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INVESTIGATIONS AND AUTHOR SUMMARIES

1291–1303 **Mapping the Genetic Basis of Symbiotic Variation in Legume-Rhizobium Interactions in *Medicago truncatula***
Amanda J. Gorton, Katy D. Heath, Marie-Laure Pilet-Nayel, Alain Baranger, and John R. Stinchcombe

Mutualisms are ubiquitous throughout all organismal kingdoms and affect many ecological and evolutionary processes. Although these interactions are known to be genetically variable, the loci underlying such natural symbiotic variation in fitness, and whether these loci are dependent on the genotype of the interacting partner, remains unknown. In this study, the authors performed QTL mapping in *Medicago truncatula* to investigate the genetic architecture of plant fitness traits when grown with one of two distinct Sinorhizobium meliloti strains and to determine the contribution of genetic variation in known mutualism signaling genes.

1305–1315 **Gal4-based Enhancer-Trapping in the Malaria Mosquito *Anopheles stephensi***
David A. O'Brochta, Kristina L. Pilitt, Robert A. Harrell, II, Channa Aluvihare, and Robert T. Alford

Anopheles mosquitoes are powerful vectors of human malaria and there is great interest in understanding the genetic basis for the physiological processes intimately involved in their susceptibility, infection, and transmission of Plasmodium. Mosquito genome sequence information is available, but the lack of powerful forward and reverse genetic technologies limits efforts to undertake functional genomic studies. Here the authors describe a transposon-based enhancer-trap system that is highly effective in the human malaria vector *Anopheles stephensi*. This system provides a new and powerful tool for manipulating the genome of Anopheles in the laboratory and for studying the genetic basis of this insect's vectoral capacity.

1317–1323 **Genetic Analysis of Vertebral Regionalization and Number in Medaka (*Oryzias latipes*) Inbred Lines**
Tetsuaki Kimura, Minori Shinya, and Kiyosi Naruse

In this study, the authors performed quantitative trait locus (QTL) analysis on 200 F2 fish to determine vertebral number and the ratio of abdominal vertebrae to vertebral number. Their results show a suggestive QTL of the ratio of abdominal vertebrae to vertebral number on chromosome 15 and 5 QTLs of vertebral number on chromosomes 1, 10, 11, 17, and 23. The authors also demonstrate that the difference in vertebral number between 2 medaka inbred lines is derived from differences in the anteroposterior length of somites. Their results emphasize that the developmental process should be considered in genetic analyses for vertebral number.

1325–1339 **A Window into Domain Amplification Through Piccolo in Teleost Fish**
Michael L. Nonet

Proteins with repeated domains have increased both in frequency and average repeat size during the evolution of vertebrates. However, the mechanisms by which domains are gained, lost, and maintained are poorly understood. Here the authors characterize a case of extensive zinc finger domain expansion via single exon amplification in the piccolo gene of teleost fish. Based on exon sequence relationships, they propose a model for the amplification and maintenance of the repeats. Their work provides a model to study both the functional and evolutionary basis of domain amplification via a combination of experimental studies using zebrafish and WGS of diverse teleost species.

1341–1344 ***Escherichia coli* Lacking RpoS Are Rare in Natural Populations of Non-Pathogens**
Emily Snyder, David M. Gordon, and Daniel M. Stoebel

Studies of natural variation must deal with the potentially confounding effects of laboratory evolution. The RpoS protein of *E. coli*, an alternative sigma factor that coordinates many stress responses, is reported to be non-functional in 20 to 30% of naturally occurring ***E. coli***. However, it is also known that routine laboratory handling can select for mutant strains that lack RpoS function. In this study, the authors report a dearth of polymorphism in RpoS and argue that previous reports of diversity are due to laboratory evolution rather than naturally occurring variation.

1345–1356 **Identification of Genes Required for Alternative Oxidase Production in the *Neurospora crassa* Gene Knockout Library**
Frank E. Nargang, Kelly Adames, Cornelia Rüb, Serena Cheung, Nancy Easton, Cheryl E. Nargang, and Michael S. Chae

Expression of alternative oxidase in *Neurospora crassa* represents a case of retrograde regulation since it is encoded by a nuclear gene that responds to signals from mitochondria. The pathway for induction of the enzyme is not well understood. Here the authors describe the screening of the ***N. crassa*** knockout library for mutants that are unable to express alternative oxidase. Sixty-two new mutants were found that affect production of the enzyme to varying degrees. The results suggest a complex mechanism or mechanisms of expression for alternative oxidase.

1357–1367 **SAGA Complex Components and Acetate Repression in *Aspergillus nidulans***
Paraskevi Georgakopoulos, Robin A. Lockington, and Joan M. Kelly

In one of the first investigations of components of the SAGA complex in *Aspergillus nidulans*, these authors describe the genetic identification and analysis of two genes encoding SAGA components. Little is known of repression of gene expression by acetate. The authors focused on the role of the complex in acetate repression, as this was the basis of the mutant screen that uncovered the Spt3 and Spt8 homologues. Their results have led to a new insight into repression by acetate in this organism.

1369–1377 **A Germline Clone Screen on the *X* Chromosome Reveals Novel Meiotic Mutants in *Drosophila melanogaster***
Kimberly A. Collins, Jonathon G. Callicoat, Cathleen M. Lake, Cailey M. McClurken, Kathryn P. Kohl, and R. Scott Hawley

In the largest screen of X chromosomes in Drosophila female meiosis reported to date, the authors identified 19 new meiotic mutants, which comprise nine complementation groups. Four mutants define two novel complementation groups that demonstrate a strong chromosome nondisjunction phenotype. The authors also identified novel alleles of known meiotic mutants, including *mei-217*, *mei-218*, *mei-9*, and *nod*. They molecularly characterized nine of ten mutants in known meiotic mutants and compared the lesions to previously isolated mutants. The authors estimate that this screen now brings the X chromosome to 95% saturation.

1379–1391 **On the Role of PDZ Domain-Encoding Genes in Drosophila Border Cell Migration**
George Aranjuez, Elizabeth Kudlaty, Michelle S. Longworth, and Jocelyn A. McDonald

Collective cell movement is important in tissue morphogenesis and wound healing, but mechanisms that control this type of migration are poorly defined. Drosophila border cells provide a tractable model for collective cell migration. Many proteins that contain the PSD95/Dlg/ZO-1 (PDZ) domain organize signaling complexes and regulate cell polarity, processes that are important for cell migration. In this study, the authors systematically targeted 64 PDZ domain-containing genes by RNAi knockdown. Of these, 14 PDZ genes identified with multiple RNAi lines affected border cell migration. These genes are expected to yield new insights into the regulation of border cell migration.

1393–1396 **A Forward Genetic Screen Identifies Eukaryotic Translation Initiation Factor 3, Subunit H (eIF3h), as an Enhancer of Variegation in the Mouse**
Lucia Daxinger, Harald Oey, Anwyn Apedaile, Joanne Sutton, Alyson Ashe, and Emma Whitelaw

In this study, the authors used an ENU mutagenesis screen to identify genes involved in transcriptional gene silencing using a GFP transgene reporter in the mouse. They identified translation initiation factor 3, subunit H (eIF3h) and found two independent mutations in this gene. One mutation disrupts splicing, and the other introduces a premature stop codon. Heterozygotes are viable and homozygotes are embryonic lethal. Mice carrying mutations in this gene have not been reported previously and its involvement in epigenetic gene silencing has not been considered.

1397–1403 **Maintaining Sufficient Nanos Is a Critical Function for *Polar Granule Component* in the Specification of Primordial Germ Cells**
Girish Deshpande, Emma Spady, Joe Goodhouse, and Paul Schedl

The authors show that a critical function of the pcg gene in the specification of primordial germ cells in early Drosophila embryos is to maintain sufficient levels of Nos protein.

1405–1413 **Shrinkage Estimation of the Realized Relationship Matrix**
Jeffrey B. Endelman and Jean-Luc Jannink

Most theoretical research on the realized relationship matrix has focused on outbred populations and the concept of identity-by-descent. Recognizing that genetic covariance is fundamentally a state property, the authors present a theoretical framework based on the concept of identity-by-state and rigorously derive estimators for arbitrary populations. They demonstrate that shrinking the estimate of the relationship matrix can improve prediction accuracy with low-density markers, but only for phenotyped individuals. For breeding applications where moderate-accuracy phenotypes are available, shrinkage has the potential to improve genetic gain.

1415–1425 **Large-Scale Screening for Targeted Knockouts in the *Caenorhabditis elegans* Genome**
The *C. elegans* Deletion Mutant Consortium

The thousands of knockouts provided by the authors' laboratories have proven to be a valuable resource within the *C. elegans* research community. Given that there are now over 1,500 papers published by the worm community using these resources, the authors believe it is time to bring direct exposure to the project. Deletion strains in this organism are an enduring community resource, as worm stocks can be frozen and then thawed when needed. Both the actual and potential applications that come from this resource are profound in several arenas, including genetics, genomics, and developmental biology.

1427–1436 **Effectiveness of Genomic Prediction of Maize Hybrid Performance in Different Breeding Populations and Environments**
Vanessa S. Windhausen, Gary N. Atlin, John M. Hickey, Jose Crossa, Jean-Luc Jannink, Mark E. Sorrells, Babu Raman, Jill E. Cairns, Amsal Tarekegne, Kassa Semagn, Yoseph Beyene, Pichet Grudloyma, Frank Technow, Christian Riedelsheimer, and Albrecht E. Melchinger

In this study, the authors examined genomic prediction for testcross performance in maize using two large data sets comprising information of 405 genotypes from 13 breeding populations evaluated in four to six environments. A detailed analysis with different cross validation schemes revealed that prediction accuracy was strongly influenced by differences in the population means. Thus, before using genomic prediction for breeding purposes, a detailed analysis of the population structure is recommended. The authors' results also demonstrate that the usefulness of genomic predictions in maize strongly depends on whether prior information on population structure is available and breeders are interested in performance prediction within or across populations.

1437–1445 **Identification of Gene Expression Changes Associated With Long-Term Memory of Courtship Rejection in Drosophila Males**
Ari Winbush, Danielle Reed, Peter L. Chang, Sergey V. Nuzhdin, Lisa C. Lyons, and Michelle N. Arbeitman

Male fruit flies previously rejected after courtship advances retain memory of this experience and do not court other females with the same vigor as naïve males. The molecular mechanisms underlying this long-term memory have not been elucidated. In this study, the authors subjected male flies to a training regimen resulting in courtship suppression that persisted for at least two days. Using RNA-sequencing, they analyzed gene and transcript-isoform expression 24 hours after training. The results enabled them to identify genes that function in chromatin modification, development, cytoskeletal dynamics, translation, olfactory behaviors, as well as genes previously shown to underlie long-term memory formation in several species.

1447–1457 ***NANOGP8*: Evolution of a Human-Specific Retro-Oncogene**
Daniel J. Fairbanks, Aaron D. Fairbanks, T. Heath Ogden, Glendon J. Parker, and Peter J. Maughan

NANOGP8 is a human retrogene expressed in cancer cells where it promotes tumorigenesis. It originated in the human ancestral lineage approximately 0.9–2.5 million years ago and is present in the Neanderthal genome. *NANOGP8* is fixed, although it originated from a mutant allele of *NANOG* that has remained polymorphic. Expression of *NANOGP8* in cancer cells may be partially explained by its

insertion into the LTR region of an SVA retroelement that may promote its transcription. Some variants utilized in cancer research to distinguish *NANOG* and *NANOGP8* transcripts are polymorphic and unreliable as distinguishing features.

1459–1472 **Evidence for Autoregulation and Cell Signaling Pathway Regulation From Genome-Wide Binding of the *Drosophila* Retinoblastoma Protein**
Pankaj Acharya, Nicolas Negre, John Johnston, Yiliang Wei, Kevin P. White, R. William Henry, and David N. Arnosti

As revealed by gene profiling experiments, the retinoblastoma (RB) tumor suppressor protein functions to transcriptionally regulate numerous targets. Identification of direct targets of RB in cultured cells has been recently accomplished in ChIP experiments. To understand potential tissue and developmental specific activities, the authors performed ChIP-seq of the Drosophila RB protein, Rbf1, in the embryo. Their results indicate that global association of Rbf1 is developmentally regulated, and that in addition to binding to cell-cycle related genes, this corepressor is implicated in regulation of numerous conserved signaling pathways.

CORRIGENDUM

1473 **High-Resolution SNP/CGH Microarrays Reveal the Accumulation of Loss of Heterozygosity in Commonly Used *Candida albicans* Strains**
Darren Abbey, Meleah Hickman, David Gresham, and Judith Berman