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INVESTIGATIONS

689–698 **A Novel Electronic Assessment Strategy to Support Applied *Drosophila* Genetics Training in University Courses**
Maggy Fostier, Sanjai Patel, Samantha Clarke, and Andreas Prokop

The advent of "omic" technologies revolutionized genetics, and classical genetics teaching at universities ought to be focused on relevant, present-day applications. Fostier *et al.* meet this demand by training students in *Drosophila* mating scheme design, a task that reflects important uses of classical genetics in modern research laboratories. They demonstrate how this training can be implemented as a flexible module for undergraduate courses, leaving substantial room for other topics. They also present a newly developed electronic assessment strategy. It combines advantages of paper and conventional computer-based examinations to assess a wide range of complex skills fairly and reliably, even in large university courses.

699–709 **Differential Regulation of Antagonistic Pleiotropy in Synthetic and Natural Populations Suggests Its Role in Adaptation**
Anupama Yadav, Aparna Radhakrishnan, Gyan Bhanot, and Himanshu Sinha

Despite being detrimental for survival, alleles contributing to various complex human disorders are selected for, possibly as a result of their advantageous effect on other fitness traits. However, little evidence exists of regulation of such antagonistic pleiotropy. Yadav *et al.* show that in yeast, while highly prevalent, this antagonism eventually gets resolved. This is brought about by preferential fine-tuning of signaling pathways during adaptation. However, in complex multi-organ systems, increased cross-talk between multiple phenotypes makes this resolution difficult. Partial resolution of this antagonistic pleiotropy could manifest as complex disorders and play a significant role in speciation.

711–718 **A Multipurpose, High-Throughput Single-Nucleotide Polymorphism Chip for the Dengue and Yellow Fever Mosquito, *Aedes aegypti***
Benjamin R. Evans, Andrea Gloria-Soria, Lin Hou, Carolyn McBride, Mariangela Bonizzoni, Hongyu Zhao, and Jeffrey R. Powell

Genetic study of the dengue and yellow fever mosquito *Aedes aegypti* is essential to understanding its evolutionary history, competence as a disease vector, and the effects and efficacy of vector control methods. Evans *et al.* have developed a genotyping chip, Axiom_aegypti1, to facilitate high-throughput genome-scale study. This chip screens for 50,000 single nucleotide polymorphisms across the genome. Here, the authors validate a subset of these markers and present evidence of the chip's efficacy in distinguishing populations throughout the world. This chip is ideal for applications ranging from population genetics to genome-wide association studies.

719–740 ***Drosophila* Muller F Elements Maintain a Distinct Set of Genomic Properties Over 40 Million Years of Evolution**
Wilson Leung et al.

The Muller F element is unusual among the autosomes in *Drosophila melanogaster*; it exhibits heterochromatic properties (high repeat density, high HP1a/H3K9me3 levels, low recombination rate) but a euchromatic gene density. Leung and the students and faculty of the Genetics Education Partnership examine how these properties affect evolution of F element domains and genes using manually improved sequences and gene annotations from *D. melanogaster*, *D. erecta*, *D. mojavensis*, and *D. grimshawi*. The authors show that the F element has maintained its distinct properties, including higher repeat density and higher rates of inversions, with genes exhibiting larger coding spans, lower codon bias, and lower melting temperature, compared to euchromatic reference regions.

741–749 **A Systematic Mutational Analysis of a Histone H3 Residue in Budding Yeast Provides Insights into Chromatin Dynamics**
Paige Johnson, Virginia Mitchell, Kelsi McClure, Martha Kellems, Sarah Marshall, Mary K. Allison, Harrison Lindley, Hoai-Trang T. Nguyen, Jessalyn E. Tackett, and Andrea A. Duina

Previous studies have shown that a mutation in histone H3 – H3-L61W – interferes with the ability of the transcription elongation factor Spt16 to properly interact with genes and causes cryptic intragenic transcription initiation events. Johnson *et al*. have carried out a systematic mutational analysis of H3-L61 to gain insights into its contribution to proper nucleosome function. The results indicate that H3-L61's hydrophobicity, rotational freedom, and size are all important for normal chromatin activity. The authors also provide evidence that the defects imparted by mutations at H3-L61 on Spt16-gene interactions and on repression of intragenic transcription are mechanistically related to each other.

751–759 **A Survey of Imprinted Gene Expression in Mouse Trophoblast Stem Cells**
J. Mauro Calabrese, Joshua Starmer, Megan D. Schertzer, Della Yee, and Terry Magnuson

Genomic imprinting is a process required for mammalian development that results in expression of genes from one parentally inherited allele over the other. Calabrese *et al*. profiled imprinted gene expression in mouse trophoblast stem cells, and found evidence for a broad range of ongoing imprinted gene expression. They found 48 genes expressed in an imprinted fashion, several of which were new candidate imprinted genes, and several of which were noncoding RNAs. An equal number of maternal and paternally biased genes were detected. These results provide a foundation upon which to dissect mechanisms that underpin imprinted gene expression in the mouse.

761–769 **Genetic Mapping of Natural Variation in Schooling Tendency in the Threespine Stickleback**
Anna K. Greenwood, Reza Ardekani, Shaugnessy R. McCann, Matthew E. Dubin, Amy Sullivan, Seth Bensussen, Simon Tavaré, and Catherine L. Peichel

Understanding the genetic contributions to complex animal behaviors has been of longstanding interest to biologists. However, it has been challenging to identify specific genomic regions that underlie behavioral variation, particularly in natural populations of vertebrates. Greenwood *et al*. use genetic linkage mapping to describe the genetic architecture of variation in schooling behavior among natural populations of threespine stickleback fish. They identify three genomic regions that are associated with differences in the extent to which fish spend time with and approach a schooling fish stimulus. These results shed light on the genetic basis for differences in social behavior in vertebrates.

771–775 **Genetic Analysis of Substrain Divergence in Non-Obese Diabetic (NOD) Mice**
Petr Simecek, Gary A. Churchill, Hyuna Yang, Lucy B. Rowe, Lieselotte Herberg, David V. Serreze, and Edward H. Leiter

Genetic divergence between recently separated substrains of mice can be an important source of phenotypic variation. Simecek *et al*. examine substrains of the non-obese diabetic (NOD) mouse, a premier animal model for the study of autoimmune insulin dependent diabetes mellitus. Using whole exome capture sequencing, the authors identified 64 SNPs and two small structural variants in coding regions.

777–801 **Modeling X-Linked Ancestral Origins in Multiparental Populations**
Chaozhi Zheng

Zheng presents a model of ancestral origin processes along two X chromosomes, which can be applied to various mapping populations, such as the advanced intercross lines (AIL), the Collaborative Cross (CC), the heterogeneous stock (HS), the Diversity Outcross (DO), and the *Drosophila* synthetic population resource (DSPR). The analytic results show that the map expansion for an X chromosome is approximately two-thirds that of an autosome in advanced intercross populations such as the AIL, the HS, and the DO, under the conditions of equal sex ratio, large population size, and large number of inbred founders.

803–817 **Condensin II Regulates Interphase Chromatin Organization Through the Mrg-Binding Motif of Cap-H2**
Heather A. Wallace, Joseph E. Klebba, Thomas Kusch, Gregory C. Rogers, and Giovanni Bosco

Chromosome organization affects essential cellular processes, including DNA replication and gene transcription. Condensin II contributes to nuclear organization by promoting chromosome unpairing and compaction of chromosomes. The authors previously showed that the Mrg15 interacts with Cap-H2 and is required for compaction and unpairing. It is unclear, however, how Mrg15 contributes to this activity. Here, Wallace *et al*. find that Cap-H2 requires Mrg15 for localization at genomic regions containing marks of active transcription. They identify a binding motif responsible for mediating the interaction between Cap-H2 and Mrg15 and show that this motif is required for Cap-H2 mediated compaction and unpairing of chromosomes.

819–827 **Diversity of Maize Shoot Apical Meristem Architecture and Its Relationship to Plant Morphology**
Addie M. Thompson, Jianming Yu, Marja C. P. Timmermans, Patrick Schnable, James C. Crants, Michael J. Scanlon, and Gary J. Muehlbauer

This study characterizes the genetic control of maize shoot apical meristem (SAM) architecture, examines its morphology across a diverse set of inbred lines, and relates SAM architecture to whole plant traits. Heterosis was observed in some F1 progeny from inbred line crosses, whereas others seemed to act in an additive fashion. Phenotypic correlations were observed between meristem morphologies and adult plant traits, revealing associations between the architecture of undifferentiated and differentiated organs. Genetic control of meristem architecture was mainly population-specific, with only one of 15 unique quantitative trait loci identified in both populations.

829–838 **Chromatin Mediation of a Transcriptional Memory Effect in Yeast**
Emily Paul, Itay Tirosh, William Lai, Michael J. Buck, Michael J. Palumbo, and Randall H. Morse

This work investigates the molecular basis for a transcriptional memory effect in yeast. The results support a mechanism in which sequences that favor or disfavor nucleosome occupancy contribute to whether or not transcription of Abf1-regulated genes continues following loss of Abf1 binding. These sequences are conserved among closely related yeast species, indicating physiological relevance. In sum, the findings support promoter-specific differences in the transcriptional response to loss of an activator, and indicate that transcriptional response to altered regulatory input can be controlled by the propensity of promoter sequences to form nucleosomes.

839–847 **Pathway-Based Factor Analysis of Gene Expression Data Produces Highly Heritable Phenotypes That Associate with Age**
Andrew Anand Brown, Zhihao Ding, Ana Viñuela, Dan Glass, Leopold Parts, Tim Spector, John Winn, and Richard Durbin

What is the relationship between our ages and the expression levels of our genes? Today's high-throughput technology offers a chance to look at the global transcriptome picture systematically. However, to achieve this one has to overcome a hurdle due to high stochastic noise in the system. This paper uses a two-step factor analysis approach both to remove effects from known covariates and learned latent artifacts, and to create succinct summaries of gene expression patterns within pathways. The authors have discovered a set of pathways significantly associated with age. Exploiting family structure, they also show that these pathways are under strong genetic control.

849–856 **Finding a Missing Gene: *EFG1* Regulates Morphogenesis in *Candida tropicalis***
Eugenio Mancera, Allison M. Porman, Christina A. Cuomo, Richard J. Bennett, and Alexander D. Johnson

The conserved APSES transcription regulator Efg1 controls several key morphological transitions that allow adaptation of the fungal pathogen *Candida albicans* to the human host. Surprisingly, when the genome sequence of the closely related pathogen *Candida tropicalis* was published, an Efg1 ortholog was missing from the assembly. Mancera *et al*. report the identification of an Efg1 ortholog in *C. tropicalis* and show that it regulates morphological transitions such as filamentation, white-opaque switching, and biofilm formation. These results highlight conservation of the role of Efg1 in this group of fungal pathogens and provide a cautionary note in analyzing "completed" genome sequences.

857–872 **Multiple Phosphatases Regulate Carbon Source-Dependent Germination and Primary Metabolism in *Aspergillus nidulans***
Leandro José de Assis, Laure Nicolas Annick Ries, Marcela Savoldi, Taisa Magnani Dinamarco, Gustavo Henrique Goldman, and Neil Andrew Brown

The dispersal and germination of conidia in a suitable environment has a profound impact on the pathogenicity of fungi and the spoilage of food it causes. The nutrient sensing mechanisms which coordinate germination are unclear. This study of *Aspergillus nidulans* identified seven phosphatases required for germination on saccharide and alcohol carbon sources, influencing morphological adaptations, metabolism, and cell cycle. In addition, this study highlighted the importance of the pyruvate dehydrogenase complex and the alpha-ketoglutarate dehydrogenase as key regulatory steps in germination. These novel insights will provide new avenues of research for the identification of inhibitors of fungal germination.

873–889 **Quantitative Genetics of Migration-Related Traits in Rainbow and Steelhead Trout**
Benjamin C. Hecht, Jeffrey J. Hard, Frank P. Thrower, and Krista M. Nichols

Understanding extant genetic variation for quantitative traits, particularly those associated with complex life histories, is critical in understanding the genetic raw material available for natural selection. Hecht *et al.* explore the heritability and genetic correlation of a suite of characters associated with migration, observing moderate heritabilities and correlations among traits in a salmonid fish that exhibits divergent migratory and resident life histories.

891–909 **Genome-Wide Association Study Based on Multiple Imputation with Low-Depth Sequencing Data: Application to Biofuel Traits in Reed Canarygrass**
Guillaume P. Ramstein, Alexander E. Lipka, Fei Lu, Denise E. Costich, Jerome H. Cherney, Edward S. Buckler, and Michael D. Casler

Tests of associations involving imputed marker data were performed in reed canarygrass, a species showing promise as a biofuel crop in the United States. To make statistically sound inferences on associations, Ramstein *et al.* used multiple imputation, which reflects imputation uncertainty by generating multiple plausible data sets from some imputation procedure. The authors show the flexibility and usefulness of this methodology and provide guidelines for its application in the context of genome-wide association studies based on linear mixed models. They also report associations that are robust to imputation uncertainty and might provide some insight into the genetic basis of important bioenergy traits.

911–920 **A Powerful New Quantitative Genetics Platform, Combining *Caenorhabditis elegans* High-Throughput Fitness Assays with a Large Collection of Recombinant Strains**
Erik C. Andersen, Tyler C. Shimko, Jonathan R. Crissman, Rajarshi Ghosh, Joshua S. Bloom, Hannah S. Seidel, Justin P. Gerke, and Leonid Kruglyak

Andersen *et al.* present an expanded collection of recombinant inbred lines and powerful new high-throughput fitness assays to enable quantitative genetic mapping experiments. Using these new resources and techniques, the authors map normal growth conditions and growth after exposure to the herbicide paraquat, discovering 56 quantitative trait loci.

921–929 **Charged/Polar-Residue Scanning of the Hydrophobic Face of Transmembrane Domain 9 of the Yeast Glutathione Transporter, Hgt1p, Reveals a Conformationally Critical Region for Substrate Transport**
Anil Thakur and Anand K. Bachhawat

The hydrophobic face of a transmembrane helix of the yeast glutathione transporter, Hgt1p, was subjected to a novel strategy of charged/polar residue scanning mutagenesis, followed by suppressor analysis to identify regions critical for substrate translocation. Among 16 charged mutants created for the hydrophobic face of TMD9, only six mutants were non-functional, revealing a surprising tolerance of charged residues in the hydrophobic part of TM helices. Only I524 in TMD9 did not tolerate any charged residue in the hydrophobic face, and suppressor analysis of I524 mutants revealed the residue G202 near TMD3, suggesting a role in substrate translocation.

931–941 **Mapping Bias Overestimates Reference Allele Frequencies at the *HLA* Genes in the 1000 Genomes Project Phase I Data**
Débora Y. C. Brandt, Vitor R. C. Aguiar, Bárbara D. Bitarello, Kelly Nunes, Jérôme Goudet, and Diogo Meyer

The HLA genes are relevant to medical and evolutionary research and are the most polymorphic in the human genome. Since next generation sequencing (NGS) is known to be problematic in such variable regions, the authors asked whether NGS data generated by the 1000 Genomes Project was reliable at these genes. Using Sanger sequencing as a gold standard, they found that the high polymorphism in this region likely impairs mapping of NGS reads, causing the observed overestimation of reference allele frequencies. This phenomenon creates biases in the 1000 Genomes data on highly polymorphic regions, which must be considered in subsequent analyses.

943–952 ***Discs large 5*, an Essential Gene in *Drosophila*, Regulates Egg Chamber Organization**
Eve Reilly, Neha Changela, Tatyana Naryshkina, Girish Deshpande, and Ruth Steward

Dlg (Discs large) proteins typically mediate signaling complex formation and localization. Vertebrate Dlg5 is responsible for polarization of neural progenitors and associates with Rab11 positive vesicles in epithelial cells. Reilly *et al*. identified dlg5 as essential in *Drosophila* embryos and germ line and follicle stem cells. Reduced levels of Dlg5 in the ovarian follicle cells leads primarily to defects in egg chamber budding, stalk cell overgrowth, and ectopic polar cell induction, and also to abnormal distribution of DE-Cadherin, a protein essential for normal organization of egg chambers.

953–962 **Genetic Interaction Landscape Reveals Critical Requirements for *Schizosaccharomyces pombe* Brc1 in DNA Damage Response Mutants**
Arancha Sánchez, Assen Roguev, Nevan J. Krogan, and Paul Russell

Brc1 protein of fission yeast, which binds phosphorylated histone H2A (γH2A) at stalled and damaged replication forks, maintains genome stability during DNA replication. A genetic screen provides new insights into the functional relationships between Brc1 and other genome protection pathways. Brc1 is especially critical in mutants lacking the deneddylation activity of the evolutionary conserved COP9/Signalosome.

963–969 **The Maintenance of Single-Locus Polymorphism by Maternal Selection**
Hamish G. Spencer and Kai X. Chiew

This work is a theoretical investigation of the ability of maternal selection – in which the fitness of an organism depends on its mother's genotype as well as its own – to maintain allelic variation. Spencer and Chiew show that even though the potential of maternal selection to maintain many alleles is greater than that of the standard model of constant viability selection, this potential is not realized via a simple process that is a combination of mutation and maternal selection.

971–981 **Resequencing at ≥40-Fold Depth of the Parental Genomes of a *Solanum lycopersicum* × *S. pimpinellifolium* Recombinant Inbred Line Population and Characterization of Frame-Shift InDels That Are Highly Likely to Perturb Protein Function**
Zoltan Kevei, Robert C. King, Fady Mohareb, Martin J. Sergeant, Sajjad Z. Awan, and Andrew J. Thompson

Tomatoes for the fresh market are predominantly produced on rootstocks. A tomato mapping population derived from a cross between two non-domesticated accessions has been previously used to identify quantitative trait loci (QTL) controlling rootstock traits. Here, the parental lines have been resequenced to provide a comprehensive description of their DNA polymorphisms; this will facilitate the identification of genes that cause the QTL effects. By systematic analysis of these polymorphisms, Kevei *et al.* have identified genes whose functions are likely to be perturbed relative to the tomato reference genome, and have described the occurrence of these alleles in other resequenced tomato genomes.

983–996 **Comparative Analysis of *Wolbachia* Genomes Reveals Streamlining and Divergence of Minimalist Two-Component Systems**
Steen Christensen and Laura Renee Serbus

Prokaryotic cell division, differentiation, and virulence are commonly controlled by "two-component regulatory systems" (TCS). Unlike free-living bacteria that can carry hundreds of TCS proteins, the streamlined bacterial endosymbiont *Wolbachia pipientis* carries only two TCS pairs: CckA-CtrA and PleC-PleD. Christensen and Serbus used comparative bioinformatics to analyze predicted TCS genes and proteins from 12 sequenced *Wolbachia* strains. The results support an ongoing role for these TCS pairs in *Wolbachia* binary fission and second messenger synthesis. Modifications in conserved domains also have implications for modular aspects of TCS protein function, cross-talk, and divergence between the regulatory systems of *Wolbachia*.

MUTANT SCREEN REPORTS

997–1006 **Leveraging DNA Damage Response Signaling to Identify Yeast Genes Controlling Genome Stability**
Jason A. Hendry, Guihong Tan, Jiongwen Ou, Charles Boone, and Grant W. Brown

Genome instability underlies oncogenesis and tumor heterogeneity, yet factors that prevent its occurrence remain incompletely understood. Hendry *et al*. have conducted a high-throughput screen in *Saccharomyces cerevisiae* to elucidate genes controlling genome stability. This screen is an adaptation of the classic "constitutive RNR3" screen originated by Stephen J. Elledge. It leverages the expression profile of *RNR3*, which is strongly induced in response to genome instability, in conjunction with high-throughput yeast genetics and a fluorescence protein reporter system. The authors relate the results to existing genome instability screens from yeast and human, and known cancer genes, identifying several novel genome maintenance factors.

1007–1019 **Rapid and Efficient Identification of *Caenorhabditis elegans* Legacy Mutations Using Hawaiian SNP-Based Mapping and Whole-Genome Sequencing**
Aimee Jaramillo-Lambert, Abigail S. Fuchsman, Amy S. Fabritius, Harold E. Smith, and Andy Golden

Temperature-sensitive mutants in any organism are a valuable resource for the study of a variety of biological processes. Many "legacy" mutants identified in *C. elegans* over 30 years ago have yet to be identified molecularly. Jaramillo-Lambert *et al*. demonstrate that Hawaiian SNP-based mapping along with whole-genome sequencing can easily help identify the molecular nature of a number of temperature-sensitive legacy mutants. Each mutant was verified by complementation assays in which they fail to complement existing deletion alleles. One cell cycle mutant was further verified by rescue with the Cas9/CRISPR technique. This mutant was also recreated with Cas9/CRISPR in an otherwise wild-type background and shown to be temperature-sensitive embryonic lethal, just like the mutation isolated some 30 years ago.