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**PROGRAM,  
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## Molecular palaeobiology of early animal evolution

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Genomic information is now available for a substantial number of organisms and understanding the evolutionary history of organisms is becoming an increasingly multidisciplinary effort, where information from multiple lines of evidence (the genomic record, the fossil record, and the phenotype of extant taxa), are being combined using sophisticated computational approaches. This new multidisciplinary endeavour is what we refer to Molecular Palaeobiology. Here I shall summarise current advances on the molecular palaeobiology of animals, and delineate how the integration of molecular and fossil data has allowed novel insights in the evolutionary history of animals.

## Small, libertine and fit: did recombination and sex promote the evolutionary success of microalgae, from the origin of life to present days?

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Microalgae are among the more ancient organisms on Earth. This group of photosynthetic unicells includes pro- and eukaryotes. The first “invented” photosynthesis, the second compartmentalized this metabolic process into plastids, derived from the intracellular domestication of cyanobacteria (endosymbiosis). Microalgae form large populations in all aquatic environments play a key role in biogeochemical cycles and include several taxa capable to proliferate at extreme, quasi-primordial conditions. The large size and consequent fitness of microalgal populations are explained by their dominant clonal reproduction and the frequent genetic mutations during DNA duplications. Nonetheless, genetic and genomic observations suggest that genetic recombination frequently occurs in both prokaryotic and eukaryotic lineages. Moreover, many eukaryotic microalgae have life cycles including a sexual phase that can provide a regular genetic reassortment. In this contribute, we review studies on natural populations of aquatic and extremophilic microalgal taxa, both pro- and eukaryotes, whose evolutionary origins span 2-0.2 Billions years ago, namely the genera *Planktothrix* (freshwater cyanobacteria), *Galdieria* (extremophilic unicellular rhodophytes) and *Pseudo-nitzschia* (marine diatoms). The data gained so far suggest that genetic recombination and sex appear to play an important role in driving microevolution and promoting population fitness in each of the above mentioned microbial organisms. In a study focused on natural populations of *Planktothrix* spp. sampled in the subalpine lake district in North Italy., recombination signals were observed by analysing four molecular loci interspersed in the genome. Computational analyses suggest that recombination introduced genetic diversity at a rate more than double than mutations. Recombination provides natural populations of *Planktothrix* spp. with a background genetic diversity from which successful genotypes can eventually emerge and conquer new ecological niches, mimicking population developments typical of pathogenic (epidemic) bacteria. A study on Icelandic strains of *Galdieria sulphuraria* showed signals of recombination at the level of plastidial genome (i.e., the gene *rbcL*) in this putatively asexual microalga. A wide post-recombination diversification was inferred in *G. sulphuraria* populations, suggesting the occurrence of an intense spreading and a high fitness of recombinant lineages. Such recombinations without proper sex (i.e., nuclear recombination), could have been maintained even after primary endosymbiosis. The usefulness of this process is that it probably promotes RuBisCo stability at extreme temperatures, as already demonstrated in other photosynthetic extremophiles (i.e., the cyanobacterium *Synechococcus*). Finally, molecular investigations on the diatom *Pseudo-nitzschia multistriata* reported signals of intra-specific recombination in natural populations (ribosomal ITS). This species showed a highly synchronized and strictly periodic sexual phase in nature, which makes it an obligate sexual organism, since geographically isolated populations would go eventually extinct in absence of sex. Nonetheless, species in the genus *Pseudo-nitzschia* also showed signals of recombination in the *rbcL* gene at both intra- and inter-specific level, suggesting that a putatively cyanobacteria-derived plastidial recombination could have been

maintained in the course of the evolution of photosynthetic lineages, besides the emergence of proper sex. In presenting and discussing these cases, we aim to develop a conceptual scenario on the origin and benefit of recombination and sex in microalgae, an ecologically and evolutionary relevant group of organisms at global scale.

## Single-gene data filtering for multi-gene phylogenetics: elucidating the higher-level phylogeny of Siluriformes

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Datasets containing taxa with highly heterogeneous evolutionary rates can lead to artifacts in phylogenetic inference, such as long branch attraction (LBA). These artifacts are produced by systematic errors resulting from the mismatch between the heterogeneous evolutionary rates on the dataset and the assumption that all taxa evolve at the same rate, an assumption that all current models of evolution make. Phylogenomic and multi-gene phylogenetic analyses have fallen short of overcoming such systematic errors, as adding genes that reinforce consistently a certain systematic error produces higher statistical support for the artifactual relationship. Normally, when rate-heterogeneity artifacts like LBA are suspected on a phylogeny, fast-evolving taxa are arbitrarily removed from the dataset, and the phylogeny reevaluated. We propose a criterion-guided methodological approach for subsampling the taxa that most closely comply with the assumption of equal evolutionary rates. The criterion used to gauge lineage rate heterogeneity is the P-value resulting from a likelihood ratio test between a phylogeny constrained to have a single molecular clock on the ingroup vs. a phylogeny in which 3 local molecular clocks are allowed. Subsampling is performed on each gene of a multi-gene dataset prior to concatenation, thus reducing evolutionary rate heterogeneity (and potentially their artifactual signal) at the gene level. After finding the subsamples with minimized lineage rate heterogeneity for each gene, the excluded taxa are filled as missing data, and all genes concatenated. Comparing the phylogeny from the concatenated full dataset with that of the concatenated dataset with evolutionary rate heterogeneity reduced on each gene permits to see the effects the removed sequences were having on the final results. We implemented our new approach to explore the controversial inter-relationships among the three main lineages at the base of the catfish order Siluriformes, where morphological and molecular phylogenies propose conflicting hypotheses. We analyzed an 11,566 bp, 10-gene dataset of 46 taxa of catfish and 6 outgroups species. Lineage rate homogeneity was reached on 7 out of the 10 genes, and a concatenated analysis of the 7 genes in which lineage rate heterogeneity biases had been presumably reduced gave a phylogeny that contrasted with previous molecular hypotheses. Our results suggest that an artifactual signal may have been at play on previous analyses, possibly due to the exceptional evolutionary rate heterogeneity present on this clade.

## NeON: an R package to estimate human effective population size and divergence time from patterns of linkage disequilibrium between SNPs

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The effective population size ( $N_e$ ) is one of the most interesting population parameter, which helps to understand how populations evolved, expanded or shrunk. Traditionally, its estimate is calculated by comparing DNA sequences diversity, so as to obtain an average  $N_e$  over many past generations without actually considering how the population size changes over generations. Linkage disequilibrium (LD) patterns contain information about these changes, and whenever a large number of densely linked markers are available, can be used to monitor fluctuating population size through time. The NeON package has been designed to explore population's LD patterns in order to reconstruct two key parameters of human evolution: the effective population size and the divergence time between populations. NeON starts with binary or pairwise-LD PLINK files, and allows a) to assign a genetic map position using HapMap (NCBI release 36 or 37) b) to calculate the effective population size over time exploiting the relationship between the average squared correlation coefficient of LD ( $r^2_{LD}$ ) within predefined recombination distance categories and  $N_e$ , and c) to calculate the confidence interval of the effective population size based on the observed variation of the estimator across chromosomes; the outputs of the functions are both numerical and graphical. This package offers also the possibility to estimate the divergence time