XLII Congreso de la Sociedad Española de Genética

SEG2021

Libro de resúmenes

14-18 de junio de 2021

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BIENVENIDA

Bienvenidos al **XLII Congreso de la Sociedad Española de Genética, SEG2021**

Estimados colegas:

Nos complace daros la bienvenida al XLII Congreso de la Sociedad Española de Genética, que se hubiese celebrado en Madrid en junio de 2020 si la pandemia no lo hubiese impedido. Ante la incertidumbre reinante, y dado que la situación no cambió sustancialmente en los meses siguientes, la Junta Directiva de la SEG decidió que el congreso se celebrase en 2021 y en modalidad virtual. Fue un reto que nos pareció necesario aceptar, a pesar de las dificultades derivadas de la novedad de su formato; creímos que era una oportunidad que debíamos aprovechar. Estamos ahora convencidos de que las reuniones parcial o enteramente virtuales se han convertido en una herramienta cotidiana de nuestro trabajo.

Hemos organizado siete sesiones a la manera tradicional, en las que se abordarán facetas de la Genética de máxima actualidad, con conferenciantes invitados, comunicaciones orales y *flash talks*. Una de estas sesiones retoma la docencia de la Genética como un tema más del congreso de la SEG. También hemos incluido en la agenda del congreso tres sesiones de pósteres, y dos sesiones satélite, tituladas “Escuela de Divulgadores de la Genética” y “Genética y COVID”.

Agradecemos vuestra participación en el congreso y deseamos que os resulte provechoso. También queremos dar las gracias a las entidades que están contribuyendo a que el Congreso sea un éxito. Esperamos que SEG2023 se celebre en un contexto totalmente distinto, compatible con la realización de actividades presenciales.

Con nuestra mejor consideración,

Rosario Linacero, Presidenta del Comité Organizador

José Luis Micol, Presidente de la Sociedad Española de Genética

COMITÉS

Congreso de la Sociedad Española de Genética

14-18 de junio de 2021

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PROGRAMA

Congreso de la Sociedad Española de Genética

14-18 de junio de 2021

14 de junio

Sesión satélite: Escuela de Divulgadores de la Genética

Moderador: José Pío Beltrán

15:00 - 15:10	Presentación de los conferenciantes
15:10 - 15:35	Isabel López Calderón – Confidencias de una divulgadora compulsiva
15:35 - 16:00	Gemma Marfany - ¿Sirvo para divulgar?
16:00 - 16:25	José Miguel Mulet – Cómo divulgar de temas impopulares
16:40 - 17:05	Lluís Montoliu – Diferentes maneras de divulgar la Genética
17:05 - 17:30	Miguel Pita - ¿Por qué divulgo?
17:30 - 17:55	Enrique Viguera – Encuentros con la Ciencia: laboratorio a la sociedad
18:00 - 19:00	Mesa redonda

15 de junio

14:00 - 14:50	Sesión de pósteres: Dinámica de Cromosomas y Expresión Génica y Epigenética

Bienvenida

14:50 - 14:55	Rosario Linacero, Presidenta del Comité Organizador
14:55 - 15:00	José Luis Micol, Presidente de la SEG

Sesión 1: Dinámica de Cromosomas

Moderadora: Aurora Ruiz-Herrera

15:00 - 15:30	Conferencia invitada: Ana Losada - Specialized functions of cohesin variant complexes carrying STAG1 or STAG2 in chromosome dynamics.
15:30 – 16:00	Conferencia invitada: Darío G. Lupiáñez – Reorganization of 3D regulatory landscapes in disease and evolution.
16:00 – 16:15	Comunicación oral: Marina Martínez García – GRAS-1 is a conserved novel regulator of early chromosome dynamics during meiosis in *C. elegans*.
16:15 - 16:30	Comunicación oral: Ángeles Cuadrado Bermejo – Advances in understanding the behavior of the nucleosome-less chromosomes of dinoflagellates.
16:30 - 16:45	"Flash Talks"
	Lucía Álvarez González - The impact of chromosomal fusions on 3D genome folding in the germ line.
	Nadia Fernández Jiménez - The plant-specific nucleoporin NUP136 is essential to ensure the obligatory crossover in Arabidopsis meiosis.
	Lucía Gómez Gil - Contribution of chromosome rearrangements to the genomic plasticity of the fungal pathogen *Fusarium oxysporum*.
	Alberto Jiménez Martín - The metazoan-like kinetochore assembly and disassembly cycle is conserved in yeast mitosis but masked by the rabl configuration.
	J. Alberto Marchal Ortega - Increased mitotic cell death in MCPH1 depleted cells upon catalytic inhibition of TOPO II.
	María Martín Peciña - B chromosomes of *Eyprepocnemis plorans* contain active protein-coding genes involved in cell division.

Sesión 2: Expresión Génica y Epigenética

Moderador: Crisanto Gutiérrez

17:00 – 17:30	Conferencia invitada: María Gómez – Functional organization of the genome: intertwined links between DNA replication and transcription
17:30 – 18:00	Conferencia invitada: Myriam Calonje - Unveiling the role of PcG-mediated histone modifications in regulating gene expression in Arabidopsis.
18:00 – 18:15	Comunicación oral: José Manuel Pérez Pérez - Dynamic hormone gradients and metabolic reprogramming link cell cycle regulation to wound-induced organ formation in plants.
18:15 – 18:30	Comunicación oral: Sara Fontalvia Ostio - Human prefoldin complex modulates co-transcriptional pre-mRNA splicing.
18:30 – 18:45	"Flash Talks"
	Laura Contreras - Translation modulates the response to Sorafenib in hepatocellular carcinoma.
	Irene Delgado Román - Early-stage replicative age contributes to proliferative heterogeneity in *Saccharomyces cerevisiae*
	Blanca Navarrete Ruiz de Clavijo - Elucidating the role of sirtuins in the control of virulence in *Ustilago maydis*.
	Ana Ramos Sáenz - Role of yeast pol5 in ribosome biogenesis.
	Ana Isabel Garrido-Godino - Rpb4 and Puf3 imprint and post-transcriptionally control the stability of a common set of mRNAs in yeast.
	Adelaida Hernaiz Martorrel - Detection of genome-wide methylation changes in the central nervous system of sheep naturally infected with scrapie.

Conferencia inaugural

Moderadora: Monsterrat Elías-Arnanz. Vicepresidenta de la SEG

19:00 - 19:05	Presentación del conferenciante
19:05 – 20:00	Andrés Moya - Simbiosis: el encuentro entre procariotas y eucariotas

16 de junio

14:00 - 15:00	Sesión de pósteres: Genética de Microorganismos y Mejora Genética

Sesión 3: Genética de Microorganismos

Moderadora: Montserrat Elías-Arnanz

15:00 - 15:30	Conferencia invitada: Iñigo Lasa – Noncontiguous operons: a new strategy for coordinating gene expression
15:30 – 16:00	Conferencia invitada: Fernando Monje-Casas - Aging from the poles: asymmetric distribution of spindle microtubule organizing centers.
16:00 – 16:15	Comunicación oral: Silvia Salas Pino - Reversible protein aggregation as cytoprotective mechanism against heat stress in Fission Yeast.
16:15 - 16:30	Comunicación oral: José Tomás Cánovas Márquez - R3B2: The exclusive RNase III of Mucorales that has evolved to cut single-stranded RNA.
16:30 - 16:45	"Flash Talks"
	Andrea Bullones Bolaños - *Salmonella* modifies the host ubiquitinome.
	Emilio Garrote Sánchez - From endosymbiont to chassis in synthetic biology: genomic engineering of *Bartonella quintana* by deleting non-essential regions.
	Antonio Joaquín Monera Girona - Unmasking the identity of plasmanylethanolamine desaturase and the role of plasmalogens in photooxidative stress signaling.
	Mª Dolores Pejenaute Ochoa - DOG9, a novel fungal protein involved in effectors secretion during plant infection.
	Chantal Renau Mínguez - Exploring the genome of *Mycobacterium brumae*, a species of bacterium with therapeutic potential.
	Manuel Sánchez López-Berges - Relevance of copper homeostasis in *Fusarium oxyspor*um pathogenicity.

Sesión 4: Mejora Genética

Moderador: Rafael Lozano

17:00 – 17:30	Conferencia invitada: Miguel Pérez Enciso – Aprendizaje automático y predicción genómica
17:30 – 18:00	Conferencia invitada: Fernando Yuste-Lisbona - ¿Cómo mejorar la productividad a través de la regulación genética de la función meristemática?
18:00 – 18:15	Comunicación oral: Cristina Menéndez Menéndez - Development of new grape cultivars better adapted to climate change.
18:15 – 18:30	Comunicación oral: Miguel Ángel Toro Ibáñez - Genetic improvement programs in aquaculture.
18:30 – 18:45	"Flash Talks"
	Andrea Arrones Olmo - GWAS analysis in the first eggplant magic population identifies strong associations for anthocyanin pigmentation.
	José A. Campoy - Haplotype-resolved, chromosome-level, reference-free assembly enabled by high throughput single-cell sequencing of gamete genomes.
	Matilde López Fernández - Exploring the genomic diversity of wheat landraces for new breeding challenges.
	Clara Pons Puig - Atlas of phenotypic, genotypic and geographical diversity underlying variability of the traditional European tomato.
	Mª Dolores Requena Ramírez - A GDSL esterase/lipase mediates lutein esterification in tritordeum being a strong candidate for the improvement of carotenoid stability in related cereals.
	Marta Isabel Terry - Identification of genes involved in volatile synthesis in the Melon genome.

Sesión abierta: Genética y COVID

Moderador: José Luis Micol

19:00 – 19:05	Presentación de los conferenciantes
19:05 - 19:45	Conferencia invitada: Fernando González-Candelas - La genética poblacional y evolutiva del SARS-CoV-2.
19:45 – 20:25	Conferencia invitada: Ángel Carracedo -El consorcio SCOURGE.

17 de junio

14:00 - 15:00	Sesión de pósteres: Genética de Poblaciones y Evolución y Genética Humana

Sesión 5: Genética de Poblaciones y Evolución

Moderadora: Amparo Latorre

15:00 - 15:30	Conferencia invitada: Josefa González - The genetic and molecular basis of environmental adaptation: a transposable element perspective.
15:30 – 16:00	Conferencia invitada: Daniel Tamarit - Novel prokaryotic lineages fuel the quest for eukaryotic ancestry.
16:00 – 16:15	Comunicación oral: Marta Álvarez-Presas - A new genomic approach to shed light on the mystery of the evolution of Bilateria.
16:15 - 16:30	Comunicación oral: Cristina López Díaz - Experimental evolution reveals mechanisms of adaptation in the fungal pathogen *Fusarium oxysporum*.
16:30 - 16:45	"Flash Talks" Paula Escuer - The chromosome-scale assembly of *Dysdera silvatica* (Arachnida, Araneae) provides insight into the origin and evolution of chemoreceptor gene families in spiders. José Luis Horreo - Global climatic changes explain long-term demography of the most-widely distributed terrestrial reptile (*Zootoca vivipara*). Eugenio López Cortegano - Evolution of mutational properties in phylogenetically closely related species. Sonia Olaechea Lázaro - Initial population structure and selection results on a new Cameroonian whole-exome sequencing dataset. Sergio Ortega Del Campo - Twenty years of evolution and diversification of digitaria streak virus in *Digitaria setigera*. Beatriz Sabater Muñoz - Fitness and mutational rate is affected by loss of groel-mutational buffering in highly bottlenecked *E. coli* populations.

Conferencia de clausura

Moderador: José Luis Micol

17:00 - 17:05	Presentación de la conferenciante
17:05 – 18:00	María Blasco -Papel de los telómeros en el origen de las enfermedades

Sesión 6: Genética Humana

Moderadora: Gemma Marfany

18:00 – 18:30	Conferencia invitada: Noèlia Fernàndez-Castillo - Bringing light to autism, from rare mutations to spiking neurons.
18:30 – 19:00	Conferencia invitada: Pablo Huertas - Defective DNA double strand break repair in Aicardi-Goutieres syndrome.
19:00 – 19:15	Comunicación oral: Sara Veiga-Rúa - Validation of new ASD-associated genomic variations using hiPSCs and zebrafish models.
19:15 – 19:30	Comunicación oral: Julián Cerón Madrigal - Mimicking human mutations in *Caenorhabditis elegans*.
19:30 – 19:45	"Flash Talks"
	Almudena Fernández López - Ratones editados genéticamente para investigar diversos tipos de albinismo.
	Unai Illarregi Insausti - Detection of fusion genes by RNA-seq in pediatric acute lymphoblastic leukemia.
	Núria Martínez-Gil - Regulation of WNT16 in bone involves upstream enhancers within CPED1.
	Juan Antonio Navarro Langa - Erastin-induced ferroptosis enhances loss-of frataxin phenotypes in a Drosophila model of Friedreich Ataxia.
	Mercedes M. Pérez Jiménez - A *Caenorhabditis elegans* model to study the role of sulfated steroid hormones in aging and aging-related diseases.
	Susana Ruiz Ruiz - Microbiota across life in a healthy cohort.

18 de junio

Sesión 7: Docencia de la Genética

Moderadora: Rosario Linacero

15:00 – 16:00	Mesa Redonda

Entrega de Premios

Moderadores: José Luis Micol y Rosario Linacero

16:00 – 17:00	Entrega de los Premios Nacionales de Genética
16:00 – 16:15	Intervención de Isabel Durán, en nombre de la Fundación Pryconsa
16:15 – 16:20	Entrega de los premios
16:20 – 17:00	Intervenciones de los premiados
17:00 – 17:15	Entrega de los Premios a los Socios Distinguidos de la SEG
17:15 – 17:30	Entrega de los premios a los mejores pósteres, comunicaciones orales y flash talks del Congreso

Clausura del Congreso

17:30 – 17:35	Clausura del Congreso

Asamblea de la SEG

17:35 – 18:30	Asamblea de la SEG

Conferencias plenarias

Congreso de la Sociedad Española de Genética

14-18 de junio de 2021

CONFERENCIA INAUGURAL

Simbiosis: el encuentro entre procariotas y eucariotas

Andrés Moya[a,b,c]

[a]Instituto de Biología Integrativa de Sistemas, Universitat de València y CSIC, València, [b]Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO), València, [c]CIBER en Epidemiología y Salud Pública (CIBEResp), Madrid.

La simbiosis, en un sentido amplio, es la coexistencia íntima entre dos o más especies, con grado diferente de beneficio o perjuicio entre ellas. De particular interés son aquellas simbiosis cooperativas o mutualistas en las que los miembros del consorcio incrementan su eficacia biológica con respecto a la situación en la que son especies de vida independiente. Aunque no es tarea sencilla probar empíricamente el mencionado incremento en eficacia biológica, con independencia de si los componentes del consorcio forman o no una nueva unidad evolutiva, una cuestión fundamental que subyace y que requiere investigación es por qué las simbiosis mutualistas, ejemplo de cooperación, parecen estar tan extendidas en la naturaleza. Aunque la cooperación pareciera plantear un dilema a la evolución por competencia darwiniana, no es el caso si el consorcio, como se acaba de indicar, mejora y evoluciona con respecto a sus componentes. Cuestión otra es la naturaleza de los cambios que deban acontecer en los actores para pasar a ese nuevo nivel, cambios que, por otro lado, pueden llegar a ser diferencialmente dramáticos entre ellos. La Genómica y otras ómicas nos permiten caracterizar propiedades genéticas y funcionales de microorganismos simbiontes no cultivables que dan apoyo, por otro lado, a la hipótesis ya formulada por ilustres predecesores, de que los procariotas están muy presentes en los eucariotas y han evolucionado de forma conjunta. En esta conferencia mostraré resultados de la investigación de mi grupo y otros en simbiosis mutualistas, desde la endosimbiosis al microbioma animal y humano.

Trabajo financiado por MICINN-PID2019-105969GB-I00 y GV-Prometeo/2018/133.

CONFERENCIA DE CLAUSURA

Papel de los telómeros en el origen de las enfermedades

Maria A. Blasco

Jefa del Grupo de Telómeros y Telomerasa

Directora Científica

CNIO – Centro Nacional de Investigaciones Oncológicas – Madrid – España

Durante los últimos años, nuestro laboratorio ha contribuido al análisis del papel de la telomerasa y de la longitud telomérica como vías moleculares clave que subyacen al cáncer y envejecimiento, así como ha estudiado el uso potencial de la activación de la telomerasa como estrategia terapéutica para síndromes teloméricos y enfermedades asociadas al envejecimiento (Blasco et al., *Cell*, 1997; Tomás-Loba, *Cell*, 2008).

Hemos desarrollado una estrategia de terapia génica basada en telomerasa que permite la activación de la telomerasa en el organismo adulto (Bernardes de Jesus et al., *EMBO Molecular Medicine*, 2012) y que ha demostrado efectos terapéuticos en ratones con patologías asociadas al envejecimiento, como por ejemplo, en los infartos de miocardio (Bär et al., *Nature Communications*, 2014) así como en modelos de ratón para síndromes teloméricos de anemia aplásica (Bär et al., *Blood*, 2016) y de fibrosis pulmonar (Povedano et al., *Cell Reports*, 2015; Povedano et al., eLife, 2018). Asimismo, hemos demostrado recientemente que la terapia génica con telomerasa no aumenta la incidencia de cáncer en los modelos de ratón, incluso en presencia de un oncogen-K-Ras activado (Muñoz et al.,*PloS Genetics,* 2018).

Igualmente, hemos logrado generar ratones con telómeros más largos de lo habitual (telómeros hiperlargos) sin recurrir a la manipulación génica, manipulando únicamente la duración de pluripotencia en células madre embrionarias de ratón. Utilizando estos ratones, hemos demostrado que los telómeros largos, *per se,* no son nocivos para el organismo (Varela et al., *Nature Communications,* 2016; Muñoz et al., *Nature Communications,* 2019). Por el contrario, los ratones con telómeros hiperlargos muestran signos de retraso en el envejecimiento y mayor protección contra el cáncer.

Como alternativa a la activación de la telomerasa y a la manipulación de las células madre embrionarias, una posibilidad para tratar los síndromes teloméricos podría pasar por intervenciones conocidas para retrasar el envejecimiento del organismo, como son la inhibición de la vía mTOR mediante el tratamiento con rapamicina. Presentaré datos recientes que muestran como tanto la inhibición farmacológica como la génica de la vía mTOR en ratones con deficiencia de telomerasa tiene efectos nocivos en contraposición a los efectos beneficiosos en los ratones normales. De hecho, hemos descubierto que la vía mTOR juega un papel protector clave en los telómeros cortos.

SESIÓN SATÉLITE

Escuela de Divulgadores de la Genética

Moderador: José Pío Beltrán

ED-01. Divulgar la ciencia

José Pío Beltrán

Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC). Valencia.

Divulgar la ciencia es una necesidad social y debe responder a la pasión de los científicos por hacer partícipes a los ciudadanos del conocimiento científico. La Sociedad Española de Genética pretende reflexionar sobre la necesidad de transmitir los avances de la genética en un contexto histórico en el que se ha puesto de manifiesto el interés social por el conocimiento científico a causa de la pandemia generada por el virus SARS-Cov-2. La Escuela de Divulgadores de la Genética pretende debatir sobre las claves del éxito de la divulgación de la genética. En general, podemos afirmar que la divulgación de la ciencia se debe fundamentar sobre tres ejes: el interés social de la temática a divulgar; la calidad científica del conocimiento que se transmite y la capacidad de comunicación del investigador divulgador. La Genética ofrece temas en número ilimitado de alto interés social y disponemos de un conjunto de investigadores excelentes. La elección del medio y de la estrategia de comunicación para cada conjunto de personas diana no es un tema menor: el medio apropiado (charlas, conferencias, radio, televisión) en directo o diferidas, las redes sociales, la duración apropiada del evento, el lenguaje apropiado a cada público libre de jerga y sin pérdida de rigor científico condicionarán en gran medida el éxito de la divulgación. La Escuela de Divulgadores de la Genética presentará distintas alternativas: las estrategias de Isabel López Calderón utilizando las metáforas apropiadas a los más diversos públicos en sus charlas; la discusión de los formatos que presentará Lluis Montoliu; la conjugación del interés social con la impopularidad de algunas temáticas que describe José Miguel Mulet; la pasión de divulgar como motivación de Miguel Pita; los Encuentros con la Ciencia de Enrique Viguera o la reflexión del camino a recorrer para convertirse en un buen divulgador de Gemma Marfany servirán como base para una reflexión conjunta con todos ellos que desarrollaremos mediante una mesa redonda.

ED-02. Confidencias de una divulgadora compulsiva

López Calderón, Isabel

Departamento de Genética. Facultad de Biología. Universidad de Sevilla

Las historias acerca de cómo cada divulgador científico ha llegado a serlo, en nuestro país y en esta época, son muy variadas, pero las de los que empezamos en la Ciencia hace más de cuarenta años creo que tienen un rasgo en común: la clandestinidad. Para la mayoría de los colegas, la comunicación de resultados e ideas era importante y evaluable, pero el hacerlo de forma efectiva, inteligible y amena, no lo era y, por tanto, no valía la pena malgastar tiempo en ello. Los que te querían bien, te aconsejaban que más laboratorio y menos preparar charlas, escritos o clases, de modo que los que teníamos vocación y pensábamos que tanto el fondo, cómo la forma deberían ser cuidados con esmero, simplemente disimulábamos el esfuerzo y admitíamos en silencio, la chanza acerca de la suerte que teníamos por ser buenos comunicadores de forma innata.

De aquella época data mi afición por las metáforas que trasladando los fenómenos y procesos, a la vida cotidiana, tanto facilitan su comprensión. También tuve que aceptar comentarios halagüeños acerca de mi facilidad, atribuida cómo no a la genética, para establecer este tipo de comparaciones, cuando la verdad es que su elaboración conlleva muchas vueltas a las ideas y mucha prueba y error en su exposición al público y a los *sparring*, esas abnegadas personas de nuestro entorno, que se prestan a la escucha o la lectura crítica de nuestra disertación. Rindo un homenaje a esos entrenadores pacientes y desinteresados.

Mucho me temo que esa práctica en la clandestinidad de la vocación divulgativa, me ha llevado poco a poco, a lo que es el tema principal de esta Presentación: la compulsividad en la transmisión del conocimiento, esa necesidad no reprimida de introducir en cualquier conversación, una "píldora educativa", una información no requerida ni necesaria, pero que, a nuestro solo criterio, va a enriquecer la cultura científica del resto de los participantes. En la Presentación, desarrollaré estos términos con mayor detalle.

ED-03. ¿Sirvo para divulgar?

Gemma Marfany

Catedrática de Genética; Delegada del Rector para la divulgación científica.
Departamento de Genética, Microbiología y Estadística, Facultat de Biologia, Universitat de Barcelona, IBUB-IRSJD, Barcelona; CIBERER-ISCIII

De científicos hay de todos tipos y condiciones, pero a todos nos une la pasión por lo que hacemos, en nuestro caso, la genética. Los hay que justo empiezan en investigación y los que ya llevan muchos años dedicados a la ciencia; los hay que trabajan en centros de investigación, donde mayoritariamente investigan, y los que están en universidades, donde la docencia va mano a mano con la investigación. Además, hay los científicos que se dedican a la gestión, o a emprender y tranferir conocimiento aplicado, y todas estas tareas tienen su reconocimiento profesional dentro de los planes de dedicación del profesorado y del investigador. Pero también hay los que, además, les gusta explicar lo que hacen y compartir con los demás el conocimiento generado, hay científicos, tanto en ciernes como más senior, a los que les gusta divulgar. Esta es una tarea que no está muy reconocida profesionalmente, pero esto cambiará, porque cada vez más se demanda que hay un retorno a la sociedad de nuestra tarea científica. Lo que sucede es que muchas veces nos parece que no estamos suficientemente preparados. Mi charla va dirigida a todos aquellos a quienes les gustaría pero todavía no se atreven. El conocimiento y la ciencia son patrimonio de todos y les debemos a nuestros compañeros de piso, a nuestra familia y amigos, a la sociedad que nos acoge, que podamos compartir nuestra pasión, buscando la manera más efectiva de poder diseminar conocimiento. La divulgación científica no solo es territorio de los excelentes divulgadores –que los hay–, de aquellos que han hecho de un hobby, un verdadero arte verbal o escénico y una profesión. Claro que hay que mirarse en su espejo para aprender qué trucos, qué recursos, qué estilo tienen, y entonces, buscar dentro de nosotros aquel espacio en que nos vamos a sentir más cómodos y explorarlo. Todos podemos divulgar si nos lo proponemos, y esta es una habilidad que se puede cultivar y que nos será muy útil, desde ayudar a exponer nuestros resultados en público –desde el más científico al más generalista–, a poder exponer con confianza un nuevo proyecto de investigación para el que pedimos financiación. Ante la pregunta ¿sirvo para divulgar?, la respuesta es siempre sí, pero que hay que esforzarse en encontrar el traje a medida de nuestras habilidades y nuestra audiencia. La divulgación ya no es la hermana pequeña de la investigación. La divulgación científica rigurosa, interesante y cercana ha llegado para quedarse.

ED-04. Cómo divulgar de temas impopulares

J.M. Mulet

IBMCP, Universitat Politécnica de Valencia-CSIC, Valencia, Spain,

En la última década la divulgación científica ha pasado de estar confinada a libros, muy pocas revistas y alguna aparición esporádica en medios de comunicación como la radio o la televisión, a popularizarse gracias a las redes sociales. Esto ha permitido que aumente la oferta disponible para la gente interesada en ciencia, ya sea en canales de you tube, twitch o diferentes plataformas y redes sociales.

Ser científico y divulgador está bien considerado por la sociedad, en gran parte por la extrañeza, ya que vivimos en un país con una tasa muy baja de inversión en I+D y de científicos por habitante, sin embargo, ¿qué pasa cuando tú divulgación se centra en temas considerado como impopulares? Divulgar de temas como la energía nuclear, los mitos relacionados con los productos naturales, la pseudomedicina o, como el tema en el que trataré en la conferencia, los transgénicos, puede tener su parte complicada, ya que el público general ya tiene una idea formada sobre tu tema, y esta es muy negativa. A esta mala imagen contribuye que el tratamiento que ha hecho la prensa de estos temas siempre ha estado sesgado, o que se ha dado voz y se ha permitido transmitir un discurso que nada tiene que ver con la realidad del tema. Ante este panorama, la divulgación científica se constituye como una alternativa para acercar la visión de la ciencia a la sociedad. ¿Cómo se puede divulgar cuando el público tiene una información que no es correcta? ¿Cuáles son los riesgos? Y por encima de todo ¿vale la pena?

ED-05. Diferentes maneras de divulgar la genética

Lluís Montoliu

CNB-CSIC y CIBERER-ISCIII, Madrid

La divulgación científica es una parte esencial de la actividad científica, especialmente cuando se realiza desde los centros y universidades públicas. Existen infinidad de formatos a través de los cuales se puede compartir algunos aspectos, simples y complejos, de la genética a la sociedad. Las charlas (ahora mismo *webinars*, por la pandemia COVID-19) son un formato habitual y efectivo con el que se consigue llegar a mucha gente, aunque no todo el mundo esté dispuesto a escuchar una charla de 30, 40 o 50 minutos sobre un tema por el que puede sentir interés pero para el cual no quiere destinar tanto tiempo de su vida. También los artículos en medios impresos y digitales, o en blogs, que se enfrentan a similares problemas. No hay tanta gente dispuesta a leer un artículo de mil palabras, por sorprendente que parezca ¿Hay alternativas? Yo creo que sí, y en esta charla ilustraré con algunos ejemplos propios como de verdad existen diferentes maneras de divulgar la genética. Como por ejemplo las piezas cortas (5-6 minutos) en programas de radio o, mejor todavía, por su carga visual, los vídeos cortos (menos de 10 minutos) en los que se puede resaltar los puntos y detalles más importantes de un tema, sin utilizar lenguaje profesional ni recurrir a conceptos complicados, para transmitir lo relevante. Y a veces uno puedo echar mano de lo que tiene por casa, sin necesidad de editar profusamente el vídeo con efectos visuales, sonoros y filtros, como se estila y hacen muy bien profesionales del ramo, *youtubers* reputados que arrastran millones de seguidores, sino recurriendo por ejemplo a los juguetes de nuestra infancia, como las piezas de colores de los juegos de construcción, como el TENTE, el LEGO español que entretuvo a varias generaciones de niños y niñas entre los años 70 y 90 antes de desaparecer.

ED-06. ¿Por qué divulgo?

Miguel Pita

Área de Genética. Departamento de Biología. Universidad Autónoma de Madrid, Madrid, España.

La carrera investigadora implica realizar distintas y variadas tareas, muchas de las cuales no implican generar conocimiento. Entre las dispares labores de un investigador se encuentra la divulgación. Sin embargo, no se trata de la tarea más extendida ni la más valorada, aunque quizá últimamente algo esté cambiando. Por ejemplo, la pandemia del SARS-CoV-2 ha dirigido repentinamente la atención hacia el conocimiento científico y se ha puesto de manifiesto que éste resulta trascendente e interesante, pero no es sencillo de transmitir. También han quedado patentes las dificultades que encuentra la ciencia para resultar más atractiva que otras fuentes más simples e infundadas de información, como la opinión. En este contexto, hoy más que nunca parece evidente que la divulgación es relevante y tiene trascendencia directa en el devenir de la sociedad.

Sin embargo, mi principal motivación para divulgar ha estado en su origen alejada de la importancia o el impacto, grande o pequeño, que pueda tener. Mi estímulo principal siempre ha sido hacer partícipes a quienes me rodean de aquello que me apasiona y que tanta satisfacción me ha reportado. Divulgando he querido compartir el inmenso privilegio que supone comprender el mundo a través del conocimiento científico. Aunque divulgar implica un esfuerzo de síntesis y *traducción* de aquello que es profundo y específico a un lenguaje más común, la satisfacción de compartir la riqueza que hay en la ciencia compensa el esfuerzo. Evidentemente, la gratificación de compartir mi vocación no es la única motivación, pero en mi caso sí ha sido el impulso original para divulgar. Más allá de lo personal, creo que aquellos que investigamos tenemos el deber de hacer *salir* a la ciencia de nuestros lugares de trabajo. Por un lado, porque de esa manera devolvemos a la sociedad parte de lo que nos da, pero también porque informamos de aquello que la ciencia, que es objetiva, descubre. De de esa manera, divulgando, podemos contribuir ayudando a nuestra sociedad a ser madura y compleja, a anticiparse a futuros problemas y a evitar caer en supersticiones, falsedades, y manipulaciones. No es fácil divulgar, pero al menos merece la pena intentarlo.

ED-07. Encuentros con la ciencia: del laboratorio a la sociedad

Enrique Viguera Mínguez, Ana Grande Pérez

[a]*Área de Genética, Universidad de Málaga, Málaga, España.*

"Encuentros con la Ciencia" nace en el año 2004 en la ciudad de Málaga con el objetivo de implicar a la comunidad científica en la difusión del conocimiento que se genera en los centros de investigación españoles. Encuentros con la Ciencia es coordinada por profesores universitarios y educadores de secundaria quienes unen esfuerzos con diferentes agentes (institucionales y privados) para que el público de todas las edades sea consciente de la importancia de la ciencia en nuestra vida cotidiana, impulsando la cultura científica y estimulando el espíritu crítico. Se trata, por tanto, de un proyecto colaborativo multidisciplinar con el objetivo principal de hacer accesible el avance de la ciencia a la sociedad.

El proyecto, actualmente en su edición número dieciocho, ha ido evolucionando en tipos y formatos de acciones a lo largo del tiempo al objeto de alcanzar a un público con diferente interés en la Ciencia. Las actividades giran en torno a dos contextos: uno general, con actividades abiertas a un público heterogéneo con diferentes intereses por la ciencia; y un segundo contexto encuadrado en el marco educativo, dirigido a los estudiantes y al personal docente.

Encuentros con la Ciencia reúne 8 acciones: Conferencias; Exposiciones; Talleres sobre ciencia; *Ciencia de cine* en colaboración con “Málaga de Festival”, actividad cultural previa al festival de cine de Málaga; Programas de ciencia dirigidos al alumnado de enseñanza media identificado con Altas Capacidades intelectuales “*GuíaMe-AC-UMA*” y "Yo de mayor quiero ser..."; Actividades de actualización científica y formación del profesorado y la base de datos de experimentos científicos *Experimenta*. Encuentros con la Ciencia ha recibido varios premios de divulgación científica locales y nacionales y generado cientos de miles de reseñas en redes sociales (@enc_ciencia; Facebook.com/encuentros_ciencia; Youtube/@enc_ciencia, www.encuentrosconlaciencia.es). La amplia repercusión mediática de esta actividad a lo largo de los años, ha abierto canales de comunicación frecuentes con los principales medios de comunicación de la ciudad (prensa, radio, televisión), de manera que ponentes y organizadores de Encuentros con la Ciencia se han convertido a menudo en fuentes habituales de información científica.

Agradecemos las ayudas recibidas de organismos públicos (FECYT, Universidades, IHSM-CSIC, etc) y privados (Ámbito Cultural de El Corte Inglés, Fundación Unicaja, Ayuntamiento de Málaga, Fundación CIEDES, Euronutra, ICOFMA, Solmark, etc) a lo largo de nuestra trayectoria.

SESIÓN 1

Dinámica de cromosomas

Moderadora: Aurora Ruiz-Herrera

I1-01. Specific contributions of STAG1 and STAG2 cohesin variants to genome folding

Ana Losada[a]

[a]*Chromosome Dynamics Group, Molecular Oncology Programme, Spanish National Cancer Research Centre*

Cohesin is an evolutionary conserved Structural Maintenance of Chromosomes (SMC) complex that entraps DNA. Although it was initially identified for its role in sister chromatid cohesion, more recent studies have shown that cohesin plays also a central role in the spatial organization of the genome. Two variant cohesin complexes containing SMC1A, SMC3, RAD21 and either STAG1 or STAG2 are present in all cell types of vertebrate organisms. While cells in culture lacking either complex are viable, murine embryos require both cohesin-STAG1 and cohesin-STAG2 to fulfill their development. In my talk, I will describe recent efforts in my group to understand the specific functions of these two cohesin variant complexes, how they are regulated and their contribution to genome folding.

I1-02. Reorganization of 3D regulatory landscapes in disease and evolution

Darío G. Lupiáñez[a]

[a]*Berlin Institute for Medical Systems Biology, Max-Delbrück Center for Molecular Medicine, Berlin, Germany*

The three-dimensional (3D) folding of chromatin is critical to accommodate the roughly two meters of DNA within the nucleus of a eukaryotic cell. This non-random process ensures a proper interaction between regulatory elements and genes, resulting in precise spatial and temporal gene expression patterns.

A fundamental unit of chromatin organization are topologically associating domains (TADs), which constitute large 3D regulatory landscapes occupied by regulatory elements and their putative genes. These landscapes are demarcated by chromatin boundaries, which limit the regulatory crosstalk between domains. TAD boundaries represent an important regulatory hallmark along the genome, as their disruption has been linked to several types of human pathologies (*1*, *2*).

In this talk, I will discuss how the reorganization of 3D regulatory landscapes can result in developmental disease or evolutionary traits (*3*, *4*). Furthermore, I will show that the molecular composition of TAD boundaries can act as an effective quantitative modulator of gene expression and phenotypes (*5*).

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4. *F. M. Real et al., Science. 370, 208–214 (2020).*
5. *C. Anania et al., bioRxiv, in press, doi:10.1101/2021.04.14.439779.*

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (GA2495/1- 1044 1) and by a Helmholtz ERC Recognition Award grant from the Helmholtz-Gemeinschaft (ERC-RA1045 0033).

O1-01. GRAS-1 is a conserved novel regulator of early chromosome dynamics during meiosis in *C. elegans*

Marina Martinez-Garcia[1], Pedro Robles Naharro[1], Saravanapriah Nadarajan[1], Nara Shin[1], Carlos G. Silva-Garcia[2], Takamune Saito[1], Sara Beese-Sims[1], Ana Castaner[1], Enrique Martinez-Perez[3] & Monica P. Colaiacovo[1].

[1]Department of Genetics, Blavatnik Institute, Harvard Medical School, Boston, MA, USA, [2]Department of Genetics and Complex Diseases, Harvard T. H. Chan School of Public Health, Harvard University, Boston, MA, USA. [3]MRC London Institute of Medical Sciences, London, UK. Corresponding author: Monica Colaiacovo (mcolaiacovo@genetics.med.harvard.edu)

Meiosis is a specialized cell division program resulting in the production of haploid gametes (eggs and sperm) from diploid germ cells. Errors in achieving accurate chromosome segregation during meiosis can result in infertility, miscarriages and birth defects such as Down syndrome. Therefore, understanding the mechanisms underlying accurate meiotic chromosome segregation is of tremendous importance for human reproductive health. Here, we uncovered a role for GRAS-1, the worm homolog of mammalian GRASP and CYTIP proteins, in coordinating early meiotic events with cytoskeletal forces outside the nucleus. GRAS-1/GRASP/CYTIP contains PDZ and coiled-coil domains and in mammals has been implicated in docking cytoskeleton components and in endosomal trafficking. A role for GRAS-1/GRASP/CYTIP during meiosis has not been previously demonstrated. *gras-1* expression starts upon entrance into meiosis and GRAS-1 localizes in close proximity to the nuclear envelope (NE)-associated protein SUN-1 starting at early prophase I. GRAS-1 IPs and MS analysis reveal it interacts with other NE and cytoskeleton proteins. *gras-1* mutants show an extended transition zone (leptotene/zygotene stage), a delay in achieving homologous pairing, the formation of aggregates with SC central region proteins that persist into pachytene while chromosome axes appear unaltered, and impaired DNA double-strand break repair progression. Importantly, these defects are partially rescued by expression of mammalian CYTIP in *gras-1* mutants, supporting functional conservation. These defects likely stem from a role for GRAS-1 in regulating chromosome dynamics given that *gras-1* mutants show accelerated chromosome movement during early prophase I. Moreover, in a *dhc-1* depleted background, *gras-1* mutants exhibit new and additional phenotypes, indicating that *gras-1* regulation of chromosome movement acts in parallel to the previously described LINC-controlled pathway. Finally, GRAS-1 undergoes phosphorylation, and analysis of a phosphodead mutant reveals that this post-translational modification is required for regulating GRAS-1 function during meiosis. We propose that GRAS-1 serves as a scaffold for a multi-protein complex coordinating the early steps of homolog search and licensing of SC assembly by regulating the pace of chromosome movement in early prophase I.

O1-02. Advances in understanding the behavior of the nucleosome-less chromosomes of dinoflagellates

Angeles Cuadrado[a], Rosa I Figueroa[b]; Marta Sixto[bc], Isabel Bravo[b], Alfredo de Bustos[a]

[a]Departamento de Biomedicina y Biotecnología, Universidad de Alcalá (UAH), Alcalá de Henare; [b]Instituto Español de Oceanografía (IEO) Vigo; [c]Campus do Mar, Universidad de Vigo, Vigo

Dinoflagellates are a large group of protists whose exceptionally enormous genome is unique among eukaryotes in terms of base composition, chromosome structure and gene expression (1). Even after decades of research, the structure and behaviour of their amazing chromosomes –which without nucleosomes exist in a liquid state– are poorly understood. A lack of appropriate cytogenetic tools has hindered studies of the dinochromosomes. We have adapted FISH protocols and developed some chromosomal markers in order to analyse the hundreds of dinoflagellate chromosomes and their behaviour during dinomitosis, the atypical nuclear division that solely occurs in the class Dinophyceae (2). This study shows the sequence of nuclear transformation and progression of dinomitosis in *Alexandrium ostenfeldii* and *Gambierdiscus australes* and follows the positioning of telomeres in relation to the nucleolus during cell division. Since dinochromosomes are always condensed during the cell cycle, and dinomitosis occurs within an intact nuclear envelope and in absence of nucleolar disassembly, the “typical” prophase and telophase phases of higher eukaryotes are not seen in dinoflagellates”. In addition, a “typical” metaphase phase is not distinguished in dinoflagellates since their chromosomes, lacking kinetochrores are attached –via their telomeres– to the inside of the nuclear envelope (3) and are not positioned in a single plane near the middle of the cell (4). Thus, in reference to the conventional mitotic phases described in higher eukaryotes, we describe dinomitosis as divided in two distinguished sequential stages, pre-anaphase and anaphase.

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2 Bhaud et al. (2000). *J. Cell Sci. 113, 1231-1239*

3 Cuadrado et al. (2019). *Sci. Rep. 9, 3072*

4 Costas and Goyanes. (2005). *Cytogenetics and Genome Res. 109, 268-275*

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FT1-01. The impact of chromosomal fusions on 3d genome folding in the germ line

Covadonga Vara[1], Andreu Paytuví-Gallart[1,2], Yasmina Cuartero[3], Lucía Álvarez-González[1], Laia Marín-Gual[1], Francisca Garcia[1], Beatriu Florit-Sabater[1], Laia Capilla[1], Rosa Ana Sanchéz-Guillén[1,4], Zaida Sarrate[1], Riccardo Aiese Cigliano[2], Walter Sanseverino[2], Jeremy B. Searle[5], Jacint Ventura[1], Marc A. Marti-Renom[3,6,7], François Le Dily[3], Aurora Ruiz-Herrera[1]

[1]Universitat Autònoma de Barcelona, Barcelona, Spain, [2]Sequentia Biotech,Barcelona, Spain, [3]CNAG-CRG, Centre for Genomic Regulation, Barcelona, Spain, [4]Instituto de Ecología AC (INECOL),Veracruz, Mexico, [5]Cornell University,Ithaca, USA, [6]Pompeu Fabra University, Barcelona, Spain, [7]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Chromosomal reorganizations such as Roberstonian (Rb) fusions are key factors for evolution, yet their impact on spatial folding of chromosomes inside the nucleus and its regulatory effects on gene expression remains unclear. Here, we take advantage of chromosome conformation capture (Hi-C) in combination with SNP genotyping and analysis of crossover events to study how the higher-order chromatin organization and recombination landscapes are affected by chromosomal fusions in the house mouse germ line. We show that chromosomal fusions alter the nuclear architecture during meiosis affecting not only chromosomes involved in them but also not-fused chromosomes. In primary spermatocytes, the presence of Rb fusions induce ectopic heterologous interactions and alterations in both chromosome synapsis and axis length, whereas in post-meiotic cells these heterologous interactions are reduced, causing an increase of intra-chromosomal interactions and therefore a reorganization of topological associated domains. Moreover, new meiotic-specific inter-chromosomal interactions related with olfactory receptor family clusters were detected in post-meiotic cells. These results suggest an adaptive role of Rb fusions by rearranging nuclear occupancy. Overall, our results provide new insights into how genome reshuffling influences chromatin folding and therefore its high-order organization inside the nucleus of germ cells.

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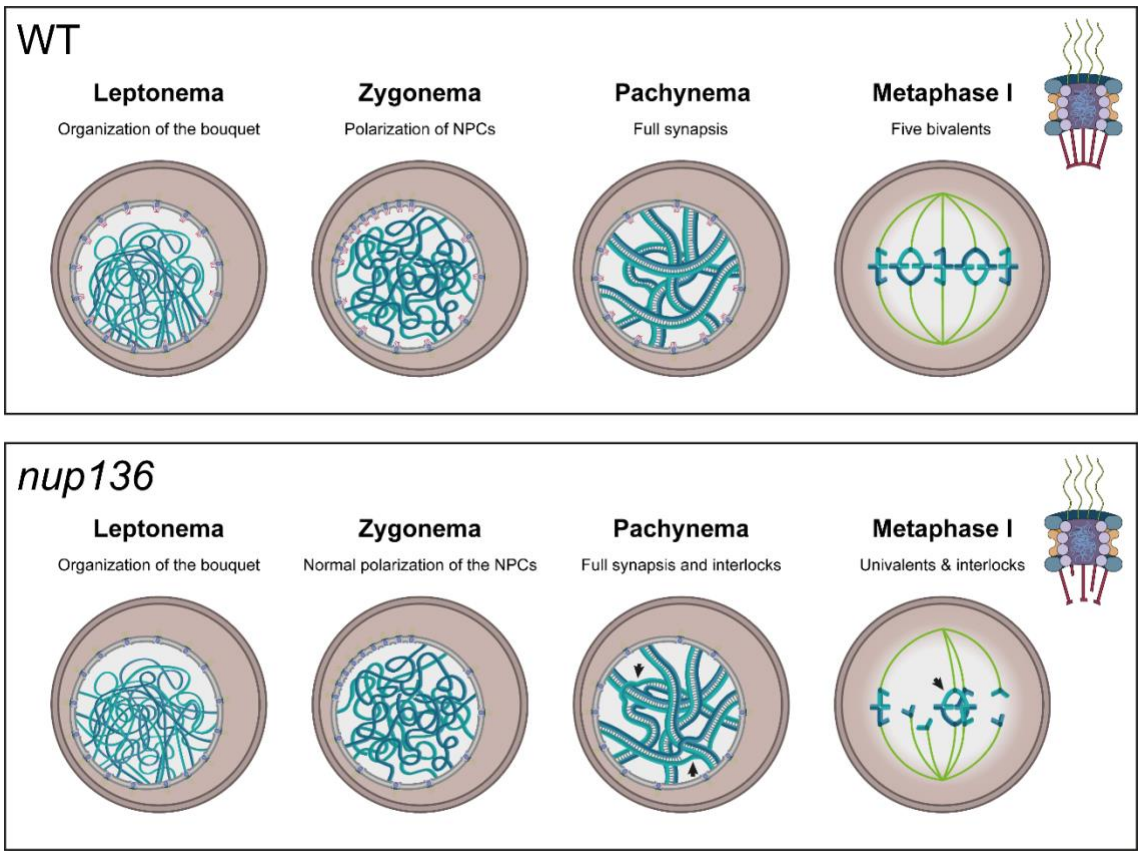
FT1-02. The plant-specific nucleoporin nup136 is essential to ensure the obligatory crossover in Arabidopsis meiosis

Nadia Fernández-Jiménez[a], Mónica Pradillo[a]

[a]*Departamento de Genética, Fisiología y Microbiología, Facultad de Biología, Universidad Complutense de Madrid, Madrid, Spain*

The nuclear envelope (NE) is a highly organized double-layered membrane that separates the nuclear content from the cytoplasm. This barrier plays a central role in meiotic chromosome movements, since telomeres attach to the inner nuclear membrane (INM) and cluster at the bouquet stage, a nearly universal event during prophase I. Plant NE is structurally similar to the NE of other kingdoms, but with several unique features. Higher plants lack centrosomes and NE acts as a microtubule organizing center before it disappears at the end of prophase I. Nuclear Pore Complexes (NPCs) are embedded into the NE and orchestrate the selective nucleocytoplasmic traffic of macromolecules. Plant NPCs are densely spaced, however, their actual role in chromatin dynamics during meiosis remains elusive. Our observations provide a meiotic function for NUP136, a plant-specific nucleoporin, likely a functional homolog of metazoan Nup153, that is located to the nucleoplasmic side, in the nuclear basket subcomplex. The absence of this nucleoporin produces alterations in nuclear morphology, reduced fertility and early flowering. The meiotic characterization of pollen mother cells (PMCs) from the corresponding mutant has revealed that this protein is required to avoid interlocks among non-homologous chromosomes and regulate crossover (CO) formation and patterning. In summary, our results identify NUP136 and NPCs as regulators of meiotic crossover in Arabidopsis.

Graphical abstract



FT1-03. Contribution of chromosome rearrangements to the genomic plasticity of the fungal pathogen *Fusarium oxysporum*

Lucía Gómez Gil[1], Cristina López Díaz[1], Dilay H. Ayhan[2], Li-Jun Ma[2], Antonio Di Pietro[1]

[1]*Department of Genetics, University of Córdoba, Córdoba, Spain*
[2]*Department of Biochemistry & Molecular Biology, University of Massachusetts Amherst, MA, USA*

Fungal pathogens represent a major threat to human health and food security. A particularly striking feature is their capacity to rapidly adapt to new environmental conditions, including new hosts. The ascomycete *Fusarium oxysporum* causes devastating vascular wilt disease in more than a hundred different crop plants as well as opportunistic infections in immunosuppressed humans. Despite lacking a known sexual cycle, this fungus displays a remarkable genomic and phenotypic plasticity. To analyze the genetic mechanisms underlying adaptation, a clonal isolate of *F. oxysporum* was subjected to experimental evolution under different environmental conditions, including the host plant tomato as well as plates with different media. Genome sequencing of evolved populations revealed both small-scale mutations such as SNPs and Indels, as well as large-scale copy number variations, including chromosome losses or duplications as described in other pathogenic fungi and yeast. Interestingly, gross chromosomal rearrangements (GCRs) recurrently occur in certain accessory or lineage specific (LS) chromosomal regions, which differ from core regions by presenting a lower gene content and a high abundance of repeated elements.

To explore the genetic mechanisms underlying GCRs, we established protocols for measuring the frequency of these events, either in long-term or short-term evolution experiments, the latter allowing to estimate GRC frequency in the near absence of selection. Studies in yeast suggested that DNA double strand breaks (DSBs) act as intermediates of GCRs and that mutations in genes involved in DSB repair via homologous recombination (HR) or non-homologous end joining (NHEJ) lead to increased rates of GCRs. To test whether this process is conserved in *F. oxysporum*, we generated knockout mutants in homologs of DSB repair genes. Preliminary results suggest that both the HR and NHEJ DSB repair pathways contribute independently to the stabilization of certain LS regions, thereby reducing their loss.

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FT1-04. The metazoan-like kinetochore assembly and disassembly cycle is conservedin yeast mitosis but masked by the Rabl configuration

Alberto Jiménez-Martín[a], Alberto Pineda-Santaella[a], Daniel León-Periñán[a], Jesús Pinto-Cruz[a], Sabas García-Sánchez[a], David Delgado-Gestoso[a], Laura Marín-Toral[a] and Alfonso Fernández-Álvarez[a] [a]
Andalusian Center for Developmental Biology (CABD), Pablo de Olavide University/Consejo Superior de Investigaciones Científicas/Junta de Andalucía, Seville, Spain

During cell cycle progression in metazoan, the kinetochore, the protein complexattached to centromeres which directly interacts with the spindle microtubules, the vehicle of chromosome segregation, is partially assembled at mitotic onset and disassembled during mitotic exit (1–3). This program is assumed to be absent in budding and fission yeast because most of kinetochore proteins are stably maintained at the centromeres throughout the entire cell cycle (4,5). In this work, we show that the assembly program at the mitotic onset of the outer kinetochore, is unexpectedly conserved in *Schizosaccharomyces pombe*. We have identified this behavior by removing the Rabl chromosome configuration during interphase, in which centromeres are permanently associated with the nuclear envelope beneath the spindle pole body (6). Hence, the Rabl configuration masks the presence of a program to recruit the outer kinetochore at mitotic onset in fission yeast, similar to that taking place in metazoan. Besides the evolutionary implications of our observations, we think that our work will help understand the molecular processes behind the kinetochore assembly program during mitotic entry using fission yeast as the model organism.

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FT1-05. Increased mitotic cell death in MCPH1 depleted cells upon catalytic inhibition of TOPO II

María Arroyo[c], Antonio Sánchez[a], Ana R. Cañuelo[a], Rosalía F. Heredia Molina[a], Eduardo Martínez Molina[a,c], Duncan J. Clarke[b], J. Alberto Marchal[a]

[a] Departamento de Biología Experimental, Universidad de Jaén, E23071 Campus Las Lagunillas s/n, Spain
[b]Department of Genetics, Cell Biology and Development, University of Minnesota, US
[c]Cell Biology and Epigenetics, Department of Biology, Technische Universität Darmstadt, Germany

The capacity of Topoisomerase II (Topo II) to remove DNA catenations that arise after replication is essential to ensure faithful chromosome segregation. Topo II activity is monitored during G2 by a specific checkpoint pathway that delays entry into mitosis until chromosomes are properly decatenated. Recently, we demonstrated that the mitotic defects characteristic of cells depleted of MCPH1 function, a protein mutated in primary microcephaly, do not depend on a weakened G2 decatenation checkpoint response. However, those mitotic defects could still be accounted for by a minor defect in the activity of Topo II during G2/M. To test this hypothesis, we have tracked at live single cell resolution the dynamics of mitosis in MCPH1 depleted HeLa cells upon catalytic inhibition of Topo II. Our analyses demonstrate that neither chromosome alignment nor segregation are more susceptible to minor perturbation in decatenation in MCPH1 deficient cells, compared with control cells. Interestingly, MCPH1 depleted cells were more prone to mitotic cell death when decatenation was perturbed. Furthermore, when the G2 arrest induced by catalytic inhibition of Topo II was abrogated by Chk1 inhibition, the incidence of mitotic cell death was also increased. Taken together, our data suggest that MCPH1 lack of function increases mitotic cell hypersensitive to catalytic inhibition of Topo II.

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FT1-06. B chromosomes of *Eyprepocnemis plorans* contain active protein-coding genes involved in cell division

María Martín-Peciña[a], Ester Sánchez-Sánchez[a], Francisco J Ruiz-Ruano[bc], María Dolores López-León[a], Josefa Cabrero[a], Juan Pedro M Camacho[a]

[a]Departamento de Genética, Facultad de Ciencias, Universidad de Granada, Granada, Spain, [b]School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, UK, [c]Department of Organismal Biology – Systematic Biology, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden

Supernumerary or B chromosomes are dispensable genomic elements found in most kinds of eukaryotic genomes. They frequently show drive mechanisms that give them an advantage in transmission, but how they achieve them remains a mystery (1). The recent finding of protein-coding genes in B chromosomes of several species has opened the possibility that their evolutionary success is based on their gene content (2). Using a protocol based on mapping genomic DNA Illumina reads from B-carrying and B-lacking individuals on the coding sequences of *de novo* transcriptomes from the same species, we identified 42 protein-coding genes in the B chromosome of the grasshopper *Eyprepocnemis plorans* from Torrox (Málaga, Spain). SNP calling comparing these mappings showed specific nucleotide changes from the B-carrying individuals that we can assign to the B chromosome. These nucleotide signatures allowed identifying B-derived transcripts in B-carrying transcriptomes, some of them showing higher frequency than the A-derived ones, thus self-disclosured as functionally subverted genes. One of them was *ndl* which, in ovaries from B-carrying females, showed more transcripts coming from B than A chromosomes. As B drive takes place through females in this species (3) and *ndl* function is related with asymmetric cell division, it is conceivable that this subverted gene might be involved in the B drive mechanism. Another active B-gene was *cdc16*, which codes for a subunit of the anaphase promoting complex or cyclosome (APC/C), an E3 ubiquitin ligase involved in the metaphase-anaphase transition. Remarkably, the gene *apc1* (coding for a different subunit of the APC/C complex) is also highly transcribed in the B chromosomes of the grasshopper *Locusta migratoria* (2). This coincidence and the fact that the *ndl* and *cdc16* genes were also present in the B chromosomes from distant *E. plorans* populations (i. e. Tanzania, Egypt and Armenia) reinforce the idea that successful B chromosomes harbor active genes involved in the regulation of cell division.

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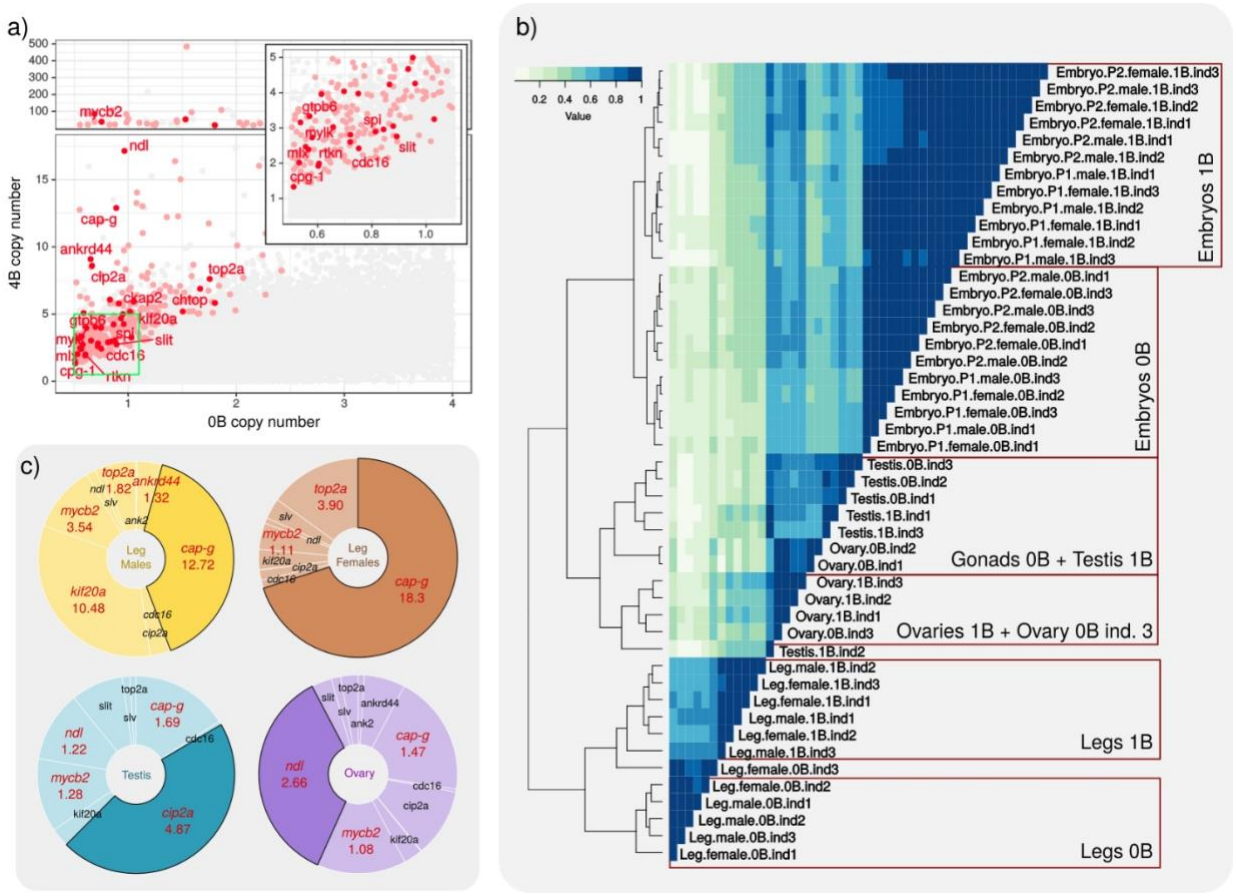
Graphical abstract:

a) Abundance in the 4B and 0B genomes of the 42 protein-coding genes residing in the B chromosome of *Eyprepocnemis plorans* (noted in dark red color; CDSs not validated by full-transcript analysis are in light red color). b) Heatmap of RNA samples using counts of Alt (B-specific SNPs) and Ref variants (located in the A chromosomes) for B-located genes in *E. plorans*. Samples are classified firstly based on tissue and then in terms of B chromosome presence. Note that 1B ovaries group together and separated from 0B ones and 1B/0B testes, suggesting specific expression patterns in ovaries due to B chromosome presence. c) Pie graphs representing the proportion of Alt/Ref counts in 1B RNA libraries for each B-gene respect to the total of ratios. Subverted genes, Alt/Ref ratio in 1B libraries>1, are highlighted in red together with the value of that ratio. Note that the *ndl* gene is strongly subverted only in the ovary, which is the organ where B drive takes place.



P1-01. Unravelling the mystery of the Arabidopsis meiotic mutant *dsy1*: a novel mutant allele of *MSH5*

Francesco Blasio[1], Bianca Martín[1], Riccardo Aiese Cigliano[2], Juan L. Santos[1] and Mónica Pradillo[1]

Departamento de Genética, Fisiología y Microbiología; Facultad de Ciencias Biológicas; Universidad Complutense de Madrid; Spain. [2]Sequentia Biotech SL, Barcelona; Spain.

The desynaptic mutant *dsy1* was isolated in a screen of T-DNA transformed Arabidopsis lines with reduced fertility more than 20 years ago. Desynaptic mutants, by definition, typically show full synapsis at pachynema, but many univalents at metaphase I. Despite the interesting phenotype of this mutant, with the lowest mean chiasma frequency known for a mutant with full synapsis, the responsible gene has not been identified. Preliminary analyses conducted in our lab, indicated the presence of several T-DNA copies in *dsy1*. For this reason, we decided to attempt whole-genome massive sequencing (WGS) to map these insertions in order to identify the gene responsible for the observed phenotype in *dsy1*. By mapping the reads against the Arabidopsis genome, it was possible to locate a homozygous insertion in locus At3g20475, encoding AtMSH5, a homologue of the MutS-homolog family involved in meiotic recombination. Given that no other insertions could be found, it is possible that multiple copies of the T-DNA were inserted in this location. In addition, a complementation test has allowed us to further confirm that *AtMSH5* is the causal gene in *dsy1*. All *AtMSH5* mutants described so far are in the Columbia (Col) accession, while *dsy1* was obtained in the Wassileskija (Ws) accession. This finding provides a valuable tool, since it allows taking advantage of having the mutation in two different genetic backgrounds for measuring meiotic recombination rates by performing fine mapping of recombinant chromosomes using SNP markers.

P1-02. *Dmrt1* gene structure analysis in the flatfish *Solea senegalensis* reveals a novel intragenic duplication

Ismael Cross, Emilio García, María E. Rodríguez, Alberto Arias-Pérez, Alejandro Centeno-Cuadros, Silvia Portela-Bens, Manuel A. Merlo, Laureana Rebordinos.

Laboratorio de Genética. Departamento de Biomedicina, Biotecnología y Salud Pública. Facultad de Ciencias del Mar y Ambientales. Universidad de Cádiz. Puerto Real (Cádiz). Spain.

Sex determination systems are especially variable in fish, and can be influenced by environmental factors (ESD) or genetic factors (GSD). Different master genes have been reported as responsible of gonadal development. The gene *dmrt1* (Doublesex and Mab-3 (DM)-related Transcription factor 1) have been to play a vital role in sex determination, differentiation and maintenance of organ functions in a variety of species, including fish, mammals, reptiles, birds and amphibians (1). *S. senegalensis* has 42 chromosomes and an XX / XY chromosome system for sex determination, although no heteromorph chromosomes nor sex master-gene has been found (2). Several cytogenetic studies suggest that the largest metacentric pair of chromosomes could be a proto-sex chromosome pair (3, 4), as they contain the gene cluster *dmrt1-dmrt3-dmrt2*. In this work, cDNA of the *dmrt1* gene from the Senegalese sole, *Solea senegalensis*, was cloned and the full genomic sequence of the gene was analised too (5). Multiple mRNA isoforms were observed, indicating a high variable system of alternative splicing in the expression of *dmrt1* of the sole in gonads. None isoforms could be related to sex of individuals. The genomic structure of the *dmrt1* gene showed a gene of 31400 bp composed of 7 exons and 6 introns. It contains an unexpected duplication of more than 10399 bp, involving part of the exon I, exons II and III. A mature miRNA of 21 bp in length was localized at 336 bp from exon V too (2). A protein-protein interacting networks analysis showed matches with *dmrt1* protein from *Cynoglossus semilaevis.* The phylogenetic study of this gene displayed a consistent evolutionary position of the species in the teleostei group. Finally, the analisys of repetitive elements in the full sequence of the gene, unvealed the presence of a SINE element, which could have played a major role in the origin and evolution of such duplication.

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P1-03. DIET EFFECTS ON MEIOTIC RECOMBINATION: IMPLICATIONS FOR RECOMBINATION STUDIES

Estefania San Martín Pérez[1], Ángela Bemonte Tébar[1], Syong Hyun Nam-Cha[2], Ana Josefa Soler Valls[3], Nadia Singh[4], Elena de la Casa-Esperón[1,5]

[1]Regional Center for Biomedical Research (C.R.I.B.). University of Castilla-La Mancha. Albacete, Spain. [2]Pathology Department. University Hospital Complex of Albacete. Albacete, Spain. [3]IREC (CSIC-UCLM-JCCM), ETSIAM. Albacete. Spain. [4]Department of Biology, Institute of Ecology and Evolution, University of Oregon. Eugene, Oregon. [5]School of Pharmacy. University of Castilla-La Mancha. Albacete, Spain.
Corresponding author: Elena de la Casa Esperón (elena.casaesperon@uclm.es)

Meiotic recombination is well known as a fundamental process that generates genetic diversity and as an essential tool for genetic mapping purposes. But is also required for proper chromosome segregation. Hence, the frequency and distribution of crossovers are genetically and epigenetically controlled in order to avoid aneuploidy and ensure fertility. We confirm that crossover frequency depends on genetic background in spermatocytes of a diverse spectrum of mouse inbred strains. However, certain environmental exposures can alter crossover frequency, such as toxicants or other stressful conditions. We have found a novel and unexpected factor affecting recombination rate: diet. The effect is both diet- and strain-dependent: while some strains are resistant to drastic nutritional changes, others are sensitive to mild differences between chows commonly used in animal facilities. We explore the origin of the observed recombination changes, as well as other effects of diet in the male germline, such as on sperm motility. Our results suggest that diet should be carefully controlled in recombination studies so as not to confound the experimental findings.

P1-04. Numtogenesis affecting chromosome segregation: a novel approach to understand immunosenescence

Sara Bravo [(a)], Víctor García-Martínez [(a)], Hikaru Kobayashi [(a)], Blanca González-Bermúdez [(b)], Gustavo R. Plaza [(b)], Mónica De la Fuente [(a)], Mónica González-Sánchez [(a, *)]

(a) Departamento de Genetica, Fisiologıa y Microbiologıa, Facultad de Biología, Universidad Complutense de Madrid (b) Departamento de Ciencia de Materiales, ETSI de Caminos, Canales y Puertos, Universidad Politecnica de Madrid Author for correspondence: mgs@ucm.es

Aging is caused by the cumulative damage produced by the constant generation of reactive oxygen species in the mitochondria (mitROS) throughout the life of the individuals. Mitochondrial DNA (mtDNA) is more vulnerable than nuclear DNA to the oxidative damage produced by mitROS. Mitochondrial fragments can escape from the mitochondria and insert into the nucleus, causing a time dependent nuclear accumulation (1). This phenomenon, called numtogenesis, has been observed in different species.

Mitochondrial fragments are preferably found at the (peri)centromeric regions, where integration is mediated by the Non-Homologous End Joining mechanism. The effect that insertions have on chromosome segregation is yet to be elucidated.

We performed a longitudinal study based on the examination of mice lymphocytes isolated from peritoneum, monitoring each animal during the adult, mature and old age. We detect a 1841bp mitochondrial sequence (1841 MT) located at definite loci in the nuclei. The amount of 1841 MT increased gradually, resulting in significant differences between each of the three groups of age analyzed. FISH in colchicine treated cells revealed that 1841MT was located at centromeres, colocalizing to Major Satellite sequence.

The Cytokinesis Block Micronucleus assay (2), which allowed for detection of chromosome instabilities leading to micronuclei formation, showed that micronuclei frequency increased progressively with aging. The majority of the micronuclei analysed were positive for centromere sequences, revealing an aneugenic origin that leads to aneuploidic cells.

The positive correlation found between mtDNA insertion and micronuclei frequency suggests that the preferential insertion of mitochondrial sequences in the pericentromeric regions affects chromosomal segregation. This new mechanism underlying immunosenescence is proposed for future studies.

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P1-05. Lymphocyte micronuclei formation is a biomarker of biological age in mice

David López, Marta Trujillo, Itziar Berruete, Mónica González-Sánchez (*)

Depto. de Genetica, Fisiologıa y Microbiologıa, Facultad de Biología, Universidad Complutense de Madrid (*) Author for correspondence: mgs@ucm.es

Since aging can occur at different speed in different individuals with the same chronological age, the concept of "biological age" has been introduced to indicate the level of aging of each individual. Nevertheless, the difficulty in estimating biological age makes it necessary to search for biological markers capable to predict life expectancy in an accurate way.

Genomic instability is a hallmark of aging. A dysfunctional centromere is likely to cause lagging chromosomes during anaphase, leading to micronuclei formation (1). Previous results from our laboratory showed an increase in micronuclei frequency associated to aging in mice lymphocyte, indicating that micronuclei formation could be considered an indicator of biological age.

The effects of various lifestyle strategies on the aging process in mice have improved immune functions and redox state, rendering positive results in the achievement of a higher mean longevity (2).

We studied the effects of some of these lifestyle strategies in improving this potential biomarker of biological age. Cytokinesis Block Micronucleus assay (3) for micronuclei quantification was conducted in peritoneal mice lymphocytes in three different strategies: a) a daily intake of the probiotic *Akkermansia muciniphila* for 4 weeks in old mice, b) the cohabitation of old animals, 15 minutes each day for 2 months, with younger mice and c) old mice subjected to the electromagnetic signals generated by the Neuralter™ system.

Treated mice showed a significant decrease in the frequency of micronuclei compared with controls of the same chronological age. Our results prove that micronuclei formation in immune cells can be altered by these lifestyle treatments, therefore, confirming this parameter as a biomarker of biological age in mice.

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This work was supported by MICINN-MAT2016-76847-R

P1-06. Cytogenetic analysis, heterochromatin characterization and location of the rDNA genes of *Hycleus scutellatus* (Coleoptera, Meloidae); a species with an unexpected high number of rDNA clusters

Laura Ruiz-Torres, Pablo Mora, Areli Ruiz-Mena, Jesús Vela, Francisco J. Mancebo, Eugenia E. Montiel, Teresa Palomeque and Pedro Lorite

Animal and Human Molecular Genetics Group (RNM-924), Department of Experimental Biology, Genetics Area, University of Jaén, 23071 Jaén, Spain
Corresponding author: Pedro Lorite (plorite@ujaen.es)

The family Meloidae contains approximately 3000 species and is commonly known as blister beetles for their ability to secrete a substance called cantharidin, which causes irritation and blistering in contact with animal or human skin. In recent years there have been numerous studies focused on the anticancer action of cantharidin and its derivatives.

Despite the recent interest in blister beetles, cytogenetic and molecular studies in this group are scarce and most of them use only classical chromosome staining techniques. *Hycleus* is one of the most speciose genera of blister beetles, including 500 species that are mainly distributed throughout the Old World. In spite of this pattern, there are currently no cytogenetic data for any species of this genus. The main aim of our study was to provide new information in Meloidae.

In this study, we performed a karyotype analysis of *Hycleus scutellatus*, an endemic species of the Iberian Peninsula. We determined its chromosome number, 2n = 20, as well as the presence of the X and Y sex chromosomes. In addition to a karyotype analysis, we carried out DAPI staining. By fluorescence *in situ* hybridization, we mapped the rDNA clusters on 12 different chromosomes. This is one of the highest numbers of rDNA sites found in the Polyphaga suborder (Coleoptera). Additionally, we isolated a satellite DNA family (Hyscu-H), which was located within the pericentromeric regions of all chromosomes, including the sex chromosomes. The results suggest that Hyscu-H is likely to be one of the most abundant satellite DNA repeats in *H. scutellatus*. The results obtained in this study may be a suitable starting point to initiate more extensive cytogenetic analyses in this important species-rich genus and in the family Meloidae in general.

P1-07. Devil is in the details - meiotic chromosome dynamics in Australian marsupials

Laia Marín-Gual[a,b], Laura Rodríguez-Rodelas[a,b], Gala Pujol[a,b], Covadonga Vara[a,b], Jesús Page[c], Andrew Pask[d], Marilyn Renfree[d], Paul D Waters[e], Aurora Ruiz-Herrera[a,b]

[a]Departament de Biologia Cel·lular, Fisiologia i Immunologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Spain. [b]Genome Integrity and Instability Group, Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Spain. [c]Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, Madrid. [d]School of BioSciences, The University of Melbourne, Victoria, Australia. [e]School of Biotechnology and Biomolecular Sciences, Faculty of Science, UNSW, Sydney, NSW 2052, Australia.

During meiotic prophase I, homologous chromosomes pair, synapse and recombine in a tightly regulated process, which ensures the generation of genetically variable haploid gametes. This process has canonical features that are highly conserved across mammals, although there are notable differences between taxa (1). The mechanisms underlying meiotic cell division have been thoroughly studied in many model species. However, our understanding of the dynamics of meiotic prophase I in non-traditional model vertebrates is still in its infancy, especially when viewed from a phylogenetic perspective (2). Here, we compare the similarities and differences in the regulation of meiosis in two previously uncharacterized Australian marsupial species: the Tammar wallaby (*Macropus eugenii*: family Macropodidae) and the fat-tailed dunnart (*Sminthopsis crassicaudata*: family Dasyuridae). We performed a cytological analysis of the meiotic prophase I, including the study of chromosome synapsis, double strand break (DSB) formation and meiotic sex chromosome inactivation (MSCI). Our results show that sex chromosomes associate forming the so-called dense plate (DP) following different strategies in both marsupial species, which correlates with differential sex chromosomes architecture and transcriptional patterns. Furthermore, we characterize a previously undescribed phenomenon in primary spermatocytes of the fat-tailed dunnart: heterologous telomeric associations accompanied with telomere transcription and elongation, which is consistent with the telomeric dimorphism characteristic of the Dasyuridae family (3). Overall, our results provide new insights into the regulation of meiotic prophase I in marsupials.

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P1-08. Cytogenomic analysis of the *hox* genes in *Solea senegalensis*

Marco Mendizabal-Castillero[a], Manuel Alejandro Merlo[a], Silvia Portela-Bens[a], Maria Esther Rodríguez[a], Laureana Rebordinos[a].

[a]*Genetics Area-Faculty of Marine and Environmental Sciences, INMAR, University of Cadiz, Cadiz, España.*

The Senegalese sole (*Solea senegalensis*, Kaup 1858) is a marine flatfish, belonging to the Pleuronectiformes order, with a high-appreciated meat in the market, which makes it a commercially important species for fisheries and aquaculture (1).

Despite the effort to optimize its culture, some bottlenecks have still to be resolved, such as skeletal deformities and high mortality during the larval and juvenile phase (2), possibly associated with genetic factors or the inability of homeotic mechanisms to compensate the environmental and nutritional stress (3).

In this research, the *hox* gene clusters were characterized molecularly and cytogenetically, due to the importance of the *hox* genes in embryonic development, morphogenesis and cell differentiation, thus being interesting to study in flatfish, especially in the Senegalese sole. To such purpose, a BAC library was used to isolate the clones that contained these genes to sequence them by NGS and to use them as hybridization probes in Fluorescent *in situ* Hybridization (FISH) analysis.

To date, it has been possible to isolate the BAC clones containing *hoxb* and *hoxc* clusters. Sequence and microsynteny analysis showed highly similarities with the same *hox* clusters of closely-related species. FISH analysis demonstrated a localization of these two clusters in different acrocentric chromosomes. This work also lay the foundation for further studies on expression during larval development, which would be valuable for future improvement programs.

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P1-09. Satellitome analysis of *Rhodnius prolixus*, one of the main Chagas disease vector species

Eugenia E. Montiel[a], Francisco Panzera[b], Teresa Palomeque[a], Pedro Lorite[a] and Sebastián Pita[b]

[a] Animal and Human Molecular Genetics Group (RNM-924), Department of Experimental Biology, Genetics Area, University of Jaén, 23071 Jaén, Spain, [b] Evolutionary Genetic Section, Faculty of Science, University of the Republic, Iguá 4225, Montevideo 11400, Uruguay. Corresponding author: Eugenia E. Montiel (emontiel@ujaen.es)

The triatomine *Rhodnius prolixus* is the main vector of Chagas disease in countries such as Colombia and Venezuela and the first kissing bug whose genome has been sequenced and assembled. In the repetitive genome fraction (repeatome) of this species, the transposable elements represented 19% of *R. prolixus* genome, being mostly DNA transposon (Class II elements). However, scarce information has been published regarding another important repeated DNA fraction, the satellite DNA (satDNA). Here we offer, for the first time, extended data about satDNA families in the *R. prolixus* genome using bioinformatics pipeline based on low-coverage sequencing data. The satellitome of *R. prolixus* represents the 8% of the total genome and it is composed by 39 different satDNA families. Only three of these satDNA families exceed the 1% of the genome. Chromosomal hybridization with these satDNAs showed dispersed signals over the euchromatin of all chromosomes, both in autosomes and sex chromosomes. Clustering analysis reveals that most abundant satDNA families configure several superclusters indicating that the *R. prolixus* satellitome is complex and that the four most abundant satDNA families are composed by different subfamilies. Additionally, transcription of satDNA families was analyzed in different tissues, showing that 33 out of 39 satDNA families are transcribed in four different patterns of expression across samples.

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P1-10. Both homologous chromosomes of a heterokaryotype were involved in the origin of *Drosophila subobscura* inversion e_9

Dorcas J. Orengo[a], Eva Puerma[a], Unai Cereijo[a,b], Montserrat Aguadé[a]

[a] Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, i Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Barcelona, Spain. [b] Present address: Centre for Research in Agricultural Genomics, CSIC-IRTA- UAB-UB, Campus UAB, Bellaterra (Cerdanyola del Vallès), 08193, Barcelona, Spain.

Chromosomal inversions are among the most common structural changes observed both as polymorphisms within species and as fixed differences between species. *Drosophila subobscura* stands out for its high level of chromosomal inversion polymorphism and for presenting several overlapping inversions complexes. Overlapping inversions unequivocally define the order of appearance of these complex chromosomal arrangements. Moreover, nucleotide variation at regions flanking inversion breakpoints can also be informative about both the inversion age and its originating mechanism, given the reduced recombination at these regions in heterokaryotypes. We analyzed nucleotide variation at a fragment flanking the most centromere-proximal shared breakpoint of several sequential overlapping inversions of the E chromosome of *Drosophila subobscura* —inversions E_1, E_2, E_9 and E_3. Surprisingly, the molecular genealogy inferred from variation at this shared fragment does not exhibit the branching pattern expected according to the sequential origin of inversions. The detected discordance between the molecular and cytological genealogies led us to consider a novel possibility for the origin of inversion E_9, and more specifically that this inversion originated on a heterokaryotype for chromosomal arrangements. Based on this premise, we propose three new chromosome models (NHEJ-4, NHEJ-3 and BIR-NHEJ) for the origin of this inversion.

P1-11. Ants and aphids, mutualistic species that also share transposable elements

Jesús Vela, Eugenia E. Montiel, Pablo Mora, Areli Ruiz-Mena, Pedro Lorite and Teresa Palomeque

Animal and Human Molecular Genetics Group (RNM-924), Department of Experimental Biology, Genetics Area, University of Jaén, 23071 Jaén, Spain
Corresponding author: Teresa Palomeque (tpalome@ujaen.es)

Aphids (Hemiptera, Aphididae) are small parasitic insects of plants, causing agricultural pests on many occasions. Many species establish mutualistic relationships with ants. Aphids produce a sugary food for the ants and ants protect the aphids from predators and parasites. Aphids represent an interesting cytogenetic model because they possess holocentric chromosomes with an X0 sex-determination system.

The genus *Aphis* contains at least 600 species, some of them important plant pests as *A. fabae* that feeds on fava beans, beets and many others species. *A. hederae* feeds on ivy (*Hedera helix*) but can also be found on other species. *A. hederae* and *A. fabae* present the same chromosome number, 2n=8, with three autosome pairs and two X chromosomes in females. Silver staining and FISH with rDNA probes showed that the NORs were located at one telomere of each X chromosome, in a DAPI-negative region.

PCR amplification assays have allowed the detection of *mariner*-like transposable elements (TE) in *A. hederae* and in *A. fabae* (*Ahedmar-Mr* and *Afabmar-Mr*). These TEs showed highly similarity with a TE previously isolated in the ants *Myrmica ruginodis* and *Tapinoma nigerrimum*. In both *Aphis* species, FISH using this TE showed spread hybridization signals along all chromosomes, except for the rDNA sites of the X chromosomes. A phylogenetic analysis and the possible involvement of horizontal transfer events in the evolution of these shared DNA transposons were also carried out.

P1-12. Loss of Kinesin-8 improves the robustness of the acentrosomal spindle

Alberto Pineda-Santaella[a], Nazaret Fernández-Castillo[a], Alberto Jiménez-Martín[a], Ángela Sánchez-Gómez[a] and Alfonso Fernández-Álvarez[a]

[a]*Biología Molecular e Ingeniería Bioquímica – Spring Board Lab (010), Centro Andaluz de Biología del Desarrollo, Sevilla, España*

Chromosome segregation in female meiosis in many metazoans is mediated by acentrosomal spindles, the existence of which implies that microtubule spindles self-assemble without the participation of the centrosomes (1,2,3). Although it is thought that acentrosomal meiosis is not conserved in fungi (4,5), we recently reported the formation of self-assembled microtubule arrays, which were able to segregate chromosomes, in fission yeast mutant where the contribution of the spindle pole body (SPB, the centrosome equivalent in yeast (6)) was specifically blocked during meiosis (7). Here, we demonstrate that this unexpected microtubule formation represents a bonafide type of acentrosomal spindle in yeast. Moreover, a comparative analysis of these self-assembled spindles and the canonical SPB-dependent spindle reveals similarities and differences: for example, both spindles have a similar polarity, but the location of the γ-tubulin complex differs. We also show that the robustness of self-assembled spindles can be reinforced by eliminating kinesin-8 family members, whereas kinesin-8 mutants have an adverse impact on SPB-dependent spindles. Hence, we consider that our system will help to clarify the molecular mechanisms behind acentrosomal meiosis, a crucial step towards better understanding gametogenesis.

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P1-13. Certain nucleoporins are key elements for chromosome dynamics during plant meiosis

Sara Poyatos-Peláez, Marta Vargas-Ramírez, Irene Hernando-Herrera, Nadia Fernández-Jiménez, Mónica Pradillo

Departamento de Genética, Fisiología y Microbiología, Facultad de Biología, Universidad Complutense de Madrid, Madrid, Spain

Nuclear pore complexes (NPCs) are aqueous channels embedded in the nuclear envelope (NE). They regulate the nucleocytoplasmic transport but are also emerging as relevant regulators of spatial chromatin organization, gene expression, and DNA damage response. NPCs are large complexes formed by a complex network of evolutionarily conserved proteins known as nucleoporins. Nucleoporins are not only acting in the context of NPCs, but also in the nucleoplasm, being directly or indirectly engaged in a variety of different cellular processes. Several evidences from non-plant species link nucleoporins to meiosis, the specialized cell division essential to produce haploid gametes. In this study, we have explored possible roles for different nucleoporins in mediating normal meiotic progression in *Arabidopsis thaliana*. For this purpose, we have isolated several Arabidopsis lines with mutations in genes coding for nucleoporins belonging to different NPC subcomplexes. The observation of the corresponding meiosis phenotypes has revealed that some nucleoporins are essential for chromosome dynamics in meiosis, while others are dispensable, either because other nucleoporins in the NPCs supply their function or because they are not involved in this cell division. We will discuss the possible causes of the functional divergence among nucleoporins in a meiotic context.

P1-14. Initial chromosome associations in wheat meiosis in the framework of plant breeding

Pilar Prieto

Plant Breeding Department, Institute for Sustainable Agriculture, Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC), Alameda del Obispo s/n, Apartado 4084, 14080 Córdoba, Spain

Wheat is one of the most important food crops in the world. Understand wheat genome organization (allohexaploid with three related subgenomes) is important for geneticists and plant breeders, particularly in a plant breeding framework. In a polyploid such as wheat is fundamental to shed light on how homologous chromosomes (equivalent chromosomes from the same genome) specifically recognize each other to pair at the beginning of meiosis, the cellular process to generate gametes in sexually reproducing organisms. Terminal regions of the chromosomes, which include telomeres and subtelomeres, play an essential role on chromosome recognition and pairing at the beginning of meiosis in wheat. Unzipping how these chromosome regions can contribute to chromosome specificity/pairing at the onset of meiosis could provide tools to facilitate chromosome manipulation in interspecific hybrids or genetic crosses, which are developed to transfer desirable agronomic traits from related species into wheat. The importance of terminal chromosome regions on recognition and pairing at the onset of meiosis will be discussed.

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P1-15. Localization of microsatellite markers on karyotype of *Solea senegalensis* (kaup 1858)

Daniel Ramírez[1], María Esther Rodríguez[1], Marco Anaya[1], Alberto Arias[1],
Roberto de la Herrán[2], Carmelo Ruiz-Rejón[2], Laureana Rebordinos[1]

[1] *Área de Genética, Facultad de Ciencias del Mar y Ambientales, Instituto Universitario de Investigación Marina (INMAR), Universidad de Cádiz, 11510 Cádiz, Spain;*
[2] *Facultad de Ciencias. Departamento de Genética, Universidad de Granada, 18071, Granada, Spain.*

The Senegalese sole (*Solea senegalensis*, Kaup 1858) is a flatfish species of great value for aquaculture. However, its farming still presents some difficulties such as the low production, poor-quality sperm in F1 male and several morphological abnormalities such as spinal malformations during metamorphosis. In previous works, a high-density integrated genetic map of *S. senegalensis* using cytogenetic and BACs (Bacterial Artificial Chromosome) sequencing data has been developed [1]. Also, a linkage map based on microsatellite markers (also known as simple sequence repeats, SSRs) belonging to 27 linkage groups (LG) has been described [2]. In this work, 34 SSRs, associates with the 27 LG, were traced in the gene library of *S. senegalensis* and associated with specific BAC clones. These BACs sequencing allowed obtaining a physical map. The multicolour fluorescence *in situ* hybridization (FISH) of 24 out of 34 BAC clones containing SSRs, using marked BAC clones of a *S. senegalensis* cytogenetic map previously described [1], allowed by signals co-localization, the association of 20 LGs to 11 specific chromosomes; thus LG1, LG4 and LG16 were located in the large metacentric chromosome 1. Taking these results into account, new 11 BACs out of 34 were used for double FISH-BAC, confirming for 4 BACs the association of LG1 and LG4 to chromosome 1 and LG8 and LG10 to the chromosomes 8 and 13 respectively. These results could be useful for the detection of regions of interest due to the association with QTLs previously located in other species LGs and its homology with the LGs described in *S. Senegalensis*.

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P1-16. Complementary functions of ap endonucleases and ap lyases during DNA repair of abasic sites arising from C:G base pairs

Marina Jordano-Raya, M. Dolores Moreno-Recio, Cristina Beltrán-Melero, M. Isabel Martínez-Macías, Teresa Roldán-Arjona, Rafael R. Ariza, Dolores Córdoba-Cañero

Department of Genetics/IMIBIC, University of Córdoba, Córdoba, SPAIN

Spontaneous base loss in DNA generates abasic (apurinic/apyrimidinic, AP) sites, but these ubiquitous lesions also arise as intermediates during base excision repair (BER) (1, 2). AP sites may be processed either by AP endonucleases or AP lyases, but the relative roles of these two classes of enzymes are not well understood (3). Specifically, it remains unexplored whether the sequence flanking the AP site and/or the orphan base on the opposite DNA strand influence the probability that the lesion is processed either by an AP endonuclease or an AP lyase. AP sites opposite G are common intermediates during repair of deaminated cytosines, whereas AP sites opposite C arise during repair of oxidized guanines (4, 5). We have analyzed the activity of plant and human AP endonucleases and AP lyases on DNA substrates containing an abasic site opposite either G or C in different sequence contexts. In all contexts the major *Arabidopsis* AP endonuclease (ARP) exhibited a significantly higher activity on AP sites opposite G. In contrast, the main plant AP lyase (FPG) showed a greater preference for AP sites opposite C. The major human AP endonuclease (APE1) preferred G as the orphan base, but only in some sequence contexts. We propose that plant AP endonucleases and AP lyases play complementary repair functions on abasic sites arising at C:G pairs, neutralizing the potential mutagenic consequences of C deamination and G oxidation, respectively.

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SESIÓN 2

Expresión Génica y Epigenética

Moderador: Crisanto Gutiérrez

I2-01. Functional organization of the genome: intertwined links between DNA replication and transcription

José Miguel Fernández-Justel, Cristina Santa-María, Alberto Ferrera-Lagoa, Sara Martín-Vírgala, Javier Isoler-Alcaráz and María Gómez

Centro de Biología Molecular Severo Ochoa (CSIC/UAM). C/ Nicolás Cabrera 1, 28049-Madrid, Spain

Copying and decoding the genomic information in a timely and accurate fashion is essential for life. Both tasks (DNA replication and transcription) are remarkably complex in scale and regulation, and the molecular machineries involved in them translocate on the same chromatin template, often in opposite directions and at different rates. This impose cells to employ efficient mechanisms to coordinate both processes. Failure of these mechanisms can lead to catastrophic effects on genome stability and cell viability, suggesting that dealing with these potential conflicts strongly influences key parameters of cellular function, including genome organization, chromatin structure or mutagenesis rates. Using a range of multigenomic approaches we have found that chromatin alterations affect different aspects of RNA metabolism, generating replicative stress and genomic instability. In particular, we show that linker histone H1 is required to prevent the accumulation of nascent non-coding RNAs, suggesting that it regulates non-coding transcript turnover on chromatin. Interestingly, we found that unscheduled non-coding transcripts have reduced levels of m6A modification, causing replication-transcription conflicts. Accordingly, impairing m6A demethylase activity rescues the replicative stress phenotype of H1 loss. This work unveils unexpected regulatory roles of histone H1 on non-coding RNA turnover and m6A deposition, highlighting the intimate relationship between chromatin conformation, RNA metabolism and DNA replication to maintain genome performance.

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I2-02. Unveiling the role of PcG-mediated histone modifications in regulating gene expression in Arabidopsis

Xiaochang Yin[a,h], Francisco J Romero-Campero[b,c,h*], Pedro de los Reyes[b], Peng Yan[d], Jing Yang[e], Guangmei Tian[f], XiaoZeng Yang[e], Xiaorong Mo[d], Shuangshuang Zhao[g], Myriam Calonje[b*], Yue Zhou[a*]

*[a]State Key Laboratory of Protein and Plant Gene Research, School of Advanced Agricultural Sciences, Peking-Tsinghua Center for Life Sciences, Peking University, 100871 Beijing, China, [b]Institute of Plant Biochemistry and Photosynthesis (IBVF-CSIC), Avenida Américo Vespucio 49, 41092, Seville, Spain, [c]Department of Computer Science and Artificial Intelligence (University of Sevilla), Avenida Reina Mercedes s/n 41012, Seville, Spain, [d]State Key Laboratory of Plant Physiology and Biochemistry, College of Life Science, Zhejiang University, 310058 Hangzhou, China, [e]Beijing Agro-biotechnology Research Center, Beijing Key Laboratory of Agricultural Genetic Resources and Biotechnology, Beijing Academy of Agriculture and Forestry Sciences, 100097 Beijing, China, [f]Academy for Advanced interdisciplinary Studies, Peking University, 100871, Beijing, PR China, [g]Key Laboratory of Plant Stress, Life Science College, Shandong Normal University, 250014 Jinan, China, [h]These authors contributed equally: Xiaochang Yin, Francisco J Romero-Campero, * Co-Corresponding authors*

Although it is well established that the Polycomb Group (PcG) complexes maintain gene repression through the incorporation of H2AK121ub and H3K27me3, little is known about the effect of these modifications on chromatin accessibility, which is fundamental to understand PcG function. Here, by integrating chromatin accessibility, histone marks and expression analyses in different *Arabidopsis* PcG mutants, we show that PcG function regulates chromatin accessibility. We find that H2AK121ub is associated with a less accessible but still permissive chromatin at transcriptional regulation hotspots. Accessibility is further reduced by EMF1 acting in collaboration with PRC2 activity. Consequently, H2AK121ub/H3K27me3 marks are linked to inaccessible although responsive chromatin. In contrast, only-H3K27me3-marked chromatin is less responsive, indicating that H2AK121ub-marked hotspots are required for transcriptional responses. Nevertheless, despite the loss of PcG activities leads to increased chromatin accessibility, this is not necessarily accompanied by transcriptional activation, indicating that accessible chromatin is not always predictive of gene expression.

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O2-01. Dynamic hormone gradients and metabolic reprogramming link cell cycle regulation to wound-induced organ formation in plants

Eduardo Larriba[1], Ana Belén Sánchez-García[1], Cristina Martínez-Andújar[2], Alfonso Albacete[2,3], José Manuel Pérez-Pérez[1]

[1]Instituto de Bioingeniería, Universidad Miguel Hernández, Elche, Spain, [2]Department of Plant Nutrition, CEBAS-CSIC, Espinardo, Spain, [3]IMIDA, La Alberca, Murcia, Spain
Corresponding author: José Manuel Pérez-Pérez (jmperez@umh.es)

Plants have remarkable regenerative capacity, which allows them to survive tissue damaging after biotic and abiotic stress. Some of the key transcription factors and the hormone crosstalk involved in wound-induced organ regeneration have been extensively studied in the model plant *Arabidopsis thaliana* (Ibáñez *et al*., 2020). We performed detailed transcriptome analyses and targeted metabolomics approach during *de novo* organ formation in tomato (*Solanum lycopersicum* cv. 'Micro-Tom') hypocotyl explants, and found tissue-specific metabolic differences and divergent developmental pathways after wounding. Our results indicate that callus growth in the apical region of the hypocotyl depends on a specific metabolic switch involving the upregulation of the photorespiratory pathway and the differential regulation of photosynthesis-related genes and of the glycolysis pathway. The endogenous patterns of reactive oxygen species accumulation in the apical and basal region of the hypocotyl were dynamically regulated during the time course. Besides, the expression of core cell cycle genes was differentially regulated in the apical and the basal region of the hypocotyl explants upon wounding, suggesting separate developmental trajectories for cell reprogramming along the main growth axis. Our findings provide a useful resource for further investigation on the molecular mechanisms involved in wound-induced organ formation in tomato.

*Ibáñez S, Carneros E, Testillano PS, Pérez-Pérez JM (2020). Advances in plant regeneration: shake, rattle and roll. Plants **9**: 897.*

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O2-02. HUMAN PREFOLDIN COMPLEX MODULATES CO-TRANSCRIPTIONAL PRE-mRNA SPLICING

Sara Fontalva[a], Laura Payán-Bravo[a], Xenia Peñate[a], Ildelfonso Cases[b], José Antonio Guerrero-Martínez[c], Yerma Pareja-Sánchez[a], Yosu Odriozola-Gil[a], Esther Lara[a], Silvia Jimeno-González[c], Carles Suñé[d], Mari Cruz Muñoz-Centeno[a], José C. Reyes[c], Sebastián Chávez[a].

[a]Instituto de Biomedicina de Sevilla, Universidad de Sevilla-CSIC-Hospital Universitario V. del Rocío, Seville, Spain. [b]Centro Andaluz de Biología del Desarrollo, CSIC-Universidad Pablo de Olavide, Seville, Spain. [c]Andalusian Center of Molecular Biology and Regenerative Medicine-CABIMER, Junta de Andalucia-University of Pablo de Olavide-University of Seville-CSIC, Seville, Spain. [d]Department of Molecular Biology, Institute of Parasitology and Biomedicine "López Neyra" IPBLN-CSIC, PTS, Granada, Spain.

Prefoldin is a co-chaperone composed of six different subunits, names PFDN1-6. It is present in all eukaryotic organisms and archaea. It is known for its role in the cytoplasm, folding actin and tubulin monomers during cytoskeleton assembly. While this first discovered function is cytoplasmic, prefoldin has also been found to play nuclear roles. In our lab, we previusly described that prefoldin have a role related to transcription elongation and chromatin dynamics in *Saccharomyces cerevisiae* (1).

In human cells, we found that prefoldin perturbation generates changes in gene expression over the genome. These transcriptional alterations are more acute in long genes with a high number of introns, which is consistent with the co-transcriptional splicing defect detected in prefoldin knockdown cells.

We detected genome-wide prefoldin binding to transcribed genes, mainly accumulated in the transcription star site (TSS), following a similar distribution to RNA pol II. Furthermore, its accumulation is correlated with the negative impact of prefoldin depleted cells on gene transcription.

Lack of prefoldin also generates a decrease in the levels of Ser2-phosphorylation of the RNA polymerase II CTD domain, and it is also implicated in the recruitment of the Ser2P kinase CDK9 to the genes. Moreover, splicing factors as PRP19 and U2AF65, which are known to be co-transcriptionally recruited, were also less present in transcribed chromatin in prefoldin Knot-out cells.

Taken together, these results signify that prefoldin contributes to human gene expression by preserving RNA pol II Ser2-phosphoryltion and thereby modulating co-transcriptional splicing.

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FT2-01. Translation modulates the response to Sorafenib in hepatocellular carcinoma

Laura Contreras-Bernal[a], Jordi Muntané[b], Stefano Biffo[c], Jesús De la Cruz[a]

[a]Department of Genetic. University of Seville, Seville, Spain [b]Deparment of Medical Physiology and Biophysics, University of Seville, Seville, Spain [c]Department of Biosciences, University of Milan, Milan, Italy

Protein synthesis is an essential cellular process and a major regulation step during gene expression. Its dysregulation is frequently associated with several types of cancer, leading to excessive cell proliferation; thus, malignant cells become addicted to an elevated protein synthesis rate (1). Moreover, translation has been associated with the occurrence of resistance mechanisms in different tumours against distinct treatments. All these make the translation machinery an attractive therapeutic target against cancer. Eukaryotic Initiation factor 4E (eIF4E), component of the eIF4F complex, is a proto-oncogene essential for cap-dependent translation. It appears that eIF4E phosphorylation enhances the rate of translation of oncogenic mRNAs, thus inducing tumorigenic transformation (2). eIF4E is phosphorylated by Mnk kinases, which acts downstream of the Ras-MAPK cascade that is also overexpressed in different cancers, including the hepatocellular carcinoma (HCC) (3). Sorafenib is a multikinase inhibitor used as first-line treatment in HCC. It targets different tyrosine-kinase receptors leading to apoptosis, cell cycle arrest and reduction of angiogenesis, but the precise mechanisms for this response remain unclear (4). Our findings suggest an important role of eIF4E for the early cellular responses against Sorafenib. Thus, Sorafenib reduces the phosphorylation status of eIF4E leading to a reduction in the translation of the specific mRNAs of genes such us those coding for Cyclin D1, C-myc or VEGFA. In turn, C-myc controls the expression of different component of the translation machinery stablishing a negative-loop that deprives cells of selected proteins essential for growth and proliferation (5). These results suggest the eIF4E phosphorylation event is an attractive potential therapeutic target for HCC.

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FT2-02. EARLY-STAGE REPLICATIVE AGE CONTRIBUTES TO PROLIFERATIVE HETEROGENEITY IN *Saccharomyces cerevisiae*

Irene Delgado-Román, Lidia Delgado-Ramos, Sebastián Chávez de Diego, Mari-Cruz Muñoz-Centeno.

Instituto de Biomedicina de Sevilla (IBiS), Hospital Virgen del Rocío-CSIC-Universidad de Sevilla, Spain.
Irene Delgado-Román (idelgado2@us.es)

Clonal cell populations exhibit proliferative heterogeneity in all kind of organisms, from unicellular microorganism to human tissues. This is especially relevant in, for example, tumoral cells, where this proliferative heterogeneity leads to drug resistance (Fidler and Hart, 1982; Marusyk and Polyak, 2010; Levy et al., 2012).

Yeast cell can be trapped in regular alginate microparticles by microfluidic microencapsulation, allowing to study proliferative heterogeneity at a single-cell level. When a clonal yeast culture is encapsulated, each single cell forms a microcolony inside the capsule. We have already shown that these microcolonies differ in size as a consequence of the heterogeneity in the proliferation capacities of their founder cells (García-martínez et al, 2016). We wonder what is causing that two clonal cells in the same environmental conditions proliferate at different rates.

Our previous transcriptomic results showed that the subset of microcolonies with the lowest proliferation rate were enriched in the expression of two gene categories: respiratory metabolism and cell cycle regulation. The highest enriched gene that we found was *WHI5*, which encodes a cell cycle repressor of the G1-S transition (Breeden, 1996) and the yeast functional homologous of human Retinoblastoma (Hasan et al., 2013).

We have also observed that the smallest microcolonies are not usually founded by newborns but by cells that have already undergone more than one division. This indicates that proliferative age, since early stages, is a potential explanation for heterogeneity of cell populations.

Since very small microcolonies are enriched in *WHI5* expression and are frequently founded by non-newborn cells, we wondered whether expression of *WHI5* increases with cell division rounds in mother cells. Our results confirmed this hypothesis, suggesting a role for Whi5 in cell aging, not just in late stages, but in the first cycles of the lifespan (Pardee, 1974; Wagner et al., 2009; Neurohr et al., 2018).

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FT2-03. Rpb4 and Puf3 imprint and post-transcriptionally control the stability of a common set of mRNAs in yeast

Ana Isabel Garrido-Godino[a], Ishaan Gupta[b], Francisco Gutiérrez-Santiago[a], Ana Belén Martínez-Padilla[a], Alisa Alekseenko[c], Manuel Martín-Expósito[a], Jorge Pérez-Fernández[a], Lars M. Steimmetz[d,e,f], José Enrique Pérez-Ortín[g], Vicent Pelechano[c] and Francisco Navarro[a,h]

[a]Departamento de Biología Experimental-Genética, Universidad de Jaén , Jaén, Spain. [b]Department of Biochemical Engineering and Biotechnology, IIT Delhi , Hauz Khas, India. [c]SciLifeLab, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet , Solna, Sweden. [d]European Molecular Biology Laboratory (EMBL), Genome Biology Unit , Heidelberg, Germany. [e]Stanford Genome Technology Center, Stanford University, Palo Alto, CA, USA. [f]Department of Genetics, School of Medicine, Stanford University , Stanford, CA, USA. [g]E.R.I. Biotecmed, Facultad de Biológicas, Universitat de València , Burjassot, Spain. [h]Centro de Estudios Avanzados en Aceite de Oliva y Olivar, Universidad de Jaén , Jaén, Spain.

Gene expression involving RNA polymerase II is regulated by the concerted interplay between mRNA synthesis and degradation, crosstalk in which mRNA decay machinery and transcription machinery respectively impact transcription and mRNA stability. Rpb4, and likely dimer Rpb4/7, seem the central components of the RNA pol II governing these processes. In this work we unravel the molecular mechanisms participated by Rpb4 that mediate the posttranscriptional events regulating mRNA imprinting and stability. By RIP-Seq, we analysed genome-wide the association of Rpb4 with mRNAs and demonstrated that it targeted a large population of more than 1400 transcripts. A group of these mRNAs was also the target of the RNA binding protein, Puf3. We demonstrated that Rpb4 and Puf3 physically, genetically, and functionally interact and also affect mRNA stability, and likely the imprinting, of a common group of mRNAs. Furthermore, the Rpb4 and Puf3 association with mRNAs depends on one another. We also demonstrated, for the first time, that Puf3 associates with chromatin in an Rpb4-dependent manner. Our data also suggest that Rpb4 could be a key element of the RNA pol II that coordinates mRNA synthesis, imprinting and stability in cooperation with RBPs.

FT2-04. DETECTION OF GENOME-WIDE METHYLATION CHANGES IN THE CENTRAL NERVOUS SYSTEM OF SHEEP NATURALLY INFECTED WITH SCRAPIE

Adelaida Hernaiz[a], Arianne Sanz[a], Sara Sentre[a], Beatriz Ranera[c], Óscar López-Pérez[a,b], Pilar Zaragoza[a], Juan J. Badiola[b], Hicham Filali[b], Rosa Bolea[b], Janne M. Toivonen[a], Inmaculada Martín-Burriel[a,b]

[a]Laboratorio de Genética Bioquímica (LAGENBIO), Facultad de Veterinaria, Universidad de Zaragoza-IA2, IIS, Zaragoza, Spain, [b]Centro de Encefalopatías y Enfermedades Transmisibles Emergentes (CEETE), Facultad de Veterinaria, Universidad de Zaragoza-IA2, IIS, Zaragoza, Spain, [c]Universidad San Jorge, Zaragoza, Spain.

Scrapie is a Transmissible Spongiform Encephalopathy (TSE) that affects sheep and goats and it is considered a good natural animal model to study prion diseases. Although changes in DNA methylation occur in many neurodegenerative diseases including human prion diseases, potential DNA methylation alterations have not yet been investigated in any animal TSE models or naturally infected cases. We present here a whole genome bisulfite sequencing analysis (WGBS) from thalamus of four naturally scrapie infected sheep and four controls. All animals were female, carried the ARQ/ARQ genotype for the *PRNP* gene and were sacrificed at similar age (4 to 6 years old). No differences were found in the genomic percentage of methylated cytosines (5mC) between scrapie and control groups. Although genomes displayed similar average methylation levels, we identified 39 differentially methylated promoters (DMP) and a total of 8,907 differentially methylated regions (DMR). Enrichment analyses revealed that hypomethylated DMRs were enriched in genes involved in transmembrane transport and cell adhesion whereas hypermethylated DMRs were related with intracellular signal transduction genes. The cellular prion protein (PrP^{C}) seems to act as an important regulator of cell adhesion and membrane barrier function. Therefore, the enrichment observed in these cellular processes when PrP^{C} has lost its function after the conversion to PrP^{Sc} could be indicative of an epigenetic regulation of these mechanisms. Moreover, a validation study using qPCR has shown differences in the expression of five genes (*PCDH19*, *SNCG*, *WDR45B*, *PEX1* and *CABIN1*) that match the methylation changes observed in the genomic study. Finally, we compared genes previously described to be differentially expressed in scrapie with the set of identified DMRs finding that some of these genes also harboured DMRs.

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FT2-05. ELUCIDATING THE ROLE OF SIRTUINS IN THE CONTROL OF VIRULENCE IN *Ustilago maydis*

Blanca Navarrete, José I. Ibeas, Ramón Ramos-Barrales.

Departamento de Biología molecular e ingeniería bioquímica, Centro Andaluz de Biología del Desarrollo, Seville, Spain

Ustilago maydis is a pathogenic fungus that causes smut disease on maize. During the infection, it secretes effector proteins at different stages of the process to colonize the plant and avoid the immune response. Many transcription factors involved in controlling the correct expression pattern of these effectors have been widely described in this fungus (1), however, how chromatin modification may affect this, has not been well established. A previous study from our group, systematically characterized Class I and Class II histone deacetylases and found one of them to be involved in pathogenesis (2). Now, we are focussed on the other group of histone deacetylases, the Class III (sirtuins), and its role controlling gene expression during pathogenesis. We have identified five sirtuins homolog in the genome of *U. maydis*, named Sir2, Hst2 and Hst4 to 6. We have focused in Sir2 as it was the only one localizing in the nucleus and not lethal. Deletion of *sir2* produced an earlier filament formation when cells were grown in filament-induced medium but not in axenic conditions. Any significant difference in gene expression by RNA-seq were found in the *sir2* deleted mutant compared with the WT strain in both axenic and filamentation conditions, suggesting that Sir2 may not be repressing the filamentation program rather making its induction more difficult. In agreement with this, we demonstrated that *sir2* was highly expressed in axenic condition and degraded during filamentation. In addition, the artificial induction of *sir2* during filamentation and the pathogenic process, where Sir2 is repressed as well, caused a reduction in filamentation and a decrease in the infection symptoms observed in the plant. An RNA-seq analysis performed in this over-expressed strain during the infection process showed a lack of the induction of only a pull of the virulence genes. Intriguingly, we have observed that the protective effect for induction exerted by Sir2 is not due to the deacetylation of the most described target of this protein in other fungi, H4K16 or H3K9. Current work is focused on determine whether its target is a different histone residue or a non-histone protein.

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FT2-06. ROLE OF YEAST POL5 IN RIBOSOME BIOGENESIS

Ana Ramos-Sáenz [1,2], Daniel González-Álvarez[3], Olga Rodríguez-Galán[1,2], Alfonso Rodríguez-Gil[1], Sonia G. Gaspar [4,5], Eduardo Villalobo[1,6], Mercedes Dosil [4,5,7], Jorge Pérez-Fernández [8] and Jesús de la Cruz [1,2]

[1]Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Spain,[2]Departamento de Genética, Universidad de Sevilla, Spain,[3]Instituto de Biología Funcional y Genómica, CSIC/Universidad de Salamanca, Salamanca, Spain, [4]Centro de Investigación del Cáncer and Instituto de Biología Molecular y Celular del Cáncer, CSIC-Universidad de Salamanca, Salamanca, Spain,[5]Centro de Investigación Biomédica en Red en Cáncer (CIBERONC), CSIC-Universidad de Salamanca, Salamanca, Spain,[6]Departamento de Microbiología, Facultad de Biología, Universidad de Sevilla, Sevilla, Spain,[7]Departamento de Bioquímica y Biología Molecular, Universidad de Salamanca, Salamanca, Spain,[8]Departamento de Biología Experimental-Genética, Universidad de Jaén, Spain.

Ribosomes are the cellular organelles that perform translation. They are composed of two subunits, the large 60S and the small 40S subunits. Ribosome biogenesis is an evolutionarily conserved process. The so-called *trans*-acting factors guarantee the speed, directionality and accuracy of this process. In the yeast *Saccharomyces cerevisiae*, it has been identified more than 300 different factors, among them Pol5.

Pol5 is a nucleolar protein, which is homologous to the human tumour suppressor Myb-binding protein 1A (MYBBP1A). Mutations in MYBBP1A are normally associated with different kinds of tumours in humans, most often in kidneys. We have shown that Pol5 is a ribosome assembly factor required for the production of 60S ribosomal subunits. Consistently, we have demonstrated that Pol5 participates in the processing of 27SB pre-rRNA to mature 25S rRNA and suggested that it could have a role in the correct folding of the domain III of 25S rRNA.

Currently, we are studying whether or not the function of Pol5 has been conserved during evolution by analysing MYBBP1A. This protein also localises in the nucleolus and it is supposed to have a role during ribosome synthesis. The heterologous expression of MYBBP1A in yeast leads to a dominant negative phenotype that seems to be related to an interference with the 60S ribosomal subunit biogenesis.

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P2-01. Arabidopsis CAX-INTERACTING PROTEIN4 (CXIP4) may play a role in RNA metabolism

Uri Aceituno-Valenzuela, Raquel Sarmiento-Mañús, Alejandro Ruiz-Bayón, Rosa Micol-Ponce, Sara Fontcuberta-Cervera and María Rosa Ponce

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

MORPHOLOGY OF AGO1-52 SUPPRESSED 2 (MAS2), the Arabidopsis ortholog of metazoan NF-kappa B Activating Protein, participates in the regulation of splicing fidelity and ribosome biogenesis. To better understand MAS2 function, we performed a yeast two-hybrid assay, identifying CAX-INTERACTING PROTEIN4 (CXIP4) as its most represented interactor (1). CXIP4 is a plant-specific protein and contains a zinc knuckle motif, a CCHC-type zinc finger, which is found in proteins involved in RNA metabolism (2). SREK1-interacting protein 1 (SREK1IP1), of unknown function, is the likely human ortholog of Arabidopsis CXIP4; these two proteins only share extensive sequence similarity in their N-terminal regions, which contain the zinc knuckle (2).

We obtained two recessive, insertional alleles of *CXIP4*, which we named *cxip4-1* and *cxip4-2*. The *cxip4-1* seedlings exhibited early post-embryonic lethality, but the *cxip4-2* plants were viable and fertile, and showed a pleiotropic phenotype, including slow growth and late flowering. Leaves of *cxip4-2* plants were pointed, a common trait among mutants affected in genes encoding ribosome biogenesis factors and ribosomal proteins. Our RT-PCR analyses suggest that *cxip4-1* and *cxip4-2* are null and hypomorphic alleles of *CXIP4*, respectively. A transgene carrying a wild-type copy of the *CXIP4* gene fully rescued the lethality of *cxip4-1* homozygous plants. We found that CXIP4 predominantly localizes to the nucleoplasm, but it is also present within the nucleolus.

The *cxip4-2* mutant exhibits nuclear accumulation of polyadenylated RNAs, suggesting that CXIP4 is required for a proper nuclear RNA maturation, degradation, or export. RNA gel blot analyses showed that 18S rRNA precursors overaccumulate in *cxip4-1*, which also occurs in mutants carrying alleles of genes encoding other MAS2 interactors. We are obtaining double mutant combinations of *cxip4-2* and alleles of genes involved in RNA export and maturation to assess if these genes are functionally related.

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P2-02. NR2E3 transcription factor and photoreceptor fate: identification of gene regulatory networks causing retinal remodeling in NR2E3-associated diseases

Izarbe Aísa-Marin[1,2], Quirze Rovira[3], Noelia Díaz[3], Juan M. Vaquerizas[3,4], Gemma Marfany[1,2].

[1] *Department of Genetics, Microbiology and Statistics, Avda. Diagonal 643, Universitat de Barcelona, Barcelona 08028, Spain*
[2] *CIBERER, ISCIII, Universitat de Barcelona, Barcelona, Spain*
[3] *Max-Planck-Institute for Molecular Biomedicine, Röntgenstrasse 20, 48149, Münster, Germany.*
[4] *MRC London Institute of Medical Sciences, Institute of Clinical Sciences, Faculty of Medicine, Imperial College London, Du Cane Road, London W12 0NN, UK.*
Corresponding author: Gemma Marfany (gmarfany@ub.edu)

Inherited retinal dystrophies (IRDs) are a group of diseases associated with mutations in more than 330 genes. NR2E3 encodes an orphan nuclear receptor with a dual function as transcriptional activator and repressor, necessary for retinal development and homeostasis (1, 2, 3). Mutations in *NR2E3* cause two different retinal diseases: Enhanced S-cone Syndrome and Retinitis Pigmentosa (4, 5, 6, 7). However, there is no clear phenotype-genotype correlation for most NR2E3 mutations. This gene produces a large protein isoform encoded in 8 exons. In addition, we found a previously unreported isoform of 7 exons. We dissected the *Nr2e3* function by performing CRISPR/Cas9 gene editing of the last exon and generated two different mouse models (8). Depending on the deleted domain, these models show two different phenotypes that correspond with the two known diseases caused by mutations in *NR2E3*. We performed single cell RNA-seq in our models to further investigate the gene regulatory networks guiding differentiation of rods and cone photoreceptors in our two phenotypes. Our results provide insight into the molecular mechanisms of the two rare diseases caused by mutations in *NR2E3* and set the basis for further epigenetic studies on the NR2E3 network imbalances that give rise to IRDs.

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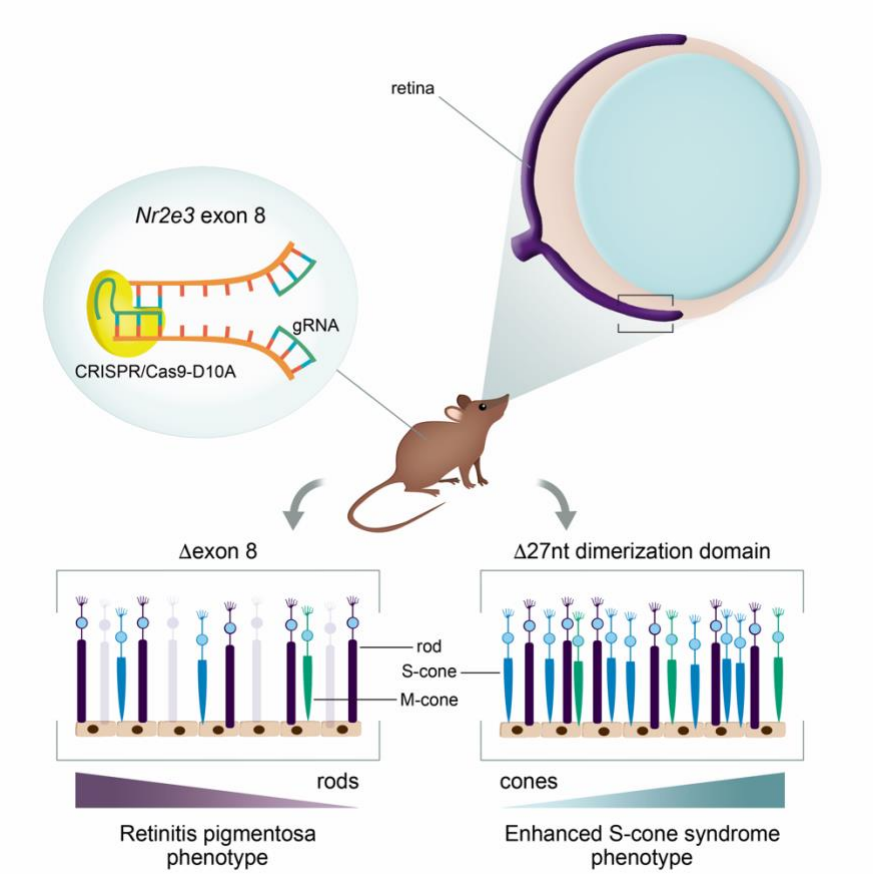
Graphical abstract



P2-03. Differential gene expression in *Chorthippus parallelus* (Orthoptera: Acrididae) related to *Wolbachia* infection

Mónica Aquilino[a], Lola Llorente[a], Patricia Jiménez-Florido[b], Óscar Herrero[a], David Buckley[b], José L. Bella[b], Rosario Planelló[a].

[a] Grupo de Biología y Toxicología Ambiental. Facultad de Ciencias. Universidad Nacional de Educación a Distancia (UNED), Madrid, Spain. [b] Departamento de Biología (Genética), Facultad de Ciencias, Universidad Autónoma de Madrid, Madrid, Spain.
Corresponding author: Mónica Aquilino (maquilino@ccia.uned.es)

Two subspecies of the grasshopper *Chorthippus parallelus* (Orthoptera: Acrididae), the Iberian endemism *C. p. erythropus* and *C. p. parallelus,* which is widely distributed throughout the rest of Europe, differ in morphological behavioural, mitochondrial, nuclear and chromosomal characters, but also in the strains of the maternally transmitted bacterial endosymbiont *Wolbachia* infecting them. The distribution of both subspecies overlaps in the Pyrenees where they form a stable hybrid zone (HZ), so representing an appropriate system to identify 'key genes' that actually maintain genetic boundaries between emerging species.

Here we show *Wolbachia* may be inducing the expression of some major genes related with development, reproduction, and other important metabolic pathways, in addition to the genes related to the reproductive barrier to which it contributes in this Pyrenean HZ. In summary, we reveal some molecular biomarkers that show the physiological responses in *C. parallelus* individuals infected by *Wolbachia*, with particular attention to hormonal pathway, immune, and stress cell responses. The expression of reporter genes in the ovaries and testicles of infected and uninfected adults of both sexes was performed by means of quantitative real-time PCR. Gonads were chosen since they are the main target of *Wolbachia* infection. Our initial, promising results show new sensitive biomarkers suitable for the study of the reproductive barrier that *Wolbachia* induces in the hybrid zone. Different transcriptional effects between sexes for all the analysed biomarkers in infected and non-infected adults are evidenced.

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P2-04. IDENTIFICATION OF THE CHANGES IN GENE EXPRESSION LEVELS THAT ARE ASSOCIATED WITH THE OUTBREAK STATE OF PEST LOCUSTS

Mohammed Bakkali

Departamento de Genética, Facultad de Ciencias,Universidad de Granada, Fuentenueva S/N,18002, Granada, Spain (mbakkali@ugr.es)

Outbreaks of pest locust populations is a recurrent problem and an existential threat to human, livestock as well as plant and animal populations in large parts of Africa and Asia. They also cause important economic lose in southern Europe, parts of south and north America and Australia.

The locust biology changes significantly between the normal (solitarious) and the outbreak (gregarious) states. The underlying cause of these important changes are gene expression, not genomic, differences due to epigenetic and gene expression regulation changes in response to the changes in population and life conditions.

Here I compare the global gene expression differences between locusts in non-outbreak and in outbreak states and I identify the genes whose changes of expression level cause, maintain or are caused by the shift of the locusts between these two states of the pest locusts.

The results are discussed at the light of the current knowledge on functional genetics (molecule functions and interactions) as well as the locust biology, including the characteristics of their population outbreaks.

P2-05. The Arabidopsis ribosomal proteins RPS24A and RPS24B are ribosome biogenesis factors with a role in 18S rRNA maturation

Adrián Cabezas-Fuster, Alejandro Selva-Mora, Raquel Sarmiento-Mañús, Rosa Micol-Ponce and María Rosa Ponce

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

MORPHOLOGY OF ARGONAUTE1-52 SUPRESSED 2 (MAS2), the Arabidopsis ortholog of animal NF-kappa B Activating Protein (NKAP), is assumed to be involved in splicing and ribosome biogenesis. In a yeast two-hybrid assay, we isolated 14 interactors of MAS2, including RPS24B, a ribosomal protein of the small subunit (40S) of the cytoplasmic ribosome (1). RPS24B and its paralog RPS24A share 95% sequence identity and are likely to be functionally redundant. Human RPS24 acts both as a ribosomal protein and as a ribosome biogenesis factor (RBF) in 18S rRNA maturation (2). To ascertain if Arabidopsis RPS24A and RPS24B act as RBFs, we analysed the processing of the 45S pre-rRNA in mutants that have mutations induced by T-DNA (*rps24b-2* and *rps24a-1*) and EMS (*apiculata6* [*api6*]). The *api6* mutation is a G to A transition that damages the splice acceptor site (3′SS) of the first intron of *RPS24B*. We found that the *rps24b-2, rps24a-1*, and *api6* mutants show strong overaccumulation of the P-A3 fragment, an 18S rRNA precursor, which indicates that RPS24A and RPS24B act as RBFs.

To examine the subcellular localization of RPS24B, we constructed a *$35S_{pro}$:RPS24B:GFP* transgene and transformed it into Col-0. Examination of transgenic cells revealed that RPS24B localizes mainly to the nucleolus, as expected from an RBF, but also to the cytoplasm, as expected from a structural component of the ribosome. We observed synergistic morphological and molecular phenotypes in the double mutant combinations of *rps24b-2* with mutations in genes encoding RBFs that participate in different steps of 18S rRNA maturation. Together, these results suggest that RPS24A and RPS24B are not fully redundant and they may act in both ribosome biogenesis and in mature ribosome function.

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Research in the laboratory of M.R.P. was supported by grants from the Ministerio de Ciencia e Innovación of Spain (BIO2017-89728-R) and the Generalitat Valenciana (PROMETEO/2019/117). R.M.P. held a postdoctoral fellowship from the Generalitat Valenciana (APOSTD/2019/001).

P2-06. NOVEL MUTANTS INVOLVED IN NERVOUS AND TRACHEAL DEVELOPMENTIN *DROSOPHILA MELANOGASTER*

Núria Corbella[1], Irene Carod[1] and Sofia J. Araújo[1]

[1]*Department of Genetics, Microbiology and Statistics, University of Barcelona, Barcelona, Spain*

The *Drosophila melanogaster* embryonic tracheal system is formed by a network of interconnected epithelial tubes that branch inside the body to allow the distribution of oxygen to all tissues. We focused on the tracheal terminal cells (TCs), which are specialized cells that form unicellular branches with a cytoplasmatic fine tube called subcellular lumen. This lumen is produced by an extensive reorganization of the cytoskeleton and the growth of a new membrane which invaginates from the adjacent stalk cell while the TC is elongating. Aimed at finding novel genes involved in subcellular lumen formation, we analysed mutants from a screen with phenotypes in tracheal and nervous system development. From this, we selected one mutant that displayed a phenotype in subcellular lumen formation and branching we named *kid kazoom* (*kkz*). This mutant displayed extra subcellular lumina (ESL) in the TCs at embryonic stages. We mapped the mutation in kkz to chromosome 2 with the Bloomington Deficiency Kit. We confirmed the position of the mutation with a complementation test and identified a previously unidentified gene in Drosophila, whose human ortholog is part of a complex involved in ribosome biogenesis and connected with a group of diseases called ribosomopathies.

P2-07. Early role of ribosomal protein eL15 during the assembly of 60S ribosomal subunits in *Saccharomyces cerevisiae*

José Fernández-Fernández[1,2], Jorge Pérez-Fernández[3] and Jesús de la Cruz[1,2]

[1]Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío / CSIC / Universidad de Sevilla, Spain, [2] Departamento de Genética, Universidad de Sevilla, Spain, [3] Departamento de Biología Experimental-Genética, Universidad de Jaén, Spain.
Corresponding author: Jesús de la Cruz (jdlcd@us.es)

Ribosome synthesis is a multistep process that includes the concomitant processing of the precursors of ribosomal RNAs and the assembly of ribosomal proteins. In eukaryotes, this complex process takes place successively in the nucleolus, the nucleoplasm and the cytoplasm. In the yeast *Saccharomyces cerevisiae*, it involves about 300 *trans*-acting factors and the 79 ribosomal proteins. The role of most ribosomal proteins in the biogenesis of each ribosomal subunit has been analysed and the timing of their *in vivo* assembly has been investigated. However, few ribosomal proteins still await functional characterization. Herein, we have analysed the contribution of ribosomal protein eL15 to ribosome biogenesis. We show that depletion of eL15 results in a severe shortage of 60S ribosomal subunits. Northern blotting, primer extension and pulse chase analyses indicate that processing of 27SA to 27SB pre-rRNAs as well as processing of 27SB to mature rRNAs is impaired upon the depletion of eL15. As a result, export of pre-60S particles from the nucleus to the cytoplasm is blocked. These phenotypes most likely appear as the direct consequence of the reduced pre-60S particle association not only of eL15 upon its depletion but also of a subset of neighbouring ribosomal proteins (e.g. eL13, eL36) and *trans*-acting factors either involved in the processing of 27SB pre-rRNA (e.g. Spb4, Nug1, Erb1, Spb1 or Has1) or $27SA_3$ pre-rRNA (e.g. Erb1, Nop7). These factors have likely not a direct role in the pre-rRNA processing reactions but a structural role in the formation of nucleolar pre-60S intermediates.

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P2-08. Potential conservation of the epitranscriptomic activity of human NKAP in its Arabidopsis ortholog MAS2

Sara Fontcuberta-Cervera, Rubén Delicado-Mateo, Sofía Riera-Vidal and María Rosa Ponce

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

We performed a screen for EMS-induced second-site mutations suppressing the morphological phenotype of Arabidopsis *argonaute1-52* (*ago1-52*), a hypomorphic and viable allele of *AGO1*, which encodes a key component of RNA-induced silencing complexes. Among the extragenic suppressors identified, nine were alleles of *MORPHOLOGY OF AGO1-52 SUPPRESSED 2* (*MAS2*) (1). *MAS2* is an essential gene that encodes the likely Arabidopsis ortholog of animal NF-kappa B Activating Proteins (NKAPs) (2).

Human NKAP is a multifunctional factor that has been described as a reader of the N^6-methyladenosine (m^6A) epitranscriptomic mark on primary microRNAs (pri-miRNAs) (3). Transgenic *amiR-MAS2* plants, which express artificial miRNAs that partially silence *MAS2*, share some morphological phenotypic traits with mutants in genes encoding core components of the epitranscriptomic machinery, such as MRNA ADENOSINE METHYLASE (MTA). MTA deposits m^6A on mRNAs and pri-miRNAs, to modulate miRNA biogenesis in Arabidopsis (4). Nuclear export of mature mRNAs depends on several factors, including the deposition (writing) and recognition (reading) of m^6A marks; we found that *amiR-MAS2* plants overaccumulate polyadenylated RNAs within the nucleus. Taken together, our results suggest an epitranscriptomic role for MAS2.

For a better understanding of the functions of MAS2, we are generating translational fusions, encoding wild-type MAS2 and several truncated versions fused to different fluorescent proteins. In addition, we are attempting to obtain hypomorphic *mas2* alleles by means of CRISPR/Cas9 editing, targeting the 5′ and 3′ UTRs of *MAS2*.

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P2-09. Genome-wide methylation analysis in *Abies alba*: outcomes of a drought-sensitive tree under a climate change scenario.

Isabel García-García[a], Belén Méndez-Cea[a], David Martín-Gálvez[b], José Ignacio Seco[c], Juan Carlos Linares[c] & Francisco Javier Gallego[a]

[a]Dpto. de Genética, Fisiología y Microbiología, UD de Genética, Facultad de CC Biológicas, UCM, Madrid, Spain.
[b]Dpto. de Biodiversidad, Ecología y Evolución, UD de Zoología, Facultad de CC Biológicas, UCM, Madrid, Spain.
[c]Dpto. de Sistemas Físicos, Químicos y Naturales, UPO, Sevilla, Spain.

A climate change-induced decline has been reported in several silver fir (*Abies alba* Mill.) populations across Europe over the last decades. Forest tree species, being sessile organisms with long generation times, are highly vulnerable to climatic fluctuations such as prolonged droughts or heat waves. Hence, their adaptation to a local changing environment may rely on epigenetic modifications when genetic frequencies are not able to shift fast enough. Nonetheless, our current knowledge about climate change-induced shifts in the epigenomes of forest trees is scarce, even though these modifications are likely to be of great importance for their survival. The current lack of knowledge on this field is mainly related to the difficulties that arise when working with these species, such as huge genome sizes, often poorly annotated, and a limited availability of bioinformatic tools to analyse the resulting data. In spite of this challenging framework, we report here the preliminary results obtained from natural populations of *A. alba* showing signs of decline. By analysing whole-genome bisulfite sequencing (WGBS) data, we were able to identify differentially methylated regions (DMR) between healthy and declining trees in forests affected by drought-induced dieback.

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P2-10. CHARACTERIZATION OF SEED DEVELOPMENT IN FAST FLOWERING MAIZE

David Hueros, Julio Perez, Joaquín Royo, Gregorio Hueros

Departamento de Biomedicina y Biotecnología, Unidad de Genética, Universidad de Alcalá, España

Our group uses the development, differentiation, and function of the maize endosperm transfer cell layer (TCL) as an experimental system. This tissue plays two roles: it facilitates the agronomically important grain filling process in maize and other cereals, and it has a crucial role as a seed-protective barrier against pathogen invasion from the mother plant. Transfer cells start to differentiate as a modified epithelium at the base of the developing endosperm 4-6 days after pollination (DAP); the goal of our studies is to analyze all physiological, cellular, and regulatory processes occurring between 4 and 16 DAP, after which the TCL becomes fully differentiated.

It is of capital importance for us to have a continuous supply of developing seeds of maize to carry out our genetic and physiological studies. Unfortunately, the size of the plants (c.a. 2 m tall) and the length of the biological cycle (c.a. 90 days from germination to flowering) restricts the possibilities of growing maize in culture chambers.

The fast flowering mini maize (FFMM) lines were synthesized in 2016 (1) through the cross of 4 early flowering parental lines, followed by 11 generations of selection for earliness, small footprint, and high yield of pollen and seeds. FFMM plants are 1 m tall, grow in 3 L pots, and flower in 30 days. In addition, the authors sequenced the genome of the line FFMM-A. FFMM lines could thus be a reliable source of developing endosperms all through the year.

In this work, we have studied the development of FFMM-A kernels using a combination of histological analyses and QRT-PCR assays with marker genes for various endosperm compartments. The aim was to set up a reference standard in terms of kernel structure and developmental dynamics. Our results indicate that, contrary to the accelerated development that characterizes the vegetative phase, the reproductive development phase of FFMM-A is assimilable to that of the standard varieties. However, the histological analyses revealed some peculiarities regarding the structure of the aleurone and transfer cell layers. We also detected a high frequency of alterations in the structure and spatial disposition of the embryos.

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P2-11. Differential recruitment to chromatin may explain the unequal functional redundancy of the epigenetic factors ICU11 and CP2 in Arabidopsis

Lucía Juan-Vicente[a], Riad Nadi[a], Yolanda Fernández[b], Vicente Rubio[b] and José Luis Micol[a]

[a]*Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.*

[b]*Department of Plant Molecular Genetics, Centro Nacional de Biotecnología (CNB-CSIC), 28049 Madrid, Spain.*

The Arabidopsis INCURVATA11 (ICU11) and CUPULIFORMIS2 (CP2) paralogs are novel components of the epigenetic machinery and belong to the 2-oxoglutarate and Fe(II)-dependent dioxygenase superfamily (1, 2). Although loss-of-function alleles of *ICU11* cause a mild morphological phenotype, and the *cp2* mutants are phenotypically indistinguishable from wild-type plants, the *icu11 cp2* double mutants exhibit postembryonic seedling lethality. The $35S_{pro}$*:CP2* transgene, but not $CP2_{pro}$*:CP2*, fully complements the mutant phenotype of *icu11-1*. Taken together, these observations demonstrate the unequal genetic redundancy of *ICU11* and *CP2*; however, the mechanism of this redundancy remains unclear.

To gain insight into the functions of ICU11 and CP2, we performed a tandem affinity purification (TAP) assay to identify their interactors. A recent study (3) showed that ICU11 demethylates H3K36me3 epigenetic repressive marks, and used a co-immunoprecipitation assay to show that ICU11 physically interacts with core and accessory proteins of the Polycomb repressive complex 2 (PRC2). In agreement with these results, the ICU11 interactors that we found included the PRC2 core or accessory proteins EMBRYONIC FLOWER 1 (EMF1), EMF2, MULTICOPY SUPPRESSOR OF IRA1 (MSI1), FERTILIZATION INDEPENDENT ENDOSPERM (FIE), SWINGER (SWN), TELOMERE REPEAT BINDING FACTOR 1 (TRB1), TRB2, and TRB3. CP2 did not interact with these proteins, but it did with other members of the TRB family, such as TRB4 and TRB5. We are conducting bimolecular fluorescence complementation assays to confirm these interactions *in vivo*.

Some of the remaining ICU11 and CP2 interactors found in our TAP assays are nuclear proteins that are known transcription factors or have a plant homeodomain. These distinct sets of interactors may differentially recruit ICU11 or CP2 to chromatin, and this differential recruitment may explain the unequal functional redundancy of ICU11 and CP2.

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P2-12. TOWARDS A COMPREHENSIVE PIPELINE TO IDENTIFY AND FUNCTIONALLY ANNOTATE LONG NONCODING RNAs (lncRNAs) IN PLANTS

Eduardo Larriba[1], José Manuel Pérez-Pérez[1]

[1]Instituto de Bioingeniería, Universidad Miguel Hernández, Elche, Spain
Corresponding author: Eduardo Larriba (elarriba@umh.es)

By definition, non-coding RNAs (ncRNAs) include any RNAs without the potential to encode proteins and that might play a key role in gene regulation in eukaryotes. A simplified classification of ncRNAs is based on their size, distinguishing two groups depending on whether their transcripts are less than 200 nt (small RNAs) or larger (long non-coding RNAs; lncRNAs). Some lncRNAs have been found to function as epigenetic regulators of gene expression in animals and plants, being able to act both in *cis* and in *trans*, interacting with DNA, RNA and proteins. Likewise, many of the functions of the lncRNAs are determined by its genomic origin, sub-classifying this group into intergenic lncRNAs, intronic lncRNAs, sense, antisense and natural antisense transcripts of protein coding genes (Wierzbicki *et al*., 2021). Although a large number of lncRNAs have been predicted and deposited in different databases, such as CANTATAdb 2.0, GreeNC, PLncDBV2.0, and NONCODEV6, there is a lack of comparative studies for lncRNAs identified by different transcriptional and computational methods in Solanaceae species. Hence, the systematic study of lncRNA expression and regulation in cultivated tomato (*Solanum lycopersicum* L.) has only just started to develop. To fill this gap, we have developed a bioinformatics pipeline for the annotation and functional classification on lncRNA in tomato during wound-induced *de novo* organ formation (Larriba *et al*., 2021). This will help future investigation of lncRNA function, especially for the dissection of the molecular mechanisms involved in tissue reprogramming.

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Wierzbicki AT, Blevins T, Swiezewski S (2021). Long noncoding RNAs in plants. Annu Rev Plant Biol doi: 10.1146/annurev-arplant-093020-035446.

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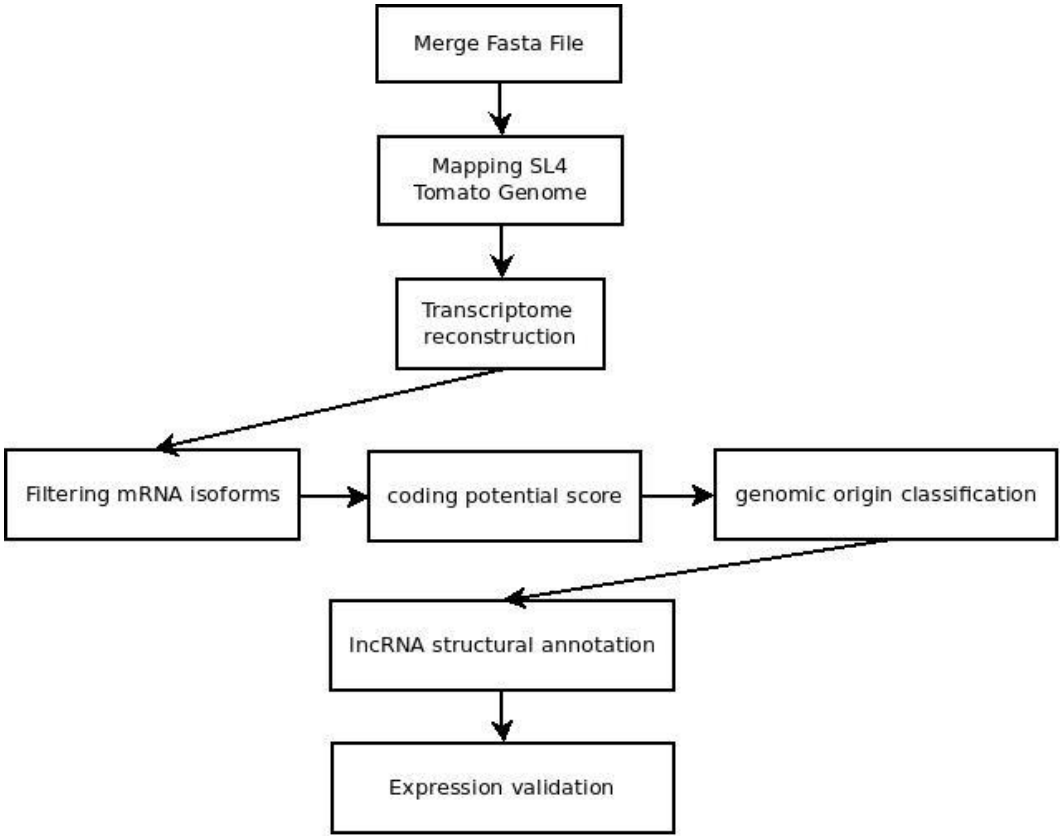
Graphical abstract:

Guidelines



P2-13. TOXIC EFFECTS OF THE PLANT SECONDARY METABOLITE 2-DODECANONE IN THE INSECT MODEL SPECIES *NASONIA VITRIPENNIS*

Lola Llorente[a*], Mónica Aquilino[a], Laureen Beaugeard[b], Óscar Herrero[a], Maria Annick[c], David Siaussat[c], Charlotte Lecureuil[b], Rosario Planelló[a]

[a]Grupo de Biología y Toxicología Ambiental, Facultad de Ciencias, Universidad Nacional de Educación a Distancia (UNED), Madrid, Spain. [b]Institut de Recherche sur la Biologie de l'Insecte (IRBI), CNRS-Université de Tours, Tours, France. [c]Institute of Ecology and Environmental Sciences of Paris, Department of Sensory Ecology, Sorbonne Université, Campus Pierre et Marie Curie, Paris, France.
[*]E-mail contact: lolallorente@ccia.uned.es

Plants synthesise a multitude of secondary metabolites that are a deterrent to pathogens and phytophagous insects. The toxicity exerted by some plant secondary metabolites means that many of these compounds could be potentially used as a "natural and healthy" alternative to the use of synthetic pesticides that can be commercially used for pest management. Methyl ketones are a group of defensive metabolites -commonly found on leaves of tomato plants- constituting up to 90% of the total of secondary metabolites secreted by glandular trichomes. However, works focused on elucidating the toxicity and the mechanisms of action of such defensive extracts are hitherto very scarce.

In the present study, the toxicity of 2-dodecanone (CAS 6175-49-1) was investigated in *Nasonia vitripennis*, a good model representative of natural enemies of pest insects. The effects of acute 48-h exposure to sublethal doses (5 µg/L and 500 µg/L) on white pupae were studied at the molecular level by analysing changes in the transcriptional activity of genes related to the endocrine system (*EcR, usp, E78, HR4, HR38* and *dronc*), detoxification pathways (*GPx* and *GST1*), and homeostasis functions (*I(2)efl*).

Our results showed that 2-dodecanone caused significant alterations in the transcriptional activity of most of the genes tested in many of the selected biomarkers. Significant induction in hormone related-genes was detected after acute exposures, in spite that no significant differences were detected in ecdysteroids levels. In contrast, the gene *I(2)efl*, which encodes a member of the heat shock 20 protein (HSP20) family, was repressed dramatically under this condition.

This study provides novel and interesting results on the toxic effects of an isolated secondary metabolite naturally present in plants in *N. vitripennis* and, highlights the potential suitability of this organism for deep into the molecular effects of plant defences in insects. These findings provide new insights into the insecticidal efficacy of 2-dodecanone, which might be explored under field conditions for plant protection and pest management to reduce reliance on synthetic pesticides.

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P2-14. Genomic Basis of Drosophila Social Memory

Francisco A Martin[a], Beatriz Gil-Marti[a], Celia G Barredo[a] Carmen Rodriguez-de Navas[a], Abhijit Das[b], Enrique Turiegano[c], Andrea H Brand[d]

[a]Cajal Institute, CSIC, Madrid, Spain, [b] School of Bioscience, Indian Institute of Technology, Kharagpury, India [c]Auotnomous University of Madrid, Spain, [d] The Gurdon Institute and Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK

The gregarism and social behavior of *Drosophila melanogaster* have been largely demonstrated. For most animals, which include flies, it is known that isolation affects behaviors such as sleep or aggressiveness. This raises the question of whether or not there is a social memory, specifically generated when individuals of the same species interact within a group. In this work we show that co-habitation increases the number of activated Kenyon Cells in the mushroom body (MB) when compared to isolated animals. As a consequence, isolated and grouped housed flies have different aggregation behavior, a phenotype dependent on Drosophila Adenylate Cyclase gene, rutabaga. In order to identify socially-activated and social memory genes we performed Targeted DamID in the MB. We show that isolation and group housing produce different transcriptional and epigenetic changes in Kenyon Cells. These data trongly suggest the existence of a memory generated by social life that affects to the final behavioral output. Besides, we also identify a epigenomic signature that may be typical of social interaction independently of memory formation. In summary, our work may shed light on genetic and epigenetic basis of social behavior, hopefully evolutionary-conserved.

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P2-15. FUS, AN ALS-ASSOCIATED PROTEIN, IS A COMPONENT OF THE CELLULAR RESPONSE TO TRANSCRIPTIONAL STRESS

M. Isabel Martinez-Macias[a,b], and Keith W. Caldecott[a]

[a]Genome Damage and Stability Centre, University of Sussex, UK, [b]Maimónides Biomedical Research Institute of Córdoba/University of Córdoba, Spain

FUS (Fused in Sarcoma). is a nuclear RNA/DNA binding protein that plays a key role in multiple steps of RNA metabolism and the DNA Damage Response. Autosomal dominant mutations in FUS have been associated with both familial and sporadic cases of Amyotrophic lateral sclerosis (ALS), the most common adult-onset motor neuron disease. It has been proposed that ALS mutations cause pathological changes in FUS-regulated gene expression and RNA processing, due either to loss of normal FUS function, toxic gain of function, or both. However, the nature of the endogenous sources of DNA damage that might trigger a requirement for FUS and/or other RNA-processing factors is unknown. Of particular threat to neural maintenance and function is DNA damage induced by topoisomerases, a class of enzymes that remove torsional stress from DNA by creation of transient DNA strand break. Here, we showed that FUS is a component of the cellular response to topoisomerase I (TOP1)-induced DNA breakage. FUS relocalised from nucleoplasm to sites of nucleolar rRNA synthesis in response to RNA polymerase II transcriptional stress induced by abortive TOP1 DNA breakage. This relocalisation was rapid and dynamic, reversing following the removal of TOP1-induced breaks and coinciding with the recovery of global transcription. The molecular role of this response is unclear, but we propose that FUS moves from sites of stalled RNA polymerase II to sites of RNA polymerase I activity either to regulate pre-mRNA synthesis and/or processing during transcriptional stress, or to modulate some yet unidentified aspect of rRNA biogenesis. Finally, we found that HeLa cells and ALS patient fibroblasts expressing mutant FUS are hypersensitive to TOP1-induced DNA breakage, highlighting the possible relevance of our findings to ALS disease pathology.

P2-16. Dominant alleles of Arabidopsis *PRE-RNA PROCESSING FACTOR 8* increase splicing fidelity

Adrián Cabezas-Fuster, Rosa Micol-Ponce, Sara Fontcuberta-Cervera and María Rosa Ponce

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

ARGONAUTE1 (AGO1) is a key factor in Arabidopsis microRNA pathways. The *ago1-52* mutant allele carries a G→A base change that creates a novel 3′ splice site (3′SS) in its last intron; this novel 3′SS is preferentially, but not exclusively, used by the splicing machinery. We performed a genetic screen for EMS-induced suppressors of the morphological phenotype of *ago1-52*, and isolated 21 extragenic suppressors, 6 of which were allelic and named *morphology of ago1-52 suppressed 5-1* (*mas5-1*) to *mas5-6* (1). These *mas5* mutations turned out to be dominant alleles of the *PRE-MRNA PROCESSING FACTOR 8* (*PRP8*) gene, which encodes the central component of the spliceosome (2). The *mas5* alleles are informational suppressors of *ago1-52*, increasing the ratio of genuine versus novel 3′SS use by the spliceosome. The *mas5-1* mutation also partially suppresses the missplicing of *incurvata13-1* (*icu13-1*), due the occurrence and use of a novel 5′SS. *icu13-1* is an allele of the *AUXIN RESISTANT 6* (*AXR6*) gene, which encodes CULLIN, a key player in protein degradation by ubiquitination (3). In contrast to its effect on *ago1-52* and *icu13-1*, *mas5-1* does not suppress the missplicing caused by mutations that eliminate genuine 5′ or 3′SSs, instead of creating novel ones.

We observed increased nuclear retention of polyadenylated RNA in the *prp8-7* mutant, which is probably caused by accumulation of aberrantly spliced mRNAs. Misspliced and other aberrant mRNAs with premature stop codons are targeted by the nonsense-mediated mRNA decay (NMD) pathway, which may mask splicing defects caused by mutated spliceosome components. We analyzed double mutant combinations of alleles of *PRP8* and *UP-FRAMESHIFT1* (*UPF1*), a core component of the NMD pathway; these double mutants exhibited synergistic morphological phenotypes, which may be caused by an enhancement of splicing defects, as it has been shown in yeast. These results also suggest that inactivation of the NMD pathway may be required for a better understanding of spliceosome function *in vivo*.

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P2-17. EXPERIMENTAL STUDY OF miRNAs INVOLVED IN GLIOBLASTOMA TUMORS

Lisandra Muñoz[1], Teresa San Miguel [1], Javier Megías [1], Concepción López [1], Silvia Calabuig[1], Daniel Monleón[1], José Manuel Morales[1], Miguel Cerdá[1].

[1]Department of Pathology, University of Valencia, Valencia, Spain. Corresponding author: Lisandra Muñoz (lisandramh@gmail.com)

Glioblastoma is the most frequent and aggressive of all gliomas because of its highly invasive and infiltrating growth properties. The epidermal growth factor receptor gene (EGFR) is often amplified, overexpressed and/or mutated in primary glioblastoma. Previous research has revealed that aberrant miRNAs expression was associated with the pathogenesis of this tumor. The miR-200 and miR-138 family is involved in the progression of different types of epithelial neoplasms, plays an important role in epithelial-mesenchymal transition (EMT) regulatory activity in which these miRNAs directly targets, and ZEB1, HIF1, VEGH. However, little is known about the potential interrelationships between *EGFR*, miR-200 and the induction of EMT processes and miR-138 is involved in angiogenesis process in hypoxia condition. We generated an experimental model of transfection with a miR-200c and miR-138 mimic, inhibitor and siRNA targeting EGFR, in different cell line and in primary glioblastoma cultures with different *EGFR* amplification levels. Our data show a significant relationship between *EGFR* amplification and miR-200c and miR-138 expression in primary glioblastoma cultures and cell line. The glioblastoma cell culture with the highest *EGFR* amplification level showed the lowest levels of miR-200c expression. This study provide evidence that miR-200c overexpression inhibits ZEB1 independently of *EGFR* amplification status, and silencing EGFR in cell cultures led to miR-200c upregulation and ZEB1 downregulation in transfected cell cultures. Likewise, miR-200c upregulation inhibits cell migration especially when the *EGFR* is not amplified, suggesting that upregulating miR-200c may serve as a novel therapeutic strategy for glioblastoma treatment.

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P2-18. The ICU11–CP2 epigenetic module and some Polycomb Repressive Complex 2 components have similar effects on the Arabidopsis transcriptomic landscape

Riad Nadi, Lucía Juan-Vicente and José Luis Micol

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

The nuclear protein INCURVATA11 (ICU11) is a component of the Arabidopsis epigenetic machinery that functions in H3K36me3 demethylation and physically interacts with Polycomb Repressive Complex 2 (PRC2) core and accessory proteins (1-3). Null alleles of *ICU11* cause a pleiotropic but relatively mild morphological phenotype: leaf hyponasty, early flowering, and reduced fertility. Mutants carrying null alleles of *CUPULIFORMIS2* (*CP2*)—the closest paralog of *ICU11*—are barely distinguishable from wild type. However, *icu11 cp2* double mutants as well as genetic combinations of *icu11* alleles with alleles of several genes encoding components of the epigenetic machinery exhibit a synergistic postembryonic lethal phenotype, reminiscent of that of strong *embryonic flower 1* (*emf1)* and *emf2* mutants (4).

We subjected to RNA-seq analysis *cp2-1* and *icu11-5* seedlings*, icu11-5 cp2-1* and *emf2-3* embryonic flowers, and inflorescences of the Col-0 wild type. Only a few genes were misexpressed in the *cp2-1* mutant compared with *icu11-5*; by contrast, we found almost 5,000 genes misregulated in the *icu11-5 cp2-1* double mutant, compared with the *cp2-1* and *icu11-5* single mutants. We also compared *icu11-5 cp2-1* double mutant and *emf2-3* single mutant embryonic flowers, and found similar numbers of misregulated genes and gene ontology enrichment profiles, as well as strongly correlated misexpression patterns. The misregulation shown by the transcriptomic profile of the *icu11-5 cp2-1* double mutant is similar to that of single and double mutants carrying loss-of-function alleles of genes encoding PRC2 core and accessory proteins. Our observations confirm the close functional relationship of ICU11 and CP2 with PRC2, and provide additional evidence of their roles as accessory proteins of this epigenetic repressive complex.

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P2-19. Cytokinins play a role in leaf margin development

Sergio Navarro-Cartagena and José Luis Micol

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

The interplay between auxin and cytokinins affects many plant developmental processes (1). However, the formation of interspersed auxin maxima has been considered sufficient to shape Arabidopsis leaf margins. These maxima form by the joint action of the PIN-FORMED1 (PIN1) auxin efflux carrier and the CUP-SHAPED COTYLEDON2 (CUC2) transcriptional regulator (2). Nevertheless, cytokinins favour compound leaf development in tomato (3), and the *Cardamine hirsuta REDUCED COMPLEXITY* (*RCO*) gene activates cytokinins and is required for the formation of compound leaves (4).

In *vasculature complexity and connectivity* (*vcc*; also named *desigual1* [*deal1*]) mutant leaf primordia, the developing leaf margin and auxin maxima distribution are both aberrant (5). Using a split-ubiquitin yeast two-hybrid membrane-based localization assay, we found that the VCC protein interacts with components of the Very-Long-Chain Fatty Acid (VLCFA) elongation complex. VLCFAs negatively regulate leaf cell proliferation by inhibiting the expression of *ISOPENTENYLTRANSFERASE* (*IPT*) genes, which encode enzymes catalyzing the first step of the cytokinin biosynthesis pathway (6).

To test whether cytokinins play a role in Arabidopsis leaf margin morphogenesis, we used the *RCO:ARR1ΔDDK* transgenic line (4), in which the *RCO* promoter drives the expression of a constitutively active form of the cytokinin effector ARABIDOPSIS RESPONSE REGULATOR1 (ARR1). The *RCO:ARR1ΔDDK* line and the *cuc2-1D* gain-of-function mutant have leaf margins with extra lobes. The *vcc-2* mutant shows ectopic expression of the cytokinin response reporter *TCSn:GFP* at leaf margins. *RCO:ARR1ΔDDK cuc2-3* and *vcc-2 cuc2-3,* but not *cuc2-3*, show lobes and sinuses caused by excess or lack of tissue. These results suggest that extra or ectopic cytokinin activity shapes the leaf margin in the absence of CUC2, and pave the way for a better understanding of the action and interactions of auxin, cytokinins, and VCC in leaf bilateral symmetry.

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P2-20. Characterization of Arabidopsis *api7-1* mutant reveals a link between ribosome recycling and auxin homeostasis

Carla Navarro-Quiles and José Luis Micol

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

In Arabidopsis, mutations in some genes encoding components of the translation machinery impair leaf development similarly: they produce pointed and dentate leaves with an aberrant venation pattern, usually ascribed to unbalanced auxin homeostasis. Some of these mutations synergistically interact with alleles of *ASYMMETRIC LEAVES1* (*AS1*) or *AS2*, which encode transcription factors involved in leaf dorsoventral patterning. The most severe double mutants produce radial leaves completely lacking dorsoventrality. Previous studies of translation machinery mutants link their phenotypes to altered post-transcriptional regulation mediated by upstream open reading frames (uORFs) within the 5′ untranslated regions (5′ UTRs) of mRNAs encoding transcription factors involved in vascular development (1, 2).

In a search for leaf mutants, we identified *apiculata7-1* (*api7-1*) a hypomorphic allele of the *ATP-BINDING CASSETTE E2* (*ABCE2*) gene, whose phenotype is reminiscent of that of translation machinery mutants. The ABCE proteins are evolutionarily conserved among archaea and eukaryotes, and are essential for ribosome recycling (3). We found that ABCE2 physically interacts with proteins that participate in translation or its regulation. One such protein is EUKARYOTIC TRANSLATION INITIATION FACTOR 3J (eIF3j), which has been described as an ABCE accessory factor during ribosome dissociation in *Saccharomyces cerevisiae* (4), supporting a role for Arabidopsis ABCE2 in ribosome recycling. We performed an RNA-seq analysis, and our results point to a potential increase in auxin biosynthesis that might cause the aberrant leaf venation pattern of the *api7-1* mutant. In agreement with evidence from *Saccharomyces cerevisiae* and human cells (4, 5), we propose that ABCE2 depletion allows ribosomes to enter into the 3′ UTR and displace regulatory factors from their target sites, impairing post-transcriptional regulation in a mechanism that differs from the mechanism described for other translation machinery mutants.

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P2-21. Identification of new *DENTICULATA* genes in Arabidopsis via mapping-by-sequencing

Àngela Ortega-Menaches, Carla Navarro-Quiles, Raquel Sarmiento-Mañús, and José Luis Micol
Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

The Arabidopsis *ASYMMETRIC LEAVES 1* (*AS1*) and *AS2* genes encode transcription factors involved in leaf dorsoventral axis specification, and synergistically interact with some genes encoding proteins involved in translation (1, 2). The *denticulata* (*den*) mutants exhibit small, serrated and pointed leaves, traits that make them candidates for carrying mutations in genes that encode proteins involved in translation (3, 4). We used mapping-by-sequencing (3) to identify the *den1*, *den4*, *den7*, and *den11* mutations. None of the candidate mutations found for the *den1* mutant seems to be related to translation. Conversely, the best candidates to be the *DEN4*, *DEN7* and *DEN11* genes encode a putative translation initiation factor, the ribosomal protein RPL34B, and a ribosomal protein of the S8e family, respectively. To confirm that these genes are causal for the phenotypes under study, we constructed transgenes carrying their wild-type alleles and transferred them into *den* plants for complementation tests. We are taking a similar approach to identify *DEN9*, *DEN14*, *DEN15* and *DEN16*.

One of the mutations that we used to obtain *as1 den* double mutants is *as1-14*, which we obtained by fast neutron bombardment of wild-type Landsberg *erecta* (L*er*) seeds and whose molecular nature had not been previously determined. PCR amplification using nested primer pairs and Sanger sequencing of the amplification products allowed us to demonstrate that the *as1-14* mutation is a 5,880-bp deletion that spans part of the *AS1* gene and the whole *ACTIN1* (*ACT1*) and *SIAMESE-RELATED12* (*SMR12*) genes, which are adjacent to *AS1*. To confirm that the mutant phenotype of *as1-14* and its genetic interactions are only due to the loss of function of *AS1*, we constructed transgenes harboring wild-type alleles of each of these genes, and obtained insertional mutants from public collections. These approaches should confirm that the phenotypes of the *as1-14* mutant result from loss of *AS1* function.

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P2-22. The transcriptomic landscape of flower phenotypic plasticity in *Moricandia*

Francisco Perfectti[a,b], José M. Gómez[c,b], Cristina Armas[c], Eduardo Narbona[d], Adela González-Megias[e,b], Luis Navarro[f], Silvia Castro[g], João Loureiro[g]

[a]Dpto. de Genética, Universidad de Granada, Granada, Spain. [b]Research Unit Modeling Nature, Universidad de Granada. [c]Estación Experimental de Zonas Áridas (EEZA-CSIC), Almería, Spain. [d]Dpto. de Biología Molecular e Ingeniería Bioquímica, Universidad Pablo de Olavide, Sevilla, Spain. [e]Dpto. de Zoología, Universidad de Granada, Granada, Spain. [f]Dpto. de Biología Vegetal y Ciencias del Suelo, Universidad de Vigo, Vigo, Spain. [g]Departamento de Ciências da Vida, Universidade de Coimbra, Coimbra, Portugal.

Phenotypic plasticity is the ability of a genotype to produce alternative phenotypes when exposed to different environments. The plant *Moricandia arvensis* (Brassicaceae) shows phenotypic plasticity for flowers which manifests as an extreme case of polyphenism (1). This species produces large, dissymmetrical, lilac flowers in spring and small, rounded, white flowers in summer. However, their close relative *M. nitens* does not show such extreme plasticity, even when forced to flower in hot summer conditions. To explore the genetic basis of floral plasticity, we analyzed the flower bud transcriptomes of five individuals of each species submitted consecutively to two experimental conditions simulating spring and summer temperature and photoperiod. We produced a the-novo transcriptome assembly for each species and compared gene expression in both conditions. For *M. arvensis*, the overall expression was different between spring and summer flower buds, with > 600 differentially expressed genes (DEGs) enriched in GO terms related to the response to stress, temperature, radiation, and light. However, the non-plastic species showed only a very small number of DEGs. The seasonal color change of the plastic species was produced by the reduction in expression of both structural and regulatory genes of the anthocyanin pathway. These changes appeared as a coordinated response to environmental cues rather than a secondary or passive response to heat. Both species diverged ~2.5 Ma (2), show the same ploidy but some differences in genome size (720 Mb vs 817 Mb). Therefore, the genetic changes promoting polyphenism in *M. arvensis* should have evolved recently, probably to facilitate plant reproduction in the harsh Mediterranean summer.

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P2-23. Novel viable alleles of the Arabidopsis *KEULE* gene allow examination of its role in postembryonic development

Alejandro Ruiz-Bayón, Carolina Cara-Rodríguez, Raquel Sarmiento-Mañús and José Luis Micol

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

Membrane fusion is required for cell-to-cell communication and subcellular organization in eukaryotes. The formation of SNARE trans-complexes, assisted by Sec1/Munc18 (SM) proteins, plays a key role in this process. Formation of the partitioning membrane in plant cytokinesis involves fusion of membrane vesicles at the cell plate in the division plane, which requires the SNARE protein KNOLLE and the SM protein KEULE (1, 2).

We isolated the *serrata4-1* (*sea4-1*) and *sea4-2* Arabidopsis mutants in a genetic screen for mutations induced by ethyl methanesulfonate, and these mutants have serrated rosette leaves (3). Through mapping-by-sequencing (4), we found that *sea4-1* and *sea4-2* are novel recessive, hypomorphic alleles of the *KEULE* gene, which is already known for its essential role in plant embryonic development.

Although all previously studied alleles of *KEULE* are seedling lethal, homozygous *sea4-1* and *sea4-2* plants are viable and fertile. These mutant plants have smaller rosettes and fewer leaves at bolting time compared with wild type. The leaves of *sea4-1* and *sea4-2* are reduced in size and undulated, have increased venation pattern complexity, develop necrotic patches, and undergo premature senescence. The *sea4* mutants allow us to examine the roles of KEULE in postembryonic development, with a focus on rosette leaf whole organ and margin patterning.

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P2-24. Arabidopsis RNA HELICASE 35 is required for leaf and flower development

Raquel Sarmiento-Mañús, Yaiza Senent-Valero, Sara Fontcuberta-Cervera and María Rosa Ponce

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

The human tumor suppressor DEAD-box helicase 41 (DDX41) and its *Drosophila melanogaster* ortholog Abstrakt (Abs) interact with NF-kappa B Activating Proteins (NKAPs). Metazoan DDX41 orthologs are components of the spliceosome, and human DDX41 is a key factor in antiviral innate immune responses. The Arabidopsis ortholog of metazoan NKAPs is MORPHOLOGY OF AGO1-52 SUPPRESSED 2 (MAS2), which is assumed to be involved in splicing and ribosome biogenesis (1). Arabidopsis has two putative co-orthologs of DDX41: RNA HELICASE 35 (RH35) and RH43. DDX41 and its animal and plant orthologs, including Drosophila Abs and Arabidopsis RH35 and RH43, share a CCHC-type zinc knuckle motif, which is present in proteins involved in RNA metabolism (2).

Seedlings of the *rh35-1* mutant produce rosette leaves with high anthocyanin levels, over-branched trichomes, rough epidermis, aberrant venation pattern, and abnormal rosette phyllotaxis. The *rh35-1* flowers lack petals and have thickened and twisted stigmata, with only a few stamens containing scarce pollen. In addition, 8% of *rh35-1* plants produced three cotyledons.

The *rh35-1* allele harbors a T-DNA insertion in its 5'-UTR and produces two mRNA variants: one identical to that of the wild type, and another retaining the single intron of the gene. Complementation with the $RH35_{pro}$*:RH35:GFP* transgene restored the wild-type phenotype in *rh35-1* plants. In transgenic $RH35_{pro}$*:RH35:GFP* plants in the wild-type background, we found RH35 to be a nuclear protein. Our results also suggest that this gene is essential in Arabidopsis, as *DDX41* and *Abs* are in humans and Drosophila, respectively.

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Research in the laboratory of M.R.P. was supported by grants from the Ministerio de Ciencia e Innovación of Spain (BIO2017-89728-R) and the Generalitat Valenciana (PROMETEO/2019/117). U.A-V. held a predoctoral fellowship from the Generalitat Valenciana (GRISOLIAP/2016/134).

P2-25. Unraveling the working mechanism of the non-canonical RNA interferencepathway in *Mucor lusitanicus*

Ghizlane Tahiri, Francisco Esteban Nicolás, Eusebio Navarro Ros, Victoriano Garre Mula

Department of Genetics and Microbiology, Murcia University, Murcia, Spain.

Mucor lusitanicus is a human pathogen that has been extensively used as a model organism of the infection known as mucormycosis. In this fungus, an RNA degradation mechanism called as Non-canonical RNA interference pathway (NCRIP), because it uses the atypical ribonuclease III R3B2, operates controlling multiple physiological processes from genome integrity to virulence. Here in this work, by sequencing small RNAs and messenger RNAs from a wild-type and an *r3b2* mutant strains in saprophyticconditions and with macrophages, and analysing their transcriptomic profile, we have discovered and described the direct targets of the NCRIP. These target genes could beused as reporters to determine when this regulatory mechanism is activated, and whichstimuli triggers this pathway. Additionally, we have studied the expression of these directtargets by analyzing the bioluminescence emission of *Mucor* strains containing the fusionof the mentioned genes to the luciferase gene, establishing a new method for gene expression analysis in this fungus. The causative agents of mucormycosis are highly resistant to common antifungals. Consequently, the discovery of the direct targets of thisRNA degradation mechanism, which is only conserved in Mucorales, could contribute tothe development of specific drugs to attack the pathogen.

P2-26. Establishment of *Nicotiana benthamiana* as a chassis for plant biotechnology in bioreactors

Fuensanta Verdú-Navarro[1,2], Juan Moreno-Cid[2], Ruth Ordóñez[2] Julia Weiss[1], Marcos Egea-Cortines[1]

[1]Instituto de Biotecnología Vegetal, Universidad Politécnica de Cartagena, Cartagena, Spain, [2] BIONET, Parque Tecnológico Fuente Álamo, 30320, Murcia

Extensive research has been performed in plant biotechnology using the Bright Yellow-2 variety of the natural tetraploid *Nicotiana tabacum*. This cell line grows at high speed in cell cultures and bioreactors. However, the scientific community is currently using *Nicotiana benthamiana*, an allotetraploid closely related to *N.tabacum* to advance in biotechnological solutions. One reason is the ease to transform, both with viral vectors for transient expression and with *Agrobacterium* for stable transformation. Despite the success of *N. benthamiana* as a model to study plant pathogen interactions and protein production in greenhouses, the growth conditions in aseptic bioreactors is not fully optimized. Our current work is centred around the relationship between the growth conditions and cell growth. This should give us a first hand understanding of the transcriptome and epigenome underlying growth speed, which is the basis of plant productivity in all environments.

This work is funded by the Catedra BIONET-UPCT

SESIÓN 3

Genética de Microorganizmos

Moderadora: Montserrat Elías-Arnanz

I3-01. Noncontiguous operons: a new strategy for coordinating gene expression

Pablo Iturbe[a], Cristina Solano[a], Iñigo Lasa[a]

[a]Laboratory of Microbial Pathogenesis, Navarrabiomed-Universidad Pública de Navarra (UPNA)-Complejo Hospitalario de Navarra, IdiSNA, Pamplona 31008, Navarra, Spain

Bacterial genes are typically grouped into operons defined as clusters of adjacent genes encoding for proteins that fill related roles and are transcribed into a single polycistronic mRNA molecule. This simple organization provides an efficient mechanism to coordinate the expression of neighboring genes and is at the basis of gene regulation in bacteria. Here, we report the existence of a higher level of organization in operon structure, named “noncontiguous operon”. The noncontiguous operon consists of operons that contain a gene(s) that is transcribed in the opposite direction to the rest of the genes of the operon. The mRNA encoded on the opposite DNA strand to the operon serves as a bifunctional mRNA. It encodes for a protein while also acting as an asRNA, which base-pairs all along its length with an internal untranslated region of the polycistronic mRNA. This genomic organization generates overlapping transcripts whose expression is mutually regulated by transcriptional interference and RNase III processing at the overlapping region. This novel transcriptional architecture is present in gram positive and gram negative bacteria. In the light of our results, the canonical view of operon structure should be revisited by including this novel operon arrangement in which co-transcription and overlapping transcription are combined to coordinate functionally related gene expression.

*1. Sáenz-Lahoya, S. et al. Noncontiguous operon is a genetic organization for coordinating bacterial gene expression. Proc National Acad Sci **116**, 201812746 (2019).*

This work was supported by the Spanish Ministry of Economy and Competitiveness grant BIO2017-83035-R. Pablo Iturbe is supported by y a F.P.I. (PRE2018-084479) contract from the Spanish Ministry of Science, Innovation and Universities

I3-02. Aging from the poles: asymmetric distribution of spindle microtubule organizing centers

Javier Manzano-López[a], Laura Matellán[b], Alejandra Álvarez-Llamas[a], José Carlos Blanco-Mira[a], Fernando Monje-Casas[a]

[a]Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), CSIC, Sevilla, Spain, [b]Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), Universidad de Sevilla, Sevilla, Spain.

The generation of asymmetry during cell division is of utmost importance for the proliferation of many microorganisms and during development and tissue morphogenesis in animals and plants (1). An asymmetric cell division requires the alignment of the mitotic spindle, a bipolar array of microtubules that facilitates the even distribution of the replicated chromosomes, along a pre-established polarity axis (2). The microtubules that form the spindle emanate from microtubule-organizing centers (MTOCs), structures located at both its poles that are known as the centrosomes in higher eukaryotes or the spindle pole bodies (SPBs) in the budding yeast *Saccharomyces cerevisiae* (2). Remarkably, MTOCs can be differentially inherited during asymmetric cell divisions. This fascinating phenomenon, which was originally described in budding yeast, was later shown to be evolutionary conserved and it has been observed during the division of stem cells from many organisms, including humans (3,4). We will present recent research from our group that provides new insights on the mechanisms that regulate the differential distribution of MTOCs during asymmetric cell divisions as well as on the consequences of disrupting the predetermined pattern of SPB inheritance in *S. cerevisiae*. Our results pave the way for a better knowledge about the processes that can lead to premature cellular stem cell aging and that can therefore be also related to the development of neurodegenerative diseases or cancer.

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O3-01. Reversible protein aggregation as cytoprotective mechanism against heat stress in Fission Yeast

Silvia Salas-Pino[1], Paola Gallardo[1,2], Paula Real-Calderón [1, 3], Ignacio Flor-Parra[1], Rafael R. Daga[1]

[1]Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide-Consejo Superior de Investigaciones Científicas, Junta de Andalucía, Seville, Spain,
[2] Current address: European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.
[3] Department of Cell and Tissue Biology, University of California, San Francisco, San Francisco, CA 94143.

Heat stress (HS) induces the unfolding and denaturation of thermosensitive proteins which promote the formation of non-native protein interactions driving phase separation and the generation of high-order molecular assemblies or biomolecular condensates [1, 2]. These condensates have been described in several organisms from yeasts to humans at different intracellular locations and with different physicochemical properties. In the last years however, it has become evident that the formation of these condensates shares a common role as protective mechanism against proteotoxic stress [2]. Here, we show in fission yeast, that acute HS results in the rapid aggregation of nuclear proteins and RNA into high-order molecular assemblies at the nucleolar region. These assemblies, named Nucleolar rings (NuRs), act as sequestering centers for several factors involved in nuclear mRNA metabolism, nuclear pore complex function, cell-cycle regulators and molecular chaperones during the HS-induced growth inhibition. Upon stress relief, NuRs disaggregate and their components relocate to their functional environments in an Hsf1- and Hsp104-dependent manner, and this is required to reactivate growth and to maintain cell viability after the stress [3]. Thus, our work highlights how reversible protein aggregation can be tuned into a protective mechanism to maintain cellular homeostasis during acute HS.

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This work was supported by the Ministerio de Economía y Competitividad (grants PGC2018-099849-B-I00 to R.R.D.) and by the Junta de Andalucia-FEDER-UPO (grant UPO-1264663 to S.S.-P.).

O3-02. R3B2: The exclusive RNase III of Mucorales that has evolved to cut single-stranded RNA

José Tomás Cánovas-Márquez[1], Sebastian Falk[2], Francisco E. Nicolás[1], Eusebio Navarro[1] and Victoriano Garre[1]

[1]Department of Genetics and Microbiology, Faculty of Biology, University of Murcia, 30100 Murcia, [2]Department of Structural and Computational Biology, Max Perutz Labs, A-1030 Vienna, Austria

The fungus *Mucor lusitanicus* has a complex RNAi system where a non-canonical RNAi pathway (NCRIP) regulates virulence and other physiological processes by degrading specific mRNAs (1–3). In this pathway, Dicer function is replaced by R3B2, an atypical class I RNase III, and small single-stranded RNA (ssRNA) are produced instead of small double stranded RNA (dsRNA) as Dicer-dependent RNAi pathways. In this work, we show that R3B2 forms a homodimer that binds to RNA molecules, but exclusively cuts ssRNA, in contrast to all known RNase III (4). The RNA substrate preference relies on its unusual RNase III domain (RIIID) as the substitution of the domain with a canonical one allows dsRNA processing. Curiously, the crystal structure of R3B2 RIIID resembles canonical RIIIDs, despite the low sequence conservation. However, the groove that accommodates dsRNA in canonical RNases III is narrower in the R3B2 homodimer, suggesting that this feature could be responsible for the cleavage specificity for ssRNA. Conservation of this activity in R3B2 proteins from other mucormycosis-causing Mucorales indicates an early evolutionary acquisition, which supposes a promising target for drug development. All together, these results describe a relevant mechanism for the pathogenic potential of Mucorales that is organized around an unusual RNase exclusive of these fungi. The thorough study of this evolutive peculiarity supposes a new way to the specific and effective treatment of mucormycosis.

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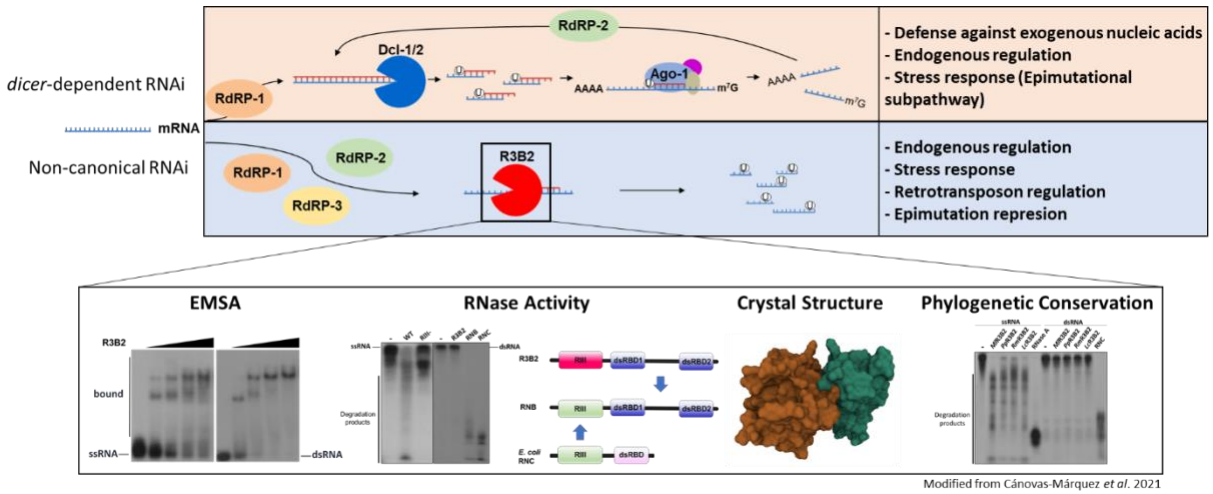
Graphical abstract:



Modified from Cánovas-Márquez *et al*. 2021

FT3-01. *SALMONELLA* MODIFIES THE HOST UBIQUITINOME

Andrea Bullones-Bolaños[1], Joaquín Bernal-Bayard[1], Francisco Ramos-Morales[1].

[1]*Departamento de Genética- Laboratory 2, Facultad de Biología, Universidad de Sevilla, Sevilla, Spain.*

Salmonella enterica is a pathogenic Gram-negative bacterium which causes many diseases in animals and humans. The virulence of these bacteria relies on two Type 3 Secretion systems (T3SS) which are protein complexes capable of translocated different effector proteins to the cytoplasm of the host cells. The effectors proteins promote the infection and survival of the bacteria inside the host cells by manipulating different cellular processes (1). Ubiquitin signalling pathways are often common targets for bacterial effector proteins (2). Our laboratory is studying a family of effectors that exhibit E3 ubiquitin ligase activity (SlrP, SspH1 and SspH2). We aim to identify the "ubiquitinome" (set of ubiquitinated proteins in the cell) in the presence of *Salmonella* E3 Ubiquitin ligases. We transfected HEK293T cells with plasmids expressing the three effectors or the empty vector, then we performed affinity purification of ubiquitinated substrates coupled to mass-spectrometry analysis. We identified 16 potential substrates of these effectors, these candidates are involved in different biological processes such as cytokinesis, cell proliferation, apoptosis, splicing, proteins folding and degradation, mitochondrial replication, and cell-cell adhesion. Ubiquitination of these potential candidates will be confirmed by *in vitro* assays and we will analyze the level of redundancy and specificity that could exist between these effectors. Our results show that *Salmonella* E3 ubiquitin ligases alter the ubiquitinome of the host cells affecting many different cellular processes.

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This work was supported by Ministerio de Ciencia e Innovación – Agencia Estatal de Investigación/10.13039/501100011033, grant number PID2019-106132RB-I00, the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement Nº 842629.

FT3-02. FROM ENDOSYMBIONT TO CHASSIS IN SYNTHETIC BIOLOGY: GENOMIC ENGINEERING OF *Bartonella quintana* BY DELETING NON-ESSENTIAL REGIONS.

Emilio Garrote-Sánchez[a], Jimena Solana-González[a], Luis Gullón-Escobar[a], Andrés Moya[a], Rosario Gil[a]

[a] *Evolutionary Systems Biology of Symbionts, Institute for Integrative Systems Biology (I2SysBio), University of Valencia and CSIC, Valencia, Spain.*

Synthetic biology has opened the possibility of designing and creating semi-synthetic cells, with applications in fields such as biotechnology and biomedicine. One common approach is the simplification of natural cells to generate a suitable chassis in which we can add the genetic modules involved in certain functions of interest. Obligatory endosymbiotic organisms, whether parasitic or mutualistic, tend to have reduced genomes compared to their free-living relatives. Their characterization, as well as the possibility of optimizing them, by eliminating superfluous genes or by adding genes to complete impaired metabolic pathways, is highly relevant in this field. However, most endosymbionts cannot be cultured in the laboratory, making it difficult to manipulate them. The endosymbiont we selected, *Bartonella quintana* str. Toulouse, can be grown in culture and has the capacity to infect mammalian cells, making this bacterium a good model to design a chassis for potential biomedical applications. We have determined the structural and functional characteristics of *Bartonella quintana*'s genome, and designed a novel pipeline called DELEAT to detect dispensable genomic regions. This tool helped us to design potential deletion regions based on the absence of essential genes and their distribution along the genome. A selected pool of deletion constructs has been obtained using the megapriming PCR method, and transferred to *Bartonella* through biparental mating conjugation, to produce mutants that lack the selected region.

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FT3-03. Unmasking the identity of plasmanylethanolamine desaturase and the role of plasmalogens in photooxidative stress signaling

Antonio J. Monera-Girona[a], Elena Pajares-Martínez[a], Aránzazu Gallego-García[a], Eva Bastida-Martínez[a], Ricardo Pérez-Castaño[a], Irene del Rey-Navalón[a], Víctor Espejo-Moya[a], Antonio Angel Iniesta[a], Marta Fontes[a], S. Padmanabhan[b], Montserrat Elías-Arnanz[a]

[a]*Departamento de Genética y Microbiología (Unidad Asociada al IQFR-CSIC), Universidad de Murcia, 30100 Murcia,* [b]*Instituto de Química Física "Rocasolano" (IQFR), CSIC, 28006, Madrid*

Plasmalogens are a special subclass of glycerophospholipids found in animals and some anaerobic bacteria but not in plants, fungi, or most aerobic bacteria. The key enzyme (plasmanylethanolamine desaturase or PEDS1) that generates the vinyl ether bond in plasmalogens remained unknown. Our discovery of the identity of PEDS1 owes, surprisingly, to the study of the light response in *Myxococcus xanthus*, an aerobic soil bacterium that makes plasmalogens (1). In *M. xanthus*, light triggers the synthesis of carotenoids to quench the reactive oxygen species formed upon illumination via two light-sensing and signaling mechanisms. In one mechanism, the membrane-associated protein CarF is required to signal formation of the highly reactive singlet oxygen, which is produced by photoexcitation of protoporphyrin IX, a photosensitizer. This leads to inactivation of the membrane-associated anti-sigma factor CarR and the release of its cognate sigma factor CarQ, thus enabling transcription of genes for carotenoid biosynthesis. We discovered that CarF and its animal homologs, including human TMEM189, but not those in plants, correspond to PEDS1. Besides unearthing an elusive human enzyme as well as novel enzymes involved in aerobic plasmalogen biosynthesis, our studies have revealed an unprecedented role of plasmalogens in signaling photooxidative stress. Local perturbation of membrane properties due to plasmalogen cleavage by singlet oxygen and/or protein adduct formation with the resulting cleavage products may underlie the plasmalogen-based signaling mechanism that leads to CarR inactivation and the induction of carotenogenesis.

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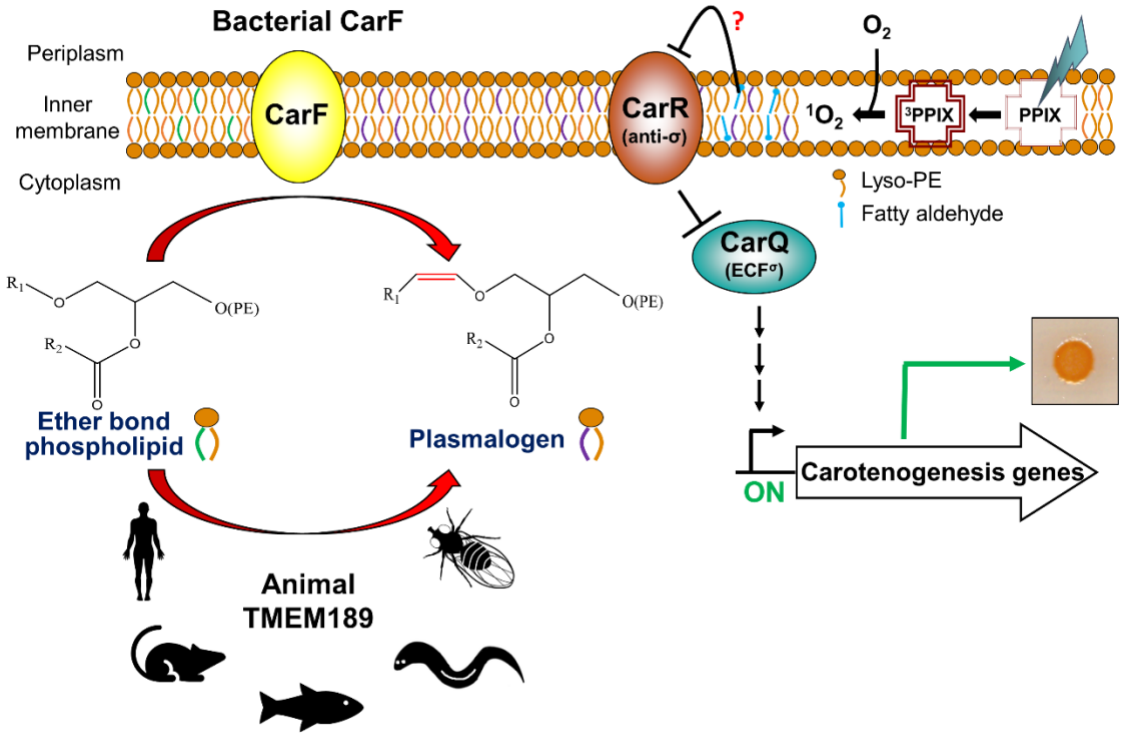
This work was supported by PGC2018-094635-B-C21 to MEA and PGC2018-094635-B-C22 to SP from the AEI and FEDER; and 20992/PI/18 to MEA from Fundación Séneca-CARM.

Graphical abstract:



FT3-04. DOG9, A NOVEL FUNGAL PROTEIN INVOLVED IN EFFECTORS SECRETION DURING PLANT INFECTION

María Dolores Pejenaute-Ochoa, Ramón Ramos-Barrales, José Ignacio Ibeas

Molecular Biology and Biochemical Engineering, Universidad Pablo Olavide, Seville, Spain.

Crop infections caused by pathogenic fungi and the increasing use of pesticides to treat them are recognized as a global threat to food safety and the environment. *Ustilago ma*ydis is a biotrophic fungus, which causes smut disease in maize. During this plant-pathogen interaction, the plant prevents fungal infection inducing defense response mechanisms.Meanwhile, the fungus undergoes changes in its cell wall and secretes effectors that allow it to penetrate and grow within the plant.

To better understand these mechanisms, our lab has been working for the last years in the identification and characterization of proteins involved in virulence with the final goal of identifying new antifungal targets. After proving the importance of glycosylation in the pathogenic process of this fungus (1), we carried out a proteomic screening to identify glycosylation dependent proteins involved in virulence. Among the twenty-five proteins identified (2), we have focused on the uncharacterized protein Dog9 (Downstream Of Glycosylation 9) because of its relevant role in infection and its specific conservation in pathogenic fungi. The Δdog9 mutant exhibited penetration defects in the maize plant. We observed that *dog9* has an early expression pattern during the virulence process; it localizes in small vesicles which move to the tip of the filament and accumulates in the appressorium, a structure that allows the fungus to penetrate inside the plant. As in *U. maydis* plant penetration seems to be facilitated by secretion of hydrolytic enzymes and effectors, we analysed and observed a role for Dog9 in effector secretion. Moreover, we have developeda phylogenetic study where we have found five orthologs in *U. maydis*, also conserved in phytopathogenic fungi, which will be analyzed for their role in virulence. Our results indicate that Dog9 is an important protein for the early stages of the infectious process of *U. maydis* and it could be a member of a family of conserved proteins involved in pathogenesis.

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This work was supported by AEI MINECO / FEDER, UE, Spain, grants number BIO2016-80180-P and PID2019-110477GB-I00 AEI/10.13039/501100011033.

FT3-05. Exploring the genome of *Mycobacterium brumae*, a species of bacterium with therapeutic potential

Chantal Renau-Mínguez[a], Paula Herrero-Abadía[b], Vicente Sentandreu[c], Paula Ruiz-Rodriguez[a],Galo A Goig[d], Iñaki Comas[e], Esther Julián[b], Mireia Coscolla[a].

[a]I2SysBio, University of Valencia-FISABIO Joint Unit, Valencia, Spain, [b]Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain, [c]Genomics Unit, Central Service for Experimental Research (SCSIE), University of Valencia, Spain,[d]Swiss Tropical and Public Health Institute, Basel, Switzerland, [e]Institute of Biomedicine of Valencia, Valencia, Spain.

Mycobacterium brumae is a fast-growing, non-pathogenic *Mycobacterium* species, originally isolated from environmental and human samples in Barcelona, Spain (1). Previous studies have shown that the implementation of non-pathogenic mycobacteria, such as *Mycobacterium bovis* BCG can improve the treatment against high-risk non- muscle invasive bladder cancer (BC) by intravesical administration. *M. brumae* has beenshown to be non-pathogenic (2) and its phenotype and immunogenic effect have been well characterized. However, the knowledge of its underlying genetic composition is stillincomplete. In this study we have sequenced the genome by means of PACBIO of the *M. brumae* strain CR-270 obtaining the most complete assembly to date. We describe its genetic content by showing evolutionary relationships between different mycobacteriaand we compare its virulence gene content with other virulent mycobacteria such as H37Rv reference strain. Furthermore, we describe the genetic variability of *M. brumae* by comparative genomics using obtained Illumina sequences. Our results contribute to increase the knowledge about the genetic bases that explain the non-pathogenic phenotype of this bacterium with therapeutic potential.

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This work was supported by RTI2018-094399-A-I00, RTI2018-098777-B-I00 and 2017SGR-229.

FT3-06. RELEVANCE OF COPPER HOMEOSTASIS IN *FUSARIUM OXYSPORUM* PATHOGENICITY

Rafael Palos Fernández[a], Antonio Di Pietro[a], Manuel Sánchez López-Berges[a]

[a]*Departamento de Genética, Universidad de Córdoba, Córdoba, Spain*

The soil-borne fungus *Fusarium oxysporum* causes vascular wilt disease on more than one hundred plant species and opportunistic infections in humans, making this species an excellent model for the study the genetic basis of fungal pathogenicity in different host kingdoms. Previous work established that MacA, a transcription factor that regulates the response to copper limiting conditions (-Cu), essential for the maintenance of copper homeostasis, is a virulence factor in animal fungal pathogens[1]. Here, we study the function of *F. oxysporum* MacA by generating a knock-out mutant lacking this transcription factor (*macA*Δ), as well as a complemented strain. We found that *macA*Δ fails to grow in -Cu, both on solid and liquid medium. Moreover, infection assays show that MacA is important for virulence of *F. oxysporum* on tomato plants. Quantification of fungal burden, both in roots and stems 3 and 7 days post inoculation, indicates that *macA*Δ does not efficiently colonize the stem of the plants. Interestingly, the expression of *ctr* genes, encoding copper membrane high affinity transporters, is low during plant infection, suggesting that the role of MacA in virulence is not linked to copper limitation. Further supporting this hypothesis, plants infected with *macA*Δ displayed similar mortality rates regardless of the presence or absence of copper in the irrigation water. In order to elucidate the role of MacA during *F. oxysporum* infection, we are studying the relationship of this regulator with oxidative stress and superoxide dismutase (SOD) activity. Although *macA*Δ grows similar to the wild-type strain under oxidative stress conditions during copper sufficiency, its SOD activity is extremely altered in -Cu. Furthermore, preliminary transcriptional data in -Cu with menadione show that *sod1* and *sod2* are overexpressed in *macA*Δ while *sod3* is deficiently activated. In summary, our results indicate that the function of MacA as regulator of copper homeostasis is conserved between *Fusarium* and other fungi; however, its role in plant pathogenesis still needs to be clarified.

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P3-01. Evaluation of the occurrence of nematocidal *cry* genes in a collection *of Bacillus thuringiensis* strains

Miguel Andrés-Antón, Yolanda Bel, Baltasar Escriche

Instituto de Biotecnología y Biomedicina (BioTecMed), Departamento de Genética, Universitat de València, Burjassot, Spain.

Plant-parasitic nematodes are crop pests found throughout the world, causing severe damage to plants and therefore serious negative effects on the environment and on the agricultural economy (1). *Bacillus thuringiensis* (Bt) is an aerobic, spore-forming, gram-positive, bacterium characterized by the production of pesticidal crystal (Cry) proteins. These Cry proteins are known to be toxic to a wide variety of pests, making Bt the most successful bioinsecticide used in the last decades (2). Seven Cry protein families toxic to nematodes have been identified so far: Cry5, Cry6, Cry12, Cry13, Cry14, Cry21, and Cry55 (2). The search and discovery of native Bt strains with nematocidal activity could represent a breakthrough in the control of pest nematodes worldwide. With this in mind, we analysed the presence of these genes in 532 Bt strains, by PCR amplification. As a result, 110 isolates (21%) contained at least one nematocidal *cry* gene. The *cry5* and *cry21* genes were the most abundant since they were amplified in 17% and 11% of the strains, respectively. For the *cry6* gene, no amplicon was obtained for the *cry6B* allele but the *cry6A* allele was found in a 3% of the Bt isolates. Bt strains harboring *cry14* or *cry12* genes were also found in 3% and 1% of the strains, respectively. However, there were no strains positive for the *cry13* or *cry55* genes. So far, we have sequenced the complete cry gene sequences of eight strains positive for *cry5*, *cry21,* and/or *cry6A*, resulting in the identification of putative *cry5Ba*, *cry21Ga*, *cry21Ha,* and *cry6Aa* genes. The expression of the nematocidal genes in the Bt strains that contained them has been examined by LC/MS-MS analysis. The results show that three of the strains produced the protein Cry5B and three produced Cry6A, but none of the strains carrying *cry21* genes was able to express them. This is the first comprehensive screening analysis that examines the presence of all Bt nematicidal genes described so far. The results obtained differ from those previously published (3,4), perhaps due to the diverse geographical origins and sources of the samples from which the Bt strains were obtained.

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P3-02. Physiological effects of peroxide stress and a novel mode of regulating the response in *Myxococcus xanthus*

Eva Bastida-Martínez[a], Irene del Rey-Navalón[a], S. Padmanabhan[b], Montserrat Elías-Arnanz[a]
[a]*Departamento de Genética y Microbiología (Unidad Asociada al IQFR-CSIC), Universidad de Murcia, 30100, Murcia,* [b]*Instituto de Química Física "Rocasolano" (IQFR), CSIC, 28006, Madrid.*

The Gram-negative soil bacterium *Myxococcus xanthus* mounts a transcriptional response leading to carotenogenesis in response to photooxidative stress, with several novel factors and an unusual phospholipid implicated in the signaling and regulation (1). How *M. xanthus* copes with oxidative stress in general and peroxide stress in particular, however, remains largely unexplored. Peroxiredoxins are a ubiquitous family of thiol-based peroxidases that remove >90% of cellular peroxides in nearly all living organisms. AhpC, a highly conserved and widespread peroxiredoxin with broad substrate specificity, efficiently scavenges low levels of H_2O_2, while catalases detoxify high levels of extracellular H_2O_2 (2-3). In most studiedbacteria, the peroxide sensors OxyR and PerR regulate expression of these enzymes through a redox switch (4). However, *M. xanthus* lacks OxyR or PerR homologs. Rather, we have found that promoters of the *ahpCD* operon (*ahpD* encodes the specific reductase required in the AhpC catalytic cycle) and of the *katB* catalase gene share irregularly interspaced direct repeats upstream of a possible σ^{54} binding site. We also found that most myxobacteria from suborder Cystobacterineae have *ahpCD* flanked by a divergently expressed gene encoding a putative enhancer-binding protein (EBP). EBPs are specific regulators of promoters that depend on the alternative σ^{54} factor. We observed that deletionof *ahpC* in *M. xanthus* markedly enhances *katB* expression and tolerance to H_2O_2, supporting a compensatory regulatory mechanism. Furthermore, the *ahpC* mutant exhibited pleiotropic effects, such as plating and motility defects, and decreased thresholds for signals that activate carotenogenesis. Our data suggest links between *ahpCD* and *katB* expression and a novel σ^{54}-dependent mode of regulation of the peroxide response.

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P3-03. An improved genome sequence of the plant pathogenic fungus *Colletotrichum graminicola*

Sioly Becerra[a], Riccardo Baroncelli[a, b], Serenella A. Sukno[a], Michael R. Thon[a]

[a]*Instituto Hispano-Luso de Investigaciones Agrarias, University of Salamanca (CIALE), Salamanca, Spain*
[b]*Department of Agricultural and Food Sciences (DISTAL), University of Bologna, Italy.*

Colletotrichum graminicola is a plant pathogenic fungus and causal agent of maize anthracnose, one of the most important maize diseases worldwide. The first version of the 55 MB haploid *C. graminicola* genome (CGRA01v1) was assembled using sequences from Sanger, 454 pyrosequencing and optical mapping technologies into 567 contigs and 13 pseudochromosomes. The new genome assembly (CGRA02v2) uses Illumina and long-read PacBio sequencing technologies. The PacBio reads were assembled using the Canu assembler and Pilon was used to improve the PacBio genome assembly using two Illumina libraries. The new assembly has 18 contigs. In the synteny between two versions, no structural rearrangements were observed. However, the distribution and organization of chromosomes provides changes with respect to the optical map assembly. The CGRA02v2 assembly contains 21 telomeric repeats (TTAGGG) suggesting that 6 complete chromosomes were identified by finding a telomeric region at both ends of the contigs. Another 9 contigs have a telomeric repeat at one end. In contrast, no telomeric repeats were found in the CGRA01v1 assembly indicating that long-read sequencing is needed to identify telomeric repeats. Gene annotation was carried out with MAKER resulting in a gene catalogue of 15.493 for CGRA02v2, whereas CGRA01v1 has 12.004 genes, an increase of 3489 genes for the new assembly. The combination of technologies used in this approach provides a genome assembly with improved quality.

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P3-04. Identification of new T6SS effectors in *Salmonella* Typhimurium

Joaquín Bernal-Bayard[a], Lilit Gevorgyan[a], Patricia Bernal[b], Francisco Ramos Morales[a]

[a]*Departamento de Genética, Universidad de Sevilla, Sevilla, España*
[b]*Departamento de Microbiología, Universidad de Sevilla, Sevilla, España*

The type 6 secretion system (T6SS) is a bacterial nanomachine used to inject effectors (toxins) into eukaryotic cells or other competing bacteria and it is considered a potent antimicrobial weapon. A toxin is a protein with antimicrobial activity which is counteracted by a cognate immunity protein or antitoxin partner (1). *Salmonella enterica* serovar Typhimurium encodes a sole T6SS, which is located within the SPI-6 (2). In addition to the genes encoding the well-characterized structural proteins (Tss), *in silico* analysis of *S.* Typhimurium T6SS cluster shows the existence of genes encoding three putative T6SS effectors-immunity pairs (EIPs). To advance our knowledge on the molecular mechanisms underlying the bacterial interference provided by *S.* Typhimurium T6SS, we have assessed the functionality of these undescribed EIPs. To do so, we cloned each gene of the pair independently into compatible plasmids and expressed them in *E. coli*. Expression of the predicted toxins in the absence of its cognate antitoxin led to a drop of *E. coli* survival while the presence of the antitoxin preserved bacterial growth. Western-blot analysis confirmed the expression of both elements, the toxins and the antitoxins.

T6SSs are tuned regulated by an assortment of regulatory systems, which enable bacteria to adapt to varied environments (3,4). *Salmonella* T6SS is switched down in laboratory conditions and the regulatory signals leading to the activation of the system remain unclear. We have performed bacterial competition assays using *E. coli* as prey and *S.* Typhimurium as predator including isogenic regulatory mutants to test the activation of this system. *hns*, *rpoS* and *fur* *Salmonella* strains present an enhanced ability to kill *E. coli* indicating that *Salmonella* T6SS is repressed by Hns, RpoS and Fur regulators. Currently, we are further investigating the potential role of T6SS in *Salmonella* virulence beyond its proven antibacterial activity.

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P3-05. Integrating genomics and infection biology to decipher differences between human and animal associated *M. tuberculosis* lineages

Guillem Santamaria[a,b], Chantal Renau[a], Carlos Gomis[a], Antonio Espert[a], Nikolina Bukša[a], Paula Ruiz-Rodriguez[a], Francisco R. Pinto[b], Mireia Coscolla[a]

[a]*I2SysBio, University of Valencia-CSIC, FISABIO Joint Research Unit Infection and Public Health, Valencia, Spain*
[b]*BioISI – Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Lisboa, Portugal*

Mycobacterium tuberculosis, which causes tuberculosis, is of the top 10 causes of death worldwide caused by an infectious disease (1). The majority of the lineages infect predominantly humans, except four lineages that infect a wide range of other mammals, and we refer to as animal-associated lineages (2). Because understanding the genetic basis of host specificity can inform about the virulence factors of *M. tuberculosis* in each host, our objective is to decipher is host specificity correlates with virulence *in vitro*, and with lineage specific genomic signatures.

We combined experiments in macrophage infection model with whole genome sequencing analysis of 35.000 clinical *M. tuberculosis* strains representing all ecotypes to determine genomic specific signatures of the bacteria during the infectious process.

We observed different genomics signatures between human and animal *M. tuberculosis* associated lineages. A differential signature was observed in three of the four genes that encode for phospholipase C in *M. tuberculosis*, lost in three out of four animal lineages, and present in the human lineages. Phylogenomic analysis showed that these deletions happened several times during evolution indicating a convergence in the loss of these regions that could be under differential selection pressure in animal and human associated strains. Because phospholipase C has been previously linked to *M. tuberculosis* virulence, we explored the differential expression of these genes between lineages infecting two different host cells.

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P3-06. The photooxidative stress regulon in the bacterium *Myxococcus xanthus*

Irene del Rey-Navalón[a], Elena Pajares-Martínez[a], Eva Bastida-Martínez[a]; Antonio Angel Iniesta[a], S. Padmanabhan[b], Montserrat Elías-Arnanz[a]

[a]*Departamento de Genética y Microbiología (Unidad Asociada al IQFR-CSIC), Universidad de Murcia, 30100 Murcia,* [b]*Instituto de Química Física "Rocasolano" (IQFR), CSIC, 28006, Madrid.*

Light, an important environmental signal for most living organisms, also causes photooxidative damage. In the Gram-negative soil bacterium *Myxococcus xanthus*, blue light triggers carotenogenesis as a photoprotective response. Our studies of the response have identified two distinct pathways and several singular factors implicated in light sensing, signal transduction and transcriptional regulation (1, 2). The more complex pathway relies on an extracytoplasmic-function σ factor, CarQ, which directly drives its own expression, and that of a carotenogenic gene and of an antirepressor that activates an operon comprising nine other carotenogenic genes. To identify new players in the photooxidative stress response, we compared global transcriptional profiles of wild-type and *carQ*-deleted strains, in the dark versus light exposure over time. This has revealed new sets of genes whose expression changes temporally in response to light in a CarQ-dependent or independent manner. Many light-activated genes are CarQ-independent and encode various proteases, oxidoreductases, chaperones, glutathione-S-transferase, permeases, and transporters. By contrast, CarQ directly activates the expression of few genes besides those mentioned earlier. Strikingly, this includes two periplasmic methionine sulfoxide reductase (Msr) systems: a bifunctional MsrBA Cys-based thioredoxin-dependent system and an MsrPQ molybdopterin-cytochrome *b*-based duo. Msr systems repair oxidized methionine residues and play central roles in protein quality control, oxidative stress resistance, and cell signaling in most domains of life (3). Our findings point to periplasmic protein methionine oxidation as collateral damage upon light exposure, whose repair contributes to cellular defense against photooxidative stress.

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P3-07. Metagenomic analysis unraveling the resistome in gut microbiota of *Blattella germanica*

Rebeca Domínguez-Santos[1], Ana Elena Pérez-Cobas[2], Carlos García-Ferris[1,3], Andrés Moya[1, 4] Rosario Gil[1] and Amparo Latorre[1,4]

[1]Institute for Integrative Systems Biology (I2SysBio), University of Valencia and CSIC, Valencia, Spain, [2]Department of Microbiology, Ramón y Cajal Institute for Health Research (IRYCIS), Ramón y Cajal University Hospital, Madrid, Spain, [3]Department of Biochemistry and Molecular Biology, University of Valencia, Valencia, Spain. [4]Genomics and Health Area, Foundation for the Promotion of Sanitary and Biomedical Research (FISABIO), Valencia, Spain.

Blattella germanica is a cosmopolitan species of omnivorous cockroach found in urban and rural environments. They are present in large numbers inside and around houses, hospitals and areas with unsanitary and insalubrious conditions. Cockroaches are the only insect known to have two types of symbiotic systems in a single individual: *Blattabacterium cuenotii* an obligate endosymbiont, located in one type of cells of the fat body (bacteriocytes), which has an important role in nitrogen metabolism, and a rich and complex gut microbiota which function is not yet well understood. We have carried out a metagenomic analysis of the gut microbiota of *B. germanica* populations treated with three antibiotics (ampicillin, kanamycin and vancomycin) and non-treated to characterize the antibiotic resistance genes (ARGs) pool of this species and to understand its temporal dynamics. Our study identifies a natural reservoir of ARGs, some of them involved in resistance to several broad-spectrum antibiotics frequently used in the clinic. Furthermore, we detected mobile element-related components suggesting a capacity to mobilize DNA, including ARGs. ARGs can increase in number in response to antibiotic treatment and be mobilized, favoring the growth of antibiotic-resistant microorganisms. Given that German cockroaches live in intimate association with humans, this can cause a biomedical problem that needs to be considered.

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P3-08. SELECTIVE NUCLEAR PORE COMPLEX REMOVAL DRIVES NUCLEAR ENVELOPE DIVISION IN FISSION YEAST

María Expósito-Serrano[a], Ana Sánchez-Molina[a], Paola Gallardo[b], Silvia Salas-Pino[a], Rafael R. Daga[a]

[a] *Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide-Consejo Superior de Investigaciones Científicas, Junta de Andalucía, Seville, Spain.*

Whereas higher eukaryotes disassemble the nuclear envelope (NE) in early mitosis and reassemble it just after chromosome segregation (open mitosis); lower eukaryotes, like yeasts, maintain NE assembled during mitosis (closed mitosis). In closed mitosis organisms, chromosome segregation is therefore achieved by the assembly of an intranuclear mitotic spindle which elongates in late mitosis generating two identically sized nuclei (1-4). However, how the site of nuclear division is determined and the underlying mechanism driving NE fission in closed mitosis remain largely unknown. In this work, using the fission yeast as a model for closed mitosis (5), we show that the microtubule bundler Ase1, localized at the spindle midzone, has a role in promoting the local accumulation of nuclear pore complexes (NPCs) in the region of the NE that surrounds the central spindle, forming a specific NE domain called midzone membrane domain (or MMD). These NPCs are competent for nucleocytoplasmic transport and locally accumulate importin-α at the MMD (6-8). Strikingly, as the spindle elongates during anaphase B, several nucleoporins of the NPCs located at the MMD are sequentially eliminated, from more peripheral to more structural ones. These events of NPC disassembly are also accompanied by the local remodeling of NE membrane and collaboratively lead to the eventual removal of NPCs, that locally fenestrate the NE and drive nuclear division. In the absence of importin-α, NPCs are not completely disassembled and no event of NE remodeling is observed. Consequently, cells fail to undergo nuclear division (8). Thus, our results highlight a new role of the central spindle as a spatial cue that determines the site of nuclear division and point to importin alpha-dependent NPC removal as the nuclear division-triggering event. This mechanism of local NE breakdown (NEBD) driving nuclear division shares mechanistic similarities with the NEBD in higher eukaryotes (3) and points to the unexpected evolutionary conservation of both processes.

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P3-09. DUPLICATED GENES UNDER CONCERTED EVOLUTION IN *Helicobacter pylori*

Jerson Alexander García-Zea, Rafael Navajas-Pérez, Francisca Robles, Juan Felipe Zapata,Roberto de la Herrán, Carmelo Ruiz Rejón

Department of Genetics, University of Granada, Spain, Faculty of Forensic Science, Technology of Antioquia, Colombia. Corresponding Author: Jerson Alexander García-Zea (alexander7719@correo.ugr.es)

Helicobacter pylori, a Gram-negative bacillus, is present in more than half of the worldpopulation. Colonizes the human stomach inducing the development of superficialgastritis, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma.
H. pylori is highly variable thanks to a great genomic dynamic promoted by mutationsand high recombination rates. Also characterized by exhibiting panmixia, genetic mosaicism, and great genomic plasticity, which indicates its ability to adapt at a micro- evolutionary level to its environment (1).

This dynamic influences in the gene duplication events and in the evolution of them. Concerted evolution (CE) between duplicate genes can occur through a genetic exchange called gene conversion (2). Few studies have analyzed the CE and the affected genes in this bacterium (3). Implementing a computational framework (including IseeCe program) (4) in 53 complete *H. pylori* genomes of five different geographical origins, 8 genes (*HopJ/Hopk, 3'-5 'exonuclease, fucosyltransferase, glycosyltransferase, oipA, RMs, 50S ribosome and cagA*) were detected showing patterns of CE. The gen *HopJ/Hopk* shows CE in the five geographical region, *3'-5 'exonuclease, fucosyltransferase* in Asia and Amerindian, *glycosyltransferase, oipA, RMs* in populations of Asia only, and finally *50S ribosome and cagA* showed CE in hybrid strains. Different test by RDP5 software evidence recombination events in the majority of the genes analyzed.

Additionally, we realized analysis of selective pressures on the groups of genes underCE by calculating the non-synonym/synonym mutations ratio (dN/dS) over the complete sequences. Proteins of these genes play crucial roles in the pathogenic life cycle of *H. pylori* such as virulence, pathogenicity, colonization of the environment, adaptation to the host.

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P3-10. Associations between the APOE genotypes and the gut microbiota composition in a Spanish population sample: A Pilot Study.

Rocío González Soltero[1], Mariangela Tabone[1], Carlo Bressa[1], Fabio Cafini[1], María Bailén[2], Maria Gregoria Montalvo-Lominchar[1] y Mar Larrosa[1]

[1]Grupo de investigación MAS Microbiota. Universidad Europea de Madrid. C/Tajo s/n. CP28670. Villaviciosa de Odón (Madrid)

[2] Departamento de Medicina Preventiva y Salud Pública y Microbiología. Facultad de Medicina. Universidad Autónoma de Madrid. Calle del Arzobispo Morcillo 4. 28029 Madrid.

Apolipoprotein E (APOE) is a protein involved in the metabolism of the fatty acids in the body. It is present in three different isoforms (E2, E3 and E4), and it the strongest prevalent genetic risk of Alzheimer disease.

In the last few years several studies have been focused on the understating of how the genetics of the individual can influence the gut microbiota, and how genetics-microbiota association features can be used to predict the onset of Alzheimer disease.

In this study, we investigated the association between APOE genotype and the gut microbiome composition in a cohort of healthy Spanish population. Allelic discrimination of the APOE gene variants was conducted with TaqMan PCR technology (7300 Instrument; Applied Biosystems) and Assay-On-Demand single nucleotide polymorphism genotyping assays (Applied Biosystems). The APOE haplotypes (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4) were determined from the alleles for the APOE single nucleotide polymorphisms rs7412 and rs429358. Bioinformatics analysis of 16S amplicon sequencing data was performed using the Quantitative Insights into Microbial Ecology (QIIME) v.2. DNA amplicons were sequenced on a MiSeq Illumina platform (Illumina, San Diego, CA) (1). Statistical analysis was carried out using SPSS software v. 22.0 (SPSS, Chicago, IL) and the R statistical package 3.5.2 (2). We founded important metabolic and microbiota-genotype associations as individuals with E3/E3 genotype and construct a Random Forest classifier model to allow as to predict some microbiota signatures from the genotype.

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P3-11. A landmark in the study of mucormycosis: stable and reproducible homologous recombination in *Rhizopus microsporus*

Carlos Lax Molina[a], Francisco E. Nicolás Molina[a], Victoriano Garre Mula[a]

[a]*Departamento de Genética y Microbiología, Universidad de Murcia, Murcia, España*

Mucormycosis is a lethal and emerging disease that lacked a genetic model fulfilling both high virulence and the possibility of performing stable and reproducible gene manipulation by homologous recombination (HR). The increase of the mucormycosis cases urges to increase the available genetic tractable models, particularly for the genus *Rhizopus*, the most frequent causal agents of mucormycosis that shows complete virulence robustness in survival assays. Here, we developed a new methodology to successfully perform HR in *R. microsporus*. First, we isolated a recipient strain showing a uracil auxotrophy phenotype without mutagenesis to reduce mutational burden. Using this strain, we followed an optimization itinerary of the critical steps in the genetic transformation of *R. microsporus*, including the time and temperature of germination, protoplast generation, and electroporation method. This was followed by an adaptation of a free-plasmid CRISPR-Cas9 system coupled with the use of microhomology repair templates. We reproducibly generated stable mutants in the genes *leuA* and *crgA* encoding an α-IMP isomerase and a regulator of several processes in fungi, respectively. The mutants in the gene *leuA* was auxotroph for the amino acid leucine as it was expected, while the mutants in the gene *crgA* showed a lack of aerial sporangiophores and an overaccumulation of melanin. Our new genetic model showed that mutations in the gene *pyrF*, a key virulence gene in several bacterial and fungal pathogens, correlated with an avirulent phenotype in an immunocompetent murine host. Moreover, ectopic integration of a wild-type allele of this gene restored virulence, demonstrating that gene complementation also works in *R. microsporus*. We propose *R. microsporus* as a reference model to study virulence and molecular genetics in the field of mucormycosis.

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P3-12. CONTROL OF CAROTENOID BIOSYNTHESIS BY THE RING-FINGER PROTEIN CarS IN *FUSARIUM*

M Carmen Limón, Julia Marente, Javier Pardo-Medina, Javier Avalos

[a]*Department of Genetics, University of Seville, Sevilla, Spain.*

The fungus *Fusarium fujikuroi* is a model in the study of the genetic regulation of carotenoid biosynthesis. The production of these pigments is stimulated by light through several photoreceptors, with the White-Collar protein WcoA playing a major role (1). The genes encoding phytoene synthase/carotene cyclase (*carRA*), phytoene desaturase *(carB*), carotenoid oxygenase for retinal biosynthesis (*carX*), and rhodopsin (*carO*) are clustered. Different mutations in a putative E3 ubiquitin ligase gene, called *carS*, provoke an accumulation of carotenoids in the dark due a strong upregulation the structural genes of the pathway. Upstream from the *carS* locus, a long non-coding RNA was found, whose synthesis is also induced by light (2). This communication is focused on our last progress in the understanding of the regulation of carotenoid biosynthesis in this fungus by CarS and its associated *carP* lncRNA.

Using the Tet-on system, transcription of the *carS* gene was induced by doxycyline, both in the wild type and in a *carS* mutant. In these strains, the carotenoid biosynthesis decreased after the addition of doxycycline due to a downregulation of the transcription of the structural *carB* and *carRA* genes. Similarly, transformants expressing the *carS* gene under the constitutive *gpdh* promoter showed an albino phenotype that demonstrates a repressor role of this regulator (3). Mutants with a deletion of the *carP* lncRNA are albino, which correlated with the downregulation of the *car* genes and the increase in the *carS* mRNA. These mutants were only complemented when *carP* gene was re-introduced at its original *locus* but not ectopically. Results suggest an interplay between the *carS* and *carP* RNAs.

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P3-13. The interplay between *Myxococcus xanthus* CRISPR-Cas systems and their link to phage defense

Alfonso López-Rojo[a], Diego Bernal-Bernal[b], María Luisa Galbis-Martínez[a], Antonio Ángel Iniesta[a], S. Padmanabhan[b], Montserrat Elías-Arnanz[a]

[a]*Departamento de Genética y Microbiología (Unidad Asociada al IQFR-CSIC), Universidad de Murcia, 30100 Murcia,* [b]*Instituto de Química Física "Rocasolano" (IQFR), CSIC, 28006, Madrid*

CRISPR-Cas systems are adaptive, small RNA-guided 'immune' systems for sequence-specific destruction of invading nucleic acids in bacteria and archaea. The bacterium *Myxococcus xanthus* has three of such CRISPR-Cas systems: a type I-C (CRISPR1-Cas), a type I-A (CRISPR2/3-Cas) and a type III-B (CRISPR4-Cas). We recently showed that an extracytoplasmic function σ factor (DdvS) and its cognate anti-σ (DdvA), together with a global regulatory complex, exert multifactorial control on the expression of all the components of the CRISPR4-Cas system (1). Given the role of CRISPR-Cas systems against bacteriophage attack, a reasonable candidate signal underlying DdvA inactivation to allow expression of the CRISPR4-Cas system could be a specific myxophage. Our current efforts are aimed at understanding the molecular mechanism underlying how the membrane-bound DdvA becomes inactivated to switch on the type III-B CRISPR4-Cas system, the links between the CRISPR-Cas systems in *M. xanthus*, and their response to attack by myxophages. The results of these ongoing studies will be presented.

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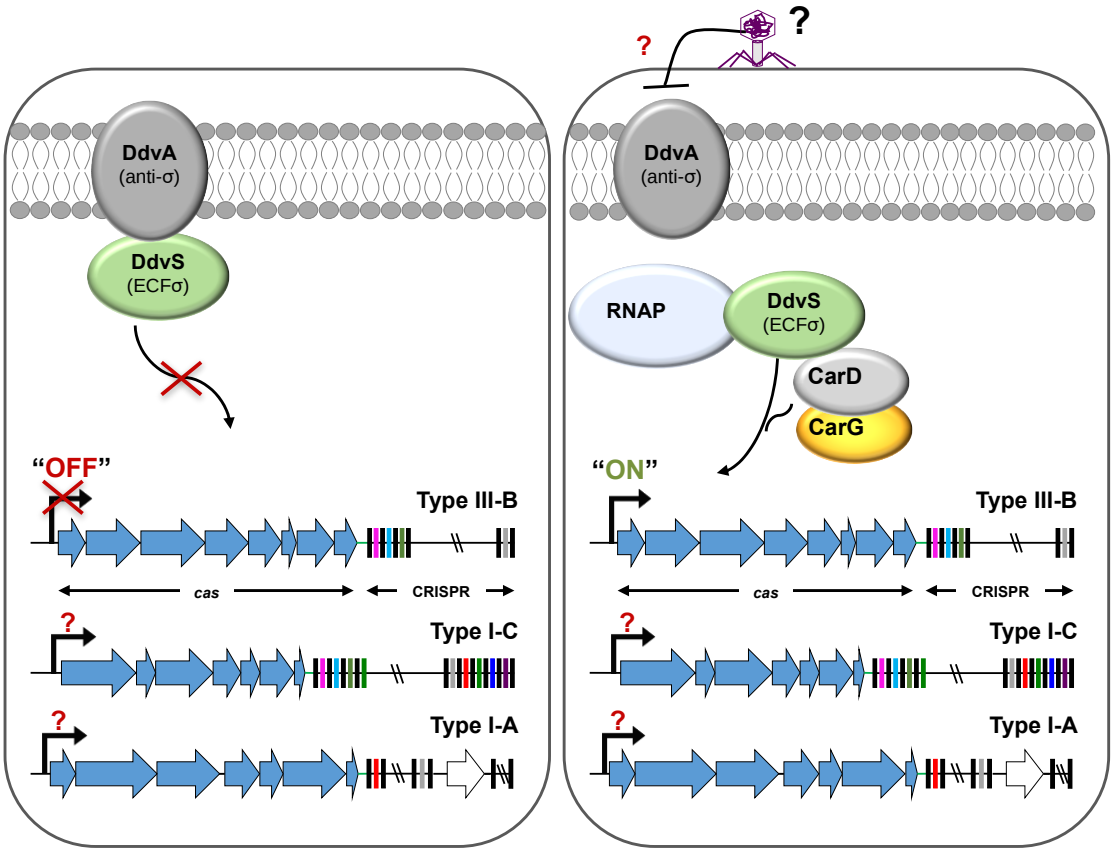
This work was supported by PGC2018-094635-B-C21 to MEA and PGC2018-094635-B-C22 to SP from the AEI and FEDER.

Graphical abstract:



P3-14. Developing new tools for genetic engineering and synthetic biology of cyanobacteria

Rafael Salas-Aparicio[a], Ignacio Luque[a] and Rocío López-Igual[a]

[a] *Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Seville, Spain.*

Cyanobacteria are the only prokaryotes with a phototrophic metabolism based on oxygenic photosynthesis like that of algae and higher plants. These Gram-negative bacteria have long been regarded as promising organisms for biotechnological applications due to their capacity to fix CO_2 into a variety of organic compounds at a low cost. Furthermore, the abundance in their genomes of gene clusters for secondary metabolites[1] attracts the attention of the pharmaceutical and biotechnological industries. Indeed, cyanobacteria are nowadays used for the production of biofuels or products of high added value like pigments or carotenoids[2]. However, the use of cyanobacteria in Biotechnology lags behind the use of heterotrophic microorganisms mainly due to the slow growth rate and the scarcity of a state-of-the-art toolbox for cyanobacterial genetic manipulation. In order to reduce this gap, we have undertaken the development of new genetic tools. Inspired by a Synthetic Biology platform developed previously[3], we have successfully engineered a system for the rapid construction of new plasmid vectors *à la carte*. This system is based in the assembly of pre-designed modules that can be joined in multiple combinations that provide the vector with distinct functionalities. A catalog of plasmids has been created that include replicative and integrative vectors for different purposes. Our results show that, despite the easy and rapid construction of these vectors further optimization steps are needed to meet the requirements for their efficient use in Synthetic Biology and Biotechnology.

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This work was supported by MICIU-AEI-PID2019-104784RJ-100 and BFU2016-77097-P.

P3-15. GENERATION OF BIOLUMINISCENT STRAINS TO ANALYZE THE ROLE OF ADENINE METHYLATION (6mA) IN MUCORALES INFECTION

Dámaris Lorenzo-Gutiérrez[a], Carlos Lax-Molina[a], José Tomás Cánovas-Márquez[a], Macario Osorio-Concepción[a], Francisco E. Nicolás-Molina[a] and Victoriano Garre-Mula[a]

[a]*Department of Genetics and Microbiology, Faculty of Biology, University of Murcia, 30100, Murcia, Spain.*

5-methylcytosine (5mC) is the main epigenetic mark in eukaryotic DNA, but N^6-methyladenosine (6mA) has recently gained relevancy (1). 6mA is involved in activating gene expression in early-diverging fungi and algae; while it is associated to gene repression, particularly of transposable elements, in animals (2). The essential machinery for 6mA is mostly based on methyltransferases which are ubiquitous proteins across the different kingdoms (3). 6mA has been related to virulence in several life kingdoms, as in bacteria (4) or fungi (5). Recent studies have revealed that 6mA abundance is variable, and it is relatively higher in early-diverging fungi species, as in Mucorales order, than in other eukaryotes. Interestingly, the genomic abundance of 6mA varies from ~0.0001−0.0003% of adenines in plants and mammals to as high as 2.8% of adenines in early diverging fungi (6). Several genes involved in adenine methylation in *Mucor lusitanicus* and *Rhizopus microsporus* has been identified and its function in DNA methylation is being characterized by the generation of knockout mutants. Due to the possible relevance of this epigenetic mark in Mucorales infection, the objectives of the present work are: i) to generate bioluminescent strains in knockout mutants in genes involved in adenine methylation in *M. lusitanicus* and *R. microsporus*, ii) to characterize the role of 6mA in the infection process in *Galleria mellonella* and murine models by tracking the bioluminescence, iii) and to evaluate the antifungal activity of 6mA inhibitors *in vivo* by bioluminescence analysis. Bioluminescent strains have been successfully generated for all mutants in genes involved in 6mA by targeted integration of a codon optimized firefly luciferase gene.

This work was supported by Fundación Séneca-Agencia de Ciencia y Tecnología de la Región de Murcia, Spain (20897/PI/18). D. Lorenzo was supported by Fundación Séneca-Agencia de Ciencia y Tecnología de la Región de Murcia, co-financing by ESF and Youth Guarantee.

P3-16. PROTEIN INTERACTION WITHIN THE *SALMONELLA* TYPE SIX SECRETION SYSTEM

Natalia Moreno de Castro[a], Francisco Ramos Morales[a], Patricia Bernal[b] Joaquín Bernal Bayard[a]

[a]*Departamento de Genética-Laboratory 2, Universidad de Sevilla, Sevilla, Spain*
[b]*Departamento de Microbiología, Universidad de Sevilla, Sevilla, Spain.*

Salmonella enterica are Gram-negative bacteria that infect different animal species, including humans. These pathogens are capable to produce from simple gastroenteritis to typhoid fever, causing about 350000 casualties per year. Specifically, *Salmonella enterica* Serovar Typhimurium is responsible for a great quantity of food-poisoning outbreaks, and it has been used as a model to study the pathogenesis of *Salmonella* infections. Previous studies have shown that one of the molecular mechanisms that *Salmonella* uses to carry out the infective process is the Type Six Secretion System (T6SS). Furthermore, it has been found that *S.* Typhimurium encodes a T6SS within *Salmonella* pathogenicity island 6 (SPI-6) (1). The main function of this machinery is interbacterial competition, but it also seems to play a role in pathogenesis, as some of the translocated effectors have eucaryotic targets. The T6SS is a multiprotein machine constituted by thirteen main components (TssA-M) that assemble in three parts: the membrane complex, the baseplate complex, and the tail complex. This last aggregation is formed by an inner tube formed by polymers of Hcp, and it ends in a spike made by VgrG. The sheath formed by TssB-TssC contracts to push the Hcp tube to the cell exterior (2). The ClpV ATPase provokes this contraction via depolymerization of TssB-TssC tubules (3). In this study we have used the Bacterial Adenylate Cyclase-based Two-Hybrid (BACTH) system to identify protein interactions among several T6SS components. While we have confirmed interactions between both Hcp proteins encoded in the cluster, we have also described protein binding between Hcp3, which is located outside the cluster, and the structural proteins TssA and TssK. In addition, we have also detected interactions between these two structural proteins. Currently, we are studying other possible interactions aiming to describe the protein interaction network that takes place in the T6SS apparatus.

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This work was supported by Ministerio de Ciencia e Innovación – Agencia Estatal de Investigación/10.13039/501100011033, grant number PID2019-106132RB-I00, The European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement Nº 842629 and *Plan de empleo juvenil de la Universidad de Sevilla*, funded by *Fondo Social Europeo* (EJ5-14).

P3-17. Plasticity in the mode of action of B_{12}-based photoreceptors

Ricardo Pérez-Castaño[a], Lucía Osete-Alcaráz[a], Jesús Fernández-Zapata[b], María Carmen Polanco[a], Marta Fontes[a], S. Padmanabhan[b] & Montserrat Elías-Arnanz[a]

[a]*Departamento de Genética y Microbiología (Unidad Asociada al IQFR-CSIC), Universidad de Murcia, 30100 Murcia*

[b] *Instituto de Química Física "Rocasolano", CSIC, 28006, Madrid*

CarH is the prototype of a new and large family of photoreceptors that are widely distributed in many bacterial species and that use the form of vitamin B_{12} known as AdoCbl (5'-deoxyadenosylcobalamin) as their chromophore (1). Typically, they repress their own expression and that of genes for carotenoid synthesis in the dark by binding to operator DNA as AdoCbl-bound tetramers, whose light-induced disassembly relieves repression (1-4). Structures of *Thermus thermophilus* CarH provided high-resolution snapshots of the dark and light states, and of a unique DNA-binding mode where three out of four DNA-binding domains contact an operator comprising three tandem 11-bp direct repeats (2). Our recent findings highlight a notable plasticity in oligomerization, DNA binding and/or operator architecture of CarH photoreceptors, which is important for their biological functions and development as optogenetic tools (3). On the other hand, genome analysis has revealed that the CarH AdoCbl-binding photosensor domain can exist as standalone proteins in many bacterial species, whose functions and modes of action are unknown. Lack of an effector domain in standalone proteins suggests that their activities may depend on heterotypic interactions with effector proteins. We will present our recent findings on the plasticity in the mode of action of these B_{12}-based photoreceptors.

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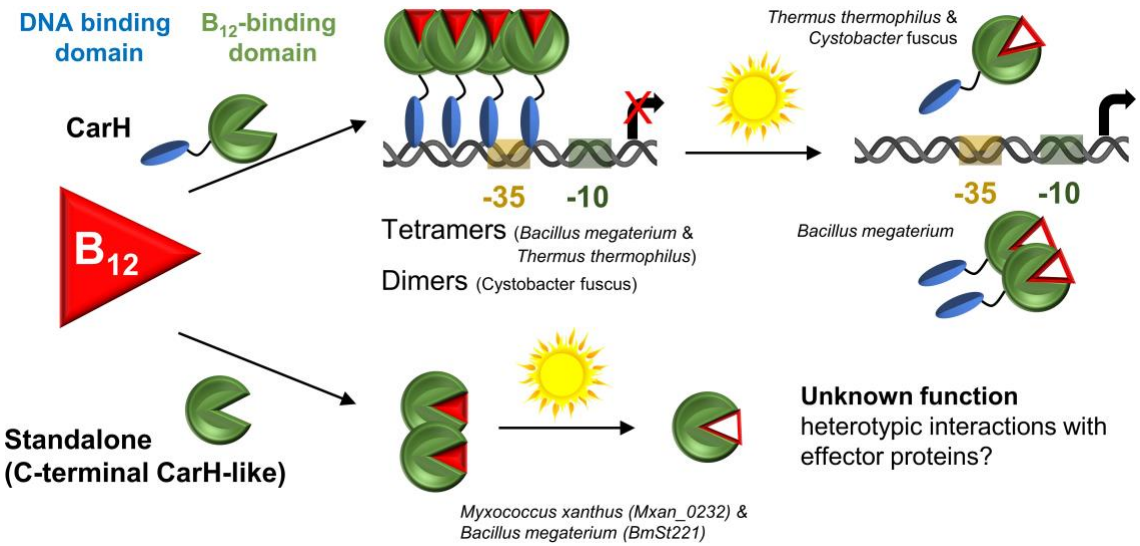
Graphical abstract:



P3-18. The role of a putative chaperone in the non-canonical RNAi pathway and the pathogenicity of *Mucor lusitanicus*

José A. Pérez-Ruiz, Francisco E. Nicolás, Ghizlane Tahiri, Eusebio Navarro, Victoriano Garre

Department of Genetics and Microbiology, Faculty of Biology, University of Murcia, Murcia, Spain.

The singularity of the non-canonical RNAi pathway (NCRIP) of *Mucor lusitanicus*, and its main protein, the ribonuclease R3B2, prompted the search of new proteins that could participate in this mechanism. R3B2 has a main role in the NCRIP, but it also participates in the canonical silencing pathway (1). This non-canonical pathway, that regulates the expression of many genes, has recently been linked with the control of genomic stability and virulence (2). Due to this interest, it was discovered a heat shock protein of HSPA12 family, which interacts with R3B2. The HSPA12 family, one of the most singular family of the entire HSP70 proteins, shows an expansion in this fungus comparing to other species and the other families of HSP70, showing the advantage that could represents these proteins for *M. lusitanicus*. On the other hand, in other fungi like *Saccharomyces cerevisiae*, this protein family is not present.

In addition, it has been discovered the implication of this HSPA12 (named as HSPA12A) in NCRIP and in the virulence of this fungus. Mutants in *hspa12a* present a reduction in the NCRIP activity levels at high temperatures (30 ºC), where HSPA12A could be participating with R3B2 in the maintenance of the pathway. Furthermore, these mutants have showed a reduction of their pathogenicity in mice, comparing with a wild strain. This decrease in the virulence of the *hspa12a* mutants may be due to the direct effect of HSPA12A on NCRIP or attributable to the affected growth that these mutants present at high temperatures.

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This research was funded by the Spanish MINECO (BFU2015-65501-P; BFU2015-62580-ERC) and MICIU (PGC2018-097452-B-I00) cofinanced by FEDER

P3-19. Sexual-driven combinatorial polimorphism leads to broad genetic diversity of *Saccharomyces cerevisiae* strains isolated from Aljarafe wineyards

Juan Quintero Blanco, Andréz Garzón Villar, Juan Jiménez Martínez

[a]*Departamento de Biología Molecular e Ingeniería Bioquímica-Área de Genética, Centro Andaluz de Biología del Desarrollo, Sevilla, España*

Natural diversity represents an inexhaustible source of yeasts for the diversification of wines and the improvement of their properties. With this objective, we isolated and characterized wine yeasts from spontaneously fermenting musts in the Aljarafe wine region of Seville. By PCR amplification of 5 microsatellite markers, we found 97 different patterns among 150 *S. cerevisiae* yeast strains isolated, a diversity greater than that normally described in equivalent analyses of natural *S. cerevisiae* yeasts to be found in musts from other regions. The physiological characterization of these 150 strains indicated that their genetics polymorphism was also reflected in characters of oenological interest such as tolerance to ethanol and high sugar concentrations. Interestingly, this enormous diversity in microsatellite patterns responds to a combinatorial diversity of the analysed markers, suggesting a sexual origin. Most of the isolates are homothallic strains with a high capacity to sporulate. Importantly, tetrad analysis of these markers in their meiotic products shows a marker combinatorics that mimics the diversity observed in natural strains, supporting that the high natural diversity of *S. cerevisiae* yeasts observed in this region is largely due to genetic combinations generated by sexual activity.

Twelve of these strains with different genetics patterns and interesting oenological profiles were selected to perform microvinifications. Each of the twelve wines obtained were organoleptically different, being one a semi-sweet frizzante wine with excellent organoleptic properties. Our data demonstrate that Aljarafe is a region with a broad genetic diversity of autochthonal wine yeasts derived from natural sexual activity that allows the isolation of *Saccharomyces cerevisiae* strains with interesting fermentative traits.

This work was supported by Bodegas Salado SL (Umbrete, Sevilla).

P3-20. UBIQUITINATION SUBSTRATES OF THE EFFECTOR SLRP FROM *SALMONELLA ENTERICA*

Francisco Ramos Morales, Andrea Bullones Bolaños, Juan Luis Araujo Garrido, Joaquín Bernal Bayard

Departamento de Genética, Universidad de Sevilla, Sevilla, España

Salmonella enterica serovar Typhimurium is a Gram-negative bacterial pathogen that possesses two type III secretion systems (T3SS) that are involved in virulence. These systems are able to translocate about 40 different proteins, known as effectors, into the cytosol of the eukaryotic host cell (1). T3SS effectors interact with host proteins and interfere with different processes and pathways.

SlrP is a *Salmonella* effector that belongs to a family of effectors with ubiquitin ligase activity. In a previous work we identified human thioredoxin as a substrate of the SlrP activity (2).

Here, we carried out a yeast two-hybrid screen to look for new interacting partners of SlrP that may be substrates of its ubiquitin ligase activity. Three million clones from a human cDNA library were screened and 588 clones were positive for the two reporter genes used. 220 corresponded to the thioredoxin cDNA, whereas sequencing of the rest revealed 30 new genes whose products were candidate to physically interact with SlrP. Five candidates were tested for *in vitro* SlrP-dependent ubiquitination and for one of them several bands corresponding to polyubiquitination products were detected. Some of these products were analysed using mass spectrometry to identify specific ubiquitinated lysine residues. This study will be complemented through *in vivo* analysis in mammalian host cells.

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This work was supported by Ministerio de Ciencia e Innovación – Agencia Estatal de Investigación/10.13039/501100011033, grant number PID2019-106132RB-I00 and The European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement Nº 842629.

P3-21. Genome annotation improvement analysing the gene KaKs ratio: A practical case with the genome of *Acinetobacter baumannii*.

Alejandro Rubio Valle[a], Juan Jiménez Martínez[a], Antonio J. Pérez Pulido[a]

[a] *Centro Andaluz de Biología del Desarrollo (CABD, UPO-CSIC-JA), Facultad de Ciencias Experimentales (Área de Genética), Universidad Pablo de Olavide, 41013 Sevilla, Spain.*

The current availability of complete genome sequences has allowed numerous comparative studies to be carried out to better understand the genome of a species. These studies are highly dependent on the quality of the used genome assemblies, so sequence errors cause poor gene prediction (1). For this reason, new tools and methods have emerged to search for new coding regions and thus be able to complete the genome annotation. An example of this is AnaABlast, developed by our group, an algorithm that discovers small and complex hidden coding sequences (2). We have already shown that the Nonsynonymous / Synonymous Substitution ratio (KaKs ratio) could effectively measure evolutionary pressure in other bacteria such as *Helicobacter pylori* (3). In this work, we propose using AnABlast and the KaKs ratio to detect regions poorly annotated by current gene finders and discover new possible protein-coding regions. For this study, we use *Acinetobacter baumannii*, an opportunistic bacterium that causes nosocomial infections with high mortality and morbidity (4), because it presents many sequenced strains allowing large-scale evolutionary analysis. We carried out a proof of concept with the ATCC 17978 strain, used as a reference of this species, to validate this approach. It presents two genome versions sequenced with different NGS tools, the most current version being the most reliable. We performed a comparative analysis between both versions, and we identified more than 200 genes that showed a wrong annotation in the first genome version of the ATCC 17978 strain. After the estimation of modifications, the evolutionary selection for each gene has been calculated using almost 2200 strains. We found that the KaKs ratio improved mostly when the most current version gene is used as a reference. Furthermore, we discovered some regions which could be new coding genes using our gene predictor. It is a preliminary result, so we must implement an even more detailed comparative analysis. All these preliminary results propose that this type of analysis could be helpful to validate and correct genetic predictions.

Graphical abstract:

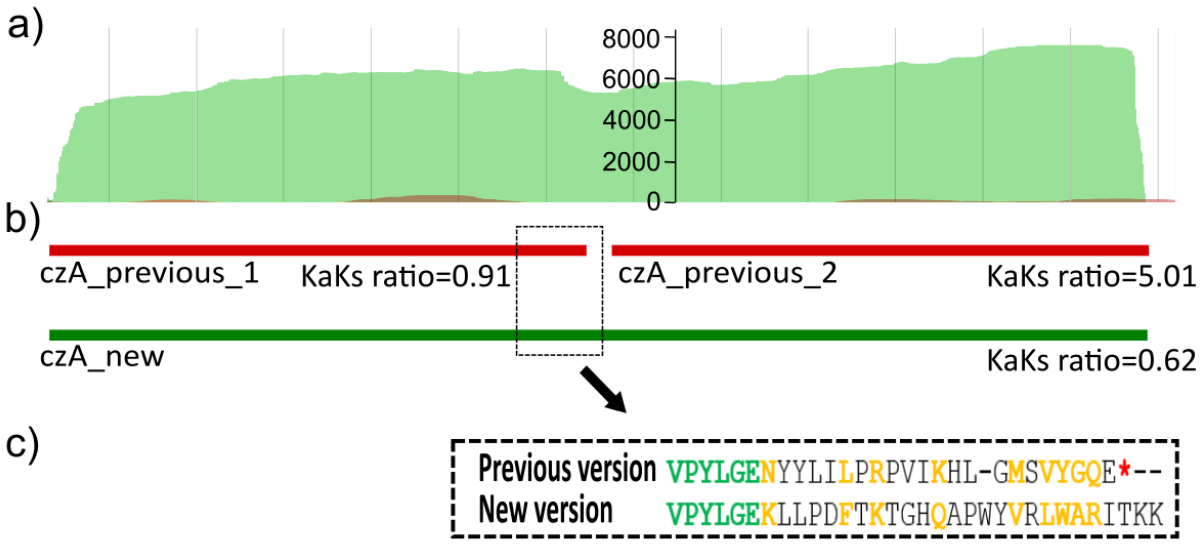

Figure 1. Proof of concept. A different annotation is observed when comparing the region 40211-47135 in both versions of the ATCC 17978 strain. The previous version has two different genes, whereas the new version has only one. The evolutionary comparison with 2200 strains indicates a better result when the most current version of the region is used as a reference since the KaKs ratio is lower. The sequence-level comparison shows changes that cause a frameshift in the previous version. (a) AnABlast profile highlighting protein-coding regions in the annotated czA gene in the ATCC 17978 strain genome. (b) Official annotation of region 40211-47135 in both versions of the ATCC 17978 strain genome and KaKs ratio values. (c) Local alignment of the C-terminal region between the czA_previous_1 and cza_new gene. We used different colours according to the degree of similarity at the amino acid.

P3-22. Role of the Nuclear Basket in proteasome localization and assembly

Gabriel Ruiz Romero[a], Paola Gallardo[a], Silvia Salas-Pino[a], Rafael R. Daga[a]

[a]*Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide-Consejo Superior de Investigaciones Científicas-Junta de Andalucía, Carretera de Utrera, Km1, 41013 Seville, Spain*

Protein levels need to be carefully regulated so that the correct amounts are present at the right place and time to perform their functions. This regulation is achieved by controlling their synthesis and degradation. In eukaryotes, there are two degradation pathways: autophagy and the ubiquitin-proteasome system (UPS), which constitutes the main pathway by removing most short-lived proteins (1). Defects in this route lead to different diseases such as cancer and neurodegenerative diseases (1, 2). The proteasome is a macromolecular complex composed of two modules, the 20S core particle (CP) and the 19S regulatory particle (RP), which confer the catalytic activity and the recognition specificity, respectively (reviewed in 3). In proliferating yeast and mammalian cells, proteasomes are present in all compartments, although accumulated in the nucleus, mainly at the nuclear envelope (NE) (4). The nuclear pore complex (NPC) forms a structure known as Nuclear Basket in the nucleoplasmic side, made up of TPR nucleoporins, which are conserved from yeasts to humans (5). In this project, we study the role of the Nuclear Basket in proteasome localization and function, making use of the fission yeast as model organism. Our results show that inactivation of Nup211, a TPR nucleoporin, using a temperature-sensitive allele (ts), leads to the accumulation of CP, but not all RP, subunits at the NE and their deprivation from the nucleoplasm. This correlates with the accumulation of proteasome intermediates represented as high molecular weight bands in native gels. Mass spectrometry analysis of these bands reveals the presence of all proteasome subunits, although the *nup211*^{ts} mutant show a significant reduction of some RP subunits. The delocalization of nuclear proteasome observed in *nup211*^{ts} is accompanied by an increase in NE localization of Cut8, a membrane-associated protein transporter, and Ecm29, a multifunctional chaperone involved in several steps of proteasome assembly and maturation. Altogether, our results suggest that the Nuclear Basket plays a role in proteasome assembly, which has an impact on its nuclear localization.

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P3-23. Studying the mechanical stress response in fission yeast

Ana Sánchez-Molina[a], Antonio J. Pérez-Pulido[a], Silvia Salas-Pino[a], Rafael R. Daga[a]

[a]*Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide-Consejo Superior de Investigaciones Científicas, Junta de Andalucía, Seville, Spain.*

Cells within tissues are constantly exposed to mechanical stress (MS), such as tension, shear stress or changes in hydrostatic pressure (1, 2, 3). Free-living organisms as yeast are also exposed to MS perturbations due mainly to changes in osmotic pressure, which cause massive cell swelling or contraction, or compressive forces, in conditions in which cell growth occurs in constrained cavities (4, 5). However, how eukaryotic cells sense MS and respond accordingly is still poorly understood. We have developed a method to apply MS to fission yeast for studying changes in the transcriptome associated to mechanical perturbations. For that, we use high-speed centrifugation of cells embedded in flexible matrices. During centrifugation, cells are exposed to compressive forces, part of which are dissipated through the agarose matrices, and cell viability is preserved. In addition, g-forces also cause changes in cellular architecture, such as nuclear displacement, which is used as readout of effective mechanical perturbation. Using this approach, we have performed genome-wide analyses by RNA-Seq to characterize how cells respond to mechanical perturbations. We detect a unique stress response in cells exposed to this MS. Among the overexpressed genes we find key regulatory genes of trehalose metabolism, genes involved in cell membrane and nuclear envelope regulation, and genes involved in oxidoreductase activities, while the most repressed genes are mainly involved in transport and metabolism. The change of expression of these genes might rewire cellular metabolism to regulate cellular viscosity and membrane endurance as a mechanism of cellular protection against mechanical perturbations. Importantly, 30 minutes after the MS, genes that were repressed immediately after MS are reactivated, while repressed genes restore the levels shown in unstressed cells. These transcriptomic changes likely comprise a characteristic response of fission yeast to mechanical perturbations.

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P3-24. CONTRIBUTION OF DNA ADENINE METHYLATION TO GENE EXPRESSION HETEROGENEITY IN *Salmonella enterica*

María Antonia Sánchez Romero[a] David R. Olivenza[b], Rocío Fernández-Fernández[b] & Josep Casadesús[b]

[a]Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain,
[b]Department of Genetics, Faculty of Biology, University of Seville, Seville, Spain.

In all domains of life, genomes contain epigenetic information superimposed over the nucleotide sequence. Epigenetic signals control DNA–protein interactions and can cause phenotypic change in the absence of mutation. A nearly universal mechanism of epigenetic signalling is DNA methylation. In gamma-proteobacteria, DNA adenine methylation has roles in genome defence, chromosome replication and segregation, nucleoid organization, cell cycle control, DNA repair and regulation of transcription. In this communication we describe how epigenetic control by DNA adenine methylation produces cell-to-cell variations of gene expression that may generate phenotypic diversity. Combinations of methylated and undermethylated GATC sites are detected at regulatory regions upstream of promoters, and single cell analysis reveals that a fraction of such loci undergo heterogeneous expression, with concomitant formation of cells in ON and OFF transcriptional states. Surveys of gene expression in pairwise combinations of Dam methylation-dependent loci reveal independent switching, thus predicting the formation of a high number of cell variants. This study thus underscores the relevance of the DNA adenine methylome as a source of phenotypic heterogeneity.

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P3-25. Fighting against microbes: The cosmopolitan German cockroach

Francisco J. Silva[a], Maria Muñoz-Benavent[a], Carlos García-Ferris[a], Amparo Latorre[a]

[a]*Institute for Integrative Systems Biology (I2SysBio), University of Valencia-CSIC, Paterna, Spain*

Insects kill pathogens through different mechanisms. One of the most relevant is the production of several types of small proteins called antimicrobial peptides (AMPs), which mainly act on microbial membranes producing the cell lysis. However, the presence of beneficial microbiota, or a permanent endosymbiont species located in specialized cells, may generate the evolutionary recruitment of one of these AMPs to control the symbiont populations. The German cockroach *Blattella germanica* requires these systems to adapt to unhealthy environments with abundance of pathogenic microbes. It also possesses a rich beneficial gut microbiota and an ancient endosymbiont (*Blattabacterium cuenoti*), whose association with the order Blattodea started more than 200 million years ago. To handle this situation, four antimicrobial gene families (defensins, termicins, drosomycins and attacins) were expanded in its genome (1). Remarkably, a new gene family (blattellicins) emerged recently after duplication and fast evolution of an attacin gene, which is now encoding larger proteins with the presence of a long stretch of glutamines and glutamic acids. Phylogenetic reconstruction, within Blattellinae, suggests that this duplication took place before the divergence of *Blattella* and *Episymploce* genera. The latter harbours a long attacin gene (pre-blattellicin), but the absence of the encoded Glx-region suggests that this element evolved recently in the *Blattella* lineage. A screening of AMP gene expression in available transcriptomic SR projects of *B. germanica* showed that, while some AMPs are expressed during almost the whole development, others are restricted to shorter periods. Blattellicins are highly expressed only in adult females. Because none of the available SR tissue projects could be associated with blattellicins' expression, RNA seq experiments with several adult tissue samples will be performed to identify where they are expressed.

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P3-26. Relationships between cell growth and cell division in the multicellular cyanobacterium *Anabaena*

Cristina Velázquez-Suárez[a], Ignacio Luque[a], Antonia Herrero[a]

[a]*Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, Sevilla, Spain.*

Anabaena sp. PCC 7120 is a multicellular cyanobacterium that performs oxygenic photosynthesis and displays a complex morphology. The organismic unit is a filament of cells, which communicate through septal junction protein channels (1). In prokaryotic organisms, cell morphology is determined mainly by the peptidoglycan that forms the cell wall. During the cell cycle, the dynamics of cell wall synthesis is essential for the determination of cell shape during growth and its maintenance during cell division (2). In rod-shaped bacteria, cell elongation is carried out by the elongasome protein complex, of which MreB, MreC and MreD determine the topology of peptidoglycan synthesis during cell growth. *Anabaena* mutant strains lacking a functional *mreB, mreC* or *mreD* gene are viable, although they showed enlarged and rounded cells (3). In bacteria, cell division begins with the polymerization of the FtsZ protein at the future division site, followed by recruitment of the divisome complex that catalyzes the synthesis of peptidoglycan to construct the division septum and the poles of the resulting daughter cells. *Anabaena* derivatives that conditionally express low levels of FtsZ (4, 5), or of ZipN (6), a cyanobacterial-specific essential cell division factor that is an organizer of the divisome, showed major alterations in cell size and morphology under restrictive conditions. In both mutants, cells progressively undergo drastic elongation and enlargement leading to very aberrant cells that finally detach from the filament and lyse. We are investigating the physiological interactions between the activities of the elongasome and the divisome in *Anabaena*. To that, we have studied the topology of peptidoglycan synthesis by staining filaments with the fluorescent compound Van-FL, which marks the sites of peptidoglycan growth, in *mreB, mreC and mreD* mutants, as well as in the above described *ftsZ* and *zipN* mutants, in comparison to the WT. Also, using GFP-protein fusions, we have investigated the localization of FtsZ and ZipN in the *mre* mutants, and the location of MreB, MreC and MreD in the cell-division mutants.

This work was supported by grant BFU2016-77097-P from Ministry of Economy and Competitiveness of Spain and FEDER.

SESIÓN 4

Mejora Genética

Moderador: Rafael Lozano

I4-01. MACHINE LEARNING AND GENOMIC PREDICTION

Miguel Pérez-Enciso

Center for Research in Agricultural Genomics (CRAG), 08193 Bellaterra, Spain

Breeders have done very well without resorting to fancy molecular and computational methods. Counterintuitively, genetic progress has continued unabated for decades. Most breeds that we observe today appeared over a century ago. Further, most of genetic progress relies on extant genetic variation, although there is evidence of new recent mutations and of extant mutations with modest effect that increased dramatically in frequency because of artificial selection.

So, why genomic selection and what about machine learning role? The reason is two-fold: First, we live in a competitive world and every gain, even if small, can make a difference; Second, breeding programs are large enterprises, molecular data and statistical techniques work by the law of big numbers even if underlying methods are only grossly accurate.

What is the fuss about machine learning? Which is its potential contribution to breeding programmes? Very roughly, machine learning comprises a wide array of algorithms designed to 'learn' regularities from the data at hand and make predictions of outcomes given new, *similar* data. The process of learning is dubbed 'training' and consists of adjusting parameters so that prediction of new data is optimum.

Genomic prediction consists of the prediction of genetic merit using genome wide marker information. Many algorithms exist, including linear models but also more recent machine learning techniques, such as 'deep learning'.

Deep learning is the most popular type of algorithms and are themselves a very heterogeneous class. All share the general principles, and consist of layers of 'neurons' (ie, non-linear transformations of the input data) and where the outputs of a layer neurons are the input of the next layer. Deep learning algorithms are very complex, they require lots of fine tuning. So far, they have not outperformed standard methods based on linear models. However, they are the best option in other problems such as in video or image analyses. They have become standard to measure new phenotypes like behavior, disease detection using images or growth modeling from satellite images.

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I4-02. Improving productivity through genetic regulation of meristematic function

Fernando J. Yuste-Lisbona

Centro de Investigación en Biotecnología Agroalimentaria (CIAIMBITAL), Universidad de Almería. 04120 Almería, Spain.

A fascinating feature of plant growth and development is that plants develop new organs continually throughout their lifetime. This ability relies on specialized groups of cells called meristems, whose function is to integrate a diversity of signals for coordinating stem cell self-renewal and differentiation into organ founder cells. Understanding the genetic regulation of meristem activity is particularly relevant in crop species, as it has a huge impact on fundamental agronomic traits such as plant architecture, the final number of flowers developed per inflorescence, and the shape and size of a mature fruit (1). Using tomato (*Solanum lycopersicum* L.) as a model species, it is shown how crop productivity can be improved and fine-tuned by exploiting combinations of selected mutations affecting meristematic function. Combinations of particular mutations affecting several components of the florigen flowering pathway have demonstrated their capability to customize plant architecture and flower production (2). The functional role of dosage balance among genes controlling inflorescence meristem maturation, and how to use genome editing technology to create a quantitative range of inflorescence architectures that translated to improved productivity will be also shown (3). Lastly, it is examined the activity of floral meristem, whose size defines the number of carpels formed in a flower and, hence, the number of fruit locules, affecting both fruit size and shape (4). In addition to the classical *CLAVATA-WUSCHEL* stem cell circuit controlling meristem size (3,4), special attention will be pay to the role of *ENO* as a key member of the transcriptional network that regulates stem cell proliferation in a flower-specific manner (5). Overall, this communication aims to illustrate how deciphering master gene regulators and genetic networks involved in controlling meristem function will provide the best scenario for the development of knowledge-driven breeding strategies to optimize yield related traits.

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O4-01. Development of new grape cultivars better adapted to climate change

Cristina Manso-Martínez, María del Mar Hernández-Álamos, María Pilar Sáenz-Navajas, Cristina M. Menéndez

Instituto de Ciencias de la Vid y el Vino (Universidad de La Rioja, CSIC, Gobierno de la Rioja) Logroño, Spain

Adaptation to climate change is a major breeding challenge in grapevine. Global warming promotes an advancement in plant growth periods, with significantly earlier *véraison* and harvest dates, resulting in incomplete berry phenolic maturity (1). Reduction in anthocyanin content, color and aroma expression leads to wines characterized by high alcohol content, low total acidity and less aroma (2).

We initiated, together with Viveros Provedo, a breeding program in Rioja 15 years ago based on generating genetic variability from intraspecific crosses among autochthonous varieties, Tempranillo, Graciano y Garnacha. Our initial hypothesis was that crosses could deliver genotypes able to produce high quality wines that would allow broadening the sensorial variability of traditional varieties and adapt to new market preferences. Moreover, selecting earlier and later genotypes with slow ripening would allow the identification of new cultivars with optimal phenolic maturity and better adapted to the new climatic conditions.

Two F1 segregating populations obtained from crossing Graciano and Garnacha as female parents with Tempranillo (pollen parent), consisting of 150 and 135 unique genotypes respectively, were studied from 2007 to 2018. Progenies and parental genotypes were evaluated for 27 agronomic and enological traits and 12 and 14 genotypes were selected based on berry weight, cluster weight, yield, anthocyanin content and ripening date (3).

Wines from twelve Graciano x Tempranillo selections were analyzed in two consecutive years, at the physicochemical level. Sensory properties and quality were evaluated by a trained panel and a group of wine experts, respectively. Wines presented high sensory variability differing in eight attributes in each vintage. Two high quality selections TG63 and TG8, consistently improved Tempranillo and Graciano, presenting high color intensity, acidity and positive aroma related to red fruit. Two early-ripening genotypes TG63 and TG43 have been submitted to Property Registry in 2020 and one late-ripening with high polyphenol content and fruity aroma, TG129, will be submitted in 2021. Other selections with roasted or dried fruit aroma notes appear as potential cultivars suitable to satisfy distinct consumer demands in the context of global warming.

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O4-02. Genetic improvement programs in aquaculture

Miguel Angel Toro[a], Jesús Fernández[b], María Saura[b] Almudena Fernández[b], Silvia García-Ballesteros[b], Beatriz Villanueva[b]

[a]Departamento de Producción Agraria, ETSIAAB, UPM, 28040 Madrid, Spain, [b]Departamento de Mejora Genética Animal, INIA, 28040 Madrid, Spain

Only about 10% of current global aquaculture production uses genetically improved stocks, so there is great potential to increase the efficiency and economic benefit through genetic improvement. An initiative in this context was he FISHBOOST project whose objective was to convert this potential into economically profitable and socially acceptable genetic improvement schemes, and promote them for each of the six target species: Atlantic salmon, common carp, European seabass, gilthead seabream, rainbow trout and turbot. In this five-year project, 14 European aquaculture RTD organizations have participated together with 7 SMEs, 4 large industries and 1 NGO.

The project has focused on disease resistance and production efficiency traits and on the development of genomic tools for these species. Genetic parameters of five important diseases in European aquaculture have been estimated. Results from GWAS and linkage analysis experiments show significant genome-wide QTL accounting for between 12 and 69% of the genetic variation in disease resistance. In general, there were no significant genetic correlations between disease resistance and production traits. The project has identified non-lethal indirect selection criteria for fillet yield and feed efficiency, which presently cannot be efficiently selected for due to the impossibility of recording those traits on live breeding candidates. Results indicated that different lipid% and fillet% showed the strongest relationships to feed efficiency. Genotyping by RAD-sequencing approaches were developed for the cost-effective discovery and population specific genome-wide genotyping of samples. Results suggested that the accuracy of genomic selection could be improved by combining information acrosss traits. Also, within-family genomic selection, which uses low-density markers and pedigree information, was found to be a cost-effective way to implement genomic selection in these species, because family sizes are large.

Finally, the project has developed software tools to assist breeders in the design of their breeding programs: BASEPOP to help aquaculture producers select strains or individuals within the strain with the overall goal of maximizing genetic variability in the breeding population, FISHBOOSTSEL is a practical tool for selection and mating of parents in aquaculture populations and GAINFISH for predicting genetic gain and inbreeding for different selection and mating strategies.

This work was supported by the European Union's Seventh Framework Programme (KBBE.2013.1.2-659 10) under Grant Agreement No. 613611.

FT4-01. GWAS ANALYSIS IN THE FIRST EGGPLANT MAGIC POPULATION IDENTIFIES STRONG ASSOCIATIONS FOR ANTHOCYANIN PIGMENTATION

Andrea Arrones[a], Giulio Mangino[a], Pietro Gramazio[b], Mariola Plazas[a], Jaime Prohens[a], Santiago Vilanova[a]

[a]Instituto de Conservacióny Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain, [b]Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan

Multi-parent advanced generation inter-cross (MAGIC) populations are of great interest for the genetic dissection of quantitative traits, addressing principal limitations of bi-parental populations (1). For the development of the first eggplant MAGIC population eight highly phenotypically diverse founders were selected including a wild *Solanum incanum* accession (2). After three generations of selfing by single seed descent (SSD), 420 S3 individuals were phenotyped for fruit anthocyanin and genotyped by means of the Single Primer Enrichment Technology (SPET) technology. The analysis shows that the population has a high degree of homozygosity (93.13%) and lack of population structure. A Genome-Wide Association Study (GWAS) revealed strong associations on chromosome 10 near to a gene similar to *MYB113* (SMEL_010g351850.1), a regulatory transcription factor known to control anthocyanin biosynthesis in eggplant (3). The ortholog of this gene in tomato has been described as the best candidate for anthocyanin fruit biosynthesis, corresponding to *SlAN2*-like (Solyc10g086290) (4). These results demonstrate the potential usefulness of the eggplant MAGIC population for shedding new light and more conclusive candidate genes for fruit anthocyanins.

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This work was supported by the Ministerio de Ciencia, Innovación y Universidades, Agencia Estatal de Investigación and Fondo Europeo de Desarrollo Regional (grant RTI2018-094592-B-I00 from MCIU/AEI/ FEDER, UE) and European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 677379 (G2P-SOL project: Linking genetic resources, genomes and phenotypes of Solanaceous crops). Andrea Arrones is grateful to Spanish Ministerio de Ciencia, Innovación y Universidades for a pre-doctoral (FPU18/01742) contract.

FT4-02. Haplotype-resolved, chromosome-level, reference-free assembly enabled by high throughput single-cell sequencing of gamete genomes

José A. Campoy[1], He-Quan Sun[1,2], Manish Goel[1,2], Wen-Biao Jiao[1], Kat Folz-Donahue[3], Nan Wang[4], Manuel Rubio[5], Chang Liu[4], Christian Kukat[3], David Ruiz[5], Bruno Huettel[6], Korbinian Schneeberger[1,2]

[1] Genome Plasticity and Computational Genetics, Chromosome Biology Department, Max Planck Institute for Plant Breeding Research, Cologne, Germany
[2]Faculty of Biology, LMU Munich, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany
[3] FACS & Imaging Core Facility, Max Planck Institute for Biology of Ageing, Cologne, Germany
[4] Center for Plant Molecular Biology (ZMBP), University of Tübingen, Auf der Morgenstelle 32, 72076 Tübingen, Germany
[5] Plant Breeding Department, CEBAS-CSIC, Murcia, Spain
[6] Genome Centre Cologne, Max Planck Institute of Plant Breeding, Cologne, Germany

The generation of haplotype-resolved assemblies in heterozygous diploid genomes remains challenging due to the presence of two similar but not identical chromosome pairs, for which traditional assembly tools work towards a simultaneous assembly of both chromosome copies. A recent solution for this problem is the separation of whole-genome sequencing reads into two sets using sequence similarity to the respective parental genomes (Koren et al., 2018), i.e., each resulting set includes reads from only one haplotype. Once this is achieved, the two haploid genomes can be assembled independently. While the method is more efficient (than others), its application is limited in species for which the paternal genomes are not accessible.

In my presentation, I will present “Gamete binning” (Campoy et al., 2020), the method which solves this problem independent of the paternal genomes or any pedigree information. We assembled both chromosome sets independently by sequencing the individual genomes of hundreds of pollen (derived from the focal individual: an apricot tree). In addition, the recombination in the pollen helped to anchor the assembly contigs to chromosomes without the need of growing a mapping population.

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FT4-03. EXPLORING THE GENOMIC DIVERSITY OF WHEAT LANDRACES FOR NEWBREEDING CHALLENGES

Matilde López-Fernández[a], Laura Pascual[a] , Isabel Faci[a], Mario Fernández[a], Magdalena Ruiz[b], ElenaBenavente[a] and Patricia Giraldo[a]

[a] Department of Biotechnology-Plant Biology, School of Agricultural, Food and Biosystems Engineering, Universidad Politécnica de Madrid, 28040 Madrid, Spain, [b] National Plant Genetic Resources Centre, NationalInstitute for Agricultural and Food Research and Technology, 28800 Alcalá de Henares, Spain.

Since the Green Revolution in the 60's, the genetic base of wheat has been narrowed as only a small set of elite cultivars has been used in the development of new varieties. Therefore, there is a need for introducing new germplasm with unexploited diversity in breeding programs. In this context, wheat landraces, specifically adapted to their region of origin and traditionally grown with less inputs, represent an important source of genetic variability (1), underlying not only adaptability, but also quality-related traits. Landraces might harbour variability for breeding programs, but their proper use requires a deep characterization for quality and agronomic traits. The main objective of this study is to analyze the variability of a collection of 189 bread wheat landraces from the Spanish NationalPlant Genetic Resources Centre (CRF-INIA) in relation to agromorphological and end-use quality traits. Previous studies have shown the wide genetic diversity represented in this setof Spanish wheat landraces compared to other germplasm collections (2). Phenotyping has been conducted under field conditions over four years. Days to heading and to maturity, plantheight, growth habit, spike length, grains per spike, thousand kernel weight, awnedness, kernel hardness, grain size and colour, grain protein content and gluten strength wererecorded. The high-throughput genotyping data (DArT-seq) obtained in a previous study (2) have been complemented with the genetic characterization for high molecular weight glutenin and puroindoline allelic composition. The results have shown the presence of novel variability for glutenins and puroindolines. The relation between glutenin composition and gluten strength has been evaluated, and several good quality alleles have been identified in the collection, some of them specific of Iberian landraces (3). We have also investigated the influence of other genomic regions in gluten strength by Genome Wide Association Studies (GWAS) analysis and some interesting associations have been detected. Currently, a GWASanalysis with all the agromorphological and quality traits analysed is being carried out in order to deep insight in the genetic control of these complex characters in wheat. This information can be very valuable in wheat breeding programs.

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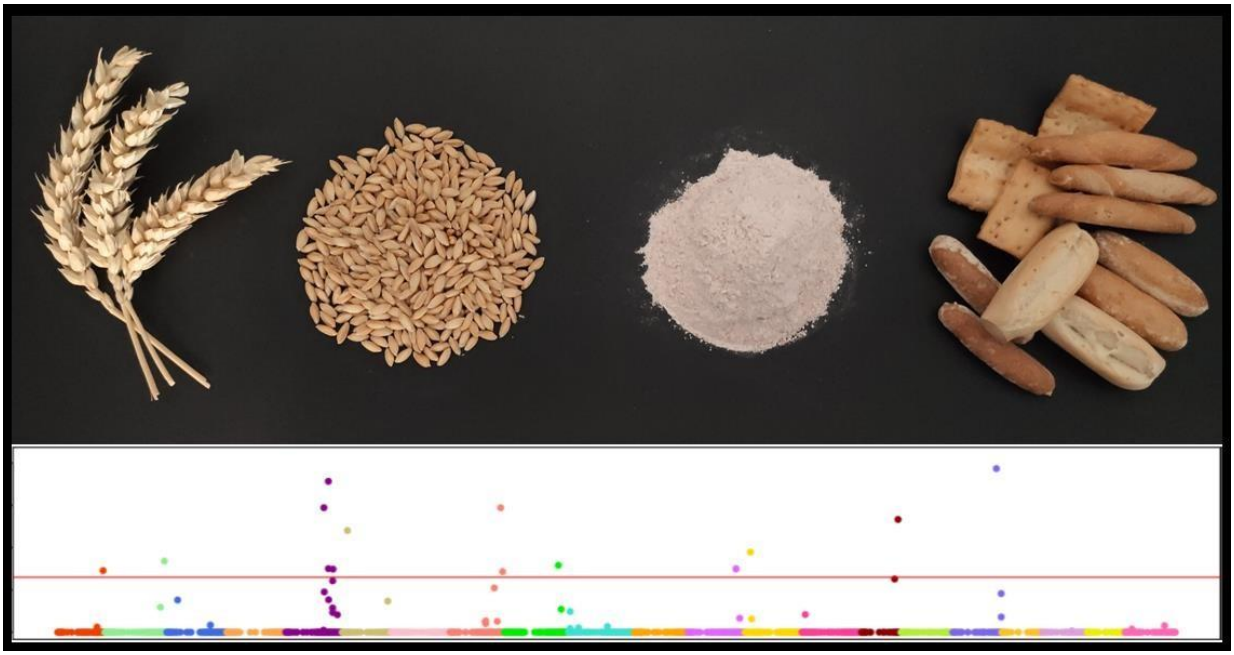
Graphical abstract:



FT4-04. Atlas of phenotypic, genotypic and geographical diversity underlying variability of the traditional European tomato

Clara Pons [a,b] on behalf of TRADITOM consortium

[a]COMAV, Universitat Politècnica de València, Valencia, Spain, [b] Plant Genomics and Biotechnology, IBMCP (CSIC-UPV), Valencia,Spain.

The large diversity of traditional European tomato (TradiTOM) existing now in the Mediterranean basin countries, a secondary center of diversification, is the result of 500 years of farmer-driven selection for culinary use and local adaptation. Traditional farmers introduced additional variability by fixation ("traditionalization") of commercial varieties after companies started to get involved in breeding around XVIII-XIX centuries. Traditional varieties are scarcely used outside local areas of cultivation, but they can be useful to improve the tomato sensory profile and contribute to a modern sustainable agriculture. The analysis of variation in tomato in previous works has been mainly focused on domestication and modern plant breeding events; however, information about the variation present in secondary centers of diversification of tomato is still limited.

To study the phenotypic and allelic diversity available in TradiTOM, we have evaluated the greatest tomato collection, including 1315 varieties that had been cultivated sometime from 1950 to 2015 in the main TradiTOM cultivation areas of South of Europe (Spain, Italy, France and Greece). Analyses of 67 agronomic traits indicated that TradiTOM displays a broad range of phenotypic variability with different distribution among countries and that tomato diversification in each country was mainly driven by gastronomical use. Furthermore, eight main phenotypic macrotypes of TradiTOM were defined. GWAS meta-analyses identified 1486 associations, mapping in 581 SNPs located in 211 loci, several of them shared between different traits. A detailed study indicated that the associations in 159 of those loci were novel, while for 99 loci were reported before for the same or related trait. Finally, multidimensional integration of GWAS meta-analysis SNPs, phenotypic, geographical and usage characteristics identified the molecular signatures for each traditional phenotypic macrotype and indicated that they were originated by differential combination of loci, in some cases with different genetic histories, that converge in the same phenotypic macrotype.

This atlas provides a unified set information covering a wide collection of European traditional tomato, revealing their value and providing a roadmap to study and exploit this untapped tomato diversity

This project has received funding from the European Union's Horizon 2020 research and innovation programmes under Grant Agreement Numbers 634561 and 101000716

FT4-05. A GDSL ESTERASE/LIPASE MEDIATES LUTEIN ESTERIFICATION IN TRITORDEUM BEING A STRONG CANDIDATE FOR THE IMPROVEMENT OF CAROTENOID STABILITY IN RELATED CEREALS.

María Dolores Requena-Ramírez[1], Sergio G. Atienza[1], Dámaso Hornero-Méndez[2], Cristina Rodríguez-Suárez[1].

[1]Instituto de Agricultura Sostenible, CSIC, Córdoba, España, [2]Instituto de la Grasa, CSIC, Sevilla, España.

Carotenoids are essential in the human diet (1), so that the development of carotenoid improvement programs in many staple crops has been promoted. Tritordeum (the amphiploid derived from the cross between *Hordeum chilense* Roem. et Schultz. and durum wheat) has a high content of carotenoids in the endosperm being most of them esterified with fatty acids (2). Esterified carotenoids have both greater capacity for accumulation in cells and stability during postharvest storage (1). We analyzed five genes, previously identified as candidates in common wheat (3), for lutein esterification in the *H. chilense* genome. All of them were expressed during the development of tritordeum grain, but only *HORCH7HG021460* was highly up-regulated. Sequence analysis of *HORCH7HG021460* revealed a Glycine to Cysteine substitution in the zero-ester accession H290 of *H. chilense* compared to the esterifying genotypes H7 and H16. Genotyping the *H. chilense* collection revealed that only H290 carried the SNP producing the Glycine to Cysteine modification (4), being also the only zero-ester accession (5). In addition, the role of HORCH7HG021460 in lutein esterification in tritordeum is supported by the findings in wheat. Indeed, HORCH7HG021460 is the ortholog of XAT-7D (6), the gene responsible for carotenoid esterification in common wheat. Therefore, *HORCH7HG021460* (*XAT-7Hch*) is a strong candidate for lutein esterification in tritordeum. This suggests a common carotenoid esterification mechanism in Triticeae and thus it would be a good candidate for the improvement of carotenoid stability in tritordeum and related cereals.

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FT4-06. Identification of genes involved in volatile synthesis in the Melon genome

Marta I. Terry[1], Iñaki Bienzobas[1], José Antonio Bernabé Díaz[2], Jesualdo Tomás Fernández Breis[2], Pau Bretó[3], Miguel Aranda Regules[4], Julia Weiss[1], Marcos Egea-Cortines[1]

[1]Ingeniería Agronómica-Genética, UPCT, Cartagena, Spain, [2]Informática y Sistemas-Lenguajes y Sistemas Informáticos, UM, Murcia, Spain, [3]Abiopep S.L., Murcia, Spain, [4]Biología del Estrés y Patología Vegetal-Patología Vegetal, CEBAS-CSIC, Murcia, Spain
Corresponding author: Marta Isabel Terry (marta.terry@edu.upct.es)

Plants synthesize, store and release a huge number of volatiles organic compounds (VOCs) in roots, leaves, flowers or fruits. These VOCs plays several critical roles including pollinator attraction and plant defence. Interestingly, this set of chemicals and volatiles differ among species and crops varieties. It is a potential trait in plant breeding due to its importance in natural disease or pest resistance. Three challenges arise when studying the genetics of VOCs synthesis. First some enzymes are promiscuous i.e. can use different substrates, and from a single substrate produce several products. Second the molecular relation between sequence and product or products is not known. And third, the enzymes and corresponding genes are not known. We have analysed volatile content in leaves of 8 melon genotypes and found a total of 89 VOCs. We found 29 compounds with known genes. These compounds were synthesized by 65 enzymes with a total of 279 transcripts found in the genome of melon. Filtering for allelic variants and mutations for the complete families identified genes identical in all varieties such as Linoleate diol synthase or Benzoic Acid Carboxymethyl Transferase. Other gene families showed a very large amount of polymorphisms such as Alpha-humulene:(-)-(E)-beta-caryophyllene synthase or Lipoxygenase 1. We used gcProfileMakeR [1], a newly developed bioinformatics package to identify constitutive and non-constitute volatiles in order to pin down possible loss of function alleles involved in distinct volatile synthesis.

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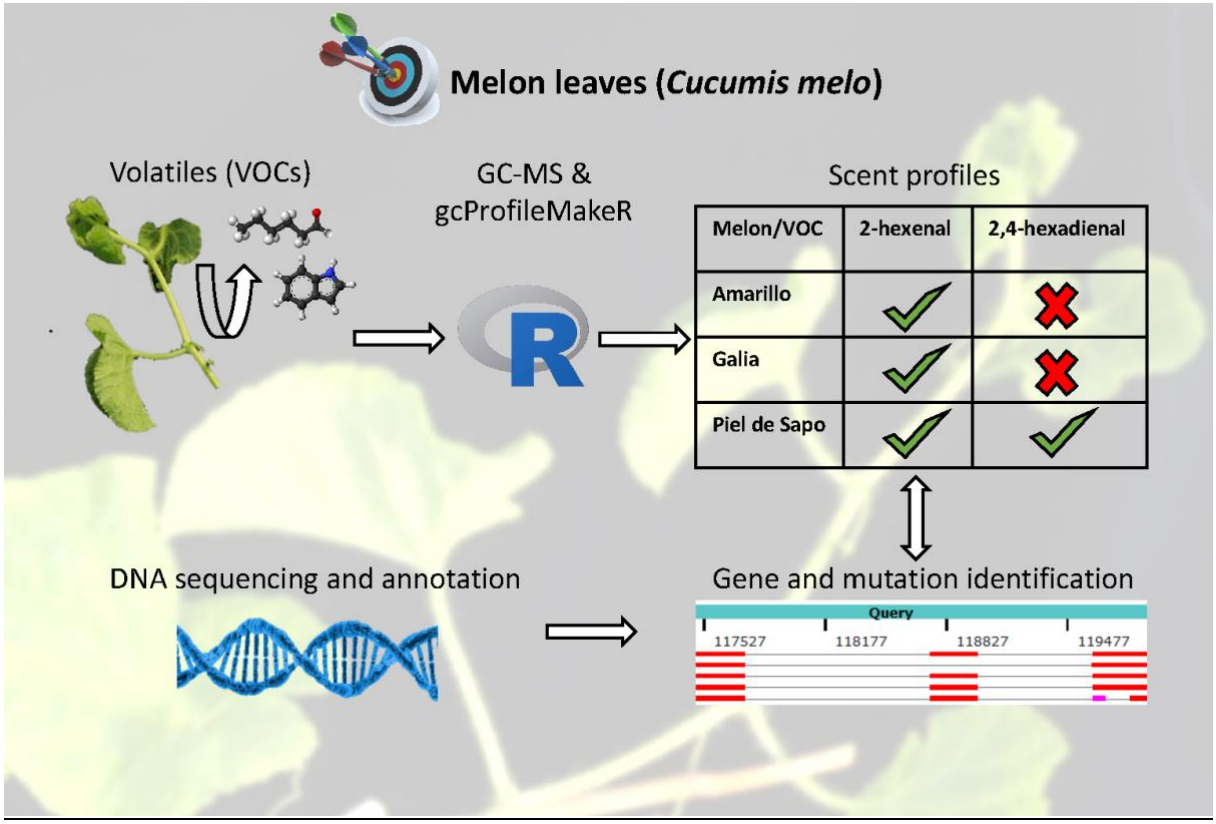
This work was funded by RIS3 MELOMUR and FEDER

Graphical abstract:



P4-01. Genotyping and fine mapping of the non-prickly trait in a *Solanum melongena* introgression line with the wild species *S. insanum*

David Alonso[a], Sergio García[b], Pietro Gramazio[c], Jaime Prohens[a], Santiago Vilanova[a]

[a]*Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022, Valencia, Spain*
[b]*Centre for Research in Agricultural Genomics, Barcelona, Spain*
[c]*Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan*.

Prickliness is an undesirable trait in cultivated eggplant (*Solanum melongena*), as prickles difficult the management of the crop, may cause wounds to field workers and, when present in the calyx, to retailers and consumers. The presence of prickles is found in different varieties, landraces, and wild species related to cultivated eggplant. For this reason, one of the objectives of breeding in eggplant is the selection of materials that display an absence of prickles. For an early selection of the most promising non-prickly varieties through molecular marker-assisted selection (MAS) it is essential to determine the genetic control of this character and to develop molecular markers linked to the trait. Several works have determined major QTLs for presence of prickles in chromosome 6 (1-3). Using a BC3S1 advanced backcross population of 45 individuals between the prickly wild species *S. insanum* and a non-prickly variety of eggplant, we have narrowed down the putative genomic region that controls prickliness to a 96 Kb located at the end of chromosome 6.

In addition, a panel of 8 SNP markers from the target region were designed and assessed on a total of 600 BC4 individuals derived from a single prickly BC3S1 line by high resolution melting (HRM). Four out of sixteen genes on this region were identified as candidate genes for the presence of prickles based on its biological functions. Evaluation of the expression of these candidate genes is currently being performed using RT-qPCR methodology. Differential genes expression in plants with and without prickles will provide insight into the regulation of the trait.

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Global Crop Diversity Trust: "Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives" initiative. RTI-2018-094592-B-I00 from MCIU/AEI/FEDER, UE. European Union's Horizon 2020 Research and Innovation Programme grant agreement No. 677379 Predoctoral grant to David Alonso (PAID-01-16).

P4-02. Characterization of tomato mutants altered in leaf development

Marybel Jáquez-Gutiérrez[a], Alejandro Atarés[a], Benito Pineda[a], Pilar Angarita[ac], Carlos Ribelles[a], Begoña García-Sogo[a], Jorge Sánchez-López[ad], Carmen Capel[b], Fernando J. Yuste-Lisbona[b], Rafael Lozano[b] and Vicente Moreno[a]

[a]Instituto de Biología Molecular y Celular de Plantas, UPV-CSIC, Ingeniero Fausto Elio s/n, Valencia, Spain. [b]Centro de Investigación en Biotecnología Agroalimentaria (BITAL), Universidad de Almería, Spain. [c]Facultad Ciencias de la Salud, Universidad Cooperativa de Colombia, Barrio Barzal, Villavicencio, Colombia. [d]Facultad de Agronomía, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, Mexico.

The characterization of mutants has been a determinant strategy to gain new insights into genes and mechanisms involved in leaf development (1). Leaf morphology of 971 tomato T-DNA lines was evaluated *in vitro* in seedlings and shoot-derived axenic plants. Following this screening *in vitro*, putative mutants altered in leaf morphology were evaluated in the greenhouse. The comparison of results in both conditions indicated a general phenotypic correspondence, showing that *in vitro* culture is a reliable system for finding mutants altered in leaf development. The main advantage of *in vitro* screening lies in its homogeneous environmental conditions, the enormous savings in time and space and the possibility of studying characters such us adventitious organogenesis (2). New mutants putatively altered in brassinosteroid metabolism, mutations determining multiple pleiotropic effects, and the first tomato mutant with helical growth were discovered (3). Studies on the association between phenotype and *nptII* gene expression showed co-segregation in 2635-MM line. This mutant has an extremely irregular number and distribution of leaflets in adult leaves and a smaller amount of viable pollen. To identify the gene tagged in this line anchor-PCR assays were performed to clone the genomic regions flanking the T-DNA insertion. Results revealed that T-DNA was located between exons 8 and 9 of the Solyc01g104410 gene, which encodes for a Sterol 3-beta-glucosyltransferase. Expression analysis suggested that abnormal leaf development might be due to the lack-off-function of this gene.

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P4-03. MOLECULAR CHARACTERIZATION OF THE *320etmm* TOMATO INSERTIONAL MUTANT IDENTIFIES A CANDIDATE GENE INVOLVED IN PLANT ARCHITECTURE AND FLORAL INITIATION

Manuel F. Blanco[a], Carmen Capel[a], Carlos Ribelles[b], Benito Pineda[b], Juan Capel[a], Vicente Moreno[b], Rafael Lozano[a]

[a]Centro de Investigación en Biotecnología Agroalimentaria (CIAIMBITAL). Universidad de Almería, 04120 Almería, Spain. [b]Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Univ. Politècnica de València, 46022 Almería, Spain.

Ten years ago, next generation sequencing approaches made possible to determine the complete sequence of the tomato (*Solanum lycopersicum* L.) genome. Since then, new structural genomics techniques have allowed the resequencing of an increasing number of genotypes, which implies a revolution for the comparative genomics of the genus. However, functional genomics needs to be improved to close the gap between gene annotation and gene functional assignment. With this aim, mutant collections are among the most valuable resources to identify novel gene functions in model agricultural species. Here, we present the characterization of a tomato mutant with altered vegetative and reproductive development, named as *320etmm*, identified as part of a tomato insertional mutant collection[1]. Mutant plants show a complete loss of apical dominance, and high proliferation of axillar branches, plants are significantly compact and develop chlorotic and variegated leaves. Also, reproductive development is altered, since they lack flowers, although occasionally micro-inflorescences that fail to complete development can be observed. The mutant phenotype co-segregates with the T-DNA selective marker that allowed us to characterize the insertional mutation. The T-DNA insertion caused a deletion of 64 pb in the promoter region of two adjacent genes transcribed in opposite directions in chromosome 2. These genes encoded members of the endoribonuclease and kinase families. Quantitative PCR analysis demonstrated that both genes were down-regulated in all the mutant tissues analysed. In order to demonstrate which of the two candidate genes affected by the T-DNA insertion was responsible for the mutant phenotype, single and double mutants were generated by CRISPR/Cas9. Taken together, these results demonstrate that insertional mutagenesis is a valuable tool for functional genomic analysis in tomato.

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P4-04. Phylogenetic relationships and conserved domain analysis of ENO homologues

Sandra Bretones, Fernando J. Yuste-Lisbona, Rafael Lozano

Centro de Investigación en Biotecnología Agroalimentaria (CIAIMBITAL), Universidad de Almería. 04120 Almería, Spain.

The APETALA2 (AP2) superfamily is one of the largest families of plant transcription factors, whose members differ by the presence of one or two AP2 domains. Thus, AP2 and Ethylene Response Factor (ERF) subfamily genes possess a double tandem-repeat and a single AP2 domain, respectively (1). The ERF subfamily genes are subdivided into 12 groups and are mainly involved in the response to biotic and abiotic stresses in many taxa (2). However, within the ERF subfamily, several members of the VIII group have also emerged as regulators of plant growth and development by modulating meristem function in diverse plant lineages and influencing key agronomic traits of several crop species; they represent potential candidates for agronomic improvement (3). *EXCESSIVE NUMBER OF FLORAL ORGANS* (*ENO*) is one of the ERF VIII subgroup members, which play a crucial function in the transcriptional network that regulates floral meristem (FM) size in tomato. Our results indicate that ENO directly regulates the expression domains of the *WUSCHEL* stem cell identity gene in a flower-specific manner. Consequently, the loss-of-function of *ENO* gives rise to alterations in FM size leading to the development of flowers with supernumerary organs and the formation of heavier and larger multilocular fruits, which in turn result in higher yield (4). Given its developmental role and agronomic significance, this work addresses the phylogenetic and evolutionary relationships of ENO homologues. A phylogram was generated using the ENO homologue protein sequences from 41 species for 12 representative phylogenetically diverse plant taxa, from bryophytes to Asterales. Phylogenetic relationship among ENO homologues and characterization of the AP2 functional domain diversity in non-model and crop species can potentially represent a basis for future crop enhancement.

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P4-05. Identification of open-pollinated Brassica lines suitable for organic farming in the Valencian region

Carla Guijarro-Real[1], Estela Moreno[1], Ana Fita[1], Noemi Uehlinger[2], Adrián Rodríguez-Burruezo[1]

[1] Instituto Conservación y Mejora Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain
2 SATIVA Rheinau AG Seeds, Winterthur, Switzerland

Brassicas include a variety of crops of high economic interest along Europe. Most of their production depends on F1 hybrids, which are based on cell-fusion cytoplasmic male sterility (1). Their use in organic farming (OF) is controversial; instead, open-pollinated (OP) populations are an alternative, which match the preferences of organic consumers (2). Here are showed the preliminary results from an agronomic characterization, farmer-collaborative, of preselected OP *Brassica* materials with potential for OF. *B. oleracea* OP varieties including 5 broccoli, 4 pointed cabbages, and 9 kohlrabi were tested under organic conditions together commercial F1 hybrids. Germination in the OP broccoli lines ranged between 67.7–85.9%. Also, most of OP lines showed low vigour and/or head formation, although one line was identified of high utility value in terms of production and head development. Pointed cabbages showed percentages of germination comprised between 65.7–82.3%, while the F1 hybrid reached 86.9%. One OP line showed high heterogeneity in vigour and on marketable heads, thus reducing its commercial value. However, the remaining lines were of medium-high utility value, although the developed heads were of minor weight and density compared to the F1 control. Finally, most of the OP kohlrabis showed similar germination ratios (>75%) and five OP lines showed commercial productions close to F1 hybrids, considering the number and weight of bulbs and crop precocity. Unlike the green hybrids, two OP lines developed purple bulbs as an added value to the crop. Overall, the results suggest that *Brassica* OP varieties may be considered as a competitive alternative for OF in the Spanish Mediterranean region, where these materials were tested, especially broccoli and kohlrabi varieties. This work represents a basis for the establishment of a competitive and more resilient OF, using diverse OP *Brassica* materials which also fit better the preferences of organic consumers (3).

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P4-06. GENOME-WIDE ASSOCIATION STUDIES OF MILK YIELD AND QUALITY TRAITS IN THE ASSAF SHEEP BREED.

Kenza Lakhssassi[a,b], María Piquer-Sabanza[a], Yasemin Öner[c], Pilar Sarto[a], Laura Pilar Iguacel[a], Olaia Estrada[a, †], Malena Serrano[d], Teresa Juan[a], Jorge Hugo Calvo[a,e]

[a] Unidad de Producción y sanidad animal, CITA - IA2 (CITA–Zaragoza University), Zaragoza, Spain, [b] INRA, Rabat, Morocco, [c] Department of Animal Science, University of Uludag, Bursa, Turkey, [d] Departamento de Mejora Genética Animal, INIA, Madrid, Spain, [e] ARAID, Zaragoza, Spain, [†] Present address: Innovation, Technology Center in Gastronomy, BCC, Donostia-San Sebastián, Spain

Knowledge of genetic markers associated with milk production traits provides an opportunity to increase the rate of genetic gain using genomic or marker-assisted selection. Within this context, a genome-wide association study (GWAS) using high-density genotyping was carried out to contribute to the knowledge of the genetic basis of milk production traits in the Assaf sheep breed. In total, 6173 records from 1894 multiparous Assaf ewes from three flocks with at least 3 test day records and aged between 2 and 7 years old were used to estimate a corrected phenotype for the fat (FP), protein (PP), lactose (LP) and total solid percentage (TSP). For milk yield (MY), 2697 records were obtained from 1001 ewes because no MY data were provided from one flock. Genotypes from 192 ewes with the Illumina Ovine HD BeadChip (680K) were used from a previous GWAS for somatic cell count in this population. The GWAS was performed with the GCTA software. No genome-wise significant results were found in the different traits after false discovery rate (FDR) multi-test correction. However, 2, 6, 3, 15, and 25 SNPs associated to MY, PP, FP, LP and TSP traits, respectively, were significant at chromosome level (FDR $p<0.1$). Subsequent validation of two SNPs (rs419686662 and rs411200126 for PP and MY, respectively) through genotyping by KASP was performed in the total population (n=1839 for rs419686662, and n= 1001 for rs411200126). According to the association results, only the SNP rs419686662 was involved in the phenotypic variation of the PP trait. Ewes with the AA genotype showed a higher PP of 0.058 $\pm$ 0.021 and 0.134 $\pm$ 0.034 than those with genotypes GA ($P < 0.05$) and GG ($P< 0.001$), respectively. This SNP is located close to the *FGFR2* gene that is related to mammary gland development.

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P4-07. Genetic and molecular causality of *fruit iterative growth* (*fig*) mutant evidence the functional role of *SlCRCa* gene in regulating floral meristem determinacy

Laura Castañeda[a], Estela Giménez[b], Trinidad Angosto[a], Ana Ortíz[a], Begoña García Sogo[c], Benito Pineda[a], Juan Capel[a], Vicente Moreno[c], Fernando J. Yuste-Lisbona[a], Rafael Lozano[a]

[a]*Centro de Investigación en Biotecnología Agroalimentaria (CIAIMBITAL), Universidad de Almería. 04120 Almería, Spain.*
[b]*Departamento de Biotecnología-Biología Vegetal, Universidad Politécnica de Madrid. 28040 Madrid, Spain.*
[c]*Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Univ. Politècnica de València, Valencia, Spain.*

Angiosperms are characterised by producing flowers as reproductive organs to ensure their survival. From their outermost to the innermost whorls, flowers typically consist of sepals, petals, stamens, and carpels that are sequentially generated from a pool of stem cells located in the floral meristems (FM) (1-3). Once a set number of floral organs has been initiated, stem cell activity is arrested, and the FM is thereby determined to form the gynoecium. The precise timing of this developmental event, also referred to as floral determinacy, is a pivotal process that establishes a defined number of floral organs arising from the FM (4-6). As the number of carpels in a flower define the final number of locules forming the mature fruit, a detailed knowledge of the factors that regulate the molecular mechanisms involved in this process in tomato (*Solanum lycopersicum* L.) constitutes a key objective for both developmental biology and agricultural production. In this study, we characterize the *fruit iterative growth* (*fig*) mutant showing developmental abnormalities in floral determinacy. *fig* plants develop a greater organ number in all floral whorls, although this increase affects more significantly to carpels (four whorl). Thus, *fig* ovaries are composed of numerous carpels that lead to the development of anomalous fruits displaying secondary fruit structures initiated from undifferentiated cells originated inside the principal fruit, and that grow in an indeterminate way. We provide evidence that a lack of function of the tomato *SlCRCa*, a homologue of the *CRC* gene, *SlCRCa*, is responsible for the phenotypic defects observed in the *fig* mutant plants. Functional analysis also reveals that *SlCRCa* is needed to regulate floral meristem determinacy by interacting with *WUSCHEL*, the best known regulator of meristem activity.

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P4-08. The functional role of EARLY FLOWERING 4 in coordination of flower opening in Petunia

María Victoria Díaz-Galián[1], Julia Weiss[1], Pedro Javier Navarro[2], Marcos Egea-Cortines[1]

[1]Instituto de Biotecnología Vegetal, Universidad Politécnica de Cartagena, Cartagena, Spain, [2]División de Sistemas e Ingeniería Electrónica, Universidad Politécnica de Cartagena, Cartagena, Spain.

Genes and environmental features interact to modulate responses via the circadian clock allowing plants to adapt to periodic changes, such as daily or seasonal alterations. *EARLY FLOWERING 4* (*ELF4)* was firstly described as a flowering time mutant in Arabidopsis. Later work concluded that the ELF4 protein takes part in the evening complex of the Arabidopsis circadian clock. We analysed the phenotypes of downregulation of *PhELF4*, in *Petunia* x *hybrida* with hairpin constructs. Initial observations indicated that RNAi:PhELF4 had more open flowers per day than WT. However, given the cymose inflorescence and the fact that plant architecture was not modified the increased floral number could not be easily explained. By using a custom artificial vision system, we uncovered an undescribed mechanism coordinating flower opening in Petunia. We called it sequential flowering as most plants opened one flower per day. This process was deregulated for *RNAi:PhELF4* flowers. Flower opening stopped during the night for those wild-type flowers which did not complete the opening process, restarting when plants were exposed to light. A decrease in *PhELF4* expression caused an immediate floral opening, resulting in several flowers opening at once during a short period of four hours in the subjective afternoon. Our results indicate that *PhELF4* is involved in controlling the distribution of flower opening throughout the day.

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P4-09. Heterotic response to salt-stress in hybrids of cultivated eggplant *(Solanum melongena)* and relatives *(S. insanum* and *S. macrocarpon)*

Neus Ortega-Albero[a], Sara Gonzalez-Orenga[a], Óscar Vicente[a], Mariola Plazas, Jaime Prohens[a], Claudia Pallotti[a], Vicente Castell-Zeising[b], Adrián Rodríguez[a], Ana Fita[a]

[a]Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politécnica de València, València, España. [b]Departamento de Producción Vegetal, Universitat Politècnica de València, València, España

Eggplant *(Solanum melongena)* is known to be moderately sensitive to salinity (1), and related species could be good candidates to improve its performance under stress (2). In this study, the response to salinity of hybrids between cultivated eggplant and its relatives *S. insanum* and *S. macrocarpon* has been studied. Young plants of *S. melongena, S. insanum, S. macrocarpon* and its hybrids *S. insanum* x *S. melongena* and *S. macrocarpon* x *S. melongena* were irrigated every three days for four weeks with solutions containing: 0 (control), 200 mM and 400 mM NaCl. Plant growth was quantified measuring the following parameters: number of leaves, plant height, stem diameter, fresh and dry weight of leaves, stem and roots, leaf, stem and root water content, leaf surface and root length. Growth reduction due to salt stress was more pronounced in the eggplant relatives than in hybrids, in parameters such as leaf fresh weight, leaf surface or plant height. Although at higher salt concentration the differences are attenuated, we found that the hybrids are more tolerant than any of the parents. These results suggest a heterotic effect for tolerance to salt stress in the hybrids. Currently, we are analysing biochemical parameters in these genotypes to establish their relation with the physiological responses and unravel the genetic control of such tolerance.

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P4-10. Inheritance of resistance to downy mildew in quinoa accession PI614911

Sara Fondevilla

Instituto de Agricultura Sostenible, Consejo Superior de Investigaciones Científicas, Córdoba, Spain

Downy mildew, caused by *Peronospora variabilis* Gäum, is the most important quinoa disease worldwide. In Spain this disease severely affects quinoa crop, infecting up to 90% of plant area and causing defoliation in susceptible cultivars under favourable conditions for the development of the disease. Despite the relevance of this trait for quinoa breeding, little is known about the genetics of resistance to downy mildew in quinoa (1). Quinoa accession PI614911 has shown high level of resistance to this disease under field conditions in Córdoba (Spain) during several seasons. The objective of this study was to unravel the inheritance of resistance to downy mildew in this accession. Towards this objective, accession PI614911 was crossed with a susceptible line and segregation of resistance analysed in the F_2 generation under field conditions in Córdoba, Spain. Differences between resistant and susceptible plants were evident. The segregation ratio in the F_2 population gave a good fit to the 3:1 ratio expected for a single gene inheritance ($\chi 2 = 0.01$; $p= 0.92$). These results demonstrate that resistance to downy mildew in quinoa accession PI614911 is controlled by a single dominant gene. Knowledge of the genetic control of resistance to downy mildew in this accession will be relevant to design the best strategy to introduce this character into new cultivars.

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P4-11. EPIGENETICS CONTROL OF TRICHOME FORMATION IN TOMATO

Rocío Fonseca[a], Carmen Capel[a], Fernando J. Yuste-Lisbona[a], Jorge Quispe[a], Cristina Gómez-Martín[b,c], Ricardo Lebrón[b,c], Michael Hackenberg[b,c], José Oliver[b,c], Trinidad Angosto[a], Rafael Lozano[a] and Juan Capel[a]

[a]Centro de Investigación en Biotecnología Agroalimentaria, Universidad de Almería, Almería, España, [b]Departamento de Genética, Facultad de Ciencias, Universidad de Granada, Granada, España, [c]Centro de Investigación Biomédica, Fundación Parque Tecnológico de Ciencias de la Salud, Granada, España

Tomato (*Solanum lycopersicum* L.) is a highly relevant vegetable crop with great advantages such as a high productivity rates and stress tolerance. Nevertheless, global climate change poses a huge challenge related with the increase of plague pressure as a result of global warming. In this scenario, the development of new tomato varieties with enhanced pest resistance becomes a key goal for breeding of this crop. Trichomes are specialized epidermal structures widely related to biotic stress tolerance since non-glandular trichomes act as a barrier preventing the spread of plagues whereas glandular ones synthetize complex molecules toxic to herbivores. In the present work, the tomato *hairplus* (*hap*) mutant, which shows a higher glandular trichome density has been characterized. The *HAP* gene has been identified by a classical genetics approach supported by a mapping by sequencing approach. Loss of function of *HAP* results in epigenomic modifications expressed as a large number of differentially methylated cytosines located along the entire genome which finally causes transcriptomic changes never before observed in tomato trichome mutants. Results obtained demonstrate that *HAP* links epigenomic modifications with tomato glandular trichome development and demonstrates that is a valuable genomic tool for tomato breeding for pest resistance.

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P4-12. DEVELOPMENT OF A PROTOCOL FOR *AGROBACTERIUM*-MEDIATED TRANSFORMATION AND *IN VITRO* REGENERATION OF *CANNABIS SATIVA* L. TRANSGENIC PLANTS

Alberto Galán-Ávila[1], Pietro Gramazio[2], Mily Ron[3], Jaime Prohens[4], Francisco Javier Herraiz[4],

[1]*Ploidy and Genomics S.L., CEEI VALENCIA, Edificio B, Módulo 20, Avda. Benjamin Franklin, nº12, Parc Tecnològic, 46980, Paterna, Valencia, Spain*
[2]*Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, 305-8572, Tsukuba, Japan*
[3] *Department of Plant Biology, University of California, Davis, CA, USA*
[4]*Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camí de Vera s/n, 46022 Valencia, Spain*
Corresponding author: Alberto Galán-Ávila (agalan@ploidyandgenomics.com)

Despite its economic interest, the development of genetically transformed plants is an elusive landmark in *Cannabis sativa* L. breeding. In the present work, we aimed at evaluating the ability of different explants such as hypocotyls, cotyledons and meristems coming from six *C. sativa* hemp varieties to produce transgenic plants. Plant transformation was evaluated after explant co-culture with *Agrobacterium tumefaciens* strain LBA4404 carrying the binary plasmid pBIN19 containing the *uidA* reporter gene and the kanamycin resistance *nptII* genes. The genetic transformation of the regenerated plants was validated through *in vitro* culture of regenerating shoots in kanamycin-containing selective regeneration medium, by GUS histochemical assay for *uidA* expression, and by PCR amplification of *uidA* and *nptII* genes. Our results showed that hypocotyls reached a higher regeneration rate (53.3%) than cotyledons (18.1%) without *Agrobacterium* co-culture. On the other hand, 100 mg L^{-1} kanamycin proved to be the best concentration in terms of regeneration rate (63.3%) and spontaneous rooting rate of hypocotyl regenerating shoots (12.2%), which displayed a 7.1% of albinism rate. After co-culture with *A. tumefaciens* and subsequent culture in antibiotic-containing selective regeneration medium, hypocotyl was the best explant type achieving 23.1% of regeneration rate, which contrasts with the 1.0% regeneration rate detected for cotyledons. Transgenic plants were obtained from all explant types studied. Although there were significant differences among varieties evaluated, hypocotyls proved to be superior to already-developed meristems, reaching a transformation rate of 5.0% and 0.8% respectively. Despite the extremely low regeneration rate of cotyledons after *A. tumefaciens* co-culture, all cotyledon-derived regenerating shoots analyzed were successfully transformed. However, due to shoot regeneration rate of hypocotyls and rooting ability of hypocotyl-derived regenerants, hypocotyl explants should be considered as the most appropriate of the evaluated explants to produce *C. sativa* transgenic plants. The present work represents a pioneering study documenting the production of *C. sativa* genetically transformed plants, which could have important connotations in *C. sativa* breeding, enabling the use of contemporary techniques like targeted genome editing by using CRISPR/Cas systems for the development of varieties with specific biochemical profiles or resistance to plant pathogens among others.

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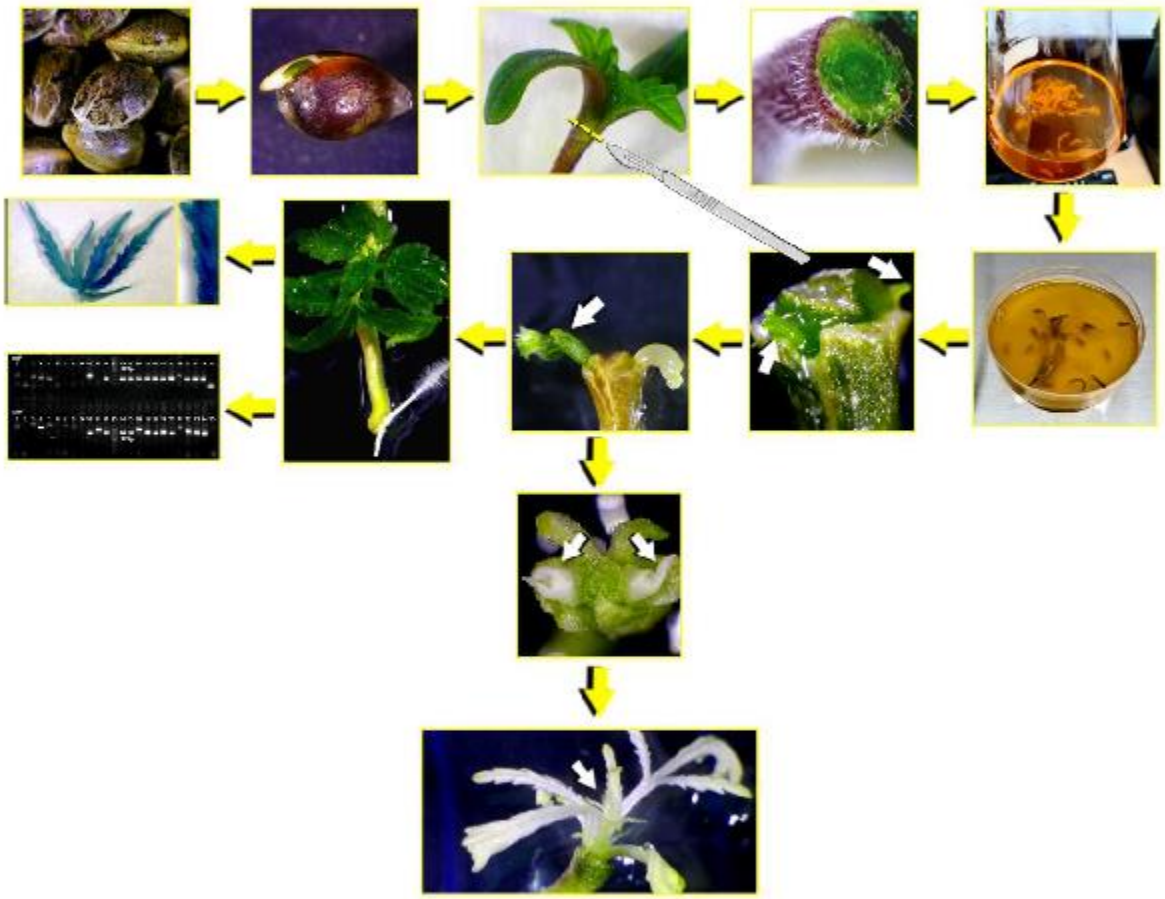
Graphical abstract:



P4-13. Searching for genetic clues of ozone tolerance in wheat

Estela Giménez[1], Laura Pascual[1], Matilde López-Fernández[1], Susana Elvira[2], Patricia Giraldo[1], Victoria Bermejo-Bermejo[2], Elena Benavente[1]

[1] Departamento de Biotecnología-Biología Vegetal, Universidad Politécnica de Madrid, Madrid, Spain
[2] Ecotoxicología de la Contaminación Atmosférica, CIEMAT, Avda. Complutense 40, 28040-Madrid.

Tropospheric ozone (O_3) is a secondary air pollutant and a greenhouse gas, whose concentration has been increasing since the industrial era and is expected to increase further in the near future. O_3 molecules can diffuse through the leaf stomata of plants, triggering significant phytotoxic damage that entail a weakening of the plant, reducing its ability to cope with other abiotic and biotic stresses. This eventually leads to a reduction in the yield and quality of crops. It poses a serious problem in the case of wheat, currently considered the most sensitive cereal to this pollutant, and the need of breeding programs with the ultimate goal of developing commercial varieties tolerant to this contaminant. With the primary aim of identifying wheat genotypes differing in their relative sensitivity to O_3, twelve varieties, including local landraces and commercial cultivars, were cultivated under four different O_3 levels. Several yield components and physiological parameters were assessed to select a subset of 6 varieties more sensitive or more tolerant to O_3. Such varieties were used to analyze the expression levels of genes previously described to be affected by high O_3 levels such as *GS1*, *GS2* (1), *rbcS* (2) and *SOD* (3). The results indicate an upward trend in the expression levels of *GS1* and *rbcS* at high O_3 levels in the tolerant varieties, suggesting that changes in the expression levels of these genes might be responsible of a favourable response of wheat plants to increased high O_3 levels.

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P4-14. GWAs in Durum wheat identified new genomic regions related with root system architecture

Laura Pascual [a], Juan M. González [b], Julián García-Abadillo [a], Yolanda Loarce [b], Rosa Mérida [c], Pilar Hernández [c], Patricia Giraldo [a], Magdalena Ruiz [d]

[a] Departamento de Biotecnología-Biología Vegetal, Universidad Politécnica de Madrid, Madrid, Spain, [b] Dpto. Biomedicina y Biotecnología. Universidad de Alcalá de Henares. Alcalá de Henares, Spain, [c] Dpto. Agronomía. Instituto de Agricultura Sostenible (IAS-CSIC). Córdoba, Spain, [d] Centro Nacional de Recursos Fitogenéticos (INIA-CSIC), Madrid, Spain

Genome wide association studies (GWAS), based on linkage disequilibrium, allow the understanding of the genetic architecture underlying traits of agronomical interest such as abiotic stress tolerance. In order to perform these kind of analysis, it is required a wide range of variation in the studied population and high-quality genomic data, which is difficult to obtain from species such as wheat, due to its complex, repetitive and polyploid genome. Nevertheless, High-quality reference wheat genomes have been published recently (1,2), which will aid in understanding the biologic mechanisms implicated in complex traits as drought tolerance, where RSA (root system architecture) play a key role due to its plasticity and adaptation to environmental factors.

In the present work a previous characterization of RSA performed in the Spanish Durum wheat core collection (3) has been extended up to 191 wheat landraces. The wide range of variation in phenotypic traits and the lack of kinship relationship between landraces (4) supported the use of this collection to perform a GWAS analysis between the SNP markers set and the RSA traits. By this analysis 51 MTAs (Marker-trait associations) have been found. The linkage disequilibrium present in the tetraploid wheat collection allowed us to cluster these MTAs in 40 MTA-QTLs (Marker-trait association – Quantitative trait loci), i.e. genomic regions potentially responsible of the studied traits. Some of these regions had been previously described in the literature (5), but most of them were novel regions which constitute a useful data set for the future exploitation of Spanish tetraploid wheat landraces diversity.

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P4-15. Increasing cis-regulatory alleles variability and GABA content in tomato by genome editing of the promoter region

Pietro Gramazio[1], Mariko Takayama[1,2], Johan Hunziker[1,3], Yu Lu[1], Yoshihito Shinozaki[1,4], Hiroshi Ezura[1,2]

[1]Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan, [2]Tsukuba Plant Innovation Research Center (T-PIRC), University of Tsukuba, Tsukuba, Japan, [3]Graduate School of Science, Technology and Innovation, Kobe University, Kobe, Japan,[4]Institute of Global Innovation Research, Tokyo University of Agriculture and Technology, Japan. Corresponding author: Pietro Gramazio (piegra@upv.es)

Hypertension is a primary risk factor for cardiovascular disease. The ɣ-aminobutyric acid (GABA) is a non-proteinogenic amino acid that has shown effective in lowering the blood pressure of hypertensive patients. Intake of GABA through the daily diet may help reduce their symptoms and might be an effective way to prevent hypertension. Tomato is one of the most produced vegetables worldwide and daily consumed in a plethora of recipes and, apart from many other beneficial compounds, it also contains high levels of GABA. However, GABA levels are higher in mature tomato green fruits and rapidly decrease in ripe fruits due to two main mechanisms in the GABA pathway. In fact, GABA is synthesized from glutamate by glutamate decarboxylase (GAD) and reversibly converted to succinic semialdehyde by GABA transaminase (GABA-T). GABA synthesis is modulated by an inhibitory domain at the C-terminus of GAD that under certain physiological cell conditions inhibits GAD activity by folding its active site, avoiding the accumulation of high GABA contents. Several studies reported that the accumulation of high GABA levels promotes severe imbalances of amino acids in cells that leads to aberrant phenotypes due to defectiveness in pollen tube growth and cell elongation causing infertility and dwarfism (Gramazio et al., 2020). Nonaka et al. (2017) developed a high-GABA content Micro-Tom line without evident morpho-agronomic defects deleting the autoinhibitory domain of *SlGAD* introducing a stop codon by CRISPR/Cas9. On the other hand, the high GABA levels obtained silencing the *SlGABA-T* gene by RNAi lead to severe dwarfism and affected vegetative and reproductive growth (Koike et al., 2013). Our study aims at further increasing the GABA content in tomato by applying an alternative CRISPR/Cas9 approach by targeting the promoter region instead of the coding region. In that way, applying multiple target gRNAs in the promoter region of *SlGABA-T*, we have produced novel *cis*-regulatory alleles that may provide a continuum of variation in *SlGABA-T* gene expression and consequently find combinations the provide a better balance between GABA accumulation and plant development. The next step is the measurement of GABA contents and the phenotyping of the new lines obtained.

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P4-16. Variability of waxy proteins in spelt wheat

Ana Belén Huertas García, Juan B. Alvarez, Carlos Guzmán

Departamento de Genética, Escuela Técnica Superior de Ingeniería Agronómica y de Montes, Edificio Gregor Mendel, Campus de Rabanales, CeiA3, Universidad de Córdoba, España.

One of the current problems in agriculture is the great homogeneity of crops, a problem that has been exacerbated by breeding programs based on the use of a few high-yielding varieties. This has led to the practical disappearance of traditional local varieties or landraces. These materials have been identified as sources of genetic variability for numerous interest traits such as resistance to diseases or abiotic stresses, or grain quality [1]. In wheat, one of the most important factors of the crop is grain quality, which is determined mainly by the grain composition. Wheat starch is the major component of the grain and represents 65-75 % of its dry weight. Starch is composed of two glucose polymers: amylose and amylopectin. The ratio of these two starch components determines the starch properties. Numerous enzymes are involved in starch synthesis, being the waxy protein one of the most important as it is solely responsible for amylose synthesis [2]. In this study, the waxy proteins variability of 180 spelt wheat (*Triticum aestivum* L. ssp. *spelta*; 2n = 6x = 42, BBAADD) accessions from 16 countries was evaluated in order to identify new variants of these proteins. Four accessions were found with the null allele for *Wx-A1* gene and nine with null allele for *Wx-B1* ones. In addition, an allelic variant of *Wx-A1* with higher electrophoretic mobility than *Wx-A1a* was detected in one accession. In conclusion, this spelt wheat collection showed polymorphism for waxy proteins. These materials could be a useful source of novel alleles in wheat quality improvement, which could be used to design new cultivars with different levels of amylose content.

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P4-17. GENOME-WIDE ASSOCIATION STUDIES OF GASTROINTESTINAL PARASITE RESISTANCE AND TOLERANT TRAITS IN THE RASA ARAGONESA SHEEP BREED

Kenza Lakhssassi[a,b], Carlos Calvete[a], Pilar Sarto[a], Malena Serrano[c], Jorge Hugo Calvo[a,d]

[a] Unidad de Producción y sanidad animal, *CITA - IA2 (CITA–Zaragoza University), Zaragoza, Spain,* [b] *INRA, Rabat, Morocco,* [c] *Departamento de Mejora Genética Animal, INIA, Madrid, Spain,* [d] *ARAID, Zaragoza, Spain*

Parasitism, especially gastrointestinal nematode (GIN) infections, have an important economic impact in ruminant livestock, primarily through subclinical disease. Thus, the identification of potential candidate host genes associated to parasite resistance or tolerance, will allow the implementation of marker/gene assisted selection to improve animal welfare and the production system. Within this context, a genome-wide association study (GWAS) using high-density genotyping was carried out to contribute to the knowledge of the genetic basis of GIN resistance and tolerance in sheep. Ewes from one single flock (n=590), raised for meat production under a semiintensive management system, were characterised as resistants (R), tolerants (T), and sensibles (S) based on their records of body condition score (BCS), serum total protein (TP), haematocrit (HCT) and faecal egg counts (FEC) before and after anthelmintic treatment using a cluster analysis approach. One hundred and six ewes (R, n=34; T, n=37; S, n=35) were genotyped with the Illumina Ovine HD BeadChip (680K). A case-control GWAS approach with the GCTA software was performed. No genome-wise significant results were found in the three contrast (R-T, R-S and T-S) after false discovery rate (FDR) multi-test correction. However, 2 (rs422152832 and rs55630359) and 1 (rs425234781) SNPs for R-S and R-T contrast, respectively, were significant at chromosome level (FDR p<0.1). For R-S contrast, the SNPs were located on chromosome 18 in an intron region of *FRMD5* gene, being upregulated in the blood of Plasmodium-infected animals. Finally, the SNP rs425234781 is located on chromosome 2 at around 37 kb from the *ARHGAP15* gene that has been associated to trypanotolerance in cattle.

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P4-18. WHOLE GENOME ANALYSIS OF RESILIENCE AGAINST PRRSV OUTBREAKS IN BREEDING SOWS

Marina Laplana[a], Roger Ros-Freixedes[a], Joan Estany, Lorenzo J Fraile[a], Romi N Pena[a]

[a]*Departament de Ciència Animal, Universitat de Lleida – AGROTECNIO-CERCA Centre, Spain*

Resilience, defined as the ability of animals to overcome internal and external stressors, is a key element in animal production. In pigs, one of the most relevant stressors is the porcine reproductive and respiratory syndrome virus (PRRSV), which causes serious health problems and loss of productivity on farms. Previous studies have shown the existence of a genetic basis in the resilience of reproductive traits such as the abortion rate and the number of live-born pigs and total lost piglets (mummified and stilborns) at farrowing in breeding sows during a PRRSV outbreak(1,2). The aim of this study was to identify and characterize at whole genome level the genetic variants that influence the stability of reproductive performance (SRP) during PRRSV outbreaks. SRP against PRRSV infection has been calculated for each sow as the number of losses during the PRRSV outbreak minus the mean losses per delivery in an endemic situation. Sows have been classified as “stable” if SRP=<1 piglet (N=22) or “sensitive” if SRP>1 piglet (N=26). 48 sows with extreme phenotypes for SRP, selected from a population of 305 Landrace x Large White sows with reproductive data collected for nearly 3 years in a PRRSV endemic farm where an outbreak occurred, have been sequenced at low coverage (7X). More than 14.5 million polymorphic variants have been analyzed. A GWAS using a genetic relationship matrix to correct for population structure has identified 13 genomic regions on 11 chromosomes that contain 44 variants ($P<10^{-6}$) associated with the “stable” phenotype. 304 genes are located in the ±1Mb regions of the relevant variants. A preliminary analysis of these genes using gene ontology has revealed the relationship of these genes with molecular functions such as binding to RAGE receptors, inflammatory processes, embryonic development, angiogenesis and cell migration or binding to growth factors. This result suggests the existence of SRP related genetic variability. These findings will be tested in the entire population and in a population with different genetic background. The identification of genetic markers can be useful to identify resilient sows that can successfully cope with a PRRSV outbreak without hampering their health status or production parameters.

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P4-19. Identification and characterization of tissue-specific non-coding RNAs in tomato (*Solanum lycopersicum*)

Ricardo Lebrón[a], Ana Ortiz-Atienza[a], Fernando J. Yuste-Lisbona[a], and Rafael Lozano[a]

[a]*Centro de Investigación en Biotecnología Agroalimentaria (BITAL). Universidad de Almería, Almería, Spain*

Most of transcripts in plant cells are non-coding (ncRNAs) and several are involved in transcription and translation regulation (1-3), although their possible tissue-specificity and implications for development have not been extensively investigated. Recently, it has been described that plant transfer RNAs (tRNAs) are processed in a tissue-specific manner to give rise to microRNA-like fragments that modulate gene expression (4). In addition, it has been shown that plant ribosomes are heterogeneous due to the presence of ribosomal protein paralogs, hinting at the existence of specialized ribosomes that selectively regulate translation (5). However, the hypothetical contribution of ribosomal RNA (rRNAs) paralogs to ribosome-mediated gene expression regulation has not yet been explored. In this study, we have analyzed the differential expression of ncRNAs between leaf and flower of *Solanum lycopersicum* (var. MoneyMaker). For this purpose, the expression levels of 2,301 *ab initio* predicted ncRNA loci were measured in poly-A enriched and total RNA-seq libraries of both tissues, following the protocol described in (6). We found 87 differentially expressed ncRNAs, standing out 23 (of 221) 25S-rRNAs, 18 (of 168) 18S-rRNAs, 3 (of 132) 5.8S-rRNAs and 5 (of 620) tRNAs down-regulated in flower relative to leaf. In contrast, only one 5.8S-rRNA and one tRNA were up-regulated in flower, suggesting that the relative abundance of certain rRNAs and tRNAs may influence translation activity in vegetative and reproductive tissues. Furthermore, certain small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), and microRNA precursors also showed significant differences at the expression level. These results suggest the presence of specialized ribosomes and tRNA-derived fragments in different tomato tissues as an additional layer of gene expression control. Further in-depth studies are needed to elucidate the functional relevance of these ncRNAs in tomato development.

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P4-20. IDENTIFICATION OF GENES EXPRESSED IN GLANDULAR TRICHOMES OF TOMATO BY A FUNCTIONAL GENOMIC APPROACH

López-Fábregas J.D[1]., Fonseca, R[1]., Jáquez-Gutierrez M[2]., Capel, C[1]., Angosto, T[1]., Yuste-Lisbona, F.J[1], Atarés, A[2], Moreno, V[2], Lozano, R[1], Capel, J[1].

[1]Centro de Investigación en Biotecnología Agroalimentaria, Universidad de Almería, 04120 Almería. [2]Instituto de Biología Molecular y Celular de Plantas, Universitat Politècnica de València-Consejo Superior de Investigaciones Científicas, 46022 Valencia.

Trichomes are epidermal structures located in the aerial organs of mostly all plant species which have been widely related to abiotic stress response, such as severe radiation or desiccation but specially with biotic stress response. Among the *Solanum* species seven different types of trichomes have been described, which can be classified in two groups, glandular (types I, IV, VI and VII) and non-glandular trichomes (II, III and V). Non-glandular trichomes form a physical barrier that prevent plagues from spreading while glandular ones synthetize a wide spectrum of specialized metabolites that usually have a toxic effect on plagues. Acyl sugars, non-volatile conjugated sugar esters that not only are toxic but also immobilize and suffocate the pest are produced by type IV glandular trichomes, scarce in adult plants of the cultivated tomato *S. lycopersicum* L., but very abundant in wild relative species such as *S. pennelli* L. Here, we present the phenotypical and molecular characterization of *S. pennelli* mutant lines obtained as part of an insertional mutagenesis programme employing an enhancer trap T-DNA strategy. All these lines show expression of the reporter *uidA* gene in type IV glandular trichomes, thus allowing the identification of the T-DNA tagged gene by an Anchor-PCR approach. Functional characterization of the tagged genes is currently under progress by the generation of knock-out alleles in the wild species as well as in the cultivated species employing the CRISPR-Cas9 methodology. The results obtained will allow to understand the complex mechanism that regulates tomato glandular trichome formation and in a future will be useful to increase tomato pest resistance.

P4-21. IDENTIFICATION OF MOLECULAR MARKERS LINKED TO THE RESISTANCE TO ZYMV IN MELON DERIVED FROM IC 274006

María López-Martín[1], Marina Albaladejo[1], Narinder P.S. Dhillon[2], Belén Picó[1], Ana Pérez-de-Castro[1].

[1]Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV-UPV), Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain, [2]World Vegetable Center, East and Southeast Asia, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand

Viral infections caused by the potyvirus Zucchini yellow mosaic virus (ZYMV) are one of the most limiting factors for the growth of melon worldwide (Martín-Hernández & Picó, 2020). The Indian accession IC 274006 has been reported as resistant to ZYMV (Sanchís, 2018) and Papaya ringspot virus (PRSV) (Dhillon *et al.,* 2007). This accession was crossed with the susceptible Spanish landrace 'Meló d´Or' (BGV016451) and their F_1 progeny was used to construct three different generations: F_2, $BC_{1\text{-IC}}$ and $BC_{1\text{-BGV016451}}$ (backcrosses to IC 274006 and BGV016451, respectively). These offsprings were inoculated with ZYMV and the segregation for the resistance fitted a monogenic recessive genetic control for this character. Furthermore, both parentals, the F_1 and a set of 26 susceptible and 24 resistant F_2 plants were genotyped with an existing set of 124 SNPs markers evenly distributed throughout the genome and implemented for their use in the Agena Bioscience platform. The genotyping allowed the identification of a region of 6 Mb in chromosome 5 associated with the resistance, as significant differences were found for the symptoms score when comparing the genotypes for marker *5m11* (*p-value* = 0.00194). A new set of SNPs markers polymorphic between IC 274006 and BGV016451 were identified by genotyping-by-sequencing (GBS) both accessions; concretely, 2.759 SNPs were obtained in chromosome 5. Five of this SNPs and marker *5m11* were transformed to high resolution melting (HRM) markers and used to genotype the three segregant generations. The genotyping allowed to narrow the candidate interval to a 913 Kb region. Progeny tests of selected plants will be performed to confirm these results and further narrowing the interval. The SNPs identified in this work will be useful in breeding programs to introgress the resistance to ZYMV in traditional landraces or elite cultivars.

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P4-22. Easymap v.2: a comprehensive, user-friendly tool for mapping mutations

Samuel Daniel Lup and José Luis Micol

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

Next-generation sequencing (NGS) technologies coupled with classical genetic mapping strategies have accelerated the rate at which researchers can establish gene to phenotype relationships (1). Many software tools have been developed to process and extract useful information from the millions of short reads that constitute each NGS sample, but the analysis of this data requires advanced bioinformatics skills and the construction of pipelines of several tools for a complete analysis. To make NGS-based mapping analyses accessible to most researchers, we developed Easymap, a user-friendly program that goes from raw NGS reads to candidate mutations with minimal user input (2).

Easymap v.1 included automated workflows to map point mutations in bulked segregant populations, and large DNA insertions such as T-DNA and transposons, and allowed the simulation of NGS experiments (3). Easymap v.2 includes additional workflows to perform QTL-seq and variant density mapping analyses, as well as a variety of new implementations, such as multithreading and alternative control sample options. Each mapping workflow can accommodate a wide variety of experimental designs, including outcrossing and backcrossing, different mapping populations (F_2, M_2, and M_3), EMS-induced mutation or natural variant mapping, DNA or RNA sequencing, alternative control samples in FASTQ or VCF formats, single-end and paired-end reads, etc. Easymap v.2 can also be used as a variant analyser to study the effect of a list of polymorphisms on annotated genes and their protein products without applying a mapping algorithm. We have validated all of our mapping workflows with over 30 published experimental datasets.

Easymap runs within UNIX environments and can be easily installed in Windows 10 within the Ubuntu app available in the Microsoft Store, in some Linux distributions (Ubuntu, AMI, RedHat, etc.), and in virtual machines running a Linux subsystem within any other operating system. Easymap includes a simple installation guide, a user-friendly web interface, and each run produces a comprehensive mapping report with high-resolution images and tabular data to ease the interpretation of the results.

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P4-23. EFFECTS OF SALINITY ON RSA AND *Dro1* GENE EXPRESSION IN DURUM WHEAT

Arturo Martín[a], Yolanda Loarce[a], Juan González[a]

[a]*Departamento de Biomedicina y Biotecnología, Universidad de Alcalá, Alcalá de Henares, Spain*

The increase in the salinity of the soils around the world is a concerning problem due to its impact in the productivity of different crops such as durum wheat (*Triticum turgidum* L.). The Root System Architecture (RSA) is known to be a fundamental factor for a plant to survive under abiotic stress such as drought or salinity (1). *Deeper Rooting 1* (*DRO1*), which was first characterized in rice (*Oryza sativa*), is a gene associated with deeper roots that may produce a yield increase under drought conditions (2, 3). We studied the effects of salinity during germination in the RSA and the root angle growth (RGA), of three lines of durum wheat belonging to the subspecies *durum* and *turgidum*. The RSA and RGA of the lines showed differences when grown in saline medium containing 100 mM NaCl compared to when grown under standard conditions. We also studied in primary and seminal roots of seedling of three genotypes of durum wheat, the expression of the *TtDro1A* and *TtDro1B* genes through a quantitative PCR (qPCR), when they grow in saline and no saline medium. We observed that salinity seems to affect the pattern expression of the copies of the *Dro1* genes present in the genomes A and B of durum wheat. Salinity also alter the ratio between the *TtDro1A/TtDro1B* genes, decreasing it when roots grown in saline medium. The changes in *TtDro1A* and *TtDro1B* genes expression may be related to the phenotypic changes observed in the RSA and RGA of the three lines when grown in the presence of salt.

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P4-24. Evaluation of different culture media for organogenesis induction in cotyledonand hypocotyl pepper explants and ploidy analysis

Marina Martínez-López[a], Edgar García-Fortea[b], Ana García-Pérez[b], Esther Gimeno-Páez[a], Jaime Prohens[a], Santiago Vilanova[a]

[a]*Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain*
[b]*Seeds for Innovation, Parque Científico-Tecnológico de Almería, Almería, Spain*

Pepper (*Capsicum* spp.) is one of the most economically important crops worldwide; however, the application of *in vitro*-based breeding strategies is limited, as *Capsicum* is considered as a recalcitrant crop (1). Although *in vitro* regeneration of pepper has been reported, it is highly dependent on the genotype, the explant used and the culture medium (2, 3). In order to overcome the regeneration bottleneck in the genetic transformation and gene editing of pepper, we have evaluated different combinations of auxins, cytokinins and micronutrients on the organogenesis induction in cotyledon and hypocotyl explants of *C. annuum* (Ca), *C. baccatum* (Cb) and *C. chinense* (Cc). In parallel, the ploidy level of the plants before and after regeneration was analysed by flow cytometry. We found variation among species and type of explant in the regeneration response, with averages of number of shoots per cotyledon explant of 1.44 (Ca), 4.17 (Cb), and 0.08 (Cc) and for hypocotyl explant of 0.28 (Ca), 3.20 (Cb), and 0.00 (Cc). Out of six media overall, the most effective medium contained BAP, IAA, copper and silver. Cytometry revealed a high number of polyploid cells in the hypocotyl tissue, compared with leaves and cotyledons (5.5 to 11-fold higher than leaf), and a tetraploid plant out of 10 acclimatized plants from hypocotyl tissue was found. This indicates that it is possibleto obtain polyploid pepper plants without using antimitotic agents by means of *in vitro*regeneration in pepper. Our results indicate that *C. baccatum* and the cotyledon explants, being respectively the species and explants with highest regeneration, could be used for *Capsicum* transformation and gene editing studies. The effect of a combination of copper andsilver in the regeneration medium seems to have had synergistic effects on regeneration. Thiswork contributes to the improvement of *Capsicum* regeneration protocols that allow reaching the levels of efficiency required for genetic transformation and gene editing technologies achieved for other crops, as well as to develop polyploid plants.

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P4-25. NATURAL VARIATION DURING WOUND-INDUCED ADVENTITIOUS ROOT FORMATION IN DIVERSE TOMATO GENOTYPES

Mariem Mhimdi[1], María Salud Justamante[1], Eduardo Larriba[1], Aurora Alaguero–Cordovilla[1], Alfonso Azorín[1], José Manuel Pérez-Pérez[1]

[1]*Instituto de Bioingeniería, Universidad Miguel Hernández, Elche, Spain*
Corresponding author: José Manuel Pérez-Pérez (jmperez@umh.es)

Tomato modern varieties have experienced a genetic bottleneck as a result of intensive breeding programs in the 20th century. We previously found substantial variation on root architectural traits during early growth in a small sample of wild tomato species and commercial cultivars (Alaguero-Cordovilla *et al.*, 2018). Here we studied wound-induced adventitious root formation in a collection of introgression lines derived from the *Solanum pennellii* × *S. lycopersicum* cross, which allow us to link specific genomic bins with enhanced rooting traits. Comparative transcriptome profiling on some of these lines revealed a conserved gene regulatory module required for adventitious rooting in *S. pennellii* that deserves further investigation. Additionally, to identify the molecular roadblocks leading to the leverage of adventitious rooting on many commercial cultivars, we studied a collection of 149 tomato accessions, including *S. pimpinellifolium*, *S. lycopersicum* var. *cerasiforme* and *S. lycopersicum* var. *lycopersicum*, that represent the genetic and morphological variability of tomato at its centers of origin and domestication (Mata-Nicolás *et al*., 2020). We found a wide range of variation on the studied rooting traits that might be later exploited in tomato breeding. Thus, our first attempt to identify SNPs associated with several of the studied rooting traits will be presented.

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P4-26. *Unifoliate* mutation in chickpea

Ana M Gálvez[a], Antonio Vico[a], Josefa Rubio[b], Teresa Millán[a], Patricia Castro[a]

[a]Universidad de Córdoba, Spain [b]Instituto de Investigación y Formación Agraria y Pesquera, Spain

Adaptation of chickpea crop to changing environmental conditions leads to explore adaptative traits that could increase final yields. Leaf shape could affect photosynthetic efficiency. The most frequent leaf morphology in cultivated chickpea is compound pseudo- imparipinnate supporting 10 to 15 leaflets on average. It has been described simple leaves or unifoliate mutants as well as differences for seed size (1). This mutation has been introduced in mexican and norht-american chickpea breeding programs due to its association with seed size (2,3). Our aim is to study the influence of leaf shape in final yield under Mediterranean conditions, and to develop molecular markers linked to that trait to facilitate the selection of best phenotypes. In this study, we used a RIL (Recombinant Inbred Line) population (RIP- 16) derived from a cross between CA2990 (unifoliata leaf) and WR315 (normal leaf). Segregation for phenotypes normal/unifoliate leaf fitted to the expected segregation for a single gene. Field evaluation of agronomic traits in 76 RIL showed a slight negative correlation between final yield and yield per plant. Bulk segregant analysis using microsatellite markers (SSR) allowed us to identify the NCPGR50 marker tightly associated with leaf type. This microsatellite was physically located in the scaffold118 (position 432718..432353) of the chickpea genome sequence. Genetics maps in previous studies revealed the location of NCPGR50 in linkage group 8 (LG8), that correspond to chickpea chromosome 8 (Ca8). The analysis of markers located in LG8 in the whole RIP-16 confirmed the location of the gene controlling normal leaf/unifoliate in Ca8. We also developed NILs (Near Isogenic Lines) for this trait that could help to re-evaluate agronomic traits with a greater accuracy.

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P4-27. Development of a ground-truth dataset for automatic phenotyping based on multispectral imaging

Leanne Miller[2], María Victoria Díaz-Galián[1], Alberto Gila-Navarro[2], Diego J. Águila[1], Marcos Egea-Cortines[1], Pedro J. Navarro[2]

[1]Instituto de Biotecnología Vegetal, Universidad Politécnica de Cartagena, Cartagena, Spain, [2]División de Sistemas e Ingeniería Electrónica, Universidad Politécnica de Cartagena, Cartagena, Spain.

The development of Artificial Intelligence algorithms and their implementation for data mining require the so-called ground truth data. This means a dataset has a number of numerical parameters that are vectorially related to each data point. This is a crucial step to advance towards absolute biology versus relative analysis. Despite the importance of image-based phenotyping, there are hardly any ground truth datasets available for algorithm development or comparison to new experimental data. We have obtained two datasets of images, one comprising 1352 grapes of five different varieties with the corresponding total anthocyanin concentration, brix index and size. The second comprised 114 flowers of *Antirrhinum majus* cv Vilmorin variee and included total anthocyanin and floral size. By using data augmentation algorithms we obtained a dataset of 12220 grape images from 1820 initial images and obtained 100% classification with ad-hoc Deep Learning architectures 3DeepM [1]. Our current effort is to obtain an additional Deep Learning architecture that will be able to classify brix index and anthocyanin concentrations based on the newly created ground truth dataset.

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P4-28. MOLECULAR CHARACTERIZATION OF GENES CONTROLLING DEHISCENCE IN PISTACHIO (*PISTACIA VERA*)

Imad Chiguen Nou, Francisco Conde, Jerson Alexander García-Zea, Francisca Robles, Roberto de la Herrán, Carmelo Ruiz Rejón, Rafael Navajas-Pérez.

Department of Genetics, University of Granada, Spain,
Corresponding author: Rafael Navajas-Pérez (rnavajas@ugr.es)

The existing commercial varieties of pistachio widely differ in the percentage of dehiscent fruits. This factor determines the destination of the product, the investment required for fruit processing operations and its final price in the market. Dehiscence is a complex biological process that involves not only environmental but also genetic factors. Two types of genes are concerned: a first group controls the valve margin identity and signals *replum* cells that eventually will constitute the dehiscence zone and the separation layer, while a second group conducts lignification of these cells and the opening of the fruit. Using WGS of three varieties of pistachio (female cultivars Batoury and Siirt, and male cultivar Bagyolu), 15 genes proved previously to be responsible for dehiscence in *Arabidopsis thaliana* (1) and *Vicia faba* (2) were characterized *in silico* and assigned to different chromosomes. Positive matches were found for all genes investigated (with similarities ranging from 96% to 99.5%, except for YAB3 (3) that was only found in female genomes. YAB3 promotes valve and valve margin formation through the expression of transcription factors (FUL and SHP) situated at the beginning of the metabolic pathway (3) and is thought to have experienced a strong selective swept during domestication (Kafkas et al., unpublished data). Linkage and gene duplication analyses showed that paralogous genes (NST1, NST3, ADPG1 y ADPG2) have been reduced to a single locus in *Pistacia vera*. Primers were designed for all 15 genes and their presence was investigated in non-dehiscent species *P. lentiscus* (lentisk) and *P. terebinthus* (terebinth).

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P4-29. The tomato *SlMAPKKK17* gene play a crucial role in explant organogenic response

Ana Ortiz-Atienza[a], Jorge Sánchez-López[b], Alejandro Atarés[b], Marybel Jáquez-Gutiérrez[b], Carmen Capel[a], Benito Pineda[b], Begoña García-Sogo[b], Fernando J. Yuste-Lisbona[a], Vicente Moreno[b], Rafael Lozano[a]

[a]*Centro de Investigación en Biotecnología Agroalimentaria (BITAL). Universidad de Almería, Almería, Spain.*
[b]*Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Univ. Politècnica de València, Valencia, Spain.*

The ability to induce morphogenesis *in vitro* is the basis of plant tissue culture techniques when they are applied to micro-propagation and genetic improvement. Therefore, with the aim to address the genetic dissection of adventitious organogenesis, an *in vitro* phenotypic screening assay was performed in a T-DNA mutant collection (1) of tomato (*Solanum lycopersicum* L.), a crop species of the utmost socio-economic importance. Here, we report the genetic, molecular and physiological characterization of the recessive mutant named as *tomato defective in bud differentiation 3* (*tdb-3*), as it lacks the ability for shoot-bud differentiation under *in vitro* normal growth conditions. Moreover, *tdb-3* plants exhibit severe alterations in both vegetative and reproductive development showing very long internodes, reduced lateral branching, abnormal flowers and seedless fruits (2). Cloning of the genomic regions flanking T-DNA insertion site revealed that T-DNA integration was located in the promoter region of two adjacent genes transcribed in opposite directions, one encoding an abscisic acid receptor homologous to the Arabidopsis *PYL6* (*SlPYL6*), and the other one a mitogen-activated protein kinase kinase kinase previously designated as *SlMAPKKK17* (3). Allele-specific primers designed from both T-DNA and genomic regions flanking T-DNA sequences were used to support that T-DNA insertion co-segregated with *tdb-3* mutant phenotype. The effects of T-DNA insertion on gene expression were determined by qRT-PCR experiments, which showed a significant downregulation of the *SlMAPKKK17* gene in *tdb-3* tissues. By contrast, *SlPYL6* transcripts were not detected in either wild-type or mutant tissues. Characterization of RNA interference lines for *SlPYL6* and *SlMAPKKK17* revealed that the *tdb-3* mutant phenotype relies on the *SlMAPKKK17* gene. Overall, results indicated that *SlMAPKKK17* may be required for the acquisition of cellular competence during the explant organogenic response, most probably in the competence phase or at the beginning of the adventitious shoot-bud differentiation process.

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P4-30. Variation for water stress tolerance among pepino (*Solanum muricatum*) cultivars

Juan Pacheco[a], Mariola Plazas[b], Ilaria Pettinari[a,], Anbu Landa-Faz[a,], Sara González-Orenga[c], Monica Boscaiu[c], Salvador Soler[a], Jaime Prohens[a,*], Oscar Vicente[a], Pietro Gramazio[d]

[a]*Institute for the Conservation and Improvement of Valencian Agrodiversity, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain*
[b]*Meridiem Seeds S.L., Paraje Lo Soler 2, 30700 Torre-Pacheco, Spain*
[c]*Mediterranean Agroforestry Institute, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain*
[d]*Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, 305-8572, Tsukuba, Japan*

Selection and breeding for drought tolerance is an important breeding objective in pepino (*Solanum muricatum*), a neglected Andean crop which is awakening an increasing interest (1). The purpose of this study was to evaluate the variation in the tolerance to water stress in seven pepino cultivars (Mur1-Mur7) with different origins and morphological characteristics. Three water stress treatments were applied: fully irrigated control (C), a moderate water stress (WS-M), and a severe water stress (WS-S). We investigated the behavior of traits related to growth, photosynthetic pigments, mono and divalent ions, osmolytes and antioxidants, during the development of water stress. Significant differences were found among cultivars in response to water stress regarding the above mentioned traits. Moderate and severe water stress affected several growth and biochemical traits, compared with control plants and, as expected, were more pronounced for WS-S treatment plants, with an important reduction of the fresh weight of leaves, stems and roots, as well as of their water content. The principal component analysis (PCA) using the relative values of growth traits, together with the ANOVA for the traits for which significant interaction cultivar × treatment was observed, revealed that cultivars Mur2 and Mur4 were the most tolerant to water stress. In addition, in general photosynthetic pigments, malondialdehyde and total flavonoids were significantly reduced by the severe water stress, and an increase in proline, Na+ and K+ contents was observed. The two pepino cultivars with better tolerance to water stress could be of interest for their use under conditions of limited water availability, as well as for breeding pepino for drought tolerance.

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P4-31. Screening of eggplant (*Solanum melongena* L.) wild relatives for growth and biochemical responses to water stress identifies sources of variation for drought tolerance breeding

Mariola Plazas[a], Sara González-Orenga[a,b], Huu Trong Nguyen[b,c], Ana Fita[a], Mónica Boscaiu[b], Oscar Vicente[a], Jaime Prohens[a]

[a] *Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022, Valencia, Spain*
[b] *Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera 14, 46022, Valencia, Spain*
[c] *Laboratory of Induced Resistance and Plant Bioprotection RIBP, University of Reims Champagne-Ardenne, 51678, Reims Cedex 2, France*

Drought is an abiotic stress that causes important yield losses in eggplant (*Solanum melongena* L.). In order to identify sources of variation for breeding among eggplant wild relatives, 16 accessions from eight eggplant wild relatives and two cultivated eggplant accessions were evaluated for drought tolerance. A fully watered control was compared to a treatment in which irrigation was arrested. The test was carried out in plantlets with at least five true leaves grown in 1.3 L pots with substrate. After 11 days of starting the water stress treatment, measurements were taken for plant growth, tissue moisture content traits, and oxidative stress-related biochemical parameters, including proline, malondialdehyde, total phenolics, total flavonoids, and catalase, and superoxide dismutase, ascorbate peroxidase, and glutathione reductase activities (1). The plant material was classified into three groups considering the reduction of the dry matter content in the aerial part of the stressed plants compared to the controls: the most tolerant (<25%), which included *S. incanum*, *S. pyracanthos*, *S. dasyphyllum*, and *S. torvum*, an intermediate group (25%-35%), and a susceptible group (>35%), in which the cultivated eggplant is included. All the accessions subjected to drought increased the concentration of proline, in particular the most tolerant. The group of more tolerant accessions also showed a greater increase in the mean level of flavonoids, however no changes were detected in the levels of malondialdehyde. The activity of the antioxidant enzymes was highly variable regardless of the group and the species to which they belonged. The results obtained reveal a high diversity within wild relatives of eggplant for drought tolerance and provide information complementary to the one obtained on variation in the cultivated species (2) on the biochemical mechanisms involved in the response to drought in wild relatives of eggplant. Our results allowed identifying sources of tolerance to drought in eggplant among wild species that are cross compatible with eggplant (3). These materials can be useful for breeding programmes to develop rootstocks and new eggplant cultivars with higher tolerance to drought.

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P4-32. BROADENING THE GENETIC BASE OF EGGPLANT THROUGH INTROGRESSION WITH WILD RELATIVES

Jaime Prohens[a], Mariola Plazas[a], Pietro Gramazio[b], Edgar García-Fortea[c], Giulio Mangino[a], Gloria Villanueva[a], Carla Guijarro-Real[a], Andrea Arrones[a], David Alonso[a], Santiago Vilanova[a]

[a]Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain, [b]Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan, [c]Seeds for Innovation, PITA, Almería, Spain

Eggplant (*Solanum melongena* L.) is an Old World crop with a narrow genetic base. Since 2013 we have followed a systematic "introgressiomics" approach (1) to broaden the cultivated eggplant genepool with introgressions with wild relatives from the primary (GP1), secondary (GP2) and tertiary (GP3) genepools (2). Multiple crosses between six accessions of cultivated eggplant and 35 accessions from 15 wild relatives resulted in 90 interspecific hybrids from 14 different wild species. A total of 48 BC1 generations towards eggplant were obtained, including the first instance of successful backcrossing with a New World species (*S. elaeagnifolium*). Subsequent backcrossing allowed developing 87 BC2S2 progenies displaying a wide diversity and novel traits. Four BC1 generations were advanced to develop new sets of advanced backcrosses (ABs) and introgression lines (ILs) with *S. insanum* (GP1), *S. dasyphyllum* (GP2), *S. incanum* (GP2), and *S. elaeagnifolium* (GP3) by marker-assisted-selection using high-throughput genotyping. The ABs and ILs developed so far cover most of the genome of the donor wild parent. First phenotyping trials of some of the ILs already obtained have allowed the selection of materials with enhanced tolerance to drought, as well as the detection of superior lines and QTLs for traits of interest (3). Given the mostly cultivated background on each AB and IL, the materials obtained are an elite resource readily available for incorporation into breeders pipelines. Our preliminary results suggest that the introgressed genes from wild species can make a significant contribution to eggplant breeding, particularly for adaptation to climate change.

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P4-33. Genetic and phenotypic characterization of the *mrd1* mutants of *Arabidopsis thaliana*

Víctor Quesada, Emilio Núñez-Delegido and Pedro Robles

Instituto de Bioingeniería. Universidad Miguel Hernández, 03202, Elche, Alicante, Spain.

The studies hitherto published reporting the effects of mutations in nuclear genes encoding mitochondrial ribosomal proteins (MRP) on plant biology are scarce (1). To increase our knowledge of MRP functions in plant development and growth, we are identifying and characterizing *Arabidopsis thaliana* mutants affected in MRP through a reverse genetics approach. In this line, insertional alleles carrying a T-DNA insertion in nuclear genes encoding MRP for which viable loss-of-function alleles had not been previously described, were identified in the wild-type accession Columbia-0 (Col-0). One of these mutants, putatively affected in a nuclear gene encoding a MRP of the large ribosomal subunit, exhibits retarded growth compared with Col-0. We named this mutant *mrd1* (*mitochondrial ribosome defective1*) and confirmed by PCR genotyping and Sanger sequencing, that the T-DNA is inserted in the first intron of the *MRD1* gene. BLAST homology searches reveal that MRD1 is a highly conserved plant protein, noticeably longer than its orthologous proteins in bacteria.

We have recently identified a second mutant allele of the *MRD1* gene carrying a T-DNA insertion in the exon 5 of this gene, as we have molecularly confirmed. Our complementation analysis between these two mutants corroborates that they are allelic and, consequently, we named them *mrd1-1* and *mrd1-2*.

Mutants *mrd1-1* and *mrd1-2* exhibit a very similar phenotype: stunted growth, reduced length of their main roots, and decreased fresh and dry weights, compared with Col-0 plants. This reduction in root size and plant weight could be attributed to the retarded growth of the *mrd1* mutants. These mutant traits are monogenic and recessive, and they are inherited with complete penetrance and constant expressivity. Interestingly, stunted growth of mutants *mrd1* is enhanced, and the differences become more evident with Col-0, when they are grown at a higher temperature (28˚C) than the one we usually use (20˚C). This result suggests that *MRD1* function may be especially sensitive to heat stress.

Taken together, our results show that *MRD1* is required for accurate plant growth in Arabidopsis. We are currently performing a molecular analysis of the *mrd1* mutants and advancing in their phenotypic characterization.

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P4-34. Loss-of-function of the tomato *SSI2* gene, encoding a stearoyl-ACP-desaturase, impairs photosynthetic machinery and plant growth

Abraham S. Quevedo-Colmena[a], Fernando J. Yuste-Lisbona[a], Ana Ortiz-Atienza[a], Marybel Jáquez-Gutiérrez[b], Alejandro Atares[b], Vicente Moreno[b], Rafael Lozano[a], Trinidad Angosto[a]

[a]*Centro de Investigación en Biotecnología Agroalimentaria (CIAIMBITAL), Universidad de Almería. 04120 Almería, Spain.*

[b]*Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Univ. Politècnica de València. 46022 Valencia, Spain.*

Galactolipids are major constituents of the thylakoid membranes in oxygenic photosynthetic organisms, including cyanobacteria, red and green algae, and higher plants. Consequently, alterations in galactolipid biosynthesis significantly influence chloroplast development (1). Galactolipids have also special biophysical features and ability to maintain the appropriate fluidity of membranes, given that they are rich in polyunsaturated fatty acids (2). Stearoyl-acyl-carrier-protein desaturase (SACPD) catalyzes the conversion of stearic acid (18:0) to oleic acid (18:1), a key step that regulates the amount of unsaturated fatty acids and the optimal fluidity of thylakoid membranes, two cell characteristics essential for photosynthetic processes (3). In this work, a new T-DNA recessive mutant, named as *pale dwarf* (*pad*), was identified as homozygous *pad* plants exhibited a dwarf and chlorotic phenotype promoted by a severe reduction of chlorophyll content, which indicates that *PAD* loss-of-function impairs photosynthetic machinery. Cloning of flanking sequences at T-DNA integration site, as well as co-segregation analysis performed using allele-specific primers, suggested that *pad* mutant phenotype was caused by a single T-DNA insertion affecting the second intron of a gene encoding a SACPD homologous to the Arabidopsis *SUPPRESSOR OF SA INSENSITIVE2* (*SSI2*). The phenotype of CRISPR/Cas9 *SlSSI2* knockout mutant lines (CR-*slssi2*) confirmed that the morphological abnormalities shown by *pad* mutant plants was due to the *SlSSI2* lack of function. Both *pad* and CR-*slssi2* plants accumulated high levels of 18:0 and reduced levels of 18:1 in leaves. Furthermore, alterations in the expression of genes related to defense response mediated by salicylic acid (SA) and jasmonic acid (JA) were found, which suggests that *SlSSI2* may play a key role in modulating crosstalk between the SA- and JA-responsive signaling cascades.

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P4-35. Identification of plant processes perturbed in the Arabidopsis *crd3-1* mutant

Pedro Robles, Eva Núñez-Delegido, Almudena Ferrández-Ayela, Javier Marchena, Adrián Bernabé and Víctor Quesada

Instituto de Bioingeniería. Universidad Miguel Hernández, 03202, Elche, Alicante, Spain.

In a search for T-DNA insertional mutants of *Arabidopsis thaliana* affected in nuclear genes encoding chloroplast ribosomal proteins, we identified a viable loss-of-function allele for a protein of the 50S large subunit, which had not been previously described. We dubbed this mutant *crd3* (*chloroplast ribosomal deffective3*). An embryo lethal allele of this gene was formerly identified in a screening of mutants defective in embryo development. We named this lethal allele *crd3-2*, after showing that it was allelic to *crd3-1*. Our phenotypic characterization of homozygous *crd3-1/crd3-1* and heterozygous *crd3-1/crd3-2* plants revealed new functions for *CRD3* in development.

We have transformed *crd3-1/crd3-1* individuals with a $CRD3_{pro}$*:CRD3-GFP* transgene in which, under the control of the *CRD3* promoter, the coding sequence of the *CRD3* gene was fused with the sequence of the green fluorescent protein (GFP). All the transgenic lines are phenotypically wild-type, demonstrating that the knocked-out gene in *crd3-1* is responsible for the mutant phenotype. Furthermore, we have analyzed by confocal microscopy, the subcellular localization of the CRD3 protein and confirmed that CRD3 is chloroplast-localized. This is consistent with the *in silico* prediction of an N-terminal chloroplast-transit peptide in the CRD3 amino acid sequence.

A massive RNA-sequencing (RNA-seq) assay revealed 586 genes significantly deregulated in *crd3-1* compared with the wild type (Col-0). We performed databases searches to identify the biological, biochemical or physiological processes in which the misregulated genes in *crd3-1* might be involved, and classified them in different categories. One of the principal categories corresponds to genes related to hormone metabolism and response. In this line, our results showed that auxin homeostasis is disturbed in the *crd3-1* mutant, because it is less sensitive than Col-0 to the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) and to the efflux auxin inhibitor TIBA. This is consistent with our previous results showing that *crd3-1* exhibited a reduced expression of the auxin response reporter *DR5:GUS.*

In order to identify potential relationships among the different translational apparatuses in the plant cells, we are currently searching for genetic interactions between *crd3-1* and other mutations affecting ribosomal proteins.

P4-36. PHENOTYPIC AND GENETIC DIVERSITY FOR YIELD AND FRUIT COMPOSITION IN A COLLECTION OF 'DE PENJAR' LONG SHELF-LIFE TOMATO UNDER TWO N FERTILIZATION LEVELS

Elena Rosa-Martínez[a], Ana M. Adalid[a], Luis E. Alvarado[a], Resurrección Burguet[a], María D. García-Martínez[a], Leandro Pereira-Dias[a], Cristina Casanova[a], Elena Soler[a], María R. Figàs[a], Mariola Plazas[b], Jaime Prohens[a], Salvador Soler-Aleixandre[a]

[a]Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain, [b]Meridiem Seeds S.L., Torre-Pacheco, Spain

The 'de penjar' tomato (*Solanum lycopersicum*) is a group of local varieties from the Mediterranean region of Spain that carry the *alc* mutation, which provides an extended shelf life (1). This type of tomato is genetically very variable, but due to its traditional cultivation under low inputs, it generally displays resilience (2). We present a comprehensive evaluation of yield and twenty-seven traits related to fruit nutritional and organoleptic quality in a collection of 44 accessions of 'de penjar' tomato grown under two levels of N fertilization (162 kg of N/ha in the high N treatment and 49 kg N/ha in the low N treatment). Significant differences among varieties were detected for all traits and a large variation was observed, with lycopene being the composition trait with the highest relative range of variation (over 4-fold) under both N treatments. The effect of reducing N fertilization was not significant for most of the traits, except for colour and sugars. In that way, fruits under low N had, on average, higher values for hue (5.9%) and lower for fructose (-11.5%), glucose (-15.8%), and total sweetness index (-12.9%). A highly significant genotype × N input interaction was found for lycopene and β-carotene. The analysis of genetic parameters showed values of broad-sense heritability lower than 0.5, except for fruit weight, malic acid and citric:malic acid ratio, which also had the highest genetic coefficient of variation. Local varieties had higher values than commercial varieties for traits related to the ratio of sweetness to acidity and for vitamin C, which reinforces the appreciation for their quality and makes interesting their use and conservation. Highest-yielding varieties under both conditions displayed wide variation in the composition and quality profiles, which may allow the selection of genotypes with high quality under low N conditions. These results revealed the potential of 'de penjar' varieties as a genetic resource in breeding for low N inputs and improving the organoleptic and nutritional tomato fruit quality.

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2. *Fullana-Pericàs et al. (2019). Agricultural Water Management 223 (1651), 1-15*

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P4-37. Identification of disease-resistant parents for potato breeding programs using molecular markers

Leire Barandalla, Lidia Mingorance, Alba Alvarez-Morezuelas, Enrique Ritter, Jose Ignacio Ruiz de Galarreta.

NEIKER-BRTA Basque Institute for Agricultural Research and Development.
PO Box 46, E-01080, Vitoria-Gasteiz, Spain.
Corresponding author: Jose Ignacio Ruiz de Galarreta (jiruiz@neiker.eus)

Neiker is performing for many years a breeding program with the aim of obtaining new adapted potato varieties. For this purpose, the choice of appropriate parents is essential, in order to obtain segregating progenies that allow the selection of new superior genotypes. DNA markers have a large potential to improve efficiency and precision of conventional plant breeding programs based on marker assisted selection (MAS). The aim of this work was to screen 150 potential parental genotypes with potato molecular markers for disease resistance to virus such as PVS, PVY, PVX, PLRV, potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*), *Phytophthora infestans* and *Synchytrium endobioticum*. A total of 11 markers, validated in previous studies were tested, namely SCAR marker RYSC3, $GP122_{564}$ related to PVY resistance genes Ry_{adg} and Ry_{sto}, respectively, as well as Nl27 for PLRV, GM339 and GM637 for PVX, and SCG17 for PVS. Also marker TG689/BCH, linked to *H1* conferring resistance to *G.rostochiensis* (Ro1/4), HC, associated with high levels of *G. pallida* (Pa2/3) resistance, markers RB and GP24 for *P. infestans* and Nl25 for *S. endobiotiucum* resistance were evaluated. Out of the 150 genotypes tested, 82 revealed some of the markers. The most frequent was TG689/BCH which is linked to the gene conferring resistance to the cyst nematode *G. rostochiensis* in a total of 70 varieties. These results suggest the possibility of using the resistant genotypes as parents in the crossing program and the application of MAS in the progenies obtained, in order to reduce the time of the selection process.

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P4-38. RECESSIVE RESISTANCE TO *tomato leaf curl New Delhi virus* IN CUCUMBER (*Cucumis sativus*) IS CONTROLLED BY A QTL IN CHROMOSOME 2

Cristina Sáez[1], Laura G.M. Ambrosio[1], Silvia M. Miguel[1], José Vicente Valcárcel[1], María José Díez[1], Belén Picó[1], Carmelo López[1]

[1]*Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain*

Tomato leaf curl New Delhi virus (ToLCNDV) generates severe damages in yield and production of cucurbit crops around the world. Resistance has been reported in sponge gourd (*Luffa cylindrica*), melon (*Cucumis melo*) and pumpkin (*Cucurbita moschata*), but at present there are no resistance to ToLCNDV in cucumber (*Cucumis sativus*). Two germplasm collections of *C. sativus* var. *sativus* with accessions from different origins were screened by mechanical inoculation with ToLCNDV. The response to the infection was evaluated by visual scoring of displayed symptoms, tissue printing hybridization and conventional PCR. The viral load of ToLCNDV was determined in a selected number of accessions using quantitative PCR (qPCR). Severe susceptibility was found in most of the assayed genotypes of *C. sativus*, including a nuclear collection of Spanish landraces and most of the accessions from different geographies. Two Indian accession (CGN22297 and CGN22986) showed mild symptoms but variable viral load, whereas three Indian accessions (CGN23089, CGN23423, and CGN23633) remained symptomless and showed a reduced viral accumulation. The resistance was confirmed by mechanical inoculation with ToLCNDV in a second assay of seedlings obtained by selfing resistant plants of the three resistant entrances. The inheritance model of the resistance to ToLCNDV was studied by crossing plants of CGN23089 and BGV011742 accessions, highly resistant and susceptible to the virus respectively. The progeny was mechanically inoculated with ToLCNDV and F_1 plants developed moderate symptomatology and showed intermediate viral load, which suggests a recessive control of resistance. Further genetic studies were conducted in F_2 and BC1 segregating populations, obtained by selfing F_1 plants and by backcrosses to each parent. All plants of these families were also evaluated against ToLCNDV by mechanical inoculation and, as was expected, segregated to symptoms severity and viral accumulation (qPCR). Proportion of resistant:susceptible plants fitted with the expected ratio for one recessive gene controlling the resistance. F_2 and $BC1_{CGN23089}$ populations were genotyped with a single nucleotide polymorphisms (SNPs) panel covering the seven chromosomes of cucumber genome. After QTL analysis, a candidate region in chromosome 2 of cucumber was identified linked to ToLCNDV resistance.

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P4-39. DNA probe design for food allergen detection using choroplast DNA markers

Africa Sanchiz[b], Isabel Ballesteros[a], Sandra Fernández-Cristobal[a], Carmen Cuadrado[b] and Rosario Linacero[a]

[a]*Departamento de Genética, Fisiología y Microbiología, Facultad de Biología, Universidad Complutense, Madrid,*
[b]*Departamento de Tecnología de Alimentos, INIA-CSIC, Madrid,*

Some tree nut proteins might be harmful to sensitive population, consequently the presence of these ingredients in foods must be indicated on the label, to prevent accidental consumption by allergic subjects. The development of suitable analytical methodologies to detect allergens in processed foods is advisable. It has been demonstrated that real-time PCR allows a specific and accurate amplification of allergen sequences in foods (1, 2). Generally, the optimal target sequences, for specific and sensitive detection, are those that show interspecific variation and high copy number. Nevertheless, some food processing methods could induce fragmentation and/or degradation of DNA that in some cases could affect the allergen detection; thus, it is an essential issue to analyze (3). In this work, we designed an assay based on a chloroplast marker (matK) for specifically detection of tree nut allergens by real–time PCR, in order to increase the sensitivity. Binary mixtures of raw and processed tree nut flour in wheat were performed at concentrations ranging from 100000 to 0.1 ppm. Different protocols for DNA isolation were carried out, to obtain total or chloroplast-enrich DNA, from tree nuts, mixtures and related species. Specificity and sensitivity of the method was assessed in peanut, pistachio and cashew using specific matK primers and TaqMan probes. Efficiency and linear correlation of calibration curves were within the adequate ranges. Moreover, the results showed a reduction of the detectability of the target after application of combined pressure and thermal processing.

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P4-40. Breeding for tolerance to Zucchini yellow mosaic virus (ZYMV) and resistance to Watermelon mosaic virus (WMV) in the Lagenaria siceraria 'Carabassa blanca Valenciana de Biar' landrace

Maria Llobell, Cristina Casanova, Maria del Rosario Figàs, Jaume Prohens, Salvador Soler-Aleixandre

Institut de Conservació i Millora de l'Agrodiversitat Valenciana , Universitat Politècnica de València, València, Spain.

The bottle gourd (*Lagenaria siceraria*) is a monoecious species, with an annual cycle, cultivated for its food and medicinal uses, as a decorative element and also as a musical instrument. This variety of uses is due to the great morphological variability of the fruit, with sizes ranging from 5 centimeters in diameter to 3 meters long. In the Valencian Community, the local variety 'Carabassa blanca Valenciana de Biar' is used to prepare the "carabassat", a pumpkin dessert that is used to make traditional sweets. In its production area, a high incidence of viruses caused by *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV) has been determined. In a screening of 15 accessions of *L. siceraria* was, we identified ZYMV tolerance and WMV resistance in the commercial variety 'Alamos'. (3,4) In the present work, a series of experiments are performed to obtain information on the genetic control of resistance to the two viruses mentioned above. For this reason, the commercial variety "Alamos" has been crossed with different selections susceptible to 'Valencian white pumpkin from Biar' (F1 generations), as well as the corresponding F2 generations. Evaluation of these generations suggests a dominant monogenic control for resistance to both viruses. In the case of WMV, a high level of resistance is observed, showing no symptoms of the disease inoculated plants of both the parent "Alamos" and the corresponding F1. With ZYMV, the results seem to suggest tolerance. Inoculated plants of "Alamos" and the corresponding F1 show absent or non-existent symptoms with very low viral loads.

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P4-41. Impact of *LC* and *FAS* alleles on cross-section fruit morphology traits in tomato (Solanum *lycopersicum* L.) germplasm

Dana Valeria Vazquez [a,b]; Javier Hernán Pereira da Costa [a,b], Vladimir Cambiaso [b], Federico Nicolás Iván Godoy [a]; Gustavo Rubén Rodríguez [a,b]

[a]*Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET-UNR), Zavalla, Argentina,* [b]*Cátedra de Genética, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Zavalla, Argentina.* vazquez@iicar-conicet.gob.ar

The *LOCULE NUMBER* (*LC*) and *FASCIATED* (*FAS*) are major genes that influence shape, size, and locule number in tomato fruits. Most fruit morphological studies focus on longitudinal section traits. The objective of this study was to characterize the impact of *LC* and *FAS* alleles on cross-section fruit morphology traits in tomato germplasm. A total of 183 cross-section fruit images from tomato accessions (n=1145) were download from https://solgenomics.net. The images were analyzed with Tomato Analyzer 3.0 (1). The evaluated traits were: perimeter (P), area (A), maximum width (MW) and height (MH), thickness (PT) and area (PA) of pericarp, and lobedness degree (LD). The locule number (LN) was visually counted. The *LC* and *FAS* data for the subset were available (2). Broad-sense heritability and phenotypic correlations were calculated. A principal component analysis (PCA) was performed. The relative frequency distribution for alleles at *LC* and *FAS* genes (*LC*/*FAS* when carrying mutated allele for both genes, *lc*/*fas* when carrying wild allele for both genes, and *LC*/*fas*, or *lc*/*FAS* respectively) for LN and LD ranges were evaluated. The broad-sense heritability was significant ($p<0.05$) for all normally distributed traits, ranged from 0.72 for PA up to 0.94 for A. The phenotypic correlations were significant ($p<0.05$) and positive for most traits. The PCA showed that variation was highly explained for the combination of *LC* and *FAS* alleles. Overall, the *lc/fas* combination had few locules and low LD, meanwhile *LC/FAS* presented high values for both traits. Also, we found that the accumulation of mutated alleles directly increased the LN but this relation was not evident for LD. On the other hand, accessions with the same combination of alleles showed contrasting LN and LD values. Our results, indicate that the the distribution of LC and FAS alleles presented a stronger impact on the variation in LN than LD, and the effect of *LC* and *FAS* genes was not enough to explain the variability present in our data for cross-section fruit traits. As conclusion, these results suggest the presence of unknown modifier genes for LC and/or FAS in tomato germplasm.

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2. *Rodriguez et al. (2011) Plant Physiology 156, 275-285*

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P4-42. SILEX: DNA extraction method suitable for recalcitrant plant species and multiple sequencing platforms used in plant breeding.

Santiago Vilanova[a], David Alonso[a], Pietro Gramazio[b], Mariola Plazas[a], Edgar Garcia-Fortea[c], Jaime Prohens[a]

[a] *Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain*
[b] *Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, 305-8572, Tsukuba, Japan*
[c] *Seeds For Innovation, PITA, Almería, Spain*

The use of sequencing and genotyping platforms has undergone dramatic improvements, becoming a standard tool in plant breeding programs (1). However, the availability of high-quality genomic DNA (gDNA) in sufficient concentrations is often the main limitation, especially for recalcitrant plant species. SILEX (SILica matrix EXtraction) is a high-throughput DNA extraction protocol, based on the standard CTAB method with a DNA silica matrix recovery, which allows obtaining NGS-quality high molecular weight genomic plant DNA free of inhibitory compounds. Manual extraction of 48 samples can be done in 96 min by one person at a cost of 0.12 €/sample of reagents and consumables. The suitability of SILEX for DNA extraction in recalcitrant species was assessed using leaves tissue of cassava (*Manihot esculenta*), grapevine (*Vitis vinifera*), loquat (*Eriobotrya japonica*), banana (*Musa* × *paradisiaca*), naranjillo (*Solanum bonariense*), and strawberry (*Fragaria* × *ananassa*). Compared with a standard CTAB extraction protocol and a common commercial extraction kit, SILEX yielded higher concentrations and better quality of DNA in a variety of species.

Also, thousands of tomato gDNA samples extracted with SILEX protocol were successfully genotyped with SPET (2) using the Illumina HiSeq 2500 platform. The reads obtained showed excellent Phred-quality scores with a mean value above 30.

Additionally, DNA extracted using this protocol was assessed by Pulsed-field gel electrophoresis (PFGE) obtaining a size that ranges between 20 to 100 Kb. These sizes are suitable for most sequencing platforms that required high-molecular-weight DNA such as Nanopore (3) or PacBio (4).

In conclusion, SILEX offers the advantages of commercial kits (high-quality DNA, fast and broad species spectrum) with those of the CTAB-based method (high yield and inexpensive), allowing to obtain DNA suitable for various next-generation sequencing platforms including Illumina and Nanopore among others.

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P4-43. EVALUATION OF ADVANCED BACKCROSSES OF EGGPLANT WITH *S. elaeagnifolium* INTROGRESSIONS UNDER LOW N CONDITIONS

Gloria Villanueva[a], Elena Rosa-Martínez[a], Ahmet Şahin[a], Edgar García-Fortea[a], Mariola Plazas[a], Santiago Vilanova[a], Jaime Prohens[a].

[a]*Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain*

Crop wild relatives (CWRs) are a fundamental resource for the development of new varieties adapted to low-input conditions and to climate change-related stresses. With this aim, in eggplant (*Solanum melongena*) we obtained hybrids and first backcrosses (BC1) between eggplant and the silverleaf nightshade (*S. elaeagnifolium*), considered a noxious invasive weed that grows in dry and nutrient-poor areas worldwide (1). In order to make the development of the introgressions lines (ILs) more efficient, successive backcrosses were genotyped and selected by means of the high-throughput genotyping platform Single Primer Enrichment Technology (SPET) (2). A set of 56 genotyped plants from advanced backcrosses (BC2 and BC3 generations) of *S. elaeagnifolium* with the recurrent *S. melongena* parent were evaluated in an open field plot under low nitrogen conditions. Morphological, biomass, yield and fruit composition traits were measured. Nitrogen (N) and carbon (C) contents were measured in fruits and leaves and nitrogen use efficiency (NUE) was calculated. A wide phenotypic diversity was found in advanced backcrosses compared to the one observed in the recurrent parent. Indeed, we have found plants with a much higher yield, NUE and phenolics content than the recurrent parent. The wide variation observed in the advanced backcrosses reveal that some materials with *S. elaeagnifolium* introgressions perform better than the recurrent parent under low N fertilization. The availability of genotyping and phenotyping data has allowed the detection of nine putative QTLs. A QTL for stem diameter in chromosome 4, three QTLs for presence of prickles in stem, leaf and fruit calyx in chromosome 6, a QTL for fruit width in chromosome 7, for chlorogenic acid content in chromosome 5, for total phenolic acid peaks area in chromosome 6, and two QTLs in chromosome 1 for chlorogenic acid peak area and phenolic acids pattern. Overall, the results show that introgressions of *S. elaeagnifolium* in eggplant may be of interest for the development of new eggplant varieties with improved adaptation to low N cultivation conditions for a more sustainable agriculture.

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P4-44. The neofunctionalization of GIGANTEA

Julia Weiss[1], Claudio Brandoli[1], César Petri[2], Marcos Egea-Cortines[1]

[1]Genética Molecular, Instituto de Biotecnología Vegetal, Universidad Politécnica de Cartagena,Spain. [2]IHMS La Mayora,CSIC, Málaga, Spain. Corresponding author: Julia Weiss (Julia.weiss@upct.es)

The gene GIGANTEA (GI) appeared in land plants. It is involved in multiple biological functions, including control over the circadian rhythm, cold tolerance, photoperiodic flowering and the emission of floral volatiles. GI is a single copy gene in the model plant *Arabidopsis* and *Antirrhinum. Petunia hybrida* (Terry et al., 2019) has two paralogs while *Nicotiana benthamiana* has three paralogs. A detailed transcriptomic and phenotypic analysis of iRNA lines of *PhGI1* and *PhGI2* in Petunia showed coinciding functions, such as changes in time period between two consecutive peaks in *PhGI1 and PhGI2*, *CHANEL* (*ZEITLUPE*) (*RNAi*:*PhGI1*) and *TOC1* (*RNAi*:*PhGI2*) expression. Silencing of both paralogs showed increased vegetative growth, reduced flower bud number, reduction in petal size due to decreased cell size, the appearance of prematurely aborting ectopic flower buds, a reduction of total volatile compounds on petal fresh weight basis and subtle changes in the volatile profile. Silencing of *PhGI1,* but not *PhGI2,* resulted in delayed flowering. Results indicate that the *PhGI*1 and *PhGI2* experienced both sub- and neofunctionalizations (Brandoli et al., 2020). On the light of previous results obtained from iRNA lines, CRISP/Cas lines will be generated for *Petunia hybrida*, *Nicotiana benthamiana* and *Antirrhinum majus* to explore the neofunctionalization of *GI* in flower development.

Brandoli C, Petri C, Egea-Cortines M, Weiss J (2020) The clock gene Gigantea 1 from Petunia hybrida coordinates vegetative growth and inflorescence architecture. Sci Rep 10: 1–17

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SESIÓN ABIERTA

Genética y COVID

Moderador: José Luis Micol

GC-01. La genética poblacional y evolutiva del SARS-CoV-2

Fernando González-Candelas [a,b,c], Mireia Coscollá [a,b], Iñaki Comas [c,d], Consorcio SeqCOVID-España

[a]Unidad Mixta Infección y Salud Pública FISABIO-Universidad de Valencia, Valencia, Spain. [b]Instituto de Biología Integrativa de Sistemas I2SysBio (CSIC-UV), Valencia, Spain.. [c]CIBER en Epidemiología y Salud Pública, Madrid, Spain. [d]Instituto de Biomedicina de Valencia (CSIC), Valencia, Spain.

Tras más de un año de pandemia de COVID-19, el estudio de la secuencia genómica del SARS-CoV-2 no ha dejado de tener un merecido protagonismo. En España, la mayoría de las secuencias del virus se han obtenido por el consorcio SeqCOVID, en el que participan más de 70 hospitales y centros de investigación. Al igual que otros consorcios y grupos de investigación, los resultados se hacen disponibles casi inmediatamente para la comunidad científica en la plataforma GISAID, en la que ya hay más de 1 millón de secuencias disponibles. Lo que en principio parecía un ejercicio meramente académico, se ha convertido en los últimos meses en una de las piezas fundamentales para el control de la epidemia. La vigilancia genómica se ha adoptado por organismos internacionales y nacionales como herramienta esencial para el control de las variantes y mutaciones, de los posibles escapes vacunales, o la confirmación de reinfecciones. Pero la información genómica también permite otras aplicaciones que nos ayudan a entender los procesos evolutivos que actúan sobre las poblaciones del virus. En esta comunicación, expondremos cómo la deriva, la migración, la expansión poblacional y, finalmente, la selección natural han ido moldeando la naturaleza y distribución de las diferentes variantes del SARS-CoV-2 que se han detectado en nuestro país.

GC-02. El consorcio SCOURGE

Angel Carracedo

Grupo de Medicina Genómica-CEGEN-CIBERER-Universidad de Santiago de Compostela
Fundación Pública Gallega de Medicina Genómica-Servicio Gallego de Salud.

Tanto los síntomas de la COVID, como la severidad y complicaciones tiene un componente genético del huésped indudable al que se le ha asignado una heredabilidad media del 50% en un estudio (1). Para encontrar el componente genético de la enfermedad se están realizando diversos estudios tanto de secuenciación de nueva generación como de GWAS y estableciéndose consorcios internacionales.

Aquí presentamos los resultados del consorcio SCOURGE, constituido por más de 80 grupos de España y países de Latinoamérica, y en el que se han recogido muestras e información clínica asociada de unos 20,000 pacientes COVID y en los que se realizó un GWAS con el array SBA (Affymetrix) que contiene 850,000 marcadores.

Además del clúster de cromosoma 3 y del gen IFNAR en cromosoma 21 ya identificados en otros estudios (2) aparecen otros hits de interés así como otros hallazgos que están siendo replicados en el consorcio HGI (Covid19 Hoste Genetics Initiative). También presentaremos los resultados de la secuenciación de genomas completos en 350 pacientes con fenotipos extremos y particularmente niños con síndrome de Kawasaki.

1. Williams et al. (2021) Twin Research and Human Genetics doi: 10.1017/thg.2020.85

2. Pairo-Castineira et al. (2021) Nature 591: 92–98

SESIÓN 5

Genética de la Población y Evolución

Moderadora: Amparo Latorre

I5-01. THE GENETIC AND MOLECULAR BASIS OF ENVIRONMENTAL ADAPTATION: A TRANSPOSABLE ELEMENT PERSPECTIVE

Josefa González

Evolutionary and Functional Genomics Laboratory. Institute of Evolutionary Biology (CSIC-UPF), Barcelona, Spain.

How organisms adapt to the environment is still an open question in Biology. Short read genome sequencing has allowed to explore the role of single nucleotide polymorphisms (SNPs) in environmental adaptation. However, SNPs alone can only explain a fraction of the existing ecologically relevant phenotypic variation. Among the structural variants that can now be studied thanks to the availability of long-read sequencing, transposable elements are likely to play a major role in adaptation due to their capacity to generate mutations that often have phenotypic effects of a complexity that is not achievable by point mutations. *Drosophila melanogaster* is an excellent model species to quantify the role of transposable elements in environmental adaptation as it has recently colonized very distinct environments. We have generated thirty-two new *D. melanogaster* reference genomes using long-read sequencing of natural populations collected in arid, cold and temperate environments. We have discovered thousands of new transposable element insertions including copies from three new transposable element families. We have also generated transcriptomic data for 18 of these genomes, which is allowing us to elucidate the role of transposable elements in expression quantitative trait loci (eQTL) variation. Finally, we are also investigating the role of the identified insertions in desiccation, oxidative and heavy-metal stress resistance, and immune response. Overall, our results will allow us to quantify the role of transposable elements in environmental adaptation and to generate testable hypothesis for follow-up functional validation studies.

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I5-02. Novel prokaryotic lineages fuel the quest for eukaryotic ancestry

Daniel Tamarit[1,3,4*], Laura Eme[1,2*], Eva F. Caceres[1,3*], Courtney W. Stairs[1], Valerie De Anda[5], Brett J. Baker[5], Thijs J. G. Ettema[1,3]

[1]Department of Cell and Molecular Biology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden. [2]Laboratoire Écologie, Systématique, Évolution, CNRS, Université Paris-Sud, Université Paris-Saclay, Orsay, France. [3]Laboratory of Microbiology, Wageningen University and Research, Wageningen, The Netherlands. [4]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden. [5]Department of Marine Science, Marine Science Institute, University of Texas Austin, Port Aransas, Texas, USA.*Equal contribution

The origin of eukaryotes is considered one of the major transitions in evolution, which took place through the symbiotic merger between an archaeon and an alphaproteobacterium. A newly discovered group, named Asgard archaea, have taken the spotlight in recent years as the probable closest archaeal relatives of eukaryotes. Yet, the limited genomic diversity available for Asgard archaea prevented a robust conclusion on the exact phylogenetic identity of the last eukaryotic common ancestor. Through the analysis of globally distributed metagenomes, we have expanded the genomic sampling of Asgard archaea, and identified four novel phylum-level clades, which we name Freya-, Gefion-, Idunn- and Vidararchaeota. Using these new genomes, we analysed two separate gene marker datasets, and applied state-of-the-art phylogenomic approaches to tackle composition-based artefacts, and explore the effects of taxon sampling, data recoding, and removal of fast-evolving sites. These results robustly placed eukaryotes within the Heimdall-Idunnarchaeota complex, and indicated a likely origin within the Heimdallarchaeota. We then used gene tree/species tree reconciliation approaches to estimate modes of evolution within Asgard archaea, which indicated significantly higher levels of gene duplication and lower levels of gene loss than other archaea. Finally, we scrutinized the genomic reconstruction of ancestral Asgard archaeal lineages to refine the gene content and metabolic potential of the last archaeal ancestor of eukaryotes, finding a likely mesophilic heterotrophic lifestyle, and access to a diverse set of eukaryotiic proteins for vesicular trafficking, membrane remodeling, N-glycosylation and informational processes. This work provides key insights into the prokaryote-to-eukaryote transition and the emergence of eukaryotic cellular complexity.

O5-01. A new genomic approach to shed light on the mystery of the evolution of Bilateria

Marta Álvarez-Presas[a], Iñaki Ruiz-Trillo[b], Jordi Paps[a]

[a]*Comparative Genomics Lab, School of Biological Sciences, University of Bristol, Bristol, United Kingdom,*
[b]*Multicellgenome Lab, Institut de Biologia Evolutiva UPF-CSIC, Barcelona, Spain*

The origin of Bilateria has been a mystery to evolutionary researchers for many years, with ongoing debates about the complex or simple nature of the ancestor of all animals with bilateral symmetry. With the advent of omics data, the phylogenetic position of some groups key to solve that question, such as the Xenacoelomorpha (composed by Xenoturbellida, Acoela and Nemertodermatida), has been subject of controversy. Two competing hypotheses support the position of xenoacoelomorphs either as sister-group to the rest of Bilateria (1,2) or as sister to echinoderms and hemichordates (within Deuterostomes), forming the Xenambulacraria group (3,4). Here we take advantage of the availability of a large number of bilaterian genomes to tackle this question. For the first time, we use genome-level evolutionary processes to infer the Bilaterian Tree of Life, generating a new dataset for this analysis, and in the application of different strategies that can shed light on such a crucial and complicated question. Among other interesting results, we have found the presence of a particular gene family that would have been a core novelty for the Bilateria on their origin.

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O5-02. EXPERIMENTAL EVOLUTION REVEALS MECHANISMS OF ADAPTATION IN THE FUNGAL PATHOGEN *FUSARIUM OXYSPORUM*

Cristina López Díaz[a], Dilay H. Ayhan[b], Li-Jun Ma[b], Antonio Di Pietro[a]

[a] *Department of Genetics, University of Córdoba, Córdoba, Spain*
[b]*Deparment of Biochemistry & Molecular Biology, University of Massachusetts Amherst, MA, USA*

Fungal pathogens represent a serious threat to human health and food safety. *Fusarium oxysporum* causes vascular wilt in more than a hundred different crops and opportunistic infections in humans (1). Despite lacking a known sexual cycle, this fungus displays a remarkable genetic plasticity (2), allowing it allows it to thrive in wide range of ecological niches. For example, a single isolate can infect hosts from both the plant and animal kingdom (3), making *F. oxysporum* an ideal model to study adaptive genetic mechanisms.

We followed an experimental evolution approach by passaging a clonal isolate through different environments, including tomato plants or plates with either complete or minimal medium. Detailed phenotyping of the evolved lines as well as competition experiments revealed significantly increased fitness in the respective selection condition compared to the ancestral clone. Interestingly, we found that independent lines passaged through plates followed similar evolutionary trajectories leading to increased growth and sporulation. Genome re-sequencing of evolved lines revealed changes at both the nucleotide and chromosome levels, many of which became fixed in the population. Two recurrent mutations were independently selected in different plate-evolved lines, including insertion of a transposable element (TE) in a gene of unknown function and a secondary mutation in the *velB* gene encoding a component of the Velvet regulatory complex that controls fungal development and secondary metabolism (4). Importantly, plate-evolved lines displayed significantly reduced fitness in other environments such as plant or animal hosts, suggesting that niche adaptation involves fitness trade-offs. Overall, our results suggest that *F. oxysporum* can adapt rapidly to new environments and that multiple mechanisms, including TEs, contribute to its remarkable genetic plasticity.

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FT5-01. The chromosome-scale assembly of *Dysdera silvatica* (Arachnida, Araneae) provides insight into the origin and evolution of chemoreceptor gene families in spiders

Paula Escuer[a], Vadim A. Pisarenco[a], Angel A. Fernández-Ruiz[a], Joel Vizueta[a,b], Jose F. Sánchez-Herrero[c], Miquel A. Arnedo[d], Alejandro Sánchez-Gracia[a], Julio Rozas[a]

[a]Departament de Genètica, Microbiologia i Estadística, Universitat de Barcelona, Spain, [b]Villum Centre for Biodiversity Genomics, University of Copenhagen, Copenhagen, Denmark, [c]High Content Genomics and Bioinformatics Unit, Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Badalona, Spain, [d]Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona, Spain

Dysdera silvatica, is a nocturnal ground-dwelling spider endemic from the Canary Islands. The genus *Dysdera* has undergone a remarkable diversification in this archipelago mostly associated with shifts in the level of trophic specialization, becoming an excellent model to study the genomic drivers of adaptive radiations. We have generated a chromosome-level assembly based on the Hi-C scaffolding technique (assembly size of 1.37 Gb; scaffold N50 of 174.2 Mb), representing a continuity improvement of more than 4,500 times with respect to the previous version (1). The seven largest scaffolds (or pseudochromosomes), which cover 87% of the total assembly size, match consistently with the seven chromosomes reported in the karyotype of this species, including the characteristic large X chromosome.

This assembly allowed the comprehensive analysis of the genomic distribution of the two main Arthropod chemoreceptor gene families, those encoding the gustatory (*Gr*) and the ionotropic (*Ir*) receptors (2). We identified 545 members distributed across all pseudochromosomes, with a notable underrepresentation in the X chromosome. Remarkably, our phylogenetic analysis uncovers some very recent gene duplication bursts. Furthermore, we found that 44% of these receptors are localized in 83 genome clusters, 17 and 66 of them including *Gr* and *Ir* genes, respectively. To estimate the level of genetic differentiation of intra and inter-clustering we defined a new gene clustering index (GCI). We obtained high GCI levels across pseudochromosomes in both gene families, ranging from 0.418 to 0.982, being highly significant (assessed by the Mann–Whitney U-test). Globally our results indicate a very recent origin of many chemoreceptors and points to the unequal crossing-over as the main mechanism of their origin. This chromosome-level assembly represents a new valuable resource to gain insights into the structure and organization of chelicerate genomes, including the role that structural variants, repetitive elements and large gene families played in the extraordinary biology of spiders.

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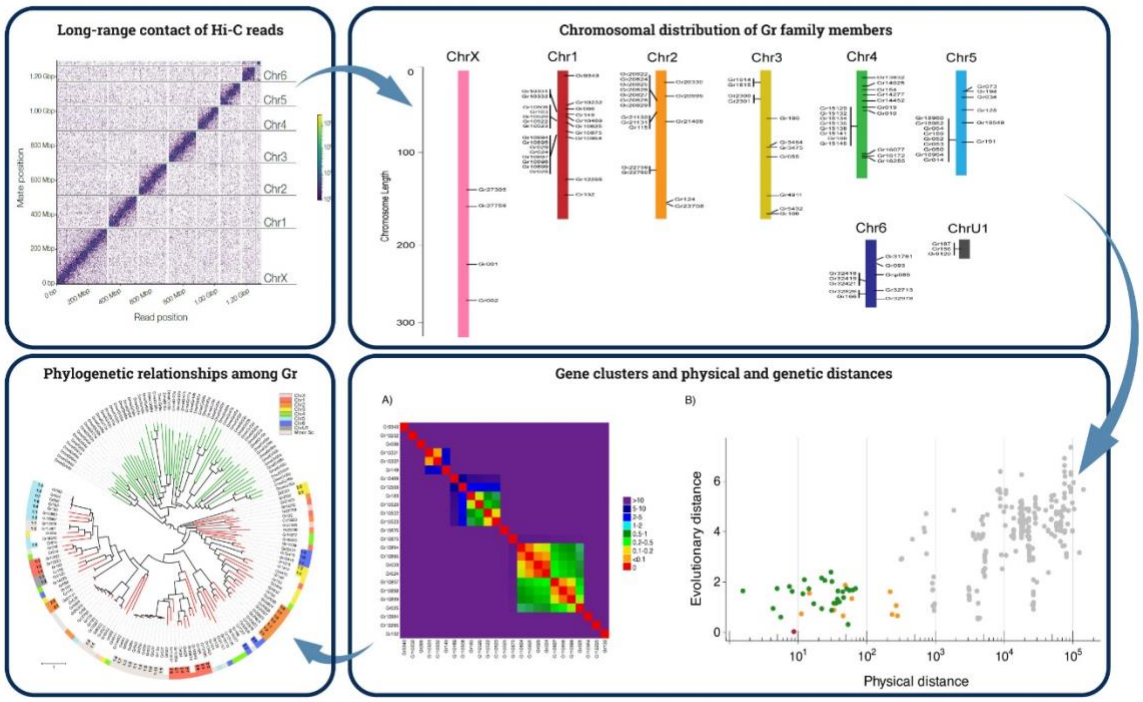
This work was supported by MICINN (PID2019-103947GB, PID2019-105794GB).

Graphical abstract:



FT5-02. Global climatic changes explain long-term demography of the most-widely distributed terrestrial reptile (*Zootoca vivipara*)

Jose L. Horreo, Patrick S. Fitze[b]

[a]*Department of Genetics, Physiology and Microbiology, Complutense University of Madrid, Madrid, Spain,*
[b]*Department of Biodiversity and Evolutionary Biology, National Museum of Natural Sciences (CSIC), Madrid, Spain*

A species' population demography depends on the local demographics of its populations, which can be compromised by local or by global phenomena. But the relevance of local and global phenomena has rarely been investigated. Here we tested for scales effects using the Eurasian common lizard *Zootoca vivipara*, the terrestrial reptile exhibiting the widest geographic distribution, as a model species. We analysed the species' ancient population demography using genetic data from its six allopatric genetic clades and tested whether its population demography depended mainly on single clades, or whether it was mainly affected by global phenomena. *Zootoca vivipara*'s population size increased since 2.3 million years ago. Population growth rate exhibited two maxima, both occurring during important global climatic changes, and the most recent one coincided with the apparition of the steppe-tundra during the Eemian interglacial. Population size and growth rate were negatively correlated with global surface temperatures, in line with global parameters driving long-term population dynamics. *Zootoca vivipara*'s ancient demography was not driven by a single clade, nor by the two clades that colonized huge geographic areas after the last glaciation. The low importance of single clades for the species' population dynamic and of the clades that colonized huge parts of Eurasia after the last glaciation, suggests that the species' high sensitivity to short-term ecological changes are responses in order to cope with changes on the short term, but what mattered on the long-term were principally the important and prolonged global climatic changes.

FT5-03. Evolution of mutational properties in phylogenetically closely related species

Eugenio López-Cortegano[a], Rory J. Craig[a], Jobran Chebib[a], Toby Samuels[a], Andrew D. Morgan[a], Susanne A. Kraemer[b], Katharina B. Böndel[c], Rob W. Ness[d], Nick Colegrave[a], Peter D. Keightley[a]

[a]Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, United Kingdom, [b]Deparment of Biology, Concordia University, Montreal, Canada, [c]Institute of Plant Breeding, University of Hohenheim, Stuttgart, Germany, [d]Deparment of Biology, University of Toronto, Ontario, Canada.

Mutation plays a key role in evolution, since it generates genetic variation, providing the raw material for selection and adaptation. However, studying *de novo* mutations is challenging, and is generally restricted to model species, meaning that we have a limited understanding of the evolution of mutational properties between closely related species. Here we present a mutation accumulation (MA) experiment in the unicellular green alga *Chlamydomonas incerta*, and perform a comparative analysis with its closest known relative *C. reinhardtii* (1), using whole-genome sequencing data. The two species' genomes are highly syntenic, with similar gene contents, but exhibit ~34% divergence at 4-fold degenerate sites, and likely diverged less than 100 million years ago (2). Our results show that the median mutation rate ($\mu \approx 6 \times 10^{-10}$), the distribution of mutations across functional genomic features, and the distribution of inter-mutation distances, are similar between the two species. The most important genomic factors explaining mutability were also shared between species, allowing for the prediction of mutability across species, and suggesting that the modeled factors were associated with the mutation rate in the species' common ancestor and have been maintained through evolutionary times. Among these factors, measures of genomic context and DNA repeatability showed the highest association with mutability. The spectrum of single nucleotide mutations (SNMs), however, substantially differed between the two species, which may relate to the occurrence of several non-synonymous substitutions at loci related to DNA repair functions, in agreement with experimental evidence from mutant strains in bacteria (3). Our results indicate that the SNM spectra have more freedom to diverge than other mutational properties, which may contribute to the early differentiation of genomic and biological characteristics.

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3. Dillon et al. (2017). Molecular Biology and Evolution 34: 93-109.

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FT5-04. INITIAL POPULATION STRUCTURE AND SELECTION RESULTS ON A NEW CAMEROONIAN WHOLE-EXOME SEQUENCING DATASET

Sonia Olaechea-Lázaro[a], Neskuts Izagirre[a], Saioa López[b], Óscar García[c], J.M. Lorenzo-Salazar[d], C. Flores[d,e,f,g], K.R. Veeramah[h], G. Hellenthal[i,j], M. G. Thomas[i,j], S. Alonso[a]

[a]*Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country (UPV/EHU), Barrio Sarriena s/n 48940 Leioa, Spain*

[b]*Department of Strategy and Data Insights, Wellcome Trust, 215 Euston Road, Bloomsbury, London, NW1 2BE, United Kingdom*

[c]*Forensic Science Unit, Forensic Genetics Section, Basque Country Police, Erandio (Bizkaia), Spain*

[d]*Genomics Division, Instituto Tecnológico y de Energías Renovables (ITER), Santa Cruz de Tenerife, Spain*

[e]*Instituto de Tecnologías Biomédicas (ITB), Universidad de La Laguna, Santa Cruz de Tenerife, Spain*

[f]*Research Unit, Hospital Universitario Nuestra Señora de Candelaria, Universidad de La Laguna, Santa Cruz de Tenerife, Spain*

[g]*CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain*

[h]*Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, 11794-5245, United States*

[i]*UCL Genetics Institute, University College London (UCL), Gower Street, London, WC1E 6BT, United Kingdom*

[j]*Research Department of Genetics, Evolution & Environment, University College London (UCL), Darwin Building, Gower Street, London, WC1E 6BT, United Kingdom*

Cameroon is considered an “Africa-in-miniature” not only due to its high genetic, ecological, and linguistic diversity, but also due to the wide variety of subsistence strategies adopted by its inhabitants. Here, we analyse Affymetrix Human Origins Array and whole-exome sequencing (WES) data to understand how environmental factors mould human genetic diversity in this context. We analysed population structure in more than 600 genotyped Cameroonian individuals with the aim of choosing 100 that were representative of the diversity present in the country and which we WE-sequenced. Then, we evaluated population structure and diversity (PCA) and selection signals (Tajima’s D, Fu and Li’s F, PBS) for this dataset. Further, given that buccal swabs were the DNA source and that ~1% of the reads were unmapped, these were used to identify the oral microbiome. Population structure analysis of our data classify Cameroon in three main genetic clusters coinciding with the North, West and South regions. Moreover, we identify putative region-specific selection signals associated to environmental factors. Of note, our initial results suggest that *SLC49A3*, a gene involved in zinc transport, has been target of positive selection. Finally, *Saccharomyces cerevisiae*, *Streptococcus pneumoniae* and *Neisseria elongata* appear in our sample as the most frequent fungi, virus and bacteria, respectively. As a conclusion, we have identified population structure and signatures of natural selection using WES, which has proved an adequate tool to assess this phenomena.

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FT5-05. Twenty years of evolution and diversification of digitaria streak virus in *Digitaria setigera*

Sergio Ortega-del Campo[1], Ioana Grigoras[2,#], Tatiana Timchenko[2,3], Bruno Gronenborn[2,3,†] and Ana Grande-Pérez[1*]

[1]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", (IHSM-UMA-CSIC), Área de Genética, Facultad de Ciencias, Campus de Teatinos, 29071, Málaga, Spain. [2]Institut des Sciences du Végétal, CNRS, 91198 Gif-sur-Yvette, France. [3]Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France.

Within the family *Geminiviridae*, the emergence of new species results from their high mutation and recombination rates. In this study, we report the variability and evolution of digitaria streak virus (DSV), a mastrevirus isolated in 1986 from the grass *Digitaria setigera* in an island of the Vanuatu archipelago. Viral DNA of DSV samples was amplified from *D. setigera* specimens, derived from the naturally infected original plant, which were propagated in different laboratories in France and Italy for more than twenty years. From the consensus sequences, the nucleotide substitution rate was estimated for the period between a sample and the original sequence published by Donson et al. in 1987, as well as for the period between samples. In addition, the intra-host genetic complexity and diversity of 8 DSV populations with a total of 165 sequenced haplotypes was characterized. The evolutionary rate of DSV was estimated to be between 1.21×10^{-4} and 3.70×10^{-3} mut/site/year, within the ranges observed in other single-stranded DNA viruses and RNA viruses. Bioinformatic analyses revealed high variability and heterogeneity in DSV populations, which confirmed that mutant spectra are continuously generated and are organized in quasispecies. The differences in variability in each of the genomic regions reflected a dynamic and modular evolution in the mutant spectra that was not reflected in the consensus sequences. However, the region encoding the movement protein (MP), showed rapid fixation of the mutations in the consensus sequence and a high dN/dS ratio in the mutant spectra, which suggests strong positive selection in this region. Phylogenetic analyses revealed a possible divergence in two genetic lineages from the original Vanuatu DSV isolate.

FT5-06. FITNESS AND MUTATIONAL RATE IS AFFECTED BY LOSS OF GroEL-MUTATIONAL BUFFERING IN HIGHLY BOTTLENECKED *E. coli* POPULATIONS

Roser Montagud-Martínez[a,b], Mario A. Fares[a,b,c], Christina Toft[d], Beatriz Sabater-Muñoz[a]

[a] Integrative and Systems Biology lab, Department of Plant stress, Institute of Plant Molecular and Cellular Biology, Spanish National Research Council (IBMCP CSIC-UPV), Valencia, Spain; [b] Program for Systems Biology of Molecular Interactions and Regulation, Institute for Integrative Systems Biology (I2SysBio CSIC-UV) Paterna, Spain; [c] Posthumous author; [d] Panomics and Evolutionary Systems Microbiology lab, Program for Systems Biology of Molecular Interactions and Regulation, Institute for Integrative Systems Biology (I2SysBio CSIC-UV) Paterna, Spain

Chaperones are involved in the folding of nascent client proteins, in the prevention of polypeptides aggregation and in the rescue of unfolded clients due to environmental stresses. The pioneering works of Rutheford and Lindquist, and those of Fares' team, and Touriki and Tawfik, have highlighted the importance of chaperones in buffering mutational effects by allowing for the adaptive evolution of its client proteins. This adaptive mechanism might be fundamental in explaining how ancient symbionts still persist even under a strong genetic drift regime. Consequently, how would an adapted consortium then deal with the loss of this key system? This can be observed in many *Mycoplasma* species, which lack this robustness system, where proteome evolution has become independent of protein folding. However, what happens when the organism's proteome depends on its chaperone folding capabilities, and this system fails?
Experimental evolution of *Escherichia coli* under high-expression rate of GroEL is only possible when the system is subjected to strong genetic drift, as overexpression is significantly costly. Despite this limitation, the loss of GroEL overexpression increases the extinction rate, leading to an equilibrium between GroEL level and fitness. By challenging *E. coli* to daily single-cell bottlenecks under high GroEL overexpression, we found that after a certain number of generations, a number of compensatory mutations arose in the system allowing for decreasing the GroEL level while not affecting the fitness. How these two parameters, structural stability and functional innovation interact, still deserves further research.

P5-01. FIRST EVIDENCE OF GENETIC CONNECTIVITY AND TEMPORAL STABILITY OF *ARISTEUS ANTENNATUS* SPAWNING FEMALE'S GROUNDS ALONG THE SPANISH MEDITERRANEAN COAST

Melania Agulló[a], Sandra Heras[a], José-Luis García-Marín[a], Manuel Vera[b], Alba Abras[a], Laia Planella[a], María Inés Roldán[a]

[a]Laboratoi d'Ictiologia Genètica, Universitat de Girona, Girona, Spain, [b]Departamento de Zooloxía, Xenética e Antropoloxía Física, Campus Lugo, Universidade de Santiago de Compostela, Lugo, Spain.

The blue and red shrimp *Aristeus antennatus* (Risso, 1816) is a marine decapod widely exploited by the deep-water bottom trawl fisheries on the Western Mediterranean Sea, mostly at depths of between 500 and 800 m (1). Mature females' aggregations on the middle slope are formed during the reproductive period (2). As a first time, we estimated the levels of genetic diversity within female's grounds, the genetic divergence among them, and the degree of temporal genetic stability (2016 and 2017 summer seasons). Twelve microsatellite loci were applied in a total of 1343 females from Gulf of Lions to Cabo de Palos (Port de la Selva, Roses, Palamós, Blanes, Vilanova i la Geltrú, Dénia and Santa Pola). The number of alleles per locus ranged from 2 to 24 with a total mean of 8.96. The mean observed heterozygosity (H_o) for each location varied from 0.427 to 0.491 in 2016 and from 0.464 to 0.492 in 2017, while the mean expected heterozygosity (H_e) varied from 0.613 to 0.643 in 2016 and from 0.628 to 0.644 in 2017. Of a total of 168 comparisons between the observed and the expected genotypic proportions under the Hardy-Weinberg equilibrium, 90 were significant after Bonferroni correction, which caused deviations from the Hardy-Weinberg equilibrium in the fourteen samples analysed. The AMOVA did not indicate significant differences among locations either 2016 (F_{ST} = 0.00128, p-value > 0.05) or 2017 (F_{ST} = 0.00033, p-value > 0.05). Likewise, no significant differentiation between the two temporal replicates (F_{CT} = 0.00020, p-value > 0.05) was detected. Thus, our study points out a scenario of high genetic connectivity and temporal stability in spawning female's grounds of *A. antennatus* during 2016 and 2017, which could be mainly caused by larval dispersal and oceanographic currents. Furthermore, although to date there is no evidence on adult females' migration, a recent genetic study of our group showed males' migration among locations.

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P5-02. HLA GENES IN AFRO-AMERICAN COLOMBIAN (SAN BASILIO DE PALENQUE): THE FIRST FREE AFRICANS IN AMERICA

Antonio Arnaiz-Villena ,Ignacio Juarez,Adrian Lopez-Nares,Paula Diaz-Candeleda,Enrique Garcia-Moreno,Fabio Suarez-Trujillo

Dept Immunology,School of Medicine,University Complutense,28040 Madrid,Spain

An Afro-American semi-isolated Colombian population is studied for its HLA genes: San Basilio de Palenque community in Colombia northern mountains. This community represents the first free Africans in America earning recognition; Spanish Crown were forced to issue a decree declaring them free (1691 AD), more than 100 years before than Haiti Republic existed. Original San Basilio de Palenque population fled from Spanish traders that carried them as slaves to Cartagena and they funded in nearby Maria mountains a fortified town (Palenque). They started helping new Africans brought as slaves to flee and join them. Nowadays, they also speak the only extant Bantu-Spanish Creole language over the World; these people have been apart from their neighbours and claim a direct African descent. Their HLA genes were compared with African, Afro-American, Amerindian and worldwide populations by using genetic distances (DA), Neighbour-Joining dendrograms and correspondences analyses. Arlequin, DISPAN and VISTA softwares were used for the completion of these computerized calculations. San Basilio de Palenque, a relatively ethnic isolate, is genetically close to other North and South Afro-Americans and to West Africa-Bantu speaking groups (Senegalese from Africa Guinea Gulf). No Amerindian or Europeans gene flow to this population was found. Five HLA extended haplotypes are found only in this population: A*02-B*07-DRB1*0801-DQB1*0301, A*02-B*35- DRB1*1304-DQB1*0301, A*02-B*15-DRB1*0302-DQB1*0402, A*01-B*51-DRB1*0301-DQB1*0201, A*68-B*15- DRB1*0102-DQB1*0501. This may be due to the maintenance of their own African culture, and even their unique Creole language and subsequent lack of genetic admixture

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P5-03. Complexity metrics trend analysis through endosymbiosis evolution

Moisès Bernabeu[a], Vicente Arnau[a,b], Wladimiro Díaz[a,b], Andrés Moya[a,c,d]

[a]Institute for Integrative Systems Biology (I2SysBio), UV-CSIC, Paterna, Spain, [b]Department of Informatics, Universitat de València, València, Spain, [c]Area of Genomics and Health, Foundation for the Promotion of Sanitary and Biomedical Research of Valencia Region (FISABIO-Public Health), Valencia, Spain. [d]Biomedical Research Networking Center for Epidemiology and Public Health (CIBERESP), Madrid, Spain.

The existence in biological evolution of a trend towards greater complexity is an open question that has generated much debate. This is mostly due to the existence of multiple definitions of complexity and their measures. Moya et al. (2020), assuming that the genome is a good record of the evolutionary history of the species in their interaction with the physical and biotic environment (Adami, 2002), carried out a study of the evolution of cyanobacteria, measuring in their genomes several metrics and genomic parameters. Through phylogenomic analysis, they estimated the values of these metrics in the internal nodes, detected a phylogenetic signal in all of them and showed that some of them presented a positive trend, that is: the genomes of the most recent species were more complex than the older species.

This hypothesis can be tested by the investigation of the evolution of an opposite case, that of endosymbionts, intracellular bacteria of eukaryotes whose genomes have undergone with respect to their free-living ancestors a drastic process of reduction and degeneration, as well as loss of metabolic diversity. These organisms should present metrics of decreasing complexity with respect to the organisms from which they are derived and, therefore, the trend should be negative. In this communication, we study a set of 192 genomes where 102 organisms are endosymbionts that belong to 13 different phyla. All the complexity metrics studied and proposed in the study of cyanobacteria were applied to the endosymbiont genomes. We derived a phylogenomic tree based on a matrix of 31 proteins to reconstruct the ancestral states of the metrics and, subsequently, we carried out the trend analysis. The metrics surprisingly show a positive slope, indicating an unexplained increase in the complexity of recent endosymbiont genomes relative to ancestral free-living bacterial genomes.

The only connection point that we found between the positive trend metrics in cyanobacteria and endosymbionts is the greater evolutionary acceleration with respect to their values in the corresponding ancestors. These results highlight the need to work on improving the metrics, or new ones, that be independent of the evolutionary acceleration, in such a way as to find those that really measure the evolution towards greater complexity, if it exists.

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P5-04. Mislabelling at sea. Applying DNA barcoding for the identification on board: The case of black hakes

Carmen Blanco-Fernandez[a], Sara Rodriguez Diego[a], Pablo Alba Gonzalez[a], Karim Erzini[b], Eva Garcia-Vazquez[a], Gonzalo Machado-Schiaffino[a]

[a]Department of Functional Biology, Universidad de Oviedo, Oviedo, Spain; CCMAR, Universidad do Algarve, Faro Portugal [b]

Fisheries sustainability relies on an accurate report of captures to manage stocks. Hence, mislabelling of catches poses a threat for sustainable fisheries. Molecular tools provide a unique method for reliable identification without the need of any previous morphological knowledge. We have studied the case of black hakes (*Merluccius senegalensis* and *Merluccius polli*). Black hakes are morphologically very similar, and have been traditionally caught together in mixed fisheries (1); therefore it would be expected that fishermen find difficulties identifying them. We selected a 450bp fragment of the Mitochondrial Control Region due to its variability (2) and identified 15 SNPs which allow distinguishing between both species. A total of 806 black hakes were captured along the coast of Mauritania and Senegal and identified both by the fishermen (visually) and using DNA forensics. Our results show high risk of mislabelling, as 31.26% of the sample was wrongly identified. Moreover, differences in directionality were found: 38.37% of the labelled *M. polli* were mislabelled, while the risk for those labelled as M. *senegalensis* was 18.37%. Furthermore, depth of capture and length of the individuals are shown to significantly explain differences in the labelling. Shorter individuals and those caught in shallower waters tend to be mislabelled more often. With this work, we would like to highlight the value of applying DNA barcoding to detect the risk of mislabelling at sea, which must be taken into consideration when making management decisions in order to guarantee the conservation of stocks.

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P5-05. Chaotic genetic patchiness in the high valued Atlantic stalked barnacle *Pollicipes pollicipes* from Iberia Peninsula: implications for fisheries management.

Marina Parrondo[a], Paloma Morán[b], Marion Ballenghien[c], Katja Geiger[d], Julio Arrontes[d], Lucía García-Flórez[e], Eva García-Vázquez[a], Alba Aguión[f], Juliette Chiss[c], Joana Fernandes[g], Gonzalo Macho[h], Teresa Cruz[i], José Luis Acuña[d], Didier Jollivet[c] & Yaisel Juan Borrell[a].

[a]Dept. Biología Funcional, Universidad de Oviedo, Spain; [b]Dept. Bioquímica, Genética e Inmunología (CIM-UVIGO), Universidade de Vigo, Spain; [c]Sorbonne Université, CNRS UMR 714, Roscoff, France; [d]Dept. de Biología de Organismos y Sistemas, Universidad de Oviedo, Spain; [e]Centro de Experimentación Pesquera, Gijon, Spain, [f]Centro de Investigación Mariña, Universidade de Vigo, Spain; [g]MARE-Laboratório de Ciências do Mar, Universidade de Évora, Portugal; [h]Estación de Ciencias Mariñas Illa de Toralla (ECIMAT), Universidade de Vigo, Spain; [i]Dept. Biologia, Escola de Ciência e Tecnologia, Universidade de Évora, Portugal.

The stalked barnacle *Pollicipes pollicipes* inhabits rocky shores from the Atlantic coasts of South England to Senegal. Because of culinary traditions of the southern Europe, stalked barnacles represent an important target for local fisheries in the Iberian Peninsula. To manage this fishery, it is important to assess the dynamics of local populations over the Iberian coast and how they are inter-connected at a wider scale using finely-tuned genetic markers. In this work, a new enriched library of GT microsatellites for *P. pollicipes* was prepared and sequenced using the Ion TorrentTM Next Gen-Sequencing technology and 1401 adults and juveniles were sampled in 15 localities of three geographic regions: Galicia and Asturias (both in Spain) and the South of Portugal. Twenty polymorphic loci were tested and validated as new molecular tools to address spatial and temporal genetic patterns. Our results reveal high genetic diversity in the adults. However, juveniles are genetically more structured than their adult counterparts, which alternatively display much more connectivity between the three studied regions. The lack of spatial genetic heterogeneity in adults may be due to the superimposition of several generations of settlers coming from different geographic origins which mainly depends upon the orientation of residual currents along the coast during reproduction. Genetic differentiation of juveniles may be indeed congruent with the Iberian Peninsula hydrodynamics which can produce chaotic genetic patchiness at small temporal scales due to sweepstakes reproductive success, collective dispersal and/or self-recruitment. In summary, high effective population sizes of *P. pollicipes* can lead to the false impression of population panmixia at the Iberian scale by masking more restricted and current-driven larval exchanges between regions. This should be taken into consideration for further specific management and conservation plans in this valuable species.

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P5-06. GENETIC CHARACTERISATION OF A REINTRODUCED POPULATION OF *Capra pyrenaica* (SCHINZ, 1838) IN THE NATIONAL PARK SIERRA DE GUADARRAMA.

Liébana[1] Alba, Lantero[1] Esther, Matallanas[1] Beatriz, Muñoz[2] Benito, Olmedo[2] Cristina, Abelenda[1] María, González[1] Eva Mª, Callejas[1] Carmen, González[1] Mª Teresa, Refoyo[2] Pablo.

[1]Dept. of Genetics, Physiology and Microbiology, Faculty of Biological Sciences, Complutense University, Madrid, Spain. [2]Dept. of Biodiversity, Ecology and Evolution, Faculty of Biological Sciences, Complutense University, Madrid, Spain. Corresponding author: Callejas Carmen (carmenca@ucm.es)

The reintroduction of Iberian ibex, *Capra pyrenaica* in the Sierra de Guadarrama National Park (PNSG) was carried out in the 1990s with few individuals from Sierra de Gredos and Las Batuecas populations. At present, the population is well established and its density has increased to almost saturation level. For an efficient management and conservation of this population, information on their genetic variability is crucial.

Currently there are few assays for high variable regions in this wild species. In this work we provide a new PCR assay specific to this species based on a highly variable region of the mitochondrial gene *cytochrome oxidase subunit I* (COI). The assay was tested in a wide number of specimens of *C. pyrenaica* from Sierra de Guadarrama. Blood samples were employed for the genomic DNA extraction and used in PCR reactions with specific primers for the COI gene. The amplicons obtained were purified and sequenced.

Sequence analysis showed quite low variability, being identified just a single SNP in the 735 bp sequence, defining two haplotypes, both at intermediate frequencies (H1, 39.13% and H2 60.86%). These results are discussed with data for other DNA markers in the context of other Iberian populations of *C. pyrenaica*. The low mitochondrial variability of this population might be the result of the pervasive bottlenecks in source populations (Sierra de Gredos and Las Batuecas), to the founder effect in the PNSG population and the subsequent action of genetic drift. The situation would compromise the health status of the individuals and their fitness.

P5-07. INTRA-POPULATION VARIABILITY IN MORPHOLOGICAL, PARASITOLOGICAL AND GENETIC TRAITS OF INVASIVE LANGUEDOC MINNOW *Phoxinus septimaniae* Kottelat, 2007 UNDER A SEASONAL COLONIZATION DYNAMIC

Inés Martín-Barrio[a], Carla Llinares[a], Gema Castillo-García[a], Pilar Arana[b], Emili García-Berthou[c], David Almeida[a], Alejandra Cruz[a]

[a]Departamento de Ciencias Médicas Básicas, Facultad de Medicina, Universidad San Pablo-CEU, CEU Universities, Campus de Montepríncipe, Madrid, Spain, [b]Departamento de Genética, Fisiología y Microbiología, Facultad de Biología, Universidad Complutense, Madrid. Spain, [c]GRECO, Instituto de Ecología Acuática, Universidad de Girona, Girona, Spain

Biological invasions are one of the main drivers of biodiversity loss at the global scale (1). This is of particular conservation concern in the Iberian eco-region, where fish fauna has a high level of endemism (>30% of native species) (2). A pivotal aspect on invasive species is the colonization capacity, which depends on a wide range of biological traits (3). Languedoc minnow *Phoxinus septimaniae* is a small-bodied cyprinid fish native to SE France. This species was introduced into the River Tordera (NE Iberia) in 2004. This is an excellent system to analyse the biological traits of non-native minnow affecting its colonization capacity because an upstream section of this river seasonally dries up (June–July), but before that drought event, a minnow sub-population migrates from the middle section to breed and spawn during late May (spring). Thus, the aim of this study consisted of comparing morphological, parasitological and genetic traits of minnow between the Intermittent and Permanent sections of the River Tordera. Specifically, a variety of biological parameters were assessed at the individual level: body condition, anatomical health status (5), fluctuating asymmetry of bilateral characters (6), parasite load, Shannon index (5) and genetic diversity (micro-satellite DNA markers). For this purpose, minnow individuals were sampled by electrofishing in mid-May 2018 and 2019, just before the spawning period. Body condition was marginally non-significant between river sections, with minnow displaying a higher adjusted body mass in the Intermittent section. Similarly, health status and developmental stability were better in the sub-population from the Intermittent section. Parasitological traits were lower in the Intermittent section, which may be related to a stronger immunological capacity to defend against diseases. Finally, the indices of genetic diversity (Internal Relatedness (7) and Homozygosity by Locus (8)) were also higher in the Intermittent fish sample. Overall, results suggest that this wide intra-population variability will contribute to the species' successful establishment throughout Iberian waters, which poses a serious risk to its highly valuable native fish fauna. These findings will aid to provide insights into the variety of mechanisms operating on the bio-invasion process.

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P5-08. ASSEMBLY AND CHARACTERISTICS OF THE FIRST GENOME DRAFT FOR THE TUNICATE SPECIES *STYELA PLICATA*

Carles Galià-Camps[1+], Tilman Schell[2], Carlos Carreras[1], Xavier Turon[3], Carola Greve[2*], Marta Pascual[1*]

*[1]Departament de Genètica, Microbiologia i Estadística - IRBio, Universitat de Barcelona, Avinguda Diagonal 643, 08028 Barcelona, Spain; [2]LOEWE Centre for Translational Biodiversity Genomics (LOEWE-TBG), Senckenberganlage 25, 60325 Frankfurt am Main, Germany; [3]Centre d'Estudis Avançats de Blanes (CEAB, CSIC), Accés Cala St. Francesc 14, 17300 Blanes, Girona, Spain. *: Both researchers have equal contribution. [+]cgaliacamps@gmail.com*

Invasive species cause important ecological and economical losses while providing natural experiments for characterizing evolutionary adaptive responses. *Styela plicata* (Lesueur, 1823) has been known for years as an introduced species in all temperate oceans worldwide. Its expansion is facilitated by its invasive potential on anthropogenic structures such as harbors and aquiculture facilities. The knowledge of the genomic bases of this invasive species, which is still not available, is key to future studies aiming to understand its biology and to predict its invasive behaviour. In this poster, we summarize the statistics of the first draft of *Styela plicata* genome, obtained by using PacBio Continuous Long Reads (CLR) and Illumina Short Reads. At the moment, our genome assembly presents high quality characteristics, such as an assembly size of 417Mb which is close to the estimated genome size of 430Mb, a N50 of 898Kb (NG50: 851Kb), a high percentage of metazoan single copy ortholog genes detected (92.3%), and a lack of contamination traces due to discarding the shortest CLR (<25 Kb) prior to building the assembly. In addition, we successfully recovered the mitochondrial genome of *Styela plicata* with a mean coverage of 381x. This mitogenome has the same structure as the two *Styela* mitogenomes available in NCBI. Now, we aim to obtain a chromosome-level genome by applying Omni-C data and annotating it with RNA transcripts from gonads, gills and mantle. This genome will be a valuable resource for investigating adaptation and invasiveness of this globally introduced species when including population genomic data.

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P5-09. Genome characterization of a new Enterococcus isolated from the gut microbiota of the German cockroach *Blattella germanica*

Rosario Gil[a], Emilio Garrote-Sánchez[a], Rebeca Domínguez-Santos[a], Vicente Sentandreu[b], Amparo Martínez[b], Giuseppe D'Auria[c], Andrés Moya[a,d], Amparo Latorre[a,d]

[a]Institute for Integrative Systems Biology (I2SysBio), University of Valencia and CSIC, [b]Genomics Unit, Central Service for Experimental Research (SCSIE), University of Valencia, [c]Sequencing and Bioinformatics Service, Foundation for the Promotion of Sanitary and Biomedical Research (FISABIO), València, [d]Genomics and Health Area, FISABIO, València

Cockroaches are omnivorous insects that harbour a rich and complex gut microbiota, similar in some characteristics to human gut microbiota. In fact, they are considered a natural reservoir of human pathogens, some of which can contain antibiotic resistance genes (ARGs) that can be transferred to humans, and its characterization may be relevant for biomedical research. Our group has been working for more than a decade with the German cockroach *Blattella germanica* as a symbiotic model system. In a previous experiment, we isolated from its hindgut a bacterium identified as belonging to genus *Enterococcus* which presented sensitivity to several antibiotics. We have carried out its genome project using a PacBio platform, in order to assign it a taxonomic status, study its genomic structure and evolution, and deduce its potential pathogenicity. Our results indicate that this bacterium is a new strain of *Enterococcus avium*, with a genome organized in two circular replicons of 3.7 and 1.2 Mb, respectively.

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P5-10. Genomic in a sea urchin across a natural pH gradient

Sara González-Delgado[1], Ricardo T. Pereyra[2], José Carlos Hernández[1], Olga Ortega-Martínez[2], Bea Alfonso[3] and Rocío Pérez-Portela[4].

[1]Departamento de Biología Animal, Edafología y Geología, Universidad de La Laguna, Canary Islands, Spain. Tjärnö Marine Laboratory, Department of Marine Sciences, University of Gothenburg, Sweden. [3]Departamento de Botánica, Ecología y Fisiología Vegetal, Universidad de La Laguna, Canary Islands, Spain. [4]Departamento BEECA del área de invertebrados e IRBIO de la Facultad de Biología de la Universidad de Barcelona, Spain. Corresponding author: Sara González Delgado (sgonzald@ull.edu.es)

Ocean acidification (OA) is one of the most important threats facing calcifying marine organisms in a near future. Recent studies in natural acidified systems, such as CO_2 seeps, have shown that some species, such as the sea urchin *Arbacia lixula*, can develop respiratory, mineralogy and reproduction tamponing systems to circumvent some of the negative effects of extreme pH drops (1). Nonetheless, the genomic information underpinning that physiological/phenotypic traits was still unavailable. In order to identify signs and or potential genes related to local adaptation to pH changes in this species, we performed a population genomic study along a steep pH gradient (along only 150 meters) in the Fuentecaliente CO_2 seeps system (La Palma Island, Canary Island). We generated 2b-RADseq genomic libraries on 74 samples collected at four different stations of the seeps system affected by different pH values (vent1 = 7.4, vent2 = 7.6 transition1= 7.8, transition2= 7.9, control = 8.1) with the aim to identify single nucleotide polymorphisms (SNPs). After high throughput sequencing of the 2bRAD libraries, we have identified and isolated a total of 14,883 variable SNPs. Genomic analyses, based on heterozygosity, genetic distances and Bayesian clustering, among others, detected differences in heterozygosity and genotypes along the pH gradient. Also, clear differences in genomic structure could be observed from the control site to the seeps sites. Although our results suggest the existence of genomic differences along the pH gradient, we were not able to identify specific genes, and their associated pathways, related to local adaption, since we lacked annotation of the outliers detected.

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P5-11. SARS-CoV-2 Genome variability in five Southern Europe urban areas

Elka Kay, Silvia Martín-Lluesma, Nuno Henriques-Gil

Departamento de Ciencias Médicas Básicas, Facultad de Medicina, Universidad San Pablo CEU Campus de Montepríncipe, Alcorcón, Madrid

Thousands of SARS-CoV-2 isolates are partial or completely sequenced in a daily basis. Indeed, the virulence may vary from one strain to another, and the phylogenetic approaches picture epidemiological relationships. Additionally, where and what mutations occur may provide important data about the constraints in SARS-CoV-2 genome evolution.

We selected five specific urban regions from Southern Europe – Athens (Greece), Central Italy, Créteil (France), Madrid and San Sebastián (Spain) – in order to test the selective models acting on this genome, and detect hypothetical recurrent mutations. We compared 121 available complete genomes, including 24 from each region, and Wuhan-1 as reference sequence.

Considering that the global diversity was quite low (π=0,00053), it is noteworthy that 252 (67,4%) out of 374 polymorphic sites were singletons. This result was expected given the strong population expansions associated with the pandemics. So, the 121 genomes corresponded to as much as 105 different haplotypes.

The phylogenetic analysis, based on the parsimony informative sites, shows that the sequences of a same region tend to cluster together, but it is also clear that any region also includes “outsiders”. These facts support both a local diversification, and that different strains of SARS-CoV-2 have been imported to a given area. None of them had a “patient zero”.

The low rates on non-synonymous against synonymous mutations, indicates that the genome de SARS-CoV-2 is subject to purifying selection. This is specially so for the long ORF1ab. By contrast, the Spike Protein Gene (gene S) had the highest rate of non-synonymous changes, although still corresponding to the neutrality model predictions.

We also detected a few sites where a same mutation occurred independently in more than one different haplotype.

P5-12. THE LITTLE ONES COULD RULE THE FUTURE OCEANS

José Carlos Hernández[1], Sara González-Delgado[1], Owen S. Wangensteen[2], Carlos Sangil[1,3], Celso A. Hernández[1], Ana Z. Soto[4], Beatriz Alfonso[3], Stefano Mariani[5] and Rocío Pérez-Portela[6].

[1]Departamento de Biología Animal, Edafología y Geología, Universidad de La Laguna, Canary Islands, Spain. [2]The Norwegian College of Fishery Science, UiT The Arctic University of Norway, Tromsø, Norway. [3]Departamento de Botánica, Ecología y Fisiología Vegetal, Universidad de La Laguna, Canary Islands, Spain. [4]Ecosystems and Environment Research Centre, University of Salford, UK. [5]School of Biological & Environmental Sciences, Liverpool John Moores University, L3 3AF, UK. [6]Departamento BEECA del área de invertebrados e IRBIO, de la Facultad de Biología de la Universidad de Barcelona, Spain. Corresponding author: Sara González Delgado (sgonzald@ull.edu.es)

Metarbacoding techniques have revolutionised ecological research during recent years. Studying biodiversity had never been as fast, easy and accurate as today with these new tools (1). Additionally, these techniques makes possible to differentiate cryptic species and previously hidden diversity. Under the current scenario of climate change and ocean acidification, biodiversity loss it one of the major threats of marine ecosystems (2), and some studies have suggested significant lost of biodiversity and ecosystem simplification in marine acidified areas. Our study intends to explore the effects of ocean acidification and pH variation on marine benthic communities using the DNA metabarcoding techniques to measure biodiversity. For that purpose, we selected as the area of study a naturally acidified system, generated by a CO_2 seeps system, found on La Palma Island (Canary Islands, Spain, see 3), Four sites were sampled at different distances of one of the natural CO_2 seep that creates a pH gradient. Six scrapings of 20x20 quadrats were collected from the rocky bottom, preserved in 96% ethanol, and stored until DNA extraction. Libraries of a universal fragment of the mitochondrial gene Cytochrome c oxidase subunit I (COI) were created and high throughput sequenced following the protocol of Wangensteen and Turon (1). The OBITools-based eukaryotic metabarcoding pipeline was used to process the sequencing reads (1). Finally, the R package vegan v2.5.5 was applied for diversity analyses, and of comparison of diversity parameters among sites along the pH gradient. Our biodiversity fine-scale analyses demonstrate, in general, non-significant changes in species diversity along a natural pH gradient. Furthermore, for the first time, we point out high levels of taxonomic diversity, both within the algal (mainly Red algae) and invertebrate community, in natural acidified areas. This result of higher levels of biodiversity in acidified areas might be due to the detection of small and cryptic species, previously undetectable by other techniques, which could actually be the real “winners” of the future oceans affected by acidification.

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P5-13. HUMAN MITOCHONDRIAL DNA, DISEASE AND LONGEVITY: A POPULATION PERSPECTIVE

Candela L. Hernández[a], Luis J. Sánchez-Martínez[a], Rosario Calderón[a]

[a]*Departamento de Biodiversidad, Ecología y Evolución, Facultad de Biología, Universidad Complutense de Madrid, Madrid, Spain*

Nowadays, human mitochondrial genome is being extensively studied from two main approaches. First, to understand its association, not only with a broad spectrum of diseases, but also with aging and longevity. Second, to reconstruct the human evolutionary history, by addressing phylogenetic relationships between populations, tracing migrations and dating gene flow events. All these issues rely on particular features of mitochondrial DNA (mtDNA) –lack of recombination, inheritance pattern, high mutation rate and high copy number per cell– that are the result of a specific cellular context. One of the most interesting focuses in modern Human Population Genetics is to link present-day human genetic variation with diseases. In an attempt to merge these two viewpoints, we present here a comprehensive revision of associations found between mtDNA haplogroups (groups of mitochondrial sequences phylogenetically related and defined by specific mutations), complex diseases and longevity phenotypes. Several studies have pointed at some specific haplogroups as susceptibility factors for certain disorders (e.g. Hg H in cardiovascular diseases), explained by the presence of specific mutations that could cause higher mitochondrial oxidative damage. Nevertheless, the association is not necessarily found when analyzing individuals with different ancestries. This pattern is also observed when trying to relate mtDNA haplogroups with an increased longevity. To explain these findings, many factors should be considered, like the inherent complexity of these traits or some phenomena as *homoplasy* (recurrent mutations) along the human mtDNA phylogeny, which implies that the same variant could define several haplogroups. However, as most associations found were population-specific, the genetic background of each population could affect the potential effect of mtDNA variants on disease and longevity. Studying specific human populations will lead us to a better understanding of the connection between genotypes and phenotypes as a central question in Biology, Anthropology and Medicine.

P5-14. GENETIC DIVERSITY OF *ARUNDO DONAX* L. (GIANT REED): A REVIEW.

Jesús Jiménez-Ruiz[a,b], Juan Pablo del Monte[a], María Inés Santín-Montanyá[b].

[a] Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid (UPM), Madrid, Spain, [b] Centro Nacional Instituto de Investigación y Tecnología Agraria y Alimentaria (INIA), Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.

Arundo donax L. (Poaceae) is one of the worst invasive plants in the world (1). Most of the 12 phylogenetic studies focusing on *A. donax* show low or no genetic diversity in the invaded range (including the Mediterranean) and genetically variable individuals in Asia. Based on plastid mini- and microsatellites, some authors found the nearest relative of the invasive clone of *A. donax* in a Middle East lineage distributed along the Indus Valley (2). They also found three other lineages in China. Only one genotype across the southern USA using SRAP markers was documented (3), a genotype in the Mediterranean with AFLPs (4), and a genotype in South Africa with SSRs (5). In Australia, using AFLP, were found the occurrence of two lineages which may have been the gathering of an invasive clone (6), and another Asian lineage expanded through Indonesia. In the Mediterranean, a very high genetic diversity (129 genotypes from 203 samples and a Nei genetic diversity of 0.929) were found and a weak diversity in North America, using 10 SSRs specifically developed (7). The authors noted that sampling confusions with *Phragmites* were possible, but they screened these putative errors with control genotypes of *Phragmites*. In comparison to the results of other studies in the Mediterranean, the results of the study calls for further uses of these specific SSRs in broader sampling in the Mediterranean as well as for Asian populations using newly collected fresh material. To date, new generation sequencing and large SNP datasets have not been used to estimate genetic diversity or resolve phylogenetic relationships within Arundo. In conclusion, in its introduced range, *A. donax* shows strong genetic uniformity and no seed production. However, in Asia, this taxon is fertile and morphologically and genetically polymorphic.

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P5-15. DIVERSITY ASSESSMENT AT *drb1* EXON II LOCUS IN A REINTRODUCED POPULATION OF *Capra pyrenaica* (SCHINZ, 1838) FOR MANAGEMENT PURPOSES.

Lantero[1] Esther, Liébana[1] Alba, Matallanas[1] Beatriz, Abelenda[1] María, González[1] Eva Mª, Muñoz[2] Benito, Olmedo[2] Cristina, Refoyo[2] Pablo, González[1] Mª Teresa[1], Callejas[1] Carmen[1].

[1]Dept. of Genetics, Physiology and Microbiology, Faculty of Biological Sciences, Complutense University, Madrid, Spain. [2]Dept. of Biodiversity, Ecology and Evolution, Faculty of Biological Sciences, Complutense University, Madrid, Spain. Corresponding author: Lantero, Esther (estherlantero@ucm.es)

The Iberian ibex, *Capra pyrenaica* (Schinz, 1838), is an Iberian endemic species of great ecological and hunting relevance. The severe decline of its populations at the end of the 19th century was owed to habitat destruction, several diseases and uncontrolled hunting. The implementation of reintroduction and conservation programmes in recent decades has rendered the Iberian ibex in a species in expansion.

Here we present some results of the first genetic study carried out in the population of *C. pyrenaica* from the National Park Sierra de Guadarrama (PNSG). Few individuals of this species were reintroduced in this area between 1990 and 1992 and currently its population attains a considerable density. An informative fragment of the gene *drb1*, from the Major Histocompatibility Complex (MHC), was analysed in a representative sample of 34 specimens. The amplicons obtained by PCR were sequenced for assessing the genetic diversity they hold.

The observed heterozygosity was 47% in the analysed sample. Four alleles were identified, A1, A2 (the most frequent), A3 and A7, out of the seven described in other Spanish populations. Allele 7 was also present in C. *aegagrus hircus*, the domestic goat, and in *C. a*. *aegagrus*, the wild goat. This may be attributed to ancestral alleles or introgression events.

Genetic characterisation of the PNSG population revealed low levels of diversity, mainly due to demographic factors. The results provided by *drb1* exon II locus allowed fruitful comparisons with previous studies on others Iberian ibex populations, which are very useful to establish management units (MU) for the species.

P5-16. Gut's microbiota role in the degradation of alkaloids in the lily borer

Jesús Marín-Miret[1], Francisco González-Serrano[1,2], Tania Rosas[2], Joaquín Baixeras[3], Amparo Latorre[1, 4], Ana Elena Pérez-Cobas[5], Andrés Moya.[1, 4]

[1]Institute for Integrative Systems Biology (I[2]sysBio), University of València and CSIC, Valencia, [2]Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Cuernavaca, México, [3]Institute Cavanilles for Biodiversity and Evolutionary Biology, University of València, [4]Genomics and Health Area, Foundation for the Promotion of Sanitary and Biomedical Research (FISABIO), València, [5]Department of Microbiology, Ramón y Cajal Institute for Health Research (IRYCIS).

Brithys crini, the lily borer, feeds exclusively on a toxic plant, *Pancratium maritimum*. In most insects, the gut microbiota plays a crucial role in the nutrition and protection of the host using enzymes that are exclusive of prokaryotes. The gut microbiome of *B. crini* can resist the toxicity of the plant's secondary metabolites by degrading or expelling them through efflux pumps. We sequenced the 16S rRNA gene of the microbiota of 114 specimens of *B. crini* including two regions of the gut, the midgut and hindgut, and predicted the samples' functional profiles using the PICRUSt2 software. We examined the dynamics of the gut microbiota diversity, composition and potential functions of this insect over three consecutive years and the four corresponding seasons. We found that the insect's core bacteria comprises a small group that belongs mainly to Proteobacteria, the most abundant phylum in Lepidoptera. Interestingly, in our samples, this phylum is the primary carrier of the enzymes related to detoxification and secondary metabolites' degradation such as alkaloids. Despite striking differences in physicochemical properties among the two gut regions evaluated, we found no significant differences regarding bacterial diversity and functional profiles. It indicates that the *B. crini's* gut is colonized by generalist species that adapt to a wide range of environments. Moreover, the gut microbiota diversity and metabolic capabilities showed significant variations through the seasons and years studied. In this regard, climatic variables related to the seasons, such as temperature and precipitation, also influence the bacterial community's diversity. These results show that the external environment exerts an unexpected influence in shaping the ecology of the gut microbiota of *B. crini*.

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P5-17. PERSISTENCE OF DISTINCT ANCIENT LINEAGES IN HORSE OLFACTORY RECEPTORS

Beatriz Matallanas [a], Alejandra Cruz[b], Javier Maraver[c], Alicia de la Peña[c], Pilar Arana[c]

[a]Departamento de Ciencias de la Salud. Facultad de Ciencias Biomédicas. Universidad Europea de Madrid. Madrid. Spain [b]Departamento de Ciencias Médicas Básicas. Facultad de Medicina. Universidad San Pablo-CEU, CEU Universities. Boadilla del Monte, Madrid, Spain [c] Departamento de Genética, Fisiología y Microbiología, Facultad de Biología, Universidad Complutense, Madrid. Spain.

Equid ancestors originated in North America, crossed the Bering Strait 200 Ky BP and colonized Eurasia and Africa simultaneously spreading and diverging into several species of horses, asses, and zebras (1). Domestication of horses occurred in the Black Sea steppes around 2000 BP (2). Molecular analyses of mitochondrial and Y-chromosome sequences of domestic horse breeds yield different pictures, whereas mitochondrial sequences show a high diversity and little or no geographical signal (3), Y chromosomes are virtually uniform (4). This situation points to an effect of the domestication practices of migrating human populations, consisting in recruitment of local mares to the domestic herds together with strong artificial selection on reproducing males.

In order to obtain information about the evolution of other nuclear highly variable sequences, we chose olfactory receptor genes, that constitute the gene family with the highest variability in mammals. We analyzed sequences of two independent OR genes in a number of horse breeds and populations of extant putatively wild horses, and other domestic and wild equids, and also compared them to available ancient sequences.

We found similar patterns of evolution for the two genes: two distinct lineages showing SNPs in strict linkage disequilibrium coexist in all horse breeds, constituting two haplogroups distant to one other as much or even more than to the haplotypes found in other equids. One of these lineages is shared with *Equus przewalskii* horses, and both are found in predomestic horse sequences from Siberian permafrost samples dating 46 and 16 Ky BP (5).

Such a situation can be explained by balancing selection on ancient lineages or by a more recent event of hybridization, both alternatives are discussed in sight of the results of the analyses.

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P5-18. Understanding drought-induced processes of genetic selection in declining Atlas cedar populations.

Belén Méndez-Cea[a], Isabel García-García[a], David Martín-Gálvez[b], José Ignacio Seco[c], Francisco Javier Gallego[a] and Juan Carlos Linares[c]

[a]Dpto. de Genética, Fisiología y Microbiología, UD Genética, Facultad CC. Biológicas, UCM, Madrid, España. [b]Dpto. de Biodiversidad, Ecología y Evolución, UD Zoología, Facultad CC. Biológicas, UCM, Madrid, España. [c]Dpto. de Sistemas Físicos, Químicos y Naturales, UPO, Sevilla, España.

The Atlas cedar, *Cedrus atlantica* (Endl.) Manetti ex Carrière, is an endangered endemic conifer species naturally located in Morocco and Algeria and presented in several reforested areas, including the South of Spain. Soil water availability is key for its survival, a requirement that is being threatened by climate change. Indeed, drought-induced dieback and mortality have been recently reported for several Atlas cedar forests. Understanding the genetic bases that underlay the response of *C. atlantica* to this changing environment could be key to guide the conservation of this drought-sensitive endangered tree. Hence, genotyping by sequencing (GBS) may provide a useful way to study large genome size species, such as conifers, and when a reference genome is not available. Here we present the results of an extensive GBS analysis of Atlas cedar, including natural and reforested populations. The analysis of the population structure provides valuable clues to know the origin and drought adaptive capacity of some Spanish reforestations. Moreover, the genome-environment association study (GEA) has shown significant relationships among bioclimatic variables and the genetic variables. For instance, our results showed a main effect of annual mean temperature and annual precipitation, suggesting drought-induced processes of genetic selection in declining Atlas cedar populations.

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P5-19. Molecular diversity and haplotype distribution in two marine crab species

Ariadna Colmenero[a], Maria Sellés[a], Eva Rojo[a], Clàudia Lagares[a], Bruna Serra[a], Víctor Ojeda[a], Elena Marco-Herrero[b], Pere Abelló[c], Francesc Mestres[a]

[a]Dept. Genètica, Microbiologia i Estadística, Secció Genètica Biomèdica, Evolutiva i del Desenvolupament, Barcelona, Spain, [b]Centro Oceanográfico de Cádiz – IEO, Cádiz, Spain, [c]Institut de Ciències del Mar – CSIC, Barcelona, Spain.

A fragment of the *COI* (*Cytochrome Oxidase subunit I*) mitochondrial gene was studied in a pair of marine crab species: *Liocarcinus depurator* and *Geryon longipes*. The two species differed with regard to the marine depth in which they inhabit: the first one is found in the continental shelf and upper slope (50 to 500 m) and the second one in the middle and lower slope and also in the bathyal bottoms (400 to 2000 m). Populations in the Atlantic-Mediterranean transition were studied. Also, *G. longipes* sequences downloaded from DNA databases were also used in the analyses. Differences in the parameters that describe the molecular diversity were observed between both species. However, the most relevant point was the distinct pattern in the haplotype distribution. In the case of *L. depurator*, two haplogroups were detected, one predominant in water masses of Atlantic origin and the other in Mediterranean waters. Three main areas with regard the two haplogroups were observed: Cadiz Gulf, Alboran Sea and Levantine/Catalan coasts. This was not the case for *G. longipes*, because the population distribution of haplotypes did not follow a geographic pattern, apparently related to the rather homogeneous conditions of the deep-sea habitat of *G. longipes*. We hypothesize that this result is due to the different habitat of these two species, since their habitat characteristics differ widely during their adult, non-larval, stage, irrespective of the geographical location of the populations.

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P5-20. R2 retrotransposons in ladybird beetles (Coleoptera, Coccinellidae)

Pablo Mora[1], Jesús Vela[1], Areli Ruiz-Mena[1], Eugenia E. Montiel[1], Teresa Palomeque[1], Pedro Lorite[1], Barbara Mantovani[2], Andrea Luchetti[2]

[1] Department of Experimental Biology, University of Jaén, Jaén, Spain.
[2] Dipartimento di Scienze Biologiche, Geologiche e Ambientali, University of Bologna, Bologna, Italy.

Transposable elements (TEs) are one of the main components in eukaryotic genomes, playing an important role in the genome evolution. There are two categories of TEs, Class I (retrotransposons) and Class II (DNA transposons). Class I TEs are classified into three different types: non-Long Terminal Repeat retrotransposons (non-LTRs), LTRs and *DIRS*. R2 is a non-LTR element, specifically integrating in the repeated 28S ribosomal RNA (rRNA) genes. Coleoptera is among the biggest and most diverse group of insects. Coccinelids (commonly known as ladybugs or ladybird beetles) comprises around 6000 species in 360 genera. Almost the 90% of them are carnivorous, being one of the most effective animals used in biological pest control. The 10% herbivorous species can be pest of important crops. In this study, we have selected a wide range of species covering different genera and both carnivorous and herbivorous species. Full-size copies of the R2 retrotransposons of selected species have been characterized using molecular and bioinformatic approaches, in order to see whether the phylogeny of R2 elements is corresponded with the phylogeny of this group of insects. Phylogenetic relationships of R2 elements from coccinellids were compared with all the R2 available from NCBI and GenBank databases.

P5-21. Strong background selection on highly pleiotropic variants for human traits and diseases

Irene Novo[a], Eugenio López-Cortegano[a,b], Armando Caballero[a]

[a]Department of Biochemistry, Genetics and Immunology, Centro de Investigación Mariña, Universidade de Vigo, Vigo, Spain, [b]Current address: Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom.

Pleiotropy, which occurs when one gene affects more than one trait, has been shown to be common in the human genome, with up to 60 % of SNPs found to be pleiotropic. Recent meta-analyses have shown that rare variants tend to be less pleiotropic than common ones, suggesting that highly pleiotropic variants of great effect might be under strong natural purifying selection (1). In addition, it has been found that the effect size of variants increases with their degree of pleiotropy (2). In this study, we used data from the NHGRI-EBI GWAS Catalog (3) to investigate whether the average effect size, frequency, heritability contribution and recombination rate associated with variants for different human traits vary with the degree of pleiotropy. We also assessed whether there is an association between the degree of pleiotropy and the strength of purifying selection, measured by the *B* statistic, which indicates the reduction in genetic variability in a given genomic region due to the action of background selection (selection on deleterious mutations) (6). For this analysis, we considered data from four studies (1; 4; 5; and our own one). Our results show that the mean effect size, frequency and heritability contribution of variants increase with their degree of pleiotropy, and that highly pleiotropic variants are enriched in regions of strong background selection.

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P5-22. Evaluating the impact of non-target species in population genomic analysis

Cinta Pegueroles[a], Sergio Pous[a], Marc Vega[a], Enrique Macpherson[b], Marta Pascual[a], Carlos Carreras[a]

[a]*Departament de Genètica, Microbiologia i Estadística and IRBio, Universitat de Barcelona, Barcelona, Spain,*
[b]*Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Blanes, Girona, Spain.*

The rise of genomics during the last decade using high-throughput sequencing has allowed the genotyping of thousands of markers on a genome-wide approach, even in non-model organisms. Despite the drop of the price of sequencing, whole-genome sequencing remains costly for population studies and therefore most population studies use techniques based on sequencing DNA fragments produced by restriction endonucleases. Of note, the call of usable loci for non-model species relies on 'de novo' assembly pipelines and, in consequence, the analysis could be very sensitive to the presence of non-target species. Here, we evaluate the impact of mixing samples from different species on the quantity and quality of the markers, using two fish datasets sequenced by Genotyping by Sequencing (GBS). In the first dataset, we combined data from five species, four of the genus *Symphodus* and one of the genus *Serranus*. Firstly, we evaluated the effect of the phylogenetic distance on loci identification by combining data from individuals from the five different species. We found that mixing samples decreases the number of shared loci when increasing phylogenetic distance although increases the total number of loci. Secondly, we evaluated the distribution in genic (exons and/or introns) and intergenic regions of the shared loci relative to the species phylogenetic distance. We found an enrichment in exonic and intergenic regions with increase phylogenetic distance. In the second dataset, we evaluated the effect of mixing samples from two different *Symphodus* species at different proportions. We found that the loss of shared loci was related to the proportion of samples from the non-target species in accordance to the threshold of missingness allowed during the filtering process. Our results highlight the importance of performing a detailed pre-processing analysis to detect and discard non-target species but also indicates the potential of this type of analysis to detect incorrectly classified samples or even new cryptic species.

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P5-23. GENETIC CONNECTIVITY BETWEEN ATLANTIC BLUEFIN TUNA LARVAE SPAWNED IN THE GULF OF MEXICO AND IN THE MEDITERRANEAN SEA

Carolina Johnstone[a], Montse Pérez[b], Estrella Malca[c, d], José María Quintanilla[a], Trika Gerard[d], Diego Lozano-Peral[e], Francisco Alemany[f], John Lamkin[d], Alberto García[a], and Raúl Laiz-Carrión[a]

[a]Centro Oceanográfico de Málaga, Instituto Español de Oceanografía, Fuengirola (Málaga), Spain. [b]Centro Oceanográfico de Vigo, Instituto Español de Oceanografía, Vigo (Pontevedra), Spain. [c]Cooperative Institute for Marine and Atmospheric Studies, University of Miami, Miami, Florida, United States of America. [d]Southeast Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Miami, Florida, United States of America. [e]Centro de Supercomputación y Bioinnovación, Universidad de Málaga, Málaga, Spain. [f]International Commission for the Conservation of Atlantic Tunas. Madrid, Spain.

Highly migratory Atlantic bluefin tuna (ABFT) is managed as two stocks, Western and Eastern. Western ABFT spawn mainly in the Gulf of Mexico (GOM) and Eastern ABFT in the Mediterranean Sea (MED) (1). Understanding connectivity between ABFT populations is important for conservation and management of this valuable fishery resource that has been exploited for centuries. ABFT are highly mixed, with multiple disciplines supporting weak structuring between Western and Eastern stocks (1). Concerning genetics, subtle structuring of ABFT populations across the Atlantic Ocean has been the conclusion of studies describing genetic tools for traceability (2,3). Larval fish provide the genetic signal of successful breeders and have occasionally been genetically characterized with juveniles (young-of-the-year, YOY) collected in nursery areas. For the first time, cooperative field collection of tuna larvae during 2014 in the main spawning area for each stock enabled us to assess the structuring of ABFT genetic diversity in a precise temporal and spatial frame exclusively through larvae (5). Partitioning of genetic diversity at nuclear microsatellite loci and in the mitochondrial control region resulted in low significant fixation indices. Individual-based clustering analysis of larval ABFT genetic diversity indicate apparent connectivity between the GOM and MED spawning grounds that could support the hypothesis of mixing of breeders belonging to different stocks.

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P5-24. LONG-TERM EVALUATION OF THE INBREEDING LOAD IN POPULATIONS OF *DROSOPHILA MELANOGASTER*

Noelia Pérez-Pereira[a], Ramón Pouso[a], Ana Rus[a], Ana Vilas[a], Eugenio López-Cortegano[a*], Aurora García-Dorado[b], Humberto Quesada[a] and Armando Caballero[a]

*[a]Centro de Investigación Mariña, Departamento de Bioquímica, Xenética e Inmunoloxía, Universidade de Vigo, 36310 Vigo, Spain, [b]Departamento de Genética, Universidad Complutense, 28040 Madrid, Spain.*Current address: Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, EH9 3FL Edinburgh, United Kingdom*

Inbreeding depression, the fitness decline observed in inbred populations, is a widespread phenomenon in nature. It is widely accepted that inbreeding depression is mainly due to partially recessive deleterious mutations which remain hidden in heterozygous state in non-inbred populations (the inbreeding load), but are expressed in homozygosis due to inbreeding (1). According to theoretical and empirical evidences, the inbreeding load can be reduced by the action of genetic purging removing the exposed deleterious mutations, especially when it refers to large effect mutations (2). For moderate or small effect mutations there is some controversy, however, as these mutations could only be removed if inbreeding progresses slowly (i.e. with moderate effective population sizes), and the effects of purging appear later in time (3). In a previous study (4), a long-term experiment was carried out with populations of *Drosophila melanogaster* including two large populations of effective size $N_e \approx 1000$ individuals and derived lines of $N_e \approx 50$, in order to detect purging and quantify its magnitude. The results proved the efficiency of purging the inbreeding load, but at the end of the study a significant inbreeding load still remained in the populations. Here we added new results on the evolution of the inbreeding load in these populations. After more than one hundred generations in the large populations and between a few tens and one hundred generations in the lines, the inbreeding load was virtually exhausted, in accordance with theoretical purging predictions. According to computer simulations, this pattern could be explained by a relatively small mutation rate accounting for large effect mutations with partially recessive gene action.

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P5-25. The characterization of polymorphic and fixed inversions in the *Drosophila subobscura* cluster unveils the origin of chromosomal inversions and their evolutionary history

Eva Puerma[a], Dorcas J. Orengo[a], Montserrat Aguadé[a]

[a]*Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, i Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Barcelona, Spain.*

Inversions are major contributors to the Drosophila genus structural variation and evolution, with extensive evidence for the adaptive character of inversion polymorphism in diverse species.The molecular characterization of the breakpoints of polymorphic and fixed inversions allows not only the detailed ascertainment of each inversion originating mechanism, but also the establishment of the corresponding chromosome ancestral state. The subobscura cluster consists of three species that with five large acrocentric chromosomes and a small chromosome (dot) retain the genus ancestral karyotype. The large chromosomes differ by a reduced number of fixed inversions.The breakpoints of nine *D. subobscura* polymorphic inversions and ten fixed inversions between *D. subobscura* and *D. guanche* have been molecularly characterized. Our results reveal: i) the staggered-breaks mechanism as the predominant mechanism originating polymorphic inversions and the impossibility to establish the originating mechanism of most fixed inversions due to the long time elapsed since their origin; ii) the strict and broad reuse of some inversion breakpoints, and iii) in which lineage each inversion occurred and became fixed, and therefore to infer one of the first chromosomal phylogenies based on the comparison of inversion breakpoint sequences.

P5-26. Evolutionary traces within metabolic networks of endosymbiotic bacteria of insects

Mariana Reyes-Prieto[a,b], David José Martinez-Cano[c], Mercè Llabrés[d], Pere Palmer[d], Carlos Vargas-Chávez[a], Luis Delaye[c], Rosario Gil[a], and Andrés Moya[a,e]

[a]Evolutionary Systems Biology of Symbionts, Institute for Integrative Systems Biology (I2SysBio), Universitat de Valencia and CSIC, [b]Sequencing and Bioinformatics Service, Foundation for the Promotion of Health and Biomedical Research of Valencia Region (FISABIO), [c]Department of Genetic Engineering, CINVESTAV, Irapuato Unit,Guanajuato, México, [d]Department of Mathematics and Computer Science, University of the Balearic Islands, [e]Genomic and Health Area, FISABIO

The description and analysis of metabolic networks (MNs) are crucial to understand the principles under which biological systems respond and adapt to varying conditions. Genome-scale MNs of free-living bacteria tend to be extremely robust to the removal of enzymes. They also evolve rapidly through mutation or deletion of redundant genes, and horizontal gene transfer. However, endosymbiotic bacteria undergo a genomic degradation process while becoming host-dependent, where they endure a rapid and sizeable loss of genes and lose any possibility of gaining new genes. Consequently, they present some of the smallest MNs known so far for any organism. Up to date, more than 160 genomes of endosymbiotic bacteria of insects with different degrees of genome degradation have been fully sequenced. We carried out a large-scale study on the MNs of these endosymbionts applying two different models, metabolite-based and reaction-based. We found a significant correlation between several MNs parameters (clustering coefficient, network diameter, and number of nodes) and the genome size of the corresponding bacterial species. We also found a correlation of taxonomic groups (at a class level) between a molecular phylogeny and a distance tree resulting from the metabolic building blocks methodology. From this, we conclude that there are evolutionary traces within the metabolic networks of endosymbiotic bacteria of insects, even in organisms that became endosymbiotic a long time ago.

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P5-27. MONITORING TRANSPOSITION EVENTS IN THE GENOME OF THE PHYTOPATHOGENIC FUNGUS *FUSARIUM OXYSPORUM*

Ana Rodríguez-López[a], Cristina López-Díaz[a], Dilay H. Ayhan[b], Li-Jun Ma[b], Antonio Di Pietro[a]

[1]Department of Genetics, University of Córdoba, Córdoba, Spain, [2]Deparment of Biochemistry & Molecular Biology, University of Massachusetts Amherst, MA, USA

Transposable elements (TEs) represent an important source of genetic variation and are thought to act as evolutionary drivers (1, 2). In pathogenic fungi, genes encoding virulence factors often reside in TE-rich genomic compartments, suggesting a potential role of TEs in pathogen-host coevolution (3, 4). We use an experimental evolution approach to study host adaptation in *Fusarium oxysporum,* a soil-inhabiting fungus that causes vascular wilt disease in more than a hundred crop plants and life-threatening infections in immunocompromised humans. The capability to infect multiple organisms from different kingdoms makes *F. oxysporum* an excellent model to explore the genetic and evolutionary mechanisms of adaptation to different environments (5). Genome re-sequencing of *F. oxysporum* populations passaged through a variety of conditions suggested an important role of TEs in adaptive evolution (6). As a next step, we are using NGS Insertion Sequencing (7) to monitor individual TE insertion/excision events under different environmental conditions. We expect that this experimental approach will advance our understanding on the mechanisms of TE dynamics in *F. oxysporum* and on their role in evolutionary dynamics of this microorganism.

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P5-28. Ancient satellite DNAs in ant genomes

Areli Ruiz-Mena, Jesús Vela, Pablo Mora, Eugenia E. Montiel, Teresa Palomeque, Pedro Lorite

Human and Animal Molecular Genetics (RNM-924), Department of Experimental Biology, Universidad de Jaén, Jaén, Spain.

Ants are eusocial insects of the family Formicidae (Hymenoptera) with 14,823 described species, but probably contains more than 22,000 species. Living ants are currently classified into 21 subfamilies and 283 genera. However, the background in terms of research on satellite DNA (satDNA) in this insect group is still very scarce. SatDNA have been analyzed in species from six genera from two different ant subfamilies. So far, the satDNAs that have been described in ants are genera-specific or shared between closely related genera.

In this study we have analyzed the satDNA in the Argentine ant, *Linepithema humile* (Dolichoderinae), using both classical satDNA isolation techniques and comparative genomics. *L. humile* is native from South America and currently is one of the most widespread invasive ant species in the world. This species usually excludes most other local ants and can heavily affect other arthropods as well. We have isolated and characterized two satDNA families: Lihu_1 and Lihu_2. The use of available genome sequencing projects demonstrated that Lihu_1 and Lihu_2 were also present in other species from the subfamily Dolichoderinae, as well as in other species of the subfamilies Ponerinae, Dorylinaeae, Myrmicinae, Pseudomyrmicinae and Formicinae. The existence of shared satDNAs between ant subfamilies indicates that they were present in the genome of these species prior to their separation. Coupling molecular phylogenetic data with the fossil record of the ants suggest that the diversification of the major ant lineages occurred from the beginning of the Early Paleocene to the Late Cretaceous, 60 to 100 million years ago (Mya), with ancestors of the major subfamilies present as early as 75 to 125 Mya. Therefore, the antiquity of the Lihu_1 and Lihu_2 satDNA families should be, at least, similar.

P5-29. CHARACTERIZATION OF REPETITIVE DNA IN THE SMALL GENOME OF FLATFISH *Cynoglossus semilaevis*

Francisca Robles, Selene García, Alexander García-Zea, Rafael Navajas-Pérez, Roberto de la Herrán, Carmelo Ruiz Rejón

Department of Genetics, University of Granada, Spain,
Corresponding author: Carmelo Ruiz Rejón (carmelo@ugr.es)

Despite their size, a main feature of eukaryotic genomes is the presence of repetitive sequences. Flatfish (order Pleuronectifoms) have smaller genomes than other teleost (381 to 1076 Mb), with C-values ranging from 0.39 to 1.10 pg (1).We used a NGS-based analysis to detect and characterize repetitive DNA in the first sequenced flatfish genome, *Cynoglossus semilaevis* (2). Genomic sequences were retrieved from the NCBI database (#PRJNA73987). The dataset was explored to estimate the nature and quantity of SSRs, mobile elements, and satellite DNAs. Assembled chromosomes were separately analyzed with WindowMasker to define low complexity sequences and short repeats. SSR analysis showed an average density of 20,559.78 b per Mb. The average number of loci per Mb was 1,134.09. All chromosomesshowed a similar number of SSR loci except W chromosome, with roughly an eight-foldincrease (8,724).

To detect interspersed repeats and tandemly-arrayed sequences, 500K paired reads were randomly selected and clustered with Galaxy Repeat Explorer, masked and annotated with RepeatMasker. Repeated DNA correspond to almost 22% of the genome.Also, up to 20 different mobile elements (10.50% of the genome) were detected: nine Ty3 elements (5.11%), seven LINEs (1.97%) and four MITEs (3.37%).

Clusters annotated as mobile elements and main clusters of tandemly-arrayed sequences (selected according to repeat unit size and abundance) were mapped *in silico*. The most represented clusters of satellite DNA (Cyse 1, Cyse 2, Cyse 8, Cyse 15,Cyse 29B, Cyse 33, Cyse 73, and Cyse 101B) accounted for a 6.84% of the genome. Cyse1 (the most abundant, ~3%) mapped at chromosomes 8 and W. Cyse29B and Cyse101B are restricted to chromosome 1 and Cyse15 is found in all chromosomes.

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This work was supported by Ministerio de Ciencias, Innovación y Universidades of Spain (RTI2018-096847-B-C22)

P5-30. Genomic determinants in antigenic variation over different *Mycobacterium tuberculosis* ecotypes

Paula Ruiz-Rodriguez[a] and Mireia Coscolla[a]

[a]*Institute for Integrative Systems Biology (I²SysBio), University of Valencia-CSIC, FISABIO Joint Research Unit Infection and Public Health, Valencia, Spain*

Nowadays infectious diseases are one of the global threats to humanity, most known pathogens usually adopt the antigenic variation strategy where the host and the microorganism establish an evolutionary arms race, where each of the individuals acquires new improvements to overcome the opponent, setting a balance between them (1). In the case of *Mycobacterium tuberculosis*, it is unknown whether the antigenic variation could be behind the ability to evade the immune system. *M. tuberculosis* was described as a clonic bacterium such as the hyper-conservation of epitopes recognized by the host (2).

In this work, we identified genomic variation in 3,480 experimentally verified epitopes of T and B cells recognized from 3 different hosts (human, bovine and murine) over 13,451 non-duplicated genomes from clinical strains representing different *M. tuberculosis* ecotypes (3) (harboring 9 human lineages, and 4 animal lineages). In these sequences, we studied how diversity could impact epitope recognition through analyzing SNPs, deletions, and disruptions by insertion sequences. Additionally, we analyzed populations by phylogenomics to identify possible evolutionary convergences within ecotypes and host range.

We have found T and B cell epitopes deleted in animal associated lineages, mostly in highly virulent and immunogenic genes which are located in ESX-1 locus. We observed that most of the deleted epitopes are in immunogenic genes: CFP-10 and ESAT-6, driven by partially overlapping deletions in different animal clades and the vaccine strains *M. bovis* BCG. We observed that regions harboring epitopes are more mutated than other regions of the genome. Moreover, we found that human-associated Lineage 4 contained the most diverse T cell epitopes compared with Lineage 2 which showed the most diverse B cell epitopes. In order to verify if highly variable epitopes have immunological consequences, analysis including immunologic experiments in individual epitopes will be conducted.

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P5-31. INSERTION/DELETION POLYMORPHISMS (INDELS) AS GENETIC MARKERS OF WESTERN MEDITERRANEAN HUMAN POPULATIONS

Luis Sanchez-Martinez[1], Candela L. Hernández[1], Pedro Cuesta[2], Jean Michel Dugoujon[3], Luisa Pereira[4], Rosario Calderón[1]

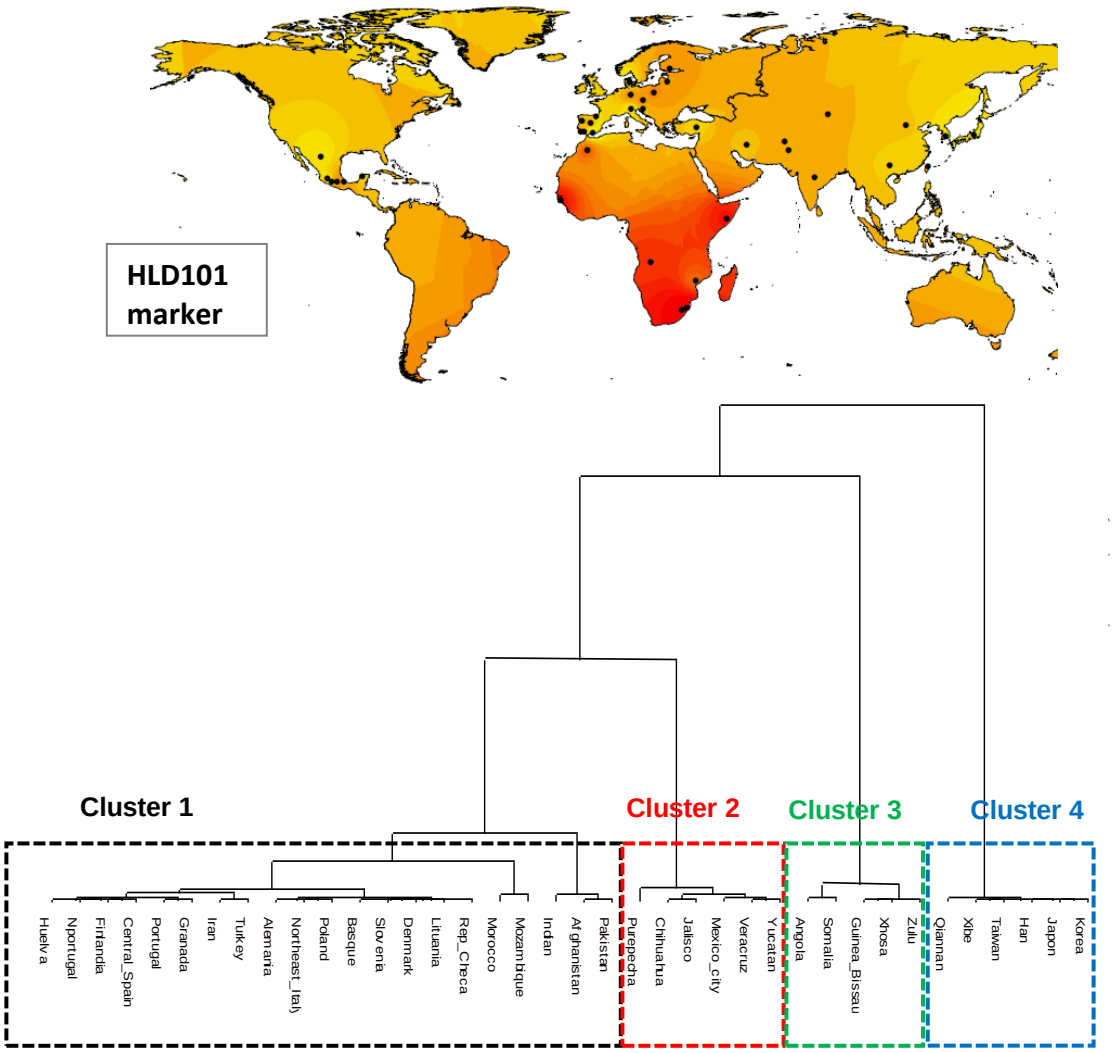
[1]Departamento de Biodiversidad, Ecología y Evolución, Facultad de Biología, Universidad Complutense, Madrid (Spain). [2]Centro de Proceso de Datos, Universidad Complutense, Madrid (Spain). [3]CNRS UMR 5288 Laboratoire d'Anthropologie Moléculaire et d'Imagerie de Synthèse (AMIS), Université Paul Sabatier Toulouse III, Toulouse (France), [4]i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto (Portugal)

Currently, informative genetic results based on Indel markers are being increasingly obtained from human populations with different continental familiar origins. Most of these surveys are mainly centered in forensic genetics and population genetics approaches. This study provides population data on biallelic insertion/ deletion polymorphisms (DIP) spread over 19 autosomes, in southern Iberians and northwestern Africans. Until the present, no information on the genetic constitution and structure of western Mediterranean populations based on these type of polymorphisms is available.

DNA samples of 124 autochthonous individuals from the Spanish Andalusian provinces of Huelva and Granada and the south of Portugal together with 44 subjects from Morocco (Berbers) have been genotyped using the *DIPplex kit*, which includes 30 biallelic Indel markers. Allele frequencies, heterozygosity levels and forensic parameters were estimated by using FORSTAT. Population genetic structure was explored through AMOVA, logistic regression analysis, and hierarchical cluster analysis (HCA). The last procedure was performed on the basis on allele frequencies of 38 worldwide populations.

Our findings revealed that Indel polymorphisms turned out to be particularly useful from a forensic perspective. No shared haplotypes were detected among the 168 subjects shaping the study population sample. The values of Power of Exclusion and Discrimination of the 30 Indel loci were 99.9999999999774 % and 99.9999999999776 %, respectively. Results emerging from population genetic structure analysis have shown that HLD118, HLD101, HLD40, HLD64 and HLD39 were the most discriminant loci among the four study western Mediterranean populations. HCA yielded four clusters and groups were defined depending on geography. Furthermore, clusters showed significant differences among them for a defined number of Indel markers. Contour maps of allelic frequencies showed particular geographic structures associated to specific insertion/deletion polymorphisms. In this line, the sub-Saharan radiation of HLD101 could reach the Iberian Peninsula, mainly through its Atlantic façade, after crossing the western fringe of North Africa.

Graphical abstract:



P5-32. PHYLOGEOGRAPHY OF THE IBERIAN ENDEMIC BUTTERFLY *EREBIA PALARICA*. PRELIMINARY RESULTS.

Laura Torrado-Blanco[a], Marta Vila[a]

[a]*Grupo de Investigación en Bioloxía Evolutiva, Universidade da Coruña, A Coruña, Spain.*

The Nymphalid butterfly *Erebia palarica* is endemic to the Northwest Iberian mountain ranges (1). Little is known about the biology and genetics of this species (2). Hence, it is vital to study the genetic diversity and structure of its populations so that convenient conservation policies can be applied (3).

We collected samples from 23 sites that cover the whole distribution. Sequencing 786 bp of the variable region of the Cytochrome Oxidase I gene (COI) (4) in 305 individuals produced 24 haplotypes. A 95% statistical parsimony network revealed two star-like patterns, typical of population expansions and previously seen on congeneric species in the same area (5). The highest level of mitochondrial diversity was found in the west corner of the Cantabrian mountain range (Somiedo). Moreover, the closest haplotype of the nearest species, *E. meolans*, also occurs in this area.

We also genotyped 494 individuals using ten microsatellite markers specifically developed for *E. palarica* (6). Ongoing research aims at determining the level of nuclear genetic diversity as well as the extent of nuclear structuring of these populations, paving the way for future studies such as the estimation of effective population size (N_e).

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P5-33. HYMENOPTERA AND ZYGENTOMA, DIFFERENT INSECT ORDERS, SIMILAR TRANSPOSABLE ELEMENTS

Jesús Vela, Olivia Sanllorente, Pablo Mora, Areli Ruiz-Mena, Eugenia Montiel, Teresa Palomeque, Pedro Lorite

Human and Animal Molecular Genetics (RNM-924), Department of Experimental Biology, Universidad de Jaén, Jaén, Spain

Azteca is a *mariner* element originally characterized in ant genomes. Transposable elements are able to transfer horizontally between different species. Horizontal transfer (HT) is evidenced when: (1) there is a high similitude in transposons from phylogenically distant hosts, (2) there are phylogenetic incongruencies between transposons and their hosts and (3) there is a discontinuous distribution of transposons among related taxa. Although HT events are not fully understood, it is clear that they could be favored by physical proximity between species. Some silverfish species are myrmecophilous and live in ant nests. Here we study the possibility of HT events between insect orders: ants (Hymenoptera, Formicidae) and silverfishes (Zygentoma). Complete *Azteca mariner* elements were characterized in four silverfish species: *Atelura formicaria, Lepisma baeticum, Neoasterolepisma delator* and *Proatelurina pseusolepisma*. Some of them conserve a complete ORF, fact that could indicate the existence of active *Azteca mariners* in that order. Phylogenetic analyses using ant and silverfish *Azteca* elements showed that ant sequences clustered together and differentiated from silverfish sequences, which were clustered in several clades. The results suggest the existence of vertical transference from a common ancestor between either orders or a very ancient horizontal transfer event. However, the results could indicate HT among silverfishes.

Graphical abstract

P5-34. STUDY OF INVASIVE SPECIES OF THE GALAPAGOS ISLANDS BY DNA BARCODING

Estefanía García Luque[a], Francisco José Villena González[a], Ana Bodoque García[a], Ana Isabel Cotilla Martín[a], Sara Gutiérrez Valderas[a], José Muñoz Martín[a], Alberto Vélez Pinela[a], Patricio Vega Mariño[b], Marilyn Cruz Bedón[b], Luis Díaz Martínez[a], Ana Grande Pérez[a], Enrique Viguera Mínguez[a]

[a]Area of Genetics, Faculty of Sciences, University of Malaga, Malaga, Spain, [b]Agency for the Regulation and Control of Quarantine and Biosafety of Galapagos (ABG), Puerto Ayora, Santa Cruz, Galapagos, Ecuador,

Invasive species are organisms that thrive outside of their own habitats, sometimes carried and introduced by humans. After a while and in the absence of predators or competitors, invasive species manage to adapt to the new environment and colonize it. Generally, native species cannot compete with foreign species, so they are displaced or become extinct. In addition to affecting the balance of the ecosystem, invasive species can be carriers of diseases that could endanger native and endemic species. Here we present the results of a volunteer program in collaboration with the Agency for the Regulation and Control of Quarantine and Biosafety of Galapagos (ABG) aimed at determining the presence of invasive species in the Galapagos Islands and establishing the sanitary status of the wild flora and fauna of the archipelago. For this, new diagnostic tools based on DNA barcoding are used. The specimens studied belong invertebrate species classified as invasive: the coffee borer beetle *Hypothenemus hampei* native to Africa, the cottony cushion scale *Icerya purchasi* original from Australia, various species of ants, the Mediterranean fruit fly *Ceratitis capitata*, the Giant African land snail *Achatica fulica* and the whitefly *Bemisia tabaci*. These pests are responsible for economic and ecological losses of local agriculture and can endanger native and endemic species. A region of the mitochondrial COI gene (cytochrome c oxidase I subunit) is used for barcoding and COI sequences are analyzed by bioinformatic tools and compared with sequences available in GeneBank and BOLDSystems to determine the origin of newly established populations. The results will help ABG to take the respective management measures and strengthen biosecurity actions in the Galapagos Islands.

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P5-35. A NEW GALL MIDGE SPECIES (DIPTERA, CECIDOMYIIDAE) AS A POTENTIAL CANDIDATE FOR BIOLOGICAL CONTROL OF THE INVASIVE PLANT CORTADERIA SELLOANA (POACEAE)

Jaime Fagúndez[a], Raymond J. Gagné[b], Marta Vila[c]

[a]BioCost research group, University of A Coruña, Spain, [b]Systematic Entomology Laboratory, Agricultural Research Service, U.S. Department of Agriculture, [c]GIBE research group, University of A Coruña, Spain.

A gall midge (Diptera, Cecidomyiidae) is reported here for the first time from spikelets of *Cortaderia selloana*, a prominent alien invasive grass species in southern Europe. The insect is described as a new genus and species, *Spanolepis selloanae* Gagné. Based on morphology and molecular genetics, the new genus and species are tentatively placed within the supertribe Lasiopteridi and tribe Dasineurini. Its effects on seed production were studied in order to ascertain its effectiveness in limiting sexual reproduction of the invasive plant species. The larvae of *S. selloanae* feed on the ovaries with a mean seed depletion of 74% in the studied population in northwest Spain. The new species is a potential candidate agent for the effective biological control against *C. selloana* (1).

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SESIÓN 6

Genética Humana

Moderadora: Gemma Marfany

I6-01. Bringing light to autism, from rare mutations to spiking neurons

Noèlia Fernàndez-Castillo[a,b,c,d]

[a]Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain. [b]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain. [c]Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, Spain. [d]Institut de Recerca Sant Joan de Déu (IR-SJD), Esplugues de Llobregat, Barcelona, Spain.

The genetic contribution to Autism Spectrum Disorder (ASD) is around 80%, one of the highest amongst neurodevelopmental disorders. Both rare and common genetic risk variants contribute to ASD, and although hundreds of genes have been related to this disorder its genetic architecture is not yet fully understood. We identified, through whole-exome sequencing, a frameshift variant (c.659-660insT, p.L220Ffs*18) in the *YWHAZ* gene, encoding the 14-3-3ζ member of this family, which was present in two siblings with ASD and ADHD. The 14-3-3 protein family are molecular chaperones involved in several biological functions acting as homo or heterodimers. This truncating mutation showed a functional impact resulting in decreased 14-3-3ζ protein solubility, loss of its ability to form heterodimers and impaired binding to tyrosine-hydroxylase, a well characterized molecular interactor. We then investigated the genetic contribution of the 14-3-3 gene family to ASD and other psychiatric disorders observing that ultra-rare variants were found enriched in ASD across the 14-3-3 genes and in schizophrenia for *YWHAZ*, whereas common variants in *YWHAE* contribute to schizophrenia. Furthermore, the expression of 14-3-3 genes was altered in post-mortem brains of ASD and schizophrenia patients (1). To further understand the role of the *YWHAZ* gene in brain function and its relation to mental disorders we used zebrafish as a model, establishing a knockout (KO) line using CRISPR/Cas9 genome engineering. Whole-brain *in vivo* calcium imaging analyses performed in larvae showed an altered hindbrain neuronal activity and functional connectivity in KO animals. Interestingly, *ywhaz* KO adults present decreased levels of dopamine and serotonin also in the hindbrain and show a freezing behaviour when exposed to novel stimuli that can be reversed with fluoxetine and quinpirole, drugs modifying serotonin and dopamine neurotransmission.

All this evidence supports a genetic contribution of the 14-3-3 family, and *YWHAZ* in particular, in ASD and neurodevelopmental and psychiatric disorders, suggesting an important role of *ywhaz* in establishing neuronal connectivity during developmental stages and show that loss of *ywhaz* function leads to an impaired dopamine and serotonin neurotransmission that may underlie behavioural alterations. Understanding the contribution of genes to ASD and neurodevelopmental disorders through comprehensive studies is crucial for unravelling the mechanisms and the development of therapeutical approaches.

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Schizophrenia: Genetics, Transcriptomics and Functional Analyses. 2020. J Clin Med. 9(6):1851. doi: 10.3390/jcm9061851.

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I6-02. Defective DNA double stand break repair in Aicardi-Goutieres syndrome

Pablo Huertas[a, b]

[a]Departamento de Genética, Universidad de Sevilla, Sevilla, 41080, Spain, [b]Centro Andaluz de Biología Molecular y Medicina Regenerativa-CABIMER, Universidad de Sevilla-CSIC-Universidad Pablo de Olavide, Sevilla, 41092, Spain

Aicardi-Goutières Syndrome (AGS) is genetically inherited rare disease characterized for an increase type I interferon activity in the cerebrospinal fluid (CSF). Its actual prevalence is still unknown, albeit a few hundred patients have been diagnosed and well characterized worldwide. It causes an early-onset encephalopathy associated with increased numbers of white cells in the CSF and accompanied by basal ganglia calcification, suggestive of an inflammatory process. Genetically, is an heterogenous disease that have been associated with mutations in up to seven different genes: TREX1 (OMIM #225750), RNASEH2A (OMIM #610333), RNASEH2B (OMIM #610181), RNASEH2C (OMIM #610329), SAMHD1 (OMIM #612952), ADAR1 (OMIM #615010) and IFIH1 (OMIM #615846). Strikingly, all seven genes encode proteins related with the metabolism of nucleic acids: TREX1 is 3'-5' cytosolic exonuclease; RNASEH2A, RNASEH2B and RNASEH2C build the RNAseH2 complex, that degrades RNA in DNA-RNA hybrids and also eliminates ribonucleotides embedded in the DNA; SAMHD1 is a dNTP triphosphohydrolase and has a ribonuclease activity; ADAR1 edits RNA sequences by deaminating adenosine into inosine; and IFH1is a cytosolic sensor of dsRNA. It has been proposed that the accumulation of nucleic acids caused by those mutations (or a hyper-activation of its sensing in the case of IFIH1) is sensed by the cells as if they were viral nucleic acids, inducing an innate immune response and causing chronic inflamation. Despite the clear evidence linking the presence of nucleic acids as the common feature eliciting a response that causes AGS, it is still a matter of debate where they come from. Our results show that AGS-associated mutations cause defects in the repair of DNA double strand breaks. Such defect relies on the formation of stable R-loops that block the early steps of the process. Additionally, we have been able to genetically and chemically suppress the DNA repair-associated defect of AGS mutants. We are currently analysing if such recovery of proficient repair could ameliorate the constitutive interferon response and, therefore, could open therapeutic avenues to treat the patients.

This work was supported by a Research Grant from the Fundación Ramón Areces.

O6-01. VALIDATION OF NEW ASD-ASSOCIATED GENOMIC VARIATIONS USING hiPSCs AND ZEBRAFISH MODELS

Sara Veiga-Rúa[a,b], Elena Goiricelaya-Seco[a], Alba Pensado-López[a,b], Ángel Carracedo[a], Catarina Allegue[a*] , Laura Sánchez[b*]

[a]Genomics and Bioinformatics, CiMUS-Grupo de Medicina Xenómica, Universidade de Santiago de Compostela (USC), A Coruña, Spain. [b]Zoology, Genetics and Anthropology Department, Veterinary Faculty, Universidade de Santiago de Compostela (USC), Lugo, Spain.

Autism Spectrum Disorders (ASD) are characterized by behavioural and communicational alterations. Although some environmental and genetic factors have been already associated to ASD, the aetiology of these disorders has not been fully described (1, 2). Next Generation Sequencing (NGS), including Whole Exome and Genome Sequencing (WES/WGS), have been used to identify new genomic variants linked to ASD (1, 2). However, the functional role and disease implication of most of them is unknown.

In this communication, we will explore how we conduct functional validation of these findings to improve our understanding of the molecular bases of the disease, genetic counselling and propose new therapeutical targets.

First, human induced pluripotent stem cells (hiPSCs) were selected as an *in vitro* model based on their differentiation capability into neuronal cell lines (3). As a first step, the CRISPR/Cas9 system was applied to knockout the selected genes. To obtain higher editing efficiencies, an inducible Cas9 was inserted into the genome of the hiPSCs, followed by the introduction of the RNA guides. After this, hiPSCs will be differentiated into neurons to study the effects of the edition in the cell function.

Second, zebrafish models were generated to validate the function of the genes *in vivo* (4). To do so, CRISPR/Cas was applied to generate new mutant lines, which will be useful to characterize the function of the genes of interest, by means of the genetic, phenotypic, and behavioural characterization of the individuals.

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O6-02. MIMICKING HUMAN MUTATIONS IN CAENORHABDITIS ELEGANS

Julián Cerón[a], Carmen Martínez-Fernández[a], Dmytro Kukhtar[a], David Brena[a], Jeremy Vicencio[a], Nuria Goya[a], Miguel Ruíz-López[a], Mariona Cots[a]

[a] *Modeling human diseases in C. elegans Group, Genes, Disease and Therapy Program, Bellvitge Biomedical Research Institute—IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain.*

Our laboratory uses the nematode genetic model *Caenorhabditis elegans* to investigate human diseases in different ways. CRISPR-Cas allows the mimicking of human missense mutations in *C. elegans* when the affected amino acid is conserved. *C. elegans* is very convenient for CRISPR-Cas genome editing thanks to its fast life cycle, hermaphroditism, and syncytial germline that permits targeting many germ nuclei in a single microinjection. Moreover, it is an animal free of ethical issues for genome editing.

We have an active research line to optimize CRISPR-Cas editing in animals (*1, 2*). Another branch of our lab mimics human mutations in *C. elegans* to investigate disease mechanisms, study the functional impact of gene variants, and perform drug screens (*3*). Most of these mutations are related to rare diseases, but we have also mimicked mutations found in human tumors (*4*). We will summarize the potential of *C. elegans* in the development of CRISPR technologies and the modeling of genetic diseases.

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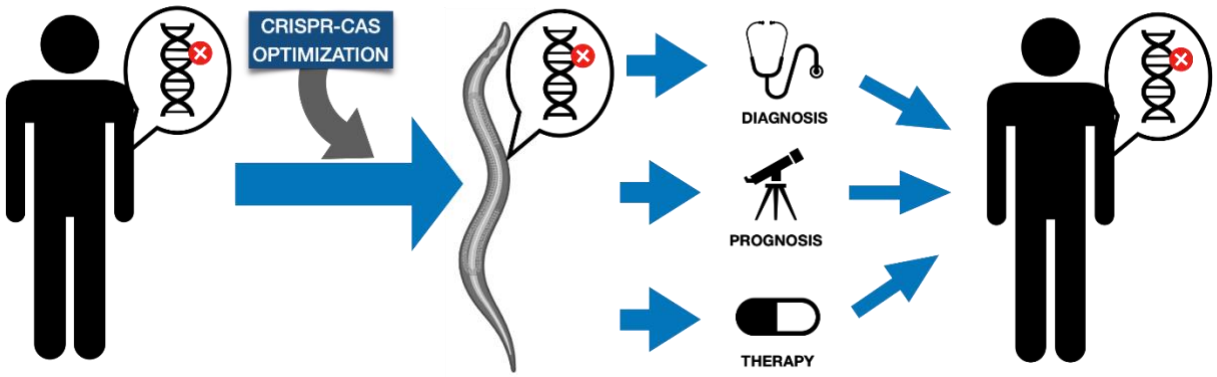
Graphical abstract:



FT6-01. Genome edited mice to study diverse types of albinism

Almudena Fernández, Ana M. Guardia, Andrea Montero, Marta Cantero, Julia Fernández, Gema Garrido, Lluís Montoliu

National Centre for Biotechnology (CNB-CSIC) and CIBERER-ISCIII, Madrid, Spain

Albinism is a human genetic condition affecting 1:10,000-20,000 newborn and, hence, it is considered a human rare disease. Albinism, beyond affecting pigmentation in most, but not all, cases, features a significant visual alteration, with severe reduction in visual acuity and altered stereoscopic vision, among other abnormalities. Genetically, albinism is heterogeneous. Currently, we know of 22 types of albinism, including non-syndromic (oculocutaneous, OCA, and ocular forms, OA) and syndromic (oculocutaneous, Hermansky-Pudlak, HPS, and Chediak-Higashi Syndromes, CHS) (1). In our group, we have applied the latest CRISPR-Cas9 methods in order to generate numerous new genome-edited mouse models to investigate several types of albinism, including oculocutaneous albinism type 1 (OCA1), OCA2, OCA4, OCA6, OCA7, ocular albinism (OA1) and FHONDA, associated with mutations in the *Tyr*, *Oca2*, *Slc45a2*, *Slc24a5*, *Lrmda*, *Gpr143* and *Slc38a8* murine genes, respectively (2, 3). With all these new mouse models we have undertaken a systematic phenotypic evaluation, at molecular, cellular and functional levels, to reveal how the diverse mutations impacting the identified genes translate into the observed retinal abnormalities. Furthermore, these animals will be used for testing safety and efficacy for proposed therapeutic approaches based on small molecules (L-DOPA), or repositioned drugs (i.e. Nitisinone) and for the development of future gene therapy scenarios. In this communication, we will report our latest findings and results in this scientific project.

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FT6-02. DETECTION OF FUSION GENES BY RNA-SEQ IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

Unai Illarregi[a], Elixabet López-López[a], Nerea Bilbao-Aldaiturriaga[a], Ángela Gutiérrez-Camino[b], Idoia Martin-Guerrero[a]

[a]Department of Genetics, Physical Anthropology & Animal Physiology, Science and Technology Faculty, IIS Biocruces Bizkaia, UPV/EHU, Leioa, Spain [b]Division of Hematology-Oncology, Research Center, Sainte-Justine University Health Center, Montreal, Canada

Acute lymphoblastic leukemia (ALL) remains the leading cause of death from disease in children. Therefore, the identification of subtypes with known prognostic value is essential for treatment stratification. However, some patients with a predicted good prognosis relapse, and 25% are not classified into any subtype, suggesting that the classification could be improved. In this context, this work aimed to improve the classification of this neoplasm by detecting fusion genes through RNA-seq technology.

A cohort of 64 Spanish patients (54 B-cell precursor (BCP) ALL, 10 T-ALL) were included in the study. RNA libraries (TruSeq Stranded Total RNA Library Prep Kit; Illumina) were processed using the Ribo-Zero Gold kit (Illumina).The resulting libraries (stranded and without ribosomal RNA) were sequenced (2 x75 bp) on the NovaSeq 6000 System (Illumina). The reads were aligned to the human genome (hg19) using STAR v2.5.3a. The fusion genes and chimeric transcripts were identified with Fusion Catcher. STAR Fusion was used to prioritize the breakpoints of the translocations of the file created by STAR.

A total of 78 fusion genes were detected in 36 patients. Remarkably, some rearrangements, such as *DUX4-IGH*, previously associated with prognosis in ALL and not detected by conventional techniques in our series, were identified in unclassified patients. This means that a more accurate classification is possible.

These results indicate that RNAseq could be an especially useful technique in the clinical diagnosis of ALL, contributing to a better patient stratification.

We extend special thanks to Pediatric Oncohematology Department from Cruces University Hospital (I Astigarraga), Niño Jesús Children's University Hospital (M Ramirez) and Hematology Laboratory from “Institut de Recerca Pediátrica Hospital Sant Joan de Déu de Barcelona” (M Camos- Guijosa). This work was supported by the Mutua Madrileña Foundation and the Basque Government (IT989-16). U Illarregi was supported by a predoctoral grant from Gangoiti foundation.

FT6-03. Regulation of *WNT16* in bone involves upstream enhancers within *CPED1*

Núria Martínez-Gil[1], Carlos Herrera-Ubeda[1], Nicola Gritti[2], Neus Roca-Ayats[1], Nerea Ugartondo[1], Natàlia Garcia-Giralt[3], Diana Ovejero[3], Xavier Nogués[3], Jordi Garcia-Fernàndez[1], Daniel Grinberg[1], Susanna Balcells[1]

1 Department of Genetics, Microbiology and Statistics, Faculty of Biology, Universitat de Barcelona, CIBERER, IBUB, IRSJD, Barcelona, Spain. 2 European Molecular Biology Laboratory (EMBL) Barcelona, 08003 Barcelona,Spain. 3 Musculoskeletal Research Group, IMIM (Hospital del Mar Medical Research Institute), Centro de Investigación Biomédica en Red en Fragilidad y Envejecimiento Saludable (CIBERFES), ISCIII, Barcelona, Spain.
Corresponding author: Núria Martínez-Gil (nuriamartinez91@ub.edu)

WNT16 stands up as an essential gene for bone homeostasis. Here, we present new evidence of the functional role of one *WNT16* region. Performing 4C chromatin conformation analysis in bone cells, we identify physical interactions between the proximal part of *WNT16* intron 2, shown here to be an active promoter in bone cells, and several putative regulatory regions within *CPED1*. Analysis of previously published RNA-seq data from hFOB cells disclosed low expression of a region located downstream of this promoter. Our results suggest a novel regulatory mechanism of *WNT16* in bone, mediated by physical interaction with various enhancer regions within *CPED1*.

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FT6-04. ERASTIN-INDUCED FERROPTOSIS ENHANCES LOSS-OF FRATAXIN PHENOTYPES IN A DROSOPHILA MODEL OF FRIEDREICH ATAXIA

Estela Selma-Soriano[a,b], María Dolores Moltó[b,c], Federico V. Pallardó[d,e,f], Juan Antonio Navarro[a,b,d]

[a]INCLIVA, Biomedical Research Institute, Valencia, Spain, [b]Department of Genetics, Universitat de València-INCLIVA, Valencia, Spain, [c]Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Valencia, Spain, [d]Department of Physiology, Faculty of Medicine and Dentistry. University of Valencia-INCLIVA, Valencia, Spain, [e] Associated Unit for Rare Diseases INCLIVA-CIPF, Valencia, Spain, [f]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain

Friedreich´s Ataxia (FRDA) is the most prevalent autosomal recessive ataxia in the European population (1:50000). The disease is caused by the reduced expression of frataxin, a mitochondrial protein involved in iron–sulfur cluster biogenesis. Deficiency of frataxin leads to a drastic reduction in the cellular energy production (1). Remarkably, the characterization of the type of cell death that affects the cells deficient for frataxin still remains unsolved. Several studies in cell culture models point towards apoptosis (2-4). However, no marker of apoptotic cell death has been observed in *in vivo* models (5). A very attractive possibility is a new type of cell death named as ferroptosis. Deregulation of iron metabolism, depletion of glutathione and accumulation of lipid peroxides are the major hallmarks of ferroptosis (6). Remarkably, these three molecular signatures have been detected in samples from FRDA patients as well as in disease models including *Drosophila melanogaster*, suggesting that loss of frataxin recapitulates ferroptotic cell death (7).

We have used 3 different known inducers of ferroptosis (buthionine sulfoximine (BSO), Erastin and Tert-Butyl Hydroperoxide) to analyse whether frataxin-deficient flies display increased sensitivity towards this stressor. Our results indicate that flies seem to react differently to all three chemicals. Erastin but not BSO and Tert-Butyl reduced locomotion of frataxin-deficient flies, affected heart function, boosted the production of lipoperoxides and impaired mitochondrial function (monitored as aconitase activity and ATP production) without enhancing longevity defects. Similar results were obtained when the fly ortholog of Glutathione Peroxidase 4 (GPTx1) was downregulated in frataxin-deficient flies. We are now assessing whether inhibitors of ferroptosis or upregulation of GTPx1 are able to alleviate frataxin-deficient phenotypes.

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FT6-05. A *Caenorhabditis elegans* model to study the role of sulfated steroidhormones in aging and aging-related diseases

Mercedes M. Pérez-Jiménez [1], José M. Monje-Moreno[1], Ana M. Brokate-Llanos[1], Mónica Venegas-Calerón[2], Alicia Sánchez-García[2], Paula Sansigre[1], Amador Valladares[1], Sara Esteban-García[3], Irene Suárez-Pereira[3], Javier Vitorica[4,5,6], José Julián Ríos[2], Marta Artal-Sanz[1], Ángel M. Carrión[3] & Manuel J. Muñoz[1]

[1]Centro Andaluz de Biología del Desarrollo (CABD)—Universidad Pablo de Olavide (UPO), Departamentode Biología Molecular e Ingeniería Bioquímica, UPO/ CSIC/JA, Sevilla, Spain.
[2]Instituto de la Grasa (CSIC), Campus Universitario Pablo de Olavide (UPO), Sevilla, Spain. [3]Departamento de Fisiología, Anatomía y Biología Celular, Universidad Pablo de Olavide (UPO), Sevilla,Spain.
[4]Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de Sevilla, Sevilla,Spain.
[5]Instituto de Biomedicina de Sevilla (IBiS)-Hospital Universitario Virgen del Rocío/ CSIC/Universidad deSevilla, Sevilla, Spain.
[6]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED),Madrid, Spain.

Working with the model organism *Caenorhabditis elegans*, we have recently described that mutations in *sul-2* gene, which encodes to the sulfatase of steroidhormones, or its inhibition with STX64 regulates aging and protein aggregation diseases.

Our research has revealed that *sul-2* acts on sulfated steroid hormones in *C. elegans*, the orthologue to STS in humans. *sul-2* mutants have an increased pool of sulfated steroid hormones, increased life-span and ameliorate symptoms of protein aggregation diseases. We managed to set up a treatment with the STX64,the specific STS inhibitor. STX64 in *C. elegans* increases longevity and ameliorates Alzheimer's, Parkinson's and Huntington's disease models. Furthermore, testosterone-derived sulfated hormones reproduce the longevity and protein aggregation diseases phenotypes of *sul-2*, supporting that the presence of sulfated steroid hormones is the responsible of the phenotypes.

Remarkably, oral STX64 treatment in acute and chronic Alzheimer's disease mammalian models suppresses the cognitive impairment and reduces the presence of senile plaques in the brain. STX64 is non-toxic in humans and together with our results open the possibility of reallocating steroid sulfatase inhibitors or derivates for the treatment of aging and aging related diseases. (Pérez-Jiménez *et al.* *Nat Commun* (2021). https://doi.org/10.1038/s41467-020-20269-y)

FT6-06. Microbiota across life in a healthy cohort

Susana Ruiz-Ruiz[a,b], Benjamí Pérez-Rocher[a], Alejandro Artacho[a], Javier Pons[a], Nuria Jiménez[a,b], Andrés Moya[a,b,c]

[a]Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana (FISABIO), València, [b]Consorcio de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBEResp), Madrid, [c]Instituto de Biología Integrativa y de Sistemas (I2Sysbio), Universitat de València y CSIC, València

Defining what constitutes a healthy microbiota is a difficult question that requires further research. In this sense, longitudinal studies of the gut microbiota could help answer the question, because they can delimit between those alterations that are a response to periodic or contingent factors of an environmental or physiological nature from those that are truly dysbiotic. In the present work, we characterize by 16S rRNA sequencing the composition and taxa conservation of the gut microbiota across life of a healthy Mediterranean cohort structured in three groups of age (infants, adults and elderly, the IAE cohort), which have been monthly followed during eight consecutive months. The average Jaccard index of shared genus for the highest time intervals (240 days) was 60%, 55% and 55% for I, A and E groups of age, respectively. On the other hand, the core microbiota consisted of 16 genera, four of which (Blautia, Faecalibacterium, Bacteroides, and a non-characterized member of the family Lachnospiraceae) were always present. Two taxa were shared at all times by I-A, two by A-E, four were unique for A, four unique for E and, interestingly, none was detected as unique in infants (I). Finally, since it is not possible to obtain the microbiota of a single individual throughout its entire life, we constructed the microbiota of virtual individuals by performing all possible combinations of all time points of any I, A, or E individual to measure, correspondingly, the degree of decay in the Jaccard index. The average Jaccard index among the highest time intervals (around 30,000 days) was about 40% of shared genera. This observation supports the hypothesis of a core and healthy microbiota across life.

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P6-01. FUNCTIONAL CHARACTERIZATION OF CPAMD8 MUTATIONS IDENTIFIED IN CONGENITAL GLAUCOMA AND ANTERIOR SEGMENT DYSGENESIS REVEALS DEFECTIVE OCULAR EXTRACELLULAR MATRIX.

José-Daniel Aroca-Aguilar [1,2], Juan-Manuel Bonet-Fernández [1,2], Marta Corton [3], Ana-Isabel Ramírez [2,4], Susana Alexandre-Moreno [1,2], María-Teresa García-Antón [4], Juan-José Salazar [2, 4], Jesús-José Ferre-Fernández [1,2], Raquel Atienzar-Aroca [1,2], Cristina Villaverde [3], Ionut Iancu [3], Alejandra Tamayo [3], Carmen-Dora Méndez-Hernández [2,5], Laura Morales-Fernández [2,5], Blanca Rojas [2,4], Carmen Ayuso [3], Miguel Coca-Prados [6], José-Maria Martinez-de-la-Casa [2,5], Julián García-Feijoo [2,5] and Julio Escribano [1,2].

[1]Área de Genética, Facultad de Medicina de Albacete/IDINE, UCLM. Albacete, Spain. [2]Cooperative Research Network on Age-Related Ocular Pathology, Visual and Life Quality (OFTARED), Instituto de Salud Carlos III, Madrid, Spain. [3]Área de Genética and Genómica, Instituto de Investigación Sanitaria-Hospital Universitario Fundación Jiménez Díaz-Universidad Autónoma de Madrid (IIS-FJD, UAM)/CIBERER, Madrid, Spain. [4]Instituto de Investigaciones Oftalmológicas Ramón Castroviejo, Universidad Complutense de Madrid, Madrid, Spain. [5]Servicio de Oftalmología/IIS, Hospital San Carlos, Madrid, Spain. [6]Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, CT, USA

Primary congenital glaucoma (PCG) and anterior segment dysgenesis (ASD) heterogeneous diseases with related genetic basis that may severely impair visual function. They result from diverse developmental abnormalities of the ocular anterior segment structures believed to be caused by defective patterning, migration, and/or differentiation of periocular mesenchyme cells. ASD patients frequently presents extracellular matrix defects and glaucomatous phenotypes. In this study we investigate the genetic alterations underlying glaucoma with ASD (CG-ASD) by full exonic DNA sequencing and bioinformatic and in vitro functional evaluation of the variants identified. We identified rare biallelic CPAMD8 variants in four patients with CG-ASD and in one case with PCG. CPAMD8 is a gene of unknown function and recently associated with ASD. We confirmed its expression by immunodetection in adult human ocular tissues involved in glaucoma and ASD (i.e., aqueous humor, non-pigmented ciliary epithelium, and iris muscles). Quantitative reverse transcription PCR and minigene analysis supported the pathogenicity of the variants identified. Optical and electron microscopy of the trabeculectomy specimen from one of the CG-ASD cases revealed ocular anterior segment defects, including altered extracellular matrix, and apoptotic trabecular meshwork cells. Our data supports that CPAMD8 mutations participates in variable recessive CG-ASD phenotypes associated with extracellular matrix disorganization and provide new insights into the normal and disease roles of this gene.

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P6-02. GENETIC SCORE FOR PREDICTING WEIGHT LOSS AFTER BARIATRICSURGERY: A 24-MONTHS FOLLOW-UP STUDY

Natalia Azcona-Granada[a], Elionora Peña[a,b], Assumpta Caixàs[c,d], Albert Lecube[e,f], Andreea Ciudín[e,g], RafaelSimó[e,g], Pere Rebasa[c,h], Patricia Mas-Bermejo[a], Paula Rovira[a], Concepción Arenas[i], Araceli Rosa[a,b,j]

[a]Secció de Zoologia i Antropologia Biòlogica, Facultat Biologia, Univ. de Barcelona (UB); [b]IBUB, [c]Institut d'Investigació i Innovació Parc Taulí, Sabadell, [d] Endocrinology and Nutrition Dept., Hospital Parc Taulí, Medicine Depart., UAB, [e] CIBER de Diabetes y Enfermedades Metabólicas Asociadas, Instituto de Salud Carlos III, [f] Endocrinology and Nutrition Depart. Hosp.Universitari Arnau de Vilanova, IRBLleida, Univ. de Lleida, [g] Institut de Recerca Vall d'Hebron, UAB, [h] Surgery Depart., Esofago-gastric Surgery Section, Hosp. Universitari Parc Taulí, Sabadell, [i]Secció d'Estadistica, Facultat Biologia, UB, [j] CIBER on Mental Health, Instituto de Salud Carlos III.

Obesity is a polygenic multifactorial trait, influenced by multiple genetic variants of minor effect. Recent genome-wide association studies have identified several common loci associated with obesity-related phenotypes (e.g: Body Mass Index, BMI). Bariatric surgery (BS) is currently the most effective long-term treatment for severe obesity. Interindividual variation in surgery outcome has been observed, and research suggests a moderating effect of several factors, including the genetic background of the patients(1,2,3). The aim of the present study was to examine the role of a Genetic Score (GS) based on 7 polymorphisms in 5 obesity-candidate genes (*FTO, MC4R, SIRT1, LEP* and *LEPR*) in relation to their effects on weight loss after bariatric surgery. We evaluated a cohort of 104 patients with severe obesity submitted to BS (Roux-en-Y Gastric Bypass or Sleeve Gastrectomy) followed-up for 24 months (loss to follow-up: 0%). A GS was calculated for all the individuals considering the number of risk alleles for the obesity related genes analyzed. During the postoperative period, the percentage of excess weight loss (%EWL), total weight loss (%TWL) and difference of BMI (ΔBMI) were evaluated. Generalized Estimating Equation (GEE) models were used for the prospective analysis of the variation of these variables in relation to GS. The longitudinal model showed a suggestive effect of the GS on the %EWL (p=0.0022), %TWL (p=0.0004) and ΔBMI (p=2.788e-05) alongtime. Whereby, individuals with a low GS seem to experience a better outcome after BS than those with a high GS. The use of GS that contemplate the polygenic nature of obesity seems to be a useful tool to better understand the outcome of patients with obesity after surgery.

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P6-03. GENERATION AND CHARACTERIZATION A CPAMD8 KNOCKOUT ZEBRAFISH LINE AS A POSSIBLE MODEL FOR ANTERIOR SEGMENT DYSGENESIS

J.M. Bonet-Fernández[1], J.D. Aroca-Aguilar[1], A.I. Ramírez[2], S. Alexandre-Moreno[1], R. Atienzar-Aroca[1], J.J. Ferre-Fernández[1], J.J. Salazar[2], J. Escribano[1].

[1]University of Castilla-La Mancha Medical School, Genetics, Albacete, Spain.
[2]Universidad Complutense de Madrid/OFTARED., Instituto de Investigaciones Oftalmológicas Ramón Castroviejo, Madrid, Spain.

Anterior segment dysgenesis (ASD) is a heterogeneous disease that may severely impair visual function, *CPAMD8* has been recently associated with ASD type 8 in human patients although gene function is mostly unknown. To better understand the role of this gene in ASD we analyzed the expression of the zebrafish *cpamd8* orthologous gene in 96 hpf larvae by fluorescent-whole mount immunohistochemistry and we generated a *cpamd8* knockout zebrafish using CRISPR/Cas9 genome editing.

Immunohistochemical analysis of tissue sections showed *cpamd8* positive signals in the periocular mesenchyme of developing dorsal and ventral iridocorneal angles, indicating that it may participate in the early morphogenesis of the iris, cornea, and lens.

CRISPR/Cas9 *cpamd8* disruption revealed that F0 (96 hpf) mosaic embryos presented varying degrees of gross developmental abnormalities, including microphthalmia, pharyngeal maldevelopment, and pericardial and periocular edemas. Optical and electron microscopy examination of these embryos showed remarkable iridocorneal angle hypoplasia (characterized by altered iris stroma cells, a primordial anterior chamber filled with abnormal cellular material, and collagen disorganized corneal stroma extracellular matrix). These data indicate that *cpamd8* disruption results in early anterior segment maldevelopment that recapitulate some ASD-8 features.

To stablish a knockout line we selected a deletion in exon 4 of *cpamd8* which was predicted to result in a frameshift and a premature stop codon (p.S136CfsTer44). Loss-of-function of *cpamd8* in F3 (96 hpf) homozygous embryos was confirmed by quantitative real-time PCR and Western blot analysis, showing that *cpamd8* mRNA was not present in knockout animals. Preliminary macroscopic studies of 96hpf embryo phenotypes have revealed no similarities to phenotypes found in F0 mosaics embryos. These results indicate the possible genetic compensation of *cpamd8* LoF in the established zebrafish line.

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Universidad de Castilla La Mancha.

P6-04. MUERTE SÚBITA DE ORIGEN ARRITMOGÉNICO EN POBLACIÓN INFANTIL-JUVENIL: AUTOPSIA MOLECULAR E IMPLICACIONES FAMILIARES

Oscar Campuzano Larrea[1,2,3], Georgia Sarquella Brugada[1,4], Anna Fernández Falgueras[2,5], Sergi César Díaz[4], Mònica Coll Vidal[2,3], Alexandra Pérez Serra[2,3], Carles Ferrer Costa[2], Elena Arbelo Lainez[6], Josep Castellà García[7], Josep BrugadaTerradellas[3,4,6], Ramon Brugada Terradellas[1,2,3,5]

1 Medical Science Department, School of Medicine, University of Girona, Girona, Spain.2 Cardiovascular Genetics Center, Institut d'Investigació Biomèdica Girona (IDIBGI), University of Girona, Girona, Spain. 3 Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares(CIBERCV), Madrid, Spain. 4 Arrhythmias Unit, Hospital Sant Joan de Déu, University of Barcelona, Barcelona, Spain. 5 Cardiology Service, Hospital Josep Trueta, Girona, Spain 6 Cardiology Service, Hospital Clinic de Barcelona, Barcelona Spain. 7 Forensic Pathology Service, Institut de Medicina Legal i Ciències Forenses de Catalunya(IMLCFC), Barcelona, Spain.. Corresponding author: Oscar Campuzano (oscar@brugada.org)

La muerte súbita en población infantil-juvenil queda sin resolver en casi un 40% de los casos tras realizar una autopsia médico-legal completa. En estos casos sesospecha una arritmia cardiaca como la causa más probable del fallecimiento. Hoy en día hay unos 100 genes causantes de miocardiopatías familiares asociadas a muerte súbita. Identificar la causa del fallecimient da una respuesta a la familia, pero también puede ayudar a prevenir otros episodios arrítmicos letales en losfamiliares. El objetivo de nuestro estudio era identificar la causa genética de la muerte súbita en 96 casos post-mortem, con evento letal antes de los 18 años de edad. Se llevó a cabo un análisis genético de todos los genes conocidos utilizando tecnología de secuenciación masiva. Se identificaron 65 casos (67.7%) portadoresde al menos una variante rara en alguno de los genes relacionados con arritmias malignas y clasificada como potencial causa del fallecimiento. Posteriormente se analizó clínica y genéticamente a los familiares. En 22 casos (22.9%) se observóuna segregación familiar positiva, reforzando el diagnóstico de la variante rara como causa de la muerte súbita. En 16 casos (16.66%) la variante rara estaba localizada en un gen que codificaba para canales iónicos del miocito o proteínas asociadas. En los familiares portadores de la misma variante rara y, por tanto, en riesgo de poder sufrir una arritmia maligna, se adoptaron las medidas preventivas terapéuticas adecuadas en cada caso para reducir el riesgo de episodios arrítmicos. Nuestro estudio apoya la implantación de la autopsia molecular en los protocolos forenses cuando se sospecha de arritmia cardiaca familiar como posible causa del fallecimiento. El estudio genético post-mortem ayuda a conseguir un diagnóstico sobre el origen del fallecimiento repentino y, además, identificar precozmente familiares a riesgo de síncope.

P6-05. *RABPHILIN* SILENCING CAUSES DILATED CARDIOMYOPATHY IN A *DROSOPHILA*'S HUMAN MODEL OF CARDIAC-NEPHROCYTE DAMAGE

Carlos Casillas-Serra [1,2], Estela Selma-Soriano[1,2], Ruben Artero[1,2,3,4], Juan Antonio Navarro[1,2], Josep Redón[1,5]

[1]Biomedical Research Institute INCLIVA, Valencia, Spain, [2]Department of Genetics, University of Valencia, Valencia, Spain, [3]Institute for Biotechnology and Biomedicine (BIOTECMED), University of Valencia, Valencia, Spain, [4]CIPF-INCLIVA joint unit, Valencia, Spain, [5]Hypertension Unit, Hospital Clínico Universitario, Valencia, Spain

Chronic kidney disease (CKD) and Heart failure (HF) have a strong correlation. Both can be cause and consequence of the other (1). Many factors can lead to the appearance and development of these diseases (2), one of them being the genetic ones. One candidate genetic factor is *Rph*, a gene that is expressed in the excretory and nervous systems in mammals as well as in *Drosophila* (3, 4, 5). This gene encodes a Rab small GTPase family effector protein, which is related to vesicular trafficking (4). We have found that *Rph* is expressed in *Drodophila*'s heart. Acknowledging this, we specifically decreased Rph levels in heart and in both heart and excretory system, and we saw that lifespan was significantly compromised as compared with control flies. Moreover, diastolic and systolic diameters (EDD and ESD, respectively, both being crucial parameters for heart function) were significantly increased, being more severe in the case of reduced Rph levels in both tissues. This suggests that Rph is important in heart and excretory system, and that nephrocyte damage contributes to the development of cardiac disease in a *Drosophila* model of human disease.

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P6-06. Longitudinal analysis of the microbiome evolution in a cohort of patients with Lynch syndrome.

Gabriela Debesa-Tur[1,2], Vicente Pérez-Brocal[1,2], Susana Ruiz-Ruiz[1,2], Adela Castillejo[1,3], Amparo Latorre[1,2,4], José Luis Soto[1,3] and Andrés Moya[1,2,4]

[1]Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana (FISABIO), València, Spain, [2]Consorcio de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBEResp), Madrid, Spain, [3]Unidad de Genética Molecular, Hospital General Universitario de Elche, Alicante, Spain, [4]Instituto de Biología Integrativa de Sistemas (I2Sysbio), Universitat de València and Consejo Superior de Investigaciones Científicas (CSIC), Paterna, València, Spain.

Lynch syndrome (LS) is a genetic condition, also known as hereditary non-polyposis colorectal cancer (HNPCC), that increases the risk of development of some types of cancer, especially colorectal cancer (CRC). It is due to germ line mutations in the mismatch repair (MMR) genes MSH2, MLH1, MSH6, PMS2 or EPCAM. It was demonstrated in a LS model mice that the intestinal microbiota has an important role in CRC development (1). We are carrying out a longitudinal study in order to evaluate the changes in the microbiota over time. We collected faecal samples every three months, beginning three years ago. Here, we analyzed the bacterial metagenomes from faecal samples at eight time points, of a cohort of initially healthy Lynch syndrome patients, some of whom developed polyps or CRC during the sampling period. Analyses of composition and abundance of the bacterial community and gene functions have been carried out on the metagenomic data based on variables such as gender, and diagnosis MMR mutation, as well as alpha diversity indexes and beta diversity analyses, such as, canonical correlation analyses, and wilcoxon tests. We also performed stability analyses, using the Jaccard index, and biomarker discovery, via Linear discriminant analysis Effect Size. We observed differences in the evolution of the microbiome composition between patients who had not developed pathological events of any kind till the last colonoscopy and patients who developed CRC.

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P6-07. *CERKL*, a retinitis pigmentosa gene, is involved in the regulation of mitochondrial function and dynamics in the mammalian retina

Rocío García-Arroyo[a,b], Gemma Marfany[a,b,c], Serena Mirra[a,b]

[a]Dept. Genètica, Microbiologia i Estadística, Universitat de Barcelona, Barcelona, Spain, [b]U718 CIBERER- ISCIII, Barcelona, Spain, [c]IBUB-IRSJD, Barcelona, Spain

The retina is the specialized region of the central nervous system that transduces light into neural signals. It is endowed with an active metabolism and displays a particular vulnerability to genetic and environmental alterations causing mitochondrial dysfunction, such as impaired energy production, mtDNA instability, disturbance of mitochondrial quality control and dynamics. These alterations make photoreceptors and retinal ganglion cells (RGCs) more susceptible to cell death (1).

CERKL mutations cause Retinitis Pigmentosa in humans, a visual disorder characterized by photoreceptors neurodegeneration and progressive vision loss. Preliminary evidences indicate that CERKL is a sensor of photoreceptor stress by contributing to the formation of RNA stress granules (2). Both in human and mouse, *CERKL* produces a wide range of mRNA isoforms that translate into proteins displaying different domains (3). Depending on the domains of each protein isoform, CERKL subcellular localization and functional role may be different.

Here we describe a pool of CERKL isoforms that localize at mitochondria in RGC primary culture. Using *Cerkl*$^{KD/KO}$ mouse models (4), we studied the impact of CERKL downregulation on the mitochondrial network organization and dynamics. Our results show that the depletion of *Cerkl* causes alterations in mitochondrial size, distribution, and trafficking. In addition, we analysed the expression of proteins regulating mitochondrial dynamics (Mitofusin2, Opa1 and Drp1), observing specific changes.

Overall, our studies indicate that *Cerkl* is a retinal neural gene involved in the regulation of mitochondrial dynamics, thus becoming a new player in the multiple pathways that control mitochondrial health in the mammalian retina.

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P6-08. ANDROGEN RECEPTOR POLYQ ALLELES AND COVID-19 SEVERITY IN EUROPEAN MEN: A REPLICATION STUDY

Rosario López-Rodríguez[a,b], Javier Ruiz-Hornillos[c], Marta Cortón[a,b], Berta Almoguera[a,b], Pablo Minguez[a,b], María Elena Pérez-Tomás[d], María Barreda-Sánchez[d, e], Esther Mancebo[f], Lorena Ondo[a], Andrea Martinez-Ramas[a], Lidia FernándezCaballero[a], Estela Paz-Artal[f,g], Encarna GuillenNavarro[b,d], Carmen Ayuso[a,b].

[a]Department of Genetics & Genomics, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid, Madrid, Spain. [b]Centre for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain. [c]Allergy Unit, Hospital Infanta Elena, Valdemoro, Madrid, Spain; Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid, Madrid, Spain; Faculty of Medicine, Universidad Francisco de Vitoria, Madrid, Spain. [d]Medical Genetics Section, Pediatric Department, Hospital Clínico Universitario Virgen de la Arrixaca, Instituto Murciano de Investigación Biosanitaria Virgen de la Arrixaca, Universidad de Murcia (IMIB-Arrixaca, UMU), Murcia, Spain. [e]Facultad de Ciencias de la Salud. Universidad Católica San Antonio de Murcia (UCAM), Murcia, Spain. [f]Department of Immunology, Hospital Universitario 12 de Octubre, Madrid, Spain; Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), Madrid, Spain. [g]Department of Immunology, Ophthalmology and ENT, Universidad Complutense de Madrid, Madrid, Spain.

Numerous evidences indicate a sex-related difference in severity of COVID-19 with a less favorable outcome observed in men. Genetic factors have been proposed as candidates to explain such difference [1]. The polyQ polymorphism in the androgenic receptor gene has been recently described as a genetic biomarker of COVID-19 severity [2].

In this study, we analyzed the involvement of the polyQ polymorphism in COVID-19 severity in our cohort of patients. A total of 1441 male patients were selected from the Spanish STOP_CORONAVIRUS cohort. Patients were classified according to their severity into four categories: 1) oligosymptomatic (19%); 2) hospitalized patients not requiring respiratory support (57.1%); 3) hospitalized patients admitted to intensive care unit (ICU) or similar and requiring respiratory support (14.6%) and 4) deceased patients (9.3%). The allele distribution, coded as shorter (number of repeats ≤22) or longer (number of repeats ≥23) alleles, did not show statistical differences between severity classes in our cohort of male patients (41-44% of frequency of the longer alleles in the four categories, Chi^2 test). Therefore, the results obtained in our study do not support the role of this polymorphism as biomarker of COVID-19 severity.

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P6-09. Looking for a possible treatment for type III galactosemia

Patricia A. Lucas-Rodríguez[a], Ana Mª Brokate-Llanos[a], Andrés Garzón[a], Manuel J. Muñoz[a]

[a]*Genetic area, Pablo de Olavide University, Seville, Spain*

Galactose metabolism is universally conserved in all living organisms, including *Caenorhabditis elegans* and humans. Galactose is metabolized by three conserved enzymes that constitute the Leloir pathway. GALE participates in the third step of the galactose metabolism pathway, catalyzing the interconversion of UDP-galactose (UDP-gal) and UDP-glucose (UDP-glu) and in some species, including *C.elegans* and humans, the interconversion of UDP-N-acetylgalactosamine (UDP-galNAc) and UDP-N-acetylglucosamine (UDP-gluNAc) (1-4). All of these UDP-sugars are required for the glycosylation of proteins and lipids. Type III galactosemia is a rare disorder caused by mutations in GALE, homologue to the *C. elegans gale-1* gen. The symptoms are due to the accumulation of intermediary galactose metabolites and the reduction of UDP-galNAc, because differently to the others, this sugar is only synthesized by GALE. Therefore, patients with type III galactosemia are recommended to follow a galactose-restrictive diet and take UDP-galNAc to avoid UDP-galNAc deficiency. This treatment is not able to improve the psychomotor retardation of these patients; it only slightly improves some of the symptoms. Our team has isolated a *Caenorhabditis elegans* mutant in the GALE homologue gene (*gale-1(pv18)*) which can be used as a model for type III galactosemia. Like humans, this mutant has an increase of UDP-gal and reduction of UDP-galNAc under regular diet. They are also sensitive to galactose rich diet and exhibit multiple developmental defects (5). We have found that the phenotype of the mutant and expression of *gale-1* gene can be modified by diet. We also observed that treatment with some sugars improves the phenotype of the type III galactosemia model. The result observed, if conserved in humans, could be of interest to improve the quality of life of patients with type III galactosemia.

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P6-10. Intracellular distribution in mammalian retinal neurons of B4GAT1 and LARGE, two proteins involved in congenital neuromuscular diseases and cancer pathogenesis

Cristina Quereda[a], Patricia Sabater[a], Mercedes Palmero[b], José Martín Nieto[a]

[a]Departamento de Fisiología, Genética y Microbiología, Facultad de Ciencias, Universidad de Alicante, Alicante, Spain. [b]Departamento de Óptica, Farmacología y Anatomía, Facultad de Ciencias, Universidad de Alicante, Alicante, Spain.

The *B4GAT1* and *LARGE* genes, encoding enzymes β-1,4-glucuronyltransferase 1 (B4GAT1) and LARGE xylosyl- and glucuronyltransferase 1, are associated with a set of congenital neuromuscular dystrophies known as dystroglycanopathies. These constitute a group of orphan, recessively-inherited diseases that course with a broad phenotypic spectrum of symptoms affecting muscles, brain and retina. Lack of function or expression of these proteins cause a loss of *O*-mannosyl glycosylation of α-dystroglycan (α-DG), a plasma membrane glycoprotein responsible for the cells' attachment to the extracellular matrix. The above enzymes participate in a complex, branched pathway where B4GAT1 is responsible for the synthesis of a glucuronic acid-β1,4-xylose-β disaccharide acceptor primer on α-DG, that LARGE elongates by adding repeating units of [-3-xylose-α1,3-glucuronic acid-β1-] to produce the so-called matriglycan structure, which is crucial for α-DG biological function (1). In the retina, α-DG *O*-glycosylation is fundamental for the establishment of functional ribbon synapses between photoreceptors and their postsynaptic, bipolar and horizontal cells (2).

In our group we are addressing the expression pattern and function of dystroglycanopathy-associated proteins in mammalian retinal neurons (2-4). In the present work we have focused on analyzing the immunolocalization of proteins B4GAT1 and LARGE by using fluorescent confocal microscopy techniques. In this fashion, we have characterized their distribution pattern in monkey and mouse retinal sections, and in the 661W immortalized cell line of mouse cone photoreceptors, by means of their coimmunostaining with molecular markers of the endoplasmic reticulum (ER) and Golgi complex. Our observations revealed that B4GAT1 was located in the ER, and LARGE in the Golgi, of retinal neurons in the monkey and mouse, including photoreceptors (inner segments and axon terminals) and 661W cells. At variance with retinal tissue, in this cell line B4GAT1 and LARGE additionally accumulated in the nucleus. These results are indicative of a relevant role of these proteins in α-DG glycosylation in the neural retina of adult mammals. Also, they suggest that B4GAT1 and LARGE could exert an additional role within the nucleus of immortalized 661W photoreceptors. In this light, a possible action of B4GAT1 (5) and LARGE (6) as tumor suppressors has been evidenced, to be further investigated.

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P6-11. Association of Schizophrenia Polygenic Risk Scores with Schizotypy and Psychotic-like Experiences in Non-Clinical Subjects

Patricia Mas-Bermejo[a,b], Sergi Papiol[c,d], Marc Via[e,f,g], Pilar Torrecilla[h], Tamara Sheinbaum[h], Anna Racioppi[h], Elionora Peña[a,b], Natalia Azcona-Granada[a], Thomas R Kwapil[i], Paula Rovira[a], Neus Barrantes-Vidal[i,j,k], Araceli Rosa[a,b,k]

[a]Secció de Zoologia i Antropologia Biològica, Dept. de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona (UB), Barcelona, Spain, [b]Institut de Biomedicina de la UB, Barcelona, Spain, [c]Institute of Psychiatric Phenomics and Genomics, University Hospital, Ludwig Maximilian University (LMU), Munich, Germany, [d]Dept. of Psychiatry and Psychotherapy, University Hospital, LMU, Munich, Germany, [e]Dept. of Clinical Psychology and Psychobiology, UB, Barcelona, Spain, [f]Institut de Neurociències, UB, Barcelona, Spain, [g]Institut de Recerca Pediàtrica, Hospital Sant Joan de Déu, Barcelona, Spain, [h]Dept. of Clinical and Health Psychology, Universitat Autònoma de Barcelona, Barcelona, Spain, [i]Dept. of Psychology, University of Illinois at Urbana-Champaign, Champaign, Illinois, USA , [j]Sant Pere Claver-Fundació Sanitària, Barcelona, Spain, [k]Centre for Biomedical Research Network on Mental Health (CIBERSAM), Instituto de Salud Carlos III, Barcelona, Spain.

Schizophrenia (SZ) is a complex disorder with a highly polygenic inheritance. It can be conceived as the extreme expression of a continuum of traits that are present in a subclinical way in the general population. The study of SZ-related endophenotypes in healthy subjects from the general population can help elucidate its aetiology. Our aim was to investigate the contribution of polygenic risk for SZ on i) self-report schizotypy and psychotic-like experiences and ii) an interviewed measure of psychosis symptoms and experiences in a sample of 252 non-clinical individuals. All participants completed two self-report questionnaires: i) the Wisconsin Schizotypy Scales (WSS) (1, 2) and ii) the Community Assessment of Psychic Experiences (CAPE) (3); and were interviewed applying the Comprehensive Assessment of At-Risk Mental States (CAARMS) (4). Polygenic Risk Scores (PRS) for SZ were calculated based on meta-analysis results from SZ GWAS (5) using the PRS-CS (Continuous Shrinkage) method (6). To test for associations between the psychometric variables and the SZ PRS, linear regression analysis were performed including age, sex and the first two ancestry principal components as covariates. The False Discovery Rate method was applied to correct for multiple testing. No association was found between the SZ PRS and the self-report questionnaires. However, when we explored the association between the SZ PRS and the CAARMS interview, we found a significant association with the Motor Change subscale (β = .59; incremental Adj. R^2 = .038; *P* = .009). Our results seem to support the association of higher PRS for SZ with the presence of motor abnormalities.

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P6-12. Changes in telomere length in relation to weight loss in obese patientssubmitted to bariatric surgery: a longitudinal approach.

Elionora Peña[a,b], Timothy R Powell[c,d], Josep Leon-Menguibar [e,f], Concepción Arenas[g], Narcís Cardoner[f,h,i,j,k], Pere Rebasa[f,l], Alexis Luna[f,l], Paula Rovira[a], Patricia Mas-Bermejo[a], Natalia Azcona-Granada[a], Assumpta Caixàs[f,m], Araceli Rosa[a,b,k]

[a].Secció de Zoologia i Antropologia Biològica, Facultat de Biologia, (UB).[b,] IBUB, [c] Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK. [d] Division of Infectious Diseases, Weill Cornell Medicine, Cornell University, New York, NY, USA. [e] Endocrinology and Nutrition Dep. Hospital Parc Tauli, Medicine Department, UAB, Sabadell, [f] Institut d'Investigació i Innovació Parc Taulí (I3PT), Sabadell, [g] Secció d'Estadística, Facultat de Biologia, Universitat de Barcelona, [h] Mental Health Department, Corporació Sanitària Parc Taulí, Sabadell, [i] Depression and Anxiety program, Department of Mental Health, Parc Taulí Sabadell, , [j] Department of Psychiatry and Legal Medicine, Universitat Autònoma de Barcelona, [k] Centre for Biomedical Research Network on Mental Health, Instituto de Salud Carlos III, Barcelona, [l] Surgery Department, Esofago- gastric Surgery Section, Hospital Universitari Parc Taulí, Sabadell, [m] Endocrinology and Nutrition Department,Hospital Universitari Parc Taulí, Medicine Department Universitat Autònoma de Barcelona, Sabadell

Background: Telomere length (TL) is one biomarker of cell aging used to explore the effects of the environment on age-related pathologies. Obesity and high Body mass index (BMI) have been identified as a risk factors for shortened TL, alongside risk for comorbid age-related diseases including type-2 diabetes (T2D), hypertension, dyslipidemia, osteoarthritis, dementia, depression and cancer [1,2]. Furthermore, it has been suggested that obesity directly increases mortality and reduces longevity via its influences on cellular processes related to ageing [3].
Objectives: To evaluate TL in different subtypes of obesity, and to examine changes in TL in relation to weight loss after bariatric surgery to elucidate how efficacious is bariatric surgery (BS) promoting TL restoration.
Methods: A cohort of 94 patients submitted to BS were followed-up for 24 months ($t24_m$: lost to follow-up = 0%) for BMI and metabolic variables. We assessed TL at each time point (i.e.: t0, $t6_m$, $t12_m$ and $t24_m$) using quantitative polymerase chain reactions and the telomere sequence to single-copy gene sequence ratio method.
Results: Patients with the severe form of obesity (grade III) showed shorter TL pre-surgery than patients with the less severe obesity (grade II) (p = 0.027). No differences in TL were found between patients with or without T2D or MetS. Furthermore, the effect of the severity was also reported in the longitudinal analysis, pointing a different evolution in TL between patients (p = 0.008). Whereby, patients with obesity grade II had longer TL after 6 months than those of grade III (Adjusted p = 0.024). However, no differences were observed at the end of the post-operative period ($t24_m$). When we reviewed systematically the literature on this topic, we found that TL seem to increase after surgery in long-term studies (>2years) presumably due to improvement in different metabolic traits.
Conclusions: Severity of obesity may have a negative effect on TL independently of co-morbidities such as T2D or MetS. Furthermore, according to our findings and previous reports, despite the numerous beneficial effects of BS for obesity patients, short-term studies seem to be unable to detect benefits in terms to TL restoration.

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P6-13. OBTAINING COMPLETE DNA PROFILES FROM LATENT FINGERPRINTS

Almudena Sánchez[a], Juan M. González[a], Pilar Rubio[b], Yolanda Loarce[a]

[a]Departamento de Biomedicina y Biotecnología, Universidad de Alcalá, Alcalá de Henares, Spain, [b]Unidad de Biología molecular, Centro de Apoyo a la Investigación de Medicina y Biología, Universidad de Alcalá, Alcalá de Henares

It has been observed that a small quantity of degraded molecules of DNA, or “Trace DNA”, are present in latent fingerprints (1). Whether they come from cells originating in the hands or exogenously to the hands, they could provide us with enough genetic material to obtain useful genetic profiles that could be used in forensic cases. There is still no specific protocol for extracting genetic material from fingerprints, and since the amount of DNA is very small and degraded, it is not possible to extract it with ordinary DNA extraction methods. This has created the need for a protocol that takes into account the material’s nature. In this study, we are testing different methods for extracting the DNA, as well as alternative quantifying methods that could be more appropriate given said state of the material. The usual quantification made with a microvolume spectrophotometer is being compared with the *Quantifiler™ Human DNA Quantification Kit (ThermoFisher).* Early results show that one of our adapted methods allows us to obtain a larger amount of DNA. The present works aims to quantify the extracted material from a single fingerprint with said commercial kit and attempt to identify the genetic profile using *the AmpFLSR™ Identifiler PCR Amplification kit (ThermoFisher),* obtaining complete or partial genetic profiles but with enough information to allow distinct identification. Furthermore, different age fingerprints will be analysed in order to explore if the DNA continues to degrade after being deposited. To examine this, we will use recent, one month, three months and five months old fingerprints, which have been stored at room temperature.

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P6-14. Benchmarking Conventional Reference Genome Databases in Human Gut Metagenomics

Samuel Piquer-Esteban[a,b], Wladimiro Diaz-Villanueva[a],Vicente Arnau[a], Andrés Moya[a,b,c]

[a]Institute for Integrative Systems Biology(I2SysBio), Universitat de València(UV) and Consejo Superior de Investigaciones Científicas (CSIC), Valencia, Spain, [b]Genomic and Health Area, Foundation for the Promotion of Sanitary and Biomedical Research of the Valencia Region(FISABIO), Valencia, Spain, [c] Centro de Investigación Biomédica en Red en Epidemiología y Salud Pública(CIBEResp), Madrid, Spain.

All metagenomics classifiers require a pre-computed database of reference microbial genomes. Among them for shotgun metagenomics the most popular reference databases are the BLAST nucleotide collection (NT Database) and the Reference Sequence Database (RefSeq Database) for high-quality nucleotide sequences (1,2). Despite their importance, there are no available comparisons in terms of classification performance between them. In this study we focus on the case of the human gut microbiota to carry out a benchmarking of different conventional reference genome databases (including NT Database and RefSeq Database at Complete Genomes and Complete Genomes + Chromosome assembly levels) and evaluate their performance using simulated and experimental datasets. These results intend to provide some insights when choosing reference genome databases in a case of special interest such as the study of the human gut microbiota.

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1.Ye et al. (2019). Cell 178(4), 779-794.

2. Breitwieser et al. (2019). Briefings in Bioinformatics 20(4), 1125-1139.

Acknowledgements & Funding:

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P6-15. Generation of Nanobodies against α-Klotho Protein in Canarian Camels

Víctor G Tagua1,2, Yeray Brito-Casillas3, Ernesto Martín-Nuñez2, Javier Donate-Correa2, Guido Santos-Rosales4, Emma Carmelo1, Pilar Foronda1, Juan F Navarro-González2

[1]Instituto de Enfermedades Tropicales, Universidad de La Laguna, Tenerife, Spain; [2]Unidad de Investigación, Hospital Universitario Nuestra Señora de Candelaria, Tenerife, Spain ; [3]Strategos-Biotech, Las Palmas de Gran Canaria, Spain; [4]Facultad de Biología, Universidad de La Laguna, Tenerife, Spain

Cardiovascular disease (CVD) is the leading cause of death worldwide. Atherosclerosis is the substrate responsible for the vast majority of cardiovascular events (1). Reductions in α-Klotho protein levels have been related with the pathophysiology of CVD, particularly in chronic kidney disease patients (2). The main objective of our project is to obtain mini- and nanoantibodies (nanobodies) from Canarian camels to be employed in the detection of human α-Klotho protein in different tissues, and serum and urine samples.

Nanobodies are variable domains of heavy chain-only antibodies (HCAbs) that can be isolated from camelids. In spite of their single domain structure, nanobodies display many unique features, such as small size, high stability, and cryptic epitopes accessibility, which make them ideal for sophisticated applications in plants and animals (3).

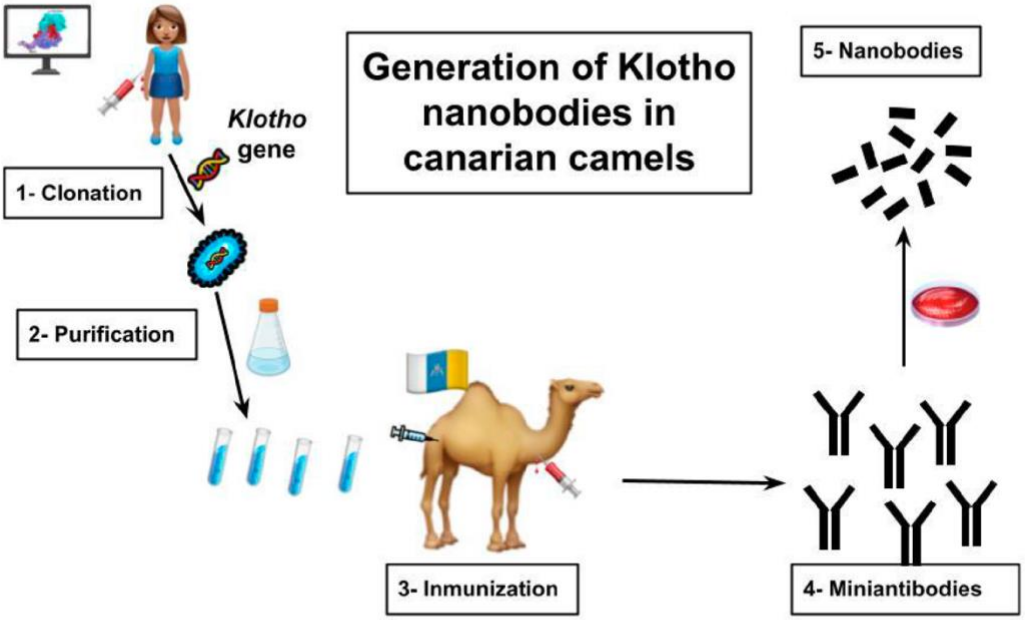
We will carry out immunizations in camels with the different regions of the protein to detect the different forms in which the protein is presented. We will isolate lymphocytes from camel blood in order to clone the DNA regions codifying for antibodies against α-Klotho, that will be further expressed in bacteria. Effectiveness and sensitivity of these new generation antibodies will be tested in clinical samples and cell lines that express α-Klotho through different approaches, e.g. western blot, ELISA and immunohistochemistry assays. These mini- and nanobodies will allow us to use α-Klotho as a biomarker with clinical applicability.

1. Ross (1999) *N Engl J Med, 340:115-26.*

2. Martín-Núñez E *et al.,* (2020) Aging (Albany NY), 11 (18): 7899-7913. 3. Wang *et al.,* (2021) *Plant Mol Biol., 105 (1-2): 43-53.*

This work is supported by Cabildo de Tenerife, Tenerife 2030, FDCAN, MEDI, in the Agustín de Betancourt programme.

Graphical abstract:



P6-16. Role of the *ADAMTSL4* gene in a juvenile glaucoma family. Zebrafish functional analysis.

Ángel Tévar Saiz[a], José Daniel Aroca Aguilar[a], Raquel Atienzar Aroca[a], Juan Manuel Bonet Fernández[a], Susana Alexandre Moreno[a], Carmen Belén Gallardo Martínez[a], María José Cabañero Valera[a], Julio Escribano Martínez[a].

[a]*Human Genetics, Neurological Disabilities Research Institute, Albacete, Spain.*

Early-onset glaucoma (EOG) is a heterogeneous, inherited and severe optical neuropathy that originates from maldevelopment of the anterior segment of the eye. It encompasses different diseases, ranging from primary congenital glaucoma (CG) to juvenile glaucoma (JG), which are usually diagnosed before three years or between 5 to 18 years of age, respectively. To identify new disease genes, we performed next generation DNA sequencing of 94 unrelated EOG and JG patients. We identified rare biallelic *ADAMTSL4* gene variants in one JG patient. In addition, rare monoallelic variants were present in 8,5% of patients. *ADAMTSL4* (a disintegrin and metalloproteinase with thrombospondin motifs-like protein-4) is a member of the ADAMTS gene family and encodes a secreted glycoprotein widely expressed in ocular tissues. Bioinformatic and segregation analysis supported a loss-of-function pathogenic mechanism for the biallelic variants. It was stablished a zebrafish *adamtsl4 knock-out* line by CRISPR/Cas9. Preliminary results in F4 generation have corroborated the disruption of this gene in zebrafish embryos (144 hpf) by qPCR. Furthermore, *adamtsl4* disruption in zebrafish embryos (144 hpf) results in a lethal phenotype in 40% of homozygous embryos, characterized by varying degrees of gross developmental craniofacial abnormalities, including microphthalmia, and pericardial and periocular edemas. Overall, our data indicate that *ADAMSTSL4* loss-of-function may underlie recessive EOG and provide evidence for its putative role in complex forms of the disease.

This work was supported by research grants from the “Instituto de Salud Carlos III/European Regional Development Fund (ERDF)” and from University of Castilla-La Mancha co-financed by Social European Fund.

P6-17. SUITABILITY OF WHOLE BLOOD SAMPLES FOR THE FLOW CYTOMETRY γH2AX ASSAY

Vanessa Valdiglesias[1,2], Natalia Fernández-Bertólez[2,3], Carlota Lema-Arranz[2,3], Raquel Rodríguez-Fernández[2,3], María Sánchez-Flores[2,3], Eduardo Pásaro[2,3], Blanca Laffon[2,3]

[1]Grupo DICOMOSA, Centro de Investigaciones Científicas Avanzadas (CICA), Departamento de Biología, Universidade da Coruña, A Coruña, Spain, [2]Instituto de Investigación Biomédica de A Coruña (INIBIC), AE CICA-INIBIC, A Coruña, Spain, [3] Grupo DICOMOSA, Centro de Investigaciones Científicas Avanzadas (CICA), Departamento de Psicología, Universidade da Coruña, A Coruña, Spain
Corresponding author: Vanessa Valdiglesias (vvaldiglesias@udc.es)

H2AX is a histone variant ubiquitously distributed and expressed throughout the genome. Phosphorylation of H2AX histone (γH2AX) represents an early event in the DNA damage response against double-strand breaks (DSB) and plays a key role in the recruitment of DNA repair proteins and signalling factors that are required downstream in the DNA damage response. Therefore, measurement of H2AX phosphorylation provides a surrogate biomarker of DSB with the potential to identify genotoxic exposures. Recently, we reported initial steps in the standardization of γH2AX assay in peripheral blood leukocytes (PBL), addressing the possibility of using cryopreserved samples, and the need of phytohaemagglutinin (PHA) stimulation prior analysis (Sánchez-Flores et al., 2015). Still, the use of whole blood samples would be particularly useful for human population studies, and this cell specimen has not been validated for this assay so far. In this study, we evaluated the suitability of fresh and frozen whole blood samples to be used in the γH2AX assay, evaluated by flow cytometry, and the convenience of PHA stimulation. Whole blood samples from 3 healthy volunteers, both freshly collected and after cryopreservation, were treated with bleomycin (BLM), actinomycin-D (Act-D) and mitomycin C (MMC), in the presence or absence of a previous 24h incubation with PHA. PBL were used as gold standard for comparison. Negative responses observed in MMC treatments were likely related to the quiescence of unstimulated cells, or to the short treatment time in PHA-stimulated cells. Responses of fresh whole blood samples to BLM and Act-D were more intense in PHA-stimulated cells, probably due to DSB indirectly produced from other less relevant types of DNA damage. According to the data obtained, PHA stimulation in frozen whole blood samples is not advisable. In conclusion, this study demonstrates that whole blood samples can be used to assess genotoxicity related to the production of DSB by the flow cytometry γH2AX assay. Further investigations in epidemiological studies will contribute to standardize the use of whole blood as a suitable sample type in the γH2AX assay and to define its utility in different experimental and clinical settings.

This work was supported by Xunta de Galicia [ED431B 2019/02], and Ministerio de Educación, Cultura y Deporte [BEAGAL18/00142 to V.V].

P6-18. FAECAL, SALIVARY AND TISSUE MICROBIOTA ASSOCIATED WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA RADIOTHERAPY

XueQian Yin[1], Andrés Moya[1,2,3], Francesc-Xavier Avilés-Jurado[4,5],Vicente Pérez-Brocal[1,2]

[1]Foundation for the Promotion of Health and Biomedical Research of Valencia Region (FISABIO-Public Health), Valencia, Spain, [2]Biomedical Research Networking Center for Epidemiology and Public Health (CIBEResp), Madrid, Spain, [3]Institute for Integrative Systems Biology (I2SysBio), University of Valencia and Spanish National Research Council (CSIC), Valencia, Spain, [4]Hospital Clínic, Barcelona,[5]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas (CIBERdem), Madrid, Spain.

In view of the potential relationship between head and neck squamous cell carcinoma (HNSCC) and microbial dysbiosis, we used a metagenomic approach and next-generation sequencing to profile the microbiome from faeces, saliva, and normal, peritumoral and tumoral tissue from patients diagnosed with HNSCC, before treatment with radiotherapy. The purpose of this study was the taxonomic and functional composition characterisation of the microbiome from samples collected before treatment in order to compare it with samples collected from the same patient after treatment, so that we can ultimately evaluate the role of the microbiome as a predictor of radiation sensitivity. After sequencing by Nextera500, and analysing 60 saliva samples, 64 faeces samples, and 135 tissue samples, we first described and also compared the taxonomic and functional differences between the samples according to their origin, including alpha and beta-diversity analyses, focusing on those taxa and genes differentially present. Likewise, we analysed differences between tissue samples according to their location, and the impact of this in the bacterial composition, abundance and diversity. This work, together with ongoing taxonomic analyses based on amplicons, provides a foundation for future studies aimed at understanding the role of the microbiome in HNSCC.

This work was funded by Plan GenT Program of the Conselleria de Sanidad Universal y Salud Pública (Generalitat Valenciana), project CDEI-06/20-E, Asociación Española contra el Cáncer (projects AECC 2017 and AECC 2018) and ISCII from the Ministry of Science and Innovation (project PI18/00844).

SESIÓN 7

Docencia de la Genética

Moderadores: José Luis Micol y María Rosario Linacero

DG-01. Adaptación de metodología docente práctica basada en trabajo cooperativo – "Puzzle de Aronson" – en la asignatura Genética Humana, Clínica y Forense

María Arroyo[a,b], J. Alberto Marchal[a]

[a] Departamento de Biología Experimental, Universidad de Jaén, E23071 Campus Las Lagunillas s/n, Spain
[b]Cell Biology and Epigenetics, Department of Biology, Technische Universität Darmstadt, Germany

Presentamos el trabajo desarrollado dentro del ámbito de un proyecto de Innovación Docente desarrollado en la Universidad de Jaén durante los cursos 2017-2019. El objetivo ha sido incorporar nuevas metodologías docentes basadas en el trabajo cooperativo al ámbito de la docencia práctica de la asignatura "Genética Humana, Clínica y Forense", de carácter optativo y correspondiente al último curso del Grado en Biología de la UJA. Se ha aplicado la técnica del "puzzle de Aronson", que implica una metodología más dinámica basada en la creación de grupos de expertos entre el alumnado, encargados del desarrollo y posterior explicación de los distintos subtemas/secciones en qué se habían dividido los contenidos prácticos. En esta comunicación se explicará la forma en qué han sido estructuradas dichas prácticas para integrarlas en la dinámica docente propuesta, así como los resultados relativos a la valoración de adquisición de competencias por parte del alumnado. Se discutirán dichos resultados junto con la valoración del profesorado respecto a la adecuación de este tipo de metodologías docentes para la mejora de la enseñanza práctica de la Genética.

Plan ID2-UJA 2016: Proyectos de Innovación e Incentivación de las buenas prácticas docentes en la Universidad de Jaén (ref. PID_11-201617)

DG-02. Using barley, instead of *Drosophila*, for Mendelian Genetics lab practices

Laura Pascual, Estela Giménez, Matilde López-Fernández, José F. Vázquez, Patricia Giraldo, Elena Benavente

Departamento de Biotecnología-Biología Vegetal, Universidad Politécnica de Madrid, Madrid, Spain

We have generated an F_2 population from the parental homozygous lines of the Oregon Wolfe Barley collection. These parental lines, OWB-D and OWD-R, differ each other for several morphological traits that can be evaluated on dry spikes and kernels as well as for many molecular markers, all of them segregating in the F_2 population (1). We are presenting a lab practice program implemented at UPM for General Genetics courses which is based on the phenotypic and genotypic analysis of around 200 individuals of that segregant population. The students evaluate spike and grain traits on dry stored material, and conduct genotyping for selected molecular markers (either dominant and codominant). For statistical data analyses, phenotype data for some traits that were recorded when plants were grown in the greenhouse (academic year 2015-16) are also provided to the students. This barley-based teaching tool allows that practice-based learning of key classical genetic concepts like dominance, epistasis and linkage can be complemented with newer concepts like gene mapping or molecular markers. The pros and cons compared to the use of *Drosophila* living individuals as practice material are also considered.

1. Giménez et al. (2021). *Plants* 10, 694.

DG-03. ADAPT YOURSELF: A TRANSVERSAL TRAINING AND SERVICE-LEARNING EXPERIENCE IN THE GENETICS DEGREE

Sònia Casillas[a,b], Gemma Armengol[c], Antonio Barbadilla[a,b], Aina Colomer[a,b], Marta Coronado-Zamora[a,d], Raquel Egea[a,b], María Pilar García-Guerreiro[a], Ruth Gómez-Graciani[b], Jesús Murga-Moreno[a,b], Alicia Roque[e], Illya Yakymenko[b]

[a]Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Bellaterra, Spain, [b]Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra, Spain, [c]Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Bellaterra, Spain, [d]Institut de Biologia Evolutiva, CSIC-Universitat Pompeu Fabra, Barcelona, Spain, [e]Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Bellaterra, Spain

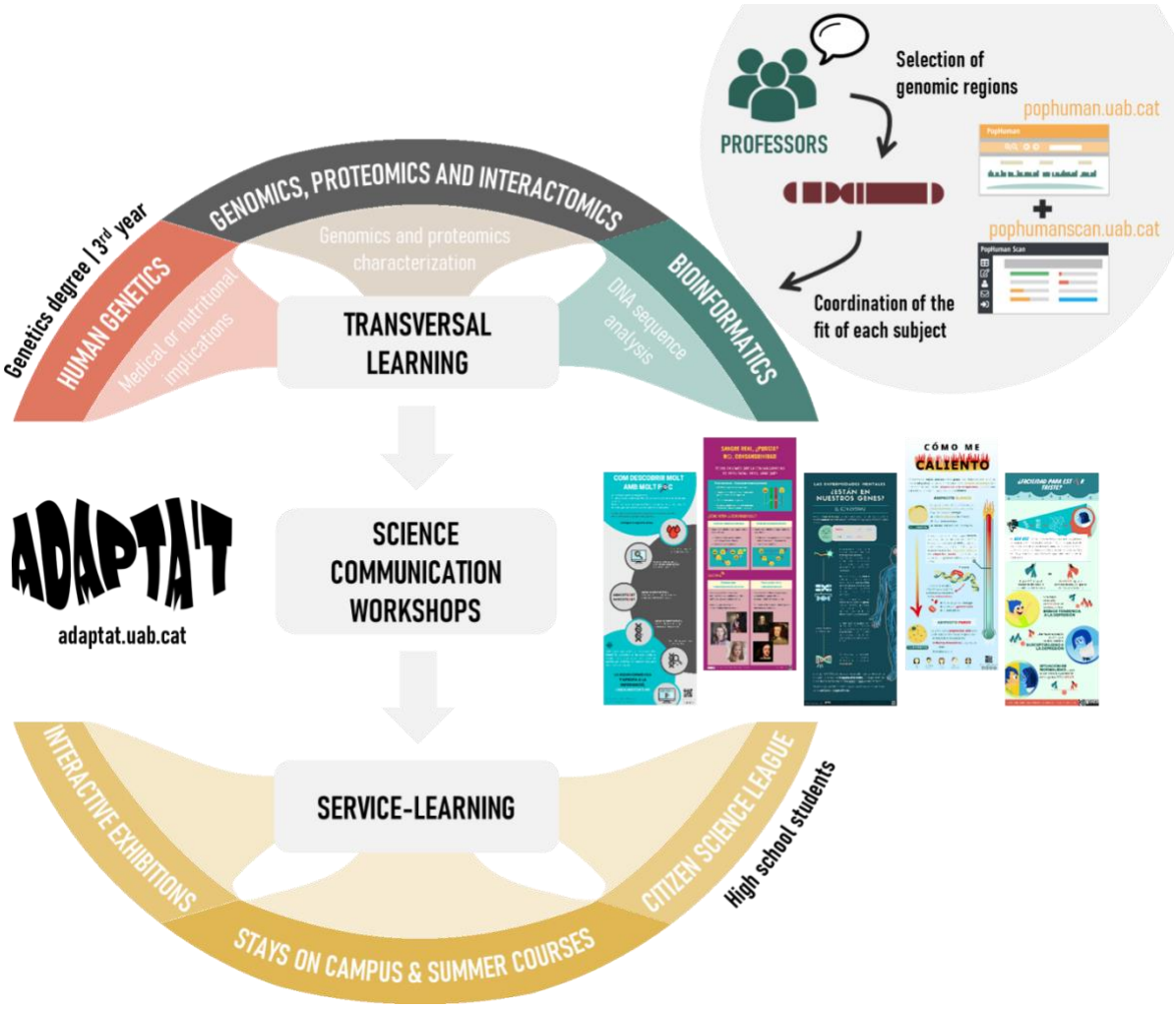
The design of learning activities throughout various academic subjects enhances both students' transversal competencies and the importance of integrating knowledge and approaches in professional research. On the other hand, service-learning opens up students' learning to society, promotes their commitment and improves their close environment outside university through their knowledge and know-how, while creating a circular relationship between learning and community service, which intensifies the effects of each.

ADAPT YOURSELF (http://adaptat.uab.cat) arises from a teaching innovation project funded by the Universitat Autònoma de Barcelona (UAB) since the 2019-20 academic year. The experience, aimed at 3rd-year students of the Genetics degree, includes the participation of three compulsory subjects: *Genomics, Proteomics and Interactomics*, *Human Genetics*, and *Bioinformatics*. Throughout the first semester, groups of students work on a real transversal problem in genetics, addressing different perspectives and integrating the different subjects. Specifically, each group studies a particular region of the human genome that shows evidence of having undergone an episode of adaptive selection in our lineage, with the goal of characterizing it. Later, in the second semester, students optionally participate in science communication workshops where they create original infographics and interactive activities based on the knowledge they have acquired during the first semester. These workshops culminate with the organization of an interactive exhibition aimed at high school students, a service-learning experience that stimulates social responsibility, autonomy and creativity in our students, enriches teaching and research in our university, and promotes the biosciences degrees at the UAB.

The holistic nature of the project has shifted the way students become aware of their learning, how they apply it to solve real-world problems, and finally transfer it to society. Finally, the high quality of the created materials and the good reception by both students and professors, encourage us to incorporate new transference and dissemination actions in future editions, including stays on campus and summer courses for high school students, as well as a cooperative citizen science league between high schools.

This work is supported by two teaching innovation grants of the UAB (calls 2019 and 2020).

Graphical abstract:



DG-04. COLLABORATIVE LEARNING FOR THE IMPROVEMENT OF TEACHING QUALITY AND THE ACQUISITION OF COMMUNICATIVE COMPETENCIES IN GENETICS STUDENTS

Carolina Gómez-Díaz and Gonzalo Machado-Schiaffino

Área de Genética, Departamento de Biología Funcional, Facultad de Medicina, Universidad de Oviedo, Oviedo, Asturias, España

Collaborative learning fosters the development of personal and teamwork skills, generates motivation and stimulates deep learning. Therefore, we have applied collaborative techniques in the subject of Genetics of the Biotechnology Degree at the University of Oviedo to improve the quality of teaching, increase interest in its study and improve presentation skills of the students. This subject has an approximate number of 40 students per year. This allowed us to make small groups for the development of two group methodologies: the Jigsaw technique and a collective Pechakucha. Each of the topics used in the Jigsaw were evaluated in the partial and final evaluations. At the end of the course, students evaluated, using a questionnaire, the perception of the usefulness of collaborative techniques for teaching improvement and knowledge integration. To evaluate the results obtained, the following indicators were used: number of students that passed the subject, the average grade of partial and final exams, the degree of satisfaction with the course compared to others where collaborative techniques are not used, using the results of a general teaching questionnaire, and the degree of perception of the usefulness of collaborative techniques by the students, by means of a Likert scale questionnaire. After analysing the results obtained, we consider that there was an improvement in teaching quality. Likewise, the high motivation of the students was reflected both in the good results obtained in the grades of the course and in the results of the questionnaire. Regarding the improvement of the students' presentation skills, it should be noted that the answers to the questionnaire indicate a positive perception of the improvement of these skills. Therefore, we consider that the introduction of collaborative techniques in the classroom is highly positive in the subject of Genetics.

This work has been carried out under a teaching innovation project of the University of Oviedo (PINN-17-A-070).

DG-05. LABORATORIO EN ABIERTO: aPrendiendo a CopiaR el ADN

F Blasio, N Fernández, I García, E Lantero, J Lira, B Méndez, M Martinez, P Arana, I Ballesteros, C Callejas, N Cuñado, FJ Espino, A Figueiras, FJ Gallego, M González, MT González, F Gil, N Gorfinkiel, JL Hórreo, M Muñoz, A de la Peña, M Pradillo, JM Vega, R Linacero

Departamento de Genética, Fisiología y Microbiología, Universidad Complutense de Madrid.

La Genética es uno de las ramas de la Biología de mayor impacto social siendo cada vez más frecuente oír hablar de conceptos relacionados con la genética, la genómica y sus aplicaciones en el campo de la salud, la alimentación o la biotecnología. Sin embargo, el conocimiento real que tiene la sociedad sobre la genética es, en general, muy limitado. Los estudiantes han crecido en la era de la información digital y saben la importancia de conocer cómo se almacena, se copia, se transmite y se interpreta la información en las redes sociales. El desafío es que los alumnos comprendan que la información genética también se almacena, se copia, se transmite, se expresa y cambia, y que la genética nos permite comprender como funcionan estos procesos en los seres vivos.

Se pretende desarrollar, en los alumnos de secundaria, habilidades para la resolución de problemas científicos a través de retos que despierten su interés y estimulen su imaginación. Todo ello combinado con el uso de recursos educativos en abierto y la enseñanza virtual, herramientas necesarias para hacer accesible el conocimiento al mayor números de estudiantes posible.

Asumiendo que la contextualización del trabajo científico favorece el proceso del aprendizaje, la propuesta se basa en la presentación de un problema contextualizado para que los alumnos lo resuelvan. Así, esperamos que los estudiantes de secundaria asuman el rol de investigador, entiendan los planteamientos del método científico y su aplicación a la resolución de problemas cotidianos. Concretamente, se presenta un problema ficticio, para que, utilizando un modelo de aprendizaje basado en la indagación, los alumnos lo resuelvan aplicando una herramienta que es indispensable en cualquier laboratorio de biología molecular, la Reacción en Cadena de la Polimerasa o PCR.

En este planteamiento resulta fundamental la intervención de los profesores de secundaria como parte activa del proyecto, para que ellos lo transmitan en sus aulas, aumentando el impacto científico y divulgativo de la Genética.

DG-06. DEL COLOR DE OJOS AL INTERIOR DEL GENOMA. NUEVAS TECNOLOGÍAS APLICADAS A LA ENSEÑANZA DE LA GENÉTICA.

N Fernández, I García, E Lantero, J Lira, B Matallanas, B Méndez, A Sanchiz, P Arana, B Beroiz, C Callejas, N Cuñado, FJ Espino, A Figueiras, FJ Gallego, M González, A de la Peña, M Pradillo, JM Vega, C Llanos, C Moreno, J Barrios, R Linacero

Departamento de Genética, Fisiología y Microbiología, Universidad Complutense de Madrid.

La Genética es una de las áreas de la Biología más difíciles de entender por los alumnos debido a su complejidad conceptual. En el ámbito de un proyecto de innovación educativa se ha diseñado una actividad práctica en la que, mediante el cambio en la metodología docente, se pretende promover un aprendizaje más autónomo. Así, los estudiantes podrán integrar los conocimientos del análisis genético, en sus diferentes niveles de complejidad, con los datos genómicos cada vez más relevantes. Este cambio de metodología conlleva el uso de las nuevas tecnologías de la información y comunicación (TIC) que permiten trabajar con los recursos almacenados en las bases de datos.

Los estudiantes, en seis sesiones de tres horas, llevan a cabo un experimento, al que hemos denominado, *Del color de ojos al interior del genoma,* que engloba desde el análisis de la herencia de un carácter morfológico hasta la localización del gen responsable en el genoma de la especie. Los alumnos se convierten en investigadores, sintiéndose más motivados en el proceso de aprendizaje y asimilando de forma sencilla los planteamientos del método científico.

La actividad incluye cuatro bloques:

I. Análisis genético de caracteres morfológicos: cálculo de las distancias genéticas.

II. Obtención de la secuencia de un gen relacionado con un carácter morfológico.

III. Análisis bioinformático: localización en el genoma de la especie.

IV. Distancia genética vs distancia física.

Al final de la actividad se analiza si los resultados, en cuanto a la adquisición de competencias y habilidades propias de Genética, tienen el impacto esperado: que los alumnos sepan relacionar los resultados del análisis genético clásico con la información obtenida usando herramientas propias de la biología molecular y la genómica.

DG-07. Personifying science: bringing students closer to the researchers behind scientific articles

Gonzalo Machado Schiaffino[a], Carolina Gómez Díaz[a]

[a]*Department of Functional Biology (Area of Genetics), University of Oviedo, Oviedo, Spain.*

New technologies nowadays offer a wide range of options to innovate within the field of higher education, generating greater motivation in students and promoting at the same time the in-depth learning of different subjects. Frequently, undergraduate and graduate students consider as absolute truths all the contents found in a scientific article SCI, however, these scientific papers are prepared by researchers, and reviewed by other people (peer review). Therefore, such scientific papers may contain flaws/biases of different kinds. In turn, scientific papers often have a history of failures or successes prior to publication that is unknown to the reader. Therefore, it is of utmost importance for students to be able to learn from such experiences. That is why only through a personal connection (e.g. online interview) with the researchers responsible for such works, it will be possible to access this type of information that reinforces the critical reading of scientific publications. At the same time, these meetings with experts will clarify key concepts and methodologies for their application in different fields of molecular biology.

This work was part of a teaching innovation project for a subject of the master's degree in marine conservation. The main objectives were I) to improve the teaching quality of the subject "Molecular techniques and their application" through the use of new technologies for online conferences with scientific experts in topics associated with the subject II) to encourage critical reading of scientific articles and III) to improve verbal interaction in non-native language (in English).

Online interviews were conducted with foreign researchers responsible for SCI articles that the students had previously read. The invited researchers were experts in different topics within the subject (e.g. use of molecular markers in species identification and commercial fraud). As a control, questionnaires were also conducted on other similar articles in which the students did not interact with the authors.

AUTORES

Índice alfabético de autores

Congreso SEG 14-18 junio 2021