT+ strain from New Zealand.
While most studies comparing genetic diversity in a diatom species have been based on a few genomic loci, this study allows to reconstruct a more detailed picture by applying whole genome sequencing to a considerable number of strains. The variety of the samples allowed so far unique

comparisons of the influence of spatial and temporal aspects on Pseudo-nitzschia genetic variation as well as investigation of bloom-season and non-bloom strains. New insights in the toxicity and sex determination genomic region of the species can inspire further research in Pseudo-nitzschia and other toxic and non-toxic diatoms.

Overwintering does not affect the microbiome diversity in the Brown Marmorated Stink Bug

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The brown marmorated stink bug (BMSB), Halyomorpha halys (Hemiptera: Pentatomidae) is an invasive agricultural insect pest from East Asia that is now widespread in North America, South Europe and Chile. This species survives temperate winters using a particular overwintering strategy including the aggregation of individuals that enter a state of dormancy and starvation. Gut microbial communities have recently been shown to play a crucial role in the biology of many organisms, but it is not known if and how the BMSB microbiota is affected by its dormancy period. To address this we investigated the short-term evolution of BMSB gut microbiota diversity by performing 16S amplicon analysis of five distinct populations, sampled before and after overwintering dormancy in the Province of Trento, northern Italy. Results indicate that the alpha and beta diversities of pre-overwintering microbiota is similar among populations . We also noted the presence of specific pathogens which might be of environmental interest and found Nosema maddoxxi (an important bee pathogen) in two populations (instead, phytoplasmas were absent). We did not find any significant differences in microbiota diversity between the pre-overwintering and overwintered individuals. It is well known that microbiotas in several organisms (e.g., Drosophila melanogaster and Homo sapiens) significantly shift with environmental and dietary changes. Our result is therefore unexpected, since overwintering is associated with starvation and metabolic shifts in BMSB However, it has been shown in some other insects (e.g., the cockroach Periplaneta americana) that microbiota diversity does not change in response to starvation. Our results indicate that BMSB maintains a stable host-symbiont relationship with its microbiota despite overwintering under stressful conditions: this strategy may be related to the previously reported highly specific associations of stink bugs with some of their symbionts. From an applied point of view, our results suggest that the use of the sterile insect technique should characterize the microbiota of original populations before insect release to avoid propagation and spread of potential pathogens such as N. maddoxxi which can be maintained after overwintering.

Large-scale genome reconstructions from human gut metagenomes to study phage-host relationships

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Bacteriophages, or simply phages, are viruses that infect bacteria. They are extremely abundant in the human gut, yet their diversity, evolution and the ecological relationships with their bacterial hosts are still underexplored. The lack of universal markers and low sequence conservation, in particular, are hindering their study through phylogenetic analysis. To increase the number of phage genomes that can be phylogenetically modelled and to test coevolution with their bacterial hosts, we mapped all known viral reference genomes from RefSeq, together with a newly developed resource of putative viral genomes from highly purified viromes, against a large set of previously assembled contigs from more than 9,000 human metagenomic samples and 3,000 viromes. We obtained more than 180,000 genomes that were grouped into nearly 4,000 phage clusters, of which only a fraction (17.5%) included a known reference. To reconstruct the molecular phylogenies of the newly characterised phages, starting from full genome alignments, we devised a new strategy combining clustering, multiple-sequence alignment, trimming, and automatic refinement of the alignment to detect and remove possible artefacts. We then used host predictions generated by CRISPR spacer matches to link the retrieved phages to their hosts, building a catalogue of phage-host pairs that cooccurred in the same samples. Our methodology allowed us to reconstruct the phylogenies of both phages and their putative hosts from the same samples, helping to clarify phage-bacteria coevolution and the general evolutionary ecology of the human metagenomes.

Extensive phylogenomic analysis of Zika virus provides an updated scenario of its origin and evolution

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The late Zika virus (ZIKV) outbreaks have been well characterized: many studies have addressed the dynamics of ZIKV circulation since its onset in Brazil in 2013. Earlier dynamics of Zika evolution in particular its origin have been instead rather neglected. To address these issues, we build a large dataset of circa 500 ZIKV genomes from all over the world and we performed recombination, molecular clock, and phylogeography studies. Results identified recent recombination between Singaporean and African lineages, suggesting that multiple distant lineages are co-circulating during outbreaks. We confirm Thailand's pivotal role in the spread of the virus in Asia. Moreover, South East Asia is suggested to be the primary source of infection for over 20 years. The analysis of the African ZIKV unveils consistent issues in dated phylogenetic analysis if employing virus samples with a cell passage history. Our dated phylogenies point toward an eleventh-century origin (Middle Age) of the ZIKV lineage, followed by a recent diversification in the mid-nineteenth century. We further explored the timing of the recent re-introduction into the African continent of the ZIKV Asian lineage revealing a strong delay between introduction and outbreak onset. Our study provides novel insight into both earlier and recent dynamics of ZIKV evolution; this data improves our knowledge of ZIKV biology and will help forecast future outbreaks of this and other Arboviruses.

Posters

Leading human skin microbiome research through interdisciplinary perspectives: SKIOME project

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The impacts of microbiota on human life are well-documented and skin microbiota is no exception. To mention one, many skin diseases are driven by the microbial community which lives upon us. Dysbioses usually arise due to common commensal microorganisms' balance alteration. Understanding these complex processes still requires the integration of multiple datasets to capture the diversity of microbiome overall, pushing research towards microbiome meta-analysis and data harmonization strategies.
Here, we present the SKIOME project, which aims to disentangle human skin microbiome through the integration of different multi 'omics techniques with an interdisciplinary perspective. Coupling a data-driven approach with wet lab strategies, we aim to identify microbial biodiversity associated with different environmental variables and skin phenotypes, characterize microbial networks, and determine microorganisms' functional properties.
To do this, we started creating a curated collection of 16S rRNA amplicon-sequencing skin metagenomics datasets (i.e. SKIOME collection), enriched with study-related metadata, which have been deposited on international public databases in the last decade. To gain meta-analysis and data harmonization, we developed a bioinformatic strategy able to facilitate and automatize datasets integration. As a first case study, we selected 16S rRNA amplicon DNA sequences belonging to healthy face samples from the SKIOME curated collection. Data were integrated, harmonized and re-analysed considering the publicly available metadata. From our preliminary results, we evidence differences between diverse face sites and we are investigating the biases introduced by the different protocols that research groups adopted.
The main goal of SKIOME is to reach a more comprehensive knowledge of what happens on our skin, starting from healthy individuals' one. Our work aims to contribute in developing microbiome-based cosmetic products and personalized medicine strategies.

Geographic structuring and divergence time frame of monkeypox virus

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Monkeypox is an emerging zoonosis endemic to Central and West Africa. Monkeypox virus (MPXV) is genetically structured in two clades (West African, WA and Congo Basin, CB), but its

evolution is poorly explored. We show that the WA population remained constant in size and experienced limited drift. Conversely, the CB population expanded recently, after a bottleneck or founder effect. Molecular dating indicated that the two clades separated during the Medieval Warm Period, which was characterized by expansions and contractions of rainforest areas, possibly creating the ecological conditions for the MPXV reservoir(s) to migrate. In the CB region, MPXV diversity is characterized by four subpopulations that show no geographic structuring. Conversely, the WA clade is spatially structured with two populations located West and East of the Dahomey Gap. We suggest that the distinct histories of the two clades derive from differences in MPXV ecology in West and Central Africa.

Coronavirus accessory proteins: homology-based classification uncovers extremely dynamic evolution of gene content.

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Coronaviruses (CoVs, family Coronaviridae, order Nidovirales) are a large family of non-segmented, positive-sense RNA viruses that infect a wide range of animal hosts. As of 2022, seven human CoVs are known, all of them zoonotic in origin: three are highly pathogenic (SARS-CoV-2, SARS-CoV, MERS-CoV), whereas the other four (HCoV-OC43, HCoV-NL63, HCoV-229E, HCoV-HKU1) usually cause mild symptoms. All these viruses belong either to the Alphacoronavirus or to the Betacoronavirus genera. Two additional CoV genera, Gammacoronavirus and Deltacoronavirus, include viruses that mainly infect birds, but also cetaceans, pigs, and other mammals.
CoVs have complex genomes that encode a fixed array of structural and nonstructural components, as well as a variety of accessory proteins that differ even among closely related viruses. Accessory proteins often play a role in the suppression of immune responses and may represent virulence factors. Despite their relevance for CoV phenotypic variability, information on accessory proteins is fragmentary. We applied a systematic approach based on homology detection to create a comprehensive catalog of accessory proteins encoded by CoVs. Our analyses grouped accessory proteins into 379 orthogroups and 12 super-groups. No orthogroup was shared by the four CoV genera and very few were present in all or most viruses in the same genus, reflecting the dynamic evolution of CoV genomes. We observed differences in the distribution of accessory proteins in CoV genera. Alpha-CoVs harbored the largest diversity of accessory ORFs, delta-CoVs the smallest. However, the average number of accessory proteins per genome was highest in beta-CoVs. Analysis of the evolutionary history of some orthogroups indicated that the different CoV genera adopted similar evolutionary strategies. Thus, alpha-CoVs and beta-CoVs acquired phosphodiesterases and spike-like accessory proteins independently, whereas horizontal gene transfer from reoviruses endowed beta-CoVs and delta-CoVs with fusion-associated small transmembrane proteins. Finally, analysis of accessory ORFs in annotated CoV genomes indicated ambiguity in their naming. This complicates cross-communication among researchers and hinders automated searches of large datasets. We suggest that orthogroup membership is used together with a naming system to provide information on protein function.

Exploring the bacterial microbiome of cantharidin-producing blister beetles (Coleoptera: Meloidae)

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Many insects are associated with mutualistic microorganisms that play important roles for the ecology and evolution of their hosts. Insect-associated microbes are known to produce secondary metabolites (Brachmann & Bode 2013, Adv Biochem Eng 135:123-155) and, in some cases, involved in the biogenesis of insect defensive terpenes (e.g., pederin in Paederus fuscipennis; Song et al. 2022, Insect Mol Biol 1-14). Blister beetles, including almost 3000 species (Riccieri et al. 2022, Syst Ent 1-12), are characterized by the production of cantharidin (CA), a toxic terpene. CA is exuded by blister beetles in yellowish oily hemolymph droplets from legs and antennal joints as a defensive strategy (Gisondi et al. 2019, Entomol Sci 22: 258-263). Although the latest steps of CA biosynthesis are still elusive (Fratini et al. 2021, BMC Genomics 22: 808), males are known to produce more CA than females and accumulate large amounts of this terpene in their reproductive organs (Carrel et al. 1993, Experientia 49: 171–174). Females are especially attracted by males with high amount of CA, which is transferred to females upon mating for protecting eggs from predators or parasites (Sierra et al. 1976, Experientia 32: 142-144). Through 16S metabarcoding we explored the bacterial composition of males and females of five species of blister beetles (Hycleus polymorphus, Mylabris variabilis, Lydus trimaculatus, Zonitis flava, Meloe proscarabaeus). Proteobacteria, Firmicutes and Actinobacteria were the most abundant (i.e. “core”) groups in both sex of all species. Among those, Firmicutes and Actinobacteria attracted our attention, because of their well-documented and extensive repertoire of secondary metabolites to engage in protective symbioses (Kaltenpoth 2009, Trend Microb, 17: 529-535). We discuss the potential involvement of these groups of bacteria in the biogenesis of CA and/or in other aspects of the ecology of blister beetles.

Serratia marcescens “breaking bad”: comparative genomics reveals signs of adaptation from environmental to pathogenic lifestyle

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Serratia marcescens is a bacterial pathogen able to cause fatal outbreaks in neonatal intensive care units. The bacterium is able to colonize animals, plants and the rhizosphere, suggesting its ability to occupy novel ecological niches. Recent studies about the S. marcescens population structure revealed that most outbreak episodes can be traced back to a specific S. marcescens lineage, but further information is needed to validate this hypothesis.
The aim of this project is to investigate the evolution and population structure of S. marcescens to identify and characterize genetic traits involved in its environment-to-clinical shift.
Our analyses included 915 genomes of Serratia marcescens strains sampled from environment, animals and nosocomial settings: 248 sampled from Italian hospitals and 667 retrieved from public databases. We performed SNP-based phylogeny, orthology analysis and genome-wide association studies (GWAS).
Phylogeny revealed the presence of five well distinguished groups, including two associated to animal/environment and one to clinical settings. Interestingly, all strains sampled during outbreaks fall within the clinical group. Pangenome/core genome analysis revealed that the clinical group displayed less steep pangenome and core-genome curves, despite the group being over represented in the dataset. Moreover, gene presence-absence clearly revealed a remarkable set of accessory genes linked to each lineage and GWAS analyses extracted 102 genes associated with the clinical group.
These results consistently suggest the existence of a lineage of Serratia marcescens with a peculiar gene composition that perhaps suggest how the bacterium shifted to a pathogenic lifestyle.
Future perspectives include dating the time of origin of each clade and functionally annotating these genes, to investigate the function carried out by these clade-specific genes and ecological factors (i.e. agriculture) that could have enhanced their selection.

SYMPOSIUM

POPULATION GENETICS USING MODERN AND ANCIENT DATA

CHAIRS: NICOLA SEGATA

Invited speaker RASMUS NIELSEN, Center for Computational Biology, University of California, Berkeley, Berkeley, USA

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Using Ancestral Recombination Graphs for Population Genetic Inferences

In this talk I will discuss the emerging use of Ancestral Recombination Graphs (ARGs) as a tool for population genetic inferences. I will show examples of the use of ancestral recombination graphs for inferences of demography and natural selection in humans, but also discuss some limitations and challenges in their use.

Talks

Using ancient genomes to investigate responses to climate and anthropogenic impacts in the Atlantic bluefin tuna

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Like many commercial marine fish populations, the eastern Atlantic and Mediterranean population of Atlantic bluefin tuna (Thunnus thynnus) has experienced dramatic changes in abundance and complexity during the past 50 years due to overexploitation. Since 2014, this population has shown signs of recovery, yet we have a poor understanding of how it was composed prior to 1970, and thus how those more recent changes reflect long-term population dynamics when exploitation rates were lower and climate conditions were different. Genomic data provides an opportunity to investigate how this species has responded to climate and the Anthropocene in terms of its demography and adaptive traits. This information is much needed to reduce the likelihood of future population declines and predict ecosystem function during dramatic climate events to come. Thus, we present the first analyses on nuclear whole-genome data of this species, which includes sample groups of archaeological and archived ABFT dated to between the 10th century and early 20th century. Furthermore, we present the first data to represent a putative Black Sea population of ABFT, which disappeared rapidly during the 1970's. We successfully sequenced medium-coverage modern and ancient genomes and applied a battery of methods to identify adaptive loci and study allele frequency changes between spatiotemporal sample groups using both hard-called genotypes and genotype likelihoods. These novel data have implications for the management of ABFT since they elucidate the current population structure and allow for the forecasting of responses following what has been observed with this robust dataset during several climatic and cultural events during the last millennia.

How do different ploidies adapt? A case of Arabidopsis in non-extreme edaphic environment.

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Considering adaptation as mostly a genetic process, whole-genome duplication (WGD), a major mutation, represents a controversial but rather promising engine of adaptive evolution. Generally, polyploid organisms have the opportunity of tapping into a larger gene pool, where alleles are multiplied resulting in higher heterozygosity which can in turn work as a buffer against deleterious mutations and can lead to a higher diversification and evolvability. Furthermore, WGD is likely to affect gene flow between species, for example weakening or creating reproductive barriers and isolation between cytotypes. While adaptation to extreme environments has been largely investigated in plants, mechanisms of evolution in less deadly conditions received less attention. Theoretically, lower mortality rate is expected to slow genetic adaptation, imposing less steep

selective clines. How different ploidies of the same species differ in their genetic evolution under such conditions was, so far, not addressed. We analyzed 608 resequenced genomes of two diploid-autotetraploid Arabidopsis species to uncover and compare ploidies' evolution of genomic basis underlying adaptation to non-extreme and common siliceous and calcareous substrates. Also coupling genomic data with ionomic results of locally sampled soil material, for each cytotype and species we identified candidate genes as mostly enriched in GO terms related to ions cellular transport and life-history traits. Finally, we assessed intraspecific ploidies' differences and parallelisms, investigated the evolutionary sources of trans-specific polymorphisms and measured the role of WGD caused interspecific gene-flow therein.

Differences in population structure between two octopus species, Eledone moschata and E. cirrhosa, in the Mediterranean Sea

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Cephalopods with their complex behavior and predatory lifestyle are an important part of the marine ecosystem. Many cephalopod species are still unknown from both the demographic and genomic point of view. Among them are octopuses of the genus Eledone, characterized by possessing only one row of suckers on their arms. The aims of the presented work were to investigate the genetic diversity, and population genetic differentiation and structure in two species, Eledone moschata and E. cirrhosa, limited to the Mediterranean Sea. Using the reduced representation genomic method (ddRADseq) we genotyped 32915+ loci in 110 specimens. We used coancestry matrix analyses to show the differences in the pattern of the distribution of these species. Our results showed genetically divergent populations in the Tyrrhenian and Adriatic Seas for E. moschata and E. cirrhosa. However, whilst the populations of E. moschata were subdivided into two main groups, corresponding to the Tyrrhenian and Adriatic Seas, there was no geographic pattern found in E. cirrhosa, with genetically even populations across the Mediterranean Sea. Our demographic histories analyses revealed stepper demographic growth only for E. moschata in the Adriatic Sea. The demographic history may have played a crucial role in shaping the present-day geographical framework, biological diversity, and the ability for colonization of new habitats. Observed different pattern of population diversity in both species, might be due to their different ecology and lifestyle. The overall pattern of divergence in both analyzed species could possibly be influenced by their ecology (e.g., larval dispersal abilities). The presented genomic study contributed to resolving still understudied population genetic relationships in cephalopods. Herein, the genomic methods demonstrate the applicability to distinguish populational differences and could be used for other non-model marine organisms. We provided the molecular base for further ecology studies of this economically important species sensitive to ecological, biotic and abiotic changes, as well as the negative impact of human overfishing and activities.

Scales of genomic patterns of selection reflect biogeographic history of a beech population

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Plant populations growing along steep environmental gradients experience strong microhabitat selection, which can lead to microgeographic adaptation despite strong gene flow. Here, we searched for signatures of natural selection in a dataset of 40594 SNPs obtained from exome capture, and mapped onto a reference genome, in four natural, recently expanded European beech (Fagus sylvatica L.) stands located at different altitudes and on different slopes of a single mountain. We found overall low pairwise genetic differentiation between stands (FST max = 0.0024, Jost's D max = 0.00665). Divergence outlier approaches found 389 SNPs differentiating stands at different elevations and/or on different slopes, and a Genetic-by-Environment Association approach identified 290 SNP-environment associations. A search for gene-level multi-locus genetic divergence signals found 728 outlier regions, which were randomly distributed across the genome; on the contrary, analyses run at the chromosome-bin level picked up only divergence between two putative refugial populations, with 130 divergent bins tightly clustered in few chromosomal regions. Overall, we detected several selective signals at different genomic scales. Selection has affected the genome of the refugial populations at the chromosome level; the expanded populations experienced new selective forces which have disrupted this ancestral genomic pattern and locally allowed for the formation of novel combinations of divergent loci across the genome. Our study indicates that the microgeographic selective pressures are strong enough to have caused genomic divergence at many loci in spite of both the gene flow and the few generations elapsed since the re-expansion.

Exploring heritable and plastical contributions to survival in individuals born under naturally unfavorable conditions using the King penguin as a study case

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Energetically demanding activities like reproduction should be synchronized with peaks of food resources for species to thrive in natural systems. Consequently, individuals born out of optimum condition peaks are expected to face developmental constraints that may lead to lower individual fitness and higher mortality. Here, we investigate the contribution of genetic and plastic components to the early development and survival of individuals born out of the species' phenological optimum, using the King penguin (Aptenodytes patagonicus) as a model. We sequenced the whole genomes of chicks born in and out of the peak of food resources (i.e., early and late in the season) to search for signals of genetic differentiation between those groups. We also analyzed the blood transcriptomes of early and late chicks that survived their first winter in order to identify early development consequences of being born under unfavorable conditions. Late chicks showed a higher expression of genes related to lipid metabolism at hatching, which may represent an adaptation to the shorter period they have to accumulate body mass before winter

fasting in comparison to early chicks, which are born one month in advance. More specifically, the most significantly upregulated gene in late chicks, the MAX Dimerization Protein 4 (MXD4) protein coding gene, is located close to two quantitative trait loci related to abdominal fat accumulation in chicken. Despite the observation of differential gene expression patterns, no signal of global genomic differentiation was detected between the two groups nor between chicks that survived and died after the first winter, suggesting that survival in the first year of life is majorly determined by plastic rather than heritable adaptations in this species. Moreover, our results point to the influence of maternal effects in the survival of offspring generated out of the phenological peak, suggesting that chick survival is more dependent on the external conditions faced by the parents than to its genomic composition.

Human-to-human transmission of bubonic plague: a lesson from the Milan epidemic of 1630.

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In 1630 a large and devastating bubonic plague epidemic struck northern Italy, killing hundreds of thousands of people and deeply affecting the demography and the economy of the region for decades. Milan, one of the most important cities of the region, suffered probably the most devastating plague epidemic of its history, an epidemic that was also described and made famous by the novel of Alessandro Manzoni "I Promessi Sposi".
In the last twenty years the analysis of ancient DNA collected all over the world has allowed to partially reconstruct the evolutionary history of Yersinia pestis, the etiological agent of plague. Unfortunately, no molecular data are available yet for the 1630 Milan epidemic. Despite this, an invaluable historical source of information is available for the city: the Milan death registers (Mortuorum libri), in which physicians of the time recorded every death that occurred inside the city from 1452 to 1801. For each death case detailed information such as name, surname, geographical location, date and cause of death were recorded.
In this work, we digitised and analysed the information contained in the Milan death registers of 1630 to reconstruct the spatio-temporal evolution of the epidemic inside the city. Our analyses revealed that the epidemic started in the peripheral areas of the city and reached the centre after the San Carlo procession of 11 June, a religious mass gathering event attended by thousands of people for about twelve hours. Transmission of bubonic plague is conventionally mediated by the rat-flea-human chain. Nevertheless, the effect of this mass gathering on the spread of the epidemic clearly shows that human-to-human transmission could have played a key role in this plague epidemic.

A genomic portrait of the Isthmus of Panama

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The Isthmus of Panama was an obligatory passage for the first peopling of the Americas and played a pivotal role during the European colonization and the African slave trade. In this context, attempts to estimate the demographic processes occurring before and during the European expansion are complicated. Historical, archeological, and genomic data have suggested that Panamanian Indigenous groups experienced a reduction in population size occurring before contact, which intensified with the conquest and was followed by a demographic recovery. However, rate estimates and detailed mechanisms are still unknown. To explore these issues, we have sequenced 10 high-coverage genomes (~25-30X) from modern Panamanian individuals belonging to Indigenous and admixed groups. In addition, new high-quality ancient genomes from colonial individuals have been produced and analyzed, together with already published pre-contact genomes, generating a unique time transect of the region. The level of resolution of high-coverage genomes and state-of-the-art methods made it finally possible to reconstruct demography and effective population size in the Isthmian area before and after European contact.

Bringing order to chaos: population genomics and forward genetic simulations explain the causal mechanisms of chaotic genetic patchiness in an Antarctic gastropod

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Elucidating the mechanisms shaping the genetic diversity of marine organisms is essential for understanding microevolutionary processes as well as for marine reserve design and fisheries management. However, it has proven challenging to explain a number of paradoxical observations of marine populations, a prime example being chaotic genetic patchiness (CGP). CGP consists in unexpected patterns of genetic differentiation which occur among populations located within fine geographic scales. These patterns are unstable over time and may disappear in as little as one generation. CGP patterns are not expected according to classical population genetic theory because they occur below the effective range of larval dispersal, meaning that genetic structuring should be counteracted by gene flow. Several distinct mechanisms have been proposed to explain CGP and discriminating among them is fundamental for understanding how marine organisms reproduce and disperse, but is extremely difficult in natural settings and has not been possible to date. Here, we report a striking empirical example of CGP in the Antarctic limpet Nacella concinna, an unusually tractable system where multiple competing explanations can effectively be ruled out. To provide novel insights into the causal mechanisms of CGP, we used high-throughput genomic data, a temporally replicated sampling design, surface drifters deployed within Ryder Bay (Antarctica), and forward genetic simulations. In this way we showed that, while selection appears to be unimportant, CGP likely arises from a combination of an extreme sweepstake event, which occurs when a very limited number of individuals contribute offspring to the next generation, coupled with collective dispersal. Our empirical findings highlight the importance of neutral demographic processes in natural populations and help to resolve a long-standing paradox in

marine evolutionary genetics with important implications for understanding the recruitment dynamics, population connectivity, local adaptation, and resilience of marine populations.

Robust demographic inference from low-coverage whole-genome data through Approximate Bayesian Computation

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The reconstruction of past demographic processes relies on the pattern of genetic variation shown by the sampled populations; this means that an accurate estimation of genotypes is crucial for a reliable inference of populations' dynamics. Approximate Bayesian Computation (ABC) is a robust and flexible approach to reconstruct past evolutionary and demographic events, which can be coupled with coalescent simulations to generate the expected level of variation, represented by known genotypes, under different evolutionary scenarios. Demographic inference is then performed by comparing the simulated data with the genotypes called in the sampled individuals. Low sequencing depth drastically affects the ability to reliably call genotypes, thus making low coverage data unsuitable for such an approach, severely limiting our ability to make inference about past population dynamics.
Here, we present a new ABC framework, based on the Random Forest algorithm, to infer past population processes using low coverage whole-genome data. We summarized the data using the full genomic distribution of the four mutually exclusive categories of segregating sites (FDSS hereafter). Under this framework, the FDSS is not directly calculated from known genotypes, but rather estimated using genotype likelihoods, so as to take into account the uncertainty linked to low-coverage data . The simulated FDSS is thus directly comparable with the observed in low coverage experiments. We assessed the inferential power of this procedure in distinguishing among different demographic models and in inferring model parameters under different experimental conditions, such as the coverage depth (1x to 30x), the number of individuals, the number and the size of the genetic loci considered in the analysis.
Our results showed that the use of genotype likelihoods integrated within the ABC framework provides a reliable inference of past population dynamics, thus paving the ground for model-based demographic inference based on low-coverage genomes data.

Posters

Reconstructing the global invasion routes of Halyomorpha halys through Approximate Bayesian Computation

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The brown marmorated stinkbug (Halyomorpha halys) is a polyphagous insect pest, which has rapidly spread worldwide causing extensive damage to global agriculture. This species, native to China and Japan, was first identified outside of Asia in 2004 in Switzerland. Since then, it has invaded 30 countries from Europe to the Americas, damaging a wide variety of crops and becoming a global economic threat for the agricultural and horticultural industry. Investigating the genetic diversity among H. halys populations is essential to understand the patterns of colonization and invasion history of local populations. Still little is known about the H.halys populations' genetic structure at a global scale; efforts have been made using partial mitochondrial DNA sequences, resolving the introduction routes only at specific geographic areas. Recently genomic data (ddRAD) from multiple worldwide populations of H. halys have been published, and preliminary analyses suggested a complex pattern of invasion from Asia to Europe and America. In this study we analyze published ddRAD sequencing data of 389 individuals from 12 worldwide populations of H. halys from Asia, Europe, and USA, with the final aim of explicitly compare different demographic models of invasion, and shed light on the dispersal process of the species. We identified 1577 high quality single nucleotide polymorphism (SNPs), that have been used to investigate the fine-scale population structure and the genetic diversity of the species. Our results showed a sharp genetic differentiation between the native Asian population of Japan and all the other populations, both from Asia, and from Europe and USA. Furthermore, we observed a peculiar genetic structure in both the native Chinese populations and in the invaded countries, suggesting multiple invasion processes from China to Europe and a single, subsequent invasion from Europe to the USA. This hypothesis will be explicitly tested simulating alternative demographic scenarios employing an Approximate Bayesian computation framework.

Ancient DNA analyses clarify population dynamics between the Late Pleistocene and Bronze Age Italian canids and the possible contribution to local dog domestication

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In the last decades, paleogenetic methodologies have greatly improved, permitting to study ancient specimens through the analysis of their whole mitochondrial and nuclear genomes. This approach makes it possible to investigate very ancient evolutionary processes, even older than 1 million years ago, and better comprehend the evolutionary patterns of modern species and their relationships with ancestors. In Europe, especially in southern areas corresponding to the historical glacial refugia during the Last Glacial Maximum, only a few data about the genetic diversity of grey wolves (Canis lupus) prior to their domestication and of early dogs (Canis lupus familiaris) are currently available. Such ancient DNA data are even rarer and more fragmented for the Italian wolves (Canis lupus italicus), which represent the only modern population to fall exclusively within the mitochondrial haplogroup diffused across Eurasian and North American wolves during the Late Pleistocene. Therefore, to fill the gap of such information, in this study, performed within the project "FIDO - Following Dog Domestication Origin and dynamics from Late Pleistocene in Italy", we applied the cutting-edge methodologies for the analysis of ancient DNA. We sequenced 30 ancient wolf and early dog complete mitogenomes with an outstanding coverage range from 22X to 946X, from biological remains collected in northern Italian archaeological sites, dated from 42.000 to 3.000 years BP. These mitogenomes were compared to ancient and modern wolf and dog sequences available in the literature to perform phylogenetic analyses. Results provided an overview of the temporal patterns of the Italian wolf genetic variability, clarifying population dynamics that started in the Late Pleistocene and contributed to the current morphological and genetic uniqueness of the Italian population. Furthermore, thanks to these results, the possible role of ancient Italian wolves in local dog domestication dynamics, was investigated.

Should I stay wild or should I go domestic?

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The wild boar (WB) arrived in Sardinia (Sus scrofa meridionalis) with the first human settlers in the early Neolithic, and has had the chance to hybridize with the domestic pig (DP, S. scrofa domesticus) throughout its evolution on the island. In order to understand the genetic relationships between the two groups, we analysed 96 Sardinian WB with several commercial pig breeds and Sardinian local pigs, along with a putatively pure WB population from Central Italy, all genotyped with a medium density SNP chip. We first identified the hybrids with different approaches: principal component analysis, model-based ancestry assignment, f3-statistics.
We found 3 hybrids within the Sardinian group, with estimated admixture time around 20 generations ago. We inferred wild and domestic windows along the genomes of the hybrids with Local Ancestry (LA) deconvolution. Furthermore, we explored the pattern of domestic introgression by looking for genomic regions of domestic origin under positive selection. Thus, we computed population branch statistic (PBS) to identify genomic regions overly differentiated in the introgressed compared to the pure Sardinian and Italian WB. Since the sampling size for the introgressed was low, we also looked for genomic regions under selection in the donor population (i.e. the pool of DP) compared to the pure Sardinian WB with cross-population extended haplotype homozygosity (XP-EHH).
We found 11 significant windows with PBS but no overlap with LA, and 82 with XP-EHH, two of which overlapped with genomic regions enriched for domestic alleles. Genes in these regions can be linked with reproductive success.
Given our results, domestic introgression does not seem to be pervasive in the Sardinian WB; nevertheless, a larger sample could reveal additional introgressed individuals to better characterize

the possible evolutionary advantages deriving from this form of anthropogenic hybridization in a rapidly changing and human-dominated landscape.

Genomic of successful lessepsian alien fishes colonising the Mediterranean Sea.

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Several threats have been recently identified with a predicted major impact on biodiversity in the Mediterranean Sea, making this environment one of the most impacted seas in the world. Since the opening of the Suez canal an increasing number of exotic species, so-called Lessepsian invaders, are entering the Mediterranean. Many of them are successfully colonising this basin, negatively interfering with native species. Currently available literature on Lessepsian species mainly focuses on ecological data. Few genomic studies carried out so far have not thoroughly investigated the role and impact of adaptive and deleterious variability in the colonisation of the Mediterranean Sea. Nonetheless, the investigation of pre-adaptations and post-invasion selection has been considered as crucial to fully understand the factors underlying the success of invasive species in the new environment and to allow the design of predictive models. The scope of this project is to investigate the dynamics of different types of genetic variability during the colonisation of new habitats, categorising the variability based on its evolutionary impact (adaptive, deleterious and neutral). Using Lessepsian fish species as a model, we are going to investigate the genomic characteristics that predispose a population to the invasion of a new environment and, in turn, which are the consequences of the invasive processes on the genetic variability.

Understanding the process of skin lightening in Europeans: a mix of selection and migration factors.

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During his spread out of Africa, Homo sapiens faced the challenge to adapt to new environments. One of the best-known adaptive features is the variation of skin pigmentation. Populations from equatorial regions have darker skin than those from higher latitudes, which is explained as an adaptation to different levels of UV radiation. Where UVR explosion is high, a high melanin content is protective against the photolysis of folate, whereas at low sunlight levels low levels of melanin facilitate the synthesis of vitamin D.
However, the details of the evolutionary process behind the observed pattern are all but clear. Skin pigmentation is a complex trait, influenced by genes involved in several aspects of melanin production, storage and transportation. From the analysis of modern populations, it seems that variants involved in skin lightening appeared at different times and geographic areas, but the

combination of selective and migration processes that lead to the current geographical distribution is still unknown.
Ancient DNA studies showed that only 10,000 years ago hunting-gathering populations living as high north as the British islands had dark skin, and the first genetic signals of lightening did not appear until the spread of Neolithic farmers, almost 3,000 years later. However, neither the pioneering farmers, nor the later Bronze Age migrants, showed the pale skin phenotype that nowadays is widespread in Europe.
Here we present an innovative approach based on Artificial Intelligence to infer skin pigmentation from DNA haplotypes. We are comparing the genetic information between modern populations and key ancient individuals, which are representative of the major prehistoric migrations into Europe. Based on state-of-art bioarchaeological approaches (aDNA, 14C dates and cultural information on the samples), we are tracking back the events that shaped the current diversity of human skin pigmentation.

SYMPOSIUM

OTHER

Talks

Population genetics of the tick Ixodes frontalis and correlation with the presence of bacterial endosymbionts.

1_Bisaglia Beatrice, 2_Sophie Melis, 3_Tiago Nardi, 4_Romain Daveu, 5_Olivier Plantard, 6_Michele Castelli, 7_Greta Bellinzona, 8_Alessandra Cafiso, 9_Chiara Bazzocchi, 10_Emanuela Olivieri, 11_Davide Sassera

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Ixodes frontalis is an understudied ornithophilic tick species widely distributed in Europe that can vector multiple pathogens. Two genetic variants of COI gene (lineages A and B) are recognizable in the populations of this tick. Investigations on other tick species showed that bacterial symbionts are fundamental for supplementing the nutritional deficiencies of a blood-based diet. While such associations are rather evolutionarily stable, closely related ticks might bear distinct symbionts, due to horizontal transfers of the bacteria. Preliminary screenings showed the presence of two symbionts in I. frontalis: Midichloria mitochondrii and Spiroplasma ixodetis. In this study, we aimed to improve the knowledge on the prevalence of bacterial symbionts in I. frontalis, as well as to correlate prevalence/abundance of symbionts with the ticks' lineages. A total of 218 immature specimens (larve and nymphs) I. frontalis were collected from vegetation, under bamboo bushes, in different areas in France and Italy. Specimens were assigned to COI lineages by PCR, with the majority belonging to the haplotype A (69,7%). Presence and load of M. mitochondrii and S. ixodetis were investigated with both qualitative and quantitative PCR. M. mitochondrii was detected with a slightly higher prevalence (18,8%) than S. ixodetis (17,8%). Interestingly, a significant portion of the examined ticks (65,5%) lacked both symbionts. The correlation between lineage and presence of symbiont showed that ticks belonging to lineage A may carry both M. mitochondrii (26,9%) and S. ixodetis (17,01%), while M. mitochondrii is absent in specimens of lineage B and S. ixodetis has a lower prevalence (18,1%). Considering the high frequency and known importance of bacterial symbionts in other tick species, a 16S rRNA gene metagenomics was performed on a subset of samples to investigate the whole bacterial community composition and identify other candidate symbionts. Data analyses are ongoing.

Molecular systematics of the enigmatic spider genus Mastigusa Menge, 1854.

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Spiders belonging to the genus Mastigusa, distributed in the Western Palaearctic, represent an interesting model for evolutionary studies for different reasons: they display extreme modifications in the morphology of both male and female genitalia, whose adaptive significance (if any) is still unknown; they also show peculiar ecological features, with diversified lifestyles ranging from free-living to cave-dwelling and even to ant association. A rigorous study of such life-traits in an evolutionary framework is hindered by severe taxonomic uncertainties that have interested this genus since it's description. Mastigusa was indeed placed in different spider families, always doubtfully, given the lack of strong morphological evidence. Three species are currently recognized: M. arietina (Thorell, 1871), M. lucifuga (Simon, 1898) and M. macrophthalma (Kulczyński, 1897), but their circumscription has always been problematic due to the inconsistency of the diagnostic characters used, leading to confusion and disagreements about their actual status and distribution. We here present the first phylogenetic placement of the spider genus Mastigusa based on both mitochondrial and nuclear markers, together with a phylogeny of its representatives with the aim of exploring and describing its actual diversity at a species level. Our analyses showed Mastigusa, currently placed in the family Hahniidae, clustering instead within Cybaeidae, with insights about its relationships with other genera. At the species level we found a significant, and mostly overlooked, genetic diversity with five strongly divergent lines and a clear geographic pattern, compatible with the great ecological variability observed across the distribution range of Mastigusa. The characterization of these lines lays the groundwork for a modern taxonomical revision of the genus.

Seascape genomics approach to describe population structure of two marine species: Solea solea and Merluccius merluccius case studies.

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The combination of complex life-history traits of marine species with environmental and spatial features generates population structure. The integration of molecular and environmental data is crucial to understand selection and demographic processes shaping population structure of marine organisms for management and conservation purposes.
In the present work, we used genomic data including hundreds of single nucleotide polymorphisms (SNPs) to illustrate the population structure of two marine fishes targeted by commercial fisheries in Mediterranean Sea, namely common sole (Solea solea) and European hake (Merluccius merluccius). Our aim was to identify patterns of neutral and potential adaptive genetic variation by applying seascape genomic framework considering spatial and environmental features. Redundancy analysis was combined with genetic differentiation to characterize spatial structure and identify factors potentially involved in local adaptation. For common sole we analysed putative populations inside the Mediterranean Sea while for European hake we targeted distinct sites within the Alboran Sea, extending into the neighboring Mediterranean Sea and Atlantic Ocean.
From all analysis performed, a significant genetic differentiation across locations was found for both species. Results consistently showed the occurrence of population structure for both case studies by detecting westward–eastward differentiation among populations, higher levels of

structure and potential distinct subgroups at a fine geographical scale using putatively adaptive SNPs. Seascape analysis on European hake revealed that environmental factors better explained the genetic variation at both neutral and putatively adaptive SNPs loci, suggesting their key contribution in shaping the genetic structure. Conversely, results for common sole revealed that spatial variables better explained the genetic variation at both neutral and putatively adaptive SNPs loci, suggesting that geographical distances between sites played the major role in the spatial distribution of genetic diversity.
These results enhance the knowledge of the identification of population units of commercially relevant species, which has a fundamental role in the management of stocks, together with the understanding of which processes and factors are playing a role in the definition of their population structure.

Multiple LINE lineages contribute to early and recent bivalve genome evolution

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Transposable elements (TEs) can represent one of the major sources of genomic variation across eukaryotes, providing novel raw material for species diversification and innovation. While considerable effort has been made to study their evolutionary dynamics across vertebrates, arthropods, and plants, molluscs represent a substantially understudied phylum, with few comparative analyses focused only on a small subset of species. Here we combined an automated TE annotation pipeline and an ORF-based extraction and tree-based classification to deeply characterize 86,488 reverse transcriptases (RVT) containing LINE across 35 molluscan genomes, of which 27 belong to bivalve species. Despite being less represented than Class I and highly variable in their genome occurrence, LINE elements constitute the most common retroposon group, covering up to 10% of bivalve genomes. We identified a rich and diverse bivalve ancestral LINE complement likely present in their most recent common ancestor and that includes elements coming from all known superfamilies. Interesting cases are CR1-Zenon, Proto2 and RTE-X lineages that underwent a bivalve-specific amplification likely associated with their diversification. Finally, we manually curated more than 800 putative autonomous families tracing their evolutionary history, genome occurrence and possible interaction with host genes. We discover a possible long - term survival ability of multiple LINE families highlighting their potentially primary role in shaping both recent and early phases of bivalve genome evolution and diversification. Overall, we provide not only the first comparative study of TE evolutionary dynamics in a wide but greatly understudied phylum such as molluscs but also a reference phylogeny and LINE library for bivalves, which could represent important genomic resources for further studies on their expression in different environments and developmental contexts.

Toxicity of sex chromosomes and evolution

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The development and improvement of genome sequencing technologies in the last decade revolutionised the entire field of biology by providing the possibility to assemble and investigate the genome of virtually any organism. Despite this tremendous progress, true genome assembly completeness is not achieved in most organisms and there are complex and repetitive genomic regions that are systematically missing from genome assemblies, commonly referred to as genomic dark matter. The presence of genomic dark matter entails that such genomic regions cannot be studied and the effects and/or functions thereof (if any) cannot be discovered. It is key in evolutionary biology to be able to explore those dark genomic corners to fully understand the evolution and physiology of organisms without biasing the interpretations.
Here, I explore the sequence and structure of one of the main components of the avian genomic dark matter, namely the female-specific non-recombining W sex chromosome. By using genomes from main avian groups, I show that the W is mostly composed of endogenous retrovirus retrotransposons (ERVs) and acts like a refugium by accumulating more than half of full-length (thus potentially active) ERVs of the entire genome. Thanks to a combination of genomic, transcriptomic, and proteomic data from male and female individuals, I found that these W-linked ERVs are actively transcribed and translated in females in higher quantities than in males. These results suggest that the W chromosome may exert a toxic effect on females through its active retrotransposons by affecting the female-specific mutational load, the genome-wide heterochromatic landscape and by giving rise to genetic incompatibilities. This W toxicity may then be an additional explanatory variable for the observed reduced female lifespan with respect to males and for the occurrence of Haldane’s rule in birds.

Posters

Relationship between salinity conditions and transposable element activity in teleosts

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Fish are an interesting taxon comprising species adapted to a wide range of environments. In this work, we analysed the transcriptional contribution of transposable elements (TEs), one of the most intriguing components of the genome, in the gill transcriptomes of three fish species exposed at different salinity conditions. We considered the giant marbled eel Anguilla marmorata and the chum salmon Oncorhynchus keta, both diadromous, that have to face changes in salinity in a defined stage of their life cycle and the marine medaka Oryzias melastigma, an euryhaline organism sensu stricto. Our analyses revealed an interesting activity of TEs in the case of juvenile eels, commonly adapted to salty water, when exposed to brackish and freshwater conditions. Moreover, the evaluation of the expression of genes involved in TE silencing mechanisms (six in heterochromatin formation, 14 known to be part of the nucleosome remodelling deacetylase (NuRD) complex, and four of the Argonaute subfamily) unveiled that these mechanisms are active. Finally, our results evidenced for the first time a krüppel-associated box (KRAB)-like domain specific to actinopterygians that, together with TRIM33, might allow the functioning of NuRD complex also in fish species. The possible interaction between these two proteins was supported by structural prediction analyses.

The distribution of regulatory mitochondrial RNAs with nuclear targets (smithRNAs): a bioinformatic approach.

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RNA interference is gaining interest in gene silencing studies, in particular in response to the discoveries regarding the extreme plasticity of small non coding RNAs, their biogenesis and their activity. Previous studies found that this regulation could involve small noncoding RNAs originating from mitochondrial genome towards nuclear targets. These elements, called small mitochondrial highly transcribed RNAs (smithRNAs), were first detected in Ruditapes philippinarum, a bivalve species with an unconventional mitochondrial inheritance phenomenon: the Double Uniparental Inheritance. The functionality of smithRNAs has been demonstrated by in vivo experiments in this peculiar system, while in silico analyses suggest a broad distribution among animals. This study aims to shed light on their presence in Metazoa using a robust bioinformatic pipeline. The analyses reveal an unequal level of smithRNAs between investigated species. A sex-linked function is likely to be the cause of such a great disparity; however, further investigation is needed to address their implications in other cellular aspects. The present study suggests that smithRNAs are fast-evolving elements able to emerge quickly on evolutionary scales. For these reasons, they candidate as the main regulation mechanism shaping the coevolution of the different genomes inhabiting the eukaryotic cell.

Evolutionary Anatomy of the Avian Quadrate Bone

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The quadrate of tetrapods plays an important morpho-functional role by allowing the articulation of the mandible with the cranium. In reptiles and mammals, this element is fundamental in the biomechanics of the skull and in the auditory systems and thus its evolution has been extensively studied. In contrast, anatomical variation in the quadrate is poorly understood in Aves. Our aim was to determine if the morphological variability of the quadrate bone was related to diet and foraging in different groups of the birds' taxa. Because the quadrate forms part of the kinetic multi-bar linkage system within the avian skull, we hypothesized that variation in. To answer this question, we compared 101 species of birds from different families including two extint taxa. We analyzed the relationship between anatomy, diet, body mass and foraging, using techniques of three-dimensional geometric morphometrics and phylogenetic comparative methods. Our results demonstrate substantial variation in birds' quadrate morphology. There is a significant phylogenetic signal in the quadrate ($K= 0.7664$, $p = 0.001$). Results also revealed that there is no significant correlation between bone morphology and diet, body size and foraging in the Aves group; but there is a significant correlation between body size and square bone morphology in the Neognathae subgroup ($p = 0.036$). Furthermore, principal component highlighted the morphological differences between the Neoaves, Paleognathae and Galloanserae groups. Members of Galloanserae are characterized by having wide, rectangular mandibular condyles whereas paleognaths have extensive triangular mandibular condyle and otic condyle. Neoaves are more variable in their quadrate morphology than non-neoavian forms, suggesting that Neoavians have more diverse jaw kinematics than the other two groups. Together, these results demonstrate that quadrate anatomy is highly variable across crown birds and evolutionary history is more important in shaping this variation than dietary or functional factors.

Microbiome evolutionary convergence among Triatominae, ticks and assassin bugs

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Reduviidae are one of the largest and most diverse families of insects, encompassing roughly 7,000 described species. Most species are hemolymphophagous and show predatory behaviour (assassin bugs), but the subfamily Triatominae evolved towards hematophagy and a parasitic lifestyle, becoming important vectors of Chagas disease in American Countries. In addition, other hematophagous vectors, i.e. hard and soft ticks, are highly diffused in the same areas. Cohabitation and co-feeding can lead to horizontal transmission of the microbiome among arhtropods. Furthermore, both hemolymph and blood lack vitamins and amino acids in their composition, which is usually supplied to the host by its microbiome. We evaluated the possibility of evolutionary convergence of the microbiomes of assassin bugs, kissing bugs and ticks towards

the same main bacterial genera to provide the nutrients lacking from their diets. Furthermore, we investigated the effects of cohabitation and niche overlap on the microbial communities.

The biogenesis of mitochondrial short non-coding RNAs: an analysis of CLIP-seq data

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Interactions between organellar and nuclear genomes have played a central role in eukaryote evolution. Mitonulear genomic coadaptation has resulted in a network of regulative processes, which are not fully understood yet. Recently, we found a set of short non-coding RNAs that are transcribed by the mitochondrial genome and are predicted in silico to target nuclear transcripts (which we named Small MITochondrial Highly transcribed RNAs, smithRNAs). In vivo functionality of some of these smithRNAs has been proved in at least one species (i.e. Ruditapes philippinarum, the Manila clam), but their regulatory pathway and maturation are still unclear. In this study, we analyzed publicly available next-generation sequencing libraries of RNA immunoprecipitation (RIP) and cross-linking immunoprecipitation (CLIP) of the Argonaute family proteins and of other related proteins. Aiming to characterize smithRNA biogenesis, we looked for mitochondrial short non-coding RNAs that were detected by co-immunoprecipitation with possible interacting proteins. Our data revealed that in Homo sapiens and Mus musculus mitochondrial tRNA-derived smithRNAs interact with AGO2, and in H. sapiens with DROSHA and DGCR8 as well. Therefore, our in silico analysis predicts that smithRNAs might follow a miRNA-like canonical maturational pathway, at least in mammals. The PIWI protein superfamily is highly differentiated in metazoans; therefore, other Argonaute proteins (Ago-like, PIWI and WAGO families) in other phyla (Arthropoda, Nematoda, Cnidaria) have been tested as candidates for the interaction with smithRNAs. Until now, only CSR-1 in Caenorhabditis elegans has been detected as a putative interacting protein.

Genetic load in the Apennine brown bear: testing deleterious variants in cell cultures

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The small and endangered population of the Apennine brown bear lives in complete isolation in the Italian Apennine Mountains. It is highly inbred and it harbors very low genomic variation. Bioinformatic predictions suggest that this population accumulated some deleterious amino acid changes. In particular, three nonsynonymous substitutions occur in the NADH dehydrogenase subunit 5 (ND5) of the mtDNA genome. One of these, E526G, is fixed in the Apennine bear, almost absent in any other organism, and shows the highest probability of being deleterious. Since mutations in the MT-ND5 gene can impair the function of the mitochondrial electron transport system, with significant consequences on energy input, we designed a set of experiments to evaluate the impact of the Apennine bear's ND5 mutations on the cell metabolism. The assessed mitochondrial bioenergetics and cellular functions were considered as fitness proxies. Our experiments showed that Madin-Darby canine kidney (MDCK) cells overexpressing the Apennine

bear ND5 subunit have significant reduction in Calcium response, a significant increase in reactive oxygen species production, and a significant decrease in mitochondrial transmembrane potential compared to the control. Interestingly, all these effects appear to be caused by the mutation E526G, while the impact of the other two amino acid changes seems irrelevant. The same parameters were measured in fibroblast cells isolated from palatal biopsies obtained from Apennine and non-Apennine brown bears (control). Similarly to what observed in MDCK, experiments conducted in primary bear fibroblasts showed that cell metabolic parameters were significantly lower in the Apennine bear. At further confirmation of the existence of mitochondrial dysfunctions in the Apennine bear, we also found a decreased mitochondrial turnover and a reduced oxygen consumption rate. This is one of the first studies in non-model species where the effects of variants predicted to be deleterious by bioinformatic methods are functionally analyzed using cell cultures of the same species. One mutation clearly weakens the mitochondrial efficiency, supporting the view that genetic load in the small Apennine brown bear population is an issue for the conservation of this endemics.

A bioinformatic pipeline for the detection of gene presence/absence variation and the construction of pan-genome assemblies: the case of the Pacific oyster Crassostrea gigas

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The recent extension of whole-genome resequencing approaches to non-model species is revealing that genomic structural variation is more widespread than originally thought in eukaryotes and that large indels, previously thought to mostly occur in intergenic regions, often target protein-coding genes, which can therefore be found in a hemizygous state in diploid organisms.
On a broader population scale, such genes may be entirely missing in some individuals (i.e. dispensable), leading to the gene presence-absence variation (PAV) phenomenon, defining on the other hand a larger set of genes invariably shared by all individuals (i.e. core genes). Altogether, core and dispensable genes define a pan-genome, whose variable fraction may provide a significant contribution to local adaptation and phenotypic diversity. Nevertheless, very little attention has been placed to its biological relevance in metazoans to date, even though the recent discovery of an open pan-genome in the Mediterranean mussel suggests that similar phenomena may be present in other marine invertebrates.
Here we here describe a straightforward bioinformatic pipeline, which could be broadly applied to identify regions subjected to hemizygosity and PAV in fully assembled diploid genomes and to build a pan-genome assembly by recovering dispensable genomic regions from whole genome resequencing data.
We used the Pacific oyster Crassostrea gigas as a case study to test the reliability of this approach, analyzing 420 individuals for which Illumina paired-end resequencing data was available. Like in the case of the Mediterranean mussel, the Pacific oyster displayed an open pan-genome, which included several thousand dispensable genes, which were significantly enriched in functions related with immune response and survival. Overall, our pan-genome reconstruction approach allowed the recovery of nearly 150Mb of dispensable genomic sequence, increasing the size of the reference genome assembly by nearly 25%."

Maize/Common bean intercropping and their interactions with soil microbiota

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Industrial agriculture relied on mono-specific/genotypic pure stands reducing agro-ecosystems complexity. Plant – plant beneficial interactions as complementarity and facilitation have been affected by reduced genetic and functional diversity. Strong use of input factors led to a loss of nutrient-mobilizing traits as root exudates production and microbial partners recruitment ability. Polycultures, instead, diversify cultivations, permits lower inputs and provide ecosystem services. Cereal/legume intercropping exploit plant complementarity for resources and facilitation in positive plant-microorganisms interactions. To identify functional microbial groups, their role and their preferential affinity for specific plant associations, we performed a two-years field experiment were five maize (Zea mays L.) and four common bean lines (Phaseolus vulgaris L.), representing breeding lines and landraces, were grown in pure stand and in full pairwise combination system. At flowering, rhizosphere and soil near roots were sampled. Total DNA was extracted and sequenced with Illumina platform. Amplification of 16S and ITS region was conducted and reads classified with the Naïve-Bayes classifier on Silva and Unite databases for bacteria and fungi, respectively. For 16S data we identified 3645 taxa, 3194 genus and 2426 species, while for ITs data 551 taxa, 469 genus and 353 species. Statistical analysis of diversity metrics and relative abundances of metagenomic data revealed shifts in microbial composition from sole to inter-crop, confirming the importance of soil microbiota in shaping plant beneficial interactions. Differential patterns in rhizosphere microbiome determined by distinct plant combinations enables a preliminary characterization of facilitative processes regulating combining ability in intercropping. This provides interesting features for the exploitation of beneficial interactions in innovative breeding programs towards an ecological intensification of agriculture.

Effects of mutator genes in a minimal genetic algorithm

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Independent

Genetic algorithms (GAs) are a family of methods for searching for and optimizing solutions to computational problems, an approach designedly inspired by Darwinian selection.
We have implemented minimal GA in an agent-based programming environment in order to simulate adaptive responses in a population of candidate solutions for an elementary arithmetical problem; then we have tested the effects produced by mutator genes on the search process and on themselves, in order to model a biological system facing an environmental challenge – here replaced by a computational challenge. Indeed, there are many clues indicating mutation rate variations (to the point of genomic chaos) as part of the response to cellular and tissue stresses;

these include pathogens and cancerous cells adapting to the microenvironment and therapies of the host, or in vitro manipulated cells.
Assuming that the mutation rate might also be under genetic control, the activity of mutator genes is treated within our simulation as a quantitative trait. When the population is initialized, the mutator genes determine by default only single point-mutations in the structural genes (codifying for the candidate solutions), but the user can set the probability of hitting the mutator gene itself, which can become stochastically an anti-mutator as well as a multi-mutator, the second leading towards the hypermutation state.
Generally, the presence of mutator genes slows down the search process: increasing the hypermutation potential, the probability of finding the best solution tends to decrease, sometimes producing a failure of the GA. Notably, sexual reproduction through crossover can mitigate the detrimental effect of genetic impairment. The parameters sweep has revealed changing incidences of two attractors (anti/multi-mutation) during the different stages of the search: under the pressure of selection, and without the aid of a preexisting regulative pathway, they can give rise to patterns of functional self-organization.

Somatotype and choice of the ideal food strategy

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Each individual has a different body shape. An accurate definition of one's body structure and composition is a useful parameter for establishing and regulating the supply of nutrients, as well as for maximizing the performance in physical activity.
The work intends to propose a method for orienting on the choice of dietary strategy, based on the evaluation of the Heath&Carter somatotype. Each patient enrolled in this work, did a nutritional visit with anthropometric measurement (weight and height, circumferences and skinfold measurement) and the BIA (Akern BIA 101). Between the first visit (T1) and the check-up (T2) usually after one month, we have evaluated the variation of data over a period of dieting.
The diets, elaborated with “Linea Dadi method”, were assigned after the calculation of the Somatotype:
ENDOMORPH (puts on fat mass easily,muscle mass easily,loses weight hardly); low glycemic index diet, increased protein intake (red dice) or ketogenic diet (keto dice) (10-20% C , 40-50% P, 50-30% F)
MESOMORPH (puts on fat mass easily,muscle mass easily,loses weight easily); low glycemic index, normo protein and lipids diet (yellow dice). (40% C, 30% P, 30% F)
ECTOMORPH (puts on fat mass hardly, muscle mass hardly, lose weight easily); medium-high glycemic index diet, increased protein intake (green dice) (50% C, 20 % P , 30% F)
The results are considerably significant with $p< 0,05$. For almost all of somatotypes there is a decrease of endomorphism and an increase of mesomorphism and BCM (metabolic mass) detected by BIA. In conclusion, the personalization of a diet through the use of analysis of somatotypes proves to be useful in accelerating the achievement of the setted targets. It would be interesting to correlate the change in the somatotype of Italians in the last 50 years and associate this with the change in eating style.

Clues of accelerated molecular evolution in gene families associated with gonad determination in bivalves

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Bivalve molluscs show a great variety of reproductive modes, ranging from strict gonochorism (i.e., species with separate male and female individuals) to simultaneous and sequential hermaphroditism. In addition, both genetic and environmental factors appear to determine sex in most bivalve species studied to date, albeit heteromorphic sex chromosomes seem to be absent. Our knowledge concerning molecular aspects of bivalve reproduction and sex determination is still poorly characterized, while bivalve physiology and biochemistry have so far gained more attention. Furthermore, most of the studies were mainly focused on single species of economic and/or environmental interests, and a broad comparative analysis is still missing. For these reasons, we are currently performing an extensive comparative genomics analysis of publicly available data. In particular, we are surveying gene families whose members have been found to be differently transcribed between female and male gonads in various bivalve species, such as Dmrt, Sox, and Fox genes, with the attempt to better understand their evolution and dynamics in a broad genomic landscape. Preliminary results show that, among these families, there are some genes showing peculiar features: for example, some of them exhibit a mosaic distribution among bivalve species , while others seem to be bivalve-specific with no clear orthologs in other molluscs. In addition, genes that have been previously found to be associated with female/male gonad determination seem to be more divergent at the level of amino acid sequence than their close relatives, suggesting that some peculiar molecular evolution mechanisms have acted throughout their evolution. Lastly, the analysis of genomic distribution of these gene families suggests heavy genomic rearrangements and reshuffling in certain bivalve clades, such as Mytilidae and Unionidae.

Citizen Science Experiment – A participatory approach to characterize and conserve food legumes genetic resources

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Food legumes play a crucial role in the development of a friendly and sustainable food system, based on varieties able to meet global community needs. The characterization, maintenance, and better use of food-legume genetic resources are the basis to gain competitive and sustainable agronomic performances.
A new approach has been proposed by the EU H2020 project INCREASE, which aims to facilitate access to well-characterized and well-managed collections of genetic resources for common bean, chickpea, lentil, and lupin: the Intelligent Collections (ICs), nested-core collections of genetically purified accessions (based on Single Seed Descent).
In order to contribute to this goal, a Citizen Science Experiment (CSE) on common bean is going on in INCREASE, where citizens using a dedicated mobile App (Increase CSA), receive a set of common bean (Phaseolus vulgaris L.) genotypes from the IC (among a set of about 1000 genetically purified accessions, mainly landraces, characterized in previous studies). Every registered citizen provides on a voluntary basis phenotypic data (morphological and, phenological)

by observing the plants growing in their field, home garden, or terraces and shares pictures, experiences, and seeds via the App.
This represents a decentralized approach in which citizens can contribute to the evaluation, conservation, and valorization of plant genetic resources, under the guidance of the INCREASE team and the partner FAO by the implementation of the SMTA (easy-Standard Material Transfer Agreement) and digital agreements in the App.
The first round of INCREASE CSE is already completed with over 3,400 registered citizens across all EU; the second round is in progress with even more participants.
The huge amount of data under collection are used to analyze the relationship between phenotypic and genetic diversities and the environments, with the aim to enhance the knowledge on food legume resources, putting in practice a decentralized conservation system.

Protecting Italian endemic species: a lesson plan to engage children

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ENDEMIXIT is a comprehensive conservation genomics project based on five Italian iconic endangered endemics as model species. Communication and dissemination are an important goal of the project. To the aim of engaging young generations, we organized two teaching activities suitable for primary and secondary schools pupils. For the primary school, the activity was organized for two fourth-grade classes in Ferrara. The session lasted about two hours and was structured in three parts. Initially, we provided the students with a presentation of the ENDEMIXIT project, the five endemic species involved (Ursus arctos marsicanus, Podarcis raffonei, Hipparchia sbordonii, Acipenser naccarii, Bombina pachypus) and we explained what it means for a species to be endangered. After that, the pupils worked in groups to build the “identity card” of each of the five species. In this way they learned more about the biology and the behaviour, and, more importantly, about the threats that make them at risk of extinction. Finally, we “opened the discussion”. Kids were allowed to ask questions and we additionally clarified the concepts mentioned earlier. For the secondary schools we designed an educational package consisting of didactic cards, lessons and questionnaires to assess the effectiveness of the dissemination. We worked with three schools in the National Park of Abruzzo, Lazio and Molise and the lessons were focused on the conservation of the Apennine brown bear, an iconic vertebrate in the area. A total of 108 children participated in this activity. Even with a few hours of interactive lessons the children understand the importance of stopping the biodiversity loss and preventing the extinction of species and populations. Morevoer, showing as examples a few species living close to them favour their engagement in nature protection. The goal now is to understand if these ideas are retained also in the long term.

Tudor protein family evolution: a metazoan-wide analysis

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Tudor domain-containing proteins, or Tudor proteins, are a set of proteins involved in a wide range of biological processes, such as chromatin remodeling, pre-RNA splicing and RNA-interference. These tasks are also allowed by the presence of the Tudor domain, that mediates protein-protein interactions by binding methylated arginines or lysines of ligands. Previously proposed functional classifications divided Tudor domains from three to four groups, that are characterized by the evolutionary stepwise accumulation of specific N-terminal structures associated to different functions.

Our study aims to clarify the evolution of Tudor domains and proteins in Metazoa through bioinformatic analyses, performed on 111 different holozoan species, covering 21 animal phyla and 4 clades of unicellular eukaryotes. We inferred a maximum-likelihood phylogenetic tree of the Tudor domains collected from the proteomes of the data set species, supporting the fact that the Tudor domain types had already diversified to current levels in the common ancestor of Metazoa. However, the tree resolution could neither confirm nor refute the evolutionary stepwise accumulation model of N-terminal structures previously proposed. Despite this, previous evidence from the Tudor domain structural architecture strongly suggests that this evolutionary model is still the most likely.

We also investigate the variability in number of Tudor proteins in our data set. We found that loss of Tudor proteins was shared by almost all endoparasite species, probably due both to the genome reduction that accompanied the structural simplification of the parasitic habit, and to variability in piRNA molecular pathway. This last reason, together with whole genome duplication events, probably guided the massive expansion of Tudor proteins that we could observe in some free-living species.