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MEETING REPORT

3237–3241 Meeting Report on Experimental Approaches to Evolution and Ecology Using Yeast and Other Model Systems

Daniel F. Jarosz and Aimée M. Dudley

This meeting report describes the fourth EMBO-sponsored conference on Experimental Approaches to Evolution and Ecology Using Yeast and Other Model Systems, which was held at the EMBL in Heidelberg, Germany, October 19-23, 2016.

GENOME REPORT

3243–3250 High-Quality *de Novo* Genome Assembly of the *Dekkera bruxellensis* Yeast Using Nanopore MinION Sequencing

Téo Fournier, Jean-Sébastien Gounot, Kelle Freel, Corinne Cruaud, Arnaud Lemainque, Jean-Marc Aury, Patrick Wincker, Joseph Schacherer, and Anne Friedrich

The exploration of the genetic diversity within non-model organisms represents a fundamental resource for biological research. The non-conventional yeast *Dekkera bruxellensis* is of particular interest, as natural isolates show different ploidy levels and extensive chromosomal rearrangements, suggesting a rapid evolution. Here, we produced a *de novo* sequence and high-quality genome assembly of *D. bruxellensis* constructed with a combination of Oxford Nanopore long-reads and Illumina short-reads. This genome represents a good frame for population genomic surveys as well as to study genome plasticity in this yeast species.

MUTANT SCREEN REPORTS

3251–3256 Exploring Potential Germline-Associated Roles of the TRIM-NHL Protein NHL-2 Through RNAi Screening

Gregory M. Davis, Wai Y. Low, Joshua W. T. Anderson, and Peter R. Boag

High throughput RNAi screening is a robust approach for investigating loss of function of particular genes. In this study, we employ an RNAi screening approach to identify genes that result in synthetic reproductive defective phenotypes when knocked down in TRIM-NHL-2 deletion mutants in *Caenorhabditis elegans*. NHL-2 is a highly conserved protein and contributes to the regulation of several biological processes by functioning as a microRNA cofactor. This screen was germline focused and has identified many candidate genes of considerable interest, including several components of the germline 22G RNA pathway. These findings may unveil novel germline functions of NHL-2.

3257–3268 High-Throughput Genetic Screening of 51 Pediatric Cataract Genes Identifies Causative Mutations in Inherited Pediatric Cataract in South Eastern Australia

Shari Javadiyan, Jamie E. Craig, Emmanuelle Souzeau, Shiwani Sharma, Karen M. Lower, David A. Mackey, Sandra E. Staffieri, James E. Elder, Deepa Taranath, Tania Straga, Joanna Black, John Pater, Theresa Casey, Alex W. Hewitt, and Kathryn P. Burdon

Cataract is a major cause of severe visual impairment in childhood. The purpose of this study was to determine the genetic cause of paediatric cataract in Australian families. Fifty one genes associated with congenital cataract were sequenced in 33 affected probands. We believe this work will be of interest to your readers as we strengthen the genotype-phenotype correlations for disease and expand the known mutation spectrum.

- 3269–3279 **Systematic Identification of Determinants for Single-Strand Annealing-Mediated Deletion Formation in *Saccharomyces cerevisiae***
Maia Segura-Wang, Megumi Onishi-Seebacher, Adrian M. Stütz, Balca R. Mardin, and Jan O. Korbel
- Different molecular processes exist for ensuring genomic integrity. Errors these mechanisms can result in different classes of mutations, including genomic structural variants (SVs). SVs play an important role in phenotypic variation and genome evolution. Here we present a systematic genome-wide assay to identify *Saccharomyces cerevisiae* gene knock-out mutants associated with single strand annealing (SSA) deletion formation. Our assay uncovered a set of novel genes that are involved in chromatin remodeling and meiosis as potential regulators of maintenance of genomic stability.

INVESTIGATIONS

- 3281–3294 **Neo-sex Chromosomes in the Monarch Butterfly, *Danaus plexippus***
Andrew J. Mongue, Petr Nguyen, Anna Voleniková, and James R. Walters
- We report the discovery of a neo-sex chromosome in Monarch butterfly, *Danaus plexippus*, and several of its close relatives. Z-linked scaffolds in the *D. plexippus* genome assembly were identified via sex-specific differences in Illumina sequencing coverage. These sequencing-coverage based assessments of Z-linkage combined with homology based chromosomal assignments provided strong evidence for a Z-autosome fusion in the *Danaus* lineage. Cytogenetic analysis further revealed a large W-chromosome that is partially euchromatic, consistent with being a neo-W chromosome.
- 3295–3303 **Intricate and Cell Type-Specific Populations of Endogenous Circular DNA (eccDNA) in *Caenorhabditis elegans* and *Homo sapiens***
Massa J. Shoura, Idan Gabdank, Loren Hansen, Jason Merker, Jason Gotlib, Stephen D. Levene, and Andrew Z. Fire
- Our manuscript highlights an understudied, mysterious fraction of the eukaryotic genomes. Here, using an unbiased genome-wide approach, we present an emerging understanding of how fluctuations in genome 3D organization, as a whole, contribute to cell fate and function.
- 3305–3315 **Increased LOH Due to Defective Sister Chromatid Cohesion Is Due Primarily to Chromosomal Aneuploidy and Not Recombination**
Dror Sagi, Evgeniya Marcos-Hadad, Vinay K. Bari, Michael A. Resnick, and Shay Covo
- It was previously suggested that cohesin suppresses LOH through channeling recombination to sister chromatids instead of homologous chromosomes. Our data suggest that prevention of LOH mediated by cohesin is mainly through prevention of aneuploidy and not interhomolog recombination. We also found a new class of instability events that are suppressed by cohesin of which only the telomeric part of the chromosome remains.
- 3317–3329 **Interchromosomal Transfer of Immune Regulation During Infection of Barley with the Powdery Mildew Pathogen**
Priyanka Surana, Ruo Xu, Gregory Fuerst, Antony V. E. Chapman, Dan Nettleton, and Roger P. Wise
- Plant immune systems exemplify multi-tiered signaling networks. Host immune receptors sense pathogen effectors, triggering defense cascades comprising thousands of genes. We used an expression Quantitative Trait Locus (eQTL) approach to address the temporal regulation of immunity in barley-powdery mildew interactions. Two major clusters of trans eQTL on chromosomes 2HL and 1HS were identified, which are associated with penetration of host epidermal cells and development of haustorial feeding structures, respectively. Regulatory control of 22% of these genes was re-prioritized from 2HL to 1HS as infection progressed. These results show that master regulators respond to pathogen attack according to temporal programs.
- 3331–3335 **The Glyphosate-Based Herbicide Roundup Does Not Elevate Genome-Wide Mutagenesis of *Escherichia coli***
Clayton Tincher, Hongan Long, Megan Behringer, Noah Walker, and Michael Lynch
- This study evaluates genome-wide mutagenicity of the glyphosate-based herbicide Roundup using the mutation accumulation/whole-genome sequencing. We find that the genome-wide mutation rate of *E. coli* decreases as the concentration of the glyphosate-based herbicide Roundup increases.

- 3337–3347 **Taxonomically Restricted Genes with Essential Functions Frequently Play Roles in Chromosome Segregation in *Caenorhabditis elegans* and *Saccharomyces cerevisiae***
Adrian J. Verster, Erin B. Styles, Abigail Mateo, W. Brent Derry, Brenda J. Andrews, and Andrew G. Fraser
- Every genome contains many genes that have no significant similarity outside very closely related species. These Taxonomically Restricted Genes (TRGs) are poorly studied and little at all is known about their functions. Here we examine a subset of TRGs that are known to be essential for the viability of either budding yeast or nematode worms and find that these yeast-specific or nematode-specific essential TRGs have many common features. Most interestingly, we find that many of these TRGs have roles in chromosome segregation, suggesting that this process is very rapidly evolving in multiple ways.
- 3349–3357 **KinFin: Software for Taxon-Aware Analysis of Clustered Protein Sequences**
Dominik R. Laetsch and Mark L. Blaxter
- Comparative analysis of protein families is central to comparative genomics, but is hard to do when millions of proteins from hundreds of species are included. KinFin solves this problem by integrating data from common file formats, including functional annotations, and delivers aggregative annotation of protein families. Analyses can be guided by any user-defined grouping of taxa, such as by phenotype or phylogeny. We illustrate the capabilities of KinFin by investigating protein families from filarial nematodes, parasites of veterinary and medical importance. KinFin can be integrated seamlessly into existing comparative genomic workflows and promotes transparent and reproducible analysis of protein families.
- 3359–3377 **CONE: Community Oriented Network Estimation Is a Versatile Framework for Inferring Population Structure in Large-Scale Sequencing Data**
Markku O. Kuismäki, Jon Ahlinder, and Mikko J. Sillanpää
- Development of high-throughput sequencing technologies has launched a need for more efficient statistical methods to infer population structure from large genetic data sets. This article presents how statistical network estimation tools combined with community detection algorithm can be used to efficiently infer population substructure from large genetic data sets. This framework is computationally efficient and does not share the same limitations with the model based clustering methods. The example analyses presented here illustrate the clear benefits and potentials of this framework including comparable population structure estimates with those of ADMIXTURE, STRUCTURE, fastSTRUCTURE, sNMF and Principal Component analysis.
- 3379–3391 **Genome-Scale Genetic Interactions and Cell Imaging Confirm Cytokinesis as Deleterious to Transient Topoisomerase II Deficiency in *Saccharomyces cerevisiae***
Cristina Ramos-Pérez, Jessel Ayra-Plasencia, Emiliano Matos-Perdomo, Michael Lisby, Grant W. Brown, and Félix Machín
- Topoisomerase II (Top2) is an essential protein for chromosome dynamics and segregation and also an important target in antitumor chemotherapy. Top2 downregulation is a strategy of cancer cells to become resistant to chemotherapy, despite it also leads to the formation of anaphase bridges. In this report, we have searched for genetic modifiers of Top2 sublethal downregulation at a genome-scale level in *Saccharomyces cerevisiae*. We have used synthetic genetic array (SGA) analysis for this purpose. We show that the most significant biological whose downregulation alleviates, in turn, the sublethal downregulation of Top2 is the Mitotic Exit Network (MEN).
- 3393–3403 **Duplication and Sub/Neofunctionalization of *Malvolio*, an Insect Homolog of *Nramp*, in the Subsocial Beetle *Nicrophorus vespilloides***
Elijah C. Mehlferber, Kyle M. Benowitz, Eileen M. Roy-Zokan, Elizabeth C. McKinney, Christopher B. Cunningham, and Allen J. Moore
- In *Drosophila* and honey bees a single copy of *Malvolio* acts as a transporter of divalent cations and influences feeding behavior. Vertebrates have two copies of the homologous *Nramp*, and each has different expression and function in different tissues. A phylogenetic analysis showed that honey bees and *Drosophila* are unusual; *Malvolio* is typically duplicated across insects. Expression of the two copies in *Nicrophorus vespilloides*, an insect that parents its offspring, was tissue specific. Expression was opposite in the two copies both during parenting and in different tissues. We suggest that *Malvolio* is undergoing neofunctionalization in perhaps influencing parenting.

- 3405–3414** **Benefits of Dominance over Additive Models for the Estimation of Average Effects in the Presence of Dominance**
Pascal Duenk, Mario P. L. Calus, Yvonne C. J. Wientjes, and Piter Bijma
- Our objective was to investigate accuracy of a sample-estimated average effect (\hat{a}) in the presence of dominance, using either a single locus A-model or AD-model. Our results show that the AD-model yields more accurate estimates of average effects from a finite sample, because it is more robust against sampling deviations from HWE frequencies than the A-model. Genetic models that include dominance, therefore, yield higher accuracies of estimated average effects at single loci than purely additive models when dominance is present. These results are important for GWAS, and potentially also for genomic prediction.
- 3415–3425** **Whole Genome Sequencing-Based Mapping and Candidate Identification of Mutations from Fixed Zebrafish Tissue**
Nicholas E. Sanchez, Breanne L. Harty, Thomas O'Reilly-Pol, Sarah D. Ackerman, Amy L. Herbert, Melanie Holmgren, Stephen L. Johnson, Ryan S. Gray, and Kelly R. Monk
- Zebrafish are a powerful tool for forward genetic screens, and the advent of next-generation sequencing approaches such as whole genome sequencing (WGS) has greatly facilitated progress in mutant gene identification. However, all currently available methods require DNA preparation from fresh tissue; thus, screens involving phenotypes observed by in situ hybridization or antibody stains are not amenable to the rapid gene discovery enabled by WGS. Here, we describe a new WGS sequencing pipeline and importantly, a new and straightforward method to prepare WGS quality genomic DNA from fixed tissue.
- 3427–3434** **High-Resolution Maps of Mouse Reference Populations**
Petr Simecek, Jiri Forejt, Robert W. Williams, Toshihiko Shiroishi, Toyoyuki Takada, Lu Lu, Thomas E. Johnson, Beth Bennett, Christian F. Deschepper, Marie-Pier Scott-Boyer, Fernando Pardo-Manuel de Villena, and Gary A. Churchill
- We have genotyped six mouse reference populations on high density genotyping array. Although the strains were presumed to be fully inbred, we found residual heterozygosity in 40% of individual mice from five of the six panels. We also identified de novo deletions and duplications, in homozygous or heterozygous state, ranging in size from 21kb to 8.4Mb, and gene conversions.
- 3435–3447** **A Comparative Analysis of Genetic Ancestry and Admixture in the Colombian Populations of Chocó and Medellín**
Andrew B. Conley, Lavanya Rishishwar, Emily T. Norris, Augusto Valderrama-Aguirre, Leonardo Mariño-Ramírez, Miguel A. Medina-Rivas, and I. King Jordan
- More than two-thirds of the ~10.7 million Africans that were forcibly brought to the New World ended up in Latin America. Despite the impact that African descendants have had on the region's demography, there have been relatively few studies on the genetic ancestry of Afro-Latinos. We present a comparative analysis of genetic ancestry and admixture in the Afro-Colombian population of Chocó. In addition to characterizing the overall extent of African genetic ancestry in Chocó, along with its patterns of European and Native American admixture, we were also able to pinpoint the specific regions in Africa from which the population originated.
- 3449–3457** **Genetic Dissection of Trabecular Bone Structure with Mouse Intersubspecific Consomic Strains**
Taro Kataoka, Masaru Tamura, Akiteru Maeno, Shigeharu Wakana, and Toshihiko Shiroishi
- Trabecular bone structure has an important influence on bone strength, but little is known about its genetic regulation. Here, we conducted extensive X-ray micro-CT analysis of mouse intersubspecific consomic and subconsomic strains. We found that the majority of mouse chromosomes contribute to structural variation of trabecular bone, and that there are pervasive non-additive phenotypic effects on trabecular bone structure. Moreover, we identified four novel QTLs on mouse chromosome 15 that affect trabecular bone structure and skeletal fragility.
- 3459–3468** **Fine-Scale Genetic Structure in Finland**
Sini Kerminen, Aki S. Havulinna, Garrett Hellenthal, Alicia R. Martin, Antti-Pekka Sarin, Markus Perola, Arno Palotie, Veikko Salomaa, Mark J. Daly, Samuli Ripatti, and Matti Pirinen
- A detailed information about genetic population structure is crucial for designing and interpreting rare variant association studies as well as for refining our knowledge about history. This study identifies rich and geographically highly clustered genetic fine-structure in Finland with striking concordance with historical borders and current dialectal regions. The results provide a geographically precisely-defined reference data that aid the interpretation and design of future sequencing studies in Finland and generate interest among general public about genetics research.

- 3469–3479 **Transcriptome Analysis Suggests That Chromosome Introgression Fragments from Sea Island Cotton (*Gossypium barbadense*) Increase Fiber Strength in Upland Cotton (*Gossypium hirsutum*)**
 Quanwei Lu, Yuzhen Shi, Xianghui Xiao, Pengtao Li, Juwu Gong, Wankui Gong, Aiyang Liu, Haihong Shang, Junwen Li, Qun Ge, Weiwu Song, Shaoqi Li, Zhen Zhang, Md Harun or Rashid, Renhai Peng, Youlu Yuan, and Jinling Huang
- 3481–3490 **Discovery of a Novel Stem Rust Resistance Allele in Durum Wheat That Exhibits Differential Reactions to Ug99 Isolates**
 Jayaveeramuthu Nirmala, Jyoti Saini, Maria Newcomb, Pablo Olivera, Sam Gale, Daryl Klindworth, Elias Elias, Luther Talbert, Shiaoman Chao, Justin Faris, Steven Xu, Yue Jin, and Matthew N. Rouse
- The disease wheat stem rust threatens global food security because a new strain of the wheat stem rust pathogen called Ug99 is virulent to the majority of wheat varieties worldwide and few genetic resources are available that confer resistance, especially in durum wheat. But, we discovered a unique stem rust resistance locus called Sr8155B1 present in durum wheat that is effective to most isolates in the Ug99 race group. Therefore we recommend the release of durum wheat varieties that possess both Sr8155B1 and previously characterized Ug99-effective Sr13 for maximum immediate protection of durum wheat from the threat of Ug99.
- 3491–3507 **Genome-Wide Association Mapping of Stem Rust Resistance in *Hordeum vulgare* subsp. *spontaneum***
 Ahmad H. Sallam, Priyanka Tyagi, Gina Brown-Guedira, Gary J. Muehlbauer, Alex Hulse, and Brian J. Steffenson
- A diverse collection of 314 wild barley (*Hordeum vulgare* ssp. *spontaneum*) accessions was evaluated for resistance to five cultures of the stem rust pathogen (*Puccinia graminis*). The frequency of resistance in the collection was relatively low, ranging from 0.6% to 19.4% to individual rust cultures. A genome-wide association study was conducted to map stem rust resistance loci using 50,842 single nucleotide polymorphic markers. Forty-five quantitative trait loci were identified for resistance across all seven barley chromosomes, several of which were novel. These novel alleles will enhance the diversity of resistance available for cultivated barley.
- 3509–3520 **A Bimolecular Fluorescence Complementation Tool for Identification of Protein-Protein Interactions in *Candida albicans***
 Ana Subotić, Erwin Swinnen, Liesbeth Demuyser, Herlinde De Keersmaecker, Hideaki Mizuno, H el ene Tournu, and Patrick Van Dijck
- Candida albicans* is a major cause of opportunistic fungal infections whose therapeutical treatment has a significant economic impact in the human healthcare system worldwide. Elucidation of the complex protein interactome involved in its virulence is of high interest for the research community. Here, we present the development of a *Candida* optimized bimolecular fluorescence complementation assay (BiFC) based on a split yEmVenus fluorophore. It represents a novel tool for in vivo PPI studies in *C. albicans*. We validated the use of this tool by demonstrating three biologically relevant interaction complexes in *C. albicans*.
- 3521–3531 **Overexpression of miRNA-9 Generates Muscle Hypercontraction Through Translational Repression of Troponin-T in *Drosophila melanogaster* Indirect Flight Muscles**
 Prasanna Katti, Divesh Thimmaya, Aditi Madan, and Upendra Nongthomba
- Many micro-RNAs play important roles in the pathogenesis of cardiac hypertrophy. Micro-RNA-9 (miR-9) has been implicated in the development of cardiac hypertrophy in murine model, but detail mechanisms and its role in the development of muscle remain obscure. In this paper, we show that over-expression of miR-9 during development of indirect flight muscles in *Drosophila* causes hypercontracted muscle phenotype, which results from dysregulated acto-myosin interaction. We show that the muscle phenotype results from miR-9 repressing the expression of major target protein, Troponin-T. Our findings have implication in understanding the cellular and pathophysiology of cardiomyopathies associated with miR-9 over-expression.

3533–3542 **Single-Step qPCR and dPCR Detection of Diverse CRISPR-Cas9 Gene Editing Events *in Vivo***
Micol Falabella, Linqing Sun, Justin Barr, Andressa Z. Pena, Erin E. Kershaw, Sebastien Gingras, Elena A. Goncharova, and Brett A. Kaufman

CRISPR-Cas9 creates numerous different alleles in founder mice, some of which are designed alleles, while others are fortuitous indels causing functional ablation. Because of the expense of mouse transgenesis, researchers frequently employ a “two-for-one” approach, which generates the need to detect any variation from the wild-type sequence. Here we present an approach that can be planned at the time of guide design to test guide targeting efficacy, extent of gene editing, and enable future genotyping. The approach leverages the benefits of internal reference probes, the specificity of locked nucleic acids, and the precision of digital PCR methods for editing detection.

3543–3556 **Genomic Relatedness Strengthens Genetic Connectedness Across Management Units**
Haipeng Yu, Matthew L. Spangler, Ronald M. Lewis, and Gota Morota

Genetic connectedness refers to a measure of genetic relatedness across management units. Genetic connectedness has been studied for pedigree based BLUP; however, relatively little attention has been paid to using genomic information to measure connectedness. We assessed genome-based connectedness across management units by applying prediction error variance of difference, coefficient of determination, and prediction error correlation to a combination of computer simulation and real data. We found that genomic information increased the estimate of connectedness among individuals from different management units compared to that based on pedigree. We contend that genomic relatedness strengthens measures of genetic connectedness across units.

CORRIGENDUM

3557–3558 **Genomic Prediction of Single Crosses in the Early Stages of a Maize Hybrid Breeding Pipeline**
Dnyaneshwar C. Kadam, Sarah M. Potts, Martin O. Bohn, Alexander E. Lipka, and Aaron J. Lorenz