

**1<sup>st</sup> PhDay Complutense**

**Faculty of Biology**

**14-12-2017**



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# Introduction



The 1<sup>st</sup> PhDay Complutense organized by the Doctoral School of Complutense University represents an uplifting initiative for PhD students as an attractive networking event. Not only is PhDay Complutense an opportunity to train PhD students in communication and presentation skills, but also in establishing social relationships. Therefore, we really want to express our gratitude to the Office of the Vice-Rector for Scientific Policy, Research and Doctorate and the Office of the Vice-Rector for Students for their support in organizing this event in conjunction of the different faculties and PhD programs.

The PhDay Complutense held in the Faculty of Biology will be formed by students from five PhD programs: Biology; Biochemistry, Molecular Biology and Biomedicine; Ecology; Microbiology and Parasitology; and Ecology, Conservation and Restoration of Ecosystems. Participants are grippingly encouraged to talk about their PhD projects from the very beginning of their thesis inasmuch as this event is considered an outstanding opportunity for their formation throughout the doctorate. Moreover, our purpose is that undergraduate and Master students will join the PhDay to learn what a PhD is, which PhD projects are addressed in our Faculty and how doctorate is organized in Complutense University. Furthermore, we have organized a Plenary Lecture about the future of science and a round table composed by formers PhD students where they will tell through their own experience along the PhD. We enthusiastically thank their participation in this event by sharing their own experience which we regard essential for young students since “we are only dwarfs standing on the shoulders of giants”.

# Organizing Committee

## **José Carlos Castillo Sánchez**

I obtained my Bachelor's Degree on Biochemistry and my Master's Degree on Biochemistry, Molecular Biology and Medicine in Complutense University in 2015 and 2016, respectively. Afterwards, I began my PhD thesis on Biophysics and Molecular Biology in lung surfactant system under the supervision of Prof. Jesús Pérez Gil and Prof. Antonio Cruz Rodriguez. I have currently begun the 2<sup>nd</sup> year of PhD thesis in the Biochemistry and Molecular Biology Department in the Faculty of Biology at Complutense University.

## **Irene Cobo Simón**

I hold a Bachelor's Degree in Biology with Evolution and Biodiversity specialization by the University of Alcalá and a Master's Degree in Evolutionary Biology by Complutense University of Madrid. Afterwards, I was collaborating for three years in the Biodiversity and Evolutionary Biology Department at National Museum of Natural History, Madrid, Spain in different research lines related to phylogeography and evolution from a molecular approach. Currently, I am a PhD student at Genetics Department in the Faculty of Biology (Complutense University of Madrid). My research pursues characterizing patterns and processes related to the climate change-related selective pressures on populations of conifers established in ecotones, and it is particularly focused on Spanish fir (*Abies pinsapo* Boiss). I have a predoctoral fellowship from the Spanish Economy Ministry in Pablo de Olavide University (Sevilla).

## **Daniel García Rincón**

I obtained my Bachelor's Degree on Biology with Neurobiology specialization in Complutense University in 2013. I did my Bachelor's Thesis under the supervision of Prof. Ismael Galve in Manuel Guzman's laboratory. Afterwards, I obtained my Master's Degree on Neuroscience and my Master's Thesis was focused on studying the regulation of neuron migration mediated by cannabinoid CB<sub>1</sub> receptors. My PhD thesis is

involved in understanding the role of Endocannabinoid System along neural development in early embryonic stages.

### **África Sanchiz Giraldo**

I hold a Bachelor's Degree in Biotechnology by the University of Leon and a Master's Degree in Plant Biotechnology by Autonomous University of Barcelona. After that, I got a predoctoral fellowship to do my PhD thesis about the influence of thermal treatments on cashew and pistachio immunoreactivity as well as in the detection of these nuts by real time PCR. I carry out these activities in Food Science Department (INIA) and Genetics Department of Faculty of Biology (UCM).

### **Elena Tena López**

I obtained my Bachelor's Degree on Biology (2014) and a Master's degree in Conservation Biology (2015) both by the Complutense University of Madrid. Currently, I am doing my PhD in José Luis Tellería's lab with distribution and habitat selection of bats in the centre of the Iberian Peninsula. My scientific interest is linked to Biology and Conservation of terrestrial vertebrates, especially mammals and birds. My expectations are focused in the study of bats across acoustic identification. Chiroptera have been affected in their interaction with human, therefore, I am worried about knowledge of their distribution, habitat selection and modelling patterns.

### **Esther Lantero Bringas**

I obtained my Bachelor's Degree in Biology with neurobiology specialization (2013) and a Master's degree on Genetics and Cellular Biology (2014) in the Complutense University of Madrid. I collaborate at Genetics Department since 2012, where I have been able to defend a *Tesina* on phylogeography of the olive fruit fly on 2014. My PhD project is focused on the genetic characterization of *Bactrocera oleae* and its application to biological control, trying to improve the olive pest management.

### **Héctor Alonso Miguel**

I began my research career when I did my Bachelor's thesis on analyzing the potential use of essential oils as fungicides. Afterwards, I obtained my Master's Degree on Applied Plant Biology (2015-16) in Complutense University, and my Master's thesis was involved in studying composition variability of essences in several plant species. I am currently doing my PhD thesis on composition and variability of essential oil in Citrus. Moreover, I am collaborator in the Botanic Department, Faculty of Biology in Complutense University and the Royal Botanical Garden "Alfonso XII".

### **María Torres Sánchez**

I obtained a Bachelor's Degree in Biology (University of Santiago de Compostela, 2007-12) and two Master's Degrees: one in Biodiversity and Conservation in Tropical areas (International University of Menéndez Pelayo, 2012-13) and another in Bioinformatics and Biostatistics (Open University of Catalunya and Barcelona University, 2015-17). I am currently finishing my PhD in comparative transcriptomics and gene discovery in Caecilian amphibians at Complutense University of Madrid.

## **Awards**

Complutense University will reward the best communications with a prize of 750€ and two second prizes of 300€ to boost PhD students research. The Committee will be compound by member of the Doctoral Commission and will consider the comprehension, content and communication of the talks. Winners will be announced in the Closing Ceremony.

# Program





- 09:00** Welcome and conference opening
- 09:15** Plenary Session. **María Saura Álvarez.** Is it possible to devote to research in 21<sup>th</sup> century?
- 10:00 Oral Communications. Session 1.
- 10:00** **Irene de Sosa Carrasco.** Bless this phylogeographic mess.
- 10:10** **Alberto Jorge García.** Possible life-history traits linked with zinc incorporation in the mandibles of the hyper-diverse Hymenoptera.
- 10.20** **Esther Lantero Bringas.** Genetics in pest control.
- 10:30** **Hyelim Park.** Analysis of worldwide fisheries catches with relation to satellite-derived environmental variables, aggregated at two spatial scales.
- 10:40** **María Torres Sánchez.** Towards the secrets of life...
- 10:50** **Alejandro Llanos Garrido.** Outlier analysis to test for local adaptation in the contact zone between two lineages of a mediterranean lizard.
- 11:00** **Javier Pineda Pampliega.** Antioxidant supplementation and telomere attrition in white stork nestlings.
- 11:10** **Elena Tena López.** Patterns of use of urban parks by a community of bats: a case study in Madrid.
- 11:20** **Irene Cobo Simón.** Climate change-related shifts in the genetic diversity of relict tree species: understanding the patterns of molecular markers in *Abies pinsapo*.
- 11:30 *Poster session 1 and coffee break*
- 12:00 Oral communications. Session 2
- 12:00** **Belén Méndez Cea.** Genomic study of the adaptive potential of Scots pine populations (*Pinus sylvestris* L.).
- 12:10** **Héctor Alonso Miguel.** Comparison of essential oils of different species of the genus Citrus.
- 12:20** **Javier Fernández López.** Local adaptations or phylogenetic restrictions: what factors drives spore morphology in fungi?

**12:30**        **Sandra Nogal Prata.** *Aleurodiscus limonisporus*, a failed attempt of Gondwanan distribution.

**12:40**        **Nadia Fernández Jiménez.** The involvement of the *Arabidopsis thaliana* Nuclear Pore Complex during meiosis and DNA repair.

**12:50**        **Elsa Franco Echevarría.** The RNA binding protein Nrd1, presents a singular recognition mode for GUAA.

**13:00**        **Yolanda Pérez Pérez.** The phytohormone auxin plays a key role in microspore embryogenesis, a biotechnological tool in crop breeding.

**13:10**        **Eduardo Berenguer Peinado.** Autophagy: a way to improve microspore embryogenesis.

**13:20**        **Carmen Yuste Calvo.** Biotechnological functionalization of viral nanoparticles derived from an elongated flexuosus plant virus for different applications.

**13:30**        **Marta García Díaz.** Mycotoxin producing fungi in different stages of maize production cycle.

**13:40**        **África Sanchiz Giraldo.** Influence of technological processing on pistachio and cashew allergens and detection by real time PCR.

**13:50**        **Daniel Marín Peñalver.** Behaviour of liposomes under various technological treatments in the gelling properties of hake muscle.

14:00        *Lunch and poster session 2*

15:00        *Oral communications. Session 3.*

**15:00**        **Marta Seijo Vila.** Antitumor effect of pure cannabinoids versus *Cannabis sativa* whole plant extracts in preclinical models of breast cancer.

**15:10**        **Sandra Blasco Benito.** HER2-CB2 heteromers as new prognostic tools and therapeutic targets in breast cancer.

**15:20**        **Irene Berenice Maroto Martínez.** Potential neuroprotective role of the endocannabinoid system in striatal astrocytes.

**15:30**        **Daniel García Rincón.** Dissecting the neurodevelopmental consequences of prenatal THC exposure: when sex matters.

**15:40**        **Noemí Ceprián Costoso.** Dietary supplementation with antioxidants ameliorates the early immune function and behavior impairment in a mouse model of Alzheimer's disease.

**15:50**        **Antonio Garrido Tarrío.** Premature aging in behavior and immune functions in tyrosine hydroxylase haploinsufficient female mice. A longitudinal study.

**16:00**        **Fernando Gómez-Aldecoa Cuadrado.** Meiotic behaviour of a new derivative of the rye B-chromosome.

**16:10**        **Victoria Gálvez Cortés.** Development and characterization of a KLK5 overexpressing skin humanized mouse model resembling Netherton syndrome.

**16:20**        **José Carlos Castillo Sánchez.** Looking through the eyes of lung surfactant system to understand how breathing takes place in alveolar spaces.

**16:30**        **Cristina García Mouton.** Emulating the respiratory surface by a biophysical model.

**16:40**        **Raquel Arroyo Rodríguez.** Structural characterization of human pulmonary surfactant protein SP-D by atomic force microscopy.

**16:50**        **Laura Botana Veguillas.** Cytokine and chemokine dynamics in serum of patients with visceral leishmaniasis and cutaneous leishmaniasis caused by *Leishmania infantum*.

**17:10**        **Claudio Alba Rubio.** Comparison of different bioinformatics tools and pipelines in the study of gastric microbiome.

**17:20**        **Ana Alaminos Torres.** Physical activity for the prevention and treatment of obesity in genetically predisposed schoolchildren.

**17:30**        **María Teresa García Jiménez.** Renewing trends in Natural Science teaching approaches and its influence in public health (1876-1936). Environmental Education and Health Education.

17:40        *Poster session 3 and coffee break*

**18:15**        Debate. *After the PhD, what is next?*

- **Guillermo Fandos Guzmán.** Postdoctoral research scientist, looking for a contract. Department of Zoology and Physical Anthropology. Complutense University of Madrid.
- **Nuria Roldán.** Postdoctoral research scientist, applying for a Marie-Curie Postdoctoral fellowship.
- **Anna Chiarlone.** In-House Clinical Research Assistant. PRA Healthscience

**19:15**        *Award and closing ceremony.*

# Abstracts



# Communications

## Session 1

## **Bless this phylogeographic mess**

Irene de Sosa

*School of Biology, Complutense University, Madrid*

Due to the influence of Atlantic Ocean and Mediterranean Sea, the Iberian Peninsula presents a range of different climates, from desertic to Atlantic. We sampled populations of the parthenogenetic and ripicolous earthworm *Eiseniella tetraedra* in two different biogeographical areas in Spain (Madrid and Galicia), in order to study their genetic diversity and test their potential taxonomic distinctiveness. Moreover, we evaluated the presence or absence of two different genetic lineages (Eurosiberian and Mediterranean) found in other parthenogenetic earthworms such as *Aporrectodea trapezoides* and *A. rosea*. We studied the molecular markers COI, 16S and 28S. *E. tetraedra* presents a high diversity in the study areas (six lineages and one COI haplotype every two individuals were found) and no clear geographical patterns. However, there is a diffuse pattern along the Guadarrama River basin in relation with a contamination gradient. On the other hand, worldwide localities were genetically more homogeneous with lower diversity. After morphological study, no correlation was found between phylogenetic relationships and the diagnostic characters for the previously described subspecies in *E. tetraedra*. After these unexpected preliminary findings, we decided to increase the sampling effort by adding populations covering the whole area of the Iberian Peninsula. This is currently being done. Furthermore, we have created a research network with international researchers. The aim of this project is to construct a European phylogeography, in order to understand the history of this species and revisit the systematics of the genus.

**Keywords:** earthworm, *Eiseniella tetraedra*, genetic variability, population structure, phylogeography, contamination

## **Possible life-history traits linked with zinc incorporation in the mandibles of the hyper-diverse Hymenoptera**

Alberto Jorge

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Within Hymenoptera (bees, wasps and ants), structures related with oviposition and feeding are known to have a cuticle hardened by incorporation of trace metals, such as Zn. Mandibles are used by adults for feeding, digging and crawling at emergence, so that their composition may be also under selection through ecological pressures. We used data on Zn incorporation in the mandibles of species of Hymenoptera to test the effect of key life-history traits (adult emergence substrate, larval feeding resource, adult foraging mode) on the observed variability. Zn lacked in the three most primitive “symphytan” superfamilies, while it was ubiquitous in all apocritan superfamilies except, within “Parasitica”, in Proctotrupeoidea (common) and, within Aculeata, in Vespoidea (rare) and in Apoidea (extremely rare). We found that parasitoids and herbivores developing into plants tend to have greater Zn-enrichment in their mandibles than pollinivorous species (bees). Zn was also higher in species developing in concealed, compared with unconcealed, sites. However, there is an effect on phylogenetic: most parasitoids are within the “Parasitica” and all of them have Zn, independently from site concealment; predatory apoid wasps are closely related to pollinivorous bees, and both almost invariably lack Zn; among herbivores developing into plants, Zn occurs in all “Parasitica” species associated with galls (Cynipidae) but only in the most derived “Symphyta”. Within Vespoidea and Apoidea, the few cases of Zn incorporation they exclusively found in primitive parasitoid lineages and in Formicidae, suggesting that metal enrichment was independently lost and re-acquired a few times during evolution of Aculeata.

**Keywords:** Hymenoptera, mandibles, zinc metal, ecological pressures

## Genetics in pest control

Esther Lantero Bringas

*Faculty of Biology, Department of Genetics. Av. José Antonio Novais 12, 28040 Madrid.*

The olive fruit fly *Bactrocera oleae* (Rossi 1790) is the major pest of olive crops (*Olea europaea*). Its larvae are monophagous and feed exclusively on olives after the adult females lay the eggs under the fruit epidermis. Many countries at the Mediterranean Basin, headed by Spain, are the main worldwide producers and exporters of olives and olive oil.

Given the economic impact caused by the presence of this pest at the olive groves, it is mandatory to increase our knowledge about this species. Thus, here we present a genetic study divided in two main parts.

- First, considering the massive use of the organophosphates (OPs) to control the olive fruit fly populations, we have screened the presence of resistant alleles to OPs at the Mediterranean populations.
- Second, given the frequency of resistant alleles at the olive fruit fly populations we have performed molecular markers to detect *B. oleae* in arthropods' gut present at the olive groves. This will allow identifying the best candidates for pest control trying to promote the biological control mediated by predators, an alternative solution to the use of chemical products

**Keywords:** molecular genetics, molecular markers, Integrated Pest Management.



# **Analysis of worldwide fisheries catches with relation to satellite-derived environmental variables, aggregated at two spatial scales**

Hyelim Park

*Department of Ecology, Faculty of Biological Sciences, Complutense University of Madrid. 28040. Madrid. Spain*

Climate change has wide-ranging effects on ocean ecosystems, affecting fisheries catches in several ways and at various spatial scales. Our work highlights the possible changes in fishery catch rate over time related with three environmental variables: Sea Surface Temperature (SST4), concentrations of Chlorophyll (CHL), and the PIC:POC (PIC: Particulate Inorganic Carbon concentration; POC: Particulate Organic Carbon concentration) ratio at two spatial scales: worldwide and the nineteen FAO Major Fishing Area (FAO-MFA). We used FAO fishery database (FishStatJ) and MODIS Aqua satellite images from 2002 to 2014.

The Vector Auto-Regressive (VAR) model for capture rate at a worldwide level included SST4 as causal variables. Varying spatial conditions in fisheries, as well as the influence of climate change variables has been detected. VAR model for capture rate at each FAO-MFA level was constructed significantly within only seven FAO areas and included different variables. In the region based VAR model, a fishery change at each FAO-MFA was represented with independent environment conditions. Also, the effects of the top-down or bottom-up mechanisms should be considered depending on the scale and in every FAO area, thus introducing a discussion of mutual effects. Furthermore, there is a need to consider the changes of various factors –not only environmental but also the economic effect such as fishery efforts– to understand future changes and availability of fisheries captures.

**Keywords:** FAO FishStatJ, MODIS Aqua, Sea Surface Temperature (SST4), Chlorophyll concentration, Carbon cycle ratio (PIC:POC ratio), Vector Auto-Regressions (VAR) models.

## **Towards *the secrets of life...***

María Torres Sánchez

*Department of Biodiversity, Ecology and Evolution, School of Biology, Complutense University of Madrid*

Many aspects of biological diversity remain unknown and understudied. With the advent of the genomic era, RNA sequencing (RNA-seq) has become one of the most powerful tools to unravel the secrets of biological adaptation and diversity in all species through their particular gene expression profiles. We studied comparatively the genes expressed in different tissues of several species of one of the least known group of vertebrates, the caecilians (order Gymnophiona). Caecilians are fossorial, limbless, tropical amphibians that constitute the sister group of frogs and salamanders. Little is known about this enigmatic animal group. To improve the understanding of caecilian ecology and evolution, we have analysed caecilian genomic functional elements at three levels: among other vertebrates, among caecilian species and among caecilian tissue types. Our study provides valuable insights about the expansion of gene machineries in vertebrates, points out protein-coding genes involved in the specific evolutionary adaptations of caecilian amphibians, and highlights important functional elements in the caecilian skin tissue type. To our knowledge, this is the first large-scale genomic characterization of the genetic functional elements of this secretive vertebrate group, and it provides the basis for future research on the molecular elements underlying the remarkable biology of caecilian amphibians.

**Keywords:** Caecilians, gene expression, gene families, molecular innovations, skin specializations, transcriptomics.

## **Outlier analysis to test for local adaptation in the contact zone between two lineages of a mediterranean lizard**

Alejandro Llanos Garrido

*Department of Zoology and Physical Anthropology, Faculty of Biology, Universidad Complutense de Madrid, Madrid, Spain.*

Local adaptation, a stepping stone to ecological speciation, shapes species diversity and distribution ranges. Next Generation Sequencing approaches allow us to investigate local adaptation by comparing allele frequencies and outlier loci along environmental clines. Here, we use such approaches to explore the key question of how is local adaptation affected by historical and current contingencies.

We sampled five populations from the contact zone between two lineages of the lacertid lizard *Psammodromus algirus*, with phenotypic differences that have been previously shown to be adaptive. We used outlier analysis to detect loci putatively under selection, and we estimated phylogeographic relationships and admixture among populations on the basis of 73,000 genome-wide single nucleotide polymorphisms (SNPs) and 21 outlier SNPs.

We found evidence of genetic structure between lineages using all loci, and of genetic structure within lineages using only outliers. We also found that populations with lower levels of genetic differentiation for outlier loci have conservative genotypes that seem to maintain the ancestral gene pool, whereas populations with high levels of local differentiation have got them by isolation.

The genetic structure inferred with outlier loci only is strongly consistent with previous knowledge about habitat-phenotype associations, to the extent of detecting not unexpected, but relatively subtle among-population differences within one of the two lineages. Our results suggest that outlier loci may be informative not only of ongoing adaptation, but also of demographic history and connectivity, which are strongly linked to a well known scenario of ecological divergence.

**Keywords:** local adaptation, outlier SNPs, population genomics, GBS, *Psammodromus algirus*.

## **Antioxidant supplementation and telomere attrition in white stork nestlings.**

Javier Pineda-Pampliega

*Department of Biodiversity, Ecology and Evolution, Faculty of Biology, Complutense University of Madrid, 28040 Madrid, Spain*

Diet is one of the main environmental traits during nestling period that have shown marked effects during adulthood. For years now, white storks are using rubbish dumps as feeding areas to feed their chicks because their constant supply of food make these areas stable and predictable food resource. The counterpart is the presence of different pollutants and additives in processed food, which added to the lack of antioxidants, makes this diet pro-oxidant. The study of the possible long-term effects required individual recapture, which sometimes is difficult or even impossible, reason why we propose instead the measurement of telomere attrition. Telomeres are conserved structure at chromosome ends with function in genome stability, which attrition has recently emerged as a candidate biomarker of ageing, phenotypic quality and expected lifespan.

To investigate the effect of feed in rubbish dumps on fitness prospects we carried out an experiment in the wild using nestlings whose parents feed them with food from rubbish dumps. We supplemented them with antioxidants because of the pro-oxidant characteristics of the food and the known correlation of antioxidants with telomere attrition. During 3 weeks we administered 2 doses of vitamins and extract 3 blood samples (at day 0, 10 and 21 of the experiment) where we evaluated telomere length using Telomere Restriction Length Assay. Our objectives are establish telomere length and the rate of telomere attrition in white storks, as well as the possible alleviative effect over this rate of the antioxidant supplementation.

**Keywords:** White stork, telomeres, rubbish dumps, antioxidants, nestlings.

## **Patterns of use of urban parks by a community of bats: a case study in Madrid**

Elena Tena

*Departamento de Biodiversidad, Ecología y Evolución, Facultad de Biología, Universidad Complutense, Madrid. Corresponding Author: [e.tena@ucm.es](mailto:e.tena@ucm.es)*

The order Chiroptera is a group of mammals relevant for its high species richness. However, studies on its distribution in urban environments are poor. This study analyzes 27 parks located in the city of Madrid that vary in size, isolation distance, vegetation structure and water availability. The parks were sampled on 4 occasions between July and October of 2015 and 2017. An ultrasound detector was used in 10 minute sample units distributed according to the size of the parks. In addition, bats were sampled during four years (2014-2017) in the Madrid Mountains with the same methodology. Eight species were recorded in the parklands and fifteen in the mountains. The main determinant for the species richness was the size of the parks, which was correlated with the presence of water points. The loss of species presented a nested pattern, with the rarest species located in the largest park and the most common distributed along the entire gradient. The nestedness rank was significant for the size and the isolation distance of the parks, which clearly represents the importance of geographical factors in the urban distribution of bats. The positive relation among the appearance frequencies of bats species in the Mountains and in the parklands, proves the role of parks as a passive sampling of a bat community.

This study was supported by the Council for Culture, Education and Sport in the Autonomous Community of Madrid and the European Social Fund.

**Keywords:** Bat diversity, Chiroptera, fragmentation, habitat selection, nestedness, richness.

## **Climate change-related shifts in the genetic diversity of relict tree species: understanding the patterns of molecular markers in *Abies pinsapo***

Irene Cobo Simón

*Department of Physiology, Genetics and Microbiology, Faculty of Biology, Complutense University, Madrid. Department of Physical, Chemical and Natural Systems, University Pablo de Olavide, Sevilla. [irenecob@ucm.es](mailto:irenecob@ucm.es).*

The Spanish fir (*Abies pinsapo* Boiss.) is a drought-sensitive conifer, endemic from southern Iberian Peninsula. This relict tree has showed widespread mortality and growth decline over the last decades, which have been related to land-use and recent climate change. It is known that relict species constitute reliable models to shed light on adaptation process to rapid environmental changes. Here we attempt to place population genetic structure in a climate change context, using as experimental system the drought-sensitive fir *A. pinsapo*. We investigated the pattern of nuclear microsatellites (nSSR), chloroplast microsatellites (cpSSR), intermicrosatellites (ISSR) and single nucleotide polymorphisms (SNP) of two drought resistance-related candidate genes (PIP1 and GORK) to the main hypothesis that selective pressure of climate change is related to molecular markers structure of *A. pinsapo* populations along a climate gradient. Our results aim of (1) obtaining information about its current status in order to design appropriate conservation strategies and (2) testing a possible effect of the recent climate change on its genetic structure. Low genetic diversity and high inbreeding were found for this species. In addition, significant differentiation among populations was obtained, together with evidences of past bottlenecks. Furthermore, weak but significant differences were found related to altitude, age and survival. Environmental Association Analysis also showed a significant relation between GORK gene and both altitude and survival, indicating a possible role of this gene facing drought in *A. pinsapo*. These results point to a possible effect of recent climate change on the genetic structure of these populations.

**Keywords:** Climate Change, relict species, trees, conifers, molecular markers

# Communications

## Session 2

# **Genomic study of the adaptive potential of Scots pine populations (*Pinus sylvestris* L.)**

Belén Méndez Cea

*Physiology, genetics and microbiology Department, Faculty of Biology, Complutense University of Madrid, Calle José Antonio Novais, 12, 28040 Madrid, Spain.*

*Pinus* is a genus of conifers with a wide range of distribution, which it is made up of more than one hundred species. *Pinus sylvestris* L. is one of the species of this genus, and it is the second most extended conifer in the northern hemisphere, with more than 14,000 km from the Iberian Peninsula to the Siberian plain. Scots pine has persisted in the Mediterranean region where it forms relict populations. Because of its ability to grow at elevations and in a geographical broad range, it is located in different types of habitats with various conditions.

Many studies have reported that Climate Change is a real threat for forests, and specifically for Scots pine. The distribution of this species is now shifting due to the effects of global warming, and specifically due to the severity of drought. For this reason, we are studying the correlation between the genotype of trees and their response to Climate Change.

Ecological genomic approaches coupled with next- generation sequencing are useful means to detect local adaptation and uncover its underlying genetic basis in tree species. We report results from a study on Scots pine using genotyping by sequencing (GBS). We analyzed subpopulations in divergent ecological habitats within Europe to uncover loci under local selection and associated with environmental–functional traits in response to Climate Change.

**Keywords:** *Pinus sylvestris*, Climate Change, GBS, Selection footprint.



## Comparison of essential oils of different species of the genus *Citrus*

Héctor Alonso Miguel

*Complutense University, Madrid*

The purpose of the study is analyze and extract of essential oils from pericarp of different species from genus *Citrus*, both of the parental species [mandarin (*C. reticulata* Blanco), pummelo (*C. maxima* Burm.), citron (*C. medica* L.), small-flowered papeda (*C. micrantha* Wester), kumquat (*C. japonica* Nagami)] and of the hybrids [sour orange (*C. × aurantium* L.), grapefruit (*C. × paradisi* Macfad.), sweet organge (*C. × sinensis* Osbeck.), lemon (*C. × limon* Osbeck.), mexican lime (*C. × aurantifolia* Swingle.). Pericarp essential oil is rich in compounds, the major is limonene in all of genus except in small-flowered papeda (*C. micranthalime*) and mexican lime (*C. × aurantifolia*), that p-Mentha-1(7),8-diene is the major compound. Moreover, small-flowered papeda (*C. micrantha*) has been found like the species more different in the composition within the set of citrus analyzed, the species more similar has been their hybrids lemon (*C. × limon*), mexican lime (*C. × aurantifolia*). Respect of the extract of essential oils, the species with more output are mandarin (*C. reticulata*) and orange (*C. × sinensis*), and the species with less output are citron (*C. medica*) and lemon (*C. × limon*)

**Keywords:** *Citrus*, limonene, p-Mentha-1,(7),8-diene, essential oil, composition.

## **Local adaptations or phylogenetic restrictions: what factors drives spore morphology in fungi?**

Javier Fernández López

*Royal Botanic Garden, Spanish National Research Council (CSIC). School of Biology, Complutense University, Madrid*

Spores morphology has been considered as key trait in order to identify and recognize fungal species. However, despite of their importance in dispersal and sexual reproduction processes, little is known about the selective forces and evolutionary adaptations driving their morphology. In this research, we study the relation between spore size and environmental variables in a corticioid group of fungi (*Xylodon*, Hymenochaetales, Agaricomycotina). We combine both, molecular tools and ecological models to explore the effects of temperature and precipitation over spore size, accounting for non-independence in our sample data due to interspecific relations and common ancestry (phylogeny).

We found a strong impact of the phylogenetic history in the spore size (phylogenetic signal). We observed that, once phylogenetic signal is controlled, the effect of precipitation over spores size varies among temperature values, while high temperatures are correlated with higher spore size. These results are according with the hypothesis that spore size in fungi is influenced by water-balance dynamics; since large spores are able to store more water and nutrients, which are essential resources during germination processes.

J.F.L. was supported by Predoctoral Grants from the Spanish Ministry of Economy and Competitiveness (CGL2012-35559; CGL2015-67459-P; BES-2013-066429).

**Keywords:** fungi, spores, phylogenetic signal, temperature, precipitation.

## ***Aleurodiscus limonisporus*, a failed attempt of Gondwanan distribution**

Nogal-Prata Sandra<sup>1,2</sup>

*Royal Botanic Garden, Spanish National Research Council (CSIC) School of Biology, Complutense University, Madrid*

The previous morphological-based taxonomy has largely underestimated the true diversity and distribution patterns of corticoid fungi. Generally, this group is characterized by morphological stasis, in which genetic differentiation has not resulted in phenotypic variation. Therefore, it is necessary evaluate this diversity through integrative taxonomy framework, including information from different sources of evidence. During a recent investigation in Huinay Reserve in the Chilean fjords region, few specimens that correspond to *Aleurodiscus* were collected. These do not fit with any *Aleurodiscus* species previously reported from Patagonia; however, it seems morphologically *A. limonisporus*, species originally described from Australia, and reported from New Zealand too. The aim of this study was identify, characterize and analyse these specimens on the basis of the morphological and molecular data. Phylogenetic analyses, based on the concatenated dataset of the internal transcriber spacer (ITS) and nuclear large subunit ribosomal RNA (nLSU) sequences, show that *Aleurodiscus* from Huinay Reserve forms a high supported monophyletic clade, which is close related to the *A. limonisporus* clade. Moreover, key morphological characters allow discriminate between them. Both species have monomitic hyphal system, paraphyses, and large citriform spores; however, the *Aleurodiscus* species from Huinay Reserve differs from *A. limonisporus* in having hyphae with clamps (absent in *A. limonisporus*), basidia larger and wider,  $150\text{--}180 \times 22\text{--}27 \mu\text{m}$  (in *A. limonisporus*:  $130\text{--}160 \times 18\text{--}25 \mu\text{m}$ ), and basidiospores with length/width ratio (Q) = 1.38 (in *A. limonisporus*: Q= 1.50). These results led us to describe a new species: *Aleurodiscus patagonicus*.

Sandra Nogal Prata was supported by Predoctoral Grants from the Spanish Ministry of Economy and Competitiveness (CGL2015-67459-P; BES-2016-077793).

**Keywords:** *Aleurodiscus* – Chile- morphology – ITS nrDNA – nucLSU – russuloid lineage

## **The involvement of the *Arabidopsis thaliana* Nuclear Pore Complex during meiosis and DNA repair**

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Nuclear Envelope (NE) is a barrier that separates the nucleus from the surrounding cytoplasm and protects the genetic material. The NE is interrupted by Nuclear Pore Complexes (NPCs) that mediate the selective nucleocytoplasmic traffic of macromolecules. NPCs also participate in cell division and transcriptional regulation, and influence in the spatial organization of the chromatin. During meiosis, there is a large-scale chromatin remodeling and the NE plays an essential role in the achievement of chromosome movement at prophase I. In order to highlight a potential role of the components of the NPCs in meiosis we have isolated several *Arabidopsis* lines with mutations in genes coding for nucleoporins. Our first approach consisted on analysing nucleoporins that belong to the structural subcomplex NUP107-160 of the NPCs, specifically AtNUP160 and AtNUP96, also called SUPPRESSOR OF AUXIN RESISTANCE1 and 3, respectively. The cytological characterization of pollen mother cells (PMCs) in sar mutants has revealed the presence of a percentage of meiocytes with altered chromatin condensation and chromosome fragmentation. These defects do not seem to be related with epigenetic modifications and appear in both first and second meiotic division. We have also confirmed that sar mutants are hypersensitive to different DNA damage agents. These findings would suggest that SAR function could be related to the homologous recombination (HR) process. This study reveals a possible relationship between NPCs, meiosis and DNA repair that has not been previously reported in plants.

For this project, I have been granted with a FPU-PhD fellowship from the Spanish Ministry of Education.

**Keywords:** Meiosis, *Arabidopsis thaliana*, NPC, nucleoporins, AtSAR.

## **The RNA binding protein Nrd1, presents a singular recognition mode for GUAA**

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Transcription termination of non-coding RNAs transcripts is regulated in yeast by the NNS complex. This protein complex consists of three RNA binding proteins: Nrd1, Nab3 and Sen1. Specifically, Nrd1 interacts with Rbp1 of RNA polymerase II, Trf4 of TRAMP and also binds to nascent RNAs, which contain the GUAA/G terminator sequences. There is no structural information for the last of these binding events. By X-ray Crystallography, we have been able to determine the structure of the RNA binding domain of Nrd1. The structure reveals that, in fact, Nrd1 presents two domains: a canonical RRM (RNA recognition motif) and a novel domain (Split domain) with a new architecture. This organization has not been described before for the RRM domains. In addition, we have also crystallized Nrd1 bound to three different RNA complexes of different lengths containing the GUAA sequence. Our results allowed us to establish that the RNA binding site is preformed and to propose a unique mode of RNA binding, in which the two domains of Nrd1 are involved and only recognizes the GUAA sequence in a specific way. This expands our knowledge on RRM domains, one of the most common ways of RNA binding in living things.

**Keywords:** Crystallography, transcription termination, RNA binding proteins, RRM.

## **The phytohormone auxin plays a key role in microspore embryogenesis, a biotechnological tool in crop breeding**

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During anther development *in vivo*, microspores develop and follow the gametophytic pathway to produce pollen grains. *In vitro*, isolated microspores can be reprogrammed by stress treatments, and they become totipotent cells, follow the embryogenesis program, and produce doubled-haploid embryos and plants. Pollen embryogenesis is a powerful biotechnological tool for plant breeding, as the fastest way for rapid generation of new varieties through double haploid plants. Our knowledge on the involvement of the phytohormone auxin, which plays a key role in the pollen reprogramming, is very limited.

In my PhD thesis I analyze auxin concentration (by liquid chromatography linked to mass spectrometry) and cellular accumulation (by immunofluorescence and confocal laser microscopy), expression of *TAAI* auxin biosynthesis gene and *PINI-like* efflux carrier gene (by qPCR), as well as the effects in microspore embryogenesis of inhibitors of auxin biosynthesis (Kynurenin), transport (N-1-naphthylphthalamic acid, NPA) and action ( $\alpha$ -(p-Chlorophenoxy) isobutyric acid, PCIB) in these two microspore developmental pathways, gametophytic and embryogenic, in two model herbaceous species (the dicot *Brassica napus* and the monocot *Hordeum vulgare*).

The results obtained indicate that auxin biosynthesis, polar transport and action were required for microspore embryo initiation and progression, while auxin progressively diminishes during gametophytic development, in the two species, the dicot rapeseed and the monocot barley. These findings indicate the key role of auxin in directing the cell fate of the microspore.

Work supported by project (AGL2014-52028-R) funded by MINECO. Work contract by CSIC-Comunidad de Madrid, European Union implicated with European Social Fund (ESF), Youth Employment Initiative (YEI).

**Keywords:** auxin, pollen, microspore embryogenesis, gametophytic development, *Brassica napus*, *Hordeum vulgare*

## **Autophagy: a way to improve microspore embryogenesis**

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Microspore embryogenesis is an important biotechnological tool that permits rapid generation of doubled-haploid plants and produce a source of new genetic variability. This system permits the microspore to reprogram, acquire totipotency and induce embryogenesis in a specific stage of development by in vitro stress treatment. Nevertheless, the new developmental program is limited by the occurrence of cell death after the inductive stress. Autophagy is the major catabolic process in eukaryotic cells wherein a portion of the cytoplasm is engulfed by a specific membrane, delivered to vacuole and finally digested by hydrolytic enzymes. In this work we have analyzed the involvement of cell-death proteases and autophagy in the initiation and/or execution of cell death during microspore embryogenesis induction in *Brassica napus*. In vivo treatments with several autophagy and protease inhibitors were performed and their effects on cell death and embryogenesis induction efficiency analyzed. Results revealed that cell death increased after stress treatment accompanied with the appearance of autophagic structures. Furthermore, preliminary assays revealed increased metacaspase activity in stress-treated microspores. This activity was inhibited in vitro by leupeptin but not by E64. In vivo treatments to inhibit autophagy reduced cell death and increased embryogenesis induction. Taken together, these results open new possibilities to improve microspore embryogenesis efficiency in recalcitrant species using chemical modulators of autophagy. Funding: Work supported by project grant AGL2014-52028-R of the Spanish Ministry of Economy and Competitiveness (MINECO) and the European Regional Development Fund (ERDF/FEDER), and STSM grant of Autophagy COST action CA15138 in SLU stay (Uppsala, Sweden).

**Keywords:** microspore embryogenesis, stress response, autophagy, metacaspase, cell death.

## **Biotechnological functionalization of viral nanoparticles derived from an elongated flexuous plant virus for different applications**

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Functionalization of viruses as nanoparticles is rapidly and strongly emerging as a powerful technology with multiple applications in many different fields such as biomedicine, biomaterial engineering, antimicrobial surfaces, image enhancers, or enzymatic immobilization among others. In this context, plant viruses show clear advantages such as biosafety, fast and massive production using plants as bioreactors, or low costing. Many applications have been developed using icosahedral viruses, but rod-shaped viruses have also attracted attention as viral nanoparticles (VNPs). In our work, we are developing the functionalization of Turnip mosaic virus (TuMV) nanoparticles, an elongated flexuous plant virus. TuMV allows the multiple-display of different molecules (more than 2000 per viral particle) on its surface. By a deep study of the structure of the TuVM coat protein (CP) and its amino acid residues, we have developed different approaches for VNP functionalization depending on the application itself or the compound of interest, approaches related with genetic fusion or chemical conjugation. So far, these applications include biomedical-related ones, such as ultrasensitive antibody detection in connection with inflammation; veterinary in relation with important animal viruses; and industrial, by the nanoimmobilization of industrially relevant enzymes. Progress towards these goals and current achievements for the different applications will be described and discussed. The main text of your abstract goes here. Please have in mind that readers may not be familiar with your research field.

**Keywords:** Nanobiotechnology, biomedicine, nanoparticle functionalization, viral nanoparticles



## **Mycotoxin-producing fungi in different stages of maize production cycle**

Marta García-Díaz

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Maize (*Zea mays* L.) is one of the main cereals present in food, feed and processed products. Contamination of these products by mycotoxins represents a significant risk to human and animal health. The objective of this work was to evaluate the presence of mycotoxin-producing fungi in order to detect the moment when contamination occurs during maize production cycle. Maize samples were evaluated in two plots located in Madrid (Spain). Four stages were sampled: anthesis or male flowering (25 male flowers and 25 female flowers), preharvest (25 cobs), post-harvest (3 kg of grain) and stored maize (1 kg of grain). DNA extraction was performed after 24 h of incubation at 28 °C in Sabouraud-cloramphenicol broth and species-specific PCR protocols were used to detect the most important mycotoxigenic *Aspergillus* and *Fusarium* species. The aflatoxin-producing species *A. flavus* and the fumonisin producer *F. verticillioides* were detected from the first stage, indicating that contamination occurred in the earliest period of the production cycle. In pre-harvest, new species were detected including fumonisin-producing species (*F. proliferatum*) and ochratoxin A producers (*A. niger* aggregate species). All of them were also detected during post-harvest and storage. The knowledge of the moment when contamination occurs is crucial to establish a correct treatment schedule to prevent mycotoxins entering the food chain. This work was supported by AGL2014-53928-C2-2R.

**Keywords:** Mycotoxins, Maize, *Aspergillus*, *Fusarium*.

# **Influence Of Technological Processing On Pistachio And Cashew Allergens And Detection By Real Time PCR**

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Food allergy is a serious health concern that affects to 1-3% of the general population. Pistachio and cashew are appreciate because of their healthy benefits; however, prevalence of these tree nuts allergy is increasing over the years and symptoms cover from mild to fatal consequences.

To assure patients safety and compliance of the European regulation, reliable and specific methodologies to detect allergenic ingredients in food products are necessary. Food processing, commonly used in industry, might modify structure of proteins and allergenic properties. Consequently, technological treatment can be used to obtain material with reduced IgE-binding capabilities.

Real time PCR assays to detect and quantify pistachio and cashew in processed foods have been developed. Primers and probes from allergen-coding sequences were designed, allowing detection up to 10 ppm from both tree nuts. The effect of thermal processing on pistachio and cashew detectability has been analyzed. Boiling did not affect amplification, whereas heat and pressure considerably reduced detectability.

The influence of boiling and autoclave treatment on pistachio and cashew immunoreactivity has been determined using traditional immunoassays, complemented with Skin Prick Test and mediator release assay to test the IgE cross-linking capability. Moist thermal treatment reduced IgE binding capacity of both nuts, especially after heat and pressure processing. Degranulation of basophils after challenge with treated cashew and pistachio samples was also diminished compared to non-processed. Boiled nuts produced an important decrease in wheal size in SPT and effector cell degranulation.

Study supported by AGL2012-39863-C02-02 (MINECO). AS is beneficiary of a grant from MINECO (BES2013065833)

**Keywords:** cashew, pistachio, allergy, real time PCR, thermal processing

## **Design, development and characterization of phosphatidylcholine liposomes to be used as carriers bioactive extracts**

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The aim of this work was to standardize the elaboration of phosphatidylcholine liposomes to be used as carriers of natural bioactive extracts. After characterization of the raw materials, diverse liposomes were elaborated from different purification-grade phosphatidylcholines (2 or 5 washes) extracted from soybean lecithin and different sonication procedures (120W-5min or 30W-2min). A control lecithin liposome was also studied. Once selected the most appropriate conditions, liposomes were loaded with bioactive products of different nature from agro-industrial waste or underutilized sources: sea fennel, pomegranate peel, crustacean bio-wastes or fish collagen.

The lipid fractionation evidenced that the purification washes promoted the phosphatidylcholine concentration (95% of the total lipids) reducing free fatty acids and neutral lipids (90%). Besides, the phosphatidylcholine signal was 164 times greater than that for other phospholipid classes. The phosphatidylcholine concentration was higher ( $p \leq 0.05$ ) in the five-washes sample, that explains the more accentuated reduction in free fatty acids and neutral lipids than in two-washes sample. These results were supported by the FTIR data.

All liposomes presented high stability ( $< -30\text{mV}$ ), being the liposome prepared from phosphatidylcholine with five-washes, and strong sonication (L5A) the smallest (87nm). The endothermic transition peaks and enthalpy changes shown by this liposome reflected its great phosphatidylcholine concentration and higher bilayer stability. The prevalence of small unilamellar vesicles was confirmed by transmission electronic microscopy.

The different bioactive extracts added to L5A-liposome did not change the mean size or morphology, and provided good storage stability and high antioxidant properties.

**Keywords:** soybean lecithin, phosphatidylcholine, phospholipids, liposomes, bioactives

# Communications

## Session 3

## **Antitumor effect of pure cannabinoids versus *Cannabis sativa* whole-plant extracts in preclinical models of breast cancer**

Marta Seijo-Vila

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Breast cancer is a heterogeneous disease sub-classified into three major subtypes: ER+/PR+, HER2+, and triple-negative. Despite the currently available targeted and non-targeted treatments, a variable percentage of patients doesn't respond to them, and many relapse with time. It is therefore obvious the need of new/complementary therapeutic approaches to manage this pathology. Cannabinoids such as  $\Delta^9$ -tetrahydrocannabinol (THC) or cannabidiol (CBD) produce antitumor effects in preclinical models of cancer. In addition, preclinical and clinical data suggest that *Cannabis sativa* whole-plant extracts are better therapeutic tools than pure cannabinoids. These preparations contain a wide variety of cannabinoid and non-cannabinoid compounds, which present therapeutic potential by themselves and interact in a synergic manner when combined.

Therefore, the aim of this project was to compare the antitumor effect of pure cannabinoids versus whole-plant extracts, in preclinical models of breast cancer. Our results showed that the THC-rich extract used in this study was more efficient than pure THC in decreasing the viability of ER+/PR+, HER2+ and TN breast cancer cell lines. On the other hand, pure CBD was as potent as the CBD-rich extract used in this project. We have also observed that pure cannabinoids produce their effects, at least in part, by activation of CB<sub>2</sub> and the production of reactive oxygen species. In the case of the THC-rich extract, additional mechanisms of action were evident, and most likely attributable to the accompanying compounds or the interaction between them.

These results support the idea that THC-rich whole-plant extracts improve the therapeutic efficacy of pure THC.

**Keywords:** Breast cancer, antitumor actions, whole-plant extracts.

## **HER2-CB<sub>2</sub> heteromers as a new prognostic tools and therapeutic targets in breast cancer**

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Breast cancer is one of the most frequent malignancies worldwide and represents an important public health problem. This disease is sub-classified in different subtypes. One of them is characterized by the overexpression of the human epidermal growth factor receptor 2 (HER2). In this context, our group has focused on two major goals: 1) understanding the role of the endocannabinoid system in the physio-pathology of HER2+ breast tumors, and 2) analyzing the antitumor potential of plant-derived cannabinoids in breast cancer.

Regarding the first aim, we have demonstrated that CB<sub>2</sub> cannabinoid receptor is overexpressed in HER2+ breast cancer, and that promotes tumor generation and progression. Moreover, we have found that CB<sub>2</sub> forms heteromers with HER2 in human breast cancer samples, and that this new structures are associated to poor patient prognosis.

Concerning the second goal, we previously found that cannabinoids induce antitumor responses in breast cancer via activation of CB<sub>2</sub> receptors. We therefore decided to study the involvement of the HER2-CB<sub>2</sub> heteromers in such actions. Our results show that  $\Delta^9$ -tetrahydrocannabinol (THC) produces antiproliferative responses that are accompanied by the reduction in HER2-CB<sub>2</sub> heteromers, followed by HER2 inactivation, ubiquitination and degradation.

Taken together, our results suggest that HER2-CB<sub>2</sub> heteromers may constitute a new prognostic tool in HER2+ breast cancer, and a new therapeutic target for this tumor subtype. Specifically, we hypothesize that pharmacological tools disrupting these structures (including THC) would produce antitumor responses.

**Keywords:** breast cancer, CB<sub>2</sub> cannabinoid receptor, HER2, cannabinoids

## Potential neuroprotective role of the endocannabinoid system in striatal astrocytes

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The endocannabinoid system exerts neuroprotective functions in many different animal models of acute brain damage and chronic neurodegeneration, which has raised hope about the possible clinical use of cannabinoids as neuroprotective drugs. However, the assessment of the physiological relevance and therapeutic potential of cannabinoids is hampered by the lack of knowledge of the cell-population specificity of CB<sub>1</sub> receptor action. Here we study the potential neuroprotective role of the endocannabinoid system elements in astrocytes of the mouse corticostriatal circuitry.

We used an adenoviral-vector delivery strategy based on the expression of CFP-tagged mutant huntingtin harboring a pathogenic polyQ repeat of 94 residues under the control of an astroglial promoter in the dorsal striatum. Selective mutant huntingtin expression in striatal astrocytes led to impairments in motor coordination and alterations of striatal markers. However, when animals were treated with THC or JZL-184, an inhibitor of monoacylglycerol lipase (MAGL), motor coordination and striatal integrity were recovered.

Next we explored the role of astroglial CB<sub>1</sub>R and astroglial MAGL in the observed neuroprotection. The deletion of CB<sub>1</sub>R in astrocytes did not produce any significant effect but it does in cortical projections enhanced mutant huntingtin-induced damage. In mice lacking MAGL in astrocytes the toxic effects of mutant huntingtin were attenuated.

These data unveil an interdependence between striatal astrocytes and corticostriatal projections in the control of striatal neuron survival. Since astrocytes are crucial for neuronal physiology and their dysregulation occurs in many neurodegenerative diseases, this cell type may represent a relevant target to design cannabinoid-based therapies.

**Keywords:** endocannabinoid system, astrocytes, corticostriatal circuitry, neuroprotection

## **Dissecting the neurodevelopmental consequences of prenatal THC exposure: when sex matters**

Daniel García Rincón

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Cannabinoid CB<sub>1</sub> receptor is an important regulator of mammalian brain development, since it modulates key processes as neural progenitor proliferation, neuronal specification and neuronal migration. Hence, embryonic manipulation of CB<sub>1</sub> receptor function results in long-lasting alterations that involve both glutamatergic and GABAergic neuron subpopulations. Dissecting how these cohorts are differently affected is a crucial step in order to address the consequences of cannabis abuse during pregnancy.

Our work shows that prenatal THC exposure induces an assortment of histological and behavioral outcomes in adult stages, including increased seizure susceptibility. In addition, embryonic THC exposure induces cognitive and neuropsychiatric alterations in humans. Our results herein present that some of the long-term consequences of prenatal THC administration exhibit a striking sexual dimorphism. Thus, among the offspring of THC-treated dams, males are more prone to pentylenetetrazole-induced seizures. This sex-selective sensitivity to prenatal THC exposure is present in other traits, such as CB<sub>1</sub> agonist-evoked hypothermia or hypolocomotion. We found that THC-exposed adult males exhibit a selective reduction of CB<sub>1</sub>-expressing cholecystinin (CCK)-containing basket interneuron density. Interestingly, no obvious cortical alterations are detected by cortical EEG-recordings and a behavioral test dependent on cortical function appears unaffected, while impaired performance in a spatial memory-dependent task was evident. Spatial memory is known to rely on hippocampal circuit activity, and in particular CCK+ basket cells play a crucial role in spatial information coding. In summary, embryonic THC exposure interferes the appropriate development of selective interneuron cell populations. This in turn disrupts hippocampal circuitry, driving long-term behavioral consequences in adult stages.

**Keywords:** cannabis, CB<sub>1</sub> cannabinoid receptor, prenatal exposure, sex differences, THC.



## **Dietary supplementation with antioxidants ameliorates the early immune function and behaviour impairment in a mouse model of Alzheimer's disease**

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Dietary supplementation with antioxidants has been shown useful for improving the immune and the nervous system functions as well as the behaviour in mice. In this regard, diets such as the Mediterranean diet has been shown as a protective factor in Alzheimer development in humans. The aim of the present work was to study the effect of an enriched diet with antioxidants on the behaviour and immune functions of a transgenic mouse model of Alzheimer's Disease (3xTgAD).

Female 3xTgAD mice received a diet supplemented with the antioxidants N-acetylcysteine and tioproline (0.1%, 3xTg-Diet). Another group of 3xTgAD (3xTgAD-Control) and a group of non-transgenic (NTg) mice received a standard diet. The supplementation started at 2.5 months of age. At 2, 4 and 6 months of age, the peritoneal cells were obtained and immune (phagocytosis, natural killer activity, basal and stimulated lymphoproliferation) and oxidative (glutathione, malondialdehyde) parameters were analysed. Also, at 6 months the episodic memory (object recognition test), the anxiety-like behaviour (elevated plus maze) and the exploratory capacity (T-maze) were analysed.

The results showed a decrease in anxiety and an improvement of exploration capacity and memory, as well as of the immune functions studied in 3xTgAD-Diet (both at 4 and 6 months of age) in comparison with 3xTgAD-Control mice. The values of parameters studied were in 3xTgAD-Diet similar to those obtained in NTg animals.

In conclusion, the early impairment of behaviour and immunity observed in 3xTgAD mice with respect to NTg animals can be avoided with a diet supplemented with antioxidants.

Support: FIS-FEDER-(PI15/01787)-ISCIII-FEDER, UCM-(910379ENEROINN)

**Keywords:** Alzheimer's Disease, antioxidants, immune function, behavior, mice, diet.

## **Premature aging in behavior and immune functions in tyrosine hydroxylase haploinsufficient female mice. A longitudinal study**

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Aging is accompanied by impairment in nervous, immune, and endocrine systems and in neuroimmunoendocrine communication. Indeed, there is an age-related alteration of the physiological response to acute stress, which is modulated by catecholamine (CA), final products of the sympathetic-adreno-medullary axis. The involvement of CA in essential functions of nervous system are consistent with the neuropsychological deficits found in mice with haploinsufficiency of tyrosine hydroxylase enzyme (TH-HZ). However, other alterations in regulatory systems have not been studied. The aim of work was to analyze whether adult TH-HZ female mice presented impairment of behavior and immunological responses that occur with aging and whether they affected their mean lifespan. ICR-CD1 female TH-HZ and WT mice were used in a longitudinal study. Behavioral tests were performed in adult and old mice in order to evaluate sensorimotor abilities and exploratory and anxiety-like behaviors. At ages of  $2\pm 1$ ,  $4\pm 1$ ,  $9\pm 1$ ,  $13\pm 1$  and  $20\pm 1$  months, peritoneal leukocytes were extracted and several immune functions were assessed. Also, several oxidative and inflammatory stress parameters were analyzed. The results indicate that adult TH-HZ mice presented worse sensorimotor abilities, exploratory capacity and greater anxiety-like behaviors than WT mice. Furthermore, adult TH-HZ mice exhibited lower values of phagocytosis, NK cytotoxicity, and lymphoproliferative response than WT mice, showing higher oxidative and inflammatory stress than their WT counterparts. This premature immunosenescence and impairment of behavior of TH-HZ mice was accompanied by shorter lifespan in comparison to WT counterparts. Thus, haploinsufficiency of *th* gene in female mice provokes premature aging of regulatory systems, affecting lifespan.

**Keywords:** Behavior, immunosenescence, premature aging, tyrosine hydroxylase, lifespan, longitudinal study.

## Meiotic behaviour of a new derivative of the rye B-chromosome

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B chromosomes (Bs) are supernumerary chromosomes that pair only among themselves at meiosis and do not recombine with the basic chromosome complement (A chromosomes). Rye Bs are mainly composed by repetitive DNA, lack functional genes and are not essential for the survival of the plant; therefore, they are used as a model system to study the structural requirements for correct chromosome transmission. Moreover, they may be modified to have useful attributes in plant biotechnology. We have analyzed by fluorescent *in situ* hybridization (FISH) the structure and meiotic behaviour of a new derivative of the rye B that arised naturally from an isochromosome of the long arm (Iso-BL).

The new derivative is an asymmetric deficient Iso-BL (adIso-BL) that has the typical structure of an Iso-BL but it is truncated in one of the arms, losing the distal heterochromatic blocks (half of block 4, and blocks 5 and 6). This deficiency abolishes the pairing of the truncated arm with the standard arm. In a plant disomic for adIso-BL, the frequency of metaphase I cells showing adIso-BL bivalents was twice the amount of standard-B bivalents observed in a plant disomic for the standard-B, 64% versus 37%, respectively. Because the formation of bivalents is the limiting factor for regular meiotic transmission, our results suggest that adIso-BL would transmit better than the standard-B to the next generation, and, therefore, has a better potential to be used as an artificial chromosome in biotechnology.

Research supported by the Spanish Ministry of Economy, Industry and Competitiveness (Grant AGL2011-28542).

**Keywords:** B-chromosomes, Meiotic transmission, Rye, FISH

## **Development and characterization of a KLK5 overexpressing skin humanized mouse model resembling netherton syndrome**

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Summary Netherton Syndrome (NS) is an autosomal recessive form of ichthyosis regarded as one of the most severe skin hereditary disorders affecting newborns, and patients present both cutaneous and immunological abnormalities. NS is caused by loss-of-function mutations in the *SPINK5* gene, encoding the serine-protease inhibitor LEKTI, whose deficiency results in unrestricted activity of kallikreins (mainly KLK5) that leads to degradation of corneodesmosomal proteins, thus producing the detachment of the stratum corneum and disruption of the epidermal barrier. Due to the limited availability of NS skin biopsies and ethical constraints of experimentation on human subjects we sought to develop a skin humanized mouse model for the disease based on the grafting of transgenic bioengineered human skin onto immunodeficient mice. In order to recapitulate the histopathological hallmarks of the condition we first considered an approach consisting in the overexpression of the KLK5 gene in human keratinocytes by lentiviral transduction. We have performed an initial characterization of the model through the analysis of histopathological markers associated to NS as well as KLK5 expression and activity. The study confirmed the alterations in the KLK5 pathway, which results in clear epidermal proliferation and differentiation alterations, similar to those observed in skin biopsies of NS patients. In a preliminary experiment, a topical formulation of a KLK5 inhibitory peptide was applied to KLK5 overexpressing grafts. The analysis of the treated grafts showed a lower activity of KLK5 and a certain degree of reversion of the main NS phenotypic markers.

**Keywords:** Genodermatoses, KLK5, LEKTI, bioengineered human skin.

## Looking through the eyes of lung surfactant system to understand how breathing takes place in alveolar spaces

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Respiratory dynamics would be hindered without the presence of lung surfactant complexes into alveolar spaces. Pulmonary surfactant is a lipoprotein complex that reduces surface tension into the alveolar air-liquid interface enabling respiratory dynamics, thus, gas exchange in the lungs. It is synthesized and packed into highly packed organelles, the so-called lamellar bodies, by alveolar type II cells. Afterwards, lamellar bodies are secreted and form a multilamellar membrane film coating the alveolar air-liquid interface. Several membrane structures coexist into the aqueous subphase including lamellar bodies, unpacked membrane film and inactive structures formed during breathing dynamics and oxidation processes. Each of the membrane structures formed along surfactant metabolic cycle differs from the others not only in structural features promoted by surfactant proteins and lipids but also in functional properties. Specially, surfactant assembled into lamellar bodies has been described to have outstanding surface-active properties. That is the reason why several approaches have been suggested to obtain or produce it for surfactant replacement therapies in premature newborns or in diseases where lung surfactant function is disrupted by inhibitory agents.

Here, we demonstrate that a surfactant purified from human amniotic fluid may well be a proper source of highly active surfactant not only because its structural and functional features, as analyzed by several biophysical techniques (Cryo-Electron Microscopy, Fluorescence, Electron Spin Resonance, <sup>31</sup>P-Phosphorus Nuclear Magnetic Resonance and Captive Bubble Surfactometer) correspond to those expected for lamellar bodies, but also because its purification is a suitable process, non-invasive and allow obtaining high enough yields of a human surfactant.

**Keywords:** Lung surfactant, biophysics, molecular biology, membrane structure, supramolecular complexes.

## Emulating the respiratory surface by a biophysical model

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In evolution, the appearance of aerial breathing meant the establishment of an air-liquid interface, a physic barrier with special physicochemical properties, like surface tension. In mammals, the pulmonary epithelium is coated with a thin aqueous layer, so the lung must handle with the surface tension at the air-liquid interface during breathing. To reduce it, type II pneumocyte synthetize, assemble and secrete a lipid-protein material, the pulmonary surfactant (PS), which is mainly composed by amphipathic molecules that allow them to form interfacial films at the alveolar surface. Furthermore, its particular, composition and biophysical properties make PS an attractive system for solubilizing and delivering different drugs and nanoparticles along the pulmonary surface.

A novel biophysical model to emulate the respiratory interface was develop in my laboratory combining a Wilhelmy and Langmuir-Blodgett balances in a double trough balance. This device, presents different applications, such as: (i) evaluate the biophysical properties of PS and its efficiency to act as a vehicle of drugs, (ii) perform compression-expansion cycles mimicking breathing dynamics, (iii) analyze the structures formed in the interfacial film and (iv) evaluate the drug exclusion process. Therefore, this new system can help to predict the effect of PS/drug combinations onto the alveolar interface and to develop and optimize procedures to administrate them.

**Keywords:** surface tension, pulmonary surfactant, air-liquid interface, drug delivery, respiratory dynamics, Langmuir-Blodgett balance.

## **Structural characterization of human pulmonary surfactant protein SP-D by atomic force microscopy**

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Pulmonary surfactant protein D (SP-D), a C-type (Ca<sup>2+</sup>-dependent) lectin, belongs to the collectin family. It is found mainly in lung surfactant, participating in the innate immune defense of the lungs.

SP-D monomer contains four structural domains: an N-terminus domain, a collagen region, an  $\alpha$ -helical coiled-coil neck and a C-terminus carbohydrate recognition domain (CRD). Monomers form trimers, which are stabilized by two disulfide bonds in the cysteine-rich N-terminal domain. Trimers associate into higher order oligomers whose size and conformation is sensitive to environmental factors and the conditions during purification and storage. Despite extensive studies carried out to characterize the oligomerization process of SP-D, the pathway and type of interactions involved in the formation of large oligomers, are not clearly understood.

In the current study, a structural characterization of recombinant human SP-D (rhSP-D), produced in mammalian CHO cells, has been carried out by Atomic Force Microscopy (AFM) and electrophoresis. The goal has been the determination of the distribution of oligomeric forms, the exploration of the possible oligomerization pathway and the description of the conformational diversity of rhSP-D. AFM experiments revealed that rhSP-D is a mixture of trimers, hexamers, dodecamers, and larger oligomeric species identified as “fuzzy balls”, with the most abundant structure being the dodecamer. We have developed a cross-linking protocol to detect the presence of SP-D dodecameric forms by PAGE-SDS, in which dodecamer is only visualized after chemical crosslinking and in the presence of denaturing agents, indicating the importance of hydrophobic interactions in dodecamer formation.

**Keywords:** SP-D, collagen, AFM, collectins, non-covalent interactions.

## **Cytokine release assay as test for exposure to *Leishmania*, and for confirming cure from leishmaniasis, in anti-TNF patients**

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Immunosuppression is a major risk factor in the appearance of overt clinical leishmaniasis; it can also alter the presentation of the disease and response to the treatment. Nowadays, the prevalence of immunosuppression conditions not associated with HIV virus is increasing. The treatments with glucocorticoids, steroids, and anti-TNF $\alpha$  affect lymphocyte function and increase the susceptibility to intracellular pathogens as *Leishmania* parasites.

The aim of our work is to characterize the cellular immune response against *Leishmania infantum* in immunosuppressed individuals residing in an area of high endemicity (Fuenlabrada, Spain). For this purpose, cytokine specific secretion was analyzed in the supernatants of PBMCs stimulated with soluble *Leishmania* antigen (SLA) and lymphoproliferative response to SLA (CPA).

During active of visceral leishmaniasis (VL) the patients did not present specific secretion of any of the cytokines studied, which was related with negative CPA and lymphocyte number below normal levels. However, after VL therapy, the patients presented a positive CPA and were within normal limits lymphocyte number, which contributes to a specific production of IFN- $\gamma$ , TNF- $\alpha$  and granzyme B. Similar results were found in asymptomatic subjects under immunosuppressive treatment.

The existence of this asymptomatic population demonstrates the need to establish a control over the time, in order to verify if this cellular response protects them in the long term from developing the disease. The combined use of CPA and cytokine secretion allows the follow-up of VL patients under immunosuppressive treatment, to assess the specific immunity to the parasite and therefore the risk of relapse.

**Keywords:** immunosuppression, leishmaniasis, asymptomatic, cytokine.



## Comparison of different bioinformatic tools and pipelines in the study of gastric microbiome

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New bioinformatic tools are developed to facilitate the microbiome deep sequence interpretation. The aim of this presentation is to compare different bioinformatics tools and pipelines in the study of gastric microbiome in pediatric patients with or without *Helicobacter pylori* (*Hp*).

51 children(16 *Hp*-positive, 35 *Hp*-negative) were studied. DNA from gastric biopsies was extracted with UltraClean Tissue & Cells DNA Isolation Kit(MoBio) including bead-containing lysing and high-throughput sequencing(Illumina) of the V4 region of the 16S rRNA was carried out.

Bioinformatics analysis was conducted combining R(v 3.2.3), Qiime(v 1.8.0) and Galaxy/Hutlab platform to perform alfa diversity, beta diversity and taxonomical analysis.

New bioinformatic approaches emerge each year providing to scientists and clinicians, a range of possibilities for the study of metagenomics. Here nearly all bioinformatic tools yield the same conclusion: *H. pylori* is the main actor in the gastric ecosystem but the rest of gastric microbiota are similar in *Hp*-positive and *Hp*-negative patients.

**Keywords:** Bioinformatic tools; Gastric Microbiome; *Helicobacter pylori*; Deep sequence interpretation.

## **Physical activity for the prevention and treatment of obesity in genetically predisposed schoolchildren**

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In the adult population, it is shown that physical exercise attenuates excess weight and adiposity in subjects genetically predisposed to it; but this association is little explored in childhood. In Spain, 23-28% of schoolchildren are overweight and only 52% perform at least one hour per day of exercise, as recommended by the World Health Organization (WHO).

This project aims to quantify the influence of the level and type of physical activity on the body composition of Spanish schoolchildren, based on their genetic risk for common obesity. The aim is to analyze a sample of 350 subjects of both sexes, between 6 and 14 years old. The nutritional condition will be assessed by anthropometry, taking measurements of weight, height, perimeters and subcutaneous fat, from which the body mass index and various parameters of body composition and fat distribution will be calculated. The genetic predisposition will be determined by a risk score developed from 10 single nucleotide polymorphisms (SNPs).

To assess physical activity, information will be gathered about participation in extracurricular sports, as well as the time dedicated to playful exercise that involves a moderate aerobic effort of at least 30 minutes in a row (running, cycling, skating, dancing, playing with a ball, etc.). The participants will be classified according to the aforementioned WHO recommendation. In addition, they will be asked about the intensity of each exercise performed in order to translate it into units of caloric expenditure (metabolic equivalents).

**Keywords:** obesity, childhood, anthropometry, genetic polymorphism.

## **Renewing Trends in Natural Science Teaching Approaches and its influence in Public Health (1876-1936). Environmental Education and Health Education.**

Maria Teresa Garcia Jimenez

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During important social crisis, new trends appear and get established in the Education System as a base for a social renovation. They nearly allways consider that the important point is to incorporate updated scientific knowledge but its not frecuent to analyze Metodological changes to promote a significant learning. The student needs to asume that learning is not only important for his profesional future but also for his life.

Santiago Ramon Y Cajal as the director of the “Junta para Ampliación de Estudios “, the CSIC precursor institution, said “la Docencia también es Ciencia” (Teaching is also Science) and provided sholarships to professors as researches to get relevant experiences abroad.

Our review is refered to the period between 1876 and 1936. It is not common to focus on international valuable past experiences because it seems that new technologies demand other type of teaching methodology. This new technologies, althought they are useful, are not indispensable and they can frecuently favor a repetitive education with a lack of adquisition of skills and habilities in the students.

The most relevant projects that we have found have common points: they are based in the knowledge of the body and the environment, the importance of taking care of them and the interaction between them. This made Natural Science the axis of the educational process.

We would like to contribute to the necessary renovation of the School Environment, specially to Secondary School and teacher training, particularly at their first steps.

**Key words:** Natural Science, Secondary School, Methodology, Health, Environment.

# Posters

## Comparison of essential oils of different species of the genus Citrus

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The purpose of the study is analyze and extract of essential oils from pericarp of different species from genus Citrus, both of the parental species [mandarin (*C. reticulata* Blanco), pummelo (*C. maxima* Burm.), citron (*C. medica* L.), small-flowered papeda (*C. micrantha* Wester), kumquat (*C. japonica* Nagami)] and of the hybrids [sour orange (*C. × aurantium* L.), grapefruit (*C. × paradisi* Macfad.), sweet organge (*C. × sinensis* Osbeck.), lemon (*C. × limon* Osbeck.), mexican lime (*C. × aurantifolia* Swingle.). Pericarp essential oil is rich in compounds, the major is limonene in all of genus except in small-flowered papeda (*C. micranthalime*) and mexican lime (*C. × aurantifolia*), that p-Mentha-1(7),8-diene is the major compound. Moreover, small-flowered papeda (*C. micrantha*) has been found like the species more different in the composition within the set of citrus analyzed, the species more similar has been their hybrids lemon (*C. × limon*), mexican lime (*C. × aurantifolia*). Respect of the extract of essential oils, the species with more output are mandarin (*C. reticulata*) and orange (*C. × sinensis*), and the species with less output are citron (*C. medica*) and lemon (*C. × limon*)

**Keywords:** *Citrus*, limonene, p-Mentha-1,(7),8-diene, essential oil, composition.

# Lessons from lung surfactant: how biophysics can help in clinical practice

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The lack, deficiency or inactivation of pulmonary surfactant is the cause of severe respiratory disorders which could be lethal both in newborns, leading to respiratory distress syndrome (RDS) or meconium aspiration syndrome (MAS), and in adults, bringing about the acute respiratory distress syndrome (ARDS).

Nowadays, surfactant replacement therapy (SRT) is a treatment that is employed to stimulate pre-term neonates' own production of surfactant. However, although early SRT (within the first 2-3h) provides the best outcomes, no assay is available to test surfactant functionality. Conversely, as for MAS and ARDS, routine SRT leads to disappointing results, as exogenous surfactant also emerges as being inactivated similarly to the host's endogenous surfactant. Therefore, the challenge to future trials of SRT is to develop new strategies that can overcome the negative effects of inactivation and guiding surfactant administration at the bedside.

Here, we propose three different approaches based on biophysical methods, that may be useful in clinical practice. 1) We created a screening test for guiding SRT in RDS by testing the time-dependent accumulation of fluorescently-labelled surfactant at the air-liquid interfaces. 2) We demonstrated the positive effect of hypothermia (33.5°C) on surfactant function by captive bubble surfactometry in the presence or absence of inhibiting molecules. 3) We used a murine model that mimic human ARDS (increased sPLA2 and phosphatidylglycerol (PG) depletion) and we obtained an *ex vivo* restoration of surfactant dysfunction by repletion of PG and SP-B.

In conclusion, our results suggest the huge potential of biophysics to seek novel strategies for patients' management.

**Keywords:** pulmonary surfactant, surfactant replacement therapy, captive bubble surfactometry, whole body hypothermia

## **Cytokine and chemokine dynamics in serum of patients with visceral leishmaniasis and cutaneous leishmaniasis caused by *Leishmania infantum***

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In Spain, leishmaniasis is caused by *Leishmania infantum*. The disease presents two distinct clinical forms: the cutaneous, which usually heals spontaneously, and, the visceral form, the most severe. The immune response of the host is the fundamental factors involved in the development of one or another clinical form.

The aim of our work is to characterize the serum production of cytokine and chemokine, in immunocompetent patients with visceral leishmaniasis and cutaneous leishmaniasis both before and after treatment. Plasma levels of IP-10, IL-8, IFN- $\gamma$  and IL-10 were evaluated.

The results show that in patients with visceral leishmaniasis, the plasma concentration of IP-10, IFN- $\gamma$  and IL-10 is high during active disease and decreases significantly after treatment. In these patients, the plasma levels of IL-8 are not related to the active or cured phase. In the case of patients with cutaneous leishmaniasis, the plasma levels of these chemokines and cytokines are not related either to the active phase or to the cured phase of the disease.

The high plasma levels of IP-10 compared with those of IFN- $\gamma$  and its decrease after the cure, postulate this chemokine as a sensitive marker for the post-treatment monitoring of patients with visceral leishmaniasis caused by *Leishmania infantum*, as previously described for *L. donovani* and others infectious diseases such as tuberculosis. This work shows that in patients with cutaneous leishmaniasis are not detected changes in the expression of plasma cytokines and chemokines do not appear systemic response to the parasite similar to that seen in visceral leishmaniasis.

**Keywords:** Visceral leishmaniasis, cutaneous leishmaniasis, cytokine, chemokine.

# Genetic and epigenetic implications in the reproductive dynamics of Mediterranean pines

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Mediterranean pines are large-sized, long living, iteroparous species. During their long life, they have to endure changing biotic and abiotic environments that favor plastic strategies in relative investment of resources in growth, maintenance and reproduction. The rhythm of change determined by global change imposes serious doubts regarding the viability of individual plasticity as a strategy of adaptation to environmental change. Life history theory predicts compromises between the different vital functions that might impose limitation in a context of new and multiple biotical and abiotical stresses. In addition, in Mediterranean climates and long living species, selective forces may change significantly from year to year and from one recruitment event to the next. The ultimate aim of this thesis is to improve our understanding of the capacity of our pinewoods to adapt to global change and to design strategies of management of forest genetic recourses that will ensure the persistence of pine forests and the maintenance of intraspecific genetic diversity. This thesis deepens in the direct repercussions of the possible adaptive syndromes on the recruitment and responses to selection that will influence the next generation. We include species with different ecological niches and contrasted life histories, aiming to progress in the understanding of the different strategies.

**Keywords:** serotiny, fire, reproductive allocation, genetic, epigenetic, global change



## Experimental manipulation of temperature reduce ectoparasites in nests of blue tits (*Cyanistes caeruleus*).

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Several models predict changes in distributions and incidence of diseases associated to climate change. However, there are few studies on how microclimatic changes could affect host-parasite relationships. In the present study, we experimentally manipulated the temperature inside nest-boxes occupied by blue tits (*Cyanistes caeruleus*) during the breeding season. The temperature was increased using heat mats located under the nest material in order to know its effect on parasitic abundance (i.e., nest-dwelling ectoparasites, blood-sucking flying insects and hemoparasites) and host condition (i.e., nestlings and adults). The experimental procedure increased the temperature 3°C on average and reduced the relative humidity about six units. The abundance of mites (*Dermanyssus gallinoides*) and blowfly pupae (*Protocalliphora azurea*) was significantly reduced in heated nest-boxes. In addition, a not significant lower prevalence of flea pupae (*Ceratophyllus gallinae*) was found in heated nests. However, hemoparasite infection of adult blue tits and the condition of adult and nestling blue tits were not affected by the heat treatment. In conclusion, the present heat treatment reduced nest-dwelling ectoparasites without any apparent benefit of the host.

**Keywords:** blue tit, ectoparasites, mites, *Protocalliphora*, relative humidity, temperature.

## **Breathing in progress: lung surfactant is composed by several membrane structures formed along respiratory physiology.**

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Pulmonary surfactant complexes are essential for breathing mechanics since they reduce surface tension at the alveolar air-liquid interface. However, lung surfactant is continuously subjected to compression-expansion breathing cycles. That is the reason why a permanent synthesis of surfactant and recycling of wasted material is necessary to maintain respiratory homeostasis. As a consequence, different surfactant structures coexist in alveoli and are related to lung surfactant metabolism from its synthesis by alveolar type II cells to its recycling by pneumocytes or alveolar macrophages. Nonetheless, lung surfactant used as reference in clinical applications and research laboratories are obtained from animal lungs, and therefore, they contain a mixture of these structures what might limit their functional properties.

Here, we demonstrate that lung surfactant is composed by a heterogeneous mixture of membrane structures with different structural and functional properties. Lung surfactant was obtained from bronchoalveolar lavages of porcine lungs and fractionated by density gradient ultracentrifugation. Interestingly, lung surfactant fractions differ in composition, structure and biophysical activity. Moreover, we propose that the different surfactant fractions might be related to the different stages of synthesis, packaging, adsorption into the alveolar air-liquid interface and recycling along surfactant metabolism. However, we did not obtain a fraction with the properties expected for pristine surfactant, which has been proposed as the most surface-active agent. Therefore, we suggest the necessity to look for an innovative source to purify lung surfactant since its purification from animal lungs might limit its properties as a consequence of having being already subjected to respiratory mechanics.

**Keywords:** Lung surfactant, biophysics, molecular biology, membrane structure, supramolecular complexes.

## Effects on immune functions in old male mice regrouped after being isolated post-weaning

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Social species, such as rodents or humans, need social bonds in order to ensure the correct function of regulatory systems (nervous, immune and endocrine) and hence, mental and physical health. Accordingly, social isolation and loneliness, especially in the elderly, is a risk factor of morbidity and mortality. This detrimental effect of isolation depends on how the individual responds, above all, by anxiety levels. Some studies have seen that re-socialization after social isolation can neutralize the cognitive and behaviour impairment. According to the above, the aim of this work was to study the effects of post-weaning social isolation on immune function in aging male mice with different anxiety-like behaviours, and if later re-socialization could reverse the negative effects of this isolation.

At the age of 17 months some immune functions and oxidative parameters were measured in peritoneal leukocytes in isolated and grouped male B6D2F1 mice. At 19 months, isolated animals were regrouped depending on their anxiety-like behaviour. And two months later, the immune function and oxidative parameters were measured.

The results showed that post-weaning social isolation impaired immune function and oxidative stress parameters, particularly in high anxiety mice. The regrouping in old age of these animals generates an immune deterioration and an alteration of the oxidative state regardless of their state of anxiety, although the individuals of lower anxiety seemed less affected. Therefore, it can be concluded, that responding with high levels of anxiety to any stressful situation produces a deterioration of the immune function and oxidative stress parameters.

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**Keywords:** social isolation, socialization, anxiety, male mice.

## Climate change-related shifts in the genetic diversity of relict tree species: understanding the patterns of molecular markers in *Abies pinsapo*

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The Spanish fir (*Abies pinsapo* Boiss.) is a drought-sensitive conifer, endemic from southern Iberian Peninsula. This relict tree has showed widespread mortality and growth decline over the last decades, which have been related to land-use and recent climate change. It is known that relict species constitute reliable models to shed light on adaptation process to rapid environmental changes. Here we attempt to place population genetic structure in a climate change context, using as experimental system the drought-sensitive fir *A. pinsapo*. We investigated the pattern of nuclear microsatellites (nSSR), chloroplast microsatellites (cpSSR), intermicrosatellites (ISSR) and single nucleotide polymorphisms (SNP) of two drought resistance-related candidate genes to the main hypothesis that selective pressure of climate change is related to molecular markers structure of *A. pinsapo* populations along a climate gradient. Our results aim of (1) obtaining information about its current status in order to design appropriate conservation strategies and (2) testing a possible effect of the recent climate change on its genetic structure. Low genetic diversity and high inbreeding were found for this species, which are characteristic of small and relict populations. In addition, a weak but significant differentiation among populations was obtained, together with evidences of past bottlenecks. Moreover, weak but significant differences were found related to altitude and age in some populations, as well as between dead and alive individuals belonging to the lower stands, where climate change-related selective pressure is stronger. These results point to a possible effect of recent climate change on the genetic structure of these populations.

**Keywords:** Climate Change, relict species, trees, conifers, molecular markers

## Development and characterization of a KLK5 overexpressing skin humanized mouse model resembling Netherton syndrome

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Netherton Syndrome (NS) is an autosomal recessive form of ichthyosis regarded as one of the most severe skin hereditary disorders affecting newborns, and patients present both cutaneous and immunological abnormalities. NS is caused by loss-of-function mutations in the SPINK5 gene, encoding the serine-protease inhibitor LEKTI, whose deficiency results in unrestricted activity of kallikreins (mainly KLK5) that leads to degradation of corneodesmosomal proteins, thus producing the detachment of the stratum corneum and disruption of the epidermal barrier. Due to the limited availability of NS skin biopsies and ethical constraints of experimentation on human subjects we sought to develop a skin humanized mouse model for the disease based on the grafting of transgenic bioengineered human skin onto immunodeficient mice. In order to recapitulate the histopathological hallmarks of the condition we first considered an approach consisting in the overexpression of the KLK5 gene in human keratinocytes by lentiviral transduction. We have performed an initial characterization of the model through the analysis of histopathological markers associated to NS as well as KLK5 expression and activity. The study confirmed the alterations in the KLK5 pathway, which results in clear epidermal proliferation and differentiation alterations, similar to those observed in skin biopsies of NS patients. In a preliminary experiment, a topical formulation of a KLK5 inhibitory peptide was applied to KLK5 overexpressing grafts. The analysis of the treated grafts showed a lower activity of KLK5 and a certain degree of reversion of the main NS phenotypic markers.

**Keywords:** Genodermatoses, KLK5, LEKTI, bioengineered human skin.

## **Mycotoxin-producing fungi in different stages of maize production cycle**

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Maize (*Zea mays* L.) is one of the main cereals present in food, feed and processed products. Contamination of these products by mycotoxins represents a significant risk to human and animal health. The objective of this work was to evaluate the presence of mycotoxin-producing fungi in order to detect the moment when contamination occurs during maize production cycle. Maize samples were evaluated in two plots located in Madrid (Spain). Four stages were sampled: anthesis or male flowering (25 male flowers and 25 female flowers), preharvest (25 cobs), post-harvest (3 kg of grain) and stored maize (1 kg of grain). DNA extraction was performed after 24 h of incubation at 28 °C in Sabouraud-cloramphenicol broth and species-specific PCR protocols were used to detect the most important mycotoxigenic *Aspergillus* and *Fusarium* species. The aflatoxin-producing species *A. flavus* and the fumonisin producer *F. verticillioides* were detected from the first stage, indicating that contamination occurred in the earliest period of the production cycle. In pre-harvest, new species were detected including fumonisin-producing species (*F. proliferatum*) and ochratoxin A producers (*A. niger* aggregate species). All of them were also detected during post-harvest and storage. The knowledge of the moment when contamination occurs is crucial to establish a correct treatment schedule to prevent mycotoxins entering the food chain. This work was supported by AGL2014-53928-C2-2R.

**Keywords:** Mycotoxins, Maize, *Aspergillus*, *Fusarium*.

## **Analysis of pulmonary surfactant as a potential drug delivery system under dynamic conditions**

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Pulmonary surfactant (PS) is a surface-active lipid-protein complex synthesized, assembled and secreted by type II pneumocytes. It can spontaneously form an interfacial film at the respiratory air-liquid interface, stabilizing it and reducing the surface tension during the breathing dynamics. Due to the particular composition and structure of PS, it possesses unique biophysical properties to adsorb and diffuse rapidly at the air-liquid interface. Therefore, using PS as a drug delivery system provides advantages to efficiently solubilise and transport different drugs and nanoparticles along the pulmonary surface.

To develop and optimize procedures to administrate PS/drug combinations is essential to analyse the biophysical properties of PS to adsorb and spread along the interface, its efficiency to act as a vehicle of drugs and how these drugs are released and incorporated into the lung. To evaluate these properties, a novel device consisting of a double trough combining Langmuir-Blodgett and Wilhelmy balances was designed and optimized in our laboratory. The vehiculization of Tacrolimus, a hydrophobic immunosuppressive, by PS has been studied in this work as a model for drug delivery.

The results of this work suggest that Tacrolimus travels along the air-liquid interface in combination with PS, and it is released during compression-expansion cycling mimicking breathing dynamics. According to this, we propose a model of how PS could facilitate drug delivery into lungs and how respiratory dynamics could promote drug release when it reaches the alveolar spaces.

**Keywords:** pulmonary surfactant, air-liquid interface, drug delivery, respiratory dynamics, Langmuir-Blodgett balance.

## **Improvements in behavior and immune function and increased lifespan of old mice cohabitating with adult animals**

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The social environment can affect the regulatory systems, and cohabitation with sick subjects is a negative factor for the nervous and immune systems, compromising the lifespan. Nevertheless, the possible beneficial effects of a positive social environment on nervous and immune functions and longevity have not yet been studied. The aim of the present work was to analyze several behavioral and immune function parameters and lifespan in old mice after their cohabitation with adult animals. Old and adult ICR-CD1 female mice were divided into three experimental groups: adult controls, old controls and a social environment experimental group. The latter contained 2 old with 5 adult mice. After two months in these conditions, mice were submitted to a behavioral battery of tests in order to analyze their sensorimotor abilities, anxiety-like behaviors and exploratory capacities. Peritoneal leukocytes were then collected and several immune functions as well as oxidative and inflammatory stress parameters were assessed. The animals were maintained in the same conditions until natural death occurred. The results showed that old animals, after cohabitation with adult mice, presented an improvement of behavioral capacities, immune functions and a lower oxidative and inflammatory stress. Consequently, they exhibited a higher lifespan.

**Keywords:** Social environment, longevity, environmental strategy.



## **Preliminary study of geographic variation on the galls of Asian chestnut gall wasp, *Dryocosmus kuriphilus*, in the Iberian Peninsula.**

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The Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae), is an invasive species from China and a severe pest of chestnut trees, causing a considerable economic damage in these trees. Gall induction in chestnut trees produces a production decrease of the normal plant structures, causing fruit loss and branch shortenings, that represent serious problems for chestnut production and wood industry sectors.

This cynipid, has been introduced in Iberian Peninsula since 2012 and has already colonized different areas of this region with an important presence of chestnut forests. Observing the external morphology and the size of the galls in these areas, it is remarkable that certain differences between them can be appreciated, *a priori*. This fact could be related to numerous variables, such climatology, meteorology or location of the different zones, the chestnut tree variety, or the number of years in which the plague has been present in the area, among others.

In this poster, we have measured and weighed a high number of galls from the different areas of Iberian Peninsula with the presence of *D. kuriphilus*, obtaining the weight, volume and surface of them, as well as the sample biomass. As a result, a characterization of the area based on these gall variables has been performed. In addition, we have obtained relationships between different climatic and geographical variables depending on the shape and the size of this cynipid galls, although it is probably that there are other types of variables (biological, ecological, etc.) that also conditioned them.

**Keywords:** Cynipid, gall, Asian chestnut gall wasp, GLM, variance analysis.

## Possible life-history traits linked with zinc incorporation in the mandibles of the hyper-diverse Hymenoptera

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Within Hymenoptera (bees, wasps and ants), structures related with oviposition and feeding are known to have a cuticle hardened by incorporation of trace metals, such as Zn. Mandibles are used by adults for feeding, digging and crawling at emergence, so that their composition may be also under selection through ecological pressures. We used data on Zn incorporation in the mandibles of species of Hymenoptera to test the effect of key life-history traits (adult emergence substrate, larval feeding resource, adult foraging mode) on the observed variability. Zn lacked in the three most primitive “symphytan” superfamilies, while it was ubiquitous in all apocritan superfamilies except, within “Parasitica”, in Proctotrupeoidea (common) and, within Aculeata, in Vespoidea (rare) and in Apoidea (extremely rare). We found that parasitoids and herbivores developing into plants tend to have greater Zn-enrichment in their mandibles than pollinivorous species (bees). Zn was also higher in species developing in concealed, compared with unconcealed, sites. However, there is an effect on phylogenetic: most parasitoids are within the “Parasitica” and all of them have Zn, independently from site concealment; predatory apoid wasps are closely related to pollinivorous bees, and both almost invariably lack Zn; among herbivores developing into plants, Zn occurs in all “Parasitica” species associated with galls (Cynipidae) but only in the most derived “Symphyta”. Within Vespoidea and Apoidea, the few cases of Zn incorporation they exclusively found in primitive parasitoid lineages and in Formicidae, suggesting that metal enrichment was independently lost and re-acquired a few times during evolution of Aculeata.

**Keywords:** Hymenoptera, mandibles, zinc metal, ecological pressures

## **Behaviour of liposomes under various technological treatments in the gelling properties of hake muscle**

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The objective of this study is to evaluate the behaviour of the addition of nano-vesicles in the gelation of hake muscle, as a matrix to develop gel-like functional foods. Nano-vesicles elaborated from phosphatidylcholine were subjected to different technological treatments such as high pressure (600MPa), freezing, freeze-drying and spray drying and then they were incorporated into hake mince. The liposome (141 nm) wasn't change by high pressure (HP) while the addition of glycerol as cryo-protector modified the final size of liposome depending of the treatment applied (freezing or freeze-drying). On the other hand, the atomized liposome by spray-dry offered a rentable alternative to the lyophilization process. All liposomes maintained a very electronegative zeta-potential (-39.6 to -49.5 mV) indicative of their high stability.

In the relaxometry  $T_2$  analyzed by low-field nuclear magnetic resonance (LF-NMR) of the batters (salt-ground muscle formulations) it is observed that the liposome-containing batters showed more water binding capacity than the control batter without liposomes, contributing to maintain the hydrated structure of the myofibrillar protein.

The temperature sweep rheological test reflected that the liposomes didn't affect negatively the thermal gelation of the muscle, and the frequency sweep test that they increased the fluidity of the corresponding liposome-containing batters by inducing less protein-protein interaction. It is in agreement with the gel strength, which shown a higher value for control gels both 60 as 80 °C, indicating a higher protein aggregation than in liposome-containing batters.

In summary, the addition of liposomes improved the structure and organization of the hake mince matrix.

**Keywords:** phosphatidylcholine, liposomes, glycerol, hake mince, protein aggregation.

## **New insect prey known in the diet of the greater horseshoe bat (*Rhinolophus ferrumequinum*)**

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The diet of the greater horseshoe bat (*Rhinolophus ferrumequinum*) has been studied in several countries of northern and central Europe, being composed mainly of lepidoptera and coleoptera. However, there is a lack of information in the countries of the Mediterranean area, where this bat is more frequent and abundant. In the last decade, summer census of this species has been carried out in the Central System and the southern Iberian System. Accumulations of remains of several insects have been observed in 10 bat roosts. As a result, there have been found deposits of wings of the Spanish moon moth (*Graellsia isabelae*), which is a new species for its diet. Also, remains of other lepidoptera have been identified: southern pine hawkmoth (*Sphinx maurorum*), *Atlantarctia tigrina* and *Dendrolimus pini* ssp. *iberica*, as well as numerous remains of beetles (*Melolonthinae*). Photographs of these evidences have been taken and samples of droppings have been collected in order to confirm by molecular analysis the presence of *G. isabelae* in the diet of *R. ferrumequinum*. The two species are cataloged respectively as Special in the Special Protection Regime and as Vulnerable in the Spanish Catalogue of Endangered Species. Both suffer the same threats such as fragmentation and destruction of their habitat and intoxication due to the use of pesticides. The trophic relationship of these two species points to the need for conservation of their habitats and the ability of bats to adapt to their feeding according to the vital cycles of their prey.

**Keywords:** Chiroptera, Coleoptera, feces, feeding, *Graellsia isabelae*, Lepidoptera.

## Three cases of JAK2 V617F-negative PV with different mutations in JAK2 12TH exon

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**Background:** The discovery of V617F mutation in Janus kinase gene (JAK2) revolutionized the classification and diagnosis of BCR/ABL- chronic myeloproliferative disorders (CMPDs), which includes polycythemia vera (PV), essential thrombocytosis (ET) and primary myelofibrosis (MF).

In great number of cases, these pathologies exist in absence of this mutation, it must be other alterations related with their origin and development.

It has been demonstrated that mutations in 12th exon of JAK2 are involved in the PV development (with a phenotype similar to that caused by JAK2 V617F mutation.)

**Methods:** We studied 43 patients diagnosed as PV according to the WHO criteria (2008). Hematological data were obtained with a hematinetric counter. JAK2 V627F mutation presence was determinate by PCR-ARMS. JAK2 12th exon presence was determinate by automatic sequencing.

**Results:** 35 patients showed the JAK2 V627F mutation, 3 had heterozygous mutations in JAK 12th exon (R54-E543delinsK and two N542-E543del). They shown middle age at diagnosis, elevated hematological parameters, no splenomegaly neither thrombotic events.

**Discussion** Most of PV patients carried mutations in JAK2, in cases with exon12 mutations, phenotype is similar but the age at diagnosis was lower, and the hematological parameters a little higher.

In other cases mutations studied were not found, but showed a clear PV phenotype: other mutations should have involved in CMPDs. That might explain phenotypic differences between patients and forms of the disease and their implications in the clinical, diagnosis and treatment of the CMPDs must be studied more deeply.

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**Keywords:** JAK2, myeloproliferative disorders, polycythemia vera, 12th exon.

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