Societat Catalana de BIOLOGIA

# XVII Jornada de Biologia Evolutiva

*Organitzada per la Secció de Biologia Evolutiva de la SCB*

**INSTITUT D´ESTUDIS CATALANS**

**Carrer del Carme 47**

Barcelona

26 de juny de 2017

# XVII Jornada de Biologia Evolutiva

## PROGRAMA

Organitzadora:

Mª Pilar García Guerreiro (UAB)

Secretaria de la SCB:
***scb@iec.cat***

9 -9:20 Registration

9:20- 9:30 Welcome

9:30- 10:20 Invited speaker talk. Epigenetic interplay between mammalian transposable elements and host genes. **Rita Rebollo** (Université Claude Bernard-Lyon)

**Moderator: Antonio Barbadilla**

10:20-10:35 Representation and analysis of a thousand *Drosophila* genomes with PopFly. **Sergi Hervas** (UAB).

10:35-10:50 Untangling chelicerate gene and genome evolution through comparative genomics and transcriptomics analyses in spiders. **Silvia Hinojosa** (UB)

10:50-11:05 Insights into the rare and the small: the first draft genome of a kinorrhynch (Metazoa, Kinorrhyncha) based on a single specimen provides a resource to illuminate Ecdysozoa evolution. **Rosa Fernández** (CRG-UPF).

11:05-11:30 Coffee break

**Moderator: María Inés Roldán**

11:30-11:45 Population genomics and early life traits variation of two sympatric congeneric Mediterranean endemisms. **Héctor Torrado** (CEAB-CSIC)

11-45-12:00 Evaluating the impact of the Cayman Island green Turtle Farm: characteristics and consequences of an ex situ conservation strategy. **Anna Barbanti** (UB).

12:00-12:15 An interplay between plasticity, epigenetics, and parental phenotype determines impacts of ocean acidification on a reef fish. **Celia Schunter** (King Abdullah University of Science and Technology)

12:15-12:30 Population History in Pan species. **Sojung Han** (IBE-UPF)

12:30-12:45 Adaptive Introgression in the Chimpanzee Genome. **Jessica Nye** (IBE-UPF)

12:45-13:00 Novel evidence of complex patterns of gene flow in chimpanzees and bonobos. **Martin Kuhlwilm** (IBB-UPF).

13-13:15 Similar genomic proportions of copy number variation within gray wolves and modern dog breeds inferred from whole genome sequencing. **Aitor Serres-Armero** (IBE-UPF)

13:15-14:45 Lunch break

**Moderator: Montserrat Aguadé**

14:45-15:00 Evaluating the performance and replicability of fecal DNA targeted sequencing. **Jessica Hernandez-Rodriguez** (IBE-UPF).

15:00-15:15 Population Genomics with Transposable Elements in Drosophila. **Maite Barrón** (IBE_UPF)

15:15-15:30 Organization and evolution of the *Hsp70* gene family in the *subobscura* cluster. **Marta Puig Giribets** (UAB).

15:30-15:45 A molecular palaeobiological investigation into the early colonization of land by arthropods. **Jesus Lozano-Fernandez** (University of Bristol).

15:45-16:00 The filasterean Capsaspora owczarzaki as an experimentally tractable system to understand the origin of animal multicellularity. **Núria Ros i Rocher** (IBE-UPF).

16:00-16:30 Break/Prevosti Prize Committee Meeting

16:30 Announcement of the Prevosti Prize winner. End of the meeting

## X Premi Antoni Prevosti de Biologia Evolutiva, any 2017

Amb l'objectiu de fomentar la participació i la discussió de la recerca dels joves investigadors, pre i postdoctoral, en tots els camps de la Biologia Evolutiva, l'any 2007 fou instaurat per primera vegada el premi Antoni Prevosti de Biologia Evolutiva per premiar la millor comunicació a la jornada presentada per un jove investigador (que faci menys de 3 anys que ha llegit la tesi).

El premi consisteix aquest any en 250 € que es lliuraran al finalitzar la darrera sessió de la Jornada. El receptor del premi haurà de ser present a la sala per rebre'l. Si no és així, el premi passarà a la comunicació que hagi quedat en segon lloc, si n'hi hagués, o podria ser declarat desert.

La comissió que decidirà el premi de l'edició de l'any 2016 estarà formada per.

| | |
|---|---|
| President: | Carme Segarra (UB) |
| Secretari: | Marta Riutort (UB) |
| Vocal 1: | Sebastian Rodrigo Najle (IBE-UPF) |
| Vocal 2: | Barbara Negre (UAB) |
| Vocal 3: | Hafid Laayouni (UPF) |

La comissió atorgarà el premi en base a la qualitat científica i presentació del treball, així com a les respostes a les intervencions dels assistents.
Els membres de la comissió no podran votar a les persones del seu grup.

**Representation and analysis of a thousand *Drosophila* genomes with PopFly**

Sergi Hervas[1], Esteve Sanz[2], Sonia Casillas[1], Antonio Barbadilla[1]

1. Institut de Biotecnologia i de Biomedicina and Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain.
2. Servei de Genòmica i Bioinformàtica, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain.

High-throughput sequencing technologies are allowing the description of genome-wide variation patterns of an ever growing number of organisms. However, we still lack a comprehensive understanding about the relative amount of different types of variation, their phenotypic effects, and the detection and quantification of distinct selection regimes acting on genomes. The recent compilation of over 1100 worldwide wild-derived *Drosophila melanogaster* genome sequences reassembled using a standardized pipeline (Drosophila Genome Nexus, DGN –Lack *et al*. 2015, 2016) provides a unique resource to test molecular population genetics hypotheses and ultimately understand the evolutionary dynamics of genetic variation in populations (Casillas and Barbadilla, 2017). Here, we present a complete characterization of the nucleotide variation landscape in the genome of *D. melanogaster* by analyzing the DGN data. We have also developed a novel approach which incorporates information on the site frequency distribution to the framework of the McDonald and Kreitman test (McDonald and Kreitman, 1991) to map five different regimes of selection acting on new mutations along the genome (strongly deleterious sites, weakly deleterious sites, neutral sites, sites that have become neutral recently, and adaptive fixations), and we have inferred the major genomic determinants of the observed rates of nucleotide diversity and adaptation. All population genomic estimates have been implemented in PopFly (Hervas *et al*. 2017; http://popfly.uab.cat), a population genomics-oriented genome browser, based on JBrowse software (Buels *et al*. 2016), which allows an easy visualization, exploration, and retrieval of such information. In summary, this work provides both a global view of evolutionary forces shaping genome variation patterns in *D. melanogaster* and a novel reference tool to the research community for future population genomic studies in this model species.

**References**

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4. Lack,J.B. *et al.* (2015) The Drosophila Genome Nexus: A Population Genomic Resource of 623 Drosophila melanogaster Genomes, Including 197 from a Single Ancestral Range Population. *Genetics*, **199**, 1229–41.
5. Lack,J.B. *et al.* (2016) A Thousand Fly Genomes: An Expanded Drosophila Genome Nexus. *Mol. Biol. Evol.*, **33**, 3308–3313.
6. McDonald, J.H. and Kreitman, M. (1991) Adaptive protein evolution at the Adh locus in Drosophila. *Nature* **351,** 652-654.

**Untangling chelicerate gene and genome evolution through comparative genomics and transcriptomics analyses in spiders**

Silvia Hinojosa[1], Paula Escuer[1], Cristina Frías-López[1], Jose F. Sánchez-Herrero[1], Joel Vizueta[1], Miquel A. Arnedo[2] , Alejandro Sánchez-Gracia[1] & Julio Rozas[1]
[1] Departament de Genètica and Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Av. Diagonal 643, Barcelona 08028, Spain
[2] Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals and Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Av. Diagonal 643, Barcelona 08028, Spain

Spiders compose the most diverse group of chelicerates (Arthropoda), including more than 45,000 described species that are the dominant predators in most terrestrial ecosystems. Given their earlier split from the other three arthropod subphyla (~470 Mya), they are excellent models to study the diverse strategies adopted by the major arthropod lineages during their independent adaptation to terrestrial environments. With this aim in mind, we designed two complementary approaches. First, we conducted a comparative RNA-Seq analysis across different body structures of the nocturnal wandering hunter spider *Dysdera silvatica* (Schmidt 1981). Specifically, we obtained the complete transcriptome of this species as well as the specific expression profile in legs and palps, which are thought to bear the chemosensory appendages in spiders.

Secondly, using the adaptive radiation of the genus *Dysdera* in the Canary Islands as a case study, we investigate the genomic signatures associated with species diversification, including the specific ecological (dietary specialization) shift processes undergone by some of its members during speciation. We use comparative transcriptomics to identify the genomic changes associated with these shifts, both at the nucleotide (in coding and non-coding sequences) and at the gene copy number levels, in an experimental design that included two pairs of generalist-specialist species (with respect to dietary specialization). To complement the study we have further sequenced the complete genome of the five above-mentioned *Dysdera* species.

**Insights into the rare and the small: the first draft genome of a kinorrhynch (Metazoa, Kinorrhyncha) based on a single specimen provides a resource to illuminate Ecdysozoa evolution**

**Rosa Fernández**
Bioinformatics & Genomics Unit
Centro de Regulació Genòmica
Carrer del Dr. Aiguader, 88
08003 Barcelona
+34 93 316 02 81
rosamaria.fernandez@crg.eu

Kinorhyncha – commonly known as mud dragons - is a phylum of small marine invertebrates of 1 mm or less that are widespread in mud or sand at all depths as part of the meiobenthos. They belong to the clade Ecdysozoa, composed by some relatively well-known phyla (such as Arthropoda, Onychophora, Tardigrada, Nematoda and Nematomorpha) and also several poorly known but fascinating taxa: Priapulida, which has both coelomate and pseudocoelomate members, Loricifera, with extraordinary morphologies, and Kinorhyncha, composed of animals with a unique type of segmentation. Despite the scientific efforts paid to resolve the phylogeny of Ecdysozoa, the interrelationships of its phyla remain unclear, with different pieces of work supporting different topologies. This is partly due to the scarce availability of genetic resources for some of these phyla, notably Loricifera and Kinorrhyncha. Here, we present our most recent efforts to sequence the genome of a kinorrhynch based on a single specimen collected in the East coast of USA. We optimized a protocol based on single cell sequencing techniques for DNA extraction and library construction. Although preliminary, this draft genome seems to be among the smallest in Metazoa. We discuss the relevance of our findings in the context of the evolution of Ecdysozoa in a prevailing quest for illuminating the Metazoa Tree of Life.

**Population genomics and early life traits variation of two sympatric congeneric Mediterranean endemisms.**

Héctor Torrado[1, 2], Carlos Carreras [2], Núria Raventós [1], Enrique Macpherson [1], Marta Pascual [2].

[1]Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Car. Acc. Cala St. Francesc 14, 17300 Blanes, Girona, Spain. Tel: +34 972 336101

[2]Department de Genètica, Microbiologia i Estadística and IRBio, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain.

E-mail: htorrado@ceab.csic.es

Environmental changes can force marine organisms to adapt and alter some of their traits, such as morphological or life history traits, in response to external conditions. Most marine fishes have complex life histories that include a pelagic larval stage that affects connectivity between their populations. Both genomic differences and phenotypic plasticity can contribute to adapting under variable conditions, whether environmental fluctuations in the same locality or across geographic environmental gradients among localities. The study of genetic and phenotypic variation associated to early life history traits across geographic gradients can provide insights into the adaptive potential of species while considering both connectivity and selection.

We study two sympatric and congeneric fish endemic species of the Mediterranean Sea, *Symphodus ocellatus* (Linnaeus, 1758) and *Symphodus tinca* (Linnaeus, 1758), with similar larval duration (7-13 days), caught along a North-South gradient in the Western Mediterranean submitted to different temperature regimes. For both species, we individually measured some early life history traits (*e.g.* date of birth, size at hatching, pelagic larval duration, size at settlement, growth rate) and obtained Single Nucleotide Polymorphisms (SNPs) by Genotyping-By-Sequencing (GBS).

Population genomic structuring differs between the two species. *Symphodus ocellatus* shows high genetic differentiation among localities and major connectivity reduction across two oceanographic barriers while *S. tinca* presents lower genetic distances and no detectable effect of the major fronts. Genome-wide association analyses (GWA) were performed in order to evaluate the relation between the candidate regions containing those SNPs and the phenotypes studied across the individuals. The highest association of SNPs was found with growth rate during the pelagic larval phase for *S. ocellatus* and with the birth's date in *S.tinca*. The differences observed in species with similar ecological traits and dispersal capabilities could be related to early life traits mediated by differences in the season of their reproductive periods.

**Evaluating the impact of the Cayman Island green Turtle Farm: characteristics and consequences of an ex situ conservation strategy.**

Anna Barbanti, Clara Martín, Marta Pascual, Carlos Carreras
Department de Genètica, Microbiologia i Estadística and IRBio, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain. Tel: 934024850. Email: anna.barbanti.es@gmail.com.

**Abstract**
Conservation of endangered species is now a priority worldwide since biologists suggest that a sixth mass extinction may be under way. Ex situ management strategies have become an important tool in species conservation programmes that aim to conserve components of biological diversity outside their natural habitats while supporting species survival in the wild. Ex situ strategies followed by a reintroduction into the wild may be the solution to optimize endangered species conservation and to preserve the maximum genetic variability within a species, considering that not all species, in particular marine ones, can be preserved in their natural habitats. However, such conservation actions need thorough population management and monitoring to minimize inbreeding depression and loss of genetic diversity and to evaluate the impact of the program into the wild populations. In 1968 the Cayman Islands government started a captive breeding program to repopulate the green turtle (*Chelonia mydas*) population of the islands recently declared extinct. This ex situ reintroduction program has been releasing green turtles for almost 40 years without a proper monitoring. The purpose of the present study is to determine the current population structure of captive individuals of the Cayman Turtle Farm and to evaluate the impact of the reintroduction program on wild populations of green turtle. Using genetic markers (microsatellites, D-loop and STR mtDNA) we were able to identify parental relationships and population structuring within the farm and between wild and captive individuals, resulting in high degrees of relatedness. These results show the positive influence and the impact of the farm on the present wild population. Genetic based analyses are helpful not just to evaluate the outcome of the reintroduction, but also to understand the present structuring of captive populations in order to improve future management to maintain genetic diversity on a long term and avoid inbreeding depression.

# An interplay between plasticity, epigenetics, and parental phenotype determines impacts of ocean acidification on a reef fish

Celia Schunter, Megan J. Welch, Göran E. Nilsson, Jodie L. Rummer, Philip L. Munday*and Timothy Ravasi

**Introductory paragraph**

The impacts of ocean acidification will depend on the ability of marine organisms to tolerate, acclimate, and eventually adapt to changes in ocean chemistry. Here we use a unique transgenerational experiment to determine the molecular response of a coral reef fish to short-term, developmental, and transgenerational exposure to elevated $CO_2$ and to test how these responses may be influenced by variations in tolerance to elevated $CO_2$ exhibited by the parental phenotype. Within-generational responses in gene expression to end of century predicted $CO_2$ levels indicate that a self-amplifying circle in GABAergic neurotransmission is triggered, explaining previously reported neurological and behavioural impairments. Furthermore, epigenetic regulator genes exhibited a within-generation specific response with some divergence due to parental phenotype. Importantly, we find a recovery pattern for the majority of within-generation responses following exposure of parents to high $CO_2$ conditions. Our result show that both parental variation in tolerance and cross-generation exposure to elevated $CO_2$ are crucial factors in determining the response of reef fish to changing ocean chemistry.

**Affiliations**

KAUST Environmental Epigenetic Program (KEEP), Division of Biological and Environmental Sciences & Engineering and Division of Applied Mathematics and Computer Sciences, King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia
Celia Schunter and Timothy Ravasi

ARC Centre of Excellence for Coral Reef Studies and College of Marine and Environmental Sciences, James Cook University, Townsville, Queensland, Australia
Megan Welch

ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland, Australia
Jodie L. Rummer, Philip L. Munday

Section for Physiology and Cell Biology, Department of Biosciences, University of Oslo, Oslo NO-0316, Norway

**Population History in Pan species**

*Sojung Han*, *Tomas Marques-Bonet*, *Martin Kuhlwilm*

Comparative genomics, University Pompeu Fabra (IBE), Barcelona, Spain

E-Mail: sojung.han@upf.edu

With high-throughput sequencing development, multiple genomes from different populations have played important roles in revealing population history. In humans, population admixture and ancestry in modern populations have been described in detail, suggesting how the peopling of the world has taken place, and a fine picture of population history has been established through studies in archaic human populations, such as Neandertals and Denisovans. This allowed us to define signatures of bottlenecks and changes of effective population sizes over time in humans.

Recently, the first evidence for admixture between the two *Pan* species, from bonobos to non-western chimpanzees (de Manuel et al., 2016), has been published. We further analyzed genomic segments inferred to be introgressed, and found that some of them appear to have amino acid changes in genes relevant for reproduction, which is possible evidence of adaptive introgression. We further study the same dataset to detect the genomic signatures of the demographic history in each population of the *Pan* clade, and find that deleterious mutations (assessed by scores such as SIFT, PolyPhen-2 and Grantham) are more effectively removed from the central chimpanzee population compared to other *Pan* populations. On the other hand, western chimpanzees, which used to have a small effective population size and went through bottlenecks, show an elevated level of deleterious load at homozygous loci. These observations agree well with predictions based on the demographic history, and add a valuable perspective to comparable findings in human populations.

**Adaptive Introgression in the Chimpanzee Genome**

Jessica Nye
Jaume Bertranpetit
Hafid Laayouni

The admixture between two closely related species, or introgression, can result in an evolutionary advantage. The sudden introduction of derived alleles into a population may allow for rapid adaptation to a certain environment or disease. This scenario has been described in the human genome after its introgression with Denisova (Huerta-Sánchez *et al*., 2014) and Neanderthals (Dannemann *et al*., 2016; Deschamps *et al*., 2016). Recently, introgression between Bonobo (*Pan paniscus*) and three subspecies of Chimpanzee (*Pan troglodytes troglodytes, P.t. schweinfurthii, and P.t. ellioti*) was described (de Manuel *et al.*, 2016). Here we interrogate these introgressed haplotypes using site frequency spectrum-based statistical tests Tajima's D (Tajima, 1989), and Fu and Li's D and F (Fu and Li, 1993) in order to explore the possible adaptive alleles gained from introgression with Bonobos and are still segregating in the Chimpanzee genome.

**Novel evidence of complex patterns of gene flow in chimpanzees and bonobos**

Martin Kuhlwilm[1], So Jung Han[1], Marc de Manuel[1], Tomas Marques-Bonet[1,2,3]

[1] Institut de Biologia Evolutiva, (CSIC-Universitat Pompeu Fabra), PRBB, Barcelona, Spain.
[2] National Centre for Genomic Analysis–Centre for Genomic Regulation, Barcelona Institute of Science and Technology, Barcelona, Spain.
[3] Institucio Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Our closest living relatives, chimpanzees and bonobos, have a highly dynamic demographic history including episodes of ancestral interbreeding among them. We have presented multiple lines of evidence for a signature of gene flow from bonobos into the ancestors of non-western chimpanzees, possibly during extended periods of time up to 550 thousand years ago. This suggests that admixture appears to have been widespread during hominid evolution. However, it seems possible that additional events of gene flow have taken place, in particular, admixture from lineages outside the known *Pan* clade. Here, we analyzed the high-coverage whole genomes of 69 wild-born chimpanzees and bonobos from ten countries in Africa to investigate the genetic traces of such archaic gene flow events by applying the S* statistic. Our findings suggest additional gene flow events into the bonobo populations. While around 0.2% of central chimpanzee genomes might carry bonobo haplotypes, we find that bonobos might carry up to 1% of haplotypes falling outside the common *Pan* clade. We conduct a detailed investigation of demographic scenarios causing these observations, and characterize these haplotypes regarding their age and their divergence patterns and impact on possible functional consequences, exposing for a first time partial genome information from an extinct great ape lineage.

# Similar genomic proportions of copy number variation within gray wolves and modern dog breeds inferred from whole genome sequencing

Aitor Serres-Armero*, Inna S. Povolotskaya*, Javier Quilez*, Oscar Ramirez, Gabriel Santpere, Lukas FK Kuderna, Jessica Hernandez-Rodriguez, Marcos Fernandez-Callejo, Daniel Gomez-Sanchez, Adam H Freedman, Zhenxin Fan, John Novembre, Arcadi Navarro, Adam Boyko, Robert Wayne, Carles Vilà, Belen Lorente-Galdos§, Tomas Marques-Bonet§

IBE, Institut de Biologia Evolutiva (Universitat Pompeu Fabra/CSIC), Ciencies Experimentals i de la Salut, Barcelona, C/Dr Aiguader 88, 08003, Spain (93 316 08 64)
aserresarmero@gmail.com

Whole genome re-sequencing data from dogs and wolves is now commonly used to study how natural and artificial selection have shaped the patterns of genetic diversity. Single nucleotide polymorphisms, microsatellites and variants in mitochondrial DNA have been interrogated for links to specific phenotypes or signals of domestication. However, copy number variation (CNV), despite its increasingly recognized importance as a contributor to phenotypic diversity, has not been extensively explored in canids.

Here, we develop a new accurate probabilistic framework to create fine-scale genomic maps of segmental duplications, compare patterns of CNV across groups and investigate their role in the evolution of the domestic dog by using information from 34 canine genomes. Our analyses show that duplicated regions are enriched in genes and hence likely possess functional importance. We identify 86 loci with large CNV differences between dogs and wolves, enriched in genes responsible for sensory perception, immune response, metabolic processes, etc. In striking contrast to the observed loss of nucleotide diversity in domestic dogs following the population bottlenecks that occurred during domestication and breed creation, we find a similar proportion of loci with variable copy number in dogs and wolves, suggesting that other dynamics are acting to particularly select for copy number variants with potentially functional impacts.

## Evaluating the performance and replicability of fecal DNA targeted sequencing.

Authors: Jessica Hernandez-Rodriguez[1], Mimi Arandjelovic[2], Jack Lester[2], Cesare de Filippo[2], Antje Weihmann[2], Matthias Meyer[2], Samuel Angedakin[2], Ferran Casals[3], Arcadi Navarro[1,4,5], Linda Vigilant[2], Hjalmar S Kuhl[2], Kevin Langergraber[6], Christophe Boesch[2], David Hughes[1,7], Tomas Marques-Bonet[1,4,5]

Authors information:

[1]Institut de Biologia Evolutiva (Universitat Pompeu Fabra/CSIC), Ciencies Experimentals i de la Salut, Barcelona, 08003, Spain

[2]Department of Primatology, Max Planck Institute for Evolutionary Anthropology, Leipzig, 04103, Germany

[3] Genomics Core Facility, Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Parc de Recerca Biomèdica de Barcelona, Barcelona, 08003, Spain

[4]Centro Nacional de Análisis Genómico (CNAG), 08028 Barcelona, Spain

[5]Institucio Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, 08010, Spain

[6]School of Human Evolution & Social Change, Arizona State University, Tempe, USA

[7]MRC Integrative Epidemiology Unit, School of Social and Community Medicine, University of Bristol, Bristol, UK

Abstract:

Target capture technologies have raised in the past years, proving to be a very efficient tool for sequencing selectively regions of interest. These methods have also allowed the use of non-invasive samples as feces (known by their low quantity and quality of endogenous DNA), for their use in conservation genomics, evolution and population genetics. Our goal in this paper was to test different protocols and strategies for exome capture with Roche SeqCap EZ Developer kit (57,5Mb). First, we captured a considerable pool of DNA libraries. Second, we assessed the influence of using more than one fecal sample, extract and/or library from the same individual, and how does that affect the molecular variability of the experiment. The set of samples we used to validate our experiments were 18 chimpanzee fecal samples, 9 from Kibale National Park (Uganda) and 9 from Loango National Park (Gabon). We have proved that at least 16 libraries can be pooled and hybridized with ¼ diluted probes, obtaining a considerable number of SNPs for popgen analysis, we also found that there is an increase of library richness when using multiple libraries from the same extract or extracts from the same sample. We conclude that with two rounds of capture the results obtained are much better, with a 7.83% off-target reads compared with the 34.15% off-target obtained performing one round of capture.

**Population Genomics with Transposable Elements in Drosophila**

Maite G. Barrón Aduriz, José Luis Villanueva-Cañas, and Josefa González

Institut de Biologia Evolutiva (IBE). CMIMA-Pssg. de la Barceloneta 37-49. 08003 Barcelona (Spain). maite.barron@ibe.upf-csic.es

Transposable elements are an abundant, diverse, and active component of virtually all genomes sequenced to date. However, TEs have been largely ignored in genomic studies mainly due to methodological limitations. The *Drosophila melanogaster* genome is one of the few in which transposable elements have been well-annotated. As such, this species is an excellent model to understand the role of TEs in genome structure, function and evolution. We have analyzed the transposable element genomic content in 61 worldwide natural populations, 37 of them reported here for the first time. For 23 of these 61 natural populations, we have seasonal samples, meaning that the same population was sequenced at least twice in the same year. This comprehensive dataset, which includes all the natural populations available for this species, allow us to investigate the geographic and temporal scale of transposable element dynamics. Our results show that the site frequency spectrum is similar in all the populations analyzed: most of the transposable element insertions are either fixed or present at very low frequencies. The levels of geographical and seasonal variation in transposable element frequencies are similar suggesting that both the spatial and temporal scales play a role in the dynamics of transposable elements. Moreover, we detect 203 transposable element insertions present at low frequencies in populations from the ancestral range of the species and at high frequencies in derived populations and thus likely to be adaptive. 80% of the candidate adaptive transposable element insertions are located inside genes or less than 1kb from a gene. Most of the insertions located inside genes are in introns or UTRs, suggesting that they might affect gene expression. Overall, these results suggest that transposable elements play a substantial role in adaptive evolution.

# Organization and evolution of the *Hsp70* gene family in the *subobscura* cluster

Marta Puig Giribets*[a], María Pilar García Guerreiro*[b], Francisco José Rodríguez-Trelles Astruga*[c], Rosa Tarrío*[d].

*Group of Genomics, Bioinformatics and Evolutionary Biology (GBBE), Department of Genetics and Microbiology, Faculty of Biosciences (Building C), Autonomous University of Barcelona. 08193 Bellaterra (Barcelona). Phone: (+34)935814705.

[a] marta.puig.giribets@uab.cat
[b] mariapilar.garcia.guerreiro@uab.es
[c] franciscojose.rodrigueztrelles@uab.cat
[d] rosamaría.tarrio@uab.cat

The temperate species *Drosophila subobscura* exhibits rich inversion polymorphisms in all its five acrocentric chromosomes throughout its range. Particularly interesting are two O chromosome arrangements with north-south clinal distributions, namely the warm-climate associated $O_{3+4}$ and the cool-climate associated $O_{ST}$. Earlier experiments in our laboratory identified the thermal-stress-inducible *Hsp70* locus, located within the $O_{3+4}$ region, as a candidate gene responsible for observed differences in thermal adaptation between the two arrangements. It was found that, flies homokaryptypic for $O_{3+4}$ exhibited increased basal levels of *Hsp70*, in contrast with $O_{ST}$ and $O_{3+4+8}$. One possible explanation is that $O_{3+4}$ carry greater number of *Hsp70* copies. To test this hypothesis, we characterized the genomic organization of the *Hsp70* family in the four most common arrangements of interest, namely $O_{ST}$, $O_{3+4}$, $O_{3+4+8}$ and $O_{3+4+16}$, plus the closely related outgroups *D. madeirensis* and *D. guanche*. By combining *in situ* hybridization and reconstruction of a 7-kb long genomic region at the *Hsp70* locus, we unveiled that the four *D. subobscura* strains do not differ in their *Hsp70* genomic organization, which consists of two palindromic, head-to-head oriented segments, each including a 1.929 bp long copy of the *Hsp70* locus. This arrangement is conserved across the two assayed outgroup species, and the more distant members of the *obscura* group *D. persimilis* and *D. pseudoobscura*. In addition, we detected signatures of concerted evolution between copies, and of that the genomic organization of the *Hsp70* family in the *obscura* group may have been mediated by transposable elements.

**A molecular palaeobiological investigation into the early colonization of land by arthropods**

Jesus Lozano-Fernandez[1,2,*], Robert Carton[3], Alastair R. Tanner[2], Mark N. Puttick[1], Mark Blaxter[4], Jakob Vinther[1,2], Jørgen Olesen[5], Gonzalo Giribet[6], Gregory D. Edgecombe[7] and Davide Pisani[1,2]

[1]School of Earth Sciences, and [2]Shool of Biological Sciences, University of Bristol, Life Sciences Building, 24 Tyndall Avenue, Bristol BS8 1TQ, UK
[3]Department of Biology, The National University of Ireland Maynooth, Maynooth, Kildare, Ireland
[4]Institute of Evolutionary Biology, University of Edinburgh, Edinburgh EH9 3TF, UK [5]Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark
[6]Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA [7]Department of Earth Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK
[*] jesus.lozano@bristol.ac.uk

Animals have marine origins and only few phyla contain fully terrestrial lineages. The process through which animals adapted to life on land is referred as terrestrialization, and it is one of the most extreme cases of adaptation. Arthropoda represent the largest majority of terrestrial biodiversity and have an extensive fossil record that suggests they were the first terrestrial animals. They colonized the land multiple times independently, which allow rigorous comparison of the alternative solutions adopted by the different groups to the same adaptive challenge.

In this study we implemented a molecular palaeobiological approach, merging molecular and fossil evidence, to elucidate the deepest history of the terrestrial arthropods. We focused on the three, independent, Palaeozoic arthropod terrestrialization events (Myriapoda, Hexapoda and Arachnida) and showed that a marine route to the colonization of land is the most likely scenario. Molecular clock analyses confirmed an origin for the three terrestrial lineages bracketed between the Cambrian and Silurian, and while molecular divergence times for Arachnida are consistent with the fossil record, Myriapoda and Hexapoda are inferred to have colonised land earlier. Recent methodological developments, such as total-evidence analyses, allow simultaneously estimate and date the relationships among living and fossil species using morphological and molecular data, I will discuss preliminary data applying these methods to understand the terrestrialization of several arthropod lineages.

**The filasterean *Capsaspora owczarzaki* as an experimentally tractable system to understand the origin of animal multicellularity**

*Núria Ros i Rocher, Helena Parra-Acero, Alberto Pérez-Posada, Aleksandra Kozyczkowska, Sebastián R. Najle, Iñaki Ruiz-Trillo*

The transition to animal multicellularity from a single-celled ancestor is one of the most transcendent events in the history of life. Recent genome data from the closest unicellular relatives to animals have shown that changes in regulatory programs involving cell signalling, cell adhesion, cell communication and transcriptional regulation were probably crucial for the emergence of metazoans. Thus, functional studies of key genes involved in these programs essential for multicellular functions in a unicellular context can give us insights into the molecular mechanisms that drove this transition.

However, traditional model systems cannot address this question. Thus, we need to develop genetic tools among the closest unicellular relatives of animals, which are the only ones with the potential to answer how regulatory programs were co-opted at the onset of Metazoa.

To this end, we have developed the filasterean *Capsaspora owczarzaki,* one of the closest unicellular relatives of animals, as an experimentally tractable system. We have optimized a classical transfection protocol with plasmid DNA, which results in a reasonable efficiency for further functional assays such as overexpression and localization experiments. In this regard, we have created a platform of multiple expression vectors tagging several cellular locations. In parallel, we are evaluating several selection strategies to achieve stable transfection. Preliminary results and implications of this study will be presented and discussed.

Institute of Evolutionary Biology
Passeig Marítim de la Barceloneta, 37-49
08003 (Barcelona)
93 230 95 00 (ext: 6037)
nuria.ros@ibe.upf-csic.es

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