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INVESTIGATIONS

- 775–780 **Genomic Regions Associated with Tolerance to Freezing Stress and Snow Mold in Winter Wheat**
Erika B. Kruse, Scott W. Carle, Nuan Wen, Daniel Z. Skinner, Timothy D. Murray, Kimberly A. Garland-Campbell, and Arron H. Carter
- This study examined tolerance to freezing stress and snow mold in a recombinant inbred line population of winter wheat derived from tolerant and susceptible parents, Eltan and Finch, respectively. Controlled freezing chamber survival assays and field-rated snow mold tolerance were measured in the population. Four total QTL were discovered; one for both freezing and snow mold tolerance, two unique to freezing tolerance, and one unique to snow mold tolerance. This information will be useful in identifying candidate genes that convey tolerance to these stresses and also to wheat breeders selecting for tolerance to these stresses.
- 781–788 **Single-Molecule Sequencing of the *Drosophila serrata* Genome**
Scott L. Allen, Emily K. Delaney, Artyom Kopp, and Stephen F. Chenoweth
- Third generation sequencing technologies, that generate very long reads, hold great promise in facilitating *de novo* genome assembly for non-model species. Here we have successfully assembled and sequenced the genome of the non-model *Drosophila* species *D. serrata* using only 65x coverage third generation single-molecule sequence data (Pacific Biosciences). The genome has a high degree of completeness in terms of gene contents and has a favorable level of contiguity compared with second generation short-read assemblies. Our study highlights the utility of long-read technology when assembling small to moderate sized genomes of non-model species.
- 789–799 **Mutants for *Drosophila* Isocitrate Dehydrogenase 3b Are Defective in Mitochondrial Function and Larval Cell Death**
Dianne M. Duncan, Paula Kiefel, and Ian Duncan
- We report that the three “type” alleles of the *Drosophila* ecdysone-induced transcription factor gene *Eip93F* (*E93*) are in fact allelic to a nearby gene, *Idh3b*, which encodes the β subunit of isocitrate dehydrogenase-3, a mitochondrial enzyme of the tricarboxylic acid cycle. These mutations cause mitochondrial depolarization and early developmental arrest, indicating a near-complete block to oxidative phosphorylation. *Idh3b*-mutant salivary gland cells fail to undergo mitochondrial fragmentation and do not initiate autophagy, early steps in the cell death program. *Idh3b* is released from salivary gland mitochondria during fragmentation, suggesting that it may play an important role in the initiation of apoptosis.
- 801–811 **Persistency of Prediction Accuracy and Genetic Gain in Synthetic Populations Under Recurrent Genomic Selection**
Dominik Müller, Pascal Schopp, and Albrecht E. Melchinger
- Synthetic populations were simulated by intermating various numbers of parents from ancestral populations differing in linkage disequilibrium (LD) and subjected to 30 cycles of recurrent genomic selection to analyze prediction accuracy and genetic gain. Persistency of accuracy was generally high and LD replaced co-segregation as dominant information source when the number of parents increased, resulting in relatively constant long- and short-term genetic gain for more than 4 parents given sufficient LD in the ancestral population. Information from pedigree relationships mainly contributed to accuracy within the first few generations. Larger TS and higher marker density improved persistency of accuracy.

- 813–822 **Novel Resampling Improves Statistical Power for Multiple-Trait QTL Mapping**
Riyan Cheng, R. W. Doerge, and Justin Borevitz
 A gene may influence some traits of interest but not others. Joint analysis of multiple complex traits (multitrait analysis) can have a better chance to identify a quantitative trait locus (QTL) if a parsimonious model is employed. The authors developed a method that excludes trivial QTL effects and achieves a gain in power for QTL identification, and also proposed a procedure for testing QTL-trait associations with an improved power.
- 823–834 **Cytoplasmic–Nuclear Incompatibility Between Wild Isolates of *Caenorhabditis nouraguensis***
Piero Lamelza and Michael Ailion
 Reproductive barriers between populations, such as the production of unfit hybrids (e.g. sterile or inviable), are essential to the evolution of new species. The Bateson-Dobzhansky-Muller (BDM) model hypothesizes that hybrids are relatively unfit due to deleterious interactions between divergent genomic loci. Identifying the genes involved in these deleterious interactions and the forces that drove their divergence is crucial to understanding the mechanism of speciation. Here we report that deleterious interactions between foreign cytoplasmic and nuclear genomes occur frequently between wild isolates of the nematode species *Caenorhabditis nouraguensis*. This system highlights the role of divergent cytoplasmic-nuclear coevolution in promoting speciation.
- 835–841 **Genetics and Adaptation of Soybean Cyst Nematode to Broad Spectrum Soybean Resistance**
Michael Gardner, Robert Heinz, Jianying Wang, and Melissa G. Mitchum
 Very few genetic studies have examined the inheritance of virulence in the soybean cyst nematode *Heterodera glycines*, a microscopic roundworm responsible for major economic losses to soybean production. This is the first report that soybean cyst nematode reproduction on resistant soybean may be specified by a combination of virulence genes inherited in a recessive fashion and moreover that different alleles at a common locus may counter-select for virulence on different resistant sources. Our findings show that the soybean-soybean cyst nematode pathosystem is highly genetically complex, advancing our understanding of host-pathogen adaptation.
- 843–850 **Linkage Mapping and Comparative Genomics of Red Drum (*Sciaenops ocellatus*) Using Next-Generation Sequencing**
Christopher M. Hollenbeck, David S. Portnoy, Dana Wetzel, Tracy A. Sherwood, Paul B. Samollow, and John R. Gold
 A dense genetic linkage map was produced for red drum (*Sciaenops ocellatus*), a marine fish species of considerable economic importance in the southeastern United States and elsewhere. Using comparative genomics approaches, we demonstrate how the map can be used to assign putative positions to candidate genes of interest, in the absence of a reference genome. The map will be a valuable tool for commercial and restoration aquaculture of red drum, as well as for conservation and management of wild populations.
- 851–858 **SHH Protein Variance in the Limb Bud Is Constrained by Feedback Regulation and Correlates with Altered Digit Patterning**
Rui Zhang, Chanmi Lee, Lisa Y. Lawson, Lillian J. Svete, Lauren M. McIntyre, and Brian D. Harfe
 Genes are transcribed and produce mRNA, which in most cases is translated into protein. Recently, it has become clear that even in identical cells mRNA expression can vary. Using the bilaterally symmetric vertebrate limb bud we demonstrate that protein variance of the evolutionally conserved signaling molecule Sonic Hedgehog (SHH) occurs during normal limb development. Our analysis suggests that failure to eliminate SHH protein variance collaborates with defects in digit patterning and digit length. These data suggest that protein variance occurs during normal development and suggests a novel mechanism potentially responsible for changes in digit patterning during evolution.
- 859–864 **Lack of Association of CD55 Receptor Genetic Variants and Severe Malaria in Ghanaian Children**
Kathrin Schuldt, Christa Ehmen, Juergen Sievertsen, Jennifer Evans, Juergen May, Daniel Ansong, Birgit Muntau, Gerd Ruge, Christian Timmann, Tsiri Agbenyega, Rolf D. Horstmann, and Thorsten Thye
 CD55, a surface molecule on human red blood cells, was recently identified as a receptor essential for red blood cell invasion of *Plasmodium falciparum*, the parasite that causes severe childhood malaria. Here, we studied human CD55 variants in a Ghanaian case-control study of more than 1,700 children and did not find any convincing evidence for an association between genetic variants in the CD55 gene and severe malaria complications. This study answers an obvious and timely question in human genetics of malaria, which represents the classical example for an impact of genetic variation on disease susceptibility and resistance.

- 865–870 **Genetic Dissection of a QTL Affecting Bone Geometry**
Olivia L. Sabik, Juan F. Medrano, and Charles R. Farber
 Parameters of bone geometry including width, length and cross-sectional area are major determinants of bone strength. These traits are highly heritable; however, few genes influencing bone geometry have been identified. In this work, we fine-mapped a QTL influencing femur length using the C57BL/6J and CAST/EiJ strains. Using an F2 cross, sequence analysis, and allelic expression in F1s, we identify candidate genes driving differences in femur length. Interestingly, we also find that the refined location of *Feml2* overlaps a cluster of six independent genome-wide associations for human height. Ultimately, this work provides the foundation to identify novel genes affecting bone geometry.
- 871–880 **De Novo Transcriptome Characterization of a Sterilizing Trematode Parasite (*Microphallus* sp.) from Two Species of New Zealand Snails**
Laura Bankers and Maurine Neiman
 We provide the first genomic resources for a textbook model of host-parasite coevolution, *Microphallus*, a trematode infecting the New Zealand freshwater snail *Potamopyrgus antipodarum*. We generated, characterized, and analyzed transcriptomes from *Microphallus* sampled from both freshwater and estuarine habitats. We used these resources and data to (1) identify genetically differentiated orthologs between these two trematodes, (2) provide functionally interesting sets of candidate genes likely involved in infection and response to salinity stress, and (3) generate a large new set of genomic resources, including variable molecular markers and primers applicable to a wide range of future research.
- 881–893 **Adapting Genotyping-by-Sequencing for Rice F2 Populations**
Tomoyuki Furuta, Motoyuki Ashikari, Kshirod K. Jena, Kazuyuki Doi, and Stefan Reuscher
 We show the application of GBS for a large F2 population derived from *Oryza sativa japonica* and the African wild rice *Oryza longistaminata*. We evaluate and optimize the performance of GBS for this heterozygous population from a wide cross. In addition, we use error correction and imputation of missing data to construct a linkage map. Finally, we test our dataset using simulated and real-world data to perform QTL analyses. We detected at least four QTL for the number of tillers and confirmed two of these in introgression lines derived from the same set of parents.
- 895–909 **Predicted Residual Error Sum of Squares of Mixed Models: An Application for Genomic Prediction**
Shizhong Xu
 Cross-validation (CV) is an essential tool to evaluate the predictability of a prediction model. Unfortunately, CV presents a substantial increase in computational load. We developed an alternative method, the HAT method, to replace CV. The new method corrects the *estimated residual errors* from the whole sample analysis using the leverage values of a hat matrix of the random effects to achieve the *predicted residual errors*. Properties of the HAT method were investigated using seven agronomic traits and 1000 metabolomic traits of an inbred rice population. Results showed that the HAT method is good approximation of the CV method.
- 911–921 **Functional Analysis of Kinases and Transcription Factors in *Saccharomyces cerevisiae* Using an Integrated Overexpression Library**
Ji-Young Youn, Helena Friesen, Alex N. Nguyen Ba, Wendy Liang, Vincent Messier, Mike J. Cox, Alan M. Moses, and Brenda Andrews
 Kinases and transcription factors (TFs) often function together in important regulatory pathways, but because of redundancy and condition-specific requirements, loss-of-function mutants of individual kinase and TFs may not be informative. In contrast, overexpression of these regulators may reveal new biology. Youn and colleagues built a collection of yeast strains expressing inducible alleles of kinases and TFs and characterized the phenotypes associated with their overexpression. To investigate the relationships between kinases and TFs, they used genetic interaction screens, identifying 94 genetic interactions between 65 overexpression alleles of TFs and nine kinases.

- 923–933 **CRISPR-Cas-Induced Mutants Identify a Requirement for dSTIM in Larval Dopaminergic Cells of *Drosophila melanogaster***
Trayambak Pathak, Deepti Trivedi, and Gaiti Hasan
- The essential process of calcium homeostasis in non-excitabile metazoan cells is maintained by a mechanism called Store-operated Ca^{2+} entry (SOCE). SOCE requires the endoplasmic reticulum membrane resident calcium-sensor STIM and the plasma membrane localised calcium channel, Orai. SOCE in neuronal calcium homeostasis and signaling has been debated because there exist diverse calcium entry mechanisms in excitable cells. Here, we generated a complete deletion and a tissue-inducible mutant for *dSTIM* and identified a critical requirement for SOCE in developing dopaminergic neurons of *Drosophila*. Thus, SOCE might maintain vertebrate dopaminergic neuron integrity, with consequences for human diseases like Parkinsons.
- 935–942 **Single-Step BLUP with Varying Genotyping Effort in Open-Pollinated *Picea glauca***
Blaise Ratcliffe, Omnia Gamal El-Dien, Eduardo P. Cappa, Ilga Porth, Jaroslav Klápště, Charles Chen, and Yousry A. El-Kassaby
- We incorporated traditional pedigree with genomic information in a HBLUP-analytical approach for estimating genetic variance components of white spruce progeny test trial. The genotyping effort was investigated through varying the number of genotyped individuals. This approach has proven to be effective in capitalizing on the pedigree connectedness resulting in better genetic variance estimates. This represents the first application of this approach in forest tree testing populations, indicating that the improvement in the estimated genetic parameters justifies the costs associated with genotyping. The method is universally applicable to progeny testing experiments.
- 943–952 **Reproduction and In-Depth Evaluation of Genome-Wide Association Studies and Genome-Wide Meta-analyses Using Summary Statistics**
Yao-Fang Niu, Chengyin Ye, Ji He, Fang Han, Long-Biao Guo, Hou-Feng Zheng, and Guo-Bo Chen
- In line with open-source movement in genetics, we report a novel linear regression technique for genome-wide association studies (GWAS), called **Open GWAS algorithm (OATH)**. Using naïve summary statistics (NSS) only, OATH can not only completely reproduce the reported results from an experimental model but also recover underreported results from other alternative models. We demonstrated the utility of OATH for 42 *Arabidopsis* phenotypes under 3 magnesium conditions. In addition, OATH can be used for consortium-driven genome-wide association meta-analyses (GWAMA), and greatly improve the flexibility of GWAMA. OATH has been realized in the software **Genetic Analysis Repository (GEAR)**.
- 953–966 **Genome-Wide Analysis of lncRNA and mRNA Expression During Differentiation of Abdominal Preadipocytes in the Chicken**
Tao Zhang, Xiangqian Zhang, Kunpeng Han, Genxi Zhang, Jinyu Wang, Kaizhou Xie, and Qian Xue
- The present study was the first to screen for lncRNAs and mRNAs regulating chicken preadipocytes differentiation by sequencing and annotating the transcriptome of preadipocyte at four differentiation stages. 3,095 differentially expressed genes were identified. The dynamic changes of DEGs expression were plotted. We identified six stage-specific modules related to A0, A2 and A6 stages using weighted co-expression network analysis. Many well-known/novel pathways associated with preadipocytes differentiation were found. We also identified highly connected genes in each module and visualized them by cytoscape. Many well-known genes related to preadipocytes differentiation were found such as IGFBP2.
- 967–981 **The Impact of Selection at the Amino Acid Level on the Usage of Synonymous Codons**
Paweł Błażej, Dorota Mackiewicz, Małgorzata Wnętrzak, and Paweł Mackiewicz
- Besides mutational pressure and selection on effectiveness of translation, biased codon usage can be also a by-product of a general selection at the amino acid level. Using an evolutionary optimization, we found that the effect is enhanced by mutational processes generating more adenine and thymine. Interestingly, this effect is observed only under an unrestricted model of nucleotide substitution and disappears when the mutational process is time-reversible. Furthermore, it can considerably interfere with other selections on the codon usage, especially in AT-rich genomes and may cause the overestimation of protein coding sequences whose codon usage is subjected to the adaptational selection.

- 983–989** **Identification of Unannotated Small Genes in *Salmonella***
Jonghwan Baek, Jiyoung Lee, Kihoon Yoon, and Hyunwoo Lee
- The annotations of sequenced bacterial genomes are suspected to miss many small genes encoding proteins equal to or smaller than 100 amino acids. Our study reports the identification of 130 unannotated ORFs in the model bacterium *Salmonella enterica* Typhimurium using ribosome profiling with the aid of *in silico* predicted putative open reading frames. The majority of ORFs identified encode peptides/proteins \leq 100 amino acids, suggesting the persistent problem of annotating small genes. These findings call for the development of an annotation pipeline that accurately detects small genes.
- 991–1000** **Regulators of Lysosome Function and Dynamics in *Caenorhabditis elegans***
Kevin Gee, Danniell Zamora, Teresa Horn, Laeth George, Cameron Upchurch, Justin Randall, Colby Weaver, Caitlin Sanford, Austin Miller, Sebastian Hernandez, Hope Dang, and Hanna Fares
- Using a forward genetic screen in *Caenorhabditis elegans*, we identified six proteins that regulate different aspects of lysosome biology. Five of the six proteins are conserved in mammals, highlighting conserved mechanisms of regulating lysosome function and dynamics.
- 1001–1010** **The *Saccharomyces cerevisiae* Cdk8 Mediator Represses *AQY1* Transcription by Inhibiting Set1p-Dependent Histone Methylation**
Michael J. Law and Michael A. Finger
- Transcriptional response to environmental changes requires extensive communication between the RNA polymerase II holoenzyme complex, transcription factors, and post-translational histone modifications. Here, we describe a role for the highly conserved cyclin C-Cdk8p kinase complex inhibiting locus-specific H3Lys4 3me in budding yeast to repress transcription. Interestingly, cyclin C-Cdk8p prevent Set1p methyltransferase recruitment to target loci. This study suggests that one way in which cyclin C-Cdk8p regulates stress responsive and developmental gene transcription is by restricting H3Lys4 methylation.
- 1011–1018** **Cellular Proteomes Drive Tissue-Specific Regulation of the Heat Shock Response**
Jian Ma, Christopher E. Grant, Rosemary N. Plagens, Lindsey N. Barrett, Karen S. Kim Guisbert, and Eric Guisbert
- The heat shock response is a stress response regulated by a network of highly conserved genes involved in protein homeostasis. Despite the essential roles of these regulators in all cells, they display extensive tissue-specificity in HSR regulation. Here, we find that the tissue-specificity is influenced by the interactions between the cellular proteome and the protein homeostasis network. For example, actin levels sensitize muscle tissue to disruption of the TRiC/CCT chaperone that is required for actin folding. Additionally, we characterize organismal phenotypes of actin overexpression and find support for the role of the actin cytoskeleton in longevity.
- 1019–1025** **Allelic Dropout During Polymerase Chain Reaction due to G-Quadruplex Structures and DNA Methylation Is Widespread at Imprinted Human Loci**
Aaron J. Stevens, Millie G. Taylor, Frederick Grant Pearce, and Martin A. Kennedy
- Loss of one allele during amplification of DNA by polymerase chain reaction (PCR) may have profound implications for molecular diagnostic and research procedures that assume biallelic amplification. Our work focusses on an unusual type of allelic drop-out which is caused by the combined effect of DNA structure formation (G-quadruplexes) and cytosine methylation, whereby the methylated allele is always completely lost. We demonstrate that this type of allelic drop-out potentially occurs at many imprinted regions of the genome, and is hence an important consideration for laboratories carrying out molecular diagnostics or genetic research that involves imprinted regions of the genome.
- 1027–1037** **A Temporal Perspective on the Interplay of Demography and Selection on Deleterious Variation in Humans**
Evan Koch and John Novembre
- We investigate how deleterious variation responds to population size change, giving special attention to nonequilibrium temporal dynamics and events relevant to recent human evolutionary history. Specifically, we consider basic growth and bottleneck models before contrasting a complex history representative of an Out-of-Africa population (bottleneck + growth) to a relatively stable population history representative of an African population. We find that even simple summaries of deleterious genetic variation can have complicated responses, and we compare our theoretical findings to patterns of deleterious variation in a large sample of human exomes.

- 1039–1048 ***Saccharomyces cerevisiae* FLO1 Gene Demonstrates Genetic Linkage to Increased Fermentation Rate at Low Temperatures**
Rebecca C. Deed, Bruno Fedrizzi, and Richard C. Gardner
- Saccharomyces cerevisiae* is often fermented in extremely stressful environments, including low temperatures. The transcriptional response of yeast to low fermentation temperatures is complex and involves hundreds of genes. Therefore, the identification of a single gene with an impact on the fermentative ability of yeast at low temperature is of interest to geneticists, as well as the food and beverage industry. We have discovered that the *FLO1* gene, encoding a flocculation protein, has genetic linkage to maximal fermentation rate (V_{max}) at low temperature (12.5 °C). This research supports current literature demonstrating that Flo1p has a role in stress tolerance.
- 1049–1060 **Differential Regulation of Cyclin E by Yorkie-Scalloped Signaling in Organ Development**
Zhiqiang Shu and Wu-Min Deng
- In the organ development of multicellular organisms, cell proliferation is tightly coordinated to achieve its proper structure and functions. In *Drosophila*, the wing imaginal disc displays differences in the nuclear size when accelerated proliferation and endoreplication were induced by Yorkie (Yki), a growth regulator, and Fizzy-related (Fzr). These differences were due to spatiotemporally expressed Yki transcription factor partner Scalloped (Sd) and Yki target gene Cyclin E (CycE). CycE promoted cell proliferation and apoptosis, and was shown to negatively feed back to transcription activity of CycE and Diap1, suggesting its role in differential proliferation and tissue integrity in organ development.