

5<sup>th</sup> Congress  
ITALIAN SOCIETY FOR  
EVOLUTIONARY BIOLOGY  
Trento, 28-31 August 2013

**PROGRAM,  
BOOK OF ABSTRACTS,  
AUTHOR INDEX.**

**EDITED BY LINO OMETTO AND OMAR ROTA-STABELLI**



V congress of the Italian Society for Evolutionary Biology. Trento, 28-31 August 2013

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## THE CONGRESS:

**+160** PARTECIPANTS,

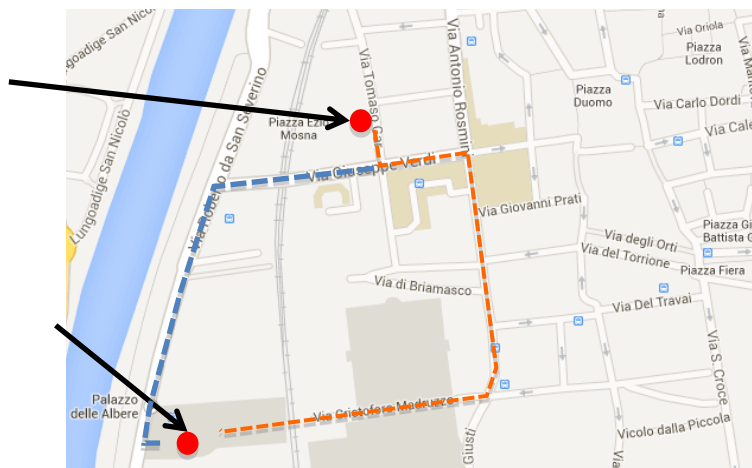
**108** SCIENTIFIC ABSTRACTS,

**30** OUTREACH TALKS AND ACTIVITIES

**ALL LECTURES TO BE HELD AT LETTERE BUILDING, DINNER AND OUTREACH AT MUSe.**

**Lettere**  
Via Gar 14

**MuSe**  
Viale del Lavoro  
e della Scienza 3



**CONTACTS**  
omar.rota@fmach.it  
lino.ometto@fmach.it

**THE CONGRESS IS A JOINT COLLABORATION AMONG SIBE (ITALIAN SOCIETY FOR EVOLUTIONARY BIOLOGY), FEM (FONDAZIONE EDMUND MACH), MUSe (MUSEO DELLE SCIENZE DI TRENTO), AND CIBIO (UNIVERSITÀ DI TRENTO)**

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# DETAILED CONGRESS PROGRAM

\* denotes talk from a PhD student.

## WEDNESDAY 28 AUGUST

- 13:00** registration opens  
**14:00** welcome from the organizers, SIBE presidency, and authorities

### EVOLUTIONARY APPLICATIONS FOR BIOSYSTEMS AND AGRICULTURE

- 14:20** Chairs introduction (Gianfranco Anfora and Claudio Varotto)

#### Part 1: Insects and ecosystems

- 14:30** *Elisa Frasnelli*: Linking the evolution of brain asymmetries and social behaviour in honeybees  
**14:50** *Alessandro Cini*: Insects social networks: from pure to applied ethology  
**15:10** *Giuliano Gasperi*: Uncovering the tracks of a recent and rapid invasion: the case of the tiger mosquito, *Aedes albopictus* in Europe  
**15:25** \* *Kaur Rupinder*: Comparative morphology and evolutionary genomics provide useful clues for management of an emerging *Drosophila* pest  
**15:40** brief outline of the related posters (Rossi-Stacconi, Giacomelli, Grzeskowiak, Baracchi, Esposito, Sofo, Kaur, Coman, Rigosi) and coffee break

#### Part 2: Plants and microbes

- 16:45** *Roberto Papa*: The cost of domestication (if any): can we get a refund?  
**17:15** *Giuseppe Vendramin*: Adaptation of Mediterranean forest species to climatic gradients: a forest genetic perspective  
**17:45** *Chris Creevey*: Evolutionary metagenomic analysis of the rumen microbial community of cattle  
**18:00** *Andrea Campisano*: How grapevine got pimples. The interkingdom horizontal transfer of an unusual symbiont  
**18:15** *Irene Stefanini*: Social insect intestines are mating nests for *Saccharomyces cerevisiae*  
**18:30** \* *Sonia Ciccazzo*: Evolution of complex microbial communities for biotransformation of tar produced by biomass pyrolysis  
**18:45** \* *Anna Sandionigi*: A hypothesis on the interactions between microbiomes in a parasitic relationship: The case of *Apis mellifera* and its parasite *Varroa destructor*

- 20:30** Event at MuSe (open to the public, in Italian): "DIMMI CON CHI VIVI E TI DIRÒ CHI SEI" with Maurizio Casiraghi, Duccio Cavalieri, Stefano Turillazzi, Marco Ferraguti.

## THURSDAY 29 AUGUST

### EVOLUTIONARY GENOMICS AND BIOINFORMATICS

- 09:00 Chairs introduction (Manuela Sironi and Dan Sargent)
- 09:05 *Francois Balloux*: **Reconstructing human genetic diversity in space and time**
- 09:45 *Monica di Paola*: **Evolution and genome divergence determine virulence traits in *Candida* spp.**
- 10:00 *Paolo Gratton*: **“Bad” species? Restriction sites associated DNA sheds light on the evolution of the *Erebia tyndarus* species complex**
- 10:15 \* *Francesco Rubino*: **A framework for the evolutionary study of uncharacterised microbial communities**
- 10:25 \* *Valentina Cappelletti*: **Systems biology approach for the identification of genetic determinants of colony morphology switch in natural *S. cerevisiae* strain**
- 10:35 **brief outline of the related posters (Vicario, Ometto, Scolari, Plazzi, Vicario, Gargiulo, Mancini, Milani, Cagliani, Hoban) and coffee break**
- 11:10 *Christian Schlötterer*: ***Drosophila* population genomics**
- 11:50 \* *Sukanya Ramasamy*: **Bioinformatic and evolutionary analysis of chemoreceptors and related proteins in a grapevine pest**
- 12:05 *Caroline Scotti-Saintagne*: **Evidence of disruptive selection in the adaptation of two closely related species of tropical trees**
- 12:20 *Marco Passamonti*: **Structure, transcription and variability of metazoan mitochondrial genome: perspectives from an unusual mitochondrial inheritance system**
- 12:35 \* *Karen Siu-Ting*: **Concatabominations: identifying unstable taxa in morphological and genomic datasets using Safe Taxonomic Reduction**
- 12:50 \* *Wasiu AJ Akanni*: **Development of a Maximum Likelihood and Bayesian Supertree method and the analysis of the Tree of Life**

13:05 **lunch box provided**

### EVOLUTIONARY MEDICINE AND HEALTH

- 14:10 Chairs introduction (Carlotta de Filippo and Heidi Hauffe)
- 14:20 *Eugene Rosenberg & Ilana Zilber-Rosenberg*: **Variation and Evolution of Holobionts**
- 15:00 *Samanta Michelini*: **Bifidobacteria in gut microbiota of *Callithrix jacchus* L. (common marmoset): their relationship with primate and non primate bifidobacterial microbial ecology**
- 15:15 *Giacinto Libertini*: **Evidence for aging theories from the study of a hunter-gatherer people (Ache of Paraguay)**
- 15:30 *Lisa Rizzetto*: **Diverse strain immune reactivity shapes fungal inflammation or tolerance**
- 15:45 *Lorenza Putignani*: **Gut microbiota in cystic fibrosis (CF) patients: a combined -omic translational workflow**
- 16:00 \* *Diego Forni*: **An evolutionary view of antigen processing and presentation across different timescales**
- 16:10 **outline of the related posters (Baracchi, Cavallero, Mezzavilla, Libertini, Fascio, Berra) and coffee break**
- 17:00 *Duccio Cavalieri*: **Systems level analysis of human fungal populations: passengers colonizers and invaders**

### NEW PHD ON THE BLOCK (SPECIAL SESSION)

- 17:30 Chairs introduction (Alessandro Gretter and Lino Ometto)
- 17:30 \* *Luisa Bresadola*: **Genetic variability in the promoter of miR397 in *Picea abies***
- 17:40 \* *Claudia Scavariello*: **Non-LTR retrotransposon R2 molecular characterization and activity in *Bacillus rossius* (Phasmida, Bacillidae)**
- 17:50 \* *Emanuele Rigato*: **Effects of cryptic genetic variation on innovability in *Escherichia coli***
- 18:00 \* *Laura del Latte*: **Towards a phylogeny of the geophilomorph centipede genus *Stenotaenia***
- 18:10 \* *Davide Guerra*: **The control regions of the male- and female-transmitted mitochondrial DNAs in *Musculista senhousia* (Bivalvia Mytilidae)**
- 18:20 \* *Johannes De Groeve*: **From evolutionary trees to behavioural ecology: exploiting SAM to visualize spatio-temporal movement patterns**
- 18:30 \* *Francesca Tassi*: **A comparison of linguistic and genomic diversity in Europe**
- 18:40 **outline of the related posters (Baccei, Bernini, Giovannini, Salvagnin, Poli, Bisanti, Portera, Andreatta, Gazzola)**

## FRIDAY 30 AUGUST

### BIODIVERSITY 3D: THE INTERRELATIONS AMONG GENES, SPECIES, AND ECOSYSTEMS

- 09:00** Chairs introduction (Ivan Scotti and Cristiano Vernesi)
- 09:10** *Mike Bruford: Genes, species and ecosystem services: do we have the tools to pursue the paradigm?*
- 09:50** *Elisabetta Versace: Experimental evolution uncovers habitat-dependent fitness dynamics among *Wolbachia* strains in *Drosophila melanogaster**
- 10:05** *David Baracchi: Intra or inter specific variability? Wing morphology and chemical epicuticular compounds reveal a cryptic species of Stenogastrinae (Hymenoptera: Vespidae)*
- 10:20** *Emanuele Serrelli: A new look at the Extended Evolutionary Synthesis*
- 10:35** brief outline of the related posters (Ruggiero, Meraner, Battocchi, Cornetti, Rivero, Hears, Bertoncini, D' Ercole, Bonosi, Benazzo, BARCODING) and coffee break
- 11:10** *Krystal Tolley: Exploring the history of Chameleons*
- 11:30** *Ulirke Obertegger: Cryptic diversity of *Synchaeta* spp. (Rotifera, Monogononta) in mountain lakes: relationships with environmental parameters*
- 11:45** *Elena Mosca: Effects of climate on fine-scale spatial genetic structure in four alpine keystone species*
- 12:00** *Saverio Vicario: Mean phylogenetic surprise: a unit of measure for describing changes across biological communities*
- 12:15** short break
- 12:20** \* *Luca Cornetti: Small effective population size and fragmentation in Alpine populations of *Bombina variegata*: the combined effects of recent bottlenecks and postglacial recolonization*
- 12:30** \* *Emanuele Fasola: Microevolution due to pollution in Amphibians*
- 12:40** \* *Alexis Marchesini: Amphibian biodiversity in an alpine region: a multi-level approach. Patterns, processes and conservation implications*
- 12:50** \* *Verena Pichler: Evidence of recombination on the "speciation island" X-chromosome centromeric region between the malaria mosquitoes *Anopheles gambiae* and *An. coluzzii**
- 13:00** lunch box provided

### LIFE THROUGH TIME: PALEOBIOLOGY AND PALEOBIODIVERSITY

#### Part 1: From fossils to biodiversity

- 14:00** Chairs introduction (Silvio Renesto and Giorgio Binelli)
- 14:05** *Mike Benton: Origin of biodiversity*
- 14:45** *Giorgio Carnevale: Cretaceous Ophidiiform fish from North-Eastern Italy: systematics and evolutionary implications*
- 15:05** *Evelyn Kustatscher: From the Palaeozoic to the Mesozoic - how did the flora change in Europe?*
- 15:25** *Stefano Dominici: Macroevolutionary emergence of onshore-offshore gradients in community turnover*
- 15:45** *Silvio Renesto: The diversity and biogeographic significance of the Norian (late triassic) terrestrial reptiles from northern Italy*
- 16:05** brief outline of the related posters (Rota-Stabelli, Bauer) and coffee break

#### Part 2: Paleo-evo-devo 16:45 Chairs introduction (Massimo Bernardi)

- 16:50** *Massimo Delfino: Developmental palaeontology from an Italian perspective*
- 17:10** *Giuseppe Fusco: Fossilized ontogenies in evo-devo: the case of trilobites*
- 17:30** \* *Kathleen Bauer: Early ontogeny in a fossil ginkgophyte from the Triassic resembles modern *Ginkgo biloba**
- 17:40** short break

#### Part 3: Molecules: from Precambrian to Present 17:45 Chairs introduction (Omar Rota Stabelli)

- 17:50** *Davide Pisani: Molecular palaeobiology of early animal evolution*
- 18:20** *Domenico D'Alelio: Small, libertine and fit: did recombination and sex promote the evolutionary success of microalgae, from the origin of life to present days?*
- 18:35** \* *Carlos J. Rivera-Rivera: Single-gene data filtering for multi-gene phylogenetics: elucidating the higher-level phylogeny of Siluriformes*
- 18:50** *Massimo Mezzavilla: NeON: an R package to estimate human effective population size and divergence time from patterns of linkage disequilibrium between SNPs*

**20:00 BUFFET DINNER AT MUSE. Awards and Toasting**

## SATURDAY 31 AUGUST

**10:00 CONCILIO DI TRENTO: Annual congress of SIBE members, Open to all SIBE members at Lettere!**

### WALLACE DAY (AT MUSe, OPEN TO PUBLIC. IN ITALIAN)

#### **14:00-16:00 Tavola Rotonda: "NON SOLO MUSEO. L'EVOLUZIONE AL TEMPO DEI BLOG E DEI SOCIAL NETWORK"**

- 1) Stefano Mazzotti (Museo di Storia Naturale di Ferrara, chairman): *Introduzione: casi, linguaggi e criticità*
- 2) Marco Ferrari: *Web, social network ed evoluzione*
- 3) Telmo Pievani (Università di Padova): *Pensare evolutivo sul web: i primi anni del portale Pikaia*
- 4) Michele Bellone: *Antidarwinismo in rete: quando e come intervenire?*
- 5) Michele Lanzinger, Michele Menegon: *MuSe: Ricerca, collezioni, tassonomia e... web*
- 6) Maurizio Casiraghi (Università di Milano-Bicocca): *Alfabetizzazione scientifica e comunità web*
- 7) discussione e conclusioni

#### **16:00-19:00 IL POMERIGGIO DELGI EVOLUZIONISTI**

**Un pomeriggio dedicato a chi vuole conoscere l'evoluzione da parte di chi l'evoluzione la studia tutti i giorni. Serie di micro interventi ripetuti durante la giornata, laboratori e attività interattive.**

- Piano -1: *Storie di mammiferi*, con Alessandro Minelli (Università di Padova)
- Piano -1: *Dinosauri italiani*, con Massimo Bernardi (MuSe)
- Piano -1: *Ediacara, Burgess e altre storie bizzarre*, con Omar Rota Stabelli (FEM)
- Piano -1: *Ritrarre Emma Wedgwood Darwin*, di Chiara Ceci (Royal Society of Chemistry, Cambridge)
- Piani -1 e +1: *Ominini, ominidi, uomini*, con Telmo Pievani (Università di Padova)
- Serra tropicale: *Evoluzione nella foresta*, con Michele Menegon (MuSe)
- Serra tropicale: *Vestiti da vespa*, con Lino Ometto (FEM)
- Piano terra: *Animali estremi? L'orsetto d'acqua*, con Roberto Guidetti (Univ. di Modena e Reggio Emilia)
- Piano terra: *La speciazione e "Il valzer del moscerino"*, con Valerio Mazzoni (FEM)
- Piano +1: *Supermarket DNA: il codice a barre della vita*, con Maurizio Casiraghi (Univ. di Milano-Bicocca)
- Piano +1: *Dimmi con chi vivi, ti dirò chi sei: come la simbiosi influenza l'evoluzione degli organismi viventi*. con Maurizio Casiraghi (Università di Milano-Bicocca)
- Piano +1: *Quando la critica non è scientifica*, con Michele Bellone (Science writer)
- Piano +1: *Darwin e Wallace*, con Marco Ferraguti (Università di Milano)
- Piano +1: *Giocare all'evoluzione*, con Matteo Bisanti (Università di Modena e Reggio Emilia)
- Piano +1: *Paleotech*, con PaLEoS (e con Fablab MuSe)
- Piano +2: *Evoluzione in musica*, con Kuru (CIBIO)
- Piano +3: *Prede e predatori*, con Rachele Nieri e Valerio Rossi Stacconi (FEM)
- Piano +3: *Vespe e castagne*, con Giorgio Maresi e Federico Pedrazzoli (FEM)

**18:00 Aperitivo entomologico** (entomological aperitif, only for congress attendees). Introduction by entomologist Valerio Mazzoni (FEM)

# BOOK OF ABSTRACTS

Sorted by Symposia: oral first (in the same order as they appear in the program) and then poster (sorted by submission order).

## Symposia:

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# EVOLUTIONARY APPLICATIONS FOR BIOSYSTEMS AND AGRICULTURE

This symposium aims at providing a general view of the impact that both man-made and natural selection had and still have on plant and animal species of agricultural relevance. The themes tackled by the symposium will range from the evolution of invasive species, the traits that human being have been selecting during plant and animal domestication, the relationship between biodiversity and the occurrence of the centers of domestication, the co-evolution of crops and the pests affecting them, the relationships between social behavior and the evolution of communication mechanisms in insects and many more topics.

**Chairs: Gianfranco Anfora and Claudio Varotto (Fondazione Edmund Mach)**

## Linking the evolution of brain asymmetries and social behaviour in honeybees

**Elisa Frasnelli<sup>1</sup>, Elisa Rigosi<sup>1,2,3</sup>, Gianfranco Anfora<sup>2</sup>, Lesley J. Rogers<sup>4</sup>, Giorgio Vallortigara<sup>1</sup>**

<sup>1</sup>CIMeC Center for Mind/Brain Sciences, University of Trento, Corso Bettini 31, I-38068 Rovereto (TN), Italy.

<sup>2</sup>Research and Innovation Center, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>3</sup>BIOtech research center, Dept. of Industrial Engineering, University of Trento, via Mesiano 77, 38123 Trento, Italy.

<sup>4</sup>Centre for Neuroscience and Animal Behaviour, University of New England, Armidale, NSW 2450, Australia.

Contact: elisa.frasnelli@gmail.com

The honeybee (*Apis mellifera* L.) has been shown to be an excellent model to investigate cognitive abilities such as learning and memory. Studying the mechanisms of learning, storage and recall of memory in bees is very important because these mechanisms are of vital importance for honeybees to be able to forage for nectar, learn and remember where flowers are located, as well as to find their way home to the hive. In fact, the failure of bees to return to the hive after foraging has been shown to be the primary symptom of the colony collapse disorder which has devastated honey bee populations worldwide since about 2006. Here a series of studies are presented to understand the cognitive abilities of honeybee foragers in learning, discriminating, memorizing and recalling different odours. A population-level asymmetry in the use of the antennae has been found: bees use their right antenna to learn and recall short-term memory, whereas the left antenna allows recall of long-term memory. Recently, it has been suggested that the alignment of lateralization at the population level may have evolved as an evolutionary stable strategy (ESS) when individually-asymmetrical organisms must coordinate their behaviour with that of other asymmetrical organisms. Game-theoretical models based on ESS suggest that population-level lateralization is more likely to evolve in social than in non-social species. This hypothesis has been tested by comparing olfactory responses of the right and the left antenna using behavioural (conditioning of the Proboscis Extension Reflex, PER), physiological (ElectroAntennoGraphy, EAG) and morphological (Scanning Electron Microscopy, SEM) measurements in three species of Hymenoptera Apoidea that show different levels of sociality: eusocial honeybees (*Apis mellifera* L.), solitary mason bees (*Osmia cornuta* L.) and bumble bees (*Bombus terrestris* L.), an annual eusocial species. The connection between the evolution of population-level asymmetries and the evolution of social behaviour is discussed on the basis of communication mechanisms in honeybees.

## Insects social networks: from pure to applied ethology

**Alessandro Cini<sup>1</sup>**

<sup>1</sup>Laboratoire Écologie & Évolution UMR 7625, Université Pierre et Marie Curie, 7 quai St Bernard, Bâtiment A 7ème étage, Paris Cedex 05, France.

Contact: cini.ales@gmail.com

Social interactions are crucial for the life of thousands of species worldwide. The most famous peaks in social organization have been reached inside the complex societies of social insects, where hundreds to thousands of individuals continuously



interact, creating a large variety of intricate social networks. The study of social behaviour is now starting to benefit from the use of a sociology-derived approach, the social network analysis, which allows meaningfully describing and analyzing such complex social landscapes. While social behaviour is a core topic of evolutionary studies since decades, it represents, on the contrary, a rather neglected subject in the field of applied entomology. In this talk I will suggest that a greater interest toward social behaviour in pest and invasive species should be developed in order to increase our ability to protect agroecosystems. First, I will use a social wasp, *Polistes dominula*, as a model organism to show how animal social relationships can be profitably studied with Social Network Analysis (SNA). I will examine a recently discovered case of altruism that occurs inside overwintering groups of social wasps and show how SNA allowed uncovering the possible evolutionary origin of this unexpected phenomenon. Then, I will suggest how the same tools can be used to tackle applied questions for agroecosystems protection using two model species: the eusocial honeybee *Apis mellifera* and the invasive weevil *Rhynchophorus ferrugineus*.

## Uncovering the tracks of a recent and rapid invasion: the case of the tiger mosquito, *Aedes albopictus* in Europe

**Mosè Manni<sup>1</sup>, Nidchaya Aketarawong<sup>2</sup>, Ludvik M. Gomulski<sup>1</sup>, Gabriella Tait<sup>1</sup>, Francesca Scolari<sup>1</sup>, Marco Falchetto<sup>1</sup>, Anna R. Malacrida<sup>1</sup>, Giuliano Gasperi<sup>1</sup>**

<sup>1</sup>Department of Biology & Biotechnology, University of Pavia, Via A. Ferrata 9, 27100 Pavia, Italy.

<sup>2</sup>Department of Biotechnology, Mahidol University, RamaVI road, Bangkok 10400, Thailand

Contact: gasperi@unipv.it

The dynamic aspects of the invasion process of *Aedes albopictus* has been inferred using different but complementary molecular markers (ITS2, SSRs and SNPs). The analyses of 79 ITS2 sequences from Asian, American and European populations indicates the absence of great differentiative processes and the presence of high degree of heterogeneity among individuals from these populations. This supports the expansion of this species. Using SSRs to analyze the dispersion of this species, China has been suggested as home range of the species by gene diversity estimates, cluster analyses, Bayesian analyses of ancestry and migration rates. It is clear that Chinese mosquitoes share their genomes both with American and European populations. It is evident that the dispersion pattern from this area was/is not due to natural step-by-step migration but the overall picture seems to be masked by a strong propagule pressure driven by human activities, by continued migration events and commercial traffic. We are also assessing whether this mosquito underwent adaptive processes during its invasion process. Because *Ae. albopictus* relies on olfactory cues for host-seeking, mating, blood feeding and oviposition, the antennal transcriptomes have been chosen for the identification and characterisation of chemosensory genes. The analysis of these genes in populations throughout the species range will allow us to detect single-nucleotide polymorphisms (SNPs) that may represent markers of adaptation. An outcome of this integrated analysis will be the development of a database of ITS2, SSRs and SNP fingerprints in ancestral and derived invasive populations as a tool for mosquito traceability in order to infer the origins of new outbreaks, and thus to develop risk maps for this mosquito.

## Comparative morphology and evolutionary genomics provide useful clues for management of an emerging *Drosophila pest*

**Rupinder Kaur<sup>1</sup>, Valerio Rossi Stacconi<sup>1</sup>, Sukanya Ramasamy<sup>1</sup>, Stefanos Siozos<sup>1</sup>, Alberto Grassi<sup>1</sup>, Lino Ometto<sup>1</sup>, Valerio Mazzoni<sup>1</sup>, Gianfranco Anfora<sup>1</sup>, Omar Rota-Stabelli<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: kaur.rupinder@fmach.it, omar.rota@fmach.it

*Drosophila suzukii* is one of the few fruit flies to lay eggs and feed on fresh fruit. Its recent outbreak in western countries, and its peculiar ecological behaviour makes it an emerging model for pest management and biology. A recent genomic survey suggested that *D. suzukii* unusual behaviour is intimately linked with an ecological pre-adaptations to temperate climates and the ability of overwinter in sexual diapause state. Here we provide comparative morphological and behavioural evidences supporting that diapause occurs preferentially in females, is temperature dependent, and is likely mediated by an enlargeable highly pigmented spermatecha. Comparative genomics reveals that a cytochrome associated with spermatecha is differentially expressed and the gene under stronger positive selection in *D. suzukii* compared to sister species. Based on these results, we advocate that an early spring trapping is key for *D. suzukii* population control as it may target the few overwintering females exiting diapause. Evolutionary genomics further identifies two genes under

positive or abnormal evolution involved in insecticide resistance and immune response to parasitoids, warning us on the feasibility of some types of control strategies. Our results show that evolutionary genomics and comparative morphology are useful tools to guide application in the field of pest management.

## From evolutionary trees to behavioural ecology: exploiting SAM to visualize spatio-temporal movement patterns

**Johannes De Groeve<sup>1,2</sup>, Omar Rota-Stabelli<sup>2</sup>, Lino Ometto<sup>2</sup>, Nico Van de Weghe<sup>1</sup>, Tijs Neutens<sup>1</sup>, Francesca Cagnacci<sup>2</sup>**

<sup>1</sup>Ghent University, Krijgslaan 281, S8 9000 Ghent, Belgium

<sup>2</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: johannes.degroeve@ugent.be

Sequence alignment methods have a long history in similarity analysis of DNA and protein sequences. However, starting from the mid-1990's sequence alignment has also been applied to a variety of other disciplines, including behavioural patterns. Although digital tracking technologies have greatly increased the availability of regular, high frequency spatio-temporal sequences of animal behavior information, sequence alignment has never been used to analyse this type of sequential data. Nevertheless, this method holds promising potential for the study of habitat selection, since contrary to standard approaches, which only consider spatial patterns, it takes also into account the temporal correlated nature of data. In this paper the applicability of sequence alignment is assessed for spatio-temporal sequences of roe deer (*Capreolus capreolus*) GPS locations and their related habitat features collected in an alpine population from Trentino (Italy.). The results can be robustly interpreted in an ecological context and are validated by previously acquired biological information. In addition, we will present a framework of a codon-based sequence alignment model which may allow to import temporal autocorrelation directly into the sequences. Prospects and current limitations of this model will be shortly discussed.

## TBA The cost of domestication (if any): can we get a refund?

**Roberto Papa<sup>1</sup>**

<sup>1</sup>Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università Politecnica delle Marche, 60131 Ancona, Italy.

<sup>2</sup>Cereal Research Centre, Agricultural Research Council (CRA-CER), S.S. 16, Km 675, 71122 Foggia, Italy.

Contact: rpapa@univpm.it

## Adaptation of Mediterranean forest species to climatic gradients: a forest genetic perspective

**Giovanni G. Vendramin<sup>1</sup>, Delphine Grivet<sup>2</sup>, Santiago C. Gonzalez-Martinez<sup>2</sup>**

<sup>1</sup>Plant Genetics Institute, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy.

<sup>2</sup>Department of Forest Ecology & Genetics, Center of Forest Research, CIFOR-INIA, Carretera de la Coruña km 7.5, E28040 Madrid, Spain

Contact: giovanni.vendramin@igv.cnr.it

Combining coalescence modelling, neutrality tests and environmental correlations, demographic history and non-neutral patterns of evolution in candidate genes related to drought stress and secondary compounds were investigated in two closely-related Mediterranean conifers, *Pinus pinaster* Ait. and *Pinus halepensis* Mill. Amplicons covering candidate genes were sequenced in a sample from the full range of these two species. Higher levels of nucleotide diversity in candidate genes for drought response were present in *P. pinaster* than in *P. halepensis*, despite its narrower range in the Mediterranean. Differences across species were also reflected in the haplotype distribution for each tree species, with *P. pinaster* showing many different haplotypes at similar frequencies and *P. halepensis* showing fewer haplotypes with only one that is common or even fixed. The low levels of nucleotide diversity in Aleppo pine are more noticeable in its western distribution where most genes were fixed or almost fixed for particular haplotypes, a probable consequence of long-range colonization of the Western Mediterranean from ancient Aleppo pine populations in the easternmost edge of its current distribution and a more acute impact of the Ice Ages in this range of the species. Molecular analyses also revealed intense and relatively recent bottlenecks in Aleppo pine as well as a time of split between North-African and Iberian populations of the species well predating the Last Glacial Maximum albeit not as old as the one estimated for maritime pine. In contrast,

maritime pine seems to harbor large amounts of diversity for these genes due to a more stable demography; in addition, because of its more mesic distribution, higher environmental heterogeneity would have resulted in contrasted selective pressures that may have increased general levels of diversity at candidate genes. Using a wide range of neutrality tests, we found some of the candidate genes studied to evolve in non-neutral patterns. Interestingly, two of them showed statistical correlation with temperature variables, in particular with extremely high or low temperatures, and may constitute valuable tools for monitoring adaptive genetic diversity in these two Mediterranean pines. Our study shows that the use of complementary approaches can help capturing different aspects of the evolutionary processes that govern molecular variation at both intra- and inter-specific levels

## Evolutionary metagenomic analysis of the rumen microbial community of cattle

**Francesco Rubino<sup>1</sup>, Matthew McCabe<sup>1</sup>, Sinead Waters<sup>1</sup>, David Kenny<sup>1</sup>, Chris Creevey<sup>2</sup>**

<sup>1</sup>Teagasc, Animal & Bioscience Research Department, Grange, Dunsany, Co. Meath, Ireland.

<sup>2</sup>IBERS, Aberystwyth University, Aberystwyth, Ceredigion, SY23 3BF, United Kingdom.

Contact: chris.creevey@gmail.com

Ruminants have evolved a mode of herbivory which utilises a multi-chambered digestive system to house a diverse community of anaerobic microbes capable of breaking down indigestible lignocellulosic material to readily absorbed volatile fatty acids. The microbial community consists of core and transitory species which fluctuate with changes in diet and even over the course of a single day. Some such as the methanogens often form stable associations with other rumen microbes to degrade complex organic compounds and supply their substrates. Metagenomic analysis of these communities have revealed that the genes for many of the core fermentative functions have been widely shared among the microbes, however the extent of variation of these genes and how it relates to their function is largely unknown. We undertook to assess the functional variation in the rumen microbial community through the metagenomic analysis of the rumen contents of 14 tube-sampled Holstein-Friesian cattle. After assembly and functional annotation of the metagenome we identified and annotated the SNPs that occurred and calculated an evolutionary rate for each gene from each taxonomic group. We found that the evolutionary variation calculated for individual taxonomic groups was highly replicable across animals. We also found that there were large differences in evolutionary rates for different microbial groups, suggesting that they may be subject to differential selective pressures. Finally, we identified genes which had differential evolutionary rates in different organisms, suggesting a link to the importance of the gene to organisms inhabiting different evolutionary niches and a mechanism of using an evolutionary approach to understanding the microbial ecosystem as a whole.

## How grapevine got pimples. The interkingdom horizontal transfer of an unusual symbiont

**Andrea Campisano<sup>1</sup>, Omar Rota Stabelli<sup>1</sup>, Michael Pancher<sup>1</sup>, Stéphane Compant<sup>2</sup>, Livio Antonielli<sup>1</sup>, Lino Ometto<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>2</sup>Austrian Institute of Technology, Vienna, Austria

Contact: andrea.campisano@fmach.it

Humans have established a long-lasting co-habitation with a variety of animals, plants and microbes. The human-gut microbe interaction has been recently explored, and cases have been reported of microbial host transfer from domesticated animals to humans. Much less is known regarding human-plant microbial transfers. Here we report a recently established symbiosis between the human-associated opportunistic pathogen *Propionibacterium acnes* and the highly domesticated grape *Vitis vinifera*. We detected *P. acnes* in many grape plants using pyrosequencing of 16S rDNA and fluorescent in-situ hybridization (FISH) which localized *P. acnes* in specific endosphere habitats (pith and bark). We also show that *recA* genes in uncultivable endophytic *P. acnes* of grape are pseudogenes, suggesting a recent obligate symbiosis which we tentatively date as being not older than 7000 years ago, an age compatible with the domestication of grape by humans. Our results represent the first documented inter-kingdom horizontal host transfer of a human symbiont.

## Social insect intestines are mating nests for *Saccharomyces cerevisiae*

**Irene Stefanini<sup>1</sup>, Leonardo Dapporto<sup>2</sup>, Luisa Bernà<sup>3</sup>, Mario Polsinelli<sup>4</sup>, Stefano Turillazzi<sup>4</sup>, Duccio Cavalieri<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>2</sup>Department of Biological and Medical Sciences, Oxford Brookes University, Headington, Oxford, OX3 0BP, United Kingdom

<sup>3</sup>NEUROFARBA Department, University of Florence, Viale Pieraccini 6, 50139, Florence, Italy.

<sup>4</sup>Department of Biology, University of Florence, Via Madonna del Piano, 6, 50019 Sesto Fiorentino, Italy.

Contact: irene.stefanini@fmach.it

Sex can be a difficult task for motionless dispersed organisms such as the yeast *Saccharomyces cerevisiae*. Albeit yeasts are known to mainly asexually reproduce in nature, they should rely on sex to generate new genetic assemblies. Recent studies on the worldwide *S. cerevisiae* wild biodiversity significantly broadened the yeast population genetics horizons. Nevertheless, indication on where the yeast sexual reproduction can occur is still lacking. Social insects, by vectoring and maintaining yeast cells in the wild and among different environments, guarantee a possible site for yeasts meeting and mating. *S. cerevisiae* strains isolated from the intestines of social insects show higher heterozygosity than any other wild and human-related isolate, indicating an occurred genome mixing of different yeast lineages. We provide experimental evidence that *Saccharomyces sensu stricto* strains co-habiting the intestines of social insects can face sexual reproduction, generating inter- and intra-specific hybrids. This indication, rising from the identification and whole-genome sequencing of *S. cerevisiae* X *S. paradoxus* and *S. cerevisiae* X *S. bayanus* hybrids isolated from the wild *V. crabroguts*, is mirrored by results obtained in *in vitro* conditions (*Polistes* spp. and *Apis mellifera*). The intestine of social insects is identified as the niche where *Saccharomyces* spp. can meet and mate, finally acting as yeasts mating nest and contributing in shaping the fungal evolutionary ecology.

## Evolution of complex microbial communities for biotransformation of tar produced by biomass pyrolysis

**Sonia Ciccazzo<sup>1</sup>, Serena Manara<sup>2</sup>, Lorenzo Brusetti<sup>2</sup>**

<sup>1</sup>Faculty of Agriculture, University of Milan, Via Celoria, 2, 20133 Milan, Italy.

<sup>2</sup>Faculty of Science and Technology, Free University of Bolzano, piazza Università 5, 39100 Bolzano, Italy.

Contact: sonia.ciccazzo@unimi.it

Biomass is an important alternative to fossil fuels, for energy production through pyrolysis and gasification. However, this thermo-chemical process leads to the formation of toxic condensable compounds (tar) mainly composed by polycyclic aromatic hydrocarbons (PAH) and volatile monocyclic aromatic compounds (BTEX). Up to now the problem of the disposal of tar from biomass gasification is far to be solved. Bioremediation of tar with a mixed microbial culture despite its complexity seems a highly promising and innovative approach for the handling of these byproducts. Microbial resource management is a new concept dealing with the use of complex bacterial and fungal communities to achieve a particular objective, such as xenobiotic degradation or mineralization. The evolution concept is at the basis of this new approach, since microbiologists can benefit of the genetic and physiologic adaptation of the living bacteria and fungi when inoculated in adverse environments. The aim of this study is to assess the degradation dynamics of three different laboratory-scale microcosms enriched with complex tars, where natural microbial consortia are used for the treatment of tars. Tars were incubated with a complex microbial consortium taken from highly polluted soils. Triplicate microcosms were setup and incubated at room temperature for 30 days. Aliquots were taken at different time intervals to assess the structure and diversity of the biodegrading bacterial communities by Denaturing Gradient Gel Electrophoresis (DGGE), Automated Ribosomal Intergenic Spacer Analysis (ARISA) and 16S rRNA gene pyrotagging. Chemical analyses were carried out by GC/MS before and after microbial growth. For all the different tars, bacterial growth could be observed after two days. However, tar color changed visibly only after 6 days. Both the bacterial growth and the change in color were not detected in the negative controls. At day 30, all the inoculated microcosms were completely odorless, whereas the negative controls emitted the characteristic tar scent as intensively as at day 0. This sensory perception was confirmed by chemical analysis as a proof of the occurred degradation of the volatile fraction. ARISA reliability of replicated microcosms was very high (97%). ARISA electropherograms and 16S rRNA gene pyrotagging confirmed that a bacterial consortia was growing and evolving in the degrading microcosms, and it was for sure feeding on tar since tar was the only carbon source available. Bacterial species grew accordingly to a "cross-feeding" behavior, involving the use of metabolic outputs of one species as an energy source for another one. The evolution of the microbial consortia and of single microbial taxa during all the time points will be presented at the conference

## A hypothesis on the interactions between microbiomes in a parasitic relationship: The case of *Apis mellifera* and its parasite *Varroa destructor*

**Anna Sandionigi<sup>1</sup>, Erica Prosdocimi<sup>2</sup>, Maurizio Casiraghi<sup>1</sup>**

<sup>1</sup>University of Milano Bicocca, Piazza della Scienza 3 Milano, Italy.

<sup>2</sup>University of Milano, Via Celoria Milano, Italy.

Contact: a.sandionigi@campus.unimib.it

*Varroa destructor* is a parasite mite that accomplishes its reproductive cycle exclusively in honey bee colonies. It was originally a symbiont of the Asian bee *Apis cerana*. After the first contact with colonies of *Apis mellifera* at the beginning of the sixties, it spread all over the world except for Australia and Madagascar. *Varroa* fed on old bee larvae, pupae in sealed cells and adult bees. It is well known that this mite can act as a vector of several pathogenic agents, both bacterial and viral, which altogether cause a syndrome called varroosis. To date, the microbiome of adult *A. mellifera* is well-known, but that of larval stages is far to be characterized, as like as the microbiome of the parasitic mite. Consequently, it is still unclear how bacterial communities characterizing the two organisms affect each other, if we exclude some works on the transmission of a specific pathogen. Our hypothesis is that varroa mites play a fundamental role in the alteration of bacterial community composition of honey bee larvae, not only being a vector, but also acting as a "stargate", an open "door" through which exogenous bacteria alter the mechanisms of primary succession in honey bee microbiome. We studied varroa and honeybee bacterial communities through barcoded amplicon pyrosequencing methods, taking advantage of the of high-throughput sequencing technologies and the opportunity to detect uncultured and uncultivable bacteria allowed by such techniques. In summary, we characterized the honey bee-associated microbiota in the larval stage and the parasite-associated microbiota, and explored the possible patterns of interaction between the two microbiomes.

## Host-adaptation of parasitoids to the newly introduced invasive species, *Drosophila suzukii*

**Marco Valerio Rossi Stacconi<sup>1</sup>, Alberto Grassi<sup>1</sup>, Daniel Dalton<sup>2</sup>, Betsy Miller<sup>2</sup>, Augusto Loni<sup>3</sup>, M'bark Ouantar<sup>4</sup>, Claudio Ioriatti<sup>1</sup>, Vaughn Walton<sup>2</sup>, Gianfranco Anfora<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>2</sup>Oregon State University, Corvallis, OR 97331-7304, United States of America.

<sup>3</sup>University of Pisa, Via S. Michele 2, 56124 Pisa, Italy.

<sup>4</sup>Mediterranean Agronomic Institute of Bari, Via Ceglie 9, 70010 Valenzano (Ba), Italy.

Contact: mvrs82@gmail.com

The reciprocal evolutionary interaction between a pest and its natural enemies leads to a dynamic runaway escalation or 'arm-race'. The final result of this co-evolutionary process between species guarantees the population control, leading to an ecological balance. This balance is no longer maintained when a pest colonizes a new environment, in which specific biological control agents are lacking. The present study focused to determine the field occurrence of indigenous parasitoids of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). Since its introduction in USA and Europe in 2008, this invasive pest caused hundred thousand dollars worth of damage to small and stone fruits business. The uncontrolled outbreaks of *D. suzukii* in the invaded regions were mainly due to the absence of specialized natural enemies. On the contrary, in the areas of *D. suzukii* origin, a number of hymenopteran parasitoids, having a co-evolutionary history with this pest, have been reported. Here we state the results of a survey aimed at determining the presence of indigenous *D. suzukii* parasitoid populations carried out from May to October 2012 in two areas negatively affected by this fruit pest: Trento Province, Northern Italy., and Oregon in the Pacific Northwest of the USA. We conducted field and laboratory studies in order to determine the status of biological control agents utilizing *D. suzukii* as a host. Our study sites included a range of commercial soft fruit and natural non-commercial habitats. In each site, sentinel traps were baited with either *D. suzukii* or *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) larvae in different food substrates. The generalist parasitoid, *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae), was collected from both *D. suzukii* and *D. melanogaster* pupae in traps deployed in a selection of these sites. This report of *P. vindemiae* in 2012 represents the first identification of *D. suzukii* parasitoids in Europe. A successive parasitism efficacy test was set up under controlled conditions confirming the ability of *P. vindemiae* to attack *D. suzukii* pupae. We discuss the possible practical implications of this finding for the biological control of *D. suzukii*.



## The contribution of network and spatial analysis in understanding how diseases pressure and ergonomic requirements moulded evolution of insect societies

**David Baracchi<sup>1</sup>, Alessandro Cini<sup>2</sup>**

<sup>1</sup>Queen Mary University of London, School of Biological and Chemical Sciences, Mile End Road, London E1 4NS, United Kingdom.

<sup>2</sup>Laboratoire Écologie & Évolution UMR 7625, Université Pierre et Marie Curie, 7 quai St Bernard, Bâtiment A 7ème étage, Paris Cedex 05, France.

Contact: david.baracchi@gmail.com

Complex societies are formed by thousands to millions of individuals which incessantly interact in the sheltered shared nest space. Insect societies are thus complex systems which face every day with different evolutionary pressures, such as infectious disease and group coordination, working at the same time. Understanding how colony members interacts in space and time is thus of overwhelming interests to unravel how natural selection mould evolution of insect societies. Indeed, the last years saw an increasing attention towards the characterization of the networks of social connections and the use of space in social insects. In the present work we investigated the spatial positions and the contacts network of workers and queen in a honeybee society thanks to high resolution recordings of individual spatial behaviour and “network connections”. We quantified the exact spatial organization (the real space) and we delineated the structure of the interaction network (the abstract contact space) of each single worker of different age and the queen. Our results clearly show that the honeybee colonial network is highly compartmentalized both at the social (association network) and at the spatial (use of space on the comb) scale. Groups of different age workers, corresponding to different task-related groups, occupy different comb areas and show limited contact among them. Foragers, who usually may introduce the contagion to the colony are also the least connected individuals in the network and those that occupy the periphery of the social network. Young workers are the individuals more connected, and those that form the network core. The queen is completely embedded within young bees and sheltered by pathogens. Our data show that segregation in the interaction network corresponds also well with a segregation of honeybees in the real space on the comb. In addition, each category insists on a different part of the comb, clearly dependent on nest content and individual specific task. Foragers are clumped together on a small area near to the entrance of the hive, while young bees and queen avoid completely this region of the comb and alighted mainly on brood cells. The comb shows a characteristic well organized pattern of brood, pollen and honey and this configuration leads to a strong spatial division among older bees and both queen and younger bees influencing the dynamics between pathogens and their hosts. Opposite pressures exert by both pathogens and ergonomics requirements could have been contemporaneously at work in shaping the network design and spatial organization into the nest and the present study furnished empirical data that social organization and the pathogens could have been resolved in an evolutionary trade-off much more than previously thought.

## Characterization and phylogenetic analysis of the *Vitis vinifera* gibberellin oxidases

**Lisa Giacomelli<sup>1</sup>, Omar Rota-Stabelli<sup>1</sup>, Claudio Moser<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: lisa.giacomelli@fmach.it

Gibberellins (GAs) are phytohormones important to regulate several aspects of plant development, such as growth, flowering and fruit-set. GAs are used in viticulture to control berry size and bunch architecture, which are important traits that often determine the economic success of a grape variety. Despite the identification of 136 GA structures in nature, only a handful are biologically active. GA<sub>1</sub> and GA<sub>4</sub> are the most common bioactive GAs in grapevine and their pool is maintained through a combination of biosynthesis and deactivation activities, which involve a family of GA oxidases belonging to three distinct groups: GA 20-oxidase (GA20ox), GA 3-oxidase (GA3ox), and GA 2-oxidase (GA2ox). In this work we identified and characterized the family of grapevine GA oxidases, and identified six GA20ox, three GA3ox and eight GA2ox proteins, the latter further distinct into five C<sub>19</sub>-GA2ox and three C<sub>20</sub>-GA2ox proteins. Our phylogenetic analyses suggest a common origin of GA3ox and C<sub>19</sub>-GA2ox and challenge previous evolutionary models. The specific activity of the identified GA oxidases was confirmed in vitro by expression of the recombinant proteins, and in vivo by ectopic expression in the model species *Arabidopsis thaliana*. This work was supported by the Autonomous Province of Trento (Call 1 - Post doc 2009 - Incoming) awarded to Lisa Giacomelli.

## Molecular evolution of flowering genes in grapevine

**Lukasz Grzeskowiak<sup>1</sup>, Silvia Lorenzi<sup>1</sup>, M. Stella Grando<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: lukasz.grzeskowiak@iasma.it

Flowering is a key adaptive trait important for plant fitness and crop production. Recently, using progenies derived from crosses of different varieties, a new QTL for flowering time was mapped in the grapevine genome. We resequenced candidate floral pathway genes located in this QTL from a collection of multiple grape accessions. These genes cooperate to regulate meristem formation and flowering transition. Two of them, FT and SVP, were suggested as major players in plant thermosensory perception. FT is a floral activator that integrates signal inputs from various pathways and SVP controls flowering time by negatively regulating the expression of FT via direct binding. This interaction is proposed as one of the molecular mechanisms evolved by plants to modulate the timing of the developmental transition to flowering under fluctuating temperature conditions. The pattern of linkage disequilibrium and population differentiation at this locus in grapevine may indicate that these flowering genes are targets of selection. We investigate possible genotype-phenotype relationships for these candidate genes by applying field observations of flowering time in the core collection recorded during several growing seasons. Our long-term objective is to provide information on the genetic variation of phenological traits and to facilitate choice of grapevine varieties adapted to atmospheric conditions of a specific geographic location

## Bacterial community structure changes during summer in high alpine fresh water springs

**Alfonso Esposito<sup>1</sup>, Michael Engel<sup>1,2</sup>, Luca Daprà<sup>1</sup>, Lorenzo Brusetti<sup>1</sup>**

<sup>1</sup>Free University of Bolzano, Piazza Università 1, Bolzano, Italy.

<sup>2</sup>European Academy (EURAC), Piazza Università 1, Bolzano, Italy.

Contact: alfonso.esposito@natec.unibz.it

Water springs in high elevation environments are one of the most endangered habitats because of their vulnerability to environmental changes. The rapid change of genetic structure of bacterial communities due to little variations of environmental parameters in water systems may reflect evolutionary processes going on throughout the season. Quantitative Amplified Ribosomal Intergenic Sequence Analysis (qARISA) was carried out on environmental DNA extracted from sediments collected in three water spring in the upper Matsch Valley (South Tyrol, Italy.; 2,300 m.a.s.l.) four times during the summer. The fingerprinting matrix was analyzed through canonical correspondence analysis (CCA), and it showed a clustering pattern that reflected a variation of community structure closely linked to the environmental parameters. Shannon ( $H'$ ) diversity index obtained from the fingerprinting matrix was analyzed statistically against the water  $^2\text{H}$  and  $^{18}\text{O}$  isotopic ratios, electric conductivity (EC) and water temperature. The latter features the highest correlation with bacterial diversity ( $r=0.75$ ), followed by EC ( $r=0.58$ ). No significant correlation was detected between diversity and isotopic ratios. Through a multiple regression analysis, bacterial diversity appears to be indeed causally related to EC and water temperature ( $\text{adj-R}^2=0.5$ ,  $p<0.05$ ). Our results suggest that in oligotrophic freshwater environments evolutionary processes may be driven by selective pressures due to water parameter variations.

## Sustainable management for increasing soil microbial diversity in a Mediterranean agro-ecosystem

**Adriano Sofo<sup>1</sup>, Cristos Xiloyannis<sup>2</sup>, Assunta Maria Palese<sup>2</sup>, Antonio Scopa<sup>1</sup>**

<sup>1</sup>SAFE - University of Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy.

<sup>2</sup>DICEM - University of Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy.

contact: adriano.sofo@unibas.it

Olive can be considered a paradigm species for Mediterranean agro-ecosystems. In conventional olive growing, adopted by the majority of the farmers, frequent soil tillage has reduced soil microbial diversity and microbiota complexity that strongly contribute to the overall soil fertility. Therefore, the conventional agronomic practices should evolve in a more sustainable olive management addressed to improve soil microbial diversity. A better understanding of the ecology of soil microorganisms could lead to identify agricultural management practices that stimulate and select the soil microorganisms having beneficial purposes in agriculture, such those interested in nitrogen cycle. The aim of this study was to evaluate the effects of sustainable practices (grass cover and pruning residues recycling) on soil microbiological quality in a Mediterranean olive orchard. The trials were carried out in a mature olive grove (*Olea europaea* L. – cv



Maiatica) located in Basilicata Region (Southern Italy.) and managed for 13 years (medium-term) according to two different soil management systems: the sustainable treatment (ST) and the conventional treatment (CT). Soil microorganisms in the two systems were monitored by both microbiological cultural-dependent and molecular methods, and by microscopy. In the ST olive orchard, soil microbiota showed a higher complexity and metabolic diversity. The adoption of 'innovative', sustainable, agricultural practices had positive effects on soil microbiota and its biodiversity, which in turn can influence soil fertility and plant growth by increasing nutrients availability and turnover. The results of this study encourage the use of sustainable agricultural practices able to enhance physico-chemical and microbial soil fertility and promote good-quality fruit production without detrimental effects on water and soil resources. The role of the identified microorganisms in the soil microbial network is discussed, in order to provide a detailed view of the impact that man-made selection had on soil microorganisms of agricultural relevance in a typical Mediterranean agronomic biosystem.

### Insights into *Drosophila*-*Wolbachia* Interactions: Cryptic *Wolbachia* strains in closely related species

**Rupinder Kaur<sup>1</sup>, Stefanos Siozos<sup>1</sup>, Gianfranco Anfora<sup>1</sup>, Iliaria Pertot<sup>1</sup>, Omar Rota-Stabelli<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: rupinderkaur@fmach.it

*Wolbachia* is a widespread group of endosymbiotic bacteria of arthropods that have diverse and complex effects on their host biology. Among them, are the protection against viruses and the mostly known reproductive effects including Cytoplasmic Incompatibility, male-killing, induction of parthenogenesis and feminization. Owing to its effects, *Wolbachia* are considered to be great candidates for their biocontrol potential. Between *Drosophila* species, *Wolbachia* are extremely common and include strains with variable phenotypic effects. Here we report on the genome sequencing of the *Wolbachia* endosymbiont "wSuzi" from the invasive pest *Drosophila suzukii*. The final assembly yielded approximately 1.35 Mbp in 110 contigs, with an average depth of coverage 60X. We found wSuzi to be closely related to the widespread *D. simulans*' wRi strain. The two strains proved to be identical on the basis of the existing typing systems, including Multiple Locus Sequence Typing (MLST) and the most recently Multiple Locus VNTR Analysis (MLVA). However, several structural variations such as InDels and genomic rearrangements were able to discriminate the two strains. Based on the differences between wRi and wSuzi, we identified variable regions that were able to recognize different strains in different populations of *D. suzukii* and in the sister species of *D. suzukii*. Our results show that the currently used marker systems for *Wolbachia* lack the resolution to discriminate and identify potential cryptic strains. This could lead to an underestimation of the true biodiversity of *Wolbachia* bacteria but could also affect the design of applied control strategies and subsequent monitoring procedures.

### The influence of fertilization in chlorophyll content of dominant legumes and grasses in a permanent pasture of low hills in Banat (Timis county)

**Marius Coman<sup>1</sup>**

<sup>1</sup>USAMVBT, Calea Aradului nr 119, Romania

Contact: comanmario81@yahoo.com

In this study, the objective was to determine the effects of mineral and organic fertilizers on chlorophyll content of dominant legumes and grasses in a permanent pasture of low hills in Banat (Timis county). The average of the annual air temperature in this region is 10.7°C and the average of the annual rainfall amount is 608 mm. The climate in the studied area is temperate continental with Mediterranean influences. The experimental field was organized by setting 9 fertilisation variants (3 organic, 3 mineral and 3 organic - mineral fertilisation variants) and a control non-fertilised variant. Every studied variant has three replicates. The importance and relevance of fertilizing the grasslands lie in the principle of restoring nutrients exported through the harvested crop, a concept developed by Bossingaul and founded by Justus von Liebig in the 19th century; it refers to the fact that crops consume nutrients from the soil to develop the crop thus depleting the soil from nutrients that are removed with the crop which results in a decrease of soil fertility. Hence the idea of recovering soil nutrients (Sala F, 2008), Chlorophyll content was determined using a portable SPAD. Legumes have a higher content of chlorophyll in all variants compared with grasses. The highest chlorophyll content of legumes case was registered at the organo-mineral variant fertilized with sheep manure 20t/ha + 50kg/ha P<sub>2</sub>O<sub>5</sub> + 50kg/ha K<sub>2</sub>O (V6). In the case of grasses, the highest chlorophyll content was recorded in chemically variant fertilized with 150 kg/ha N50 kg/ha

P<sub>2</sub>O<sub>5</sub> 50kg/ha K<sub>2</sub>O (V9). The results confirm a positive correlation between chlorophyll content, dry matter content and crude protein in under the influence of fertilization.

## **Asymmetric social interactions through left and right antenna in honey bees**

**Elisa Rigosi<sup>1,2,3</sup>, Lesley J. Rogers<sup>4</sup>, Elisa Frasnelli<sup>1</sup>, Giorgio Vallortigara<sup>1</sup>**

<sup>1</sup>*CIMEC Center for Mind/Brain Sciences, University of Trento, CorsoBettini 31, I-38068 Rovereto (TN), Italy.*

<sup>2</sup>*Research and Innovation Center, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.*

<sup>3</sup>*BIOtech research center, Dept. of Industrial Engineering, via Mesiano 77, 38123 Trento, Italy.*

<sup>4</sup>*Centre for Neuroscience and Animal Behaviour, University of New England, Armidale, NSW 2450, Australia.*

Contact: elisa.rigosi@gmail.com

Left-right specialization of brain and behaviour is not unique property of vertebrates but has been recently observed in invertebrate species as well. Interestingly, a link between lateralization and sociality has been suggested, together with capabilities of mastering high cognitive tasks, at least among vertebrates. The study of invertebrates is important from an evolutionary and comparative perspective. In particular, honey bees are useful models to investigate the evolution of brain asymmetries and social traits. To date, though a lateralization has been revealed at the electrophysiological level in single individuals during olfactory processing, nothing is known about asymmetric bias and social behaviours. Here we report for the first time a strong lateral preference in honey bees to use their right antenna in social interactions with conspecifics. We analyzed different social behaviours (latency to contact, numbers of proboscis extension (PER), number of C-responses, number of mandibulations) in pairs of bees coming either from the same colony or from different colonies. We found a directional bias in the use of antennae for three measures of social interactions such as latency, PER, and C-responses. In particular in bees with only right antennae social behaviour is more context-appropriate compared to left antennae bees, the later possibly due to an impairment in distinguishing between hive mates and bees from another hive. This highlights a direct association of lateral biases during social interactions in honey bees and raises the question whether it might have evolved as a putative adaptation to eusociality.

# EVOLUTIONARY GENOMICS AND BIOINFORMATICS

The symposium will feature contributions dealing with any aspect evolutionary genetics and genomics in any living organism, including viruses. Descriptions of adaptive events at the genetic level, new methodological advances in the field, analysis of complete genomes or gene sets from an evolutionary perspective are invited. Bioinformatics approaches to analyze large-scale genetic data for selection signature inference will also be welcome. The aim of the symposium is providing insight into the manifold pathways to genetic adaptation and into the methodological advances to study the evolutionary history of genes and genomes.

**Chairs: Manuela Sironi (IRCCS E. MEDEA) and Dan Sargent (Fondazione Edmund Mach)**

## Reconstructing human genetic diversity in space and time

**Francois Balloux<sup>1</sup>**

<sup>1</sup>*UCL Genetics Institute, Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK*

Contact: f.balloux@ucl.ac.uk

The worldwide distribution of human genetic variation is characterized by smooth clines of isolation by distance and a decrease of genetic diversity with increasing geographic distance from Sub-Saharan Africa along likely colonization routes. While these striking patterns strongly suggest a recent out-of-Africa expansion for Anatomically Modern Humans (AMHs), they shed little light on the more subtle aspects of the past demography of our species. Thus, we developed a spatially explicit model of the expansion of AMHs and using climate reconstructions over the past 120k years to quantify the possible effects of climate on human demography. The combinations of demographic parameters compatible with the current genetic makeup of worldwide populations indicate a clear effect of climate on past population densities. Our estimates of this effect, based on population genetics, capture well the observed relationship between current climate and population density in modern hunter-gatherers worldwide, providing supporting evidence for the realism of our approach. Furthermore, while we did not use any archaeological and anthropological data to inform the model, the arrival times in different continents predicted by our model are also broadly consistent with the fossil and archaeological record. Our framework arguably provides the most accurate spatiotemporal reconstruction of human demographic history but still offers considerable scope for improvement. In the last part of the presentation, I will present unpublished results including a reconstruction of the colonisation of the Americas based on whole modern and ancient genomes. Finally I will discuss the potential and limitations of explicit spatiotemporal models for inferring past demography.

## Evolution and genome divergence determine virulence traits in *Candida* spp.

**Luisa Bernà<sup>1</sup>, Monica Di Paola<sup>1</sup>, Lisa Rizzetto<sup>2</sup>, Manuel Santos<sup>3</sup>, Duccio Cavalieri<sup>2</sup>**

<sup>1</sup>*Department of NEUROFARBA "Neuroscience, Psychology, Drug Research and Child's health", Viale G. Pieraccini, 6 50139 Florence, Italy.*

<sup>2</sup>*Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.*

<sup>3</sup>*RNA Biology Laboratory, Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal.*

Contact: monica.dipaola@unifi.it

*Candida albicans* and *Candida parapsilosis* are known as the most causative agents of human fungal infections, but in healthy conditions they colonize the human gastrointestinal tract as commensals. While the pathogenicity of *C. albicans* is associated to the phenotype switch -with conversion from white to opaque colony and from cell to hyphae- affecting a variety of virulence-related genetic factors, for *C. parapsilosis* the genetic makeup associated to the virulence is still unknown. In order to investigate the intra and inter-species variability involved in the phenotypical changes we analyze the whole genome sequences of 7 candida strains (3 of *C. albicans* and 4 of *C. parapsilosis*) isolated from fecal samples of healthy and Crohn's disease patients, a severe inflammatory bowel disease. The results show two different population

structures: while *C. albicans* present an extreme variability and divergence between strains, *C. parapsilosi* seems to be more clonal, with very low number of polymorphisms. Notably, in *C. albicans* intriguing genomic plasticity have been observed. Indeed, over the high polymorphism, strain-specific gene losses, acquisition, and several miss-sense genes were found. Moreover, in *C. albicans* isolates, the most polymorphic genes codify proteins related to the cell wall and hyphal formation, external encapsulation structure and cell periphery, suggesting a continuous adaptation to adverse environments or stress conditions. Our data are confirmed by phenotypical characterization that show changes in virulence related traits (invasive growth, hyphal formation and switch from smooth to myceliated colonies) for the majority of *C. albicans* strains. Also in two *C. parapsilosis* strains similar pathogenic traits were found. Interestingly, the cytokine milieu released by human PBMCs and DCs showed that specific inflammatory responses are produced by strains with the most virulent phenotype. Overall these results provide significant insights regarding the link between host adaptation, pathogenesis and evolution.

## “Bad” species? Restriction sites associated DNA sheds light on the evolution of the *Erebia tyndarus* species complex

**Paolo Gratton<sup>1</sup>, Alessandra Trasatti<sup>1</sup>, Giorgio Riccarducci<sup>1</sup>, Emiliano Trucchi<sup>2</sup>, Silvio Marta<sup>1</sup>, Giuliana Allegrucci<sup>1</sup>, Donatella Cesaroni<sup>1</sup>, Valerio Sbordoni<sup>1</sup>**

<sup>1</sup>Dipartimento di Biologia, Università di Roma "Tor Vergata", Via Cracovia, 1 Roma, Italy.

<sup>2</sup>Centre for Ecological and Evolutionary Synthesis (CEES), University of Oslo, Blindernveien 31, Oslo, Norway.

Contact: paolo.gratton@gmail.com

The *Erebia tyndarus* species complex is a cluster of closely related alpine butterflies, representing an intriguing riddle for taxonomists, evolutionary biologists and biogeographers and a still underexploited model for understanding the role of neutral and adaptive processes in speciation. Members of the ‘tyndarus’ group have been characterized so far by morphological and ecological characters, cross-breeding experiments, karyology, and molecular data from allozymes and mitochondrial DNA (mtDNA). However, the delimitation of species, their relationships, as well as the origin of their peculiar geographic distribution, are still unclear. So much so that these taxa were cited as an example of ‘bad species’, for which the establishment of a phylogenetically ‘correct’ taxonomy could represent a desperate and almost meaningless endeavour (Descimon & Mallet, 2009). Up to five different species belonging to the ‘tyndarus’ group have been described in the Alps: *E. tyndarus*, *E. calcaria*, *E. nivalis*, *E. cassioides* and *E. carmenta* (with the latter often considered a subspecies of *E. cassioides*) all of them belonging to a unique “Alpine” clade (Albre et al., 2008). Figure 1 illustrates the distribution range of these taxa. Three species (*E. tyndarus*, *E. nivalis*, *E. calcaria*) are endemic to the Alps, while *E. cassioides* stretches its patchy distribution from Eastern Alps into the Balkans and *E. carmenta* from Western Alps into Apennines and Pyrenees. This distribution pattern includes instances of allopatry, parapatry and quasi-sympatry; the latter case represented by *E. nivalis* narrowly overlapping along an altitudinal gradient with *E. cassioides* or (in a single site) *E. tyndarus*. In this ongoing study, we are employing restriction sites associated DNA (RAD) along with more traditional mtDNA sequencing in order to clarify the systematic and genetic relationships among taxa of the *E. tyndarus* “Alpine” clade, as a basis for further evolutionary studies. Our preliminary analyses show that our RAD data are able to provide, for the first time, a clear molecular support for the recognition of four well-defined genetic units, corresponding to the species *E. tyndarus*, *E. nivalis*, *E. calcaria* and *E. cassioides+carmenta* (Figure 1c). Interestingly, these lineages do not show any synapomorphies at 1200 bp of mtDNA (Figure 1b). In striking contrast, the distribution of mtDNA haplotypes highlights a strong geographical structure within *E. cassioides+carmenta*, where RAD shows a rather continuous genetic gradient from Pyrenees to Alps and Apennines, highlighting the historical role of nuclear gene-flow and recombination in maintaining the genetic cohesion of a single evolutionary unit. Finally, RAD data are providing provisional evidence for gene flow between *E. tyndarus* and *E. nivalis* (whose range overlap is very limited), while hybridization between *E. cassioides* and *E. nivalis* (occurring in quasi-syntopy at several sites) seems to lack altogether, which suggests an intriguing correlation between reproductive isolation and ability to coexist by niche-splitting.

## A framework for the evolutionary study of uncharacterised microbial communities

**Francesco Rubino<sup>1</sup>, Christopher Creevey<sup>1</sup>**

<sup>1</sup>IBERS, Aberystwyth University, Aberystwyth, Ceredigion, SY23 3BF, United Kingdom.

Contact: fr11@aber.ac.uk

Complex microbial environments such as those analysed in metagenomic studies present a challenge in how these communities change and adapt to external stimuli. One such environment is present in the rumen of herbivores, which use

a multi-chambered digestive system to host a complex microbial community that can break down the lignocellulosic material they feed on. There are both core and transitory species that can change with diet and animal condition over the course of a single day. Approaches based on identification of the OTU have several limitations, such as the number of the unknown species that colonise the environment, the difficulty to characterise their metabolic pathways and their mutual interactions. We took a different approach that aims to be used in studying the relationship in the community and to understand the patterns of the interactions between the organisms. We developed a framework that can be used in a number of different studies, without knowing a prior knowledge of the organisms that compose the community, taking a functional approach at the analysis, using evolutionary rates as the guide to understand the microbial ecosystem analysed. The framework has been used to study the microbial community found in the rumen of 14 tube sampled Holstein-Friesian cattles as a test for the framework and work is underway to study the differences in the microbial communities that colonise the solid and liquid phases that are present in the rumen.

## Systems biology approach for the identification of genetic determinants of colony morphology switch in natural *S. cerevisiae* strain

**Valentina Cappelletti<sup>1</sup>, Irene Stefanini<sup>1</sup>, Luisa Bernà<sup>2</sup>, Matteo Ramazzotti<sup>2</sup>, Alessandro Cestaro<sup>1</sup>, Ivo Gut<sup>3</sup>, Misha Kapushesky<sup>4</sup>, Attila Csikász-Nagy<sup>1</sup>, Duccio Cavalieri<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>2</sup>Università degli Studi di Firenze, Viale Pieraccini 6, Italy.

<sup>3</sup>Centre Nacional d'Anàlisi Genòmica (CNAG), Baldiri Reixac 4, Spain.

<sup>4</sup>European Bioinformatics Institute (EBI), Cambridge, CB10 1SD, United Kingdom.

Contact: valentina.cappelletti@fmach.it

Colony morphology is a fascinating phenotype described in unicellular organisms as a possible step towards multicellularity. The spreading of filamentous structures is used by some pathogenic fungi, as *Candida albicans*, to invade human tissues thus causing infection. This phenotype, rarely observed in *Saccharomyces cerevisiae* colonies, is present in heterozygosis in the M28 *S. cerevisiae* strain, isolated from damaged grapes of the Montalcino area (Italy). Meiotic segregants of M28 show mendelian inheritance of colony morphology, thus making this strain the best suitable model to study the genetic determinants of structured colonies (filigreed). We analyzed cellular and colony morphology of M28 meiotic segregants in several different carbon sources. The addition of ethanol as the only carbon source lead to an increase in filamentation: in this perspective the stable and uniform morphotype, induced by ethanol, could reflect an adaptation to stress. In order to investigate the correlation between invasiveness, filamentous morphotype and pseudohyphal growth in *S. Cerevisiae*, we assessed the ability of this natural strain to invade solid media. Transcriptional analysis by means of microarrays on cells grown in fermentable and not-fermentable carbon sources and Functional Enrichment Analysis identified the genes involved in the regulation of colony morphogenesis. Our results support the hypothesis of an ecological function of filamentous phenotype in creating a community adaptable to the shifts of the environmental conditions. Whole genome comparative analysis on 12 M28 sporal derivatives of three different tetrads, with Next-Generation Sequencing (NGS) approach, allowed to discover mutations in genes candidate to be the genetic determinants of the colony morphology phenotype. We demonstrate that a number of three tetrads is sufficient to map a genetic trait with mendelian inheritance. RNA-seq based transcriptomics on the all M28 sequenced genome allowed to identify a gene expression profile associated to the filamentous morphotype and to confirm the candidate morphogenesis regulatory genes.

## *Drosophila* population genomics

**Christian Schlötterer<sup>1</sup>**

<sup>1</sup>Institut für Populationsgenetik, Vetmeduni Vienna, Veterinärplatz 1, 1210 Wien, Austria

Contact: christian.schloetterer@vetmeduni.ac.at

The analysis of next generation sequencing data from pooled individuals (Pool-Seq) is a cost effective approach to study allele frequency changes in natural and experimental populations. I will provide some case studies in *Drosophila* demonstrating the power and limitations of Pool-Seq.



## Bioinformatic and evolutionary analysis of chemoreceptors and related proteins in a grapevine pest

**Sukanya Ramasamy<sup>1</sup>, Gianfranco Anfora<sup>1</sup>, Omar Rota Stabelli<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: sukanya.ramasamy@fmach.it

*Drosophila suzukii* is an invasive pest of fruits such as grapevine, berries and cherries. *D. suzukii* lays eggs on ripening, unwounded fruits, while most other *Drosophila* oviposit on rotting fruits. This switch in ecological behaviour is reflected by morphological adaptations such as a serrated ovipositor, but should also include adaptations for tracking the odor of fresh fruits. In *Drosophila*, like in all other insects, odor(chemo) reception is mediated by multi-gene families of olfactory and gustatory receptors (Ors, IRs and GRs) sometimes assisted by two diverse protein families, odorant binding and chemosensory proteins (OBPs and CSPs). Knowledge of these genes may not only help in developing agricultural controlling strategies, but also offer an interesting model for the study of evolution of behavioral innovation. We extensively blasted the newly sequenced genome of *D. suzukii*, identified its likely full set of chemosensory receptors, and conducted a comparative genomic analyses of these genes in various sister species. Results show that repertoire of chemosensory genes in *D. suzukii* is different to that of sister species, but that this variation follows a likely birth death process as in other *Drosophila*s. Exception is the OBP family where the gene loss is significantly higher in *D. suzukii* than in sister species. Our results reveal some specific gene gains and losses in *D. suzukii* that may be responsible for its innovative behavior.

## Evidence of disruptive selection in the adaptation of two closely related species of tropical trees

**Caroline Scotti-Saintagne<sup>1</sup>, Maxime Casalis<sup>2</sup>, Henri Caron<sup>1</sup>, Luise Brousseau<sup>3</sup>, Valerie Troispoux<sup>1</sup>, Bruno Ferry<sup>4</sup>, Astrid Tempestini<sup>5</sup>, Ivan Scotti<sup>1</sup>**

<sup>1</sup>INRA - UMR ECOFOG campus agronomique BP 709, 97387 Kourou cedex, France.

<sup>2</sup>Université Antilles Guyane (UAG) - UMR ECOFOG campus agronomique BP 709, 97387 Kourou cedex, France.

<sup>3</sup>Université de Lorraine - UMR 1137 «Ecologie et Ecophysiologie Forestières», 54280 Champenoux, France.

<sup>4</sup>UMR INRA-AgroParisTech 1092, Centre INRA de Nancy, Champenoux, France.

<sup>5</sup>Université Pierre et Marie Curie - UMR ECOFOG campus agronomique BP 709, 97387 Kourou cedex, France.

Contact: caroline.scotti@ecofog.gf

Amazonian forest is highly diverse and the presence of closely related species still exchanging genes is not rare involving that gene flow may play a significant role in the speciation processes. Here, we present a population-genomic approach, using both AFLP and SNP genome scans, to analyze the genetic differentiation between the tree species *Symphonia globulifera* and its closely related species *S. sp1*. We first demonstrated the significant role of disruptive selection at a small proportion of AFLP loci at both the population and the regional level. In addition, we showed that at least one AFLP locus was linked to environmental features related to the water soil content. Secondly, we present the first results of the high-throughput AFLP sequencing which permitted to obtain more than 15000 contigs for 40 individuals (20 per morphotypes). We will see how the inter-species genetic differentiation is distributed in the genome and we will discuss the possible role of key genes in adaptation to the environment.

## Structure, transcription and variability of metazoan mitochondrial genome: perspectives from an unusual mitochondrial inheritance system

**Fabrizio Ghiselli<sup>1</sup>, Liliana Milani<sup>1</sup>, Davide Guerra<sup>1</sup>, Peter L. Chang<sup>2</sup>, Sophie Breton<sup>3</sup>, Sergey V. Nuzhdin<sup>4</sup>, Marco Passamonti<sup>1</sup>**

<sup>1</sup>Dipartimento di Scienze Biologiche, Geologiche ed Ambientali (BiGeA), Università di Bologna, Via Selmi 3, Bologna, Italy.

<sup>2</sup>Program in Molecular and Computational Biology, Department of Biological Sciences, University of Southern California, Los Angeles, USA.

<sup>3</sup>Département de Sciences Biologiques, Université de Montréal, Montréal, Québec, Canada, Canada.

<sup>4</sup>Program in Molecular and Computational Biology, Department of Biological Sciences, University of Southern California, Los Angeles, USA.

Contact: marco.passamonti@unibo.it

Despite its functional conservation, mtDNA presents strikingly different features among eukaryotes, such as size, rearrangement frequency, and amount of intergenic regions. The reasons beyond this diversity have been object of extensive studies that have investigated the correlation between different mtDNA evolutionary patterns and body mass, metabolic rate, reactive oxygen species production, lifespan, etc. The fundamental role

of non-adaptive processes such as random genetic drift and mutation rate in genome evolution has been recently highlighted by the work of several Authors. Under this light, mitochondrial bottlenecks and number of germ line replications are critical factors for mtDNA evolution, and different patterns of germ line differentiation could be responsible for the mtDNA diversity observed across eukaryotic lineages. Among metazoans, bivalve mollusc mtDNAs show unusual features, like hypervariable gene arrangement, high mutation rate, large amount of intergenic regions and, in some species, an unique inheritance system, the Doubly Uniparental Inheritance (DUI). In DUI species, two mitochondrial lineages are present: one is transmitted maternally (F-type), the other paternally (M-type). We exploited the DUI species *Ruditapes philippinarum* to study intergenic mtDNA functions, and to assess mitochondrial transcription and polymorphism in gonads. The coupling of high mutation rate with quite large genomes (i.e., large proportion of intergenic DNA) would be in contrast with the Mutation Pressure theory, but this is not true if most of that intergenic DNA has a function. We observed the presence of conserved functional elements and novel ORFs that could explain the evolutionary persistence of such regions and possibly be involved in DUI-specific aspects. Accordingly, the RNA-Seq analysis showed that mtDNA transcription is lineage-specific and it is independent from the nuclear background. This is consistent with an involvement in transcriptional control of the lineage-specific structures found inside the intergenic regions. We also found that male-transmitted and female-transmitted mtDNAs have a similar amount of polymorphism, but of a different kind. In contrast with organisms that show male-driven evolution, in this species male and female germ cells undergo a similar number of replication events due to gonad physiology, and this is reflected by the number of SNPs in M and F mtDNAs. The different kind of polymorphism is instead an effect of a different population size (different bottlenecks) and of a different efficiency of selection on the two mitochondrial lineages. Our results are consistent with the hypothesis that mtDNA evolution is strongly dependent on the dynamics of germ line formation. The DUI system offers the possibility to study the evolutionary dynamics of mtDNAs that, despite being in the same organism, experience different genetic drifts (due to different mitochondrial population size in gametes), but also different selective pressures. Actually, DUI animals are the only known organisms in which the mtDNA (i.e. the M-type) is subject to selection for male function. In a broadcast spawning organism the performance of spermatozoa is extremely important and selection for viable sperm, as well as sperm competition, is more efficient on a mtDNA which is transmitted through males. For this reason, we think that even if DUI probably arose by chance through completely stochastic events, its evolutionary persistence may be adaptive, because an increased male fitness would be beneficial for the whole species.

## Concatabominations: identifying unstable taxa in morphological and genomic datasets using Safe Taxonomic Reduction

**Karen Siu-Ting<sup>1</sup>, Christopher Creevey<sup>2</sup>, Mark Wilkinson<sup>3</sup>, Davide Pisani<sup>4</sup>**

<sup>1</sup>National University of Ireland, Maynooth, Biology Dpt., NUI Maynooth, Co. Kildare Ireland.

<sup>2</sup>IBERs, Aberystwyth University, Aberystwyth, Ceredigion, SY23 3FG, United Kingdom..

<sup>3</sup>Natural History Museum, Cromwell Road, London, SW7 5BD, United Kingdom.

<sup>4</sup>University of Bristol, Woodland Road, Bristol BS8 1UG, United Kingdom.

Contact: agalychnica@gmail.com

Missing data is one of the sources of uncertainty in the resolution of nodes in phylogenetic trees. Under parsimony, the strategy of excluding the poorly known taxa has been commonly used to minimize the number of most parsimonious trees and maximise resolution. In this sense, Wilkinson's (1995) Safe Taxonomic Reduction (STR) is useful to determine the potential instability of taxa a priori, and to determine which taxa are safe to exclude from an analysis. STR has been mostly applied in palaeontological phylogenies but also in the context of supertree reconstruction. Even though it has been widely used, it presents some limitations, especially with a particular type of "taxonomic equivalence". In the present work, we introduce an extension to this method, called "Concatabominations". This is based on the recoding of pairs of taxa that are found to be "potential taxonomic equivalents" but that still do not have enough data to place them unequivocally in a tree. By recoding potential equivalents we create a new "concatabominated" taxon that is replaced in the original matrix. A compatibility based approach is then used to determine whether two potential equivalents are true equivalents and then these are tallied based on the number of taxonomic equivalents they present. This new strategy was implemented in a pipeline and tested using gap-rich paleontological datasets and genomic MRP (Matrix Representation with Parsimony) matrices. The output of the pipeline can be visualized in a network to identify the most unstable taxa to delete first. This approach is helpful in reducing the number of unstable taxa due to missing data and as a result improve resolution in trees, and also will be useful in supertree analyses when dealing with gap-rich phylogenomic datasets.



## Development of a Maximum Likelihood and Bayesian Supertree method and the analysis of the Tree of Life

**Wasiu Akanni<sup>1</sup>, Davide Pisani<sup>2</sup>, Mark Wilkinson<sup>3</sup>**

<sup>1</sup>*N.U.I Maynooth, Kilcock Rd. Maynooth, Co. Kildare, Ireland.*

<sup>2</sup>*University of Bristol, School of Biological Sciences, Bristol, United Kingdom.*

<sup>3</sup>*National History Museum, London, United Kingdom.*

Contact: waakanni13@gmail.com

The concept of using a tree to show the evolutionary history of all the organisms based on the analysis of their full genome is the pivotal concept underlying the field of phylogenomics and dates back all way to Darwin, himself. The literature is littered with many attempts to reconstruct the tree of life (e.g Delsuc et al. 2005). Many past attempts have been faced by two common problems. The first problem is that of the amount of full genome sequence data available. The recent advancement of next generation sequencing techniques means that we are now fully in the era of phylogenomics. The number of complete genomes available is at an all time high and this number is increasing with each passing day. The second problem other researchers have faced when reconstructing the Tree of Life is that of an available and appropriate phylogenetic reconstruction method. This is where the development of new robust and parametric supertree reconstruction methods have come to find their niche. We have developed the first software implementation of a parametric Bayesian supertree method and a Maximum likelihood supertree method. The Bayesian supertree method has the advantage to allow the support for the clades in a supertree to be properly estimated. We have lend the ability of our Bayesian MCMC supertree method the questions of the reconstruction of the tree of life and compare our phylogeny to that are currently accepted as the best in the literature.

## GeoKS: an automatic tool for estimating burnin and assessing convergence in MCMC Phylogenetic Inferences

**Saverio Vicario<sup>1</sup>, Simone Battagliero<sup>2</sup>, Giuseppe Puglia<sup>3</sup>, Teresa Maria Creanza<sup>4</sup>, Giancarlo Tria<sup>5</sup>, Gaetano Scioscia<sup>6</sup>, Pietro Leo<sup>6</sup>**

<sup>1</sup>*Istituto di Tecnologie Biomediche, Consiglio Nazionale delle Ricerche, Italy.*

<sup>2</sup>*Exprivia S.p.A., viale A. Olivetti 11/A, 70056 Molfetta, Italy.*

<sup>3</sup>*Department of Biological, Geological, and Environmental Sciences, University of Catania, Italy.*

<sup>4</sup>*Istituto di Studi sui Sistemi Intelligenti per l'Automazione, Consiglio Nazionale delle Ricerche, Italy.*

<sup>5</sup>*Biological Small Angle Scattering, European Molecular Biology Laboratory, Germany.*

<sup>6</sup>*IBM Italia S.p.A., GBS BAO Advanced Analytics Services, Via Pietro Leonida Laforgia 14, 70125 Bari, Italy.*

Contact: saverio.vicario@gmail.com

An open problem of Bayesian methods for phylogenetic inference is the burn-in detection and convergence assessment. In fact, several solutions were proposed (Nylander et al. 2008, Beiko 2006) but none give objective criterion to accept or reject convergence of the Markov Chain Monte Carlo used to perform phylogenetic inference. This puts several limitations in the use of Bayesian phylogenetic inference within an automatic pipeline of analysis. Further, it's still very difficult to diagnose which aspect, poor mixing or too few generations, caused the lack of the convergence, given the difficulty to apply all the statistical tools developed for general MCMC integration (that involves scalar parameters) to phylogenetic inference (where the main parameter to be estimated is the topology). We propose an automatic method for addressing burn-in and convergence assessment, grounded on the theoretical framework given by the space of phylogenetic trees (given by topology and branch lengths). This space gives the possibility to calculate distances between trees but also to estimate a mean tree, that unlike a consensus tree includes also branch lengths. By means of tree distances and the mean tree, we estimate the autocorrelation across a tree series, which could be used to compare the efficiency of mixing across different runs and models. Further, with this statistics we are able to estimate the effective sample size of the sampled trees and to calibrate a Kolmogorov-Smirnov test that detects differences between independent runs of the MCMC integration. To increase the detection power, before the convergence test we discard from each run an initial number of generations, given by our burn-in detection algorithm. We tested the power of the convergence test by using two different strategies: 1) by artificially increasing the distances between two sets of trees 2) by observing the effective number of trees necessary in order to detect convergence on several slices of increasing size of a run that reached convergence thanks to a very large number of generations. In the latter tests we observed the empirical rule, which appears to be dataset-independent, that 300 effective trees should be obtained before convergence can be detected with no bias. The algorithm is implemented in a software written in C available as a web application (<http://mblabproject.it/geoks/>) or as a step of a larger pipeline. The web application is able to calculate the geodesic distance, the mean tree, the burn-in and the p-value of convergence; in the second deployment, the tool is included in a series of workflows for phylogenetic inference with the program MrBayes (<http://www.myexperiment.org/packs/371.html>) that can be played on the portal of the FP7

project BioVeL (<http://tavlite1.biovel.eu/>). This web service implements only the burn-in estimation and convergence test procedures, but is wrapped in a Python script that implements a Fisher procedure to combine p-values from several pairwise comparison of runs: this allows to generalize the approach to more than two runs.

## The draft genome of *Cardamine resedifolia*: genomic tools to investigate plant adaptation to high altitude

**Lino Ometto<sup>1</sup>, Mingai Li<sup>1</sup>, Claudio Varotto<sup>1</sup>**

<sup>1</sup>Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: lino.ometto@fmach.it

*Cardamine resedifolia* is a perennial species usually growing on siliceous substrates in open spaces between 1,500 and 3,500 meters above sea level. The environmental and ecological factors associated to this habitat evidently impose distinct selective pressures to the species, as revealed by an in-depth analysis of the patterns of molecular evolution in genes involved in cold response in photosynthesis (Ometto *et al.* 2012). However, both protein sequence and gene regulation may be involved in adaptation. Thus, a comprehensive knowledge on the genome structure and evolution of the species is necessary to gather precious insights in the pattern of coding genes and regulatory sequence evolution. To this aim, we present the draft genome sequence of *C. resedifolia* and its partial annotation, which represent the first step to characterize the genetic basis of adaptation to high altitudes for this and also other plant species.

## Mating-responsive genes from the testes and male accessory glands of the Mediterranean fruit fly, *Ceratitis capitata*

**Francesca Scolari<sup>1</sup>, Ludvik M. Gomulski<sup>1</sup>, José M.C. Ribeiro<sup>2</sup>, Paolo Siciliano<sup>1</sup>, Alice Meraldi<sup>1</sup>, Marco Falchetto<sup>1</sup>, Angelica Bonomi<sup>1</sup>, Mosè Manni<sup>1</sup>, Paolo Gabrieli<sup>1</sup>, Alberto Malovini<sup>1</sup>, Riccardo Bellazzi<sup>1</sup>, Serap Aksoy<sup>3</sup>, Giuliano Gasperi<sup>1</sup>, Anna R. Malacrida<sup>1</sup>**

<sup>1</sup>University of Pavia, Via Ferrata 9, Italy.

<sup>2</sup>National Institute of Allergy and Infectious Diseases, Rockville, Maryland, United States of America.

<sup>3</sup>Yale School of Public Health, New Haven, Connecticut, United States of America.

Contact: gasperi@unipv.it

Insect seminal fluid is a complex blend of spermatozoa, proteins, carbohydrates and lipids, which are produced in the male reproductive tract. This mixture is transferred during copulation and induces post-mating responses in the female. Molecular characterization of the protein components of seminal fluid in the Mediterranean fruit fly (medfly), *Ceratitis capitata*, is limited, even though different studies suggest that some of these proteins are biologically active. We report the functional annotation of 8448 expressed sequence tags (ESTs) derived from the testes and male accessory glands, to identify transcripts encoding putative secreted peptides that might elicit post-mating responses in females. The ESTs were assembled into 3344 contigs, of which over 33% produced no hits against the nr database, putatively representing novel medfly sequences. Extraction of the coding sequences resulted in a total of 3371 peptides. Four hundred peptides were found to have putative secretory activity, including odorant binding proteins, protease inhibitor domain-containing peptides, antigen 5 proteins, mucins, and immunity-related sequences. Quantitative RT-PCR on transcripts expressed in the accessory glands revealed that several changed in abundance after one or more copulations, compared to virgin males of the same age. Our results suggest the presence of transcriptional switch points possibly related to the need to replenish transcripts encoding secreted proteins with an active role in the seminal fluid. This represents the first large-scale dataset for novel studies on functions and processes associated with the reproductive biology of this invasive pest species. The identified genes may help the study of the evolution of the genome, in the light of its high adaptive potential. In addition, studies of male recovery dynamics in terms of accessory glands gene expression profiles and correlated remating inhibition mechanisms will permit the improvement of the current pest management approaches.

## The complete mitochondrial genome of *Solemya velum* (Mollusca: Bivalvia) and its relationships with Conchifera

**Federico Plazzi<sup>1</sup>, Anisa Ribani<sup>1</sup>, Marco Passamonti<sup>1</sup>**

<sup>1</sup>University of Bologna, Via Selmi, 3, 40126 Bologna (BO), Italy.

Contact: federico.plazzi@unibo.it

Bivalve mitochondrial genomes exhibit a wide array of uncommon features, like extensive gene rearrangements, large sizes, and unusual ways of inheritance. Species pertaining to the order Solemyida (subclass Opponobranchia) show many peculiar evolutionary adaptations, such as extensive symbiosis with chemoautotrophic bacteria. Despite Opponobranchia are central in bivalve phylogeny, being considered the sister group of all Autobranchia, a complete mitochondrial genome has not been sequenced yet. We characterized the complete mitochondrial genome of the Atlantic awning clam *Solemya velum*: A-T content, gene arrangement and other features are more similar to putative ancestral mollusks than to other bivalves. Two supranumerary open reading frames are present in a large, otherwise unassigned, region, while the origin of replication could be located in a region upstream to the *cox3* gene. We show that *S. velum* mitogenome retains most of the ancestral conchiferan features, which is unusual among bivalve mollusks, and we discuss main peculiarities of this first example of an organellar genome coming from the subclass Opponobranchia. Mitochondrial genomes of *Solemya* (for bivalves) and *Haliotis* (for gastropods) seem to retain the original condition of mollusks, as most probably exemplified by *Katharina*.

## The BioVel Project: foolproof phylogenetic workflows for Biodiversity studies

**Saverio Vicario<sup>1</sup>, Bachir Balech<sup>2</sup>, Giacinto Donvito<sup>3</sup>, Pasquale Notarangelo<sup>3</sup>, Graziano Pesole<sup>2,4</sup>**

<sup>1</sup>Consiglio Nazionale delle Ricerche, Istituto di Tecnologie Biomediche, Italy.

<sup>2</sup>Consiglio Nazionale delle Ricerche, Istituto di Biomebrane e Bioenergetica, Italy.

<sup>3</sup>Istituto Nazionale di Fisica Nucleare, Italy.

<sup>4</sup>Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica, Università degli Studi di Bari "Aldo Moro", Bari, Italy.

Contact: saverio.vicario@gmail.com

Biologists and environmental scientists are asked to provide decision support for managing biodiversity components at multiple scales (genomic, organismal, habitat, ecosystem, landscape, etc...) to prevent and mitigate environmental losses. The BioVeL project ([www.biovel.eu](http://www.biovel.eu)) has been designed to address these needs by offering a series of robust and reliable web services that could be managed with the tools suite of myGRID project. The project proposes building workflows exploiting these services to ensure the best practice and efficiency of use. These workflows provide the end users easily accessible capabilities to execute their applications through several kinds of resources, such as European Grid Infrastructure (EGI), local batch farm or other dedicated servers. Within the first round of services produced by the project, we describe here the phylogenetic inference workflows. The workflow (Figure 1) starts from a user defined collection of biosequences (homologous nucleotide protein coding), accesses an alignment Web Service that implement HMMER3 align algorithm (Eddy, 2011) and uses, conditioned on the biosequences as queries, the correct PFAM as guiding profile chosen with 'HMMER3 search' function. Using a supplied user threshold, amino acids and consequentially DNA sites with lower posterior probability are filtered out. The MrBayes (Huelsenbeck et al., 2001) model block is built following user supplied request or using PartitionFinder, while the Metropolis-coupled Markov Chains Monte Carlo (MCMCMC) numerical integration options are in part specified by the user and in other part are fixed to maximize MPI efficiency by the farm system. MCMCMC numerical integration convergence is assessed by GeoKS (Battagliero et al., 2011) software that estimates the burn-in value and the reached convergence based on tree parameter. The convergence information is then used to build the consensus representation of the phylogenetic inference. To control the molecular evolution model fit to the data, a web service implements a posterior predictive test which uses as input the samples from the posterior distribution to simulate data sets and compare the original data entropy with the distribution of simulated ones. Finally, data are visualized using the iTOL services (<http://itol.embl.de/>). The workflow is built within Taverna Workflow Management System, where each of the described steps are executed in a distributed computational environment. This was possible thanks to REST-FUL web service that exploits the usage of JST (Job Submission Tool) (Donvito et al. 2012) allowing job submissions and monitoring over the grid. The solution described in this work will allow also the end user to exploit the power of a computing grid infrastructure like EGI, without the burden to learn how to exploit it. Further the workflow procedure could be commented and/or modified, with the help of Taverna desktop, in the public repository ([www.myexperiment.org](http://www.myexperiment.org)), while original and modified workflows could be played in the portal of the project (<http://tavlite1.biovel.eu>). Acknowledgements: we would like to thank FP7 grant '283359' that allowed to fund this work.

Figure 1. The Phylogenetic Inference Workflow scheme showing the underlying web services and computational resources. Gray arrows indicate real data flow, black arrows specify logic and symbolic links.

## Phylogeny of *Asperula* L. sect. *Cynanchicae* (DC.) Boiss.: a starting effort

**Roberta Gargiulo<sup>1</sup>, Emanuele Del Guacchio<sup>2</sup>, Olga De Castro<sup>1</sup>, Paolo Caputo<sup>1</sup>**

<sup>1</sup>University of Naples "Federico II", Via Foria 223, Naples, Italy.

<sup>2</sup>Bagnolifutura SpA, Via E. Cocchia 28, Naples, Italy.

Contact: roberta.gargiulo@unina.it

One of the most puzzling issues in phylogenetics and systematics is the reconstruction of interspecific relationships between entities extremely similar from a molecular point of view; this affinity is not always associated to a morphological resemblance. We faced this problem in the investigation of phylogenetic history of the genus *Asperula* L. (Rubiaceae) sect. *Cynanchicae* (DC.) Boiss.; for many members of the section, interspecific differentiation is truly subtle and discrimination is too often founded on obscure quantitative traits. Moreover, a hybrid and/or introgressive nature of some entities is suspected (e. g., Bernardo et al., 2010). Phylogenetic analysis was conducted mainly in a Bayesian framework (Ronquist & Huelsenbeck, 2003), which gave a higher support for several clades, but methods based on maximum parsimony and maximum likelihood were also used. Basically, first outcomes indicate a global weak resolution of the relationships (i.e., various clades are collapsed or barely resolved). Unsurprisingly, clades including taxa with distinctive morphological traits (e. g., various Russian and some Greek taxa) exhibit a satisfactory resolution. A possible explanation for this low molecular variability could be sought in the relatively recent divergence of some entities; however, the presence of various autapomorphies in some terminal taxa may even suggest the opposite. An additional interpretation, especially in conditions of sympatry, would be the occurrence of hybridization and introgression. Apart from the necessity to find discriminating markers which may lead to a more resolved (and robust) phylogenetic hypothesis, we started to develop a microsatellite library to investigate the most difficult cases, in terms of low interspecific variability (Yao et al., 2008). In these cases, we will be going to compare the interspecific differentiation with the pattern of variations between populations of the same species, in the effort to reach a phylogeographic hypothesis.

## Allelic variation at the immunity gene TEP1 in an area of hybridization between the malaria mosquitoes *Anopheles gambiae* and *An. coluzzii*

**Emiliano Mancini<sup>1</sup>, Maria Ida Spinaci<sup>1</sup>, Beniamino Caputo<sup>1</sup>, Verena Pichler<sup>1</sup>, José Luis Vicente<sup>2</sup>, Amabelia Rodrigues<sup>3</sup>, Marco Pombi<sup>1</sup>, Vincenzo Petrarca<sup>1</sup>, João Pinto<sup>2</sup>, Alessandra della Torre<sup>1</sup>**

<sup>1</sup>Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma "Sapienza", Rome, Italy.

<sup>2</sup>Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal

<sup>3</sup>Instituto Nacional de Saúde Pública, Bissau, Guinea-Bissau.

Contact: emiliano.mancini@uniroma1.it

*Anopheles gambiae* (Giles) and *A. coluzzii* (Coetzee & Wilkerson sp. n.) - the most important cryptic mosquito species transmitting malaria in sub-Saharan Africa - are defined on rDNA-IGS specific SNPs (Fanello et al. 2002 Med Vet Entomol. 16: 461-4) and distinguished for the specific insertion of a retrotransposable element (~1Mb from IGS) in *A. coluzzii* (Santolamazza et al. 2008 Malar J. 25 7:163). *A. gambiae* and *A. coluzzii* are reproductively isolated by pre-mating barriers (Diabaté et al. 2009 Proc Biol Sci. 7 276:4215-22; Pennetier et al. 2010 Curr Biol. 26 20:131-6) and, although these are not absolute, ecologically based divergent selection has been hypothesized to contribute substantially to the lack of gene flow in sympatric areas in West and Central Africa (< 1% of hybrids in nature) (Coetzee et al. 2013 Zootaxa 3619: 246-274). However, unusual high rates of putative hybrids have been recorded in The Gambia (up to 7%) and Guinea Bissau (>20%), leading to suppose that the westernmost African regions may represent an area of secondary contact between the two species (Oliveira et al. 2008, J Med Entomol 45:1057-63; Caputo et al. 2011, PLoS One 15 6:e16415). Genome-wide data highlighted that diversification between *A. gambiae* and *A. coluzzii* in West and Central Africa has occurred despite some ongoing gene flow: in fact, some genome regions resist introgression - mostly in the low-recombinant centromeric areas of chromosomes X and 2 - while others continuously homogenize (Turner et al. 2005 PLoS Biol. 3:e285). Genome scans also revealed a significant peak of divergence in an area of chromosome 3 where the innate-immunity gene TEP1, a complement-like opsonin whose binding triggers killing of gram-negative bacteria and protozoa via phagocytosis, is located (Lawnickzak et al. 2010 Science 330:512-4). It has been shown that the 'R1' allele of TEP1 - which confers resistance to Plasmodium and bacterial infections (Blandin et al. 2009, Science, 326: 147-50; Yassine et al. 2012 PLoS Pathog. 8:e1003029) - is confined to *A. coluzzii* in West and Central Africa, whereas the other two allelic classes ('S' and



'R2', which confer 'susceptibility' and an 'intermediate resistant phenotype' to infections, respectively) are shared with *A. gambiae* (White et al., 2011 Proc Natl Acad Sci U S A 108:244-9). In order to investigate how reduced reproductive isolation in the hybrid zone affected divergence at genome regions known to be segregated between the two species, we assessed TEP1 allele segregation, evaluated the genetic polymorphism in TEP1 catalytic region (i.e. 'TED' region, ~ 500 bp) and tested for adaptive evolution in ~250 individuals of *Anopheles gambiae*, *Anopheles coluzzii* and putative hybrids collected in 2010 in five localities along a west-to-east transect in the secondary contact area in Guinea Bissau. Our results indicate that both resistance-associated alleles occurred at lower frequencies ('R1': 22%; 'R2': 8%) than susceptible ones ('S': 70%), but that all three allelic classes were maintained in almost all Guinean populations. Noteworthy, hybridization process allowed 'R1' allele introgression from *A. coluzzii* into the hybrids and *A. gambiae* genomes, allowing speculating on potential consequences of introgression on mosquito immunity and malaria transmission in the hybrid zone.

## A comparative analysis of Mitochondrial ORFans: new clues on their origin and role in species with Doubly Uniparental Inheritance of Mitochondria

**Liliana Milani<sup>1</sup>, Fabrizio Ghiselli<sup>1</sup>, Davide Guerra<sup>1</sup>, Sophie Breton<sup>2</sup>, Marco Passamonti<sup>1</sup>**

<sup>1</sup>Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, Università di Bologna, Via Selmi 3, Bologna, Italy.

<sup>2</sup>Département de Sciences Biologiques, Université de Montréal, Montréal, Québec, Canada.

Contact: marco.passamonti@unibo.it

Despite numerous comparative genomics studies revealed that animal mitochondrial genomes (mtDNAs) are highly conserved in terms of gene content, supplementary genes are sometimes found. Many mechanisms are responsible for the origin of such new genes. For example, novel mitochondrial Open Reading Frames (ORFs) can arise from gene duplication. In bivalve molluscs, a *cox2* duplication is found in the clam *Ruditapes philippinarum* and in the mussel *Musculista senhousia*. Moreover, *nad2* duplication are at the origin of two novel ORFs in the oyster genus *Crassostrea*. Extra-elements are also found in cnidarian mtDNA, some attributed to the duplication of extant genes others to an ancient invasion by a linear plasmid. Interestingly, mitochondrial ORFans (ORFs having no detectable homology and unknown function) were found in bivalve molluscs with Doubly Uniparental Inheritance (DUI) of mitochondria. In DUI animals two mitochondrial lineages are present: one transmitted through females (F-type), the other through males (M-type), each showing a specific and conserved ORF. The analysis of 34 mitochondrial major Unassigned Regions of *Musculista senhousia* F- and M-mtDNA allowed us to verify the presence of novel mitochondrial ORFs in this species and to compare them with ORFs from other species with ascertained DUI, with other bivalves and with animals showing new mitochondrial elements. Overall, 17 ORFans from 9 species were analyzed for structure and function. Our results generally excluded an origin from gene duplication for such sequences, instead many clues suggest that the analyzed ORFans arose from endogenization of viral genes. The co-option of such novel genes by viral hosts may have determined some evolutionary aspects of host life cycle, possibly involving mitochondria. These putative proteins may have a role in the maintenance of sperm mitochondria during embryo development, possibly masking them from the degradation processes that normally affects sperm mitochondria in species with strictly maternal inheritance. It is well established that the nucleus regulates mitochondrial gene expression through anterograde regulation. On the other hand, several studies have recently demonstrated that signals from organelles regulate nuclear gene expression by retrograde signalling. It appears likely that, given the complex cross talk between the nucleus and mitochondria, not only chemical messengers but also exported proteins may participate in transducing signals from mitochondrion to nucleus, as, for example, the retrograde signalling that characterize plants with Cytoplasmic Male Sterility. Moreover, the presence of mitochondrial proteins in diverse cellular extra-mitochondrial sites supports the existence of specific export mechanisms by which certain proteins exit mitochondria. In our case, given the presence of a signal peptide in many of the analyzed ORFs, this N-terminal sequence may be used to target the proteins to sites outside mitochondria. The structure similarity of DUI ORFans within evolutionary lineages may indicate that they originated from independent events. If these novel ORFs are in some way linked to DUI establishment, a multiple origin of DUI has to be considered. We can hypothesize that viral selfish elements may have colonized the mitochondrial genome in male bivalves promoting its segregation into primordial germ cells thus allowing the transmission to next generations, and leading to DUI achievement. If this is true, the insertion event and the appearance of DUI might be causally linked, and some implications on the origin and evolution of DUI become evident. Finally, the general mechanism proposed above for the transmission of selfish elements would imply that bivalves are in some way prone to viral integration in the mitochondrial genome and therefore in DUI establishment, and

maybe that other animals can have experienced such kind of mitochondrial transmission modification but no evidence has been found so far.

## **Photoperiod-driven selection at human circadian regulatory loci and at risk variants for neuropsychiatric diseases**

**Rachele Cagliani<sup>1</sup>, Diego Forni<sup>1</sup>, Uberto Pozzoli<sup>1</sup>, Claudia Tresoldi<sup>1</sup>, Stefania Riva<sup>1</sup>, Giorgia Menozzi<sup>1</sup>, Giacomo Comi<sup>2</sup>, Mario Clerici<sup>3</sup>, Manuela Sironi<sup>1</sup>**

<sup>1</sup>IRCCS E. Medea, Via Don L. Monza 20, Bosisio Parini, Italy.

<sup>2</sup>Dino Ferrari Centre, Department of Physiopathology and Transplantation, University of Milan, Fondazione Ca' Granda IRCCS Ospedale Maggiore Policlinico, Via F. Sforza, Milano, Italy.

<sup>3</sup>Chair of Immunology, Department of Physiopathology and Transplantation, University of Milan, Via F.lli Cervi 63, Segrate (MI), Italy.

Contact: manuela.sironi@bp.inf.it

Day/night cycles represent a major circadian synchronizing signal and vary widely with latitude. We applied a geographically explicit model to show that human out-of-Africa migration determined adaptive evolution at circadian regulatory loci. The SNPs we identified using this model display consistent signals of natural selection using tests based on population genetic differentiation and haplotype homozygosity. Signals of natural selection driven by annual photoperiod variation were detected for schizophrenia, bipolar disorder and restless leg syndrome risk variants, in line with the circadian component of these conditions. Our data provide insight into human evolutionary history and suggest genetic modulators for chronotherapies.

## **SPOTG: planning online a conservation/population genetics study**

**Sean Hoban<sup>1</sup>, Oscar Gaggiotti<sup>2</sup>, ConGRESS Consortium<sup>3</sup>, Giorgio Bertorelle<sup>1</sup>**

<sup>1</sup>Department of Life Sciences and Biotechnology, University of Ferrara, Via L. Borsari 46, 44100 Ferrara, Italy.

<sup>2</sup>School of Biology, Scottish Oceans Institute, University of St Andrews, St Andrews, Fife, KY16 8LB, United Kingdom.

<sup>3</sup>European Consortium ConGRESS: Conservation Genetic Resources for Effective Species Survival.

Contact: shoban@alumni.nd.edu

We constructed an online software to help scientists determine the power of their genetic marker and sampling strategy when five relevant topics in population/conservation genetics are investigated: bottleneck, connectivity, individual assignment, hybridization, and monitoring. This tool is called SPOTG, Sample Planning Optimization Tool for conservation and population Genetics. Here we briefly present the five modules of SPOTG and we focus more specifically on detecting bottlenecks. Detecting bottlenecks is an important task in evolutionary biology. Several tests are available to detect bottlenecks based on genetic data from a number of individuals in a population. While such methods exist, their ability to detect bottlenecks has only been evaluated for severe declines, to some tens of individuals. Furthermore, previous evaluations of these methods have not been performed for realistic situations across a range of bottleneck times and severity. In light of increasing use of next generation sequencing for marker discovery, we used SPOTG to investigate power of large numbers of markers to detect moderate, bottlenecks (e.g. to  $N_e \sim 100$ ), which is realistic for many conservation situations. For the realistic situations that we examined, we show that (i) doubling the number of loci shows equal or better power than tripling the number of individuals, (ii) increasing the number of markers (up to 100) results in continued additive gains in power, and (iii) recovery after a moderate amount of time or gradual change in size reduces power, by up to one-half. Our software tool and simulation study show that bottleneck tests can have good power for realistic situations in the genomic age. Furthermore we show more generally that population genetic simulations can be very valuable when planning a genetic study. SPOTG is part of a EU project dedicated to conservation genetics and it is available at the address: [www.congressgenetics.eu](http://www.congressgenetics.eu).

# EVOLUTIONARY MEDICINE AND HEALTH

Evolutionary Medicine is a relatively new, innovative field of research, applying evolutionary theory to the study of human health and disease. This symposium will feature a series of invited and submitted talks on these issues, with a special focus on the role of symbiotic microbial communities in immunity and human health, including the evolution of diet, autoimmune disease, immune system development, and host-parasite interactions.

**Chairs: Carlotta de Filippo and Heidi Hauffe (Fondazione Edmund Mach)**

## Variation and Evolution of Holobionts

**Eugene Rosenberg<sup>1</sup>, Ilana Zilber-Rosenberg<sup>1</sup>**

<sup>1</sup>*Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Tel Aviv 69978, Israel.*

Contact: eros@post.tau.ac.il

## **Bifidobacteria in gut microbiota of *Callithrix jacchus* L. (common marmoset): their relationship with primate and non primate bifidobacterial microbial ecology**

**Samanta Michelini<sup>1</sup>, Monica Modesto<sup>1</sup>, Verena Stenico<sup>1</sup>, Loredana Baffoni<sup>1</sup>, Paola Mattarelli<sup>1</sup>**

<sup>1</sup>*University of Bologna, Viale Fanin 44, Bologna, Italy.*

Contact: samanta.michelini2@unibo.it

Bifidobacteria are an important commensal group of the gut microbiota of animals. They belong to key bacterial groups playing symbiotic and probiotic role in promoting health activities for the host. The distribution of bifidobacterial species in non-human primates is currently poorly investigated. The occurrence of bifidobacterial species in animals is characterized by a peculiar trend where there is a cluster of species typical for non-primates animals and a different cluster typical of man (human primates). Recently 5 new bifidobacterial species have been isolated from non-human primates such as New World monkeys (common marmoset, *Callithrix jacchus* L., and red-handed tamarin, *Saguinus midas* L.) (Endo et al., 2012). Due to the novelty of these species they cannot be assigned to any bifidobacterial species cluster. Studies currently under development in our lab show that bifidobacterial isolates from orangutan (*Pongo pygmaeus*), and chimpanzee (*Pan troglodytes*) belong to human primates bifidobacteria cluster: this is in agreement with the strict evolutionary link between Great Human apes such as orangutan and chimpanzee and humans. The aim of the present study is to investigate the biodiversity of bifidobacterial species in primates more distant to human from an evolutionary point of view such as common marmoset, New World monkey, in order to establish where does the bifurcation between the two bifidobacterial clusters can occur. Faecal samples were collected from 5 infants of common marmoset. Colony counts and isolation of bifidobacteria were performed in mTPY (modified TPY) added with mupirocine (100 mg/l). The isolates were tested for the activity of fructose-6-phosphate phosphoketolase (F6PPK) in order to confirm the genus attribution. Rep-PCR using primer BOXAR1 was conducted and the R software with the algorithm developed by Ishii et al. (2009) was used to perform the cluster analysis. For the species identification, the 16S rRNA gene amplification and sequencing of one representative strain from each cluster were performed. Bifidobacteria were detected in all sampled animals. The faecal Bifidobacterium spp. counts in infant common marmosets ranged from 7.42 to 9.48 log<sub>10</sub> CFU/g. The analysis of BOX profiles showed richness of species revealing about 5 clusters in each subject. Sequencing analysis of 16S rRNA gene amplified with genus-specific primers are currently on going. The partial results indicated the presence of two species recently isolated and described by Endo et al. (2012), *B. callitrichos* and *B. reuteri*. Furthermore one of the most representative cluster represent a novel species which could not be related to known Bifidobacterium species as its high sequencing similarity shared 94.6% to *B. scardovii* and 94.5% to *B. stellenboschence*.



## Diverse strain immune reactivity shapes fungal inflammation or tolerance

**Lisa Rizzetto<sup>1</sup>, Monica Di Paola<sup>2</sup>, Daniela C. Ifrim<sup>3</sup>, Gloria Giovannini<sup>4</sup>, Mihai G. Netea<sup>3</sup>, Luigina Romani<sup>4</sup>, Duccio Cavalieri<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>2</sup>Department of Neuroscience, Psychology, Drug Research and Child's, University of Florence Firenze, Italy.

<sup>3</sup>Nijmegen Institute for Infection, Inflammation and Immunity, Radboud University Nijmegen Medical Centre Nijmegen Nijmegen, The Netherlands.

<sup>4</sup>Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Perugia, Italy.

Contact: lisa.rizzetto@fmach.it

For over a century microbiology and immunology have classified microorganisms in pathogenic or non-pathogenic. This definition, clearly relevant at the level of strain and species for most bacteria, has never been probed in fungal species. Understanding the nature of fungal pathogenesis will result in developing more effective therapies for fighting invasive fungal infection. Currently, several studies attempt to address pathogenicity mechanisms using different strains as a model. This study was designed to explore the immune-based diversity of *Aspergillus* spp strains and *Saccharomyces cerevisiae* fungal strains comparing different fungal life stages, from conidia to hyphae to spores. Our results show a wide strain-dependent variation of the immune response elicited indicating that different isolates possess diverse virulence and infectivity. Moreover, in contrast to the *S. cerevisiae* yeast cell-induced Th1 response, dendritic cells stimulated with yeast spores induce cellular responses shifted towards Th17 differentiation. The switch between spores and yeast is crucial for the commensalism of *S. cerevisiae* and depends on the use of a different receptor repertoire. We demonstrate that the differential recognition of specific mannan structures is one of the master regulator of the discrimination between harmful and harmless fungi. The in-vitro preliminary classification and characterization of fungal biodiversity in inducing immune responses led us to start the investigation on how/if the different cell mediated immunogenicity could result in differences in trained immunity properties of the tested *S. cerevisiae* strains. Thus, the definition of markers of inflammation or pathogenicity cannot be generalized. Understanding the role of cell wall composition in different strains and variation in the balance between tolerance and inflammation responses might lead to fully understand the boundaries between safety and pathogenicity.

## Evidence for aging theories from the study of a hunter-gatherer people (Ache of Paraguay)

**Giacinto Libertini<sup>1</sup>**

<sup>1</sup>Independent Researcher, Via Cavour 13, 80023 Caivano, Italy.

Contact: giacinto.libertini@tin.it

For the study of a species, it is fundamental to observe it under natural conditions. For the human species, the closest condition to the natural one is that of the residual hunter-gatherer populations, which is equivalent to the human condition in the Paleolithic period. One of the few studies of this kind was conducted in late seventies on a small tribal population of Paraguay, the Ache. Data from this population turn out useful for considerations about evolutionary hypotheses on aging phenomenon. In natural conditions, after juvenile ages, Ache show an age-related increasing mortality, as "civilized" populations, and a mean duration of life (ML) of 38.8 years. Without the age-related increase of mortality, ML would be 87.75 years. The ratio between the two values is 2.260 and is in accordance with the values from other studies on mammal and bird species. In short, age-related increasing mortality limits strongly ML in natural conditions for many vertebrate species. Moreover, data for our species are also in agreement with the observation about an inverse correlation between extrinsic mortality (0,01%/year for our species) and the deaths due to the age-related increasing mortality (about 67% for our species), a relation that disproves non-adaptive aging theories and supports adaptive aging hypotheses, which predict a direct and an inverse relation, respectively. Another important information is given by the causes of death for Ache. When this people lived in the wild, the main causes of deaths for modern populations (heart attacks, diabetes, hypertension, etc.) were absent, and almost all deaths derived from accidents, violence, intoxications and infections. Cases of death by cancer were not reported, and only in the group aged 60+ years (age reached by about 31% of the population) some isolated cases of illness attributed to unspecified causes or to "old age" could have been the result of neoplastic diseases. For many gerontologists, the age-related decline of vital functions is well explained as a consequence of the gradual decline of cell turnover, genetically determined and regulated by the declining duplication capacities of stem cells. For the adaptive interpretation of aging, this is not a difficulty, and indeed it is a necessary mechanism to reduce life span. In contrast, for non-adaptive theories of aging, the progressive decline of cellular capabilities

of duplication requires an explanation. The current explanation is that these restrictions are a general defense against cancer, which limits and contrasts the pathological proliferation of any tumoral mass. However, among wild Ache (and other natural populations), cancer is virtually unknown in non-elderly subjects, and only among older individuals there are deaths attributable to oncological diseases. Moreover, fitness decline begins long before oncological diseases have fatal effects in significant numbers: when there are the first possible cases of deaths by cancer, fitness decline has already caused the death of most individuals. This completely disproves the hypothesis that the reduction of cell duplication capacities would be a defense against cancer, because a supposed defense against a deadly disease cannot exterminate a population before the disease begins to kill. On the contrary, according to the adaptive hypothesis of aging, the decline of defense against cancer is in function of the decline of cell replication capacities, which undermines immunological defenses against it, and the cases of cancer in old age are part of aging effects. Moreover, according to current theories on aging, very few or no individual reach old age and, so, aging cannot be directly influenced by natural selection. However, data from our population in the wild show that a significant proportion of the population reaches 60 and 70 years of age (31% and 19%, respectively). These data are consistent with similar data from other species studied under natural conditions and invalidate the main theoretical tenet of current evolutionary hypotheses about aging. Evidence from a human population studied in the wild, therefore, brings new arguments against the non-adaptive interpretation of aging.

### Gut microbiota in cystic fibrosis (CF) patients: a combined -omic translational workflow

**Pamela Vernocchi<sup>1,2</sup>, Mariacristina Valerio<sup>3</sup>, Luca Casadei<sup>3</sup>, Federica Del Chierico<sup>2</sup>, Antonietta La Storia<sup>4</sup>, Francesca De Filippis<sup>4</sup>, Fabio Majo<sup>5</sup>, Cristiano Rizzo<sup>6</sup>, Ersilia Fiscarelli<sup>6</sup>, Cesare Manetti<sup>7</sup>, Maurizio Muraca<sup>6</sup>, Vincenzina Lucidi<sup>5</sup>, Danilo Ercolini<sup>4</sup>, Bruno Dallapiccola<sup>8</sup>, Lorenza Putignani<sup>1</sup>**

<sup>1</sup>Interdepartmental Centre for Industrial Research-CIRI-AGRIFOOD, Alma Mater Studiorum, University of Bologna, Piazza Goidanich 60, 47521 Cesena (FC), Italy.

<sup>2</sup>Unit of Parasitology and Unit of Metagenomics, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165 Rome, Italy.

<sup>3</sup>Department of Chemistry, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy.

<sup>4</sup>Department of Agriculture, Division of Microbiology, University of Naples Federico II, Via Università 100, 80055 Portici, Napoli, Italy.

<sup>5</sup>Cystic Fibrosis Unit, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165 Rome, Italy.

<sup>6</sup>Laboratory Medicine, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165 Rome, Italy.

<sup>7</sup>Department of Chemistry, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy.

<sup>8</sup>Scientific Directorate, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165 Rome, Italy.

Contact: lorenza.putignani@opbg.net

Cystic fibrosis (CF), is an autosomal recessive disorder affecting the exocrine glands of the respiratory, digestive and reproductive systems and its lethality ranging from the first year of life to the third (and later) decade. This lethal hereditary disorder leading to respiratory infections, chronic inflammation, repeated antibiotic treatments and swallowing of infected respiratory mucus has known or suspected links to the gut microbiota, including a possible association with its dysbiosis. This correlation which has been assumed now need to be proved and fully documented. Intestinal malabsorption is a severe and early characteristic of CF patients, probably due to the CF-related gut dysbiosis. High-throughput meta-omics-based approaches may actually assist in unveiling this complex network of symbiosis modifications. The aim of this work was to investigate the gut microbiota composition and modulation of CF patients by metagenomic and metabolomic combined analyses in relation with healthy children. Thirty faecal samples from either CF young patients and healthy children (age range 0-6 years), respectively, were collected at Bambino Gesù Children's Hospital. The metabolomic analyses were performed by gas-chromatographic (GC)-mass spectrometer (MS)/solid-phase microextraction (SPME) and proton nuclear magnetic resonance spectroscopy (<sup>1</sup>H-NMR), while metagenomic analysis was carried out by 454 pyrosequencing platform. About two hundred volatile organic compounds (VOCs), 150 shared between healthy and CF children and 50 belonged only to FC patients were detected and quantified (with area interpolation versus internal standard) by GC-MS/SPME. VOCs were described and grouped according to chemical classes: esters, alcohols, sulphur compounds, ketones, alkanes, alkenes, aldehydes, phenols, indoles, aromatic organic compounds, short chain fatty acids (SCFA) etc. <sup>1</sup>H-NMR metabolic profiles of faeces, after water extraction, showed a variety of different compounds including SCFA, organic acids, amino acids, uracil, trimethylamine, glycerol, glucose, phenolic acids, cholate and formate. The inter-individual variability of VOCs levels resulted high. Compared to healthy children, the level of esters (i.e., ethyl acetate, butyl butyrate), alcohols (i.e., ethanol, 1-butanol) and aldehydes (i.e., hexanal, 4-methyl benzaldehyde)

were higher in faecal samples from CF patients. On the contrary, SCFA such as acetic, propionic and butyric acids were higher in healthy children than CF. From the <sup>1</sup>H-NMR analysis, the CF patients showed lower levels of SCFAs (acetate, propionate, butyrate and isobutyrate), amino acids (leucine, valine, alanine, glutamate, tyrosine and phenylalanine) and uracil than healthy children. Preliminary metagenomic results on 15 CF samples indicated Firmicutes as most abundant phyla, while the abundance of Bacteroidetes and Proteobacteria varied according to the sample analyzed. Moreover, this analysis identified a remarkable degree of variability of the operational taxonomic units (OTUs) (from 200 to 1000) confirming the high level of inter-individual metabolic variability. By this integrated approach it's possible to generate personalized "omics" charts that can be used for the monitoring of the nutritional state of the child and for the evaluation of gut absorption in CF patients, hence provide a translational medicine tool

## **An evolutionary view of antigen processing and presentation across different timescales**

**Diego Forni<sup>1</sup>, Rachele Cagliani<sup>1</sup>, Claudia Tresoldi<sup>1</sup>, Uberto Pozzoli<sup>1</sup>, Giulia Filippi<sup>2</sup>, Stefania Riva<sup>1</sup>, Giacomo P. Comi<sup>3</sup>, Nereo Bresolin<sup>3</sup>, Mario Clerici<sup>4</sup>, Manuela Sironi<sup>1</sup>**

<sup>1</sup>Scientific Institute IRCCS E. MEDEA, Bioinformatics, Bosisio Parini, Italy.

<sup>2</sup>Department of Biotechnology and Biosciences, University of Milan-Bicocca, Milan, Italy.

<sup>3</sup>Dino Ferrari Centre, Department of Physiopathology and Transplantation, University of Milan, Fondazione Ca' Granda IRCCS Ospedale Maggiore Policlinico, Milan, Italy.

<sup>4</sup>Chair of Immunology, Department of Physiopathology and Transplantation, University of Milan, Milan, Italy.

Contact: diego.forni@gmail.com

The antigenic repertoire presented by MHC molecules is generated by the antigen processing and presentation (APP) pathway. We analyzed the evolutionary history of 45 genes involved in APP and show that: 1) 11 genes (including CD1D, langerin, CYBB, ERAP2, tapasin, TAP1) evolved adaptively in mammals, and 2) several positively selected sites involve positions of fundamental importance to the protein function. Thus, positively selected sites in TAP1 are located at peptide-binding domains, and a selection target in CD1D affects a trafficking signal in its cytoplasmic tail. For CYBB, positive and negative selection was found to act on different sites in the same region, as assessed by analysis of GCD mutations. Using a novel approach we also determined that 9 genes underwent local adaptation in human populations and that most causal variants are located within noncoding regions with regulatory function in myeloid cells. Conversely, for TAP1, NCF4, and langerin aminoacid-replacement variants were found to represent balancing selection targets. Notably, selected variants in PSMB10 and langerin contribute to human phenotypes (Crohn's disease and HIV-1 susceptibility, respectively), supporting the view that adaptive events underlie phenotypic variability in humans. Data herein provide novel insight into APP and a list of sites to prioritize in follow-up analyses.

## **Systems level analysis of human fungal populations: passengers, colonizers, and invaders**

**Duccio Cavalieri**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

## **The role of the nest in the evolution of collective defence against pathogens in social insects**

**David Baracchi<sup>1</sup>, Giuseppe Mazza<sup>2</sup>, Stefano Turillazzi<sup>2</sup>**

<sup>1</sup>Queen Mary, University of London, Queen Mary University of London, School of Biological and Chemical Sciences, Mile End Road, London E1 4NS, United Kingdom.

<sup>2</sup>Università degli Studi di Firenze, Università degli Studi di Firenze, Dipartimento di Biologia, Via Madonna del Piano 6, 50019 Sesto Fiorentino (FI), Italy.

Contact: david.baracchi@gmail.com

The evolution of sociality in insects led to an increase in the number of individuals living in high densities as well as a decrease in genetic variability. Both trends, together with the high levels of spatial proximity and physical interactions occurring within the nests, significantly increase the vulnerability of colonies to pathogens and disease epidemics. However, as a result of co-evolutionary dynamics between parasites and hosts, social insects have evolved the ability to counteract potential epidemics. The first defence against infection of the colony is represented by the use of antimicrobial

secretions and in bees, wasps and thrips, the antimicrobial compounds progressively increase their efficacy with the enlargement of group size. In addition to individual immunity, social insects have also evolved sophisticated collective defences against disease. Accordingly, since natural selection often innovatively develops old traits in new ways we hypothesized that chemical defences would switch from individual immunity to complex forms of social immunity as colony size and sociality level increased. The family Vespidae provides an optimal opportunity to examine this scenario since it includes subfamilies, like the Eumeninae, with only solitary species and subfamilies, such as Stenogastrinae, Polistinae and Vespinae, with species presenting facultative, primitive and advanced eusociality. In order to define a possible relationship between the use of venom as an antimicrobial and the level of social organization, we first carried out microbiological tests on parts of the nests and venom extracts belonging to several wasp species of four subfamilies of Vespidae. We then analysed, by means of mass spectrometry techniques, fragments of the same nests focussing our search on the presence of venom compounds, previously detected in the venom reservoir of female wasps. We finally considered the possible interaction between the use of venom as an antimicrobial with another hygienic behaviour involved in the elimination of larval faeces from the nests by adults. Our data show that all the studied species, be they solitary or social, have strongly active venoms against gram-positive and gram-negative bacteria. More importantly we found that the nest, a simple workplace for adults and a nursery for brood in solitary and facultative social species, plays a key role for social immunity in the primitive and advanced eusocial species. Despite the venom of the solitary Eumeninae and the facultative social Stenogastrinae showing antimicrobial activity, their nests lack antiseptic properties and do not contain any venom compound, regardless of the nature of building material used. By contrast the nests of all the primitive and advanced eusocial species studied are coated with venom compounds, making them antiseptic homes. Primitive eusociality represents the critical point that requires collective chemical defences to counteract infections and colony size seems to be a relevant element in determining the use of different strategies in disease defence. Our findings shed further light on how eusocial insects, especially the more numerous species, were able to reduce the costs imposed by parasitic pressure and the threat of disease epidemics to establish themselves worldwide. This also suggests that in the evolutionary transition towards more complex societies, natural selection also began to act at the colony level.

## Genetic variability and phylogeography in *Ascaris* spp. using nuclear and mitochondrial markers

Serena Cavallero<sup>1</sup>, Barbara Gambetta<sup>1</sup>, Vitantonio Perrone<sup>2</sup>, Francesca Pacella<sup>1</sup>, Viliam Snabel<sup>3</sup>, Massimo Dutto<sup>4</sup>, Stefano D'Amelio<sup>1</sup>

<sup>1</sup>Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy.

<sup>2</sup>Az. ASL Roma B, Servizio Veterinario, Dip. di Prevenzione, Rome, Italy.

<sup>3</sup>Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, Kosice, Slovakia.

<sup>4</sup>Medical Entomology and Zoology, Local Health Unit CN1, Cuneo, Italy.

Contact: serena.cavallero@uniroma1.it

Human Ascariasis is a soil-transmitted helminthiasis infecting more than one billion people, but is considered a Neglected Tropical Disease. The infestation is caused by the nematodes *A. lumbricoides* (Linnaeus 1758) and *A. suum* (Goeze 1782) their significant similarities in morphological, biological and genetic features arise a controversial species delimitation. Moreover, evidences for hybridization and gene flow motivate the investigation on molecular systematic (Anderson, 2001, Trends Parasitol, 17:183; Peng and Criscione, 2012, Infect Genet Evol, 12:227; Li et al, 2012, Gene, 492:110). Nowadays, there is a strong debate on the taxonomic status and origin of the two entities in their respective hosts (Leles et al. 2012, Parasite vectors, 5:42): a) *A. suum* and *A. lumbricoides* are two valid species; b) *A. suum* is the ancestor of *A. lumbricoides*; c) *A. lumbricoides* the ancestor of *A. suum*; d) *A. suum* and *A. lumbricoides* are variants of a single polytypic species. Previous molecular epidemiological studies described two main scenarios: the first showed two different transmission cycles corresponding to host affiliation in highly endemic regions (Anderson et al; 1993, Parasitology, 107:319; Peng et al; 2005 Electrophoresis 26:4317); the second indicated a mixed transmission in which *A. suum* is the main etiological agent of Ascariasis in non-endemic areas (Anderson, 1995, Parasitology 110:215; Richard et al., 2011, Emerg Infec Dis 17:10). Conversely, recent evidences underline the zoonotic potential of *A. suum* in endemic region as China (Zhou et al. 2012, Infect Genet Evol 12:1172). The main aims of the present study were to investigate the molecular epidemiology of human and pig *Ascaris* spp. from Italy, a non-endemic region, and to infer the phylogenetic and phylogeographic relationships among the samples with reference to existing data (Cavallero et al. 2013, Plos NTD 7:e2170). Ninety-seven specimens (90 from pigs and 7 from humans) have been analyzed using PCR-RFLP on nuclear ribosomal ITS to identify nematodes at species level. Moreover the *cox1* mitochondrial partial gene has been sequenced to infer the genetic variability and phylogeography of individuals of the two taxa by comparing samples from both endemic and non endemic regions (GenBank retrieved data). A representative sub sample from a single farm (25 nematodes from pigs and 1 from the farmer) has been analysed to infer the genetic variation on a single local population. Molecular characterization using PCR-RFLP has confirmed that most pig nematodes displayed the typical *A. suum* pattern, as most human nematodes, but heterozygote genotypes and cross-infections has been proved in both hosts. The genetic variation observed in the entire dataset was significantly higher than variation observed in the local population, where high homozygosity and inbreeding



are likely to occur. Phylogeny and phylogeography described three taxonomic units previously named A, B and C (Anderson and Jaenike, 1997, *Parasitology*, 115:325; Snabel et al. 2012, *Jpn J Infect Dis* 65:179) where neither host affiliation or geography contribute to modulate clades. The lack of fixed genetic differences and admixture of haplotypes confirm a close evolutionary relationship of *A. suum* and *A. lumbricoides*, that may be variants of the same species. Further analyses would be useful to test their taxonomic status.

### Insight into genetic determinants of resting heart rate.

**Massimo Mezzavilla<sup>1</sup>, Annamaria Iorio<sup>2</sup>, Marco Bobbo<sup>2</sup>, Marco Merlo<sup>2</sup>, Sheila Ulivi<sup>1</sup>, Paolo Gasparini<sup>1</sup>, Gianfranco Sinagra<sup>2</sup>**

<sup>1</sup>*Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", University of Trieste, Via dell'Istria 65/1, Trieste, Italy.*

<sup>2</sup>*Cardiovascular Department, University of Trieste, Via Valdoni 7, Trieste, Italy.*

Contact: mzzvilla@gmail.com

Recent studies suggested that resting heart rate (RHR) might be an independent predictor of cardiovascular mortality and morbidity. Nonetheless, the interrelation between RHR and cardiovascular diseases is not clear. In order to resolve this puzzle, the importance of genetic determinants of RHR has been recently suggested, but it needs to be further investigated. The aim of this study was to estimate the contribution of common genetic variations on RHR using Genome Wide Association Study. We performed a Genome Wide Association Study in an isolated population cohort of 1737 individuals, the Italian Network on Genetic Isolates – Friuli Venezia Giulia (INGI-FVG). A significant level of association ( $p < 5 \times 10^{-8}$ ) was detected for Single Nucleotide Polymorphisms (SNPs) in two genes expressed in the heart: *MAML1* and *CANX*. Moreover, a haplotype analysis was performed, identifying one haplotype region that encompasses both genes. We categorized RHR into 4 classes, representing our phenotype, according to the distribution in quartiles in our sample. A regression tree analysis was run to highlight the effect of each haplotype combination on the phenotype. Interestingly we found that the three different variants of the haplotype yielded a phenotypic correlation in our cohort. Indeed, a haplotype in homozygosity is significantly associated with the lower quartile of RHR (RHR  $\leq 58$  beats per minute). Moreover no significant association was found between cardiovascular risk factors and the different haplotype combinations. Common genetic variants were found to be associated with RHR. Moreover, we have demonstrated a relation between a haplotype and the lower quartile of RHR in our populations. Our findings highlight genetic determinants of RHR that may be important to understand mechanisms underlying cardiovascular diseases and could allow a better risk stratification.

### Prevention of refractive defects of vision by means of Evolutionary Medicine

**Giacinto Libertini<sup>1</sup>**

<sup>1</sup>*Independent Researcher, Via Cavour 13, 80023 Caivano, Italy.*

Contact: giacinto.libertini@tin.it

Evolutionary Medicine is not an alternative medicine, but a medicine that is more thoroughly scientific as it includes the concepts of Evolutionism. The most useful concept of Evolutionary Medicine is that of the diseases caused by a “mismatch” between the adaptation of the individuals of a species and changed conditions of life. Here, I apply to the refractive defects of vision a methodology proposed for the diseases caused by a “mismatch” phenomenon. Step 1 - Epidemiological study of modern populations. Refractive defects (myopia, astigmatism and hyperopia) are a group of related diseases with frequency: a) very common; b) varying greatly from population to population; c) varying greatly within the same population with changing conditions; d) varying greatly within a population over a short time. Step 2 - Comparison between the frequency of a disease in modern populations and the frequency of the same disease in populations in primitive conditions. Studies on primitive populations show that these defects are very rare or nonexistent among them. So, evidence from here and previous step indicate that refractive defects are likely caused by a “mismatch” condition. Step 3 - Hypotheses on the possible changes in the ecological niche underlying the disease and on possible pathogenetical mechanisms. In children, a reduced exposition to natural light, a mismatch condition, is a documented cause of refractive defects. Excessive near work, especially with artificial lighting, another mismatch condition, appears to contribute as secondary factor to refractive defects. Step 4 - Study of the mechanisms that connect the alteration of the ecological niche and the pathogenesis of the disease. In chickens, alterations of vision conditions for few days determine astigmatism associated with myopia or hyperopia. In control birds, astigmatism is normal at the birth and disappears or decreases in the next days. In our species too, astigmatism – together with myopia or hyperopia - is normal at the birth and disappears in the first years of life. The genetic program of eye development allows to obtain at birth an eye with only approximate vision. Immediately after birth, it is activated another program that modulates the further development of the eye in order

to obtain an optimum sight. Step 5 - Possible restoration of the normal, alias primeval, conditions or possible compensatory conditions. In modern conditions of life, particularly in urban areas with high population densities, it is "normal" that babies do not grow outdoors with exposure to natural light. It is a widespread belief that babies should be protected as much as possible from the external environment, in particular from the direct exposure to sunlight, as this will damage them in some way. Many infants spend the first months of life almost exclusively indoors, only exposed to artificial lighting, with uneven and weak brightness, and however under conditions quite different from those to which our species is adapted. Babies, for proper eye development, should be exposed as much as possible to natural light conditions, not avoiding their exposition to sunlight but, on the contrary, looking as possible to make them live in conditions similar to the original ones. Step 6 - Analysis of the results achieved and ideation and proposal of further improvements. Such measures should be applied on a large scale, because evidence indicates that the current epidemic of refractive defects is caused by alterations in the exposure rate to natural light in infants and children. Selected groups of infants and children, grown under various conditions of compliance with these guidelines, should be carefully monitored by an ophthalmologic point of view and compared with control groups that fail to comply with them. It would be essential to quantify the exposure to natural light needed to prevent the occurrence of refractive defects. Keywords evolutionary medicine myopia astigmatism prevention

## **Malocclusions and urolithiasis in the context of Evolutionary Medicine**

### **Giacinto Libertini<sup>1</sup>**

<sup>1</sup>*Independent Researcher, Via Cavour 13, 80023 Caivano, Italy.*

Contact: [giacinto.libertini@tin.it](mailto:giacinto.libertini@tin.it)

Evolutionary Medicine is not an alternative medicine, but a medicine that is more thoroughly scientific as it includes the concepts of Evolutionism. The most useful concept of Evolutionary Medicine is that of the diseases caused by a "mismatch" between the adaptation of the individuals of a species and changed conditions of life. Here, I apply to malocclusions and urolithiasis a methodology proposed for the diseases caused by a "mismatch" phenomenon. Step 1 - Epidemiological study of modern populations. Malocclusions are very common in modern populations (up to 85%) with a frequency that is various in different populations and varied from time to time. The probability of urolithiasis differs in various parts of the world (up to 20% in Saudi Arabia) and changes with times. Step 2 - Comparison between the frequency of a disease in modern populations and the frequency of the same disease in populations under primitive conditions. Malocclusions and urolithiasis are very rare in primitive populations. So, evidence from step 1 and 2 indicate that these diseases are likely caused by "mismatch" conditions. Step 3 - Hypotheses on the possible changes in the ecological niche underlying the disease and on possible pathogenetical mechanisms. Changes of the diet, compared with the habits of primitive societies, are the documented cause of malocclusions and urolithiasis. For malocclusions, a critical factor is the amount of dietary vitamin D and of sun exposure for the formation of additional vitamin D, as an insufficient intake and absorption of dietary calcium in the early years of life determines, among other things, insufficient development of facial bones and an improper development of the set of teeth. Urolithiasis frequency is inversely related to dietary calcium intake, even though supplemental calcium may increase the risk. Dietary calcium reduces oxalate absorption and the urinary excretion of oxalate and this lowers the risk of kidney stones of calcium oxalate, the prevalent type of stones. Other factors correlated with a lower frequency of urolithiasis are potassium intake and fluid intake. The intake of fiber and plant foods reduces oxalate absorption and urinary calcium excretion and thus the frequency of the stones, while carbohydrate intake has the opposite effect. A higher protein intake is associated with a weakly increased risk of urolithiasis. Step 4 - Study of the mechanisms that connect the alteration of the ecological niche and the pathogenesis of the disease. For malocclusions - The proper development of facial bones and set of teeth is optimal when the values of dietary calcium and of vitamin D absorption and production are those to which our species is adapted. Modernized alimentation has severely altered these factors, and perhaps others that are more or less important to a correct development. For urolithiasis - There is hypercalciuria in 95% of patients with nephrolithiasis and this increases the risk of renal stones. Some foods reduce calcium absorption, and therefore the urinary calcium (K, PO<sub>4</sub>, fiber, Alkali Load alias fruits and vegetables), while others have the opposite effect (Na, Mg, Carbohydrates, Acid Load alias animal flesh). An increase in dietary calcium reduces calcium excretion in the urine and thus reduce the frequency with which they form calcium oxalate stones, the most common type of calculations. Step 5 - Possible restoration of the normal, alias primeval, conditions or possible compensatory conditions. It is essential to compare the ancestral diet with that of contemporary Western populations. It is certainly useful to correct as much as possible the changes of primeval diet and

lifestyle that to a greater extent show to raise disease frequencies, in particular increasing: a) the intake of dietary calcium, potassium and vitamin D; b) the exposure to sunlight; c) the intake of foods and elements that reduce oxalate absorption and calcium absorption, and therefore urinary calcium (K, PO<sub>4</sub>, fiber, Alkali Load alias fruits and vegetables); d) the intake of plain water; and e) reducing the intake of the foods and elements that increase oxalate absorption and calcium absorption, and therefore urinary calcium (supplemental Ca, Na, Mg, Carbohydrates, Acid Load alias animal flesh). Step 6 - Analysis of the results achieved and ideation and proposal of further improvements. Afterwards, it will be indispensable to evaluate the results obtained with different types of diet more or less suited to these principles.

## The Expression of the emotions: Darwin's scientific photographs

### Fascio Umberto<sup>1</sup>

<sup>1</sup>C.I.M.A. - Università degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy.

Contact: umberto.fascio@unimi.it

"The Expression of the Emotions in Man and in Animals" was written by Darwin in four months: a very short time. It was one of the first scientific books ever published with photographic illustrations, although at that time the technical limitations hindered the use of photography in scientific research. The photographic illustrations were inserted in the book pages with a lengthy process that greatly increased the cost of publication. For this reason Darwin used a method of photomechanical reproduction, the heliotype, which allowed a production in series of images that could be bound directly with the pages of the text. The use of heliotype allowed Darwin to include an unprecedented number of photographic plates and especially not to rely on artists who hand-copied photographs on lithographic sheet. For Darwin, it was important to obtain photographs of a gesture, a fast-action, to analyze and study the fleeting movements of the facial muscles. Only the snapshot, with its objectivity, allowed a careful observation of these movements. Unfortunately, at that time, snapshots were impossible and the normal exposure times varied between several seconds and 2 minutes. The images from a patient of Guillaume-Benjamin-Amand Duchenne greatly interested Darwin. Duchenne found that his patients could maintain expressions induced by a steady stream of electricity long enough to be compatible with the long exposure times required by the photographic technique. But Darwin needed as many details as possible for his work and the occasional encounter with Rajlander helped in resolving his problems. In fact, the ability of Rajlander in photo manipulation resulted very useful for the production of illustrations which Darwin considered suitable for publication. Anyway, the publication of Darwin's book marked the birth of the scientific photography. In fact, the distinction between documents and scientific illustration was not there, because there were no clear rules regarding the acceptance of photographs as scientific data. The publication of "The Expression of the Emotions in Man and Animals" began creating these rules.

## What Does Reciprocal Altruism Have to Do with Language?

### Irene Berra<sup>1</sup>

<sup>1</sup>University of Messina, Via Concezione 6-8, 98121 Messina, Italy.

When non-human primates are observed over months, the time frame of reciprocation turns out to be longer than expected considering their cognitive capacities. The emotional track of past interactions can motivate the long-term exchange of grooming, agonistic support and other currencies, easily avoiding the cognitive efforts required by episodic memory and strategic foresight. In contrast to the social intelligence hypothesis, this emotional bookkeeping (Schino and Aureli, *Advances in the Study of Behavior* 39:45-69, 2009) could have been foundational for language, more than Machiavellian intelligence, i.e. the anticipation and manipulation of the behaviour of others. Grooming time requirements in hominin evolution may have pushed for an emotionally based impulse to speak, rather than for a tactical exchange of information. Also, trust and companionship provide an explanation for those birds, pinnipeds, cetaceans and elephants imitating human voice. Above all, vocalizations actively strengthen emotional bonds, probably through those neuroendocrine mechanisms shared by mother-infant relationship, pair bonding and social attachment. As an example, maternal comforting speech releases oxytocin in girls, just as physical contact. Vocal language could have been originally an adequate means for expressing both positive, negative and neutral emotions. The full range of emotions expressed by infant vocalizations in the first year supports this hypothesis. Although we currently dress social life with hierarchical and impersonal meanings, the pleasure in understanding each other can still be considered as a reward reinforcing closeness and intimacy between the speaker and the receiver.



# BIODIVERSITY 3D: THE INTERRELATIONS AMONG GENES, SPECIES, AND ECOSYSTEMS

Biodiversity has been officially described (Convention on Biodiversity, Rio de Janeiro, 1992) as the variation of all living organisms measured at three different levels: genes, species and ecosystems. While the concept of species richness has become quite popular in the vast audience of non specialists, genes and ecosystems still struggle to be perceived as key biodiversity components. Very little is known yet about the interrelations among gene, species and ecosystem richness, despite the general acceptance of the importance of their interplay. In this symposium, we aim at gathering specialists in the three dimensions of biodiversity: genetic, specific and ecosystemic. We will welcome speakers studying the relationships among the three dimensions and the mechanisms underlying them. We wish to foster dialogue on a common ground among scientists from the three backgrounds; such a multidisciplinary approach is much needed to support our efforts to halt the current and expected biodiversity loss.

**Chairs: Cristiano Vernesi (Fondazione Edmund Mach) and Ivan Scotti (INRA)**

## Genes, species and ecosystem services: do we have the tools to pursue the paradigm?

### Mike Bruford<sup>1</sup>

<sup>1</sup>Cardiff School of Biosciences, Cardiff University, The Sir Martin Evans Building, Museum Avenue, Cardiff, CF10 3AX, United Kingdom.

Contact: BrufordMW@cardiff.ac.uk

## Experimental evolution uncovers habitat-dependent fitness dynamics among *Wolbachia* strains in *Drosophila melanogaster*

### Elisabetta Versace<sup>1</sup>, Christian Schlötterer<sup>1</sup>

<sup>1</sup>Institut für Populationsgenetik, Vetmeduni Vienna, Veterinärplatz 1 1210 Wien, Austria.

Contact: elisbettaversace@hotmail.com

Natural populations of *Drosophila melanogaster* host different *Wolbachia* strains. The habitat-specific fitness of different strains is not yet understood, mainly because of the complexity of natural environments. We use experimental evolution in controlled environments to investigate the responses of different *Wolbachia* strains. We investigated a *D.melanogaster* population sample from Portugal in which four of the six described *Wolbachia* clades are present. While exposing the fly population to two different environments (hot and cold) we traced the rate of infection and the frequency of different *Wolbachia* strains during the experiment. In both environments *Wolbachia* infection was fixed in less than 37 generations. We found that in the hot environment the *Wolbachia* diversity remained remarkably stable over 37 generations. In the cold environment, however, two *Wolbachia* strains were lost, one decreased in frequency and another became the most abundant. To distinguish between host and *Wolbachia* mediated effects, we exposed to the cold environment flies that had previously evolved for 42 generations in the hot environment. Interestingly, we observed the same pattern of the previous experiment, showing that *Wolbachia* from clade V have a fitness advantage in the cold environment. Moreover, the high reproducibility of our results suggests that experimental evolution studies are key to study the functional basis of habitat-specific fitness among *Wolbachia* strains and host-symbiont interactions.

## Intra or inter specific variability? Wing morphology and chemical epicuticular compounds reveal a cryptic species of Stenogastrinae (Hymenoptera: Vespidae)

**David Baracchi<sup>1</sup>, Lucia Pizzocaro<sup>2</sup>, Atikah Nurul<sup>3</sup>, Stefano Turillazzi<sup>2</sup>**

<sup>1</sup>Queen Mary, University of London, Queen Mary University of London, School of Biological and Chemical Sciences, Mile End Road, London E1 4NS, United Kingdom.

<sup>2</sup>Università degli Studi di Firenze, Università degli Studi di Firenze, Dipartimento di Biologia, Via Madonna del Piano, 6 50019 Sesto Fiorentino (FI), Italy.

<sup>3</sup>University of Malaya, University of Malaya, Institute of Biological Sciences, 50603 Kuala Lumpur, Malaysia.

Contact: david.baracchi@gmail.com

The Stenogastrinae represent a sub-family of social wasps belonging to the family Vespidae. Stenogastrinae are endemic to the Indo-Malaysian area and comprise 57 described species belonging to seven genera. Data concerning the phylogenetic relationship among species within the group are still missing except for the one recently obtained for a 15 species belonging to the *Liostenogaster*, *Eustenogaster* and *Parischnogaster* genera through chemical taxonomy and wing morphology. This subfamily shows an astonishing variability in the nest architecture and, even in the same species, the nests are sometimes various. The challenge is understood if these various nest architectures represent intra or inter specific variability. Indeed, in the Stenogastrinae, characters of nest architecture have been used in the past to discriminate among very similar species impossible to distinguish by classic taxonomic methods based on differences in morphological characters. In the present work, we studied three wasp species (*Liostenogaster flavolineata*, *Parischnogaster alternata* and *P. striatula*) that commonly show two different nest architectures with the aims to understand whether each species conceals cryptic species. To do that we collected 12 colonies for each species (6 for each subgroup) and we analysed the wasp fore-wing morphology (by means of geometric morphometrics analysis) and their epicuticular polar compounds (by means of mass spectrometry technique). The results suggest that both *L. flavolineata* and *P. alternata* represent single species with a deep intra specific variability in nest architecture probably required to exploit at maximum the few available nesting sites. By contrast, the wasps belonging to *P. striatula*, up to now considered a single species with two noticeable nest architectures, represent two cryptic species. Furthermore, multivariate statistical analyses showed unexpected similarity between one *P. striatula* subgroup and the *P. alternata* group. This finding confirms the intraspecific distance between the two groups of *P. striatula* but it also opens a new question about their systematic position. With the aim of clarify their systematic relationship, we are currently completing genetic analyses of these wasps. If genetic data will confirm our results, the two *P. striatula* subgroup will be defined as etho-species, and the description of a new species will be required.

## A new look at the Extended Evolutionary Synthesis

**Emanuele Serrelli<sup>1</sup>**

<sup>1</sup>University of Milano Bicocca, Piazza dell'Ateneo Nuovo 1, 20126 Milano, Italy.

Contact: emanuele.serrelli@epistemologia.eu

I present the initiatives, papers, and ideas of Pigliucci, Müller, and others, who are proposing an Extended Evolutionary Synthesis (EES). I then advance some reasons for concern raised by those claims, including uncertainties in timing, historical inaccuracies, lack of a theoretical structure, arbitrariness and instability of the included concepts, stereotypical characterization of the Modern Synthesis, and dissent among evolutionary biologists. Then I mention the studies by historian of the Modern Synthesis, Joe Cain, who is very detailed and careful in explaining that Mayr, Dobzhansky, Huxley & co. who claimed they were part of a Modern Synthesis, they did also for strategic and political reasons, related to their own careers and to more general cultural battles of the time. What I want to argue is not that the Modern Synthesis was an invented product of a marketing operation; rather, it is that the social and interactive dynamics of science are very important in understanding what is going on. The same could be true for the EES in our years. I maintain the primary importance of understanding how biology is today, how it has changed, what future expects us. Pigliucci's question, "Do we need an EES?", thus suggests very important issues. But I propose that we shouldn't take at face value what the protagonists of evolutionary biology see and say. The 'expert review' or the 'small group of architects' methods cannot work. No solution either comes from a traditional philosophical approach of 'describing the structure of evolutionary theory', because scientists don't work 'inside' theories; they use them in different ways. Correct methods for answering could be developed, with the help of advanced technology for analyzing the scientific literature, the ways of doing science, the 'hot topics', the birth and death of fields, etc., through time. This would mean to look seriously at the scientific community, avoiding, on the one hand, the authority principle, and, on the other hand, the surrender to an 'all flows, everything ever changes' perspective. In the context of such an endeavour, I suggest a specific look at the Italian evolutionary biology community as important for the future prospects of this science in our country.

## Biodiversity 3D: Exploring the History of Chameleons

**Krystal Tolley<sup>1</sup>**

<sup>1</sup>South African National Biodiversity Institute, Rhodes Drive, Newlands 7700 Private Bag X7, Claremont 7735 Cape Town, South Africa.  
Contact: k.tolley@sanbi.org.za

Biogeographic patterns are often explained by spatial shifts that match changing environmental conditions to which species are adapted. Biogeographic patterns however, can also be explained through the adaptation of organisms *in situ* to changing environments over time, rather than purely through shifting distributions as a response to those changes. Combining dated phylogenies with information on habitat and morphology suggest that most chameleon genera radiated in the Oligocene, resulting in a phylogeny dominated by paleo-endemic lineages which correlate to the reduction of the Pan African forest and concomitant increase in fine structured habitats (grassy savannah, grassland, heathlands) since the Oligocene. Recent Plio-Pleistocene radiations are scarce, and occur in lineages that occur in fine structure habitats. The combination of morphological and performance data (bite force, gripping, sprinting) suggest that adaptation to these fine-structure habitats has resulted in the evolution of small body size, limb lengthening, hand/feet reduction, and ornamentation reduction. In contrast, paleo-endemic lineages have not radiated substantially since the Oligocene-Miocene, and these lineages retain morphological features that are adaptive in habitats that lack fine-structure vegetation (i.e. bushes/trees, or leaf litter). This work suggests that biogeographic patterns of chameleons can be explained primarily by lineage loss that corresponds with forest habitat reduction since the Oligocene, but that certain lineages in this family have adaptively radiated to new habitats since the Pliocene.

## Cryptic diversity of *Synchaeta* spp. (Rotifera, Monogononta) in mountain lakes: relationships with environmental parameters

**Ulrike Obertegger<sup>1</sup>, Diego Fontaneto<sup>2</sup>, Giovanna Flaim<sup>1</sup>**

<sup>1</sup>Department of Sustainable Agro-ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>2</sup>National Research Council, Institute of Ecosystem Study (CNR-ISE), Pallanza (VB), Italy.

Contact: ulrike.obertegger@fmach.it

Cryptic species (i.e. distinct species that are morphologically similar) may have different ecological requirements and their merging into one species can bias the assessment of the main ecological drivers of biodiversity. We investigated the environmental parameters influencing the occurrence of *Synchaeta* species (*S. pectinata*, *S. grandis*, *S. lakowitziana*, *S. tremula/oblonga* - monogonont rotifers) in 17 waterbodies of the Trentino-South Tyrol region in the Eastern Alps. To improve taxonomic resolution in phylogenetic analysis, a marine *Synchaeta* (i.e. *S. cf. cecilia*) from the United Kingdom was also sampled and sequences from GenBank were downloaded. While *Synchaeta* species were morphologically identified based on trophi structure, cryptic species were identified based on the generalised mixed Yule coalescent (GMYC) model. We performed multivariate ordination both for morphospecies (i.e. unresolved complexes of cryptic species), a common practice in limnological studies based on morphological taxonomy, and for putative cryptic species, made possible by DNA taxonomy. We expected that resolving complexes of cryptic species could provide more information than using morphospecies. Out of the overall phylogenetic tree with 48 haplotypes from 203 individuals, the GMYC model indicated the presence of 14 GMYC entities, 11 from lakes in the Eastern Alps, 2 from Canada, and one from the U.K. *Synchaeta pectinata* showed five GMYC entities and *S. tremula* showed two; all individuals, for which the identification to *S. tremula* or *S. oblonga* was not possible, clustered in one monophyletic clade, here called *S. tremula/oblonga*, that showed additional evidence of three GMYC entities. Environmental-based multivariate ordination on cryptic species explained a significantly higher proportion of variance than that based on morphospecies. Occurrence of putative cryptic species was mainly related to total phosphorus. Moreover, different cryptic species within the same morphospecies showed different, and even opposite, preferences for total phosphorus. In addition, the same GMYC entity of *S. tremula/oblonga* found in Trentino-South Tyrol was also present in Canada and the same haplotype of *S. pectinata* found in Trentino-South Tyrol was also found in the U.K. This wide geographical distribution of haplotypes and cryptic species indicated the absence of dispersal barriers in *Synchaeta*.

## Effects of climate on fine-scale spatial genetic structure in four alpine keystone species

**Elena Mosca<sup>1</sup>, Erica A. Di Pierro<sup>1</sup>, Katharina Budde<sup>2</sup>, David B. Neale<sup>3</sup>, Santiago C. González-Martínez<sup>2</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>2</sup>INIA, Forest Research Centre, Carretera de La Coruña km 7.5, 28040 Madrid, Spain.

<sup>3</sup>University of California at Davis, Department of Plant Sciences, 95616 Davis CA, United States of America.

Contact: elena.mosca@fmach.it

Genetic responses to environmental changes can take place at different spatial scales. While the effect of climate on the wide-range distribution of genetic diversity has been the focus of several recent studies, studies of genetic responses to climate at local scales are relatively scarce. Fine-scale spatial genetic structure (SGS) was investigated in four Alpine conifers (4 to 8 natural populations per species) in the Eastern Italian Alps. SNP assays were used to characterize SGS in *Abies alba* and *Picea abies* (384 SNPs), *Larix decidua* (528 SNPs) and *Pinus cembra* (768 SNPs). Significant SGS was found for 11 out of 25 populations tested, varying from Sp of 0.0018 in *P. cembra* to 0.0035 in *Larix decidua*. Several linear models were constructed to associate SGS with climate variables. Once corrected by confounding effects (e.g. differences of SGS across species due, for instance, to dispersal capability), the best model identified April minimum temperature and spring precipitation as the most relevant climatic variables associated with differences in SGS across populations. To study the potential effect of winter temperature in relation to plant physiology, two ecological indexes related to vegetation growth (chilling-degree-day, CDD, and freezing-degree-day, FDD) were also tested for association to SGS. A significant association was found between SGS and CDD across species. This study provides new insights on the expected genetic responses of four coniferous species to climate change at local scales, suggesting that climate change, through altering SGS, could also have relevant impacts in plant microevolution.

## Mean phylogenetic surprise: a unit of measure for describing changes across biological communities

**Saverio Vicario<sup>1</sup>, Bachir Balech<sup>2</sup>**

<sup>1</sup>Istituto di Tecnologie Biomediche, Consiglio Nazionale delle Ricerche, Italy.

<sup>2</sup>Istituto di Biomebrane e Bioenergetica, Consiglio Nazionale delle Ricerche, Italy.

Contact: saverio.vicario@gmail.com

The availability of NGS technology increased the amount of communities described by environmental sequencing, while the large effort of the biodiversity scientific community mobilized and exposed very large data sets on the presence of organisms in public repository as the Global Biodiversity information facility or Sistema Ambiente 2010 in Italy. These two sources of check lists and species abundance data could be used to build knowledge on how communities are organized and how they correlate with abiotic factors. But, no real consensus exists on how to compare communities both with scalar (Jost, 2006) or phylogenetic indices (McCoy and Matsen, 2013). Generally, phylogenetic indices are expected to be more insightful. In fact, the presence of a given organism within a community depends on its ecological attributes built through the history of evolutionary adaptations and innovations of its lineage. So related organisms are likely to stay in a given community for shared reasons. This consideration makes clear that the correct level of taxonomic classification to study the correlation between abiotic factor and communities is not always at species level. This strategy of including the contribution of higher level classification decreases the effect of low counts (McCoy and Matsen, 2013). Until now, all phylogenetic approaches are within the frame of index based approach where no clear statistical framework links the index to the phenomenon of interest, making difficult to include replicates within the experimental design and in general to interpret the results. In this work, we show the phylogenetic entropy of degree one, a family member of diversity indices, proposed as by Chao et al. (2010), is indeed a true measure of information, that describes the mean surprise of an observer, sampling organisms and classifying them using a given rooted phylogenetic tree. This measure is used to build a formal measure of beta diversity that takes into account phylogenetic structure of communities that is measuring the decrease of surprise from the marginal to the conditional distribution of observation. This phylogenetic beta diversity measure is used to produce an ANOVA-like procedure which uses a phylogenetic tree of all organisms found in all sampled communities and highlight the contribution of each branch of the phylogeny and each group of communities present in the experimental design to the diversification across groups. This framework allows to include replicates (technical or biological) within the pipeline of analysis given that each groups could be represented by several sample communities. The proposed metric is numerically validated as information measure by testing different required properties of information metrics. Precision, accuracy and power of the procedure was tested using simulation of community with log normal distribution of abundance in which each group of community differs in the abundance of a given clade. Results are compared with Shannon based beta diversity estimates. An example use case is used to illustrate the web implementation of the procedure. The figure shows how the method correlates abundances of the leaf in the different groups with branch contribution to beta diversity in the phylogeny.

## Small effective population size and fragmentation in Alpine populations of *Bombina variegata*: the combined effects of recent bottlenecks and postglacial recolonization

**Luca Cornetti<sup>1</sup>, Andrea Benazzo<sup>2</sup>, Cristiano Vernesi<sup>1</sup>, Giorgio Bertorelle<sup>2</sup>**

<sup>1</sup>Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>2</sup>Department of Biology and Evolution, University of Ferrara, Via Borsari 46, 44100 Ferrara, Italy.

Contact: lucacornetti@gmail.com

Amphibians are experiencing population declines in all continents due to anthropogenic factors. Evidence of demographic reduction and local extinction have been also reported for the yellow-bellied toad, *Bombina variegata*, along all its distributional range, which includes the Italian Alps. Here we genotyped at the mtDNA *cytb* and at 11 nuclear microsatellites 200 individuals of *B. variegata* from 9 populations sampled in Trentino (north-eastern Italy.). We investigated the fine-scale population structure and we tested for genetic traces of population decline using different methods. We found that all populations showed low level of genetic diversity in comparison with other studies, low estimates of effective population size, and clear evidence of demographic decline. When the age of the decline is estimated, contrasting results are found. Some methods suggest a recent reduction of population size possibly associated with anthropogenic environmental changes, and others support a more ancient bottleneck dating back to the postglacial recolonization of the Alps. We suggest that both demographic processes occurred in the evolutionary history of the yellow-bellied toad populations, and we are now testing this hypothesis by simulations.

## Microevolution due to pollution in Amphibians

**Emanuele Fasola<sup>1</sup>, Rui Ribeiro<sup>2</sup>, Isabel Lopes<sup>1</sup>**

<sup>1</sup>University of Aveiro, Campus Universitário de Santiago, department of Biology, Aveiro, Portugal.

<sup>2</sup>University of Coimbra, Universidade de Coimbra, Department of Life Sciences, Coimbra, Portugal.

Contact: vandervas@alice.it

Introduction: Genetic variability is important for biological diversity, thus requires conservation measures. Population's genetics is shaped by: 1) Historical events (e.g. postglacial migration); 2) Present environmental stresses (e.g. pollution). Conservation procedures try to mitigate/eliminate nowadays environmental pressures. For example, pollution exerts a great impact through the elimination of sensitive genotypes: contaminant-driven genetic erosion. A population exposed to contaminants show an increase in frequency of resistant genotypes, due to loss of sensitive ones. This can be explained by: 1) physiological alterations: acclimation; 2) appearance of new gene/s underlying resistance mechanisms; 3) loss of sensitive individuals and reduction of the genetic variability through genetic erosion. Only scenarios 2 and 3 lead to a microevolutionary outcome. To distinguish between the two scenarios, researchers should establish if: resistant genotypes are present at reference and impacted site but sensitive genotypes are absent at the latter. Therefore microevolution took place through genetic erosion and not through the arising of new resistance-genes. Focus on the amphibians: Pollution-induced genetic erosion is a central issue for species preservation. This is particularly true for amphibians: their small populations are highly susceptible to genetic drift, inbreeding and erosion. Amphibians are the most globally threatened vertebrates group. Habitat destruction, climate change, increasing UV, diseases, introduction of allochthonous species, and pollution are some causes of amphibians global decline. Furthermore decreased genetic variation can reduce fitness and adaptability to these challenging environments. Little is known about contaminant-driven genetic erosion in amphibians. Nevertheless there is some evidence of its detrimental effects through: 1) reduced fitness, 2) reduced environmental plasticity, 3) absence of protective co-tolerance effects, 4) arising of fitness/resistance trade-offs, 5) increased susceptibility to pathogens. -FitnessGenetic variability should be positively correlated with a higher fitness: genetic-fitness-correlations (GFCs). Amphibians show reliable fitness proxies (developmental rate, growth rate and survival) used to investigate GFCs. Indeed in some species heterozygous individuals show increased fitness in comparison to homozygous conspecifics-environmental plasticity. It is the ability of a genotype to generate different phenotypes in stressed environments. Genetic erosion may be harmful for populations' viability because phenotypic plasticity has a genetic basis and genetic variation for plasticity is generally observed. Cotolerance mechanisms arise from the use of the same metabolic detoxification pathway facing different stressors. It is generally accepted that decreasing genetic variability lowers the tolerance towards other stressors. -Fitness trade-off costs Responses to pollution-induced stresses can be metabolically costly, arising trade-offs. Some studies show how microevolution due to pollution can bear fitness costs associated with the altered physiological processes enabling resistance. -Resistance to pathogens Genetic variability has a role in resistance to pathogens: inbred populations, possibly tolerant to one pathogen, are likely to be susceptible to most other unrelated pathogens. Heterozygosity is very important for the functioning of the immune system. Heterosis improves immune function against *Batrachochytrium dendrobatidis*, a widespread amphibians' parasite.



Conclusion: Effects of genetic erosion are poorly understood. Amphibians' populations often naturally occur as highly inbred. These represent the perfect possibility to measure the impacts of contaminant-driven genetic erosion.

## **Amphibian biodiversity in an alpine region: a multi-level approach. Patterns, processes and conservation implications**

**Alexis Marchesini<sup>1</sup>, Cristiano Vernesi<sup>1</sup>, Andrea Battisti<sup>2</sup>**

<sup>1</sup>Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>2</sup>University of Padova, Department of Agronomy, Food, Natural Resources, Animals and Environment, Viale dell'Università 16, 35020 Legnaro (Padova), Italy.

Contact: alexis.marchesini@fmach.it

The present study aims at investigating the patterns and processes affecting the different levels of amphibian biodiversity (genes, species and ecosystems), focusing on a South-Eastern Alps region (Trentino, Italy). Worldwide biodiversity is threatened by an ongoing crisis, caused directly or indirectly by human activities. Global change continuously stresses populations, ecological communities and natural environments, exposing them to new adaptive challenges. Amphibians are particularly sensitive to perturbations due to their complex life histories, susceptible physiology and low dispersal abilities. Since the '80s they are facing a dramatic decline, known as Global Amphibian Decline. Numerous studies have been carried out in order to monitor the conservation status of populations, their genetic diversity levels, as well as the availability and integrity of amphibian habitats. A growing body of evidence is showing that the different levels in which biological diversity may be divided are broadly linked and ecological processes results from the complex interactions between these parts. Despite of these intrinsic connections, the different aspects of biological diversity have so far been confined to separate lines of research, corresponding to the different fields of evolutionary biology and ecology. To overtake this limitation, modern conservation biology is now increasingly recognizing the need for an integrative approach in the study of the structural and functional components of biodiversity, with the ultimate goal of developing effective strategies to counteract its loss. With this study we made a general attempt in this direction, trying to investigate the evolutionary and ecological processes affecting amphibians populations and communities within a systemic perspective. We chose a widespread amphibian, the common frog (*Rana temporaria*), as target species for the evaluation of genetic diversity between and among populations. Genetic diversity provides the evolutionary potential for populations to adapt to environmental changes, and its preservation is of crucial importance in the context of a widespread amphibian decline. Species distribution records of amphibians species were collected for the whole study area. In addition, accurate information about amphibian communities composition for several selected sites belonging to the Natura2000 network were acquired, together with habitat heterogeneity data. Our goal is to shed light on the interactions between habitat heterogeneity (a proxy for ecosystem diversity) and amphibian diversity, both at the species and genetic level. It is generally accepted that habitat heterogeneity acts as a diversifying force and species richness and genetic diversity are hypothesised to co-vary with a positive correlation. Nevertheless, it is not always clear whether habitat heterogeneity affects the species and genetic level in similar or different patterns, and empirical data are sometimes contradictory. The described research is currently at its initial stage and we presented here some preliminary findings, together with possible conservation outcomes. In addition, some examples (taken from the recent literature) of the effects of one level of biodiversity on the others via several mechanisms are illustrated, with the aim of highlighting their consequences in terms of ecosystem stability and ecosystem services. Lastly, important conservation implications of a good/bad understanding of the nature of these interactions are discussed.

## **Evidence of recombination on the "speciation island" X-chromosome centromeric region between the malaria mosquitoes *Anopheles gambiae* and *An. coluzzii***

**Verena Pichler<sup>1</sup>, David Weetman<sup>2</sup>, Emiliano Mancini<sup>1</sup>, Marco Pombi<sup>1</sup>, José Vicente<sup>3</sup>, Amabelia Rodrigues<sup>4</sup>, Joao Pinto<sup>3</sup>, Alessandra Della Torre<sup>5</sup>, Beniamino Caputo<sup>5</sup>**

<sup>1</sup>Dipartimento di Sanità Pubblica e Malattie Infettive, Università "Sapienza", Rome, Italy. <sup>2</sup>Liverpool School of Tropical Medicine, Liverpool, United Kingdom. <sup>3</sup>Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa Lisbon, Portugal. <sup>4</sup>Instituto Nacional de Saúde Pública - Bissau, Guinea Bissau. <sup>5</sup>Dipartimento di Biologia e Biotecnologie "C. Darwin", Rome, Italy. Contact: vere.pichler@gmail.com

*Anopheles gambiae sensu stricto* (Giles) and *An. coluzzii* (Coetzee & Wilkerson sp.n.) are two of the most important mosquito species transmitting malaria in sub-Saharan Africa. The two species are morphological identical, but can be

identified by specific SNPs in the multi-copy IGS rDNA region (Fanello *et al.* 2002 *Med Vet Entomol.* 16: 461-4) and the specific insertion in *An. coluzzii* of a SINE retrotransposable element located approximately 1 MB apart from the X-centromeric IGS (Santolamazza *et al.* 2008 *Malar J.* 25 7:163). It has been proposed that the two species have diverged despite ongoing gene flow: indeed some genomic regions continue to homogenize, while others, such as the centromeric regions of chromosome 2 and X, resist introgression and might contain the genes responsible for divergence (Turner *et al.* 2005 *PLoS Biol.* 3:e285). This hypothesis has led to define these genomic regions as “Islands of Speciation” and, in particular, the reduced level of recombination on centromeric regions is believed to have promoted the process of genetic divergence between *An. gambiae s.s.* and *An. coluzzii*. In West and Central Africa hybrids between the two species are found at very low frequencies (1% or less) (Coetzee *et al.* 2013 *Zootaxa* 3619: 246–274), but in the westernmost part of Africa, such as The Gambia and Guinea Bissau, an area of putative secondary contact hybrids have been detected with frequencies exceeding 20% (Oliveira *et al.* 2008, *J Med Entomol* 45:1057-63; Caputo *et al.* 2011, *PLoS One* 15 6:e16415). Previous data in female samples have suggested that after the hybridization process and consequent introgression, recombination between the two X-linked centromeric markers (i.e. IGS and SINE) and intra-individual IGS repeat units shuffling, have occurred in this area (Caputo *et al.* 2011, *PLoS One* 15 6:e16415). In order to confirm and better evaluate the frequency of recombination events along the X-centromere (and discriminate this event from heterozygosity) we examined 277 male specimens (which are hemizygous for the sexual chromosome X) of *Anopheles gambiae s.s.*, *Anopheles coluzzii* and putative hybrids collected in 2010 in three sites along a west-to-east transect in *Guinea Bissau*. To this aim, the samples have been genotyped for the two X-linked diagnostic markers (i.e. IGS and SINE) as well as for a third marker, an insertion of 57 bp present in *An. gambiae s.s.* (but absent in *An. coluzzii*) occurring at the intron 4 of cytochrome *CYP4G16* gene (Turner *et al.* 2005 *PLoS Biol.* 3:e285), distant less than 7 kb from the retrotransposable element. Moreover, on a subset of males (n=59) a long range haplotype analysis using 18 SNPs situated on the X centromere has been performed in order to estimate levels of recombination and to evaluate the extent of haplotype homozygosity along the chromosome in *Guinea Bissau* compared to *Burkina Faso*, where the two species are expected to be well differentiated. Our results confirm the presence of intra-individual mixed IGS arrays in this area (nearly 30% of the whole male sample) and show an exceptionally high level of recombination on the X-centromeric area; in fact more than 24% of all the samples analyzed show signs of recombination events. Noteworthy, a high percentage of individuals (>13%) show recombination between the SINE and *CYP4G16* gene, despite the close proximity of these two loci. The long range haplotype analysis show a higher haplotype diversity in Guinean samples compared to *Burkina Faso*, and an intermediate haplotype diversity in hybrids compared to the two parental species. These data indicate that in hybrid zone, recombination has frequently occurred within the most divergent genome region of *An. gambiae s.s.* and *An. Coluzzi* (i.e. X-centromere) and may have been the consequence of a reduced reproductive isolation.

## Metabarcoding a diatom genus: cryptic diversity and Isolation by Time

**Maria Valeria Ruggiero<sup>1</sup>, Diana Sarno<sup>1</sup>, Lucia Barra<sup>1</sup>, Wiebe H.C.F. Kooistra<sup>1</sup>, Marina Montresor<sup>1</sup>, Adriana Zingone<sup>1</sup>**

<sup>1</sup>Stazione Zoologica Anton Dohrn, Villa Comunale 1, 80121 Naples, Italy.

Contact: mariavaleria.ruggiero@szn.it

Diatoms constitute the most abundant and diversified group of marine eukaryotic phytoplankton. Their ecological role is of paramount importance; they are responsible for approximately 40% of the photosynthetic production in the ocean and their contribution to the production of organic matter on a global scale is comparable to that of tropical rain forests worldwide. *Pseudo-nitzschia* (Heterokonta, Bacillariophyceae) is a cosmopolitan diatom genus, present along coastlines worldwide. The genus includes about 37 species, many of which (at least 12), can produce domoic acid, a neurotoxin responsible for Amnesic Shellfish Poisoning (ASP) in humans through bioaccumulation in bivalves. Cryptic and pseudo-cryptic diversity in the genus have been revealed by molecular markers or subtle ultrastructural differences. In the Gulf of Naples (Italy), *Pseudo-nitzschia* is present all year round with different species or morphospecies, as recognised by light microscopy, known to bloom in different seasons. Some morphospecies, however, show more than one peak of abundance per year. Are those temporally distinct peaks due to cryptic species, to different populations of the same species or to different pheno/ecotypes of the same population? To get a comprehensive picture of the seasonal pattern of *Pseudo-nitzschia* species diversity, 19 environmental clone libraries were constructed using genus-specific LSU rDNA primers along one year of sampling at the Long Term Ecological Research (LTER) station MareChiara (Gulf of Naples, Mediterranean Sea). Eighty-four distinct ribotypes were detected within the 15 species so far identified in the Gulf of Naples, demonstrating a much higher diversity for this genus in nature than previously known. In addition, around 40% of these ribotypes showed a strong phenological signal, being present in restricted periods of the year, while only 6% were shared among seasons. The possibility that a reduction of the gene flow on a temporal scale due to different heritable reproductive times (isolation-by-time) could be an important speciation driver in diatoms is discussed.

## Conservation genetics of the Adriatic grayling. Recent and historical exotic imprints on native Adriatic *Thymallus thymallus* populations

Andreas Meraner<sup>1</sup>, Luca Cornetti<sup>1</sup>, [Andrea Gandolfi](#)<sup>1</sup>

<sup>1</sup>Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: andrea.gandolfi@fmach.it

Patterns of population structure and possible genetic introgression have not exhaustively been investigated so far for the 'Adriatic grayling', *Thymallus thymallus*, despite its conservation critical risk status owing to stocking-induced secondary contact. We analysed 683 grayling from 30 Adriatic sites and from Danubian and Atlantic reference samples, merging mtDNA sequence and microsatellite genotypic data and applying model-based clustering as well as Approximate Bayesian Computation (ABC). Remnant Adriatic populations displayed different frequencies of exotic genetic signatures, spanning from marginal genetic introgression to the total collapse of native genepools. Genetic introgression involved multiple exotic source populations of Danubian and Atlantic origin and evidenced the negative impact that few decades of stocking provoked on the original genetic architecture of Adriatic grayling. Within the Adige River system, a contact zone of Western Adriatic and Eastern Danubian grayling populations was highlighted, with ABC analyses suggesting a historical anthropogenic origin of Eastern Adige populations, most likely founded by medieval stocking. Based on genetic results, we propose a catalogue of management measures, including the immediate legal prohibition of stocking exotic grayling strains and the use of marker-assisted genetic selection in supportive- and captive-breeding programs. Finally, we point to substantial river-specific population substructure within the 'Adriatic Grayling' Evolutionary Significant Unit, worth to be considered in future restoration programs.

## DNA barcoding and phylogenetic relationships in marine toxic dinoflagellate genus *Ostreopsis* based on mitochondrial genes

Cecilia Battocchi<sup>1</sup>, Samuela Capellacci<sup>1</sup>, Santiago Fraga<sup>2</sup>, Katerina Aligizaki<sup>3</sup>, Cristiano Vernesi<sup>4</sup>, [Antonella Penna](#)<sup>1</sup>

<sup>1</sup>Department of Biomolecular Sciences, Section of Environmental Biology, University of Urbino, Viale Trieste 296 61121 Pesaro, Italy.

<sup>2</sup>Centro Oceanografico de Vigo (IEO), Subida a Radio Faro 50, Vigo, Spain.

<sup>3</sup>Laboratory Unit on Harmful Marine Microalgae, University of Thessaloniki, Thessaloniki, GR-54124, Greece.

<sup>4</sup>Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: antonella.penna@uniurb.it

The marine dinoflagellate genus *Ostreopsis* includes species producing potent toxic compounds, such as palytoxin and palytoxin analogs, which cause toxic blooms in Mediterranean and tropical or other temperate areas. Phylogenetic and phylogeographical analyses based on ribosomal data set revealed the existence of distinct species and, within them, clades related to geographical distribution. Due to a high morphological variability, species belonging to this genus are very difficult to identify and cryptic species could be present. Thus, developing a standardized DNA barcode approach for this marine dinoflagellate can allow confirming known species and uncover hidden variability with consequent description of new species. These information, besides leading to a better understanding of species diversity in natural environment, could assist in identification and detection of different species directly in field sample. This study represents the first attempt to assess the suitability of mitochondrial *COI* (cytochrome c oxidase 1) and *cob* (cytochrome b) as an identification tool for *Ostreopsis* species. For this purpose, we designed specific primers to amplify and sequence mitochondrial *COI* and *cob* genes from several *Ostreopsis* spp. isolates from worldwide areas. Phylogenetic analyses of single and concatenated mtDNA genes within genus *Ostreopsis*, as well as their intra and inter-specific divergences, were compared to those based on nuclear ribosomal genes LSU and 5.8S-ITS regions. Phylogenetic analyses of the genus *Ostreopsis* using mtDNA genes resulted uninformative, being not able to distinguish different species. Moreover, the so called 'barcode gap' that is interspecific variation exceeding intraspecific variation, was detected only in ribosomal genes. Thus, our original goal to apply the mtDNA barcode to recognize species within this toxic marine dinoflagellate was not accomplished.

## NGS approach for investigating evolutionary transition from oviparity to viviparity in squamate reptiles

**Luca Cornetti<sup>1</sup>, Andrea Benazzo<sup>2</sup>, Mike Bruford<sup>3</sup>, Giorgio Bertorelle<sup>2</sup>, Cristiano Vernesi<sup>1</sup>**

<sup>1</sup>Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>2</sup>Department of Biology and Evolution, University of Ferrara, Via Borsari 46, 44100 Ferrara, Italy.

<sup>3</sup>Cardiff School of Biosciences, The Sir Martin Evans Building, Museum Avenue, Cardiff, CF10 3AX, Wales, United Kingdom.

Contact: lucacornetti@gmail.com

Some squamate reptile species provide a unique model system for gaining crucial information about the evolutionary transition from oviparity to viviparity in vertebrates. The lizard *Zootoca vivipara* is one of the few species with distinct reproductive modalities in different subspecies; in particular, *Z. v. carniolica* is an egg-laying lizard while *Z. v. vivipara* is a live-bearing one; they both live in the Eastern Italian Alps, sometimes in syntopy. This provides an interesting natural setting for studying the evolutionary shift in reproductive mode. Some populations were analysed using classical genetic markers (mitochondrial, nuclear DNA sequences and autosomal microsatellites). The mtDNA results indicated a marked divergence between the two subspecies (around 5% at the *cytochrome B*), as well as nuclear microsatellites. Possible existence of hybrid individuals in Carnic Alps, as recently reported by morphological evidences, has boosted the interest on this topic. RAD-tag sequencing, a next-generation sequencing technique that allows simultaneously discovering and analyzing hundreds of thousands of SNPs, was then applied to *Zootoca vivipara* subspecies in order to identify mutations correlated with the reproductive modality and with related adaptive traits.

## The touch of death: Killer Mediated Contact Inhibition and Hsp12p Secretion determines Differential Fitness in a Community of Grape Yeast Isolates.

**Damariz Rivero<sup>1</sup>, Luisa Bernà<sup>1</sup>, Enrico Baruffini<sup>2</sup>, Irene Stefanini<sup>3</sup>, Duccio Cavalieri<sup>3</sup>**

<sup>1</sup>University Of Florence, Viale Pieraccini 6, 50139 Florence, Italy.

<sup>2</sup>University Of Parma, Viale Usberti 11, 43100 Parma, Italy.

<sup>3</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: damarizrivero@gmail.com

The budding yeast *Saccharomyces cerevisiae* represents the best-understood and most powerful genetic model systems as its genome was the first, out of all the eukaryotic organisms, to be completely sequenced. Recently it has been possible to establish an interaction between genotype and environment to describe fitness in terms of better adaptation to stress. Lately, several disciplines have converged to turn *S. cerevisiae* into a model for the genetic study of ecology and evolution. During wine production, the yeast strains with the best fitness establish strategies to compete for spaces and resources, dominating the natural microbial ecosystems, although it is also possible that they establish behaviours that contribute to intraspecies coexistence. Little is known about the genes and their transcripts responsible for ecological interactions (cooperation and competition) among yeasts. To fill this gap, we performed a genome-wide analysis of two natural yeast strains. Our findings demonstrate that both Heat Shock Protein 12 (*Hsp12p*) and *PAU* genes are not only important for the fitness of the yeast during fermentation, but are also fundamental for cooperation and competition behaviours, respectively. The results highlight the existence of previously unknown cooperative compartment dependent on the secreted *Hsp12p*, counter-balanced by contact-dependent killer inhibition. This work represents a good approach to the use of the yeast *S. cerevisiae* as a model to improve our understanding in the study of the genetics of the ecology.

## Stochastic gene expression and fitness

**Thomas Heams<sup>1</sup>**

<sup>1</sup>AgroParisTech, INRA, 16, rue Claude Bernard, 75231 Paris Cedex 5, France.

Contact: thomas.heams@agroparistech.fr

With the development on gene expression studies on single cells, it appears that stochastic gene expression (SGE) is not only a background 'noise', but a documented constitutive parameter of all living beings, that deserves scrutiny at the evolutionary level. This widespread phenomenon, from prokaryotes to eukaryotes, may challenge the classic view of adaptive evolution at the molecular level as a fine-tuned process of precise regulations, in particular in multicellular organisms. It has conversely also been described as a source of flexibility and even a counter intuitive source of



intercellular synchronization, let alone a potential driver of gene evolution. Some experimental evidences can lead to encompass transcriptional stochasticity as a component of darwinian-like chance/selection processes at work in clonal cell populations. This can have several consequences relative to evolutionary studies. First, intercellular relations might be described, at least partially, in terms of short-term adaptations relying on variable and/or unpredictable behaviours of single cells, allowed by SGE in a supposedly homogeneous environment. In this framework macroscopic (tissue, organ) reproducibility would result for a big part from variations and selection in elementary (cellular, intracellular) events. This has been indeed documented in several contexts, whether it is a general or an infrequent situation remaining an open question. Mechanisms of heritability of adapted individual cellular behaviours within clonal populations may have several and non-mutually exclusive components at different scales, from simple statistical reproducibility to internal constraints that would stabilize individual cells in a given state. Second, such 'cellular darwinism' would be, to some extent, an additional level of selection, alongside individuals and populations. This has to be taken in consideration in a context where group selection has triggered many debates in the recent years. Thus, how such an expanded Darwinism would unify (and therefore strengthen) the theory in life sciences or on the contrary generate interferences between levels (and possibly weaken it) would then need careful investigation in the context of a modern evolutionary synthesis. An orthogonal issue that needs to be addressed is how SGE actually relates with classical deterministic pathways. Indeed, SGE has mainly been studied so far as a perturbation of gene expression, but it has also underlined that some deterministic pathways (e.g. bistable equilibrium) can themselves produce unpredictable elementary events with desirable functional consequences. Therefore, models are needed to describe and predict how selective pressures can take advantage of either flexibility or reliability of cellular events in different contexts. Finally, SGE occurs alongside other sources of somatic variations leading to heterogeneity within multicellular organisms whether they are due to genetic (mutations, rearrangements, mobile elements, copy number variations), epigenetic (methylation, acetylation, chromosome territories) and expression (non-coding RNAs, RNA interference) factors, which all operate at various time and space scales. Here again, the actual relation between these mechanisms and SGE, from synergy to competition, is a promising field in terms of evolutionary studies.

## **Genetic diversity in a small population of Endangered *Eulemur collaris* from South-East Madagascar: a longitudinal study**

**Stefania Bertoncini<sup>1</sup>, [Jacopo D'Ercole<sup>1</sup>](#), Francesca Brisighelli<sup>2,3,4</sup>, Cristian Capelli<sup>4</sup>, Sergio Tofanelli<sup>5</sup>, Giuseppe Donati<sup>6</sup>**

<sup>1</sup>Department of Biology, Università di Pisa, Pisa, Italy.

<sup>2</sup>Forensic Genetics Laboratory, Institute of Legal Medicine, Università Cattolica del Sacro Cuore; Rome, Italy.

<sup>3</sup>Unidade de Xenética, Facultade de Medicina, Instituto de Ciencias Forenses, Universidade de Santiago de Compostela, Santiago de Compostela, Spain.

<sup>4</sup>Department of Zoology, University of Oxford, Oxford, United Kingdom.

<sup>5</sup>Department of Biology, Università di Pisa, Pisa, Italy.

<sup>6</sup>Department of Anthropology and Geography, Oxford Brookes University, Oxford, United Kingdom.

Contact: gdonati@brookes.ac.uk

The collared brown lemur, *Eulemur collaris*, is an endangered prosimian inhabiting the littoral and lowland rainforests of south-eastern Madagascar. In the littoral forest, in particular, the species is threatened by severe habitat fragmentation and thus only small populations survive in tiny fragments. These lemurs have been shown to exhibit an extreme degree of social and ecological flexibility by adopting different group size and ranging areas depending on the level of habitat disturbance. In order to support eco-ethological data and to investigate gene flow dynamics, we analyzed ~300 bp of D-loop mitochondrial DNA and 8 microsatellite loci from 50 faecal samples of collared brown lemurs collected from the littoral forest fragments of Mandena over a period of 11 years (2000, 2004, 2008, 2011). Since these lemurs were relocated in Mandena in 2000 and there were no resident animals in this area before, we can account for 60% of the genetic initial variability of this area. We investigated changes in genetic variability within and between groups over the last 11 years and, as support of field data, parenthood. Preliminary results from mtDNA analysis show the arrival of new lineages in 2008. Similarly, autosomal results indicate an increase of diversity during the last 8 years. This suggests the occurrence of gene flows after the lemur relocation despite strong ecological barriers (i.e. the matrix of savannah, exotic plantations, and roads that today surrounds the area). These findings offer encouraging clues for the future conservation management of this endangered lemur.



## Phenomics of *Picea abies*. Characterization of phenotypic traits involved in long-term adaptation to climate change and their genetic base

**Lorenzo Bonosi<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.  
Contact: lorenzo.bonosi@fmach.it

Norway spruce (*Picea abies* (L.) Karst) is one of the ecologically and economically (timber, tourism) most important tree species in the Trentino-Alto Adige region as well as in the whole of Europe. Considered the current climate change scenarios, a full understanding of the functioning of given complex traits and their genetic base appears to be the only effective way to cope with effects that may be a menace to the performance and the survival of this species. Adaptation to drought stress is among the greatest challenges to forest trees in the current climate change scenario.

In spring 2013, a Norway spruce common garden was established at the Casteller (Trento) nursery run by the Forest service of the Provincia Autonoma di Trento. In early May seeds from 520 certified families covering the entire Alpine range (in cooperation with WSL Switzerland, BFW Vienna, INRA Avignon) seeds were planted as to obtain a half-sib population of 7750 individuals, 15 individuals per family. Mother trees were selected so as to best capture the ecological and specific diversity of Alpine *P. abies* ecosystems. Growth and phenology traits assessed were: germination time, bud burst and bud set, height at the end of the vegetation period. Parallel to the establishment of the common garden, DNA was extracted from needles and from mega gametophytes (IGV- CNR, Sesto Fiorentino) so as to perform the genotyping of a chip of 384 single-nucleotide polymorphisms (SNP) designed from previous work on *Picea* spp. (Chen *et al.* 2011; Canadian Arborea Project (<http://www.arborea.ulaval.ca>); Scalfi *et al.* submitted). A candidate gene-based approach will be then used to search for genetic association between single-nucleotide polymorphisms (SNP) markers detected in candidate genes putatively involved in the control of the measured adaptive traits and the observed phenotypic variation. The discovery of polymorphisms that underlie adaptive phenotypic traits is a fundamental goal of molecular genetics. Elucidation of the genetic components for ecologically relevant traits through association mapping at markers in candidate genes has been achieved for a variety of adaptive phenotypes in other tree species, and it has important putative applications ranging from marker assisted breeding to gene conservation in the face of climate change.

## Evidence for past and present hybridization in three Antarctic icefish species provides new perspectives in the study of an evolutionary radiation

**Andrea Benazzo<sup>1</sup>, Ilaria Anna Maria Marino<sup>2</sup>, Cecilia Agostini<sup>2</sup>, Massimo Mezzavilla<sup>3</sup>, Sean M Hoban<sup>1</sup>, Tomaso Patarnello<sup>4</sup>, Lorenzo Zane<sup>2</sup>, Giorgio Bertorelle<sup>1</sup>**

<sup>1</sup>Department of Life Sciences and Biotechnology, University of Ferrara, Via L. Borsari 46, 44100 Ferrara, Italy.

<sup>2</sup>Department of Biology, University of Padova, Via U. Bassi 58/b, 35121 Padova, Italy.

<sup>3</sup>Institute for Maternal and Child Health- IRCCS Burlo Garofolo and University of Trieste, Via dell'Istria, 65/1, 34137 Trieste, Italy.

<sup>4</sup>Department of Comparative Biomedicine and Food Science, University of Padova, Viale dell'Università 16, 35020 Agripolis, Legnaro (Padova), Italy.

Contact: andrea.benazzo@unife.it

Determining the timing, extent, and underlying causes of inter-specific gene exchange during or following speciation is central to understanding species' evolution. Antarctic notothenioid fish, thanks to the acquisition of antifreeze glycoproteins during Oligocene transition to polar conditions, experienced a spectacular radiation to >100 species during Late Miocene cooling events. The impact of recent glacial cycles on this group is poorly known, but alternating warming and cooling periods may have affected species' distributions, promoted ecological divergence into recurrently opening niches, and/or possibly brought allopatric species into contact. Using microsatellite markers and statistical methods including Approximate Bayesian Computation, we investigated genetic differentiation, hybridization and the possible influence of the last glaciation/deglaciation events in three icefish species of the genus *Chionodraco*. Our results provide strong evidence of contemporary and past introgression by showing that: i) a substantial fraction of contemporary individuals in each species has mixed ancestry; ii) evolutionary scenarios excluding hybridization or including it only in ancient times have small or zero posterior probabilities; iii) the data support a scenario of interspecific gene flow associated with the two most recent interglacial periods. Glacial cycles might therefore have had a profound impact on the genetic composition of Antarctic fauna, as newly available shelf areas during the warmer intervals might have favoured secondary contacts and hybridization between diversified groups. If our findings are confirmed in other notothenioids, they offer new perspectives for understanding evolutionary dynamics of Antarctic fish, and suggest a need for new predictions on the effects of global warming in this group.

## **The Italian Consortium for DNA barcoding ([www.barcodingitaly.it](http://www.barcodingitaly.it))**

maurizio.casiraghi@unimib.it, michele.cesari@unimore.it, roberto.guidetti@unimore.it, massimo.labra@unimib.it

DNA barcoding is a molecular method that allows the identification of biological entities, exploiting the variability of a small portion of DNA. DNA barcoding is so defined because the sequence of nucleotides that make up the fragment of standard DNA specifically defines a given species, as well as the barcode in supermarkets defines a specific product. The DNA barcoding technique has given satisfactory results in most unicellular and multicellular tested organisms, from animals to plants and fungi. The identification of living organisms by means of the DNA barcoding technique has become in a few years a widespread reality in the world of research. It has also given rise to ambitious international initiatives, such as the International Barcode of Life (iBOL), which is a great opportunity to study biodiversity both locally and globally. One of the main features of iBOL is the creation of a network of organizations and research laboratories. In Italy, several laboratories perform researches in which the DNA barcoding is used to study biodiversity at local and global levels, for food traceability, and for identification of commercial frauds. Up to now these laboratories have worked independently, but recently the Italian Consortium for DNA barcoding was created in order to build a network of private and public institutions and organizations that use, or are planning to use, this method. The main aims of the Italian Consortium for DNA barcoding are: 1) characterization of a network among the Consortium participants to increase collaborations and the synergic activities; 2) identification of reference laboratories for training activities, aimed principally at young researchers; 3) identification of the main topics of active researches in Italy, with the aim of planning common projects; 4) connection with international entities that operate in the field of DNA barcoding to share issues, researches and results; 5) connection between the research institutions and commercial entities that deal with the identification of living organisms. The research units participating to the Consortium are public institutions (such as Universities, Custom Agency, Council for Research and Experimentation in Agriculture, National Research Council, Museums), and several private laboratories. Among the first activities of the Consortium are the creation of a digital platform ([www.barcodingitaly.it](http://www.barcodingitaly.it)), the organization of a “Basic course of DNA barcoding” (June 11-12, 2013, Modena, Italy), and of a workshop “Application of DNA barcoding in the identification and traceability of organisms” (October 3, 2013, Modena Italy). The Consortium of DNA barcoding was created thanks to the support of the Italian Society for Evolutionary Biology (SIBE), the Italian Zoological Union (UZI) and the Italian Botanical Society (SBI). For information on the Consortium please log on the website [www.barcodingitaly.it](http://www.barcodingitaly.it), and write to [barcodingitaly@gmail.com](mailto:barcodingitaly@gmail.com), or to the reference persons above.

# LIFE THROUGH TIME: PALEOBIOLOGY AND PALEOBIODIVERSITY

The "first time" of paleontology at the SIBE congress aims to bridge the still existing (particularly in Italy) gap between Biology and Earth Science departments. The session will be constituted by paleontological or paleontology-related contributions with deep roots in paleobiology. Phylogenetic reconstructions, paleobiodiversity, evolutionary trends through time, developmental paleobiology, paleo evo-devo and molecular clocks will be under the focus of this interdisciplinary meeting. We aim to shed light on those research groups working on evolutionary paleobiology in Italy and abroad and to create a network of wisdom in this field. Contributions on all taxonomic branches are welcomed.

**Chairs: Silvio Renesto, Giorgio Binelli (University of Insubria), Massimo Bernardi (MUSE) and Omar Rota Stabelli (Fondazione Edmund Mach)**

## Origin of biodiversity

### Mike Benton<sup>1</sup>

<sup>1</sup>*School of Earth Sciences, University of Bristol, Wills Memorial Building, ♀Queen's Road, Clifton BS8 1RJ, Bristol, United Kingdom.*  
Contact: mike.benton@bristol.ac.uk

## Cretaceous Ophidiiform fish from North-Eastern Italy: systematics and evolutionary implications

### Giorgio Carnevale<sup>1</sup>, G.D. Johnson<sup>2</sup>

<sup>1</sup>*Dipartimento di Scienze della Terra, Università degli Studi di Torino, Via Valperga Caluso 35, 10125 Torino, Italy.*

<sup>2</sup>*Division of Fishes, Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, 10th and Constitution Ave, NW Washington, D.C. 20560-0159, United States of America.*  
contact: giorgio.carnevale@unito.it

Percomorph fishes [Division Percomorphacea *sensu* Wiley & Johnson (2010)] represent the largest and most successful evolutionary lineage of vertebrate animals, with more than 15.000 described species characterized by astonishing morphological and ecological diversity. Percomorphs first appeared in the record at the base of the Upper Cretaceous, in the Cenomanian stage, around 99 Ma (Patterson, 1993). The first 35 million of years of history of these fishes, however, are largely unknown because of their relatively low abundance in the record, which possibly reflects their original reduced ecological relevance in the upper Mesozoic aquatic ecosystems. A recent analysis of the historical trends of morphospace occupation suggests that Cretaceous percomorphs were characterized by a very low anatomical diversity and that the origin of modern body plans occurred only in the early Cenozoic in the aftermath of the K/T mass extinction, primarily to fill vacated functional roles (Friedman, 2010). This hypothesis, however, does not appear to be consistent with the results of a study of the tempo and mode of vertebrate diversification that identified percomorphs as the fastest radiating clade of gnathostomes (Alfaro et al., 2009), with most of the modern lineages originating during the Cretaceous. The fish assemblage from the Cretaceous paralac deposits of Trebiciano (Campanian-Maastrichtian; Venturini et al., 2008), a few kilometres east of Trieste, north-eastern Italy, seem to support the latter hypothesis. Among a large number of basal neopterygians, ellimmichthyiforms, and goniorhynchiformes is a moderately diverse assemblage of derived percomorphs, thereby demonstrating that this group was represented by a relatively broad anatomical diversity before the K/T biotic crisis. Accordingly, a detailed comparative analysis of these Cretaceous percomorphs is necessary in order to properly interpret their anatomical structure and phylogenetic relationships. The high scientific value of these fossils lies in the fact that they may provide a strong interpretative key for the understanding of the origin of body plan diversity within percomorphs. One of the most interesting specimens of this assemblage is a well-preserved articulated skeleton of an ophidiiform fish. To date, the Cretaceous existence of ophidiiform fishes has been demonstrated by very rare Maastrichtian otoliths characterized by a plesiomorphic morphology. In view of that, the Cretaceous articulated skeleton from north-eastern Italy possibly represents the earliest evidence of the Ophidiiformes in the record.

This Cretaceous ophidiiform exhibits a peculiar set of features (e.g., maxilla posteriorly expanded; presence of a single large supramaxilla; presence of 7 branchiostegal rays; possession of an ossified supraneural; 13 caudal-fin rays; high ratio vertebral number/dorsal-fin rays; etc.) that clearly evidence its basal phylogenetic position within the group and it is thus tentatively interpreted as a stem-group member of the Ophidiiformes basal to all other extant and extinct genera.

## From the Palaeozoic to the Mesozoic - how did the flora change in Europe?

### Evelyn Kustatscher<sup>1</sup>

*1Museo Scienze Naturali dell'Alto Adige, Via Bottai 1, Bolzano, Italy.*

Contact: Evelyn.Kustatscher@naturmuseum.it

The Permian-Triassic boundary, boundary between the Palaeozoic and the Mesozoic, marks the largest mass extinction in Earth history, primarily in the marine realm. The terrestrial plant record from this time period is scarce and mainly restricted to palynological studies. Moreover, several groups long regarded as typical Mesozoic (e.g., cycads, conifers) are now known from the uppermost Carboniferous and Permian, appearing first in drier or temporary dry (sub)tropical areas. The disappearance of many arborescent lycopsids, cordaites and some pteridosperm groups during the late Carboniferous to Permian is one of the most important floral changes of the Palaeozoic, but did not occur simultaneously worldwide. Thus it seems that part of the floral change from the Palaeozoic to the Mesozoic occurred already during the end of the Palaeozoic and not only at the Permian-Triassic boundary. Also the time constrain and dynamics of the biotic recovery of the flora after the end- Permian mass extinction are still open for discussions. It was always thought, that the earliest floras from Europe were dominated by the herbaceous lycophytes called Pleuromeia; also the palynomorph assemblages were devoid of gymnosperms. If most plant groups were indeed missing or restricted to small refugial areas is, however, still open to debate. Even more, since restudy of historical floras as well as discoveries of new floras and palynological studies at high latitudes suggest that Early Triassic floras were more diversified than previously thought: lycophytes were associated with sphenophytes, ferns, seed ferns (Peltaspermales) and conifers. Already in the Anisian the flora is very diversified, much more than during the Late Permian, with lycophytes, sphenophytes, ferns, seed ferns, cycads, ginkgophytes and conifers. This could suggest that actually the change in the flora from the Palaeozoic to the Mesozoic was much more gradual than previously understood.

## Macroevolutionary emergence of onshore-offshore gradients in community turnover

### Adam Tomašových<sup>1</sup>, Stefano Dominici<sup>2</sup>, Martin Zuschin<sup>3</sup>, Didier Merla<sup>4</sup>

*<sup>1</sup>Geological Institute, Slovak Academy of Sciences, Dúbravská cesta 9, 84005 Bratislava, Slovakia.*

*<sup>2</sup>Museo di Storia Naturale, Università di Firenze, Via La Pira 4, 50121 Firenze, Italy.*

*<sup>3</sup>Department of Palaeontology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria.*

*<sup>4</sup>Département Histoire de la Terre, UMR CNRS 7207, CR2P, CP 38, Muséum National d'Histoire Naturelle, 8 rue Buffon, 75005 Paris, France.*

Contact: stefano.dominici@unifi.it

In contrast to deeper habitats, onshore environments exhibit high taxonomic turnover at macroevolutionary time scales (>10 Myr), but the mechanisms generating this pattern remain unclear. Here we test for the first time whether onshore-offshore decline in turnover of molluscan communities holds at shorter time scales (<1 Myr). We find that turnover does not change with depth within Eocene and Pliocene at short scales and shows significant regional-scale stasis in species abundance at all depths. It demonstrates that onshore-offshore differences in ecosystem dynamic at short scales cannot be extrapolated to macroevolutionary gradients in turnover. Turnover, however, significantly declines with depth at long scales between Eocene and Pliocene. This decline is generated by a higher persistence in per-family species abundance in deeper habitats, but also by a higher persistence in per-family species richness in deeper habitats. We argue that at macroevolutionary time scales, lineages in onshore habitats are more exposed to the risk of regional-scale habitat loss and fragmentation owing to marked climatic shifts not experienced over short time scales. In contrast, offshore habitats with milder latitudinal temperature variation provide more opportunities for recolonisation and survival. Onshore-offshore gradients in turnover thus emerge only when assessed over temporal scales exceeding several Myr, and can be related to higher probability of extreme environmental shifts in onshore environments.

## The diversity and biogeographic significance of the Norian (late triassic) terrestrial reptiles from northern Italy

### Silvio Renesto<sup>1</sup>

<sup>1</sup>Dipartimento di Scienze Teoriche e Applicate, Università degli Studi dell'Insubria, Via Dunant 3, I 21100 Varese, Italy.

Contact: [silvio.renesto@uninsubria.it](mailto:silvio.renesto@uninsubria.it)

New evidence and recent studies have significantly improved knowledge about the life habits and diversity of reptiles collected from the Norian (Late Triassic) localities of northern Italy. The fossils are found in marly limestones which deposited at the anoxic bottom of intraplatform basins surrounded by ephemeral islands with freshwater reservoirs on which a diverse reptile fauna thrived. The findings comprise few aquatic reptiles (placodonts, thalattosaurs, phytosaurs) and many terrestrial reptiles: rhynchocephalians, archosaurs, protorosaurs and drepanosaurids showing a high diversity of ecological adaptations. Comparisons with other coeval faunas from both Europe and the USA suggest that the Late Triassic reptile fauna of the Italian localities does not consist of endemic taxa with little relationship to other reptiles collected in coeval localities as previously thought, but rather they are the result of the rapid diversification in an insular environment of taxa also well represented at other localities.

## Developmental Palaeontology from an Italian perspective

### Massimo Delfino<sup>1,2</sup>, Marcelo Sánchez<sup>3</sup>

<sup>1</sup>Dipartimento di Scienze della Terra, Università di Torino, Via Valperga Caluso 35, I-10125, Torino, Italy.

<sup>2</sup>Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain.

<sup>3</sup>Paläontologisches Institut und Museum, Universität Zürich, Karl Schmid-Strasse 4, CH-8006 Zürich, Switzerland.

Contact: [massimo.delfino@unito.it](mailto:massimo.delfino@unito.it)

The last few years were marked by a growing interest in the fossil record of the ontogenetic processes. The launch of the website Developmental Palaeontology, a large, searchable database focusing on the literature related to fossils of non adult vertebrate specimens, was followed by a monographic issue of the journal Seminars in Cell & Developmental Biology dedicated to fossil ontogenies, and then by the popular book *Embryos in Deep Time* dealing with different aspects of growth as preserved in the fossil record. More recently, the front cover of the Journal of Vertebrate Paleontology featured the paper Developmental biology enriches palaeontology and the prestigious journal Nature titled the front cover Jurassic Embryology. Besides these remarkable examples, a number of papers dealing with the topic were published in a wide variety of journals. While investigating the subject for a chapter to be added to the Italian translation of *Embryos in Deep Time*, we have been asking ourselves: is there an Italian contribution to the Developmental Palaeontology? Does the Italian fossil record provide significant fossils of non-adults? Is the Italian palaeontological community focusing on the topic? The only Italian palaeontologists working regularly with non-adult organism are palaeobotanists. Pollens, spores and seeds are frequently studied by Italian researchers, but the aim of their studies is to taxonomically identify the organism that produced them in order to provide climatic and palaeoenvironmental reconstructions. They do not focus on the growth process itself. Due to their abundance and small size, fossil foraminiferans represent an interesting material for developmental studies. X-ray microtomography applied to a variety of taxa revealed different growth patterns and, just to mention an unexpected result, it was recently proposed that chamber size of some extinct taxa oscillated during ontogeny with a periodicity that was correlated to tides, moon cycles and seasonal variations. Trilobites also offer a unique opportunity to explore postembryonic development within the fossil record of arthropod evolution and it has been demonstrated that hypotheses concerning the developmental pattern and control can be tested in organisms belonging to an extinct clade. The fossil record of vertebrates is much less complete and does not offer material as abundant as that of invertebrates. Besides several juvenile reptile specimens coming from Mesozoic outcrops of the Alpine area, worth mentioning are spectacular fossils as *Besanosaurus leptorhincus* and *Scipionix samniticus*, respectively represented by ichthyosaur embryos fossilized within the mother's body and by a superbly preserved juvenile theropod. The morphological features associated to a non-adult condition has been the focus of studies on these animals, and developmental subjects could be further addressed with them, for example using palaeohistological tools. Insular dwarfism as a process related to peculiar ecological interactions has been the topic of some interesting works, but late Cenozoic insular environments have been the habitat of several forms that because of their singular size, such as giant hedgehogs and rodents, could be excellent subjects of study on growth patterns. Even if there are a few remarkable examples of non-adult fossil organisms coming from Italy, and some researchers dedicated part of their efforts for studying these and other remains or topics related to growth and ontogeny, the Italian contribution to the Developmental Palaeontology could be much expanded. However, the fact that in Italy, Palaeontology and Palaeobiology are associated to the SSD GEO 01 and researchers committed to a University career usually work in Earth Sciences Departments appears to constraint the intellectual environment in which the study of fossils is approached by involving the researchers more in Geology oriented projects than in integrative Geo-Biological programs.



## Fossilized ontogenies in evo-devo: the case of trilobites

**Giuseppe Fusco<sup>1</sup>, Paul S. Hong<sup>2</sup>, Nigel C. Hughes<sup>3</sup>**

<sup>1</sup>Department of Biology, University of Padova, 35131 Padova, Italy.

<sup>2</sup>School of Earth and Environmental Sciences, Seoul National University, Seoul 151-747, Korea.

<sup>3</sup>Department of Earth Sciences, University of California, Riverside, Riverside CA 92521, United States of America.

Contact: giuseppe.fusco@unipd.it

A key feature of the evolutionary developmental biology (evo-devo) approach to the study of evolutionary change is a dialectical interplay between the research on how developmental systems evolve and the exploration of how developmental processes affect organismal evolution (Müller 2007). By providing information on the primitive states of developmental characters, the study of ontogenetic series of extinct organisms, or 'fossilized ontogenies', can complement developmental studies on living organisms in the fulfillment of both tasks (Fusco et al. 2012). Despite the potential of a developmental approach to phenotypic evolution in extinct clades, in general the fossil record does not permit deep inspection of the development of extinct organisms, but for a few cases, among which is a major clade of extinct arthropods, the Trilobita. In these arthropods, early post-embryonic biomineralization of the exoskeleton has resulted in a record of fossilized ontogenies that is among the most comprehensive for any extinct group (Hughes 2003). In recent years, several studies have shed light on ancestral features of trilobite post-embryonic development. Beyond numerous descriptive, taxon-specific studies (e.g., Dai and Zhang 2013, Kihm et al. 2013, Zhu et al. 2013), a few broader comparative studies of trilobite growth, segmentation and tagmata differentiation have permitted outlining a 'space of developmental pathways' for this group (e.g., Minelli et al. 2003, Hughes et al. 2006, Fusco et al. 2012). In addition, the remarkably rich preservation of the Czech middle Silurian trilobite *Aulacopleura koninckii* has allowed investigation of the control mechanisms of growth and segmentation in this species, providing a model system for the study of regulation of developmental dynamics in primitive arthropods (Fusco et al. 2004). Here we present some preliminary results on the ongoing research. On the whole, these studies show that through morphometric analysis of appropriate datasets it is possible to address questions of high interest for evolutionary developmental biology using data from fossils. By revealing detailed developmental features at deep nodes of the phylogenetic tree, these studies can contribute to illuminating the evolution and evolvability of early organism body patterning.

## Early ontogeny in a fossil ginkgophyte from the Triassic resembles modern *Ginkgo biloba*

**Kathleen Bauer<sup>1,2</sup>, Evelyn Kustatscher<sup>1,2</sup>, Lea Grauvogel-Stamm<sup>3</sup>, Michael Krings<sup>2</sup>**

<sup>1</sup>Bindergasse 1, 39100 Bolzano/Bozen, Italy;

<sup>2</sup>Department für Geo- und Umweltwissenschaften, Paläontologie und Geobiologie, Ludwig-Maximilians-Universität, and Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Straße 10, 80333 München, Germany.

<sup>3</sup>4 Place du Marché aux Poissons, 67000 Strasbourg, France.

Contact: Kathleen.Bauer@naturmuseum.it

Evidence of gymnosperm ontogeny is exceedingly rare in the fossil record. The only known specimens include a putative *Glossopteris* seedling from India, seedlings of the herbaceous conifer *Aethophyllum* from the Triassic of France, and seedlings assigned to the Araucariaceae and Welwitschiaceae from the Cretaceous of Brazil. However, no information is available to date about the ontogenetic development of fossil ginkgophytes. Some 20 exquisitely preserved compressions of Early Anisian (Middle Triassic) gymnosperm seedlings assignable to the ginkgophytes with almost absolute certainty have recently been discovered in a collection of fossils from the Voltzia Sandstone of the Vosges (northeastern France) assembled by the late Louis Grauvogel (Strasbourg, France). The seedlings provide the first insights into the early ontogeny of a fossil ginkgophyte. The specimens display different stages of the ontogenetic development, from germination to the appearance of the first "true" leaves. The developmental stages represented by the fossils are remarkably similar morphologically to the ontogenetic stages observed in the extant *Ginkgo biloba*. This suggests that the mechanisms governing ontogeny in this Triassic ginkgophyte are comparable (if not identical) to those in *G. biloba*. The precise systematic position within the ginkgophytes of the seedlings from the Voltzia Sandstone remains unresolved. Nevertheless, they represent an important discovery because they add a new complement of data to our understanding of the evolution of ginkgophytes by permitting a direct correlation of ontogenetic stages of an extinct taxon with its sole modern relative.

## Molecular palaeobiology of early animal evolution

### **Davide Pisani**<sup>1</sup>

<sup>1</sup>University of Bristol, School of Biological Sciences, Bristol, United Kingdom.

Contact: [davide.pisani@bristol.ac.uk](mailto:davide.pisani@bristol.ac.uk)

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?

### **Domenico D'Alelio**<sup>1</sup>, **Andrea Gandolfi**<sup>2</sup>, **Nico Salmaso**<sup>2</sup>, **Claudia Ciniglia**<sup>3</sup>, **Manuela Iovinella**<sup>4</sup>, **Marina Montresor**<sup>1</sup>, **Maria Valeria Ruggiero**<sup>1</sup>

<sup>1</sup>Stazione Zoologia Anton Dohrn, Villa Comunale, 1, 80121 Napoli, Italy.

<sup>2</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>3</sup>Seconda Università di Napoli, Via Antonio Vivaldi, 43, 81100 Caserta, Italy.

<sup>4</sup>Università di Napoli Federico II, Via Cintia, 21, 80126, Napoli, Italy.

Contact: [dalelio@szn.it](mailto:dalelio@szn.it)

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

**Carlos J. Rivera-Rivera<sup>1</sup>, Juan I. Montoya-Burgos<sup>1</sup>**

<sup>1</sup>*Université de Genève, 30 quai Ernest Ansermet, 1211 Geneva, Switzerland*

Contact: carlos.riverarivera@unige.ch

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

**Massimo Mezzavilla<sup>1</sup>, Silvia Ghirotto<sup>2</sup>**

<sup>1</sup>*Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", University of Trieste, Via dell'Istria, 65, Italy.*

<sup>2</sup>*Department of Life Sciences and Biotechnologies, University of Ferrara, Via Luigi Borsari, 46, Italy.*

Contact: mzzvilla@gmail.com

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

between populations given the  $N_e$  values calculated from the within-population LD data and a matrix of between-populations FST. These routines have been designed to human populations, but they can easily be adapted to other species whenever genetic map positions are available. The functions contained in the R package NeON aimed to shed light on effective population size of human chromosomes from LD patterns of genome-wide SNPs data, describe demography and estimate the time of divergence between populations. The NeON package enables to accommodate variable numbers of individuals, populations and genetic markers, allowing to analyze them using standard personal computers. The performance of this package was tested estimating the demography and the time of divergence between all the populations of the POPRES database, which includes nearly 6,000 subjects, and over 500,000 single-nucleotide polymorphisms.

## Is *Megachirella wachtleri* (Reptilia, Diapsida) a squamate?

**Massimo Bernardi<sup>1</sup>, Silvio Renesto<sup>2</sup>**

<sup>1</sup>MuSe - Museo delle Scienze, Corso del Lavoro e della Scienza 3, 38123 Trento, Italy.

<sup>2</sup>Dipartimento di Scienze Teoriche e Applicate, Università degli Studi dell'Insubria, Via Dunant 3, I 21100 Varese, Italy.

Contact: massimo.bernardi@muse.it

*Megachirella wachtleri* Renesto et Posenato, 2003, a well preserved partial reptile skeleton from the Middle Triassic of the Dolomites (N Italy) was originally considered a lepidosauromorph, but no phylogenetic analysis was carried out. Consequently, the taxon was overlooked in subsequent later phylogenetic analyses of the Diapsida. The holotype and only known specimen of *Megachirella wachtleri* was recently redescribed (Renesto and Bernardi, 2013), allowing an investigation of its phylogenetic relationships. Phylogenetic analyses confirm that *Megachirella* is a lepidosauromorph close to the crown group lepidosaurs (Squamata+Rhynchocephalia). *Megachirella* enhances our knowledge on the series of morphological modifications that led to the origin of the Lepidosauria, the most diverse clade of extant reptiles, but highlights the problem of taxon sampling in phylogenetic analysis of basal lepidosauromorphs.

## The exemplar co-radiation of insects and plants: phylogenomic perspectives, paleo-ecological implications, agricultural applications

**Omar Rota Stabelli<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: omar.rota@fmach.it

Insects are the most numerous and specious animals on earth, and played a key role in structuring most past and Present ecological niches, including the highly anthropized agricultural ones. Here I outline some of the results of a Marie Curie project aimed at investigating how insects and other ecdysozoans evolved during earth history, adapted to key paleo-ecological events and to agricultural niches, and evolved in relation to plants. The most interesting result is about the colonisation of land by arthropods (Rota Stabelli et al. 2013 Current Biology) which happen, according to molecules, toward the end of the Cambrian, much before fossils suggests and in a period that has been always considered incompatible with life, but that recent findings make plausible.

## Intraspecific variability of *Ginkgo biloba* leaves: implications for assessing ginkgophyte diversity in the fossil record

**Kathleen Bauer<sup>1,2</sup>, Evelyn Kustatscher<sup>1,2</sup>, Michael Krings<sup>2</sup>**

<sup>1</sup>Bindergasse 1, 39100 Bolzano/Bozen, Italy;

<sup>2</sup>Department für Geo- und Umweltwissenschaften, Paläontologie und Geobiologie, Ludwig-Maximilians-Universität, and Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Straße 10, 80333 München, Germany.

Contact: Kathleen.Bauer@naturmuseum.it

The ginkgophytes are a group of gymnosperms that originated in the late Palaeozoic. The group radiated greatly during the Mesozoic, but today is represented by only a single species, *Ginkgo biloba*. *Ginkgo biloba* is characterized by considerable intraspecific variability with regard to leaf size, basal angle and dissection depth – parameters that can also

be measured in fossil ginkgophyte leaves. The fossil record of ginkgophyte foliage largely consists of impressions and compressions, only a few of the latter also yielding cuticles. Based on the presumption that ginkgophytes represent a relatively conservative group of gymnosperms, it has been speculated that all taxa within this group have a comparably high intraspecific variability with regard to leaf morphology. However, many fossil ginkgophyte taxa are defined (exclusively or in part) based on leaf macro-morphology. *Ginkgo biloba* leaves from different localities worldwide, including China, Germany, Italy, Japan, USA, and Russia, have been measured and compared to literature data on fossil ginkgophyte leaves. Comparison of the fossil leaf parameters with the measured range of morphological leaf variability in *G. biloba* suggests that many historical foliage taxa in fact range within the intraspecific variability of one species. On the other hand, two shoot fragments to which are attached *Baiera digitata* leaves have been described from the late Permian Kupferschiefer flora of Germany. These two important specimens suggest that the *Baiera digitata* plant produced leaves distinctly less variable than those seen in the extant *G. biloba*. As a result, leaf morphological parameters such as the dimensions, basal angle and dissection depth cannot be completely rejected as a valuable tool in the identification of fossil ginkgophyte taxa.



# NEW PhDs ON THE BLOCKS

The symposium benefits from the collaboration with FIRS>T, the PhD programme of FEM. This symposium is reserved for young scientists who would like to talk about their research, their results (no matter at which stage) and their passion towards evolutionary biology in a friendly atmosphere.

**Chairs: Lino Ometto and Alessandro Gretter (Fondazione Edmund Mach)**

## Genetic variability in the promoter of miR397 in *Picea abies*

**Luisa Bresadola<sup>1</sup>, Mingai Li<sup>1</sup>, Silvio Schueler<sup>2</sup>, Caroline Heiri<sup>3</sup>, Lorenzo Bonosi<sup>1</sup>, Claudio Varotto<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

<sup>2</sup>Bundesforschungszentrum für Wald, Hauptstrasse, 7 A-1140 Vienna, Austria

<sup>3</sup>Swiss Federal Institute for Forest, Snow and Landscape Research, Zuercherstrasse, 111 8903 Birmensdorf, Switzerland

Contact: luisa.bresadola@fmach.it

Norway spruce (*Picea abies* Karst.) is a tree species that belongs to conifers, a taxon that is extremely important both from an ecological and an economical point of view. Being perennial, this species has often to face suboptimal environmental conditions and to adapt to them: microRNAs are a fundamental class of regulatory molecules, often involved in stress responses and therefore, potentially very important for plant adaptive processes. The focus of this study is on miR397: in *Arabidopsis thaliana* miR397 was shown to be involved in the regulation of copper homeostasis and of the transcription of laccases. These enzymes operate during lignin biosynthesis and therefore their regulation is really important in woody plants like Norway spruce, in order to react to mechanical stress and to resist to the attack of pathogens. In order to understand miR397 regulatory mechanisms, its promoter was isolated in this species and putative regulatory elements were identified. This region, together with the microRNA stem-loop region, was sequenced in seeds produced by individuals originating from different alpine populations in Italy, Austria and Switzerland. In the mature miR397, the most important part for microRNA regulatory function, no polymorphism was found in the analyzed samples. This result suggests that purifying selection is probably acting on this sequence in order to preserve microRNA functionality. As regards the promoter region, several single nucleotide polymorphisms (SNPs) and some insertions/deletions were identified. Some of them are located in the putative regulatory elements, therefore they are good candidates to test if they influence the microRNA expression level and if they have consequences on phenotype that can be relevant in the process of adaptation. This will provide deeper insights into the adaptive role of microRNAs in Conifers.

## Non-LTR retrotransposon R2 molecular characterization and activity in *Bacillus rossius* (Phasmida, Bacillidae)

**Claudia Scavariello<sup>1</sup>, Livia Bonandin<sup>1</sup>, Andrea Luchetti<sup>1</sup>, Barbara Mantovani<sup>1</sup>**

<sup>1</sup>Università degli Studi di Bologna, Via Selmi, 3 Bologna, Italy.

Contact: claudia.scavariello2@unibo.it

The non-LTR retrotransposon R2 is one of the most analyzed transposable elements (TEs), its presence being recovered from diploblastic organisms to lower vertebrates. It inserts in the sequence 5'-TTAA↓GGTAGC-3' of the 28S ribosomal gene, thus affecting the production of functional rRNAs. The evolutionary relationship between retrotransposon activity and reproductive biology of the host species is still debated: while some studies suggest that genomes with limited effective recombination (unisexuals and asexuals) accumulate TEs with a low capacity to eliminate them, gonochoric organisms better manage their proliferation (Muller's ratchet). In order to go through this issue, we are studying R2 distribution and dynamics in the facultative parthenogenetic stick-insect *Bacillus rossius*. In Italy, *B. rossius rossius*, spreading along the Western peninsular coasts and in North-Western Sardinia, and *B. rossius redtenbacheri*, distributed along the peninsular eastern coasts, in Sicily and in South-Eastern Sardinia, occur with gonochoric and unisexual (parthenogenetic) populations. The R2 complete sequence was PCR amplified and sequenced from gonochoric populations of *B. r. rossius* from Capalbio (Tuscany) and of *B. r. redtenbacheri* from Patti (Sicily). The R2 activity was studied through the 5' end deletions analysis in selected parental individuals and in a sample (10-20 individuals) of their offspring. In particular we analyzed the progeny of two females each of the parthenogenetic *B. r. redtenbacheri* populations from

Curcuraci, Massa San Nicola and Castanea delle Furie (Sicily) and the male progeny of crosses involving parental females either from parthenogenetic populations (*B. r. redtenbacheri* Curcuraci ♀ X *B. r. rossius* Anzio ♂; two crosses) or from *B. r. rossius* gonochoric ones (Anzio ♀ X Anzio ♂, one cross; Capalbio ♀ X Capalbio ♂, two crosses). The R2 complete sequence in the Capalbio population is 3,515 bp long and has an A+T content equal to 47%. The ORF is 3,165 bp long and encodes for 1,054 amino acids. The R2 complete sequence in the Patti population is 3,717 bp long and exhibits an A+T content equal to 49%. The ORF is 3,176 bp long, but it is degenerated having several stop codons and a frameshift mutation. Therefore, for a selection of parthenogenetic and gonochoric populations of both subspecies, a R2 sequence survey has been performed to verify the ORF structure. From this analysis, it emerged that *B. r. rossius* populations have R2 elements with an intact ORF, *B. r. redtenbacheri* Sicilian populations have elements with a degenerated ORF and *B. r. redtenbacheri* peninsular populations have both kinds of elements without any relationship to the reproductive strategy. The insertional activity analyses showed new insertions in the thelytokous offspring of parthenogenetic females and in the amphimictic progeny of crosses (1-6 and 1-8, respectively), indicating that the element is actively transposing. However, no new insertions have been observed in the offspring of crosses between parthenogenetic females and gonochoric males. The deletion of truncated variants was also detected: one event in the progeny of a parthenogenetic female (Massa San Nicola) and one event in the progeny of the cross Anzio ♀ X Anzio ♂. Contrary to Muller's ratchet expectations, this pattern of insertion indicates that genomic turnover mechanisms seem active even in parthenogenetic genomes.

## Effects of cryptic genetic variation on innovability in *Escherichia coli*

**Emanuele Rigato<sup>1</sup>, Giulio Bertoloni<sup>2</sup>, Giuseppe Fusco<sup>1</sup>**

<sup>1</sup>Department of Biology, University of Padova, Via U. Bassi 58/B, Italy.

<sup>2</sup>Histology, Microbiology and Medical Biotechnologies Department, University of Padova, Via A. Gabelli 63, 35121 Padova  
Contact: leistus9@yahoo.it

Widespread phenotype resistance to genetic mutation ('phenotype robustness') allows accumulation of neutral genetic variation. This variation, called "cryptic genetic variation" (CGV), has no effects on phenotypes in a particular genetic or environmental context, but can become phenotypically expressed as a consequence of genetic mutations or environmental changes. Thus CGV might have a key role in the kinetic of adaptation. Since evolutionary adaptation by natural selection requires phenotypic variation, the fraction of variation emerging from CGV could enhance innovability (sensu Wagner). This effect has been demonstrated with theoretical studies, using computational models, and with experimental studies on ribozymes. However, this has not been investigated yet in more complex evolving systems, as in whole organisms, where this phenomenon depends on a number of little known parameters of the genotype→phenotype map, such as the amount of epistasis, pleiotropy and neutrality. The aim of the present study is to test for the role of CGV in the adaptative performances of a whole organismal system, the prokaryote *Escherichia coli*, in exploiting different carbon sources (glycerol and lactate). The experimental design consists in generating populations with different amounts of CGV by subjecting the same genotype (BW30270) to EMS and UV light and subsequently exposing the clones to stabilizing selection in the context of their native carbon source (glucose). These populations with identical phenotypes, but different amount of CGV, are then tested for hundreds of generations for Darwinian adaptation in metabolizing new carbon sources. The kinetic of adaptation is measured and interpreted with appropriate models. Here, some preliminary results of the ongoing experiments are presented.

## Towards a phylogeny of the geophilomorph centipede genus *Stenotaenia*

**Laura Del Latte<sup>1</sup>, Francesca Bortolin<sup>1</sup>, Lucio Bonato<sup>1</sup>, Giuseppe Fusco<sup>1</sup>**

<sup>1</sup>Department of Biology, University of Padova, Via Ugo Bassi 58b, 35131 Padova, Italy.

Contact: laura.del.latte@gmail.com

In the context of a research investigating possible correlations between segmental modularity and developmental stability in geophilomorph centipedes, this study aims at reconstructing the phylogenetic relationships within the genus *Stenotaenia*. This will provide a basis for a forthcoming comparative analysis aimed at testing the existence of a trade-off between the number of body segments and the precision of their phenotypic expression. *Stenotaenia* is mainly spread in the central-eastern part of the Mediterranean region. It exhibits high interspecific variability in the number of trunk segments and adult body size, while other morphological characters show very little variation. Presently, about five different morphospecies can be recognized, but the taxonomy of the genus is not well established. Many species are sampled only rarely and collection specimens suitable for DNA extraction are infrequent. We managed to sample a dozen populations from throughout the distribution range and representative of different morphospecies. Ongoing phylogenetic

analysis is based on four genetic markers: two from the mitochondrial genome, cytochrome c oxidase I (*COI*) and 16S rDNA, and two from the nuclear genome, 18S and 28S rDNA. These markers have been successfully used in previous phylogenetic studies at different taxonomic levels in centipedes. Levels of intraspecific and interspecific genetic variation are estimated through the analysis of COI sequences.

## The control regions of the male- and female-transmitted mitochondrial DNAs in *Musculista senhousia* (Bivalvia Mytilidae)

**Davide Guerra<sup>1</sup>, Fabrizio Ghiselli<sup>1</sup>, Marco Passamonti<sup>1</sup>**

<sup>1</sup>University of Bologna, Dipartimento di Scienze Biologiche, Geologiche e Ambientali (BiGeA), Via Selmi 3, Bologna, Italy.

Contact: [davide.guerra7@unibo.it](mailto:davide.guerra7@unibo.it)

*Musculista senhousia* is a marine mussel with Doubly Uniparental Inheritance (DUI). In DUI species two types of mitochondria are present, one inherited through eggs (F), the other through sperm (M). F and M mitochondrial DNAs (mtDNAs) may differ in length, sequence and gene content. Their control regions (CRs) usually diverge in sequence and length, but may also share regions with relatively high sequence similarity. Both *M. Senhousia* mtDNAs contain lineage-specific sequences and a large unassigned region (LUR) with highly similar subunits. We analyzed the F and M LURs of *M. senhousia* and we found molecular signatures typical of a CR, i.e. the presence of shared secondary structures and sequence motifs. Moreover, by comparing *M. senhousia* CRs to other Mytilids, we demonstrated that signals for basic mtDNA functions are retained over evolutionary times even among the fast-evolving mtDNAs of DUI species. We also found that *M. senhousia* F-mtDNA CR is duplicated in tandem, with the two copies being functional and evolving in concert. Duplication and concerted evolution are not uncommon in metazoan mtDNAs, but this is the first case for a duplicated and tandemly repeated CR in a Bivalve species. F CR was found more variable than M, the opposite of what is commonly found in DUI. This can be due to the sampling of a population whose genetic structure is the result of a recent founder-effect, as the specimens used for this study were retrieved from an invading population in Venice Lagoon. Sequences from the original geographic area are needed to confirm this scenario. On the other hand, previous studies proposed that a replication mechanism involving two CRs might be less accurate, leading to a higher mutation/replication rate of mtDNA. Usually, in DUI species, the M-mtDNA is thought to be the faster replicating one: this to account for the M capacity of invading germline during male gametogenesis. If we accept that the higher variability of F is the outcome of a higher replication rate, still M succeeds in invading germline in *M. senhousia* males, so that a higher duplication rate is not necessary to a successful invasion of the male germline by M. An active selection of M mitochondria to be transmitted through germline seems more sound here. We also discuss on how similarities between *M. senhousia* LURs and differences in gene content between its F and M mtDNAs may be explained in the context of DUI evolution and phylogeny. It is still unclear if DUI evolved once or many times in Bivalvia. Accepting a single origin, the analogies between *M. senhousia* FLUR and MLUR may be explained by an inter-genomic recombination. However, this alone cannot simultaneously account for: i) similarities between *M. senhousia* FLUR and MLUR; ii) absence of M-specific sequences in the MLUR (apart from small spacers), and iii) presence of lineage-specific coding sequences in the two mt lines (i.e. a lineage-specific ORF in the F and a duplicated *cox2* in M). On the other hand, if we consider independent origins of DUI, the above mentioned characteristics can be explained by a common origin of F and M from an ancestral maternally transmitted mtDNA, while their different organization may have been acquired after the gain of DUI. *M. senhousia* and other three DUI species (*Brachidontes exustus*, *Geukensia demissa* and *Mytella charruana*) cluster in the Mytilid phylogeny in a taxon-joining pattern, i.e. the F and M sequences of a species cluster separately from those of the others. This further complicates the reconstruction of the origin of DUI among Mytilids, as we have to consider either multiple route-reversals (i.e. M and F may switch their transmission routes) or independent origins of DUI to explain the clustering pattern. A clear answer is not yet available, and it emerges from this latter point a pressing need for more complete mt genomes from as many DUI species as possible, to help our comprehension of DUI origin and evolution in bivalves.

## A comparison of linguistic and genomic diversity in Europe.

**Silvia Ghirotto<sup>1</sup>, Francesca Tassi<sup>1</sup>, Andrea Benazzo<sup>1</sup>, Cristina Guardiano<sup>2</sup>, Guido Barbujani<sup>1</sup>, Giuseppe Longobardi<sup>3</sup>**

<sup>1</sup>University of Ferrara, Department of Life Sciences and Biotechnologies, Via Borsari 46, 44100 Ferrara, Italy.

<sup>2</sup>University of Modena and Reggio Emilia, Department of Communication Sciences and Economics, Viale A. Allegri 9, 42100 Reggio Emilia, Italy.

<sup>3</sup>University of York, Department of Language and Linguistic Science, Heslington, York, YO10 5DD, United Kingdom

Contact: [tssfnc@unife.it](mailto:tssfnc@unife.it)

The notion that patterns of linguistic and biological variation may cast light on each other and on population histories dates back to Darwin's times, but turning this intuition into a proper research project has met with serious methodological difficulties. New approaches using measures of linguistic diversity based on syntactic (as opposed to lexical) features have the potential to bypass these difficulties. In this study, we first validated the method, showing that the well-established set of relationships among European languages can actually be reconstructed with good statistical confidence from syntactic comparisons. We then compared the linguistic structure of Europe with its genetic structure, inferred from the analysis of >229,000 SNPs in 15 populations, 12 of them speaking Indo-European languages. We found a highly significant degree of correlation between measures of syntactic and genomic diversity and, contrary to what observed in studies based on smaller datasets, we found that geographic distances are poorer predictors of genomic differences than linguistic distances. In addition, a good correlation was found between syntactic and lexical distances in the subset of the data (among Indo-European speakers) where the comparison was possible. A discriminant analysis of principal components identified a genetic clustering of populations in remarkable agreement with their linguistic affiliation. We conclude that by focusing on structural linguistic features larger-scale comparisons are now feasible, which may cast light on processes of both biological and cultural change. The research leading to these results has received funding from the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013)/ERC grant agreement n° 295733.

### Epigenetics and symbiosis. The effect of *Varroa destructor* parasitic mite on honeybee (*Apis mellifera*) DNA methylation

**Sara G. Baccei<sup>1</sup>, Valentina Mangoni<sup>1</sup>, Maurizio Casiraghi<sup>1</sup>**

<sup>1</sup>University of Milano Bicocca, Piazza della Scienza 2, Milano, Italy.

Contact: s.baccei@campus.unimib.it

DNA methylation is a key epigenetic modification which consist in the addition of a methyl group on a cytosine in a CG dinucleotide. The mechanism of DNA methylation is shared among all organisms and provides a source for the switching off of gene activities, the maintenance of stable phenotypes and the integration of environmental and genomic signals. In honey bees, DNA methylation is found exclusively in transcribed regions and predominantly in exons with low G+C content and few CpGs overall. Moreover in honeybees, the major functional role of DNA methylation is the regulation of splice variants diversity rather than silencing gene transcription. The honeybee (*Apis mellifera*, Insecta: Hymenoptera) has become an important model for genetic study, especially as its genome has been sequenced. It is also an important economic insect as it is the world's principal crop pollinator and honey producer. These activities have been threatened by the spread of the mite *Varroa destructor* (Acari: Parasitiformes). Varroa mites are ectoparasites of honeybees, parasitising both immature and adult bees and reproducing in cells in the honeycomb that contains brood. Varroa mites impair the honeybee immune system and in some cases boost the amplification of bee viruses. In this study, we are exploring the hypothesis that varroa mite infestation might modify DNA methylation pattern of honeybee pupae infested. DNA methylation pattern are studied using sodium bisulfite treatment that convert methylated cytosine into uracil whereas unmethylated cytosines remain as cytosine in the sequence. Allowing discrimination between methylated and non-methylated nucleotides, sodium bisulfite treatment provides a reliable and detailed picture of the methylation state of DNA. Moreover, the overall level of genome methylation was compared between parasitized and non-parasitized individuals by FT-IR measures of genome extracts.

### A molecular approach to conservational issues: the case of *Austropotamobius italicus* (Crustacea: Decapoda) in Northern Italy.

**Guido Bernini<sup>1</sup>, Adriana Bellati<sup>1</sup>, Irene Pellegrino<sup>2</sup>, Alessandro Negri<sup>2</sup>, Paolo Galeotti<sup>3</sup>**

<sup>1</sup>Department of Earth and Environmental Science, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy.

<sup>2</sup>Department of Science and Technological Innovation, University of Oriental Piedmont, Viale T. Michel 11, 15121 Alessandria, Italy.

<sup>3</sup>Department of Earth and Environmental Science, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy.

Contact: guido.bernini@gmail.com

The Italian white-clawed crayfish *Austropotamobius italicus* (Crustacea: Decapoda) is a freshwater crustacean once widely distributed in Italy., now classified as "endangered" in the "IUCN Red List of Threatened Species" and threatened by a multitude of anthropogenic factors such as habitat destruction, pollution, invasion of exotic crayfish and climate change. Phylogenetic studies conducted in Italy using the mitochondrial genes coding for 16S ribosomal RNA and for Cytochrome c oxidase subunit I as molecular markers showed a complex phylogeographic pattern, with 4 different subspecies proposed



(*A. i. carsicus*, *A. i. carinthiacus*, *A. i. italicus* and *A. i. meridionalis*) and under this scenario conservational efforts need a genetic background, particularly when dealing with restocking or reintroduction actions. Indeed, maintaining genetic variability is a key factor to ensure long-term survival of endangered species and to avoid the loss of biodiversity. The main aim of this work is to analyze the phylogenetic relationships and sequence polymorphism of white-clawed crayfish populations in Lombardy Alpine foothills area and in northern Apennines, providing in this way a useful genetic knowledge basis for management actions and future conservation projects. Nearly 230 tissue samples of *A. italicus* were collected in the field from more than 50 different streams by removing a portion of a pereopod from the fifth pair. Samples were stored in 95% ethanol filled 1.5 ml microtubes and stocked at -20°; tissue lysis and total DNA extraction were performed, and aliquots of each sample were used to carry out PCR selective amplifications of both 3' and 5' adjacent portions of the mitochondrial gene Cytochrome c Oxidase subunit I (*COI* mtDNA, nearly 1170 base pairs long) using two couples of primers specifically designed for this study. Sequences were analyzed under a parsimony approach to build a haplotype network and phylogenetic inference was inferred with Bayesian, Maximum Likelihood approaches, selecting best substitution models using jModelTest. Homologous sequences of *A. pallipes* were obtained in the laboratory with the same procedure and used as outgroup. Genetic diversity parameters such as haplotype diversity, nucleotide diversity and mean number of pairwise differences were computed, and population genetic structure was investigated by AMOVA and Mismatch distribution analysis. Overall, 64 haplotypes were found. Preliminary results show the presence of an area of sympatry for *A. i. carsicus* and *A. i. carinthiacus* in the Adda river catchment. Moreover, haplotypes from eastern Lombardy appear well differentiated from all the other *A. i. carsicus* haplotypes, showing a higher-than-expected diversity for this area. On the other hand, populations from Northern Apennines show the lowest diversity values. In conclusion, this work contributes to define the status of different evolutionary significant units (ESUs) for *A. italicus* in Northern Italy., with the perspective to find application in future conservation projects.

## Physiological and biochemical adaptive responses and defense mechanisms to withstand increasing ultraviolet radiation and temperatures in an Antarctic meiofauna organism

**Iliaria Giovannini<sup>1</sup>, Roberto Guidetti<sup>1</sup>, Tiziana Altiero<sup>2</sup>, Michele Cesari<sup>1</sup>, Gigliola Montorfano<sup>3</sup>, Angela Maria Rizzo<sup>3</sup>, Roberto Bertolani<sup>2</sup>, Lorena Rebecchi<sup>1</sup>**

<sup>1</sup>Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia-X; <sup>2</sup>Dipartimento di Educazione e Scienze Umane, Università di Modena e Reggio Emilia; <sup>3</sup>Dipartimento di Farmacologia e Scienze Biomolecolari, Università di Milano  
Contact: [ilaria.giovannini@unimore.it](mailto:ilaria.giovannini@unimore.it)

Climate changes, are leading to an increase in ultraviolet radiation (UV), by reducing the amount of tropospheric ozone. All life forms are susceptible to UV and their effects are reinforced by rising temperatures, which lead to consequences on human and ecosystems health. This has become an important and emerging issue, particularly for the Polar regions. Antarctic micrometazoans are especially vulnerable to the synergistic effects of these two environmental factors, as their growing season happens in the spring period of ozone depletion. Tardigrades, together with rotifers, represent the main terrestrial invertebrate components of Antarctic fauna in terms of distribution, number of specimens, and colonized substrates. They survive adverse environmental conditions (i.e. dehydration or freezing) by temporally suspending their metabolism. This adaptive strategy, called cryptobiosis, allows to maintain cell integrity and to repair damaged proteins, lipids, and nucleic acids (Guidetti *et al.* 2011, *J. Insect Physiol.* 57:567–576). During cryptobiosis, harmful reactive oxygen species (ROS) are found to be one important source of cell damage. Tardigrades developed natural countermeasures to cope with the oxidative stress, showing a highly efficient antioxidant system (Rizzo *et al.* 2010, *Comp. Biochem. Physiol. B* 156:115-121). In addition, recent studies indicate that the ability to repair DNA damage may be central in tolerating desiccation and also harmful UV (Gladyshev & Meselson 2008, *PNAS* 105:5139–5144). For these reasons, tardigrades are good animal models to study the combined effects of UV and temperature changing on Antarctic organisms, and to detect molecules acting as bio-protectants for future bio-prospecting studies. We analyzed the physiological and biochemical responses of *Acutuncus antarcticus*, one of the most abundant tardigrade species colonizing bryophytes and freshwater sediments of Antarctica. Animals were exposed to increasing UV and temperatures, in order to evaluate the separate and synergistic effects of these two factors. Animal dehydration under controlled lab conditions has shown that *A. antarcticus* is able to enter anhydrobiosis with a high survival rate (92.8%), which is unusual for a tardigrade species that generally colonizes aquatic environments. Antioxidant molecules and enzymes do not seem responsible for this unexpected survival as, with the exception of catalase, there were no significant differences in the activity/amount of antioxidant molecules comparing hydrated and desiccated specimens. Instead, significant differences in antioxidants between hydrated and desiccated specimens have been detected in the semiterrestrial tardigrade *Paramacrobiotus richtersi*, living in temperate regions (Rizzo *et al.* 2010).



Experiments to evaluate the ability of *A. antarcticus* to tolerate increasing temperatures (from 8°C to 41°C) have shown that its capability to withstand high temperatures (33°C = 100% of survivorship; 37°C = 35% of survivorship), even though for a short time. This species is more thermo-tolerant than the true freshwater *Borealibius zetlandicus*, a boreal-alpine tardigrade species very common at high altitudes of temperate regions and in Greenland (Rebecchi *et al.* 2009, J. Limnol. 68:64-70), but less tolerant than *P. richtersi*. Both hydrated and desiccated of *A. antarcticus* specimens showed a good tolerance to UV. The hydrated specimens survived up to a UV dose of 61.9 kJ m<sup>-2</sup> (5% of survivorship), while the desiccated specimens tolerated up to a dose of 74.8 kJ m<sup>-2</sup> (7.5% of survivorship). The exposition of active specimens to a LD50 UV dose (28.6 kJ m<sup>-2</sup>), showed that the negative effects of UV on *A. antarcticus* survival increase in combination with the increase of temperature values (8°C = 42.6% of survivorship; 15°C = 1.7% of survivorship). These data suggest that *A. antarcticus* has the potential to overcome the environmental changes due to increasing temperature and UV radiation.

## A genetic engineering approach to study the adaptation of the grapevine moth *Lobesia botrana* from its wild hosts to *Vitis*

**Umberto Salvagnin<sup>1</sup>, Mickael Malnoy<sup>1</sup>, Stefan Martens<sup>1</sup>, Gianfranco Anfora<sup>1</sup>**

<sup>1</sup>Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy.

Contact: umberto.salvagnin@fmach.it

The grapevine moth *Lobesia botrana* is an extremely polyphagous insect, endemic of the Palearctic region and known across all the Mediterranean area as an economically important pest in the vineyards. Since the chemical ecology of the moth has been a major topic of research for decades, much is known about its behavior. Larvae of the moth can feed up to 40 plant species belonging to 27 different families and the major targets of feeding, if available, are always the reproductive tissues (flowers, fruits). A crucial role in host finding and egg-laying behaviors is played by specific volatile compounds, released by the plant and perceived by the insect olfactory system. Since the adaptation to *Vitis* is recent (the first intense damages were recorded only from the early 20th century), experiments were carried out in the last years comparing the emission of volatiles between grapevine and *Daphne gnidium*, considered the first host of *L. botrana* in the wild: as expected, the volatiles profile partially overlapped. Recent wind-tunnel studies have also shown that a blend of the 3 specific terpenoids (E)- $\beta$ -caryophyllene, (E)- $\beta$ -farnesene and (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) elicits in laboratory assays the same *L. botrana* attraction than the complete grapevine headspace collection. Of these terpenoids, only one ( $\beta$ -caryophyllene) is common between the two plants, while the others are present only in *Vitis* and were thus the target of the recent adaptation. To better understand the evolution of the plant-insect interaction, we decided to take a non host plant - the model *Arabidopsis thaliana* - and by means of genetic engineering to insert the genes responsible for the production of the three terpenoids, in order to mimic the volatile profile of the known hosts. Recently we also undertook experiments aimed at the genetic engineering manipulation of the pathway of the three terpenoids in *Vitis*: the alteration of the volatile profile will be studied as a possible starting point for the creation of a new pest control strategy.

## Sperm-seminal fluid interaction in a scenario of sperm competition

**Federica Poli<sup>1</sup>, Lisa Locatello<sup>2</sup>, Maria Berica Rasotto<sup>1</sup>**

<sup>1</sup>University of Padova, Department of Biology, Via U. Bassi 58/B, 35121 Padova, Italy.

<sup>2</sup>University of Padova, Department of Comparative Biomedicine and Food Science, Viale dell'Università 16, 35020 Legnaro (PD), Italy.

Contact: fedepolipoli@hotmail.it

Sperm competition, occurring whenever the ejaculate of rival males compete to fertilize the same group of eggs (1) is a powerful evolutionary force, shaping male behaviour, morphology and physiology (2-3). To date theoretical and empirical studies on the effects of sperm competition have primarily focused on how sperm characteristics affect the fertilization success of competing males (3). However, seminal fluid, often making up a large part of an ejaculate, influences own sperm performance and may potentially influence the outcome of sperm competition, by also affecting that of rivals (4-6). Recent theoretical analyses predict that selection should favour phenotypic plasticity in male expenditure on the ejaculate components that affect its competitive weight. In particular, variability in seminal fluid investment, in relation to sperm competition risk, is expected where mating modalities allow ejaculates to incapacitate other males'

sperm or to exploit other males' seminal fluid, enhancing own sperm performance (7). Two fish species, the grass goby (*Zosterisessor ophiocephalus*) and the black goby (*Gobius niger*), provide a great model to assess the influence that seminal fluid could have in a sperm competition scenario. Indeed both species show territorial-sneaker mating tactics, where sperm competition risk varies according to the tactic adopted (8). However, they differ in the opportunity for sneakers to release their ejaculate in proximity to those of territorial males and to eggs. We tested the role of seminal fluid in influencing sperm performances (in terms of velocity and fertilization success) of competing males by experimentally manipulating male ejaculates, separately combining sperm and seminal fluid from territorial and sneaker males. In the grass goby, where sperm of males adopting different tactics have similar performances (velocity, ATP content, viability) and ejaculate may be released in close proximity, we found that seminal fluid influences differently the sperm performance of male adopting a different tactic. Indeed, the performance of territorial males' sperm is negatively affected by the seminal fluid of sneaker males, while sneakers' sperm perform significantly better in the presence of territorial males' seminal fluid. By contrast, preliminary tests, show that in the black goby, where sneakers' sperm are faster and more viable than those of territorial males and the ejaculates of competing males are released far from each other, the presence of the seminal fluid of male adopting a different tactic does not affect sperm performances. These findings indicate that phenotypic plasticity in male expenditure on both sperm and non-sperm components of the ejaculate may depend critically on, which component of the ejaculate, sperm or seminal fluid, influences its success in a competition contest. References (1) Parker, Biol Rev 1970 (2) Birkhead & Møller, Ac Rev 1998 (3) Snook, Trends Ecol Evol 2005 (4) Pojani, Behav Ecol Sociobiol 2006 (5) Holman, Funct Ecol 2009 (6) Simmons & Beveridge, PLoS ONE 2011 (7) Cameron *et al.* Am Nat 2007 (8) Locatello *et al.* Funct Ec 2007

## **“Play with evolution”: evolutionary games with “daily life” tools – activities for primary and secondary school teachers and students**

**Matteo Bisanti<sup>1</sup>, Aurora Pederzoli<sup>1</sup>, Roberto Guidetti<sup>1</sup>**

<sup>1</sup>Dipartimento di Scienze della Vita, Università degli Studi di Modena e Reggio Emilia, Via Campi 213\d, 41125 Modena, Italy.

Contact: [matteo.bisanti@unimore.it](mailto:matteo.bisanti@unimore.it)

Evolution is a fundamental topic in school and in science teaching. Unfortunately at school it is very difficult to explain it. The main problem in teaching evolution is that evolution has both a conceptual dimension and a historical dimension, difficult arguments to explain especially in primary school. First of all we thought about activities that deal with evolution in school and that can help teachers to present evolution in a different way respect to the classic pedagogical approach. This approach, based on frontal lessons, unlikely produces a constructivist view of knowing in students. With this in mind, we developed a project of alternative didactics that using inductive methods directs student to understand evolutionary topics. The students are involved and stimulated in learning processes by creative activities that teachers can use, not only as a way to teach, but also as a way to capture students' interest. The project “Evolutionary games with daily life tools” (Giocare all’Evoluzione) was funded by the European Society for Evolutionary Biology (ESEB) in 2011. The project aimed to produce educational materials on evolutionary topics for primary and secondary school teachers and other educational staff that can be used without the assistance of an expert. We produced twenty cards in PDF format with games and activities or scientific demonstrations that use common tools and objects easy to find and linked to everyday life. These games help teachers to talk about evolution in an easy and a funny way. We proposed many topics related to animal adaptations, DNA, phylogeny, human evolution and evolutionary processes and mechanisms. We wrote these activities thinking that people use them as a play. So students and players can use only “poor” tools, as pens, straws, pins, toothpicks, coloured bottle caps, rubber bands, sheets, markers, in order to allow everyone to use these cards in all educative conditions, formal and informal, and to allow everyone to test and to change every activities. Evolution remains the main topic in the project and we created in every card a very simple and short introduction that is fundamental to understand the issues of the cards. Teachers can integrate these activities with tales helping contextualization and interest of students. We developed tools easy to use and read that do not need an expert that explain them. These tools have been transposed in files written in Italian and English, they are available and spread through workshops and internet to the teacher communities. These educational tools have been tested with teachers and students. We had constant relationships and feedbacks from teachers about the project in order to improve the cards. Teachers have tested playable cards at school within class activities and we have presented our project in teacher education courses. Now we are translating the activities in English, we would improve games thanks to feedbacks from schools, we would create other activities and we would publish them on internet and other media.

## Evolutionary Aesthetics: How did the aesthetic attitude originally arise in the course of evolution?

**Mariagrazia Portera<sup>1</sup>**

<sup>1</sup>University of Florence, Department of Philosophy, Via Bolognese 54, 50139 Firenze, Italia

Contact: mariagrazia.portera@unifi.it

In recent years Aesthetics, a branch of philosophy officially founded in the eighteenth century (Baumgarten 1735, 1750; Kant 1790), has made significant progress towards a genuine dialogue with the evolutionary theory: Evolutionary Aesthetics is a recent specialization of Aesthetics, but nonetheless lively and promising (Volland, Grammer 2003; Menninghaus 2003). The main purpose of the Evolutionary Aesthetics is to explore and define the evolutionary origins of the human aesthetic attitude, that is to say how, when and why humans have evolved the faculty to perceive aesthetically, to orient themselves aesthetically toward the world and to formulate aesthetic judgements (“this is beautiful/this is awful” etc.) about objects (not necessarily works of art), spending a considerable amount of time in aesthetic behaviors (Desideri 2004, 2011). According to Darwin (1871), whose famous book “The Descent of Man and Selection in Relation to Sex” is considered the “foundation stone” of Evolutionary Aesthetics, also animals and not only humans (*Homo sapiens*) have a genuine aesthetic faculty, which operates in the context of sexual selection (Cronin 1991). Females of several species are able to judge aesthetically which of their suitors is the most attractive and beautiful (Welsch 2004, 2013). Only in the latest decades were performed the first empirical studies showing that male ornaments are really favored by female choice (Andersson 1982), that female choice is not a mere decrypting of male's value of fitness (Burley 1986) and that female aesthetic judgment is essentially a judgment based on pleasure (Welsch 2013). Darwin, in his book, did not clarify explicitly the evolutionary transition from the animal aesthetic sense to the human one: this is a topic of a rather heated debate in Evolutionary Aesthetics. Is the human aesthetic attitude a direct development of the animal (sexually connoted) aesthetic sense? Miller (2001) believes that not only aesthetic behaviors but also the most fundamental human cognitive and mental abilities are a product of sexual selection (cfr. Fischer 1915; Menninghaus 2003). Evolutionary psychologists (Tooby, Cosmides 2001) think on the other hand that the human aesthetic attitude is an adaptation specifically shaped by natural selection: thanks to their aesthetic competence our pleistocenic ancestors should have been able to select the most adaptive habitat (Kaplan, Kaplan 1989; the “savannah-hypothesis”), the best genetically equipped sexual partner (Buss 2012) and to “tune” their cognitive abilities (Tooby, Cosmides 2001). Moving from a more ontogenetic and developmental perspective, Dissanayake (2000, 2001) suggests that the roots of our aesthetic attitude are in the mother-child early interactions, which are not sexually connoted and very common among mammals. In my research poster I will try to integrate these different models, following two steps: 1) reviewing examples (from not-human animals) of non-functional, auto-telic, intrinsically motivated and pleasurable behaviors (like in playing: Burghardt 2005), which I assume as “precursors” of the human aesthetic faculty; 2) defining the various ontogenetic, phylogenetic, historical and cultural constraints (Gould 1977, 2002) that could have contributed to transform (an exaptation Gould, Vrba 1982) that animal “precursor” in the human aesthetic attitude as today usually understood.

## Unraveling the nature of dormancy: has diapause evolved from a starvation-like response in *Drosophila melanogaster*?

**Gabriele Andreatta<sup>1</sup>, Rodolfo Costa<sup>1</sup>**

<sup>1</sup>Department of Biology, University of Padova, Via Ugo Bassi 58B, Padova, Italy.

Contact: gabrieleandreatta@gmail.com

Coping with adverse environmental conditions is one of the most crucial challenges for all living beings. One of the best examples of physiological strategies triggered by environmental stimuli is diapause, an inducible pre-programmed developmental arrest, which characterizes the life cycle of different species, from *Caenorhabditis elegans* to mammals (1). In insects, the diapause phenomenon exhibits characteristics of a stress-induced physiological response, like a downregulation of systemic insulin signaling (2) and a reduced metabolic rate (3), but nothing is known about the humoral factor/s which trigger or maintain diapause in *Drosophila melanogaster*. First we decided to characterize, in the fruit fly, the role of dopamine (a biogenic amine involved in different stress-related responses) (4-5) in diapause. Our preliminary results confirm the conserved role of dopamine in regulating diapause in insects and in turn the nature of diapause as a stress-induced phenomenon. This suggests the hypothesis that, in *D. melanogaster*, diapause could have evolved from another stress-related response, similar to a starvation-like one, given the tropical origin of this species. For this reason, we decided to focus on TOR (Target of Rapamycin) signaling in the fat body (FB), an organ analogous to vertebrate white fat and liver. Our results show that downregulating the TOR pathway in the FB increases diapause incidence, while an activation of the TOR signaling cascade triggers an exit from diapause. Taken together, these results shed new light on the role of the FB and nutritional status in *D. melanogaster* diapause trajectory, and provide a first insight into the evolutionary origin of insect diapause.

## Phenotypic plasticity: with a little help from a friend. Neurobiology supports Evolutionary Ecology

**Andrea Gazzola<sup>1</sup>, P. Galeotti<sup>1</sup>, F. Brandalise<sup>2</sup>, P. Rossi<sup>2</sup>**

<sup>1</sup>Department of Earth and Environmental Science, Pavia University, Pavia, Italy

<sup>2</sup>Department of Biology and Biotechnology, Pavia University, Pavia, Italy.

Contact: [andreagazzolalor@gmail.com](mailto:andreagazzolalor@gmail.com)

Phenotypic plasticity is the process by which organisms modify their development, behavior and physiology in response to a changing environment. Due to an extreme sensitivity to several environmental stimuli during their development, the eggs and larvae of amphibians represent an optimal model for studying phenotype modifications. There are two important, still open questions about phenotypic plasticity: 1) what is the actual nature of the signal causing a plastic response; 2) how the information carried out by the signal is processed at neuronal level? To answer these questions, we conducted a two-phase experiment: first, we exposed agile frog (*Rana dalmatina*) eggs to chemical cues obtained from larvae of dragonfly *Anax imperator* to evaluate the effect of the kairomone on the embryonic development; second, two weeks after hatching, we exposed the tadpoles to the same dragonfly cue and recorded their behavioral responses. Simultaneously, we investigated the effects of these chemical cues on the pattern of activity of olfactory receptor neurons (ORNs) by electrophysiological techniques applied to thin slices of the tadpoles' olfactory nerves and main olfactory bulb (MOB). Our results showed that 1) the eggs exposed to the dragonfly cue hatched statistically later than the controls, 2) larvae originating from conditioned eggs were smaller and lighter than those hatched from control eggs. In addition, conditioned tadpoles showed reduced activity and changed their circadian rhythm. Finally, neurophysiological analyses showed a strongly different basal synaptic activity in the tadpoles exposed to the chemical treatment as embryos compared to control groups.

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