Microsatellites in toads and whole genomes in bears: two examples to describe the transition from conservation genetics to conservation genomics

Giorgio Bertorelle

Department of Life Sciences and Biotechnology, University of Ferrara

The opportunity offered by NGS (Next Generation Sequencing) platforms to study the genetic variation at thousand of DNA fragments, or even at full genomes, combined with increased computation power and specific statistical techniques, is changing the field of genetics applied to conservation biology. Here I compare a conservation genetics study on the yellow-bellied toad, based on classical microsatellite markers, to a conservation genomic study on whole genomes in the brown bear, with the main goal to discuss pros and cons of the two approaches. First, I argue that the analysis of a relatively low number of classical markers still represents a useful tool to help the conservation and the management of threatened species. For example, if appropriate statistical methods are used, the reconstruction of population structure and demographic events with a reasonable power does not require genomic data, as shown by the toad study. Second, I discuss the large amount of additional information and evolutionary inferences that can be obtained when whole genomes are sequenced, as for example in the Apennine bear population, but also the additional problems to face. Problems include data filtering and management, difficulties to implement monitoring studies, and the current availability of computational methods with properties only partially known. The main advantage is the possibility, studying genes and regulatory regions, to go closer to the variation that really matters in evolution and conservation, i.e. adaptive variation. I conclude that genomic studies in conservation, considering their decreasing costs and the large amount of relevant information they provide, will soon become a standard. However, at least for now, the analysis of classical genetic markers is still recommended in many situations.

Extraoral expression and function of taste genes

Daniele Campa[1]

[1]*Dipartimento di Biologia, sezione di Genetica, Università di Pisa;*

Taste raceptors (*TAS*) genes in humans are expressed in a plethora of organs and tissues, including lung, colon, pancreas, pancreas skin and testis. These organs and tissues are not related to taste sensing highlighting the fact that TAS genes must have other function other than oral perception. The genes belonging to this family are highly polymorphic and several genetic variants are associated with many human phenotypes including complex diseases, longevity and male infertility once again underlining their pleiotropic role.

Yeast model of mitochondrial involvement in Aβ amyloidotic neurodegeneration

Cristina Dallabona

Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Italy

Mitochondrial dysfunction is increasingly recognised as a hallmark of neurodegeneration. Not only is the brain a major target in primary, genetically determined mitochondrial disease, but mitochondrial dysfunction is also a prominent feature in many of the most prevalent neurodegenerative diseases including Parkinson's and Alzheimer's disease.

The pitrilysin metallopeptidase 1 (PITRM1) is a mitochondrial matrix enzyme, which digests oligopeptides. Two siblings carrying a homozygous PITRM1 missense mutation were identified presenting a slowly progressive syndrome characterised by mental retardation, spinocerebellar ataxia, cognitive decline and psychosis.

The pathogenicity of the mutation was tested in the yeast *Saccharomyces cerevisiae* that presents the orthologous gene of PITRM1, named *CYM1*. We created an *ad hoc* model introducing the equivalent mutation found in patients in the *cym1Δ* strain. Our results showed a temperature-sensitive impairment of mitochondrial functions demonstrating the pathogenic role of the identified mutation. Furthermore we modified the yeast strain inducing the expression of a mitochondrial targeted amyloid beta peptide ($A\beta_{1-42}$).

The impairment of *CYM1* (and PITRM1) activity results in Aβ accumulation, thus providing a mechanistic demonstration of the mitochondrial involvement in amyloidotic neurodegeneration.

Regulation of cytokinesis by mitotic kinases

Pier Paolo D'Avino

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, United Kingdom

Cell division is essential for growth, development and reproduction in many organisms, as it controls the proper segregation of genomic and cytoplasmic materials into daughter cells. Furthermore, the orientation of the plane of division is crucial for determining cell fate and tissue organisation. Complex control and surveillance mechanisms have evolved to ensure the fidelity and robustness of mitotic and meiotic processes. As chromatin is compacted into chromosomes during cell division, these mechanisms rely in large part on post-translational modifications (PTMs) of proteins involved in all aspects of cell division, from mitotic spindle assembly to cytokinesis. Two types of PTMs are mainly used to regulate cell division: ubiquitylation and phosphorylation/de-phosphorylation. While ubiquitin-mediated protein degradation ensures accurate and timely progression through different stages of cell division, phosphorylation/de-phosphorylation controls the activity, localisation and interactions of a wide variety of mitotic players.

I will present our current knowledge of the regulation of cytokinesis by two mitotic kinases, Aurora B and Citron kinase, with particular emphasis on their roles in the assembly and function of an organelle located at the intercellular bridge, the midbody, which controls the physical separation - *i.e.* abscission - of the daughter cells and has been shown to contribute to cell fate and polarity.

Think globally, act locally: facing the data deluge in biological sciences.

Ferdinando Di Cunto

Department of Molecular Biotechnology and Health Sciences, University of Torino

In the last few years, next generation sequencing technologies have dramatically changed the information throughput of biological research. Indeed, current sequencing methods allow to rapidly and efficiently obtain global descriptive views of genome sequences, genomes' expression and epigenetic modifications, in different experimental conditions and even at single-cell level. The forthcoming third-generation single-molecule sequencing techniques promise to further, significantly expand these capabilities. However, despite undeniable progress, the current possibility of transforming genome-wide biological data into deep knowledge appears still very limited by the complex nature of physiological and pathological processes that characterize living organisms. I will here illustrate how this problem may represent the principal bottleneck for the implementation of efficient 'precision strategies' for the diagnosis and therapy of human disorders, in particular cancer and genetic diseases. Moreover, I will provide a perspective about the paradigm shifts still required to complete the transition from traditional 'reductionist' biological approach to 'systems biology'.

Unexpected features of the circadian clock in *Drosophila*

Dr Ezio Rosato,

University of Leicester, UK

One of the best studied circadian phenotypes is the rhythmic locomotor activity shown by the fruit fly *Drosophila melanogaster*. I am interested in understanding how the circadian clock of the fly organises this rhythmic behaviour.
In my talk I will discuss the organisation of the circadian neurons in the brain and I will present some experiments we have used to identify the 'logic' of the network they form. Based on these data I will present a new model of the clock as an interconnected and diffuse (rather than hierarchical) network made of several types of neurons, some of which have been overlooked to date. I will conclude that such a design is in agreement with the observed plasticity of circadian.

Forensic genetics beyond humans: applications to crimes against animals

Luisa Garofalo and Rita Lorenzini

Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Centro di Referenza Nazionale per la Medicina Forense Veterinaria. Via Tancia, 21 - 02100 Rieti, Italy.

In the common imaginary Forensic Genetics is usually associated with the resolution of criminal investigations against humans. Animal DNA Forensics originates in the '90s as a scientific support in human forensic cases requiring the analysis of non-human biological traces. Following the numerous national and international laws for protection of wildlife and domestic animals, currently Animal Forensics is widely used in investigations involving animals as victims and humans as perpetrators. In this presentation, we highlight similarities and differences between the two branches of the Forensic Genetics discipline, from the evidence sampling to the statistical treatment of the data. A summary of diagnostic queries usually submitted to our laboratory is provided, as well as an overview of the molecular markers and techniques adopted. Finally, real caseworks (inquiries dealing with poaching of wildlife, poisoning, cruelty to animals, frauds, illegal trade of protected species) solved by our laboratory are explained and illustrated for each type of diagnosis: species identification, sex assessment, determination of the population origin, hybrids detection, individual identification and DNA match, kinship analyses.

Following human expansions in East Asia and the Americas through Helicobacter pylori

Yoshan Moodley[1], Silvia Ghirotto[2], Andrea Brunelli[2], Vera Chelysheva[3], Andrey Klyubin[3], Ayaas Maady[4], Kuvat Mominyalev[5], Bodo Linz[6], Mark Achtman[7]

1 Department of Zoology, University of Venda, Republic of South Africa

2 Department of Life Science and Biotechnology, University of Ferrara, Italy

3 Research Institute for Physico-Chemical Medicine, Russia

4 Department of Endoscopy - Republic Hospital No.1, Kyzyl City, Russia

5 National Nuclear Centre of the Republic of Kazakhstan, Konechnaya, Kazakhstan

6 Department of Veterinary and Biomedical Sciences, Pennsylvania State University, USA

7 Warwick Medical School, University of Warwick, United Kingdom.

The gastric pathogen *Helicobacter pylori* has been associated with anatomically modern humans for at least 100,000 years and it is currently infecting more than half of the world population. Due to a high mutation and recombination rate, *H. pylori* strains show a clear phylogeographic signal and a pattern of genetic diversity that mirrors the one of their hosts. While *H. pylori* strains from Africa, Europe and Southeast Asia have been thoroughly investigated, sequences from northern Asia have yet to be reported. Here we analyze 400 new *H. pylori* sequences from 16 Siberian populations characterized by different lifestyles and spoken languages. Once this data was considered in a worldwide context we discovered several unrecognized strains: Siberia1, Siberia2, Ket and Altai. We also report a more than 100 new hpAmerind sequences, that for the first time have been found outside America, thusexpanding the known distribution of this strain to Northeast Siberia and Western Eurasia. We explicitly simulated different demographic scenarios, developed to explain the origin of the new strains, employing an Approximate Bayesian computation framework. Our results highlighted a recent origin for all of the newly identified strains, and their current distribution supported the hypothesis of a late recolonization of Siberia by humans following the LGM.

Molecular basis of circadian clocks in mammals

Achim Kramer

Charité Universitätsmedizin Berlin, Laboratory of Chronobiology, Charitéplatz 1, 10117 Berlin, Germany

Circadian clocks are endogenous oscillators that regulate the temporal organization of physiology, metabolism and behaviour. They provide organisms, ranging from unicellular algae to humans, with an internal representation of local time, thus allowing them to anticipate daily recurring chances and challenges in their environment. Circadian clocks oscillate in a self-sustained manner with an endogenous (free-running) period close to 24 hours. In a natural environment, these free-running rhythms are synchronized (entrained) to external time cues (Zeitgeber), such as 24-hour light-dark and ambient temperature cycles. In almost every cell of mammals, molecular circadian oscillations are essentially generated by a negative transcriptional-translational feedback loop: A multi-protein complex is rhythmically built up in the cytoplasm, undergoes posttranslational changes and – after a delay of several hours – translocates to the nucleus to inhibit the transcription of some of its key components at the appropriate time. Critical to the properties of this oscillator is the delay between the production of the complex components (such as PER and CRY proteins) and their auto-repression. Posttranslational events such as complex formation, nuclear import and export, regulated degradation, and modulation of transcriptional activity have been implicated in the generation of this delay.

Phylogeography of roe and red deer: focus on Italy as a hotspot of diversity

Rita Lorenzini[1], Luisa Garofalo[1], and Sandro Lovari[2]

[1]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Centro di Referenza Nazionale per la Medicina Forense Veterinaria, Via Tancia 21, 02100 Rieti;[2]U.R. Ecologia Comportamentale, Etologia e Gestione della Fauna, Dipartimento di Scienze della Vita, Università di Siena, Via P.A. Mattioli 4, 53100 Siena

Roe deer (*Capreolus* spp.) and red deer (*Cervus* spp.) are the only cervids that are native to Italy. Here, we summarize molecular phylogeny and evolutionary history of the two genera, starting from their appearance in the Miocene – Pliocene, to postglacial dispersal in the Pleistocene and current distributions of the species across their ranges. Hypotheses on phylogeographic patterns are proposed from the distribution of contemporary and ancient genetic lineages. The Italian Peninsula is classically regarded as a Mediterranean refugium for temperate species during the glacial phases of the Pleistocene. We suggest that, in the postglacials, different mammal populations failed to spread northwards, and remained as geographic isolates in the central-southern areas of the peninsula, unable to cross the Alpine barrier. This is one of the mechanisms that led to the intraspecific genetic differentiation that we observe today in our cervids and other species, underlining Italy as a hotspot of European mammalian diversity.

Out of anywhere.

The evolution of the genus *Homo* as a succession of geographical dispersals

Giorgio Manzi

Sapienza Università di Roma, Dipartimento di Biologia Ambientale

Our understanding of the evolutionary pattern followed by our own genus greatly changed in the last decades. Based on the evaluation of a fossil record that is much richer than before and diluted along the entire Pleistocene, it is now viewed as a bushy tree (according to the renowned S.J. Gould's prediction). Frequent dispersals across Africa and Eurasia as well as the isolation of small groups in eco-geographical refuges both played crucial roles for regional adaptation processes, combined with genetic drift effects and population admixture, either at the intra-specific or inter-specific level. This pattern is not at all in contrast with the available archaeological signals and with the molecular evidence. In this framework, the time window bracketed between 1.0 million years ago and 500 thousand years ago is of crucial importance for the emergence of a now well known – at least better than before – polymorphic species, *Homo heidelbergensis*, which emerged in this period, then spread across wide areas of Africa and Eurasia, diversifying in a number of incipient species (i.e., subspecies), and was ultimately ancestral to Neanderthals, modern humans, and the so-called Denisovans.

Migration and admixture in the Neolithic of Southeastern Europe

Iain Mathieson

Department of Genetics, Harvard Medical School, Boston 02115 MA USA

The extent of the relative contributions of migration, admixture, and cultural transmission in the European Neolithic is a source of ongoing debate. Ancient DNA from the populations directly involved provides new information about this old question. We report new genome-wide ancient DNA data from 204 individuals–65 Paleolithic and Mesolithic, 93 Neolithic, and 46 Copper, Bronze and Iron Age–who lived in southeastern Europe and surrounding regions between about 12,000 and 500 BCE. This region is critical because it is the place where migrating Farmers first encountered and interacted with the indigenous hunter-gatherers of Europe.

With our new data, we are able to investigate the dynamics of this interaction at fine scale. In particular, we demonstrate that the mesolithic populations of Southeastern Europe, the Baltic, and the North Pontic Steppe were distinct from those of Western Europe, with a West-East cline of ancestry. We show that the people who brought farming to Europe were not a single homogeneous population, as early farmers from southern Greece are not directly descended from the Neolithic population of northwestern Anatolia that was ancestral to all other European farmers. We also show that some groups of farmers in the region mixed extensively with local hunter-gatherers, with relatively sex-balanced admixture compared to the male-biased hunter-gatherer admixture that prevailed later in the North and West.

Finally, we show that there was contact between the Steppe and the Balkans during the Chalcolithic, and Bronze Age, but without the large-scale and dramatic Steppe migrations found in Northern Europe. These results show the power of ancient DNA to reveal fine-scale population changes as well as dramatic shifts.

A full list of authors and affiliations can be found at
http://www.biorxiv.org/content/early/2017/05/30/135616

The Tell-Tale Genome

Eugenio Bortolini[1,2,3*], Luca Pagani[4,5*], Enrico Crema[6], Stefania Sarno[3], Chiara Barbieri[7], Alessio Boattini[3], Marco Sazzini[3], Sara Graça da Silva[8], Gessica Martini[9], Mait Metspalu[5], Davide Pettener[3], Donata Luiselli[3], Jamshid J. Tehrani[9].

[1]Complexity and Socio-Ecological Dynamics Research Group, Department of Archaeology and Anthropology, IMF-CSIC, Spanish National Research Council, 08001 Barcelona, Spain;[2]Department of Humanities, Universitat Pompeu Fabra, 08005 Barcelona, Spain;[3]Department of Biological, Geological and Environmental Sciences, University of Bologna, 40126 Bologna, Italy;[4] APE lab, Department of Biology, University of Padova, Via Ugo Bassi 58/B, Italy;[5]Estonian Biocentre, 51010 Tartu, Estonia;[6]McDonald Institute for Archaeological Research, CB2 3ER Cambridge, UK;[7]Department of Linguistic and Cultural Evolution, Max Planck Institute for the Science of Human History, 07745 Jena, Germany;[8]Institute for the Study of Literature and Tradition, Faculty of Social Sciences and Humanities, New University of Lisbon, 1069-061 Lisbon, Portugal;[9]Department of Anthropology, Durham University, DH1 3LE Durham, UK

Observable patterns of cultural variation are consistently intertwined with demic movements, cultural diffusion, and adaptation to different ecological contexts. For the first time, we make use of worldwide whole-genome sequences to assess the impact of processes involving population movement and replacement on cultural diversity, focusing on the variability observed in folktale traditions (N=596) in Eurasia. We find that a model of cultural diffusion predicted by Isolation by Distance alone is not sufficient to explain the observed patterns, especially at small spatial scales (up to ~4000 km). We provide an empirical approach to infer presence and impact of ethnolinguistic barriers preventing the unbiased transmission of both genetic and cultural information. After correcting for the effect of ethnolinguistic boundaries we find that, of the alternative models we propose, the one entailing cultural diffusion biased by linguistic differences is the most plausible one. Additionally, we identify 15 tales which are more likely to be predominantly transmitted through population movement and replacement, and locate putative focal areas for a set of tales which are spread worldwide.

The genetics of Food Preferences

Nicola Pirastu

Centre for Global Health Research, Usher Institute of Population Health Sciences and Informatics, University of Edinburgh

Text: Food preferences are the first factor driving food choice and thus nutrition. They are influenced by numerous different senses such as taste and olfaction as well as various other factors such as personal experiences and hedonistic aspects. Although it is clear that all of these have a genetic basis, up to now very limited studies have been conducted. Therefore, we have carried out one of the first large scale (4611 individuals) GWAS on food likings assessed for 20 specific food likings belonging to 4 different categories (vegetables, fatty, dairy and bitter). In particular 2311 Italian subjects were used for the discovery step while 1755 from Europe and Central Asia for replication.

Association analysis revealed 16 independent GWAS significant replicated loci/genes (combined $p<5x10^{-8}$).

Surprisingly none of the identified genes belong to known taste or olfactory receptors. In particular some of the identified genes can be linked to the reward system pathway underlying the importance of this system in food choice. Most of the identified variants show non-additive inheritance suggesting the need of considering alternative genetic models in GWAS. Testing for possible effects of the identified variants on other related traits revealed that some of these variants affect also caloric intake and alcohol consumption.

Our results represent a first step towards unraveling the genetic bases of food liking, and in understanding the its impact on human nutrition in general.

Intergenerational inheritance in *Drosophila* female germ line

Sandy Mietzsch, Matthias Walther, Thomas Rudolph and Gunter Reuter

Institute of Biology, Developmental Genetics, Martin Luther University Halle-Wittenberg, D-06120 Halle/Saale, Germany

In *Drosophila* chromatin of primordial germ line stem cells is in a transcriptionally repressed state, shows abundant histone acetylation but only low levels of histone methylation. Like somatic nuclei also the nuclei of primordial germ line stem cells show polar chromatin differentiation.

In primordial stem cells the H3K4 demethylase dLSD1 and the H3K9 methyltransferase SETDB1 are required in maintenance of pluripotency and the corresponding mutations cause strong defects in ovarian stem cell development. Chromatin differentiation in primordial germ line stem cells is under the control of ecdysone signaling as revealed by an analysis of *taiman* and *EcR* mutations.

In order to assess chromatin differentiation in germ line stem cells directly we established genetic systems monitoring silencing processes of transgenes containing the germ line specific *fs(1)K10* gene. In these studies the function role of the basic chromatin proteins HP1, SU(VAR)3-9, SU(VAR)3-3 (dLSD1), LID, SUV4-20, E(Z), RPD3 and SU(VAR)2-1 in germ line chromatin organization was evaluated. With SU(VAR)2-1 we identified a new abundant chromatin protein controlling histone acetylation levels by recruitment. Many of the studied epigenetic factors display strong intergenerational effects between successive germ line generations.

Evolution and variability of taste receptor genes

Davide Risso

Department of Genome Sciences, University of Washington School of Medicine, Seattle, Washington 98195, USA

Vertebrates can perceive at least five taste qualities, each of which is thought to have played a specific role in the evolution of different species. The avoidance of potentially poisonous foods -generally bitter or sour tasting- and the search for more palatable ones -with high-fat and high-sugar content- are two of the most well-known examples. The study of taste genes, encoding receptors that recognize ligands triggering taste sensations, has helped reconstructing several evolutionary adaptations to dietary changes. In addition, an increasing number of studies has been focusing on pseudogenes, genomic DNA sequences that have traditionally been considered defunct relatives of functional genes, mostly because of deleterious mutations interrupting their open reading frames. In particular, the study of taste receptor pseudogenes has shown how the evolutionary history of taste in vertebrates has been the result of a succession of gene gain and loss processes. This dynamic role in evolution has been explained by the "less-is-more" hypothesis, suggesting gene loss as a mechanism of evolutionary change in response to dietary shifts. In this talk, I will give an overview of taste receptor genes and depict the major pseudogenization events that occurred in different lineages, illustrating their inter- and intra-specific variability, stressing their evolutionary importance and recapitulating signatures of natural selection.

Unexpected features of the circadian clock in *Drosophila*

Dr Ezio Rosato, University of Leicester, UK

One of the best studied circadian phenotypes is the rhythmic locomotor activity shown by the fruit fly *Drosophila melanogaster*. I am interested in understanding how the circadian clock of the fly organises this rhythmic behaviour.

In my talk I will discuss the organisation of the circadian neurons in the brain and I will present some experiments we have used to identify the 'logic' of the network they form. Based on these data I will present a new model of the clock as an interconnected and diffuse (rather than hierarchical) network made of several types of neurons, some of which have been overlooked to date. I will conclude that such a design is in agreement with the observed plasticity of circadian behaviour.

LINE-1-encoded Reverse Transcriptase mediates transgenerational inheritance

Corrado Spadafora

Institute of Translational Pharmacology, CNR
Rome, Italy

Spermatozoa of virtually all species have the spontaneous ability to take up exogenous DNA or RNA molecules and internalize them into nuclei. We have shown that a LINE-1-encoded reverse transcriptase activity, present in sperm heads, can reverse-transcribe the internalized molecules in multiple cDNA copies. Both RNA and cDNA molecules are delivered from sperm cells to oocytes at fertilization as non integrated extrachromosomal copies, further propagated throughout embryogenesis and inherited in a non-Mendelian fashion in tissues of adult animals. Nanovesicles, such as exosomes, play a central role in this flow of information.

Building on apparently unrelated results, here I propose that information-containing nanovesicles, predominantly small regulatory miRNAs and tsRNAs, are released from somatic tissues in the bloodstream, cross the Weismann barrier, reach the epididymis, are taken up by spermatozoa, further delivered to oocytes at fertilization and propgated in early embryos. This transgenerational flow of regulatory RNAs can progressively reshape the transcriptional landscape of early embryos generating, on the long run, the emergence of novel phenotypes. This phenomenon is compatible with a Lamarckian-type view and closely resembles Darwinian pangenesis

PINK1: a key protein at the crossroad of multiple neuroprotective pathways

Enza Maria Valente, MD, PhD

Neurogenetics Unit, IRCCS Santa Lucia Foundation, Rome and Department of Molecular Medicine, University of Pavia.

Mutations in the *PINK1* gene are the second most frequent cause of autosomal recessive parkinsonism after Parkin, and can represent a risk factor towards sporadic Parkinson's disease. The PINK1 gene [Phosphatase and tensin homolog (PTEN)-induced putative kinase 1] encodes a serine/threonine kinase with mitochondrial localization. The PINK1 protein product has been implicated in several functions, mostly aimed at protecting neuronal cells against different types of stress. Extensive studies identified PINK1 as a crucial player in the mitochondrial quality control pathway, required to label damaged mitochondria and promote their elimination through an autophagic process (mitophagy). Mounting evidences now indicate that PINK1 activities are not solely restricted to mitophagy, and that different subcellular and even sub-mitochondrial pools of PINK1 are involved in distinct signaling cascades to regulate cell metabolism and survival. In particular, we recently showed that, in conditions of mitochondrial damage, PINK1 selectively relocalizes at mitochondria-associated membranes (MAMs), where it recruits the proautophagic protein Beclin1, enhances mitochondria-ER contact sites and promotes the formation of omegasomes, that represent autophagosome precursors. Interestingly, other proteins implicated in neurodegeneration (such as alpha-synuclein, parkin and DJ-1) were also found to localize at MAMs, and these specialized districts have been recently implicated in many key cellular events. In this light, the observed prevalent localization of PINK1 at MAM may well explain other neuroprotective activities of this protein, such as modulation of mitochondrial calcium levels, mitochondrial dynamics, and apoptosis.