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MUTANT SCREEN REPORT

3559–3570 **Genetic Screening for EMS-Induced Maize Embryo-Specific Mutants Altered in Embryo Morphogenesis**

Dale C. Brunelle, Janice K. Clark, and William F. Sheridan

Maize (corn) is the most economically important crop plant grown in the US and is the third most important crop plant in the world. This species has much potential for increased productivity and enhanced nutritional value to help feed the world. Here we report our progress in identifying genes in corn that control the formation of the embryonic plant within the mature kernel. Understanding how these genes work may help enable the modification of corn so that it can be sustainably cultivated in a wider range of environmental conditions and have a better balance of amino acids in its kernels.

INVESTIGATIONS

3571–3586 **Genomic Prediction Within and Across Biparental Families: Means and Variances of Prediction Accuracy and Usefulness of Deterministic Equations**

Pascal Schopp, Dominik Müller, Yvonne C. J. Wientjes, and Albrecht E. Melchinger

To analyze the empirical accuracy of genomic prediction within and across biparental families, we simulated families of double-haploid lines, using models trained with full-sib, half-sib or unrelated families. Prediction accuracy was high and robust within full-sib families, yet lower and highly variable across families and traits, which we subjected to a causal analysis. Two modified deterministic equations enabled us to forecast the mean prediction accuracy across traits, but deviations for individual traits could not be accounted for. Our study provides important information for plant breeders to optimize genomic prediction within and across bi-parental families.

3587–3595 **Mapping Loci That Control Tuber and Foliar Symptoms Caused by PVY in Autotetraploid Potato (*Solanum tuberosum* L.)**

Washington L. da Silva, Jason Ingram, Christine A. Hackett, Joseph J. Coombs, David Douches, Glenn J. Bryan, Walter De Jong, and Stewart Gray

Strains of Potato Virus Y have emerged that cause tuber necrosis in some cultivars. Crossing two potato cultivars that differ in susceptibility to PVY yielded 236 progeny that segregated for tuber and foliar disease expression. Genetic information was obtained for each of the progeny and compared with disease symptom reactions allowing regions of the potato genome to be linked to symptom expression. Molecular markers diagnostic for these regions may allow breeders to select potatoes that are resistant to tuber necrosis.

- 3597–3604 **The Transcriptional Response of *Candida albicans* to Weak Organic Acids, Carbon Source, and *MIG1* Inactivation Unveils a Role for *HGT16* in Mediating the Fungistatic Effect of Acetic Acid**
Fabien Cottier, Alrina Shin Min Tan, Marina Yurieva, Webber Liao, Josephine Lum, Michael Poidinger, Francesca Zolezzi, and Norman Pavelka
- Yeast and bacteria colonise human body niches according to a precarious equilibrium which can result in fungal infection if unbalanced. Bacteria control yeast growth by producing weak organic acids which impact on yeast was shown to be increased in absence of glucose as carbon source. We now identified, using RNA-sequencing and a multi-parametric experimental design, genes of the human pathogen *Candida albicans* to be targeted to increase yeast sensitivity to acids. This knowledge can be exploited to improve control of yeast colonisation in human body niches.
- 3605–3620 **Comparison of Single Genome and Allele Frequency Data Reveals Discordant Demographic Histories**
Annabel C. Beichman, Tanya N. Phung, and Kirk E. Lohmueller
- Inferring demographic history from genetic data is a major goal of population genetics. Our article compares popular inference methods to determine which can recapitulate multiple summaries of empirical human data. While no method perfectly recapitulated all summaries of the data, some performed better than others. Our results have bearing on human population history, but may be even more important for the study non-model organisms, as we find that whole genome-based inference methods commonly used on wild populations do not recapitulate several summaries of the data, challenging the literal interpretation of the resulting demographic histories.
- 3621–3635 **Genome-Wide Analysis of DNA Methylation During Ovule Development of Female-Sterile Rice *fsv1***
Helian Liu, Ya Wu, Aqin Cao, Bigang Mao, Bingran Zhao, and Jianbo Wang
- Early investigations have demonstrated that DNA methylation participated in plant reproductive process. To explore the potential effects of DNA methylation on the female gametophyte abortive mechanism, whole-genome bisulfite sequencing were used to report a methylome analysis of ovules in high frequency female-sterile rice line (*fsv1*) and rice wild type line (Gui 99). As a result, 3471 differentially methylated regions (DMRs) were found between *fsv1* and Gui99. These DMRs were associated with many biological pathways such as reproduction, metabolic and transcription factor regulation and they may provide important clues for revealing the female gametophyte abortion mechanism.
- 3637–3647 **A New Diagnostic Resource for *Ceratitis capitata* Strain Identification Based on QTL Mapping**
Sheina B. Sim, Raul Ruiz-Arce, Norman B. Barr, and Scott M. Geib
- Sterile male medfly released for the purpose of pest control through the sterile insect technique (SIT). A fluorescent powered dye is used to distinguish SIT flies from wild incursions but can be unreliable. By integrating classical genetic and modern genomic techniques, we developed a new assay that can discriminate between wild and SIT flies. This additionally resulted in the identification of loci tightly linked to a genetic sexing trait maintained in SIT lines that will enable future identification of the gene which can be used to identify genes in other species and develop novel SIT strains for emerging tephritid pests.
- 3649–3657 **Linkage Analysis and Association Mapping QTL Detection Models for Hybrids Between Multiparental Populations from Two Heterotic Groups: Application to Biomass Production in Maize (*Zea mays* L.)**
Héloïse Giraud, Cyril Bauland, Matthieu Falque, Delphine Madur, Valérie Combes, Philippe Jamin, Cécile Monteil, Jacques Laborde, Carine Palaffre, Antoine Gaillard, Philippe Blanchard, Alain Charcosset, and Laurence Moreau
- Identification of loci (QTL) involved in hybrid performance is of key importance for crosspollinated species such as maize (*Zea mays* L.). Using a factorial design between two multiparental segregating populations from two different genetic groups, we compared the efficiency of three QTL detection models that differed in terms of allelic codings. Models taking into account differences of allelic effects in the two groups were best adapted, consistent with their high divergence. Models assuming biallelic QTL detected more QTL but did not always explain the highest percentage of variance, suggesting the presence of complex allelic series in each genetic group.

- 3659–3668 **The Effect of Common Inversion Polymorphisms *In(2L)t* and *In(3R)Mo* on Patterns of Transcriptional Variation in *Drosophila melanogaster***
Erik Lavington and Andrew D. Kern
- Large chromosomal inversion polymorphisms in *Drosophila* have been associated with phenotypic variation, including body size and stress resistance. Recently, Fuller *et al.* published a study on the effect of inversions in *Drosophila pseudoobscura* on 3rd chromosome transcription variation. We found that two different inversions in *Drosophila melanogaster* have a moderate effect on the expression of hundreds of genes across the genome. Further, we show that the majority of the transcription variation is the result of either allelic variation in LD with the inversions, trans-effects of regulators that also are in LD with the inversions, or indirect effects of the inversions.
- 3669–3679 **Genome Dynamics of Hybrid *Saccharomyces cerevisiae* During Vegetative and Meiotic Divisions**
Abhishek Dutta, Gen Lin, Ajith V. Pankajam, Parijat Chakraborty, Nahush Bhat, Lars M. Steinmetz, and Koodali T. Nishant
- We measured base mutation rates and changes in heterozygosity of a hybrid baker's yeast strain (S288c/YJM789) propagated in vegetative and meiotic modes. The meiotic lines showed rapid loss of heterozygosity (LOH) and improved spore viability. Surprisingly a few of the vegetative lines also showed extensive LOH, suggesting the occurrence of abortive meiosis and return to growth events in laboratory conditions. The average base substitution rates for the vegetative lines (1.82×10^{-10} per base per division) and the meiotic lines (1.22×10^{-10} per base per division), are the first genome wide mutation rate estimates for a hybrid yeast.
- 3681–3692 **Differential Expression of miRNAs in the Respiratory Tree of the Sea Cucumber *Apostichopus japonicus* Under Hypoxia Stress**
Da Huo, Lina Sun, Xiaoni Li, Xiaoshang Ru, Shilin Liu, Libin Zhang, Lili Xing, and Hongsheng Yang
- In present study, high-throughput sequencing was applied to investigate alterations of miRNA expression in sea cucumber under different dissolved oxygen levels. A total of 26 miRNAs differentially expressed at significant levels were obtained. Highthroughput sequencing, RT-qPCR, and hierarchical clustering identified key miRNAs, including Aja-miR-1, Aja-miR-2008, Aja-miR-184, and Aja-miR-31-5p, that may be significant in regulating adaptive hypoxia-associated gene expression. Notably, three novel miRNAs were specifically clustered and upregulated in severe hypoxia, which may be useful for novel “hypoxamiR” identification. These results will provide a basis for future studies of miRNA regulation and molecular adaptive mechanism in sea cucumbers under hypoxia stress
- 3693–3698 **Highly Efficient, Rapid and Co-CRISPR-Independent Genome Editing in *Caenorhabditis elegans***
Harriet Prior, Ali K. Jawad, Lauren MacConnachie, and Asim A. Beg
- We developed and optimized a streamlined method to rapidly and efficiently generate point mutations in *Caenorhabditis elegans* using CRISPR-Cas9 ribonucleoproteins. The tools, methods and workflow we present are readily adaptable to other nematode species and likely amenable to other experimental model systems in which CRISPR-Cas9 editing is utilized.
- 3699–3704 ***Caenorhabditis elegans* HIF-1 Is Broadly Required for Survival in Hydrogen Sulfide**
Irini Topalidou and Dana L. Miller
- Animals generate hydrogen sulfide endogenously that is used in diverse cellular signaling pathways. The enzymes that produce and metabolize hydrogen sulfide are conserved from bacteria to humans. In *Caenorhabditis elegans*, the HIF-1 transcription factor is essential to survive exposure to hydrogen sulfide. To explore the function of HIF-1 in hydrogen sulfide we performed tissue-specific rescue experiments. We find that expression of hif-1 from the unc-14 promoter is sufficient to survive hydrogen sulfide. Although unc-14 is used as a pan-neuronal-specific promoter, we show that it is active in many non-neuronal tissues as well. Together, our studies indicate that HIF-1 is required in many cell types to survive exposure to hydrogen sulfide.

- 3705–3718 **Machine Learning Analysis Identifies *Drosophila Grunge/Atrophin* as an Important Learning and Memory Gene Required for Memory Retention and Social Learning**
Balint Z. Kacsoh, Casey S. Greene, and Giovanni Bosco
- In this study, we utilize using a data-mining algorithm, PILGRM (the Platform for Interactive Learning by Genomics Results Mining) in concert with a functional predictor, IMP (integrative multi-species prediction), to predict novel genes involved in *Drosophila* learning and long-term memory. We find that our top candidate, a histone deacetylase, *Grunge (Gug)*, is involved in memory retention. Our study provides a previously uncharacterized role for *Gug* as a possible regulator of neuronal plasticity at the intersection of memory retention and memory extinction. We present a model where the maintenance of the memory is dependent on histone deacetylase activity.
- 3719–3730 **Mechanisms of Transmission Ratio Distortion at Hybrid Sterility Loci Within and Between *Mimulus* Species**
Rachel E. Kerwin and Andrea L. Sweigart
- Transmission ratio distortion is often observed in hybrid offspring of recently diverged species, but the underlying mechanisms and evolutionary significance are not always clear. In this study, we identified multiple contributors to transmission ratio bias in genomic regions linked to two *Mimulus* hybrid sterility loci. We found that hybrid transmission bias is caused, in part, by gametic action of the incompatibility itself, but these effects are partially counteracted by opposing mechanisms. Strikingly, we found little evidence of transmission bias within species, suggesting that it is limited to hybrid genetic backgrounds and is not driven by selfish evolution.
- 3731–3743 **The Ubiquitin Ligase (E3) Psh1p Is Required for Proper Segregation of both Centromeric and Two-Micron Plasmids in *Saccharomyces cerevisiae***
Meredith B. Metzger, Jessica L. Scales, Mitchell F. Dunkleberger, and Allan M. Weissman
- Loss of the yeast ubiquitin ligase Psh1p increases the steady-state levels of proteins expressed from *CEN* plasmids without affecting their turnover or total cellular protein synthesis. Instead, Psh1p is required for proper segregation of both *CEN* and 2 μ m plasmids. When cells are grown in selective media, increased *CEN* plasmid missegregation in *psh1 Δ* cells leads to higher plasmid DNA, mRNA, and protein. Surprisingly, this effect is distinct from that seen upon overexpression of the only known Psh1p substrate, the centromeric histone H3 variant Cse4p. These results strongly suggest a novel Psh1p substrate or function critical to faithful plasmid inheritance.
- 3745–3755 **Serotonin Drives Predatory Feeding Behavior via Synchronous Feeding Rhythms in the Nematode *Pristionchus pacificus***
Misako Okumura, Martin Wilecki, and Ralf J. Sommer
- The nematode *Pristionchus pacificus* shows a predatory feeding behavior against other nematodes using teeth-like denticles that open prey cuticle. To investigate the neural mechanism of predatory feeding behavior in *P. pacificus*, we generated mutants using CRISPR/Cas9 engineering and established a genetic cell ablation system utilizing reconstituted caspases. The loss of serotonin or the loss of a subset of serotonergic neurons reduced the predation efficiency and disrupted the timing and the coordination of pharyngeal pumping and tooth movement during predatory feeding behavior. Our results suggest a role of serotonin in the regulation of pharyngeal rhythms during predation in *P. pacificus*.
- 3757–3774 **Localization of Cdc7 Protein Kinase During DNA Replication in *Saccharomyces cerevisiae***
Daniel Rossbach, D. Suzi Bryan, Jay R. Hesselberth, and Robert Sclafani
- DDK is a conserved protein kinase composed of Cdc7 kinase and Dbf4 regulatory subunits. DDK is required for a number of important chromosomal regulatory processes, most notably, the initiation of DNA replication. Although it has generally been assumed DDK binds to origins of replication during S phase, there is little empirical evidence to support it. Using the “Calling Cards” genomic method to monitor Cdc7 binding to yeast chromosomes, Cdc7 was found bound throughout the genome with a preference for active origins. Loss of Dbf4 binding to Cdc7 reduced, while loss of ATP hydrolysis by Cdc7 increased binding to chromosomes.

- 3775–3787 **Whole Genome Sequence Analysis of Mutations Accumulated in *rad27Δ* Yeast Strains with Defects in the Processing of Okazaki Fragments Indicates Template-Switching Events**
Sumita Omer, Bar Lavi, Piotr A. Mieczkowski, Shay Covo, and Einat Hazkani-Covo
- Okazaki fragments that are formed during lagging strand DNA synthesis include an initiating primer consisting of both RNA and DNA. In order to ligate one Okazaki fragment to another the RNA fragment must be removed. A key player in this step is the structure-specific flap endonuclease, Rad27p (human homolog FEN1). In order to understand what types of mutations are created when Rad27 is not functioning we sequenced five colonies after 25 passages from two *Saccharomyces cerevisiae* lineages and compared the sequence to the colonies at the first passage. Out of the 455 changes observed in ten colonies isolated the two most common types of events were insertions or deletions (INDELs) in simple sequence repeats (SSRs) and INDELs mediated by short direct repeats. Surprisingly, we also detected a previously neglected class of 21 template-switching events. These events were mediated by palindromic sequences. The formation of these events is best explained by folding back of the stalled nascent strand and resumption of DNA synthesis using the same nascent strand as a template. Out of the 455 events, 55 events appeared in multiple isolates, further analysis indicate that these loci are mutational hotspots. We note that our results open new ways of understanding template switching that occur during genome instability and evolution.
- 3789–3796 **Pervasive, Genome-Wide Transcription in the Organelle Genomes of Diverse Plastid-Bearing Protists**
Matheus Sanitá Lima and David Roy Smith
- Publicly available RNA-sequencing (RNA-seq) data are an immeasurable but untapped data source for studying organelle genome transcription. We sought to use this free resource to survey mitochondrial and plastid transcription. Our RNA mapping analyses revealed pervasive, genome-wide organelle transcription among disparate plastid-bearing protists. For every species analyzed, transcripts covered at least 85% of the mitochondrial and/or plastid genomes, indicating that most of the organelle DNA—coding and non-coding—is being transcribed. Our findings suggest that non-coding organelle DNA can be transcriptionally active, raising questions about the underlying functions and evolutionary consequences of these transcripts.
- 3797–3808 **Filamentation Involves Two Overlapping, but Distinct, Programs of Filamentation in the Pathogenic Fungus *Candida albicans***
Jahaun Azadmanesh, Austin M. Gowen, Paul E. Creger, Nichole D. Schafer, and Jill R. Blankenship
- Filamentation is one of the most widely-studied biological processes in the pathogenic fungus *Candida albicans* due to its strong correlation to pathogenesis. We used phenotypic and transcriptomic approaches to compare common in vitro model systems for filamentation and found wide variation between these models. Our comparative approach allowed for the identification of genes with model-independent roles in filamentation and a model-independent transcriptional response to filamentation. Our data furthermore suggests that physical cues drive distinct programs of filamentation in *C. albicans*.
- 3809–3820 **Architecture and Distribution of Introns in Core Genes of Four *Fusarium* Species**
Mmatshapho M. Phasha, Brenda D. Wingfield, Martin P. A. Coetzee, Quentin C. Santana, Gerda Fourie, and Emma T. Steenkamp
- In this study we show that automated gene prediction software can make errors during annotation processes. This is especially the case for lower eukaryotes such as fungi for which very little information is available regarding gene cis-elements found in their genomes. We show that even within genera, cis-elements can vary and therefore, need to be incorporated into gene prediction software.
- 3821–3830 **Using Linkage Maps as a Tool To Determine Patterns of Chromosome Synteny in the Genus *Salvelinus***
Matthew C. Hale, Garrett J. McKinney, Courtney L. Bell, and Krista M. Nichols
- This article describes the construction of a linkage map for Brook trout. The linkage map was used to compare patterns of chromosome organization with other salmonids. The results are informative with respect to patterns of genome organization, as well as the identification of tetrasomically inherited chromosome arms.

- 3831–3836 **First Draft Genome Sequence of the Pathogenic Fungus *Lomentospora prolificans* (Formerly *Scedosporium prolificans*)**
Ruibang Luo, Aleksey Zimin, Rachael Workman, Yunfan Fan, Geo Perte, Nina Grossman, Maggie P. Wear, Bei Jia, Heather Miller, Arturo Casadevall, Winston Timp, Sean X. Zhang, and Steven L. Salzberg

Lomentospora prolificans is fungus that causes a wide variety of infections in both people and animals. *L. prolificans* is distributed throughout the world and primarily found in soil and plants. Here we describe the first effort to sequence the genome of this pathogen, using a combination of sequencing technologies including long-read nanopore sequencing. The final genome assembly is approximately 37.6 million bases long, the vast majority of it contained in 26 long scaffolds. Initial annotation identified 8,896 protein-coding genes. The genome and annotation have been deposited in GenBank under BioProject PRJNA392827.

CORRIGENDUM

- 3837 **Third Chromosome Balancer Inversions Disrupt Protein-Coding Genes and Influence Distal Recombination Events in *Drosophila melanogaster***
Danny E. Miller, Kevin R. Cook, Alexander V. Arvanitakis, and R. Scott Hawley