VI ENCONTRO NACIONAL BIOLOGIA EVOLUTIVA

LISBOA

22 de Dezembro de 2010

Faculdade de Ciências
Universidade de Lisboa

Organizado por:
Centro de Biologia Ambiental
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DE LISBOA
Centro de Biociências

VI Encontro Nacional de Biologia Evolutiva

09:00 – 10:00 Registo e inscrições

10:00 – 10:30 *Nota de abertura do Encontro e de Homenagem ao Professor Carlos Almaça* pelo Exmo Sr. Director da Faculdade de Ciências da Universidade de Lisboa

10:30 – 11:30 *Conferência de Homenagem ao Professor Carlos Almaça* pelo Prof. Luís Vicente (Centro de Biologia Ambiental, Departamento de Biologia Animal, FCUL)

11:30 – 12:00 CAFÉ (30')

12:00 **1ª SESSÃO COMUNICAÇÕES ORAIS** **Moderadora** **Marta Santos**

12:00 **Pedro Branco** Evolution of nutrient uptake reveals a trade-off in plant-herbivore interactions

12:20 **Gabriel A. B. Marais** Can intra-Y gene conversion oppose the degeneration of the human Y chromosome? A simulation study

12:40 **Marta D. Santos** Evolution of cooperation under the N-Person Snowdrift Game

13:00 – 14:30 ALMOÇO (90')

14:30 **2ª SESSÃO COMUNICAÇÕES ORAIS** **Moderadora** **Susana Varela**

14:30 **Lília Perfeito** Fitness landscape of a metabolic pathway

14:50 **Sara Carvalho** Patterns of life-history evolution of inbred and outbred populations of *C. elegans*

15:10 **Pedro Simões** Molecular genetic content of a clinal chromosomal inversion polymorphism

15:30 **Susana A. M. Varela** Mate choice through non-genetic information – implications to the study of speciation

15:50 – 16:30 SESSÃO DE POSTERS & CAFÉ (40')

16:30 **3ª SESSÃO COMUNICAÇÕES ORAIS** **Moderadora** **Teresa Nogueira**

16:30 **Vera S. Domingues** Evolution and genetics of adaptive pigmentation in beach mice

16:50 **Bruno F. Simões** Molecular evolution of the color vision in bats

17:10 **Cristina Gamba** The Neolithic impact in Eastern Spain: the ancient genetic perspective

17:30 **Teresa Nogueira** Genome mobility as a modulator of evolution of cooperation in bacteria

17:50 – 18:30 DISCUSSÃO: LOCALIZAÇÃO PRÓXIMO ENBE, OUTROS ASSUNTOS

Homenagem ao Professor Carlos Almaça

*Na abertura do **6º Encontro Nacional de Biologia Evolutiva** será prestada uma homenagem ao Professor Carlos Almaça, grande figura da Biologia Evolutiva em Portugal. Para essa homenagem irá contribuir uma palestra pelo Prof. Luís Vicente (CBA)*

Grande Auditório da FCUL, 22 de Dezembro de 2010 pelas 10h00

cba Centro de Biologia Ambiental

FACULDADE
DE CIÊNCIAS
UNIVERSIDADE DE LISBOA

Palestra em Homenagem ao Professor Carlos Almaça

Luís Vicente (lmvicente@fc.ul.pt)

Centro de Biologia Ambiental, Faculdade de Ciências, Universidade de Lisboa, Portugal

Carlos Alberto da Silva Almaça nasce em Lisboa a 29 de Dezembro de 1934.

Estuda Ciências Biológicas na Faculdade de Ciências da Universidade da mesma cidade onde vem a licenciar-se em Outubro de 1957, pouco antes de completar 23 anos. Envereda então por uma carreira académica que viria a mostrar-se brilhante a todos os níveis. Doutora-se na mesma instituição em Março de 1968, com uma tese sobre o *Estudo das Populações Portuguesas do Gén. Barbus Cuvier, 1817 (Pisces, Cyprinidae)*.

Todo o seu trabalho científico se desenvolve sob o quadro conceptual neo-darwinista da Teoria Sintética da Evolução de Ronald A Fisher, J.B.S. Haldane e Sewall Wright. Dedica-se com entusiasmo e profundo saber a várias áreas da Biologia, como a Evolução, a Sistemática, a Biogeografia e a Ecologia. Os seus modelos animais referenciais são fundamentalmente os peixes das águas continentais ibéricas, mas não deixa também de abordar outros grupos animais, dos Invertebrados aos Mamíferos. O seu trabalho científico tem o merecido reconhecimento internacional.

Paralelamente, profundamente preocupado com a história da ciência em Portugal e com as raízes do conhecimento actual, desenvolve investigação profunda sobre vários assuntos dentro desta temática, sendo de realçar a cultura biológica medieval, as explorações portuguesas durante a colonização da América Latina, o pensamento filosófico dos séculos XVII e XVIII ou, mais recentemente, os trabalhos dos naturalistas portugueses no século XIX e nos limiares do século XX.

Teve ainda uma actuação interveniente e militante nos desafios que a Conservação da Natureza lançava e lança aos cidadãos conscientes, ainda mais a todos aqueles cuja cultura biológica confere uma particular clarividência sobre os perigos que a Natureza corre no mundo moderno.

Nunca pactuando com o facilitismo, toda a sua actividade de investigação foi acompanhada por uma profunda preocupação pedagógica, com a consciência de uma Universidade formativa muito para além da mera transmissora de informação acrítica, procurando dotar os seus alunos da consciência do seu papel de cidadãos intervenientes na transformação da sociedade. Formou e formatou gerações da biólogos que hoje honram a sua memória e prosseguem os trabalhos de que ele foi pioneiro em Portugal.

De trato muito afável, o seu gabinete tinha sempre a porta aberta a todos aqueles que com ele desejavam aprender e discutir os mais variados assuntos.

Contribuiu também para a gestão de várias instituições ligadas à Zoologia em Portugal, desempenhando com toda a competência funções de direcção.

Académico (Classe: Ciencias; Secção: 5ª Ciências Biológicas) desde 29 de Junho de 2000, Carlos Almaça, mais que qualquer outro, foi assim integralmente merecedor do título de PROFESSOR, que soube honrar e desempenhar melhor do que ninguém.

Cabe-nos a todos homenageá-lo e honrar a sua memória neste ano do seu desaparecimento.

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Evolution of nutrient uptake reveals a trade-off in plant-herbivore interactions

Pedro Branco[1] (p.m.m.branco@uva.nl), M. Stomp[1], M. Egas[2], J. Huisman[1]

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Nutrient limitation determines the primary production and species composition of many ecosystems. Here we apply an adaptive dynamics approach to investigate evolution of the ecological stoichiometry of primary producers and its implications for plant-herbivore interactions. The model predicts a trade-off between the competitive ability and grazing susceptibility of primary producers, driven by changes in their nutrient uptake rates. High nutrient uptake rates enhance the competitiveness of primary producers but also increase their nutritional quality for herbivores. This trade-off enables coexistence of nutrient exploiters and grazing avoiders. If herbivores are not selective, evolution favors runaway selection toward high nutrient uptake rates of the primary producers. However, if herbivores select nutritious food, the model predicts an evolutionarily stable strategy with lower nutrient uptake rates. When the model is parameterized for phytoplankton and zooplankton, the evolutionary dynamics result in plant-herbivore oscillations at ecological timescales, especially in environments with high nutrient availability and low selectivity of the herbivores. High herbivore selectivity stabilizes the community dynamics. These model predictions show that evolution permits nonequilibrium dynamics in plant-herbivore communities and shed new light on the evolutionary forces that shape the ecological stoichiometry of primary producers.

Can intra-Y gene conversion oppose the degeneration of the human Y chromosome? A simulation study

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The human Y is a genetically degenerate chromosome, which has lost about 97% of the genes originally present. Most of the remaining human Y genes are in large duplicated segments (= ampliconic genes) undergoing intense Y-Y gene conversion. It has been suggested that Y-Y gene conversion may help these genes getting rid of deleterious mutations that would inactivate them otherwise. Here we tested this idea by simulating the evolution of degenerating Y chromosomes with or without gene conversion using the most up-to-date population genetics parameters for humans. We followed the fate of a variant with Y-Y gene conversion in a population of Y chromosomes where Y-Y gene conversion is originally absent. We found that this variant gets fixed more frequently than the neutral expectation, which supports the idea that gene conversion is beneficial for a degenerating Y chromosome. Interestingly, a very high rate of gene conversion is needed for an effect of gene conversion to be observed. This suggests that high levels of Y-Y gene conversion observed in humans may have been selected to oppose the Y degeneration. We also studied with a similar approach the evolution of ampliconic regions on the Y chromosomes and found that the fixation of many copies at once is unlikely, which suggest these regions probably evolved gradually unless selection for increased dosage favoured large-scale duplication events. Exploring the parameter space showed that Y-Y gene conversion may be beneficial in most mammalian species, which is consistent with recent data in chimpanzees and mice.

Evolution of Cooperation under the N-Person Snowdrift Game

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The evolution of cooperation has been gathering increasing attention during the last decades, a problem to which Evolutionary Game Theory (EGT) has been able to provide fundamental insights. One shot, symmetric 2-person games are the traditional approach adopted to investigate the emergence and evolution of cooperation; however, one cannot ignore many situations that are actually associated with collective action based on joint decisions made by groups involving more than 2 individuals. In the animal world, very commonly performing a given task which is beneficial to an entire group requires the cooperation of several individuals of that group, who often share the workload required to perform that task. To many of those cases, the N-Person generalization of the Snowdrift Game (NSG) is the most suitable model. Here we show, making use of the NSG, how spatial populations affect the average levels of cooperation, when compared with the results obtained under conventional EGT, that is, for well-mixed populations.

Fitness Landscape of a Metabolic Pathway

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Genes are regulated because their expression involves a fitness cost to the organism. The production of proteins by transcription and translation is a well-known cost factor, but the enzymatic activity of the proteins produced can also reduce fitness, depending on the internal state and the environment of the cell. Here, we map the fitness costs of a key metabolic network, the lactose utilization pathway in *Escherichia coli*. We measure the growth of several regulatory *lac* operon mutants in different environments inducing expression of the *lac* genes. We find a strikingly nonlinear fitness landscape, which depends on the production rate and on the activity rate of the *lac* proteins. A simple fitness model of the *lac* pathway, based on elementary biophysical processes, predicts the growth rate of all observed strains. The nonlinearity of fitness is explained by a feedback loop: production and activity of the *lac* proteins reduce growth, but growth itself also reduces the density of these molecules through dilution. This nonlinearity has important consequences for pathway function and evolution. It generates a critical activation threshold of the *lac* operon, above which bacterial growth abruptly drops to low values. Furthermore, the nonlinearity determines how the fitness of operon mutants depends on the inducer environment. We argue that fitness nonlinearities, growth thresholds, and gene-environment interactions are generic features of fitness landscapes for metabolic pathways and discuss their implications for the evolution of regulation.

Patterns of life-history evolution of inbred and outbred populations of *C. elegans*

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The diversity of life forms we find in space and time ultimately stems from genetic differences among individuals within breeding groups. Natural selection operates on the phenotypes generated by such genetic differences leading to changes in the genetic composition of populations with time. Mutations and recombination events are important phenomena in generating new genotypes upon which natural selection can act. We investigated the influence of both mutation and rates of recombination from standing genetic variation on patterns of phenotypic evolution and rates of adaptation to a novel environment. We performed experimental evolution in populations of *C. elegans* which differed in their

starting levels of heterozigocity and breeding-mode – androcioecious or dioecious. Replicate populations were maintained in a laboratory environment at high and constant population sizes ($N=10^4$) during 100 discrete and non-overlapping generations. The survivorship and reproduction fitness components were measured during experimental evolution, as well as the genotype frequencies at 9 microsatellite loci. Evolved populations reveal extensive phenotypic evolution for most of the measured phenotypes, with differences in phenotypic changes being on average more prominent between inbred vs outbred populations than between populations with different mating systems. The different patterns and rates of phenotypic evolution revealed by the experimental populations will be discussed in the light of the laboratory ecology, mutation rates and effective recombination rates estimated from the molecular data.

Molecular genetic content of a clinal chromosomal inversion polymorphism

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*Filiação actual: Centro de Biologia Ambiental, Faculdade de Ciências,
Universidade de Lisboa, Portugal

There is compelling evidence supporting an adaptive explanation for the evolution of inversion polymorphisms in *Drosophila subobscura*. Latitudinal clines for several chromosomal arrangements of *D. subobscura* in Europe and the repeated clinal patterns found both in North and South America clearly points to an adaptive cause. Nevertheless, the molecular mechanisms that underlie the maintenance and evolution of these polymorphisms are still in debate. Furthermore, there is little knowledge about specific regions within inversions subject to selection. Using as scenario the well-known latitudinal inversion clines in European *Drosophila subobscura* populations we characterized the molecular genetic content of these chromosomal inversions in several European populations. Interestingly, very high genetic differentiation was found between different inversions of the same population while very low genetic differentiation was observed for the same inversion across different populations. Our results also indicate clear linkage disequilibrium (LD) between microsatellite alleles and inversions with loci located inside inverted regions presenting the stronger LD patterns. Overall, our findings clearly point to an important role of inversions in shaping the molecular variation in natural populations of *D. subobscura* and highlight their effect in harbouring the genetic content within inversions due to reduced gene exchange within these genomic regions. The existence of specific regions associated with selective pressures within inversions will be discussed.

Mate choice through non-genetic information – implications to the study of speciation

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2- Laboratoire d'Évolution et Diversité Biologique, Université de Toulouse, France; 3- Konrad Lorenz Institute for Ethology, Austrian Academy of Sciences, Austria

Organisms require information to make decisions about fitness-affecting resources, such as mates. They may choose either by relying on the information encoded in their own genes (innate preferences), or by relying on the social information derived inadvertently from the mating performance of conspecifics (learned preferences). One direct consequence of using social information for mate selection is the copying of the successful conspecific choices. Mate copying by females may reduce uncertainty about male quality, allowing more adaptive decisions. Experimental studies have produced evidence that female mate copying occurs in several species of fish, birds and mammals, including humans.

Our paper in fruit flies reports the first evidence that a female invertebrate can exploit social information to select mates, suggesting that such a strategy is probably widespread in nature. With two sets of experiments we were able to demonstrate that *Drosophila melanogaster* female prospectors appraised potential mates by their performance with other females, reversing their choices if contradicted by the choices of the model females, and that they could generalize socially learned information to other males of the same successful phenotypes.

Our findings have implications for evolution given that socially learned mate preferences can spread through a population within a single generation and, like genetically inherited information, cause important skews on male reproductive success. Variation in learned preferences may, in turn, lead to reproductive isolation, setting the stage for speciation.

Studying how the genetically and non-genetically inherited information balance each other is, therefore, determinant to our understanding of how the social environment in which a female grows may actually influence the course of sexual selection and affect genetic evolution.

Evolution and genetics of adaptive pigmentation in beach mice

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Understanding the evolutionary origin of adaptive traits is a central question in evolutionary biology. In particular we would like to know which genes and alleles underlie adaptive phenotypes and how interactions among loci (epistasis) influence adaptive change. The oldfield mouse, *Peromyscus polionotus*, is a unique system to study these questions. This species shows extreme variation in color pattern throughout its range in the southeastern United States. In particular, mice inhabiting the coastal dunes and barrier islands of the Gulf Coast of Florida exhibit an extremely pale coloration, which has been driven by natural selection for crypsis. We have identified three pigmentation genes (*Mc1r*, *Agouti* and *Corin*), which together contribute to color variation in this species. We identified and functionally characterized a derived amino acid change in *Mc1r* that contributes to these color differences. In contrast, there are no differences in the *Agouti* coding region, but *Agouti* mRNA expression was highly correlated with pigmentation. A combination of fine-scale mapping and allele-specific assays suggest that the functional mutation(s) are in Agouti's *cis*-regulatory region. We are using association studies in natural populations to narrow in on the functional mutation. Using complementary approaches such as classical genetics, association mapping and developmental biology in natural and laboratory populations, we are reconstructing the evolutionary history of allelic variation at these loci, providing a model in which to study the evolution of diversity in nature.

Molecular Evolution of the Color Vision in Bats

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Within mammals bats exhibit the greatest level of sensory specialization. The complex and highly evolved process of laryngeal echolocation, allied with the capability of flight have given bats the ability to exploit most ecological niches throughout the globe. The Old World Fruit Bats have large eyes specialized for nocturnal vision, whereas the microbats rely more on laryngeal echolocation and have questionable visual capabilities. It has been argued that bats developed their echolocation at the expense of other senses such as vision, however this hypothesis has never been fully explored. Genetic data suggest that the ancestor of modern bats was most likely capable of echolocation and that the Old World Fruit Bats may have lost this sense "trading" hearing for vision and olfaction. To date the molecular analysis of color vision in bats and other mammals has linked changes in vision genes to differences in ecology as well as other sensory capabilities. With the aim of establishing the evolutionary patterns and functionality of the photoreceptor visual pigments, the UV/blue opsin gene (SWS1) and the yellow/green opsin gene (MWS/LWS) were amplified, cloned and sequenced in all bat families. This molecular data set covered species that exist in very divergent ecological niches (with different feeding and roosting habits) and that possess divergent sensory capabilities. Our data show that the UV/blue opsin has undergone gene defects leading to monochromacy in several yinpterochiropteran and yangochiropteran lineages. We will evaluate the genetic consequences of nocturnality, laryngeal echolocation type and ecological niche specialization on vision in bats.

The Neolithic impact in Eastern Spain: the ancient genetic perspective

Cristina Gamba[1,2] (cristinagamba@med.ucm.es), E. Fernández[1], M. Tirado[1], S. Palomo[1],
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Recovering ancient DNA from human samples makes it possible to test archaeological, anthropological and demographic hypotheses. In particular, by comparing modern and ancient DNA it has become possible to evaluate genetic continuity or discontinuity between different periods, and/or infer ancient human migrations. We analysed 17 Neolithic samples from three Eastern Spanish sites (Can Sadurní, Chaves and Sant Pau) to locally evaluate the demographic impact associated to the spread of the Neolithic in Europe from the Near East. We obtained ancient sequences (HVI region) from mitochondrial DNA, which is more abundant in cells than nuclear DNA, allowing us to trace maternal lineages. Criteria of authenticity have been observed from the DNA extraction, amplification, bacterial cloning and experiment replication. Currently, we are also developing a simulation approach to determine which among several models explains best the ancient and modern DNA obtained. Preliminary simulation results will thus be presented. Our results suggest some continuity between Neolithic and modern populations, hence favouring a Demic over a Cultural Diffusion model.

Genome mobility as a modulator of evolution of cooperation in bacteria

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Microbes interact with each other, within their communities, engaging a remarkable array of cooperative behaviours, by secreting shared proteins that are essential for foraging, shelter, microbial warfare and virulence. These proteins are costly, rendering populations of co-operators vulnerable to exploitation by non-producing cheaters arising by gene loss or migration. In such conditions, how can cooperation persist? Our model predicts that more mobile loci generate higher relatedness, and therefore allow the maintenance of more cooperative traits via kin selection. We have characterized the secretome of E. coli’s issuing from different specimens of the human microbiome, either commensals and of different pathogenic types. By using a bioinformatic approach we have inferred the sub-cellular localization of proteins of these genomes; analysed their genomic close co-occurrence with mobile elements, and either in the highly or weakly mobile regions of chromosomal genome, or in plasmids; and classified them either as belonging to the core or ancestral genome, or as being recent genes. We thereby confirm that genes coding for the secretome are very frequently lost and gained, supporting our major prediction that gene mobility drives bacterial cooperation. Secreted and outer membrane proteins are biosynthetically cheaper than the other proteins and even than highly expressed proteins, suggesting selection to lower the cost of cooperative traits. This analysis suggests that horizontal transfer promotes cooperation, as transmission increases local genetic relatedness at mobile loci and enforces cooperation on the resident genes. As a result, horizontal transfer promoted by agents such as plasmids, phages or integrons, drives microbial cooperation.

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A research project on zooarchaeogenetics: tracing back animal domestication and breeding in Iberia

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Iberia is considered a biodiversity *hotspot* and a Glacial *refugium* that suffered the influence of several cultures. Native animal breeds were developed which are locally adapted to specific environmental conditions. Genetic data has been gathered for Portuguese cattle and dogs which harbor high genetic diversity and are genetically structured. No phylochronological study has been conducted to investigate past genetic diversity of Iberian breeds and the occurrence of local domestication.

We propose a phylochronological approach and a combination of archaeological and genetic data to investigate: a) origins of Iberian cattle and dogs; b) occurrence of local domestication of wild ox and wolves; c) hybridization between cattle and dog domesticates with their wild counterparts; d) past animal breeding strategies.

Ancient DNA (aDNA) analysis of wild and domestic animal remains from Iberia will be done spanning over 15,000 years before present back to the Mesolithic or Superior Paleolithic. A multi-marker approach will be used to describe mitochondrial, Y-chromosomal and autosomal single nucleotide polymorphism (SNP) variation.

Although remains are buried under sub-optimal conditions, aDNA retrieval was successful and authentic mitochondrial sequences were generated for a few samples. DNA has been extracted from 20 metacarpal bones and for which osteometric data was collected. A sex discriminating SNP in the *ZFXY* gene has been analysed to determine the gender of these specimens. A Y-chromosome specific SNP (UTY19) was genotyped to determine the Y-haplogroup of male specimens. Preliminary results suggest that we will be able to implement a fine-scale genetic analysis of Iberian domestic cattle and dogs throughout time.

Icelandic lampreys (Petromyzon marinus) belong to the European stock based on molecular data

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The recent discovery of sea lamprey wounds on salmonids in Icelandic rivers prompted an investigation on the origin of sea lamprey of Icelandic waters. Using a mitochondrial DNA fragment, the origin of these lampreys is assigned to the European stock.

Biogeography of Batrachoididae

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Toadfishes (family Batrachoididae) occur in all tropical and sub-tropical ocean regions. A few lineages have invaded fresh waters. The lack of a planktonic stage and aspects of their life history suggest they have a relatively reduced dispersal ability. We studied the biogeography of the Batrachoididae by constructing a presence/absence database of the distribution of all species in the family (80 species) across 15 marine biogeographic regions. We analyzed the data using MDS and cluster analysis for marine species. Quantitative analyses supported the following conclusions:
(1) biogeographic regions indicate a strong separation between species composition of
the "New World" and "Old World";
(2) species composition share greater commonalities along longitudinal shorelines;
(3) eastern and western Pacific and Atlantic coasts differ strongly in species composition, indicating transoceanic important barriers affecting batrachoidid distribution;
(4) the rise of the Isthmus of Panama shaped the evolution of some batrachoidid taxa;
(5) Contrary to previous belief, batrachoidids are present in islands never connected to
the mainland.

Phylogenetic and phylogeography in the spittlebug *Philaenus spumarius* and related species from the same genus Philaenus.

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The spittlebug *Philaenus spumarius* (Linnaeus, 1758) is a Holartic species which has been studied for its adult colour/pattern balanced polymorphism. Here we investigate the phylogenetic and phylogeographic patterns in this species and related species from the same genus *Philaenus* by analysing populations throughout Europe and North America. Mitochondrial genes cytochrome oxidase I, cytochrome oxidase II and cytochrome b were used as molecular markers for the phylogenetic and phylogeographic analysis. The results suggest at least two routes for postglacial colonization in Europe in *P. spumarius*: a western European route from the Iberian and Italian peninsulas to the UK and an eastern route from Turkey to Finland. A lineage present in Turkey, Italy and Cyprus, also represented in the eastern part of the Iberian Peninsula, suggests an east-west or west-east colonization. The analysis also indicates that the haplotypes of the Azores and North America are most likely derived from populations related to the British ones. Our findings enable a better understanding of the historical events involved in shaping evolutionary patterns in these species suggesting so far a typical southern refugia species.

Whence freshwater *Salaria*

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The phylogenetic relationships of the marine blenny *Salaria pavo* and the freshwater *S. fluviatilis* and *S. economidisi* were analyzed using four molecular markers: the mitochondrial 12S rRNA, 16S rRNA, and the control region and the nuclear first intron of the S7 ribosomal protein. The monophyly of *Salaria* is supported, as well as that of *S. pavo* and that of all the freshwater members of *Salaria*. Thus, the present results support a single origin for all freshwater Mediterranean blenniids. *S. fluviatilis* is a paraphyletic entity with *S. economidisi* nested within it. A Moroccan population of *S. fluviatilis* is more divergent than *S. economidisi*, both in nuclear and mitochondrial genes. Fish from Israel together with some Turkish samples represent the second oldest split. It is argued that these populations may represent cryptic species. Thus, further studies on the taxonomy of these freshwater blennies are urgently needed.

The impact of the social structure on patterns of genetic diversity: a simulation approach

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Genetic data are increasingly used to study natural populations in the field and understand their ecology and behavior. In most population genetics studies it is customary to assume that samples are obtained from populations at Hardy-Weinberg equilibrium. While the assumption of random mating may be a good approximation for many species and/or for many questions it is increasingly recognized that populations are rarely isolated and are part of a network of populations. Moreover, many species are further subdivided in social groups, with different breeding strategies. Relatively little work has been done to assess the implications of these departures from random mating. Real data from different species such as marmots, prairie dogs or deer show departures from HW at the social group level, but not at the overall population level. This is sometimes interpreted to be due to inbreeding avoidance.

In order to quantify how genetic variability is influenced by the social structure (*i.e.* the existence of social groups within populations) and by the connections between social groups (*i.e.* the network structure) we performed simulations under different social group conditions. We used published data on the social structure of several vertebrate species to validate the demography and computed commonly used population genetics statistics. Preliminary results suggest that genetic diversity obtained in real data (negative F_{IS} values within groups) can be reproduced when males and females have different gene flow patterns, without inbreeding avoidance.

How to tackle evolutionary processes looking at chromosomes? Ongoing studies on comparative cytogenetics in vertebrates

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Since the end of the 19th century, researchers have been trying to retrieve valuable information from chromosomes – the physical support of heredity – using increasingly powerful methods. Species' diploid numbers (2n), chromosome patterns and the recognition of sex chromosomes (whenever present) are examples of such information. Technical advances mostly driven by human / mammals cytogenetics, were initially framed on chromosome banding procedures to unravel specific chromosome markers (constitutive heterochromatin, nucleolar organizer regions, telomeres, GC and/or AT rich segments location, etc). Comparative karyological analysis of closely related species, allowed the recognition that chromosome rearrangements may accompany or even lead to species diversification. Though, big challenges remain in precisely assessing the processes of genome reorganization and chromosomal evolution. More recent tools as Fluorescence In-Situ Hybridization (FISH) and Genomic In-Situ hybridization (GISH), flow-sorting and microdissection, are a window-of-opportunity to get cross-species painting on a genomic-wide scale and help answer questions like these. Herein, we present a selection of results of our team that illustrate the application of such techniques, specifically to unravel i) genome reshaping mechanisms in hybrids, both at homoploid and polyploid condition in Iberian fish, and ii) a taxonomic redefinition of a wide-spread carnivore species.

Genetic structure of *Squalius* populations from rivers with contrasting histories, drainage areas and climatic conditions.

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The genetic structure of Squalius populations from Portuguese Atlantic- and Mediterranean-type streams (assigned to six distinct morphoclimatic regions) was compared using sequences of the cytb and beta-actin genes. The drainage area was significantly correlated to all the genetic diversity indices, pointing to the need to control for this effect in comparing populations with different histories and paleoecologies. A significant correlation was also found between genetic diversity and the morphoclimatic regions to which the rivers were assigned, with the highest diversity in warmer, lower latitude, Mediterranean-type streams. This relationship was not due to idiosyncratic characteristics of the clades compared. When the drainage area and phylogenetic effects were removed, the southern Mediterranean streams harboured significantly more genetic diversity even when compared with much larger northern streams. It is argued that these results are likely caused both by the metapopulation structure of the Mediterranean streams and by the severe reduction or local extinction of populations in the northern rivers during glaciations.

Evidence of extensive mitochondrial introgression with nearly complete substitution of the typical *Squalius pyrenaicus*-like mtDNA of the Squalius alburnoides complex

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The first occurrence of massive mitochondrial introgression of *Squalius aradensis* genes in *Squalius alburnoides*, a hybridogenetic complex that usually carries mtDNA of its maternal ancestor (*Squalius pyrenaicus*) is reported. Possible implications of such introgressions for the history of the complex are discussed.

Projecto FISHATLAS: demografia actual e histórica e filogeografia comparada de peixes como instrumento para a conservação de espécies criticamente ameaçadas

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A região Mediterrânica é conhecida como sendo um hotspot de biodiversidade e endemismos e, simultaneamente, como uma das regiões que será mais afectada pelas alterações climáticas globais. O risco de extinção é particularmente elevado para os taxa que não podem migrar para regiões mais favoráveis, como é o caso dos peixes de água doce primários, que estão confinados aos rios onde ocorrem. Uma vez que Portugal beneficia de uma localização geográfica que está na zona de transição entre os climas Atlântico e Mediterrânico, o projecto FISHATLAS tem como principal objectivo caracterizar exaustivamente a estrutura genética das populações existentes em rios tipicamente Atlânticos e Mediterrânicos, com condições hidrológicas contrastantes, e prever os possíveis efeitos das alterações climáticas nessas populações.

Improvement of Portuguese cattle by the Christians following the "reconquista" – evidence based on osteometry and molecular sexing

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The sexual composition of livestock in antiquity is important in understanding the development of animal husbandry.

Combining osteometry and genetics of archaeological cattle bones we investigate evidence for animal improvement. 95 metacarpals were measured from three Portuguese archaeological sites – Moslem Santarém and Silves, and Christian Beja. Seven measurements were taken: length (GL), minimum shaft width (SD), distal width (BFd), width of medial and lateral condyles (wcm, wcl), depth of medial and lateral trochleae (DEM, DEL). In parallel, we extracted DNA from a subset of 20 measured metacarpals from Beja and studied a sex discriminating single nucleotide polymorphism in the *ZFXY* gene.

Osteometric data revealed a size increase in cattle between Moslem and Christian periods, probably due to agricultural improvements. The bimodal distributions of BFd in the Santarém and Beja samples were interpreted in terms of gender – the smaller being females and the larger males - an interpretation now confirmed via ancient DNA.

We conclude that the post-Moslem size increase was not due to sex ratio variation and reflects cattle improvement. DEL *versus* BFd and SD/GL *versus* BFd/GL provide reasonable gender separation and are therefore useful in zooarchaeology. This is one of the few studies combining osteometry and molecular sexing. Osteometry and sex ratio information are useful in inferring animal husbandry strategies in the past as will also be illustrated at medieval and post-medieval Launceston Castle, Cornwall, in England. At this site we can corroborate evidence for a shift towards specialised veal and milk production.

Pattern of burrow sharing, sociality and female-biased dispersal in the common wombat: insight from a non-invasive genetic study

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In mammals, in which 50% of the species are burrowers, the construction and use of burrows have been linked to sociality. It has also been suggested that the occurrence of the uncommon female-biased dispersal pattern in some burrowers may be related to burrow-sharing. However, the influence of the use and sharing of burrows on sociality and dispersal is still not clear. Wombats (marsupials) provide an ideal opportunity to investigate this issue. First, although they are commonly considered as solitary because they are not group-living and forage alone, they can share burrows. Sociality in wombats may therefore be driven by burrow-sharing. Second, all the three wombat species exhibit female-biased dispersal. This could be related to male fitness benefits by sharing burrows with other males and/or to female bequeathing of burrows to offspring. The aim of this study was to use a non-invasive genetic approach in a Tasmanian population of common wombats (*Vombatus ursinus*) to investigate: (i) the population's social network based on the patterns of burrow-sharing, (ii) the role of kinship in burrow-sharing and the occurrence of female-biased dispersal. Our results showed that common wombats exhibit a unique form of cryptic sociality based on burrow-sharing and that males play a central role within the social network. In addition, kinship is an important determinant of burrow-

sharing. Relatedness between females sharing burrows was significantly lower than between females not sharing burrows, but it was not the case in males, which suggests the occurrence of male philopatry and female dispersal in our Tasmanian population.

A origem do cão (*Canis familiaris,* L.) em Portugal: contribuição dos restos encontrados nos concheiros de Muge (Salvaterra de Magos)

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O cão foi o primeiro mamífero a ser domesticado pelo homem. Estudos genéticos referem a domesticação do lobo como provavelmente não anterior a 16.000 anos BP sendo o número de eventos e a sua localização geográfica menos consensual, podendo ter tido origem no Médio Oriente ou China.

Os vestígios mais antigos de canídeos domésticos remontam aos 12.000 anos no Médio Oriente, 14.000 anos BP na Europa e até alguns restos de canídeos possivelmente domésticos com cerca de 31.000 anos encontrados em Goyet, Bélgica.

Portugal é inserido nesta rota através da descoberta de um esqueleto de cão datado de c. 8.000 anos BP encontrado nos concheiros de Muge (Salvaterra de Magos).

Os vestígios osteológicos de cães, no Paleolítico e no início da domesticação, são difíceis de distinguir morfologicamente dos seus parentes selvagens. Os critérios limitam-se a características morfométricas (diminuição de tamanho) e de contexto arqueológico (associação a enterramentos humanos).

Nos mamíferos o processo de domesticação provoca, numa fase inicial, uma diminuição do tamanho. O mesmo se passa com o cão, que para além disso, apresenta alterações ao nível da face, com encurtamento do focinho, característica que é fiável mesmo em cães de grande porte.

Este trabalho pretende demonstrar como os restos de cão encontrados em Portugal contribuem para a compreensão do aparecimento desta espécie.

Insights from a multi-locus approach on the origin of *Colletotrichum kahawae* and phylogenetic relationship within the group species *Colletotrichum gloeosporioides*

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Colletotrichum kahawae causes the devastating Coffee Berry Disease in Arabica coffee, hitherto restricted to the African continent. The disease was first reported in Kenya, where the pathogen hypothetically emerged from the closely related group species *C. gloeosporioides*. Previous population genetic studies were unsuccessful in drawing evolutionary or demographic inferences from *C. kahawae* populations due to an absence of variability and thus, most of our current knowledge about its origin and dispersal is based on historical data which can be misleading. Here, a phylogeographical analysis using ITS, Beta-tubulin, Gluthamine Sintethase and MAT5L was conducted with *C. kahawae* isolates from nine geographical locations as well as a collection of isolates of *C. gloeosporioides* and other closely related taxa to detect patterns of population structure and dispersal and analyze their phylogenetic relationships. Results confirm the low genetic variability in *C. kahawae*, though with a slight but consistent population structure, suggesting that populations from countries in the West coast of Africa are ancestral and that those from East African countries, such as Kenya, are derived and more recent, contradicting historical data on the origin of the pathogen. This emphasizes the importance of molecular approaches when addressing the origin and evolution of pathogens.

Spatial structure of communities and interaction of species traits with environmental heterogeneity: example of a landscape-scale assemblage of large African herbivores

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How environmental heterogeneity and co-evolved species traits interact to spatially structure communities has been little studied in large herbivores. This question is important to understand how environmental changes may locally affect the distribution patterns of this community and the ecological processes associated to it.

The models of food selection based on eco-physiological traits of species assume that animals adopt a foraging behaviour maximising both the quantity of ingested energy and of nutrients. Movements and spatial distribution patterns resulting from this behaviour is expected to be influenced by the availability of food resources and intrinsic constraints often correlated to allometric physiological requirements to the ability of the animals to perceive and discriminate between structures of their environment.

We modelled the spatial variations of the composition of the community of large herbivores of a savanna ecosystem in West Africa at a period of high resources heterogeneity. The community exhibited a spatial gradient of composition well correlated to the metabolic mass of the herbivore species. This gradient was also explained by the spatial variations of the growing vegetation measured with NDVI images. As expected, the large-size species, that theoretically require higher quantities of forage, occupied more productive areas with good quality forage than the smaller species.

The large herbivore assemblage finally appears to spatially adjust to the quality of resources defined by the specific energetic requirements and the vegetation heterogeneity. This empirical result seems to confirm the importance of bottom-up forces in structuring wild herbivore communities at the landscape scale.

Evolutionary dynamics of cooperation under the distributed Prisoner's Dilemma

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Humans contribute to a broad range of cooperative endeavors. In many of them, the amount or effort contributed often depends on the social context of each individual. Recent evidence has shown how modern societies are grounded in complex and heterogeneous networks of exchange and cooperation, in which some individuals play radically different roles and/or interact more than others. We show that such social heterogeneity drastically affects the behavioral dynamics and promotes cooperative behavior, whenever the social dilemma perceived by each individual is contingent on her/his social context. The multiplicity of roles and contributions induced by realistic population structures is shown to transform an initial defection dominance dilemma into a coordination challenge or even a cooperator dominance game. While locally defection may seem inescapable, globally there is an emergent new dilemma in which cooperation often prevails, illustrating how collective cooperative action may emerge from myopic individual selfishness.

Mutualistic Parasites - when hosts make use of their parasites and pathogens

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Parasites or pathogens and their hosts are expected to have conflicting interests, simply because parasite reproduction and transmission is done at the expense of the host's fitness. However, this may not always be the case Infected hosts can use their parasites/pathogens to harm others, and increase their own relative fitness.

Evolutionary dynamics during local adaptation: what are control populations doing behind the scenes?

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Experimental Evolution requires the use of control populations in order to disentangle environmental noise or confounding evolutionary patterns from evolutionary changes due to a new selective regime. Most studies involve the analysis of the effect of a new selective scenario. The expectation is that there will be divergent evolution between the populations under this new environment and the populations from where they derived directly. On the other hand Experimental Evolution may also analyze how much differentiated populations converge to similar values, either between them or relative to other populations already 'stabilized' in a common environment (control populations). In order to be able to obtain accurate readings of how populations are evolving it is necessary to know the dynamics of the control populations, because fluctuations in these, which are not due to environmental noise, can be misleading of the real evolutionary trajectories. In this study we will explore the evolutionary dynamics of fecundity traits during laboratory adaptation of populations of *Drosophila subobscura* founded from wild collections in Sintra. For this we will use different control populations with a varying number of generations in the laboratory (but all with more than 80 generations). By comparing linear-loglinear models involving different control populations we will address the important issue: Do inferences of the evolutionary dynamics of populations during local adaptation vary as a function of the populations used as controls?

Mutation accumulation in bacteria in the light of Fisher's geometrical model

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In a mutation accumulation (MA) experiment genetic drift is intensified over natural selection, such that mutations accumulate at approximately neutral rates and mean fitness declines as the population evolves. Classical models to interpret these experiments assume that the rate and effects of deleterious mutations is constant and independent of the genetic background in which they arise. Evidence for epistasis between mutations has increased recently and novel models of analysis of the MA data are required. Fisher's geometrical model offers a framework to start doing just that. Here we study the dynamics of fitness decline and increase in fitness variance between populations that undergo severe reductions in size under Fisher's framework. Using data from a MA in Escherichia coli we find good support for this model and provide new estimates of the rate and effects of fitness altering mutations.

Dynamics of adaptation from standing genetic variation in *Drosophila melanogaster* experimental populations

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In sexual organisms adaptation to different environments can occur by the sorting of favourable alleles present in a population. This independency of new mutations provides a quick way to respond to the demands of changing environmental conditions and may predominate in wild populations. We analyzed genotypic changes in Drosophila melanogaster experimental populations during adaptation to ancestral conditions, after being selected in several diversifying environments. Genotyping of single nucleotide polymorphisms, mostly located on the 3rd chromosome, provide longitudinal data on allele frequencies and allows us to estimate the contribution of selection and drift for the evolution of these populations. Selection occurs in different loci without general loss or gain of allele diversity and with changes in allele's fitness effects. Adaptive evolution in sexual organisms can be based on the recombination of extant genotypes and contingency in evolution can arise from the non-additive effects of alleles at different loci.

Phylogeography of *Symphodus melops*: preliminary results.

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The corkwing wrasse (*Symphodus melops*) belongs to the family Labridae which is the third largest family of marine fishes. *S. melops* is a rocky shore species inhabiting temperate Atlantic waters from Norway to Morocco and the Azores. We analysed the phylogeographic patterns of this species using mitochondrial and nuclear markers in populations throughout its distributional range. We found that *S. melops* presents a relatively shallow genealogy with a clear differentiation between Atlantic and North Sea populations, probably due to isolation by distance. There is no apparent differentiation among all North Sea populations but data on microsatellites is required to confirm this finding. There is a steep decline in the genetic diversity from the Atlantic to the North Sea populations. Nevertheless the North Sea colonization seems to be dominated by a haplotype not present in the Atlantic populations. The possibility of a glacial refugium in or near the North Sea is discussed.

Phylogeography of *Taurulus bubalis*: preliminary results.

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The longspined bullhead (*Taurulus bubalis*) belongs to the family Cottidae and is a rocky shore species that inhabits the intertidal zones of the Eastern Atlantic since Iceland, southward to Portugal and also from the Baltic Sea northward to the Gulf of Finland with some occurrences in the northern Mediterranean coasts eastward to the Gulf of Genoa. We analysed the phylogeographic patterns of this species using mitochondrial and nuclear markers in populations throughout its distributional range in west Europe. We found that *T. bubalis* has a relatively shallow genealogy with a clear differentiation between Atlantic and North Sea. Contrary to expectations from conventional phylogeographic thoughts the genetic diversity is not very different in all populations studied, being remarkably high at the populations that represent the distributional limits of our samples (Portugal and Norway). The possibility of a glacial refugium in the North Sea is discussed. It is argued that the pattern of a high diversity in the South declining to the recent colonized habitats at the North of a species range, so common in terrestrial animals and plants, does not hold to many marine organisms.

Unraveling genetic variation in Cork Oak Natural Populations

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Cork oak (*Quercus suber* L.) woodlands have an outstanding economical and ecological value in the Western Mediterranean region, particularly in Portugal, where they sustain a strong cork industry. In the context of a prospective management of these sustainable ecosystems, it is vital to better understand how the genetic variation of *Q. suber* natural populations is spatially organized and how gene flow and population admixture have contributed in shaping the extant population structure. Although a great deal of details on the genetic divergence of the Mediterranean cork oak populations has been uncovered and several hypotheses have been advanced concerning its evolutionary history, there is still much to unravel. To achieve this goal a different and complementary analysis of intraspecific variable cpDNA regions and nuclear microsatellite (SSRs) polymorphisms was initiated under a phylogeographical framework. Using samples from populations across the range of the cork oak species, we show the first results of the analysis of several populations for two chloroplastidial intergenic spacer regions and four EST (expressed sequence tags)-derived microsatellites (EST-SSRs). A low level of variability has been found so far for EST-SSRs and some populations appear differentiated on the cpDNA sequences analysis in a way that suggest a different evolutionary history than the one previously assumed for this species.

Disentangle the events of early plant evolution

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Plant chloroplasts are derived from an ancient endosymbiotic capture of a cyanobacterium by a heterotrophic eukaryotic ancestor, an event which lead to the diversification of three primary plant lineages, namely, the glaucocystophytes, red algae, and green plants, and the eventual emergence of terrestrial ecosystems. The endosymbiosis marked a crucial episode in cell and Earth history, yet our understanding of the phylogenetic context surrounding this momentous event remains obscure. Particularly, two major phylogenetic challenges in plant evolution remain unresolved: the identity of the closest living cyanobacteria to the plastid lineage, and the relationships among the three primary plant lineages. Recent phylogenomic analyses of early plant evolution show a lack of robust resolution of relationships and phylogenetic conflict, that can be related to the application of overly simplistic substitution models which fail to take account of heterogeneities known to be present in the data. This can lead to a well-characterised phylogenetic artefact where taxa are incorrectly joined due to their similar base compositions. We suggest applying novel non-stationary and data-heterogeneous substitution models, namely, the NDCH and CAT models respectively, in a Bayesian phylogenomic context to clarify the early evolutionary history of plastids and plants. I will present an analyse using data from the small and large subunits of ribosome of approximately 42 taxa representing the diversity of plant lineages and their ancestors. We believe that it is only through the application of models that better reflect the underlying substitutional properties of the data that the initial promise of phylogenomic analyses will be met.

Evolution of chromosomal polymorphisms during adaptation to a novel environment

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The role of chromosomal inversions in adaptation is well documented. In particular the clear latitudinal clines that evolved independently in Drosophila subobscura on three continents render most likely that natural selection plays a role. Nevertheless, what evolutionary processes maintain chromosomal polymorphisms remains an open question. Two main hypotheses are: (1) the superiority of heterokaryotypes due to 'coadaptation' *sensu* Dobzhansky; and (2) the stabilization of locally-adapted alleles, advantageous even without epistasis, due to reduced recombination with other populations (model of 'local adaptation'). The 'coadaptation' hypothesis leads to the expectation that chromosomal polymorphism should be maintained even in isolated populations, adapting to a specific environment. On the other hand, both genetic drift and 'local adaptation' without gene flow may lead to fixation of inversions. The study of the evolution of chromosomal polymorphism during the adaptation to a novel, controlled environment, may clarify these issues. With this study we characterize the frequency of chromosomal inversions of laboratory populations of Drosophila subobscura at different times after foundation from natural populations. A comparison between recently introduced and long-term laboratory populations, founded from the same natural location, reveals marked differences in the degree of chromosomal polymorphism, much higher in the recently founded populations. Part of these differences may involve selection of favored inversions, though founder effects and genetic drift will likely contribute too. A direct study of the temporal changes of inversion frequencies in populations during laboratory adaptation may shed light on the mechanisms maintaining chromosomal polymorphisms in natural populations.

Protocol Testing in Evolution of a conjugative plasmid and the mutualism between bacterial cells and plasmids

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The mutually beneficial association between bacteria and conjugative plasmids – mobile genetic elements – can be an important model system for the study of the evolution of cooperation in mutualisms. One unsolved question is what mechanisms are involved in the maintenance of that mutualism since plasmids frequently impose a fitness cost to their bacterial hosts? One interesting hypothesis relies on cooperative traits. Bacteria produce metabolites (“public goods”) that are secreted to the environment and can be used by neighbouring bacteria. These metabolites are costly, and thus, producer-bacteria are vulnerable to exploitation by non-producing cheater bacteria. Genes coding for those “public goods”, such as for antibiotic resistance, are commonly present in conjugative plasmids, then producer-bacteria can use horizontal transference of these mobile genetic elements to enforce cheaters to cooperate in “public good” production. By transferring genes, bacteria would avoid the spread of cheaters and this would provide an explanation for the maintenance of the bacteria/plasmid mutualism. Empirical exploration of this theory is lacking. In this work, we use a conjugative plasmid that codes for an enzyme that degrades an antibiotic outside bacterial cells. Our objective was to investigate how the evolution of such plasmid facing a high selective pressure (increasing levels of antibiotic), affects the social behaviour between cooperative and cheater bacterial cells.

Evolution through polyploidization: assemble and reshaping of parental genomes

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Allopolyploidization is a major evolutionary process where two or more different genomes are joined into the same nucleus. Genome adjustment to the same nuclear environment leads to genetic and epigenetic modifications. We used PCR-based methodologies to assess genetic rearrangements in triticale and wheat-rye addition lines involving repetitive genome fractions, namely retrotransposon, microsatellite and rDNA. Addition lines are obtained by controlled backcross of octoploid triticale to hexaploid wheat and represent the starting point for the stable introgression of rye chromatin in wheat background increasing wheat genome diversity. The presence of rye chromosomes was confirmed in all wheat/rye addition lines using molecular and cytogenetic approaches. Comparative analysis of wheat-rye addition lines, triticale, rye, and wheat banding profiles from retrotransposon and microsatellites sequences disclosed an extremely high level of sequence elimination involving preferential rye-origin bands. PCR experiments using primers designed to internal fragments of rye-origin sequences showed that the absence of those bands in wheat–rye addition lines results from sequence elimination rather than restrict changes on primer annealing sites, as noted in triticale. rDNA sequences presented however a distinct dynamics than that presented by retrotransposons and microsatellites. The results obtained emphasize the role of genome evolution through polyploidization and further remodeling induced by the disturbance of initial parental genomes for the introgression of reduced amounts of alien chromatin.

Is variability of neutral markers a good predictor of evolutionary potential? A case study study in *Drosophila subobscura*

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The capacity to respond to environmental changes is expected to depend on standing genetic variation of fitness-related traits. Nevertheless, it is unclear to what extent is the genetic variability of neutral molecular markers a good predictor of the evolutionary potential of populations. This is an issue of extreme relevance for both Evolutionary and Conservation Biology. Here we characterize several sets of laboratory populations of Drosophila subobscura on their genetic variability and differentiation at an early generation after foundation from the wild, through the analysis of 10 microsatellites. We have also estimated their subsequent adaptive rate to the laboratory analyzing several life-history traits. We found general similarities in gene diversity and allele number across foundations, in spite of significant genetic differentiation between most of them. We have also found different rates of adaptation to the laboratory between some of the sets of populations derived from different foundations. Mainly, there was no clear association between the initial genetic variability and the adaptive dynamics. There was also no consistent significant correlation between the early genetic differentiation, as measured by neutral markers, and differences in the rate of adaptation across foundations, measured in several life-history traits. These findings call for a word of caution when inferring the evolutionary potential of a population from the genetic variability estimated with molecular markers.

Small RNA profiling in Squalius alburnoides complex

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The *Squalius alburnoides* complex (Steindachner), a small endemic Iberian cyprinid fish, is among the most intricate hybrid polyploid systems known in vertebrates. This complex was produced by hybridization between female *Squalius pyrenaicus* (Gunther) (P genome) and a hypothetical paternal ancestor related to *Anaecypris hispanica* (Steindachner) (A genome). It is widely distributed in the Iberian Peninsula and in the southern basins of Portugal (*e.g*. Tagus and Guadiana) occurs in sympatry with the parental species *S. pyrenaicus* that interact directly with the complex, acting as sources of genetic material. In the *S. alburnoides* complex there is a continuous shifting between forms, being both P and A nuclear genomes cyclically lost, gained or replaced by new genomes, as a result, the complex is composed of forms with different ploidies and genomic constitutions, including diploids (PA, AA) and triploids (PAA). Interesting, gene copy silencing was already reported in the triploid forms (PAA). The aim of this study is to investigate the impact of allopolyploidy in the mechanism of gene expression regulation by sRNAs in the *Squalius alburnoides*. Thus, a sRNA profiling in the different forms and the parental *S. pyrenaicus* species was obtained by next generation sequencing technology (Illumina). In overall, results do not point to a big exchange between the expressions profiles. However we could find some particular differences. Therefore, although other epigenetic mechanisms are probably involved, we believe that these differences could contribute for the remodeling of gene regulation between forms and have a functional relevance in the maintenance of the evolutionary success of this complex.

Protocol testing in *Drosophila melanogaster*: developing tools for better & faster science

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In order for an experiment to be successful, one has to develop good methods for acquiring data. With recent developments in technology, especially computer software, the monotonous, repetitive, and very time consuming tasks performed in our laboratory can be improved. In this project, we present alternative protocols for studying two life-history traits in *Drosophila melanogaster*: fecundity and starvation resistance. Fecundity can be operationally defined as the average number of eggs a female lays per unit of time. Starvation resistance can be operationally defined as the number of hours a fly can survive in a nutrient-deprived environment. Here two different protocols to characterize the traits mentioned above were explored, in a well-defined and replicated experimental design. The aims of this project were (1) to determine the accuracy and efficiency of two different methods of egg counting, during fecundity assays, and (2) to analyze the accuracy of a new protocol for exposure to starvation.

Interactions of macrophages with *Escherichia coli*

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During millions of years "arms-race" shaped evolution not only between species across the planet but also inside the human body. Innate immune system is constantly evolving different strategies to detect and destroy intrusive microbes, while microbes evolving herewith. What are the strategies that microbes can develop to avoid and circumvent immune recognition?

We study the evolution of the model organism *Escherichia coli* under the selective pressure of the macrophages (RAW264.7) that play major role in the innate immune response to foreign intruders.

In approximately 100 generations all bacterial lines, evolved in the presence of the macrophages, showed a remarkable polymorphism, which did not occur in the controls. The relative abundance of the new morphs fluctuated stochastically over time and reached fixation after 500 generations. Those genotypes are marked by morphological differences clearly seen in the *E. coli* forming colonies and FACs measurements. Furthermore, the first observed morphs that persist better inside the macrophages because of slow growth and deficiency in metabolism have the same characteristics as SCVs (small colony variants) sampled from patients with recurrent and persistent infections. Other morphs became resistant to engulfment and killing by phagocytes, and display mucoid phenotype. Both morphs show an advantage *in vivo* and mucoid morph possibly exhibit deleterious effects in mice.

This interdisciplinary research has implications on applied biomedicine furthermore it will be able to generate predictions concerning the nature of adaptations of microorganisms to multiple infections and to the immune system.

Population genetic structure in *Iberochondrostoma lemmingii* (Steindachner, 1866): disentangling species fragmentation and colonization processes in Mediterranean river courses

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Iberochondrostoma lemmingii is an endangered cyprinid species of the southwestern area of the Iberian Peninsula. This family presents complex phylogeographic patterns, which some studies have already associated with the complex geological and climatic history of the Peninsula. Also, Iberian river habitats are particularly vulnerable to climatic changes and antropogenic pressures, that model freshwater populations. Here, we analyze the population structure and demographic history of *Iberochondrostoma lemmingii*, a species with a wide distribution range comprising several independent water drainages, using cyt b and microsatellite loci genetic diversity in order to disentangle fragmentation and colonization processes in Mediterranean river courses at different time scales. Strong evidences of population structure were found within and between river basins. With the exception of Quarteira, high levels of mitochondrial diversity were found among all drainages, probably due to ancestral polymorphism (Robalo et al., 2007). There were significant levels of differentiation between drainages and even within drainages in some cases (Caia and Foupana, both within the Guadiana drainage). Microsatellite data also revealed significant population structure among and within drainages, although some of the observed patterns were different from those observed with mtDNA, with some inter-drainage comparisons presenting unexpected low levels of microsatellite differentiation. Finally, no evidences of recent bottleneck events were detected. In fact, the results appear to show evidences of sudden expansion in all populations.

Addressing the genetic response of Bornean elephants to habitat loss and fragmentation using next generation sequencing approaches

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Habitat destruction is one of the main threats to biodiversity. Over the years there has been a tremendous increase in degradation of the rainforests of Borneo due to a rise in oil palm plantations and timber logging. Forest fragmentation can have a dramatic effect on landscape connectivity and the dispersal of animals, potentially reducing gene flow between populations, which could lead to significant inbreeding and loss of genetic diversity within fragments. This study is focused on studying the effect of habitat fragmentation on the genetic diversity of Borneo pygmy elephants (*Elephas maximus borneensis*). They are critically endangered; smaller than other Asian elephants and are found only in the north of Borneo, in the State of Sabah, Malaysia, and across the border of Sabah and Kalimantan, Indonesia. We observed high FIS values (i.e, excess of homozygotes) in most of the elephant populations by using microsatellites isolated in other elephant subspecies. This could be caused by several factors including Wahlund effect, or allelic dropout as we employed mainly, elephant dung in our study. In order to test whether such deficits were found in other genomic markers, we developed and standardized *de novo* markers (microsatellites and SNPs) using next generation sequencing methods. We present the approach chosen including details of the methodology, together with a discussion on the limitations of these approaches for non invasive samples.

Genetic differentiation and phylogeny of several of Dilta species from the Canary Islands

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Dilta Strand, 1911 (Archaeognatha, Machilidae) is a genus of primitive, slender, elongated and wingless insects, with close-fitting dull scales. The genus Dilta has a strictly palaearctic distribution and a particular set of species is found at the Canary Islands archipelago. These insects live usually on well vegetated habitats, commonly associated with various endemic trees but can also be found above the timber-line. A previous study of a different set of Dilta species showed a maximum sequence divergence of about 22% at the 12S rRNA gene. In this work, we assessed the genetic differentiation among several Dilta species using the mitochondrial gene 12S rRNA. The results from the Canary Islands Dilta species indicate lower overall differentiation, with the highest value of 13.2% suggesting, nonetheless, a considerable divergence among the island species. The set of species studied in this work shows phylogenetic trees concordant with their distribution through the islands of this archipelago and that appears to be congruent with the history of the islands.

The transvalley leak and mid-way closure of the ring species *Ensatina*: the evolutionary history of *Ensatina eschscholtzii xanthoptica*

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The Central Valley of California has constituted an important geographic barrier for many terrestrial organisms, affecting their distribution and diversification. In the salamander *Ensatina eschscholtzii* this barrier was responsible for the formation of a ring species complex. Here, we investigate the diversification process of the only subspecies within *Ensatina* that has breached the Central Valley barrier (*E. e. xanthoptica*) – the so-called “transvalley leak” by *E. e. xanthoptica*. Our multilocus analysis shows that *xanthoptica* initially diversified along the coast during mid Pleistocene. Subsequently, it colonized the foothills of the Sierra Nevada, and then split during mid Pleistocene, some 640,000 to 800,000 years ago to leave an isolate in contact with the inland subspecies *platensis*, at the mid-way closure of the ring. Evidence of post-divergence migration in *Ensatina* suggests that the formation of an ecological corridor across the Central Valley was a repeated event, which may explain disparate estimates of colonization times across taxa with similarly disjunct distributions. The antiquity of the transvalley leak corroborates the ring-species interpretation because it demonstrates that the narrow hybrid zone between *xanthoptica* and *platensis* has been acting as an effective barrier to gene flow over multiple climatic cycles. In contrast with genetics, morphological evolution does not appear to be independent at the mid-closure of the ring, and taxa converge in morphometric space. This suggests that processes related with the macro-habitat, such as phenotypic plasticity and local adaptation, might be more determinant to morphologic evolution than phylogeographic history.

Why do Bacteria have in their genome so many genes that can potentially kill them?

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Toxin-antitoxin (TA) loci are abundant and widely distributed among the genomes of Bacteria and Archea. They are composed by a toxin gene, that encodes a bactericid or bacteriostatic protein, and an antitoxin gene, that can prevent the effect of the toxin. Though these two genes are adjacently located and jointly expressed, their expression, under certain circunstances, can kill or prevent the growth of the cells that harbor them.

The biological role of these loci is widely debated:

It is known that when they are located in a plasmid, their action stabilizes the plasmid in the cells by post-segregation killing; but their role when located in the chromosome is unclear. Actually, different types of TA systems can have very different roles, from behaving as purely selfish genetic elements, to being “domesticated” and incorporated in important genetic networks.

To address the origin and dynamics of TA loci, we are analyzing complete genomes of Enterobacteriaceae and studying the number, position and organization of TA loci. Moreover, this comparative approach, coupled with phylogenetic analysis, allow us to study the dynamics of TA loss and gain in the lineages. We will present our current knowledge of the dynamics of a particular set of TA gene families within these genomes.

On the move: modelling sex-biased migration

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Several Human Population Genetics studies suggest that mtDNA and Y-chromosome data can exhibit different patterns of genetic differentiation. It is not clear whether these differences are due to stochastic or deterministic factors.
In this talk, I will focus on how different cultural practices, such as asymmetrical post-marital residence, between males and females have an impact on human genetic diversity patterns. To simulate sex-biased migration, I used a new forward spatial simulation program, developed within our group, which incorporates both geographical and demographic data, as well as several types of genetic markers.

New anomodont taxon from a largely unexplored basin: on the presence of cf. Diictodon in the Mozambican Karoo (Niassa Province), Late Permian

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The largely unexplored Metangula Graben (Mozambique) after nearly half a century of insufficient collecting, is now providing new anomodont specimens that may increase knowledge on Karoo basins outside South Africa. Here we report a new taxon for the Mozambican Karoo: cf. Diictodon. Based on a complete ilium and upon the reduced development of the preacetabular process, modest development of the supracetabular buttress, longer preacetabular process relative to the postacetabular process. This specimen represents the first well determinable Diictodon outside South Africa and Zambia, which reinforces the cosmopolitan hypothesis of this taxon in the Late Permian.

Additional resistance determinants often increase bacterial fitness –sign epistasis involving plasmids and chromosomal mutations

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Multidrug-resistant bacteria arise mostly by the accumulation of chromosomal mutations and plasmids. Typically, these resistant determinants are costly to the bacterial cell. Yet, we report that the acquisition of an additional resistance mutation or of a resistance plasmid often increases the competitiveness of a bacterial cell. This amelioration occurs in almost one third of combinations between ten antibiotic-resistance mutations and five antibiotic-resistance plasmids. These conclusions were taken by performing experiments with natural drug-resistance conjugative plasmids and bacterial strains bearing drug-resistance mutations commonly found among pathogens. This work explains, at least in part, how multidrug resistance evolved so rapidly.

In the dark: counting endangered nocturnal lemurs

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Madagascar is one of the highest biodiversity conservation priorities on Earth. Lemurs, endemic to Madagascar, belong to the most threatened primate *taxa* in the world. With increasing habitat fragmentation and deforestation, lemur surveys of the remaining forests of Madagascar are essential. Furthermore several genera have seen their number of recognized species grow rapidly in the last decade. Species identification and density estimates are essential to determine conservation priorities. However, in many regions of Madagascar this information remains unknown, particularly for recently described species. More specifically, identifying presence and estimating density of nocturnal lemurs continues to be a challenge due to their solitary foraging patterns and typically small size.
In this study we used Sherman traps to capture over 250 mouse lemurs (*Microcebus spp*) in two forest fragments from northern Madagascar to estimate population sizes and to determine the species present in this region. We also estimated population sizes of another nocturnal species belonging to the *Lepilemur* genus.
Standard morphological measurements were taken between July and September 2010 for the *Microcebus* individuals. The data suggest that the individuals captured in Daraina are closer to *Microcebus tavatra* than to other *Microcebus* species found in the north of Madagascar.
Preliminary results from density estimation procedures suggest high densities of both species. We further used this study to investigate to what extent different density estimation methods provided different results. This is a crucial issue as different methods are often used by different research teams.

Serpentine soils and the ecology and evolution of fungal-plant symbiotic partners

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Extreme environments tend to host depauperate and specialized biological communities. Serpentine soils, stressful due to high heavy metal levels, poor nutrient contents, and unbalanced calcium to magnesium ratios, exemplify this phenomenon by imposing well-known constraints on plants. I studied the effects of serpentine soil on the ecology and evolution of a fungal-plant symbiotic system to investigate adaptive responses in symbiotic partners under the same environmental constraint. I focused on serpentine ectomycorrhizal (ECM) fungal communities associated with *Quercus ilex* subsp. *ballota* and used a combination of field, greenhouse, and molecular approaches to examine community- and population-level differentiation patterns in the fungi and the plant. I found rich and phylogenetically diverse serpentine fungal communities, as well as widespread serpentine tolerance in ECM fungi. Although serpentine soil is a distinct environment hosting different fungal communities, I found no indication of it being physiologically challenging. I also report the some evidence supporting local adaptation in *Q. ilex* subsp. *ballota*, with serpentine ecotypic differentiation evidenced by disproportional root allocation. My results suggest that serpentine soils influence the ecology and evolution of symbiotic partners differently, with differential adaptation patterns.

Genetic and morphological variation of two littorinid gastropods along an East African latitudinal gradient

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The family Littorinidae is a group of marine gastropods widespread around the world and widely used in evolution and adaptation studies. Some of their most interesting aspects include large morphological variation and local adaptation that seems to depend on plastic or genetic cues under the influence of microenvironmental factors. Further, different populations and species seem to show different dispersal abilities. The mangrove periwinkle *Littoraria scabra* (Linnaeus, 1758) and the rocky shore *Littoraria glabrata* (Philippi, 1846) were investigated through genetic and geometric morphometry methods along an eastern African latitudinal gradient in order to assess the effect of habitat and geographic distance in their differentiation.
Specimens were sampled from 8 mangrove and 9 rocky shore sites. Replicates were made for geometric morphometric analysis. Segments of the mitochondrial genes 12S, 16S and COI were sequenced for a subset of samples. The results showed a greater variability of shell shape in *L. scabra* among mangroves that could be habitat-related. Nevertheless, the data also indicated a substantial variation in shell morphology in *L. glabrata*. However, we observed little genetic differentiation between populations showing that morphological adaptations are not correlated with the genetic structure of populations. This suggests the effect of phenotypic plasticity within these populations, and reopens the discussion about the capacity of marine populations to maintain the homogeneity over a scale of thousands of kilometers.

Multilocus phylogenetic analysis of the genus *Atherina* (Pisces: Atherinidae)

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Sand-smelts are small fishes inhabiting inshore, brackish and freshwater environments and with a distribution in the Eastern Atlantic and Mediterranean Sea, extending south into the Indian Ocean. Here, we present a broad phylogenetic analysis of the genus *Atherina* using three mitochondrial (control region, 12S and 16S) and two nuclear markers (rhodopsin and 2nd intron of S7). Phylogenetic analyses fully support the monophyly of the genus. Two anti-tropical clades were identified, separating the South African *A. breviceps* from the north-eastern Atlantic and Mediterranean fishes. In European waters, two groups were found. The first clade formed by a well supported species-pair: *A. presbyter* (eastern Atlantic) and *A. hepsetus* (Mediterranean), both living in marine waters; a second clade included *A. boyeri* (brackish and freshwater environments) and two independent lineages of marine punctated and non punctated fishes, recently proposed as separate species. Sequence divergence values strongly suggest multiple species within the *A. boyeri* complex.

Genomic signatures of adaptation to fast growth in Bacteria and Archaea - uses in ecological metagenomics.

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Minimum generation time is a key life-history trait in bacteria and archaea. In stable, low-resource or crowded environments, competition implies efficient scavenging (oligotrophy) and very fast growth is impossible or selected against. However, where resources are sporadically abundant, high growth rates may be advantageous to quickly compete for nutrient and space (copiotrophy), whenever conditions become favorable. Growth rates of bacteria and archaea heavily depend on their capacity to optimize the transcription and translation machineries. This optimization leads to selective patterns of gene organization and composition.

Working on a dataset of 214 fully sequenced genomes of bacteria and archaea with experimentally determined minimum generation times, we confirmed that the number of transcription/translation machinery genes, their position on the chromosome, and indices of codon usage bias are all highly correlated to growth rates. Highly expressed genes select for "optimal" codons and even more so in fast growers. This suggests a common selective pressure. We also found a strong association between deviations from the general patterns and exploitation of extreme habitats (psychrophily and thermophily).

We used this knowledge to build a sequence-based predictor of minimum generation time to be used on non-assembled genomes or subsets of genes. Applied to different metagenomic datasets, the results obtained followed expectations given the stability and richness of these habitats and their successional stage. This work synthesizes how growth rates of prokaryotes imprint and are in turn shaped by genomes and how these signatures can be used to enlighten the most frequent resource exploitation strategies in complex environments.

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