Complex Trait Community 12th Annual Meeting, May 28-31, 2013
Memorial Union, UW–Madison, 800 Langdon St, Madison, Wisconsin
ctc2013.org

Schedule Overview

Tuesday, 28 May
5:00 – 7:00pm  Registration and Opening Reception  Tripp Commons

Wednesday, 29 May
7:30 – 8:30am  Registration and Continental Breakfast  Great Hall Foyer
8:30 – 8:45  Welcome message  Great Hall
8:45 – 9:45  Keynote: John Doebley  Great Hall
9:45 – 10:25  Session 1: Resources  Great Hall
10:25 – 11:00  Break  Great Hall Foyer
11:00 – noon  Session 1 (continued)  Great Hall
noon – 1:00  Box lunch  Great Hall Foyer
1:00 – 2:40  Session 2: Systems genetics  Great Hall
2:40 – 3:00  Break  Tripp Commons
3:00 – 5:00  Posters (odd numbers)  Tripp Commons
6:00pm  Social night out  Meet at Great Dane, 123 E Doty St

Thursday, 30 May
8:00 – 8:30am  Continental Breakfast  Great Hall Foyer
8:30 – 9:30  Keynote: Eleanor Feingold  Great Hall
9:30 – 10:10  Session 3: From QTL to gene  Great Hall
10:10 – 10:40  Break  Great Hall Foyer
10:40 – noon  Session 3 (continued)  Great Hall
noon – 2:00  Lunch  Great Hall
2:00 – 3:20  Session 4: Applications of the Collaborative Cross  Great Hall
3:20 – 4:00  Break  Tripp Commons
4:00 – 6:00  Posters (even numbers)  Tripp Commons
6:00 – 7:00  Reception  Tripp Commons

Friday, 31 May
8:00 – 8:30am  Continental Breakfast  Great Hall Foyer
8:30 – 10:00  Panel Discussion  Great Hall
10:00 – 10:20  Break  Great Hall Foyer
10:20 – noon  Session 5: Computational methods  Great Hall
**Wednesday, 29 May**

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<td><em>Genetics of Hippocampal Gene Expression in Diversity Outbred Mice</em></td>
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<td><em>Genomic networks of hybrid sterility</em></td>
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<td>Christian Deschepper</td>
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<td><em>Gene co-expression modules, chromosome domains and cardiac left ventricular mass</em></td>
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**Thursday, 30 May**

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8:30 – 9:30  **Keynote:** Eleanor Feingold  Great Hall
  
  *Chair:* Karl Broman

9:30 – 10:10  **Session 3:** From QTL to gene  Great Hall
  
  *Chair:* Christian Deschepper

  9:30  Cheryl Ackert-Bicknell
  
  Identification of Slc9a9 as a candidate gene for a bone mineral density QTL on mouse chromosome 9

  9:50  Charles Farber
  
  Systems Genetics Identifies Bicc1 as a Novel Genetic Determinant of Bone Mass and Osteoblastogenesis

10:10 – 10:40  Break  Great Hall Foyer

10:40 – noon  **Session 3** (continued)  Great Hall

  10:40  Beverly Mock
  
  Biologically-Integrated Network Analyses Reveal Synergistic Action of a Drug Combination Targeting Cancer Susceptibility Pathways

  11:00  Amanda Henning
  
  Functional Characterization of the Mammary Carcinoma Susceptibility 5c Locus

  11:20  Vivek Kumar
  
  QTL analysis utilizing closely related mouse substrains identifies Cytoplasmic FMRP Interacting Protein 2 (CY-FIP2) as a regulator of cocaine response.

  11:40  Alan Attie
  
  Genetic Identification of Nrjc2 as a Critical Regulator of Pancreatic Islet Function

noon – 2:00  Lunch

2:00 – 3:20  **Session 4:** Applications of the Collaborative Cross  Great Hall
  
  *Chair:* David Threadgill

  2:00  John Didion
  
  A novel meiotic drive system gives rise to transmission ratio distortion in the CC and a selective sweep in the DO

  2:20  Narayanan Raghupathy
  
  Allele Specific Gene Expression in F1 Mice

  2:40  Natalia Gonzales
  
  Replication of GWAS results in mice using the criteria of both human and model organism genetics.

  3:00  David Aylor
  
  Genetic reproductive incompatibilities in the mouse Collaborative Cross

3:20 – 4:00  Break  Tripp Commons

4:00 – 6:00  Posters (even numbers)  Tripp Commons

6:00 – 7:00  Reception  Tripp Commons
Friday, 31 May

8:00 – 8:30am  Continental Breakfast  
Great Hall Foyer

8:30 – 10:00  Panel Discussion  
*Systems genetics: Past, present, and future*  
*Chair:* Bret Payseur  
*Panelists:* Abraham Palmer, William Valdar, Gary Churchill, Jake Lusis

10:00 – 10:20  Break  
Great Hall Foyer

10:20 – noon  Session 5: Computational methods  
*Chair:* William Valdar

10:20  Steven Munger  
*RNA-seq alignment to individualized genomes.*

10:40  Daniel Gatti  
*Quantitative Trait Locus Mapping in Diversity Outbred Mice*

11:00  Anna Tyler  
*Epistatic networks influencing diabetes-related phenotypes in mice*

11:20  Paul Schliekelman  
*Uncovering trans-eQTL Relationships in Gene Networks*

11:40  Karl Broman  
*Interactive graphics for high-dimensional genetic data*
Keynote speakers

**John Doebley, Wednesday, 8:45am**

John Doebley is George W. Beadle WARF Professor of Genetics at the University of Wisconsin–Madison.

Prof. Doebley has transformed our understanding of how maize was domesticated. He has discovered genetic changes responsible for the evolution of maize from its wild ancestor (teosinte), including several specific genes and mutations. Doebley’s research provides a model for the genetic dissection of complex traits and the domestication process. Doebley is a member of the National Academy of Sciences.

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**Eleanor Feingold, Thursday, 8:30am**

Eleanor Feingold is Professor of Human Genetics and Biostatistics and Associate Dean for Education in the Graduate School of Public Health at the University of Pittsburgh.

Prof. Feingold uses statistical genetics to dissect complex disease variation in humans. She is a thoughtful critic and practitioner of genome-wide association studies, and plays leading roles in consortia unraveling the genetics of Down’s syndrome, cancer, dental disease, and meiotic recombination. Feingold is a fellow of the American Statistical Association.
Complex Trait Community 12th Annual Meeting

ctc2013.org

Posters

Odd numbers: Wednesday, 3:00 – 5:00pm
Even numbers: Thursday, 4:00 – 6:00pm

1. Sushant Bhatnagar
   Glucose, cAMP, and phorbol ester-activated cell signaling pathways phosphorylate tomosyn-2 to regulate insulin secretion in pancreatic beta-cells

2. Andrea Bilger
   Identification of the Ifi202b candidate liver cancer modifier by linkage, microarray, and CGH analysis

3. Kari Buck
   Confirmation that Mpdz expression impacts ethanol withdrawal severity and may affect ethanol consumption: Novel MPDZ/Mupp1 transgenic and knockout heterozygote model analyses

4. Melkam Kebede
   Diabetes-Associated SORCS1 Gene Regulates Renal Uptake and Degradation of Insulin

5. Michal Pravenec
   Loss-of-function mutation in endonuclease G (Endog) is associated with metabolic disturbances and oxidative stress in the SHR

6. Jean-Jacques Panthier
   Genetic control of susceptibility to infection with Rift Valley fever virus in mice.

7. David Samuelson
   Rat Mammary carcinoma susceptibility-1b single-nucleotide-variant A074-SNV-17 is a candidate Meso1b quantitative trait nucleotide

8. Amy Hart
   SNPs associated with stronger positive subjective response to d-amphetamine are significantly enriched for SNPs that increase risk for schizophrenia and ADHD.

9. George Sutphin
   A Multi-Organism Approach to Selecting Strong Candidates for Mammalian Longevity Genes

10. Michael Johnson
    Endothelin Signaling Promotes Osteogenesis by Changing the Cellular miRNA Environment Leading to Derepression of WNT Signaling and IGF-1 Induction

11. Reinmar Hager
    Genomic imprinting effects can depend on social environment

12. David Threadgill
    Modeling Genetic Heterogeneity to Understand Susceptibility to Chemical Combinations Using Trichloroethylene (TCE) and Inorganic Arsenic (iAS)
13 Samantha Praktiknjo
Novel effects of chromosome Y on cardiac regulation, chromatin remodeling and neonatal programming

14 Jason Bubier
Effect of genetic diversity of collaborative cross mice on intestinal microbial communities and their association with disease related traits in mice.

15 Laura Sittig

16 Kwangbom Choi
Identifying Significant Molecular Events in Osteoblast Development by RNA-seq Time Course Analysis

17 Shyam Gopalakrishnan
eQTL identification using RNA-Seq analysis of three mouse brain regions

18 Michael Massett
Genetic analysis of exercise capacity and training responses in 129S1/SvImJ and NZW/LacJ mice

19 Nikki Walter
Mitochondrial Respiratory Chain Suprastructure Exhibits Genetic Dependence in Mouse Brain

20 Brittany Baur
Genome-Wide Fine-Mapping of Post-prandial Glucose in Heterogeneous Stock Rats

21 Stephen Flink
Genomic variants in the parental strains of the rat HxB/BxH recombinant inbred panel

22 Musa Hassan
Transcriptional and linkage analyses identify loci that mediate differential macrophage response to inflammatory stimuli and infection

23 Arimantas Lionikas
Dissection of the Genetic Architecture of Musculoskeletal Traits in Collaborative Cross

24 Abraham Palmer
Genome-wide association study of the attribution of incentive salience in outbred Sprague Dawley rats

25 Clarissa Parker
Genome-wide association study of behavior in outbred mice

26 Brian Parks
Genetic Control Of Obesity In Response To High-Fat/High-Sucrose Feeding: A Systems Genetics Study In The Mouse

27 Luanne Peters
Identification of QTL for Hematological Traits in Diversity Outbred Mice
28  Laura Saba  
Identifying transcriptional signatures of brain region-specific volume from whole brain RNA-Seq data

29  Leah Solberg Woods  
Fine-mapping diabetes-related traits within rat chromosome 1 in heterogeneous stock rats

30  Lauren Brooks  
Sources of Natural Selection on Body Size in Island Populations of Wild Mice

31  Melissa Gray  
Evolution of Extreme Body Size in a Natural Population of House Mouse.

32  John Hvala  
Detecting Epistasis Through Analyses of Genomic Ancestry Tracts in Admixed Populations

33  Erica Larson  
Polymorphism for hybrid male sterility during the early stages of speciation in house mice

34  Michelle Parmenter  
The evolution of skeletal variation in an island population of house mouse exhibiting extreme body size

35  Richard Wang  
Variation in meiotic recombination rate between WSB and Gough mice

36  Jean Jaubert  
Wild-derived Mus spretus strains: a resource for genetic dissection of resistance to Plague

37  Jasmin Kristianto  
Role of ECE1 in Mediating Maternal Cardiovascular Adaptation to Pregnancy

38  Christina Zheng  
Splicing Landscape of 8 Founder Strains

39  Howard Dene  
Tools for QTL Analysis in the Mouse Genome Informatics (www.informatics.jax.org) Resource

40  Sue McClatchy  
Independent Studies in Computational Biology: Engaging Talented Youth in Authentic Research

41  Beth Wilmot  
Reading the Whole Transcriptome

42  Greg Carter  
Deciphering Genetic Complexity with the Combined Analysis of Pleiotropy and Epistasis

43  Il-youp Kwak  
Mapping quantitative trait loci underlying function-valued phenotypes.
Jeremy Sabourin
*Haplotype Association Mapping in Multiple Founder Crosses with LASSO-based Resample Model Averaging*

William Valdar
*Genetics of response to drug in the diallel: heritable architecture of adverse reactions to haloperidol a mouse model*
Status of The Diversity Outbred Population

Gary Churchill, Elissa Chesler, Karen Svenson, Marge Strobel and Dan Gatti
The Jackson Laboratory, Bar Harbor, ME 04609

The Diversity Outbred (DO) population is a heterogeneous stock derived from the same eight founder strains as the Collaborative Cross inbred strains. This talk will summarize the status of DO colony, which is in the 14th generation of outbreeding as of May 2013. Several large projects are underway using DO mice to investigate genetics of complex traits. New genotyping resources and analytical tools have been developed to support these studies. Accumulation of recombination events in the DO is tracking predicted rate. However there was a major distortion in the founder allele frequencies driven by a selective sweep of WSB allele on chromosome 2. Corrective action was taken to restore allelic balance in this region. We will present empirical results and simulations to support sample size and other study design decisions. The DO is thriving and ready for widespread adoption by this community.
Access to the Collaborative Cross Population through the UNC-Chapel Hill Distribution Center

Darla R Miller\textsuperscript{1,2}, Catherine E Welsh\textsuperscript{3}, Chen-Ping Fu\textsuperscript{3}, Jeremy Wang\textsuperscript{3}, Katy Kao\textsuperscript{3}, Kenneth F Manly\textsuperscript{1,3}, David W Threadgill\textsuperscript{4}, Leonard McMillan\textsuperscript{3}, and Fernando Pardo-Manuel de Villena\textsuperscript{1,2}

\textsuperscript{1–3}University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 \textsuperscript{1}Department of Genetics \textsuperscript{2}Lineberger Comprehensive Cancer Center \textsuperscript{3}Department of Computer Science \textsuperscript{4}Department of Genetics, North Carolina State University, Raleigh, NC 28695

The Collaborative Cross (CC) is a panel of recombinant inbred lines derived from eight genetically diverse laboratory inbred strains. The CC External Advisory board recommended establishing a small set of CC lines that can be rapidly accessed by researchers for pilot studies, initial phenotyping and/or proof of concept experiments. To that end, we established a set of 12 CC lines (hereby named Tier 1 lines) in which every line has all eight founders represented, the average residual heterozygosity is 7.6\% (range 2.6 to 10.9\%) and the average litter size is 4.1 (range 3.0 – 5.6). Tier 1 includes lines from all three original populations: six from UNC, three from TAU and three from Geniad. These lines have been renamed starting with CC001 and are listed at csbio.unc.edu/CCstatus/index.py? The number and identity of Tier 1 lines will not change. The UNC distribution center has another 34 CC lines that are currently available based on the criteria described previously\textsuperscript{1}. These additional lines consist of 13 from UNC, 4 from TAU and 17 from Geniad. The number of available lines should increase as more lines reach the inbreeding threshold. Here we provide a description of the status of the CC in the US and distribution efforts by the UNC at Chapel Hill Systems Genetics Core. Recent changes in the breeding, husbandry and genotyping will be discussed.

Successful scientific exchange, through publication or discourse, assumes common understanding of the object(s) of study. For biologists, this means clear and unambiguous identity for genes, genetic/genomic variants and mutations, and genetically defined strains. Naming based on laboratory shorthand, field-of-study jargon, or common use continues to impede search effectiveness in repositories and resources where nomenclature standards are not enforced.

Nomenclature committees, such as those for mouse (International Committee on Standardized Genetic Nomenclature for Mice), rat (Rat Genome and Nomenclature Committee), and human (HUGO Gene Nomenclature Committee), work closely to harmonize ortholog naming among these well-studied species. Databases for these and other organisms, including ZFIN (zebrafish), FlyBase (Drosophila), SGD (yeast), and others, provide nomenclature guidance for their respective species.

Whole genome sequencing and assembly refinement over the years have established the number of protein-coding genes in human and mouse at 25-30,000. Discovery of new functional RNAs, a large array of regulatory elements, and highly conserved non-coding regions continues to reveal the complexity of the mammalian genome. Nomenclature continues to adapt to encompass these genomic elements and novel technology-enabled gene and chromosome modifications. Genetic background, particularly defined strains, on which specific mutations, quantitative trait loci (QTL), or transgenes are analyzed, comprises the totality of genetic inputs contributing to specific phenotypes.

We present examples of the benefits of standardized nomenclatures and how curation and application of nomenclature standards facilitate data integration and searching across online resources. Official Nomenclature Guidelines for mouse genes, alleles, chromosomal aberrations and strains are accessible from MGI’s Nomenclature Page (www.informatics.jax.org/nomen).

Supported by NIH grant HG000330
Genetic resources for accelerating understanding of the bread wheat genome

Emma Huang¹, Colin Cavanagh², Rohan Shah¹, Stuart Stephen², Andrew George¹, Matthew Hayden³, Matthew Morell²

¹CSIRO Mathematics, Informatics and Statistics and Food Futures National Research Flagship, ²CSIRO Plant Industry and Food Futures National Research Flagship, ³Department of Primary Industries Victoria

The large size and hexaploid nature of the bread wheat genome have thus far made it difficult to gain a deeper understanding of its structure. However, with advances in sequencing capability, the number of available genetic markers in wheat has increased dramatically in recent years. Now strategies are required to transform these data into knowledge useful for gene discovery. We have been developing two large Multi-parent Advanced Generation Inter-Cross (MAGIC) genetic resource populations to more quickly achieve these goals. We will describe our research in these populations towards developing a high-density genetic map and discuss progress in characterizing the genetic landscape in bread wheat.
The Drosophila Synthetic Population Resource


*Department of Molecular Biosciences, University of Kansas. **Department of Ecology and Evolutionary Biology, University of California, Irvine.

Genetic dissection of complex, polygenic trait variation is a key goal of medical and evolutionary genetics. Attempts to identify variants underlying complex traits have been plagued by low mapping resolution in traditional linkage studies, and an inability to identify variants that cumulatively explain the bulk of standing genetic variation in genomewide association studies (GWAS). We have developed a novel resource for the Drosophila community consisting of two sets of recombinant inbred lines (RILs), each derived from an advanced generation intercross between a different set of eight highly inbred, completely resequenced founders. The Drosophila Synthetic Population Resource (DSPR) has been designed to combine the high mapping resolution offered by multiple generations of recombination, with the high statistical power afforded by a linkage-based design. We have assayed the 1,700 RILs for a range of traits, including drug-resistance phenotypes, mapping a large number of quantitative trait loci (QTL) to small intervals (<0.5Mb). Many of the QTL we identify are rare - the minor allele is unique to a single founder line, and several do not show a simple biallelic pattern, suggesting multiple causative factors may frequently underlie QTL. To further characterize QTL contributing to a single trait - starvation resistance - we dissected the trait using three additional mapping designs. Over 3,000 heterozygous genotypes were generated by crossing pairs of RILs, and by independently backcrossing RILs to two isogenic tester strains. This work revealed a number of novel, potentially background-dependent resistance loci, and a surprisingly complex architecture for this important life history trait.
Genetic regulation of human adipose microRNA expression and its consequences for metabolic traits

Mete Civelek¹, Raffi Hagopian¹, Calvin Pan¹, Nam Che¹, Wen-pin Yang², Paul Kayne², Niyas K. Saleem³, Henna Cederberg³, Johanna Kuusisto³, Peter Gargalovic⁴, Todd Kirchgessner⁴, Markku Laakso³, Aldons J. Lusis¹,⁵,⁶

Departments of ¹Medicine, ⁵Human Genetics, ⁶Microbiology, Immunology & Molecular Genetics, University of California, Los Angeles, CA 90095, USA Departments of ²Applied Genomics, ⁴Cardiovascular Drug Discovery, Bristol-Myers Squibb, Pennington, NJ 08534, USA ³Department of Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland

The genetics of messenger RNA expression has been extensively studied in humans and other organisms, but little is known about genetic factors contributing to microRNA (miRNA) expression. We examined natural variation of miRNA expression in adipose tissue in a population of 200 men who have been carefully characterized for metabolic syndrome phenotypes as part of the METSIM study. We genotyped the subjects using high-density SNP microarrays and quantified the mRNA abundance using genome-wide expression arrays and miRNA abundance using next generation sequencing. We reliably quantified 356 miRNA species that were expressed in human adipose tissue, a limited number of which made up most of the expressed miRNAs. We mapped the miRNA abundance as an expression quantitative trait and determined cis regulation of expression for 9 of the miRNAs and of the processing of one miRNA (miR-28). The degree of genetic variation of miRNA expression was substantially less than that of mRNAs. For the majority of the miRNAs, genetic regulation of expression was independent of the host mRNA transcript expression. We also showed that for 108 miRNAs, mapped reads displayed widespread variation from the canonical sequence. We found a total of 24 miRNAs to be significantly associated with metabolic syndrome traits. We suggest a regulatory role for miR-204-5p which was predicted to inhibit ACACB, a key fatty acid oxidation enzyme that has been shown to play a role in regulating body fat and insulin resistance in adipose tissue.
Genetics of Hippocampal Gene Expression in Diversity Outbred Mice


The Diversity Outbred (DO) mouse population is produced from a pseudorandom intercross of the eight founders of Collaborative Cross (CC) inbred mouse strains. The resulting population has over 45 million segregating SNPs, high-recombinational precision and high behavioral diversity. Initial studies demonstrate that the DO mice provide high-resolution genetic mapping. To examine the genetics of gene expression and behavioral phenotypes, we sequenced the transcriptome from hippocampus of 258 DO mice using Illumina HiSeq 2000, genotyped at over 7500 SNPs across the genome. In the face of high genetic diversity in DO we employed an individualized diploid DO genome for read alignment and expression quantitation. These transcript abundances were subject to expression QTL analysis (eQTL) and combinatorial, genome wide gene co-expression analysis. The eQTL analysis shows that the majority of linkages are local, and that even when conditioning on these major local eQTL effects distal modifiers of expression were rarely detected with the present sample size. Gene co-expression analysis reveals a large number of small clusters relative to those observed in lower resolution populations. These clusters are functionally cohesive and can be related to behavioral phenomena and expression QTLs.
Hybrid dysfunction, a common feature of reproductive barriers between species, is often caused by negative epistasis between loci (Dobzhansky-Muller incompatibilities). Identifying both (or multiple) interaction partners is challenging using traditional genetic approaches, hence little is known about the nature and complexity of hybrid incompatibilities. Male hybrids between recently diverged subspecies of house mice (Mus musculus) often show reduced fertility. Previous studies have identified quantitative trait loci on the X and autosomes that contribute to phenotypes associated with sterility, and several X-autosome interactions. We used a systems genetics approach to identify disruptions in gene networks associated with sterility. We collected genome-wide testis expression data from 305 male F2s from a cross between wild-derived inbred strains of M. musculus musculus and M. m. domesticus. We identified several thousand cis- and trans-acting quantitative trait loci (eQTL) contributing to expression variation. Using a conditional mapping approach, we identified eQTL dependent on interactions between loci, revealing a complex pattern of epistasis. Many trans eQTL cluster into ten ‘hotspots,’ seven of which co-localize with QTL for sterility phenotypes identified in this cross. Functional annotation of transcripts with eQTL provides insights into the biological processes that are disrupted in sterile hybrids and guides prioritization of candidate genes. The systems genetics approach we employed is applicable in a broad range of organisms and we advocate for widespread adoption of a network-centered approach in speciation genetics.
Gene co-expression modules, chromosome domains and cardiac left ventricular mass

M.P. Scott-Boyer, S. Picard and C.F. Deschepper
Institut de recherches cliniques de Montréal (IRCM)

Using a panel of mouse AxB/BxA recombinant strains (RIS), we have previously identified on chr13 Lvm1, a QTL for cardiac left ventricular mass (LVM). After profiling gene expression in hearts from the same RIS and mapping expression QTLs (eQTLs), we found eight physically clustered cis-eQTLs and five trans-eQTLs matching the locus and profile of Lvm1 on chr13. By weighted gene co-expression network analysis, we found 49 gene co-expression modules. For the one correlating best with LVM, we detected a module QTL (mQTL) whose profile also matched that of Lvm1. Genes belonging to that module: 1) originated predominantly from the same chromosome that harbored the mQTL; 2) correlated with LVM in a fashion that was directly proportional to their connectivity; and 3) contained among its most connected genes the above 8 cis-eQTLs and 5 trans-eQTLs. Further analysis revealed that 21/49 modules showed evidence of being driven by a chromosome domain, since: 1) they showed linkage to one main mQTL located on one chromosome contributing a disproportionately high number of genes to the module, and 2) the genomic regions surrounding the peaks of these mQTLs showed enrichment in structural variants and polymorphic transposable elements. Thus, chromosome domains with particular physical properties and functional features constitute one principal component of certain co-expression modules, some of which have a QTL that matches that of a quantitative trait at the same location. The interconnectivity of genes in such modules greatly extends the number of candidate genes to consider as contributors to a phenotype within QTLs.
Comprehensive dissection of a complex genetic interaction

Matthew B. Taylor and Ian M. Ehrenreich
Molecular and Computational Biology Section, Biological Sciences Department, University of Southern California

Genetic interactions are likely an important contributor to complex trait variation in many species. However, because genetic interactions are difficult to detect in traditional mapping studies, general understanding of the types of genetic interactions that can occur remains incomplete. While many studies have detected pairwise genetic interactions, evidence suggests that more complex genetic interactions involving three or more loci can also contribute to traits. By examining segregants from a cross of two yeast strains, we identified a colony phenotype that appears to be entirely determined by a complex genetic interaction. To dissect the genetic basis of the phenotype, we screened nearly 10,000 segregants produced by multiple advanced crossing designs and sequenced 200 segregants with the trait. Based on these data, we identified five loci at which all individuals with the phenotype share the same alleles. We are now working to resolve the interacting loci to causal genes and variants, and are optimistic that this research will shed light on how complex genetic interactions are mediated at the molecular level.
Identification of Slc9a9 as a candidate gene for a bone mineral density QTL on mouse chromosome 9

Cheryl L. Ackert-Bicknell, Dana A. Godfrey, Rong Yuan, Gary A. Churchill, Kwangbom Choi, Matthew A. Hibbs and Daniel M. Gatti

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Bone mineral density (BMD) is a strong predictor of osteoporotic fracture risk and over 80% of the variance in peak bone mass is due to heritable factors. In this study, 588 mice from a reciprocal cross between KK/HJ and PL/J were phenotyped for whole body areal BMD by dual X-ray absorptiometry at 16 weeks of age. Using standard techniques QTL for this phenotype were mapped and 8 highly significant loci were identified, including a locus with a peak at 45.3 cM on Chromosome 9. Loci for BMD had previously been identified in other crosses in this region including: MRL × SJL (42.3 cM), B6 × C3H (44.24 cM) and NZB × SM (46.08 cM). The boundaries of these overlapping QTL were used to define the most likely genomic interval to contain the underlying gene(s). The alleles for SNP in this interval were examined to identify genes wherein all strains contributing the high BMD allele for the QTL shared the same SNP allele and vice versa. Only three SNP within the interval met this allele distribution pattern and all were found within the Slc9a9 gene. This gene codes for one of the many solute transporter transmembrane proteins, but little is known about the function of this gene in particular. The Slc9a9 gene shows abundant expression in osteoblasts, the cell responsible for bone formation and across osteoblastogenesis, expression of this gene increases sharply coincident with mineralization. In sum, Slc9a9 is a strong candidate for a BMD QTL on mouse chromosome 9 and may be associated with osteoblast function.
Osteoporosis is a complex disease of bone fragility that affects millions of individuals in the U.S. Here, we set out to identify novel genes influencing bone mineral density (BMD), a highly heritable osteoporosis-related trait. Specifically, our goal was to identify the gene responsible for Bmd43, a quantitative trait locus (QTL) in the mouse. Bmd43 is located on Chromosome 10 and harbors 351 genes. To narrow this list, we identified genes whose expression was regulated by a local expression QTL (eQTL) that was predicted to be causal for Bmd43. Bicaudal C homolog 1 (Bicc1), a putative RNA-binding protein, was the only gene fitting this criterion. Mice heterozygous for a Bicc1 null allele had decreased femoral BMD and cortical thickness, confirming that Bicc1 dosage influences bone mass. We began to determine how Bicc1 is involved in the regulation of BMD by evaluating its membership in a bone co-expression network. Bicc1 belonged to a co-expression module enriched for genes involved in the differentiation of bone-forming osteoblasts. Consistent with this observation, knockdown of Bicc1 impaired the differentiation of primary osteoblasts. In the co-expression network, Bicc1 was most strongly connected to the polycystic kidney disease 2 (Pkd2) gene and knockdown of Bicc1 decreased Pkd2 transcript levels. Pkd2 knockdown also decreased osteoblastogenesis. Overexpression of Pkd2 in Bicc1 null osteoblasts rescued impaired differentiation, whereas overexpression of Bicc1 in Pkd2 null osteoblasts did not, suggesting that Bicc1 acts upstream of Pkd2. This study identifies Bicc1 as a novel genetic determinant of BMD and osteoblastogenesis, possibly by regulating Pkd2 transcript levels.
**Biologically-Integrated Network Analyses Reveal Synergistic Action of A Drug Combination Targeting Cancer Susceptibility Pathways**

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Synergistic drug combinations have proven effective in cancer therapy. Defining drug synergy at the molecular level has proven challenging, yet, identification of synergy relevant response targets could be used to understand mechanism of combination action and define treatment responsive patient subsets. The combination of entinostat (Class I HDACi) and sirolimus (mTORi) was used to target pathways underlying genetic predispositions identified in a mouse model of myeloma. Weighted gene co-expression network analysis (WGCNA) of gene expression profile data from a human multiple myeloma (MM) cell line treated with single agents and their combination was used to identify a distinct module of 126 genes cooperatively affected by the drug combination; 37 of these genes were found to be differentially expressed in plasma cells from normal donors vs. MM patients, and were predictive of survival by multivariate analyses. Protein and mRNA target validations were performed. Pharmacodynamic responses of the signature to the drug combination were confirmed (Nanostring) in several cell lines from multiple tumor types and in ex vivo-treated primary patient samples before and after treatment. Ingenuity transcription factor (TF) analysis predicted 6 TFs upstream of the 37 responsive genes; CHIP-seq dataset mining confirmed that two TFs bound the promoters of 32 of the 37 genes. Further experiments have been performed to elucidate functional links between the two TFs, the 37 gene signature, and the drug response.
Functional Characterization of the Mammary Carcinoma Susceptibility 5c Locus

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Identifying genetic factors that contribute to breast cancer risk is critical for both risk assessment and the development of new therapeutics. It is thought that many such risk factors are high-frequency, low-penetrant alleles, which are largely found to reside in non-coding regions, making functional studies difficult. Using a rat model of breast cancer, our lab has identified many such loci, including Mammary Carcinoma Susceptibility 5c, (Mcs5c), which was found to decrease tumor multiplicity by 55% in congenic rats possessing the resistant allele. The Mcs5c locus spans 170kb on rat chromosome 5 and lies in a 1Mb gene desert. Mammary gland transplant experiments found that Mcs5c acts in a mammary cell autonomous manner. Expression analysis on surrounding genes indentified PAPPA as a likely target of Mcs5c action. Expression of PAPPA is reduced by 30-50% in the mammary gland of resistant rats at 6, 7, and 9 weeks of age. This represents a critical time during mammary gland development when animals are most susceptible to cancer initiation. Mcs5c may mediate changes in gene expression through an enhancer element that interacts with its target gene via chromatin folding. Chromosome conformation capture was used to detect such folding, and found that Mcs5c interacts with PAPPA at its proximal promoter in 12 week samples. PAPPA is an important regulator of IGF bioavailability and its reduced expression in resistant animals is consistent with a role in tumor reduction. At this time, it appears that Mcs5c mediates tumor resistance via modification of PAPPA expression in the mammary gland.
QTL analysis utilizing closely related mouse substrains identifies Cytoplasmic FMRP Interacting Protein 2 (CYFIP2) as a regulator of cocaine response.

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Identification of QTLs at the single gene or nucleotide level has been difficult due to low mapping resolutions achieved by traditional F2 or N2 mapping approaches. Frequently confounding numbers of polymorphisms within the QTL interval make identification of the causative gene or nucleotide difficult. One alternate approach is to use closely related mouse substrains with high phenotypic but low genotypic variance. Due to high relatedness between substrains, there should be limited polymorphisms within the QTL interval allowing for the identification of the causal gene or even nucleotide. Here we use two C57BL/6 substrains, C57BL/6J from Jackson Labs and C57BL/6N from NIH, to map and clone a QTL for psychostimulant response. We developed a SNP marker panel that can be used to map QTLs between several C57BL/6 substrains including C57BL/6Cr, C57BL/6Tac and C57BL/6NJ. This marker panel was used to genotype the F2 cross and map a single locus mediating cocaine response (LOD 6.4). We sequenced the C57BL/6N genome and discovered a single missense mutation in CYFIP2 within the 1.5 LOD QTL support interval. Biochemical and physiological analysis revealed this mutation destabilizes CYFIP2 leading to changes in dendritic spine morphology shown to regulate behavior. Mice harboring heterozygous gene deletion of Cyfip2 have acute and sensitized cocaine response phenotypes, confirming CYFIP2 as a regulator of psychostimulant response in mammals.

Our data will be of interest to the general mouse community because embryonic stem cells from C57BL/6N are being used for the Mouse Knockout Project. Our approach will be of interest to the QTL community because there are hundreds of mouse substrains, many with documented phenotypic differences, all of which are amenable to the approach we pilot here.
Genetic Identification of Nfatc2 as a Critical Regulator of Pancreatic Islet Function

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We mapped numerous diabetes-related traits in an F2 inter-cross between obese (Lepob/ob) C57BL/6 (diabetes resistant) and obese BTBR (diabetes susceptible) mice. We obtained a strong linkage for plasma insulin to a locus on chromosome 2. We also mapped eQTL traits for 6 tissues (pancreatic islets, liver, adipose tissue, skeletal muscle, kidney and hypothalamus). In islets, the expression of \(~600\) transcripts mapped in trans to the same region on chromosome 2 as plasma insulin. These transcripts included 36 genes previously identified in human GWAS as having an association for T2D. We asked if the islet genes mapping to the chromosome 2 locus were enriched for a motif in their promoter that is consistent with any known transcription factor. The promoter region of 85 genes were enriched for a motif recognized by Nfatc2 \((p<10^{-5})\), which is physically located at the locus. Finally, a formal test for causality \((QTLChr2 \rightarrow Genecis \rightarrow Genetrans)\) identified Nfatc2 as the strongest candidate regulator of the trans-mapping expression traits at the chromosome 2 locus. The overexpression of a constitutively active form of Nfatc2 in mouse islets significantly augmented insulin secretion in response to several insulin secretagogues. In summary, four lines of evidence converged on Nfatc2 as a critical regulator of beta-cell function.
A novel meiotic drive system gives rise to transmission ratio distortion in the CC and a selective sweep in the DO

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Statistically significant departures from the expected Mendelian inheritance ratios (transmission ratio distortion, TRD) have been observed in interspecific crosses in plant and animal species, including mice and humans. A variety of mechanisms may give rise to TRD, including differential viability of embryos or gametes of either sex, and unequal segregation of chromosomes during female meiosis (meiotic drive). TRD can have profound consequences on allele frequencies, especially in closed-breeding groups such as the CC and DO. We used CC and DO genotype data across more than 20 generations to identify and characterize maternal meiotic drive. We observed significant and reproducible TRD in favor of WSB/EiJ alleles within an ~8 Mb region of chromosome 2, approximately 50 cM from the centromere. WSB/EiJ alleles are quickly sweeping the candidate region in the DO; we have implemented a strategy to purge these alleles and recover the diversity at this locus. Analysis of transmission frequency data in the CC, DO and several other crosses has shown that TRD occurs exclusively through the female germine. These results strongly indicate a mechanism of unequal chromatid segregation at the second meiotic division. The reproducibility of the CC and the existence of multiple chromosomes that are recombinant in our region of interest provide us the opportunity to develop a model system in which to characterize the cellular and molecular mechanism underlying meiotic drive. This novel meiotic drive system, along with the select others that are known, suggests that exceptions to Mendel’s First Law are more common than previously thought.
Allele Specific Gene Expression in F1 Mice

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Allele specific gene expression (ASE), the preferential expression of one allele over the other, is an important phenotype of gene expression and regulation. ASE could be due to local genetic variations or due to parent-of-origin effects. In model organisms, recent studies on ASE using RNA-seq technology have shown conflicting degrees and causes. A key challenge in using RNA-seq technology for understanding ASE is accurately quantifying it, as alignment biases can lead to wrong estimate of allele specificity. We present a novel approach EMASE, based on expectation-maximization (EM) algorithm, to accurately quantify ASE from RNA-seq data from F1 cross. Our approach utilizes the genetic variations in the transcriptomes of the parental strains and uses RNA-seq alignments to both the parental transcriptomes to accurately estimate ASE. To understand the extent of allele specific expression due to local genetics and imprinting, and effect of diet and age on ASE, we crossed two divergent inbred mouse strains NOD/ShiLtJ and PWK/PhJ in both directions under two diets; vitamin D and Methionine, and age groups, with 6 biological replicates. We sequenced the liver mRNA samples using Illumina GAIIx. Our results show that ASE is prevalent across the genome. The primary driver of ASE is local genotype. Most of the genes showing parent-of-origin effect are known imprinted genes. Methionine diet had a strong effect on ASE, while the vitamin D diet had little effect on ASE.
Replication of GWAS results in mice using the criteria of both human and model organism genetics.

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Replication of genome-wide association studies (GWAS) is considered a necessary step in the field of human genetics. Replication is defined as observing the same significant association in at least two independent populations. In model organisms replication can be accomplished using the same standard or by directly manipulating the implicated gene and observing the effects on the relevant phenotype.

We have previously performed a GWAS using an advanced intercross line (AIL) that was produced by randomly mating the progeny from an F2 cross between LG/J × SM/J mice until the F34 generation. Because of additional recombinations that accumulated during the intervening generations, the F34 AILs provided much better QTL mapping resolution as compared to the corresponding F2 cross. Using an approach that accounted for relatedness in the F34 we mapped QTLs for various behavioral and physiological phenotypes to sub-centiMorgan intervals. In particular we identified a highly significant \( \text{(P < } 10^{-10}) \) QTL for locomotor activity in a novel environment that contained just one gene: \textit{Csmd1}. However, we have not previously attempted to replicate that finding.

In an effort to fully exploit the advantages of our model system, we have now performed both possible replication studies: we have established that the same association is observed in mice from the F39-F43 AIL generations, and we have also obtained \textit{Csmd1} null mutant mice and demonstrated the null allele recapitulates the phenotype. Thus, we have replicated our previous GWAS finding using two complimentary approaches, which together reflect the power of a model system. Interestingly, since our original identification of \textit{Csmd1} in mice, it was identified as one of 5 genome-wide significant GWAS results for schizophrenia by the psychiatric genetics consortium, which reflects the translational potential of our system.
The Collaborative Cross (CC) project is creating hundreds of new inbred laboratory mouse strains. Each new strain descends from eight existing inbred strains, which among them capture an extraordinary amount of genetic diversity and represent three distinct mouse subspecies. Over three-quarters of incipient lines cease to produce offspring during inbreeding. We expect this observation to be explained by epistatic incompatibilities between subspecies, consistent with the Dobzhansky-Muller hypothesis. To assess the genetic basis and mechanism of these incompatibilities, we measured fertility and reproductive parameters in male mice from 358 independent extinct CC lines. Our fertility testing results indicated multiple causes of extinct lines, with male infertility accounting for almost half (46%). We mapped novel loci associated with fertility, sperm count variation, variation in testis weight, and sperm quality.
RNA-seq alignment to individualized genomes.

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Genetic variation that deviates from a reference genome sequence can create biases in the alignment of short sequencing reads and can distort transcript abundance estimates in RNA sequencing (RNA-seq). Error tolerant alignment does not correct this problem. We hypothesized that alignment of sequence reads to the imputed genomes of individuals (individualized genomes) from which tissue samples were obtained would reduce alignment errors and improve transcript quantification. We developed a method, implemented as the software package Seqnature, that constructs individualized genomes of experimental model organisms including inbred mouse strains and genetically unique outbred animals. We show that alignment of mouse liver RNA-seq reads to individualized genomes increases read mapping accuracy and improves transcript abundance estimates. In an application to expression QTL mapping in a large population of genetically heterogeneous Diversity Outbred (DO) mice, this approach corrected erroneous linkages and unmasked thousands of hidden local associations. Individualized genomes can be extended to human short-read sequencing and other sequencing applications including ChIP-seq.
Quantitative Trait Locus Mapping in Diversity Outbred Mice

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The search for genes underlying complex phenotypes has been greatly aided by genetic mapping in the mouse. Traditionally, mapping has been carried out in two parent intercrosses with limited mapping resolution. Relatively few of these studies have led directly to the discovery of a gene that regulates the phenotype of interest. In order to improve mapping resolution, advanced intercrosses and multi-founder crosses have been developed. Diversity Outbred (DO) mice were developed to overcome these limitations by combining high genetic diversity and fine recombination block structure in order to increase the chances of mapping a phenotype to a small region. The DO mice are derived from the same set of eight founder strains as the Collaborative Cross and are maintained as an outbred population. The task of reconstructing DO genomes and mapping requires specialized methods and software. Here, we describe software uses a hidden Markov model to provide a probabilistic reconstruction of individual DO genomes from intensity based analysis of genotyping microarray data. Genotype probabilities are used to map phenotypes in a mixed model that adjusts for the kinship among DO mice. The model outputs additive effects for each founder allele that can be used to reduce the number of candidate genes under a mapping peak. We provide a complete analytical pipeline, implemented as a freely available R package, to go from phenotypes and genotypes to candidate gene list.
Epistatic networks influencing diabetes-related phenotypes in mice

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Genetic interactions influencing complex traits are typically weaker than single-locus main effects and can be difficult to detect even in populations sufficiently powered to detect main effects. Moreover, partial correlation of traits often confounds interpretation of epistatic interactions. Here we present a method to infer and interpret epistasis in the context of partially correlated complex traits. This method, the Combined Analysis of Pleiotropy and Epistasis (CAPE), increases power to detect interactions by leveraging complementary information across related traits. CAPE infers interactions as directed influences between genetic variants. Positive influences, indicating enhancement, and negative influences, indicating suppression, link gene variants together in a network that predicts intuitively how variants interact to affect traits. Here we demonstrate the capabilities of CAPE by reanalyzing a previously published study of obesity and diabetes. The original analysis examined a reciprocal backcross between non-obese diabetic (NOD) mice and New Zealand obese (NZO) mice to discover main-effect and epistatic QTL affecting traits associated with obesity-related diabetes. Here we show that we have increased power to infer an epistatic network influencing multiple diabetes-related traits. In addition to QTL associated with the coincidence of high plasma glucose levels and obesity, we find QTL involved in the divergence between plasma glucose levels and obesity. We also find that maternal effects modify a subset of QTL associated with body weight, plasma glucose and insulin levels.
Uncovering trans-eQTL Relationships in Gene Networks

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System genetics approaches are a key strategy for uncovering the genetics of complex traits. However, we still have limited understanding of how genetic information is transmitted through gene networks to complex traits. One key obstacle is the difficulty in identifying trans-eQTL relationships. We can readily identify cis relationships, but it has proven difficult to trace the influence of genetic variants deeper into gene networks than their cis gene. Low power to detect trans effects is presumably a major factor. The very large multiple testing correction inherent in eQTL mapping makes eQTL effects only be detectable at rather large effect sizes. In this talk, we demonstrate an approach for reducing the multiple testing burden and thereby increasing power for mapping trans eQTLs. We follow a two-part strategy that is based on the hypothesis that cis effects for one gene will often be trans effects for genes that interact with that gene in gene networks. Consider a set of genes that have been clustering into co-expression modules. First, we conduct eQTL mapping of each gene only to its cis markers in order to identify cis effects. Next, each gene is then mapped against only markers that were found to have significant cis effects for other genes in the same module. This greatly reduces the multiple testing burden and focuses the tests on markers most likely to have trans effects. Using a mouse obesity data set, we show that this approach greatly increases the number of trans-eQTL effects detected. Many co-expression modules are found to have large number of trans-eQTL relationships. We give an overview of the trans relationships thus uncovered. The most common pattern is that gene modules contain large numbers of genes with reciprocal trans-eQTL relationships.
Interactive graphics for high-dimensional genetic data

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The value of interactive, dynamic graphics for making sense of high-dimensional data has long been appreciated but is still not in routine use. I will describe my efforts to develop interactive graphical tools for applications in genetics, using JavaScript and D3. I will focus on an expression genetics experiment in the mouse, with gene expression microarray data on each of six tissues, plus high-density genotype data, in each of 500 mice. I argue that in research with such data, precise statistical inference is not so important as data visualization.
Glucose, cAMP, and phorbol ester-activated cell signaling pathways phosphorylate tomosyn-2 to regulate insulin secretion in pancreatic beta-cells

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We previously mapped a type 2 diabetes (T2D) locus on chromosome 16 (Chr 16) in an F2 intercross between the diabetes susceptible BTBR T (+) tf (BTBR) Lepob/ob and diabetes resistant C57BL/6 (B6) Lepob/ob mouse strains. Using a panel of sub-congenic mice, T2D locus was narrowed to a 1.6 Mb region. The congenic mice containing 1.6 Mb fragment of the BTBR Chr 16 into lean B6 mice (B6.16BT36–38) were hyperglycemic and hypoinsulinemic, and islets from these mice were defective in insulin secretion compared to islets from B6 mice. Within this region, we identified that a non-synonymous coding single nucleotide polymorphism (SNP) in the syntaxin-binding protein 5-like (Stxbp5l or tomosyn-2) was associated with the hyperglycemia and hypoinsulinemia in the B6.16BT36–38 mice. Our results showed that tomosyn-2 is a negative regulator of insulin secretion and that the SNP in tomosyn-2 gene affects its proteasomal degradation. Tomosyn-2 is relatively uncharacterized protein and contains a syntaxin-binding domain; syntaxin is a member of the SNARE complex that regulates insulin secretion. Preliminary results show that tomosyn-2 is phosphorylated and degraded by glucose-, cAMP-, and phorbol esters-activated signaling pathways in pancreatic β-cell line. Furthermore, by using mass spectrometry and molecular biology approaches we have identified an E3 ligase, Hrd1, which binds and regulates the abundance of tomosyn-2. These results led us to hypothesize that phosphorylation of tomosyn-2 regulates insulin secretion by modulating its protein abundance. Herein, we propose that alterations in tomosyn-2 phosphorylation will lead to inappropriate insulin secretion from the beta-cells. This will result in increased susceptibility towards T2D and can also lead to potentially life threatening hypoglycemia during fasting.
Identification of the Ifi202b candidate liver cancer modifier by linkage, microarray, and CGH analysis

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The mouse Hcs7 liver cancer susceptibility locus on distal Chr 1 differentially affects tumor growth in the susceptible C3H/HeJ (C3H) and resistant C57BL/6J (B6) strains. The C3H allele of Hcs7 confers an approximately five-fold increased susceptibility to carcinogen-induced HCC, as well as increased susceptibility to spontaneous HCC. Hcs7 does not appear to affect apoptosis or mitosis during initiation, but by 16 weeks preneoplastic lesions in mice carrying the C3H allele are 3.4-fold larger than those in B6. Hcs7 maps to a 3.3 Mb region that carries 44 genes, of which many respond to immune stimuli.

We have identified a strong candidate for the Hcs7 susceptibility locus: the interferon-inducible gene Ifi202b. Ifi202b is upregulated by androgen and downregulated by estrogen, as is susceptibility to HCC. The Hcs7 locus, including Ifi202b, is orthologous to parts of human chromosome 1q23 and 1q43 that are amplified in ~70% of human liver tumors. Human IFI16 is a candidate for the human susceptibility locus: its transcripts closely resemble those of Ifi202b, it is upregulated by androgen, and it is expressed in growing liver cells.

Ifi202b expression in the liver is 50-fold higher in the C3H strain (by microarray and qRTPCR) than in the B6 strain. The CBA/J strain, also highly susceptible to liver tumorigenesis due to a locus on distal Chr 1, likewise expresses high levels of Ifi202b. Importantly, a de novo mutation in Ifi202b in a B6.C3H congenic line correlates with this line’s unexpected resistance to HCC and with a 50% reduction in Ifi202b expression.
Confirmation that Mpdz expression impacts ethanol withdrawal severity and may affect ethanol consumption: Novel MPDZ/Mupp1 transgenic and knockout heterozygote model analyses


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Previously, we identified a QTL accounting for ~26% of the genetic variance in ethanol withdrawal (EW) convulsions in mice. Positional cloning narrowed this to a 1.8 Mb interval syntenic with human 9p24-p22.3; resident gene analyses identified allelic variation in Mpdz, resulting in different multiple PDZ domain protein (MPDZ/Mupp1) expression as potentially causal (Nat Neurosci 7:699, 2004), but rigorous testing of this hypothesis has been lacking due to the dearth of targeted genetic models. Toward this end, we created Mpdz transgenic (MPDZ-TG, DBA/2 background) and knockout heterozygote (Mpdz+/-, C57BL/6 background) models. QPCR confirmed that target expression is reduced by 47% in Mpdz+/- brain, and increased 2.9-fold in MPDZ-TG, compared to wildtype littermates (WT). Neither baseline (pre-ethanol) nor PTZ enhanced handling-induced convulsion (HIC) scores differ between WT and their respective model, demonstrating that Mpdz does not affect seizure susceptibility in general. As predicted, EW scores were lower in MPDZ-TG than WT (6.2±0.7 and 9.6±1.2), and higher in Mpdz+/- than WT (3.9±0.7 and 1.9±0.6) (p<0.03). Thus, varying Mpdz gene dosage regulates EW, with an inverse relationship between Mpdz expression and EW severity. The strengths of the transgenic approach complement the limitations of the knockout approach, and vice versa, so both supporting MPDZ’s role in EW is compelling. Mpdz+/- consume less 6%, 10% and 20% ethanol (p<0.05) than WT, but do not differ in water consumption across ethanol self-administration (ES) days. These are the first data to implicate Mpdz in ES and the genetic relationship between EW and ES.
Diabetes-Associated SORCS1 Gene Regulates Renal Uptake and Degradation of Insulin

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We positionally cloned SorCS1 as the gene underlying a QTL on mice chromosome 19 that affects fasting insulin levels. Human genome-wide association studies (GWAS) and linkage studies have shown that SORCS1 is associated with human diabetes. The Diabetes Control and Complications Trial conducted a large GWAS and found that SORCS1 was strongly associated with hemoglobinA1c in type 1 diabetic subjects injecting insulin. There was also weak association with diabetes complications; neuropathy, retinopathy, and nephropathy. SorCS1 is expressed in the brain, spinal cord, retina, pancreatic islets and kidneys. To identify the direct role of SorCS1 gene in diabetes susceptibility and complications, we generated a whole-body SorCS1 knockout (KO) mouse on a C57BL/6 background. Female SorCS1 KO mice have increase fasting plasma c-peptide to insulin ratio, suggesting a higher insulin turnover rate. Furthermore, when mice were injected with radiolabelled insulin (125-I-Insulin), it disappeared more rapidly from the circulation in the SorCS1 KO mice. Since SorCS1 is expressed in the kidney and not the liver, we hypothesized that the SorCS1 protein plays a role in renal insulin degradation. In order to test this hypothesis, insulin was coupled with a ‘trapped’ label: 125I-tyramine-cellobiose (125I-TC) and injected intravenously. As expected, liver uptake of insulin was not different between the genotypes. However, 125I-TC-insulin uptake into the kidney of SorCS1 KO mice increased two-fold. Therefore, our data demonstrate that Sorcs1 plays a role in renal uptake and degradation of insulin. Studies are now underway to investigate the role of SORCS1 in insulin uptake into kidney proximal tubules.
Loss-of-function mutation in endonuclease G (Endog) is associated with metabolic disturbances and oxidative stress in the SHR

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Recently, loss-of-function mutation in endonuclease G (Endog) has been identified as a genetic determinant of increased left ventricular mass and impaired mitochondrial function in the spontaneously hypertensive rat (SHR). In the current study, we analyzed the effects of mutant Endog on parameters of lipid and glucose metabolism, oxidative stress, and mitochondrial function in the SHR vs. SHR.BN congenic strain with wild type Endog. Four month old SHR males compared to age-matched SHR-Endog congenic rats exhibited changes in mitochondrial membrane potential and respiratory control index (RCI) in left ventricles indicating lower efficiency of oxidative phosphorylation. The SHR showed increased adiposity (relative weight of epididymal fat, ectopic fat accumulation in liver and heart), and higher serum triglyceride and NEFA levels. Adipose and muscle tissues in the SHR were resistant to insulin action when compared to congenic rats. In addition, SHR had significantly reduced palmitate oxidation in brown adipose tissue. These metabolic disturbances were associated with significantly increased oxidative stress when activities of antioxidant enzymes were reduced and concentrations of lipoperoxidation products were increased in SHR hearts and kidneys when compared to congenic rats. These results provide compelling evidence that mutant Endog is a novel determinant of metabolic disturbances in the SHR.
Genetic control of susceptibility to infection with Rift Valley fever virus in mice.

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Infection of Rift Valley fever (RVF) virus can cause mild to severe diseases in humans and animals. Host genetic determinants seem to play an important role in modeling RVF outcome. At present, it is impossible to dissect host genetic determinants of RVF severity in humans as no one has access to the large numbers of cases that would be needed to perform a genome-wide association study. This also held true for livestock. Studies in livestock are also problematic because domestic animals do not share the same environment thus the analysis of the susceptibility of livestock to RVF are influenced to some degree by aspects of the environment that cannot be controlled by the investigator. The use of the mouse seems particularly advantageous in this context. We previously demonstrated that MBT/Pas mice (Mus m. musculus) are highly susceptible to RVFV and die within 4 days post-infection (dpi), whereas BALB/cByJ mice survive on average for 7 dpi, indicating the existence of host genetic determinants to infection with the RVF virus (RVFV) (do Valle et al., J Immunol., 2010). A linkage analysis with a MBT/Pas × BALB/cByJ intercross infected with the virulent RVFV ZH548 strain found three QTLs associated with survival time. The loci were named Rvfs (Rift Valley fever susceptible locus)\textsuperscript{-1}, -2 and -3 on chromosome 2, 11 and 5, respectively. To test their contribution to susceptibility, Rvfs loci from MBT/Pas were introgressed into the BALB/cByJ background. C.MBT-Rvfs congenic mice were challenged with RVFV; they exhibited significantly shorter survival times in one or both of the two genders. RVFV was detected at 3 dpi in both C.MBT-Rvfs\textsuperscript{2} and BALB/cByJ mice. However, higher peaks of viral RNA load were observed in whole blood from C.MBT-Rvfs\textsuperscript{2} than in BALB/cByJ mice. To narrow the candidate gene list, we performed microarray analysis in macrophages from C.MBT-Rvfs\textsuperscript{2} and BALB/cByJ mice, and searched for non-synonymous SNPs or Indels in MBT/Pas exome. Combining these different approaches will allow us to prioritize candidate genes for Rvfs\textsuperscript{2}. 
Rat Mammary carcinoma susceptibility-1b single-nucleotide-variant A074-SNV-17 is a candidate Mcs1b quantitative trait nucleotide

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Mammary carcinoma susceptibility-1b (Mcs1b) is a mammary-gland cell-autonomous ortholog of a human genome-wide association-study identified breast cancer risk locus at 5q11.2. Risk associated genetic variation is located in non-protein coding DNA for these concordant loci. Mesoderm induction early response 1, family member 3 (Mier3) is an Mcs1b candidate transcript exhibiting differential levels between cancer susceptible and resistant mammary glands. We hypothesize that Mcs1b is one or more noncoding genetic variants that function as gene regulatory elements. Targeted sequence capture and massively parallel sequencing of Mcs1b resistant and susceptible strain alleles was used to identify 70 single nucleotide variants (SNVs) and 2 insertion-deletions (INDELs) as potential quantitative trait nucleotides (QTNs). Sixty-seven SNVs and one INDEL were ruled out as independently acting susceptibility QTNs by genotyping congenic lines that were previously determined to not contain Mcs1b, but had either proximal or distal donor-strain alleles that bordered Mcs1b. To determine if SNVs in Mcs1b were regulatory elements we used luciferase reporter assays. Each SNV plus flanking sequences was independently cloned into pGL3-Promoter and transiently transfected into T47-D breast cancer cells. This epithelial-like cell line expresses endogenous MIER3. Immunohistochemical staining of mammary glands revealed that Mier3 protein was present in ductal epithelial cells. One SNV named A074-SNV-17 resulted in luciferase activities similar to differential mammary gland expression profiles between cancer susceptible and Mcs1b resistant genotypes. Oligonucleotides containing A074-SNV-17 sequence bound nuclear proteins. Mass spectrometry will be used to identify proteins that might bind to this SNV. In conclusion, A074-SNV-17 is a candidate Mcs1b QTN.
SNPs associated with stronger positive subjective response to d-amphetamine are significantly enriched for SNPs that increase risk for schizophrenia and ADHD.

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Genome-wide association studies (GWAS) provide an unbiased approach to investigating the contribution of genetic variation throughout the genome to a phenotype of interest. However, an underlying assumption of GWAS is that all variants (single nucleotide polymorphisms, or SNPs) are equally likely to have an effect on a phenotype, despite the fact that most SNPs do not have functional consequences. We previously performed a GWAS for the acute response to d-amphetamine in 381 healthy human volunteers. In an effort to interrogate the numerous non-significant associations we identified, we sought to identify enrichment of functional classes of SNPs among our top GWAS associations with response to d-amphetamine. We found that SNPs with modestly low P-values (P<0.01) for response to d-amphetamine were significantly more likely than random to have similarly low P-values for schizophrenia and Attention Deficit Hyperactivity Disorder (ADHD). Furthermore, the source of this enrichment was due to an excess of alleles that increased sensitivity to the subjectively positive effects of d-amphetamine and that also increased risk for these diseases. We found no enrichment for negative control phenotypes such as height and inflammatory bowel disease. These results suggest that alleles identified using an acute challenge with a dopaminergic drug can be used to identify alleles that confer risk for psychiatric diseases that are associated with dopaminergic abnormalities. Moreover, they demonstrate the utility of the enrichment approach as an alternative to stringent standards for genome-wide significance.
A Multi-Organism Approach to Selecting Strong Candidates for Mammalian Longevity Genes

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The search for genetic factors involved aging has identified hundreds of genes for which altered expression is capable of increasing life span in one or more model organisms. As the first pharmacological agents targeting these genes begin to be translated into clinical trials for treatment of age-associated disease, it will be useful to prioritize potential clinical targets from the growing list of candidate aging factors that are likely to influence longevity in mammals. We have devised a candidate-gene approach to combine recent genomic methods in mammals with the powerful genetic tools available in invertebrates to identify evolutionarily conserved longevity genes with a high likelihood of impacting mammalian aging. An initial list of longevity-associated genes was selected based on a meta-analysis of human and mouse genome-wide association studies. Orthologs of each gene were then selected in both Caenorhabditis elegans and Saccharomyces cerevisiae. A screen is currently underway to determine whether RNAi knockdown or deletion of each ortholog increases life span in worms or replicative life span in yeast. In cases where life span extension is observed, knockdown of the ortholog will be combined with knockdown of genes in commonly studied aging pathways to look for epistatic interaction. Interesting candidates will be carried forward for longevity studies in mice. Here we provide a detailed description of our screening strategy and report preliminary results.
Endothelin Signaling Promotes Osteogenesis by Changing the Cellular miRNA Environment Leading to Derepression of WNT Signaling and IGF-1 Induction

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Endothelin (ET1) promotes the growth of osteoblastic breast and prostate cancer metastases, previously shown to be due in part to derepression of WNT signaling. Conversion of big ET1 to mature ET1, catalyzed by endothelin converting enzyme 1 (ECE1), is necessary for ET1 activity. We exposed TMOb osteoblasts to 25 ng/ml big ET1 for 6 days in growth medium and for an additional 15 days in mineralization medium. Cells and conditioned media were harvested every three days. TMOb cells exposed to big ET showed greater mineralization than control cells (N 6, p 0.008). The difference was specific to ET1 signaling, as it was blocked by inhibition of ECE1 or endothelin receptor A. Ece1 mRNA expression showed no change over the course of mineralization, ET1 was repressed and endothelin receptor A was induced. Addition of big ET1 repressed expression of all three genes. We measured mRNA levels of genes involved in the ET1 signaling axis, production of paracrine factors involved in osteogenesis, and miRNA expression. IGF-1 levels were significantly (1.3-1.8X) higher over time in the presence of big ET (p<0.001). Big ET1 repressed anti-osteogenic miRNAs, while miRNAs that target proteins involved in bone catabolism were induced by big ET1 exposure. Modulation of WNT signaling could not fully account for ET1’s osteogenic effects, as big ET1 produced a greater mineralization than treatment with LiCl. Moreover, our data suggest that ET1’s osteogenic effects are mediated by changes in the miRNA environment and IGF-1 induction, previously unrecognized ET1 osteogenic mechanisms.
Genomic imprinting effects can depend on social environment

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Epigenetic effects are increasingly recognized as an important source of phenotypic variation in addition to genetic and environmental factors. Among epigenetic effects, genomic imprinting, resulting in parent-of-origin-dependent gene expression, is among the best studied of epigenetic effects. While interactions between environment and genetic variation have been amply demonstrated very little is known about the degree to which genomic imprinting effects can be modulated by the environment, in particular the social environment given by siblings and mothers. Is it possible that patterns of imprinted gene expression depend on the number of siblings and on the type of mother? To address this question, we studied an intercross between the Large and Small mouse strains using a cross-fostering design in which mouse pups were nursed by either their own or an unrelated mother. We scanned the entire genome to search for imprinted quantitative trait loci whose effects on body weight depend on cross-fostering and detected four of such loci. Further, we demonstrate that effects of sibling number on body weight depend on individual genotype at seven loci, over and above the general negative litter size effect. Overall, these litter size by genotype interactions considerably modified the degree to which increasing litter size caused reduced weight but only one imprinted locus showed an interaction effect. Our results demonstrate that genomic imprinting effects may often be modified by the maternal environment and that such interactions can impact key fitness-related traits suggesting a greater plasticity of genomic imprinting than previously assumed. However, imprinting effects on weight that depend on the number of competitors are small. Thus, we conclude that part of the way in which offspring development is affected by maternal environment may be through modification of imprinted gene expression but the social environment provided by siblings does not have effects of similar magnitude.
Modeling Genetic Heterogeneity to Understand Susceptibility to Chemical Combinations Using Trichloroethylene (TCE) and Inorganic Arsenic (iAS)

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Recent epidemiology data suggests that exposure to trichloroethylene (TCE) is associated with increased susceptibility to a variety of diseases including elevated incidence of kidney cancer. To investigate genetic susceptibility to TCE, we exposed a genetically diverse mouse population to two dose levels of TCE with or without two dose levels of inorganic arsenic (iAS), a toxicant naturally found in the environment and in contaminated food sources. We observed cooperation between TCE and iAS in the induction of a gene expression signature in kidneys indicative of a tumor promoted environment. After one year of exposure, subsets of mice developed kidney cancers, some of which became metastatic to the lung. The results demonstrate that these chemicals can elicit additive and synergistic toxicities in susceptible individuals. Furthermore, the target organs in the genetically heterogeneous mouse populations are similar to those in humans, indicating that susceptibility alleles in the mouse population model will inform on human susceptibility. Genetic analysis of the exposed mouse population should reveal alleles contributing to susceptibility in sensitive individuals.
Novel effects of chromosome Y on cardiac regulation, chromatin remodeling and neonatal programming

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Little is known about the functions of chromosome Y (chrY) genes beyond their effects on sex and reproduction. We compared C57BL/6J mice to consomic C57.YA counterparts where chrY originates from A/J. In adult hearts, testosterone affected the size of cardiomyocytes in C57BL/6J, but not in C57.YA. In neonatal pups, anogenital distances showed that testosterone exposure was stronger in C57BL/6J than in C57.YA, but when testosterone was antagonized in fetal C56BL/6J, testosterone no longer affected the size of adult cardiomyocytes. Since the amounts of androgens produced by fetal testes were not different in the two strains, we tested whether strain-specific differences in the programming effect of perinatal testosterone could result from epigenetic differences: we studied the distribution of androgen receptors (AR) in cardiac chromatin by ChIP-Seq, and that of accessible chromatin regions by FAIRE-Seq. Both in neonatal and adult hearts, AR ChIP peaks: 1) were mostly strain-specific; 2) were validated (as they showed significant enrichment for consensus AR binding sites); and 3) contained genes that were most significantly enriched for GO or KEGG terms corresponding to heart development or hypertrophy. In neonatal hearts, we also found (by FAIRE-Seq) strain-specific differences for chromatin in open conformation. Gene expression profiling revealed that two chrY-encoded histone demethylases (Uty and Kdm5d) showed strain-specific expression around birth time. Altogether, the effects of chrY on adult cardiac phenotypes appear to result from an interaction of chrY with the organizational programming effects of perinatal testosterone, and show several characteristics of being mediated by an epigenetic remodeling of chromatin.
Effect of genetic diversity of collaborative cross mice on intestinal microbial communities and their association with disease related traits in mice.

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Many human gastrointestinal disorders including Crohn’s disease, peptic ulcer formation, and irritable bowel syndrome (IBD) have been associated with the presence, absence or over-abundance of various normal microbiota. In addition, the connection between the gut, the brain and behavior is becoming increasingly apparent. Mice possess microbial populations similar to those of humans at high levels of taxa, The host genetic diversity is an important selection on the environmental habitat of the microbiota. Together the genetic diversity and the microbial diversity may contribute to the complex heterogeneity of diseases seen in the host pathophysiology. To assess the relation between host genetic variation and microbial population composition across the whole genome and microbiome, we sampled intestinal mRNA and luminal microflora from of the inbreeding funnels of the Collaborative Cross. In addition, for these same mice, we acquired various metabolic phenotypes and basal behavioral measures that were correlated to the genetic diversity of the population, the gene co-expression networks in the gut and the individual microbiomes. Intestinal gene expression QTLs and microbial abundance QTL were mapped. In addition, microbe to gene co-expression was analyzed and integrated with GeneWeaver.org. Using the integrated online tools, the networks of microbe and gene co-expression sets were examined to identify molecular relations among various biological processes and various disease states. For example one co-expression group containing the genes associated with three different families from the Clostridiales and Bacteroidales orders is similar to a group of differentially expressed mucosal genes in IBD patients. Integrative analyses of these data reveal the relation among microbial diversity and host genetic variation.

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There are numerous well-documented phenotypic differences among closely related mouse substrains. Since there are relatively few genetic differences between these strains, identification of causal alleles is somewhat analogous to identification of de novo mutations in humans. NextGen sequencing technologies in conjunction with linkage mapping can in principal be used to rapidly identify the casual alleles. We sought to apply this approach to two substrains that have been extensively phenotyped over the years: BALB/cJ and BALB/cByJ. These strains were separated in 1935 when some BALB/cJ stock was moved to NIH and maintained independently, resulting in the BALB/cByJ substrain. Random mutation events are presumed to be the only source of genetic differences between these strains. We reviewed all previously reported genetic differences between these strains and attempted to replicate them in inbred BALB/cJ and BALB/cByJ mice. We also performed whole-genome re-sequencing on both strains. Surprisingly, for most of the traits examined we found either no difference between the strains or that the direction of the difference was opposite to what had been reported previously. Of particular interest, the robust difference in aggression was not confirmed. We performed genotyping for a known CNV to confirm that the strain identities were correct. Our results are similar to those of another group that has observed inconsistent differences in brain morphology between these two substrains. We conclude that the causal genetic differences may not have reached fixation or that there may be environmental or gene by environment interactions that negate the apparent simplicity of this model.
Identifying Significant Molecular Events in Osteoblast Development by RNA-seq Time Course Analysis

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Osteoblasts are mononuclear cells that differentiate from mesenchymal stem cells (MSCs). They are responsible for the formation and the mineralization of the osteoid matrix to produce healthy bone tissue. Understanding the genetic and molecular bases of normal osteoblast development is a key for developing new anabolic treatments for osteoporosis.

To investigate the maturation process of osteoblasts, we generated a time course RNA-seq dataset at nine equally-spaced time points from purified murine MSC samples. The primary cell cultures were derived from 5 diverse genetic backgrounds so we can characterize the differences and commonalities across the different strains. We also measured the mineralization levels of these cultures, as well as in vivo histomorphometric data at each time point in order to coordinate the timings of the transcriptional events among the 5 examined strains.

We targeted our analysis efforts to the transcriptional dynamics, for example, temporal variation of isoform-specificity or allele-specificity, associated with the phenotypic changes we measured. For this purpose, we developed a new Expectation-Maximization (EM) algorithm to estimate isoform-specific and allele-specific expression abundance from F1 mice. Through the application of our new approach followed by the differential co-expression analysis, we identified core transcriptional responses conserved across multiple strains, as well as unique expression events possibly relevant to the phenotypic variation among the particular genetic backgrounds. We believe that our systems biology perspective on the new level of transcriptional details offered by RNA-seq analysis contributed to a better understanding of the osteoblastogenesis.
eQTL identification using RNA-Seq analysis of three mouse brain regions

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Mice are one of the primary model organisms used to study behavior. We used gene expression in the mouse brain as an intermediate phenotype to better understand the mechanisms underlying behavioral traits. We sampled 80 animals from an extant outbred population (CFW). We dissected 3 brain regions: hippocampus, pre-frontal cortex and striatum and quantified the mRNA in each tissue using short read RNA sequencing (RNASeq). We used genotype-by-sequencing approach to genotype these mice at ~100,000 single nucleotide polymorphisms spread across the genome. We are performing an eQTL analysis in each tissue separately. We will use allele-specific expression differences to validate cis-eQTLs when possible. We will consolidate these results and compare the eQTLs across the tissues to identify genes that exhibit differential regulation between these regions and to increase power when regulatory differences are common to multiple tissues. We supplement this analysis by identifying genes that are differentially expressed in these 3 regions. We hope to characterize the expression landscape in these 3 brain regions, along with variants that affect them.
Genetic analysis of exercise capacity and training responses in 129S1/SvImJ and NZW/LacJ mice

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Genetic factors determining exercise capacity and the magnitude of the response to exercise training are poorly understood. The aim of this study was to identify quantitative trait loci (QTL) associated with exercise training in mice. Based on marked differences in training responses in inbred NZW (-0.65 ± 1.73 min) and 129S1 (4.31 ± 2.79 min) mice, a reciprocal intercross breeding scheme was used to generate 288 F2 mice. All F2 mice completed an exercise performance test before and after a 4-week treadmill running program, resulting in an increase in exercise capacity of 1.54 ± 3.69 min (range -10 to +12 min). Genome-wide linkage scans were performed for pre-training, post-training, and change in run time. For pre-training exercise time, suggestive QTL were identified on Chromosomes 5 (57.4 cM, 2.5 LOD) and 6 (39.8 cM, 2.8 LOD). Suggestive QTL for post-training exercise capacity were identified on Chrs 1 (52.0 cM, 2.2 LOD) and 5 (43.4 cM, 3.3 LOD). A suggestive QTL for the change in run time was identified on Chr 6 (37.8 cM, 2.9 LOD). To identify shared QTL, this data set was combined with data from a cross between B6 and FVB strains. In the combined cross analysis, a significant novel QTL for pre-training exercise time was identified on Chr 12 (54.0 cM, 3.45 LOD) and a suggestive QTL for post-training exercise capacity on Chr 8 (23.9 cM, 2.92 LOD). These data indicate that one or more QTL determine exercise capacity and training responses in mice. Supported by HL-085918.
Mitochondrial Respiratory Chain Suprastructure Exhibits Genetic Dependence in Mouse Brain

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Arrangement into stable and specific multicomplex assemblies is a fundamental property of the mitochondrial respiratory chain (RC) with significant functional implications. Genetic RC defects and associated reactive oxygen species (ROS) production underlie the debilitating consequences of the most prevalent group of inborn errors of metabolism and are central pathogenic themes in a multitude of progressive conditions, e.g., aging, cancer, and neurodegeneration. While many causal genes are now known, elucidation of their etiological mechanisms is just beginning. We assessed brain RC complexes with blue native electrophoresis (BNE) and in-gel activity (IGA) staining in two highly relevant genetic mouse models known to diverge in ROS-related traits, the C57BL6/J (B6) and DBA/2J (D2) inbred strains. These studies reveal marked qualitative and semi-quantitative differences in complex III and IV interactions affecting multiple assemblies, including respirasomes, and suggest overall organization is relatively less fixed in B6 than D2. To our knowledge, these are the first comparative analyses of RC suprastructure in mammalian brain, particularly in a genotype-dependent context. Furthermore, these data suggest brain RC may exist within a range of genetically influenced plasticity that is relevant both to adaptive energetic and oxidative homeostasis in mice, and potentially mitochondrial-related disease in humans.
Heterogeneous stock (HS) rats are derived from eight inbred founder strains and maintained in a breeding strategy that minimizes inbreeding. HS rats have a highly recombinant genome, which allows for rapid fine-mapping of complex traits genome-wide. However, this results in a complicated set of relationships between animals that is non-existent in traditional genetic mapping methods. We collected multiple diabetic phenotypes in 1,038 HS male rats and genotyped these animals using the Affymetrix 10K SNP array. Following ancestral haplotype reconstruction, a mixed modeling approach with sibship as a random effect was used to identify genetic loci. We report results for glucose area under the curve after a glucose tolerance test, the first of several diabetic traits that will be analyzed. Genome-wide significant marker intervals were detected on rat chromosomes 1, 3, 10 and 13, with the average 95% confidence interval for these loci being only 3.15 Mb. Most of these loci fall within regions previously identified for glucose tolerance using traditional mapping methods (e.g., F2 intercross studies). A multilocus modeling technique involving resample model averaging is currently underway and will be used to determine how frequently each locus is detected when resampling a portion of the original data-set, thus reducing potential false positives. Follow-up studies using gene expression and sequence variation will be used to narrow candidate genes within these loci. These data demonstrate the utility of HS rats for detecting genetic loci for diabetic traits genome-wide.
Genomic variants in the parental strains of the rat HxB/BxH recombinant inbred panel

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Panels of recombinant inbred strains are an invaluable tool for genetical/genomic analyses of complex traits. Continuing refinements in genome and transcriptome sequencing provide increasingly detailed sets of markers for genome-wide analyses including differences in RNA expression responsible for phenotypic diversity.

The parental strains of the HxB/BxH rat RI panel are the spontaneously hypertensive rat strain, SHR/OlaIpcv, and the BN-Lx/Cub, a Brown Norway congenic strain with polydactyl-luxate syndrome. These two strains are believed to be among the most genetically diverse among laboratory rat strains in general use, and show physiological and behavioral differences beyond those traits for which they were originally bred.

We sequenced the DNA of the BN-Lx/Cub and SHR/OlaIpcv strains and mapped the resulting sequences to the latest Brown Norway (BN) reference genome (RGSC 5.0/rn5). We identified over 3.2 million single-nucleotide polymorphisms and over 600,000 small (<15 nt) genomic insertions or deletions between the parental strains using a standard samtools/bcftools pipeline. We also identified larger genomic variants including insertions and deletions of length 15nt, inversions, and differences in copy number between the parental strains and the reference genome using more novel analysis methods. Many of the variants between the strains may have a significant impact on protein production and function, with as many as 50,000 small variants occurring within or near protein-coding genes. Data from this study are available at our website: http://phenogen.ucdenver.edu/PhenoGen/ Supported by NIAAA (AA013162, AA013162-08S1) and the Banbury Fund.
Transcriptional and linkage analyses identify loci that mediate differential macrophage response to inflammatory stimuli and infection

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Individual variation in macrophage responsiveness to cytokines, such as interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα), or conserved pathogen-associated molecular patterns, such as LPS and CpG, is thought to result in variable disease phenotypes between individuals with divergent genetic background. Therefore, we aimed to elucidate the molecular mechanisms underlying variation in macrophage response to inflammatory stimuli and to infection with the obligate intracellular pathogen Toxoplasma gondii, a common opportunistic pathogen in immunodeficient individuals.

By combining transcriptional and linkage analysis of bone marrow-derived macrophages obtained from recombinant inbred mice, we show that in resting macrophages, differential transcriptional profiles are mostly regulated in cis, while the differential responsiveness to stimulation or Toxoplasma is largely determined by a small number of loci with large effects on the expression of many genes. Additionally, we identified a locus on mouse chr 2 that regulates both mouse and Toxoplasma gene expression, and a locus on chr 3 that regulates parasite growth in vitro. Finally, by leveraging the power of RNA-seq, we identified and provide evidence for genetic linkage to differential alternative splicing and mRNA editing. We have validated some of these results using used shRNA-mediated knockdown assays.

Because activation of macrophages by IFNγ and TNFα confers resistance to many pathogens, many of which have overlapping susceptibility loci, we expect that the data generated in this study will help to identify genes important in mediating response to other pathogens.
Dissection of the Genetic Architecture of Musculoskeletal Traits in Collaborative Cross

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The founder strains of the collaborative cross (CC) differ substantially in body size. Because skeletal muscles and bones are major determinants of body size, we initiated QTL mapping of the weights of five hindlimb muscles: (tibialis anterior (TA), extensor digitorum longus (EDL), gastrocnemius, plantaris and soleus) as well as femur length in 4 founder strains (A/J, 129, NOD/Lt, C57BL/6) and 28 CC strains. Male mice (n 155, between 2 and 13 per strain) aged between 40 and 59 days were phenotyped. HAPPY and DOQTL software were used for QTL mapping. The genetic correlations between the age-adjusted strain means ranged from 0.37 (soleus weight and femur length, p < 0.05) to 0.89 (weight of TA and gastrocnemius, p < 1 × 10⁻¹⁰). Muscle weight and bone length were lowest in NOD/Lt strain and largest in WAB2_DH (TA, plantaris), C57BL/6 (EDL, gastrocnemius and soleus) and BEM_AG (femur) strains. Differences of 2.3- to 3.3-fold were observed between the extremes for muscle weight (ANOVA for strain effect P < 1 × 10⁻¹³) and 1.4-fold for the femur length (P < 1 × 10⁻⁸). The most robust QTLs (95% confidence) for muscle weight and femur length mapped to chromosomes 1 and 10, respectively. Chromosomes 1, 2, 3, 4, 6, 8, 13 and 16 also harboured QTLs (90% confidence) affecting various phenotypes. We conclude that the CC provides an excellent model for mapping genes affecting variation in musculoskeletal traits.

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Genome wide association study of the attribution of incentive salience in outbred Sprague Dawley rats

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Much of our daily behavior is controlled by stimuli (cues) associated with rewards; these cues can promote either adaptive or maladaptive behavior. Cues associated with rewards (conditional stimuli, CSs) powerfully motivate behavior only if they are attributed with incentive motivational properties (incentive salience), and thus acquire the ability to act as incentive stimuli. Among outbred Sprague Dawley rats there is large variation in the propensity of individual rats to attribute incentive salience to food and drug cues. Using a behavioral paradigm called Pavlovian Conditioned Approach (PCA), Sprague Dawley rats can be assigned a quantitative score indicating their tendency to behave as sign trackers, meaning that they assign incentive salience to the cue (sign) or goal trackers, which do not assign incentive salience to the cue. As part of an ongoing program project grant we (Flagel, Robinson) are already phenotyping several thousand Sprague Dawley rats. We (Parker, Palmer) are now in the process of genotyping DNA from these rats using a genotype by sequencing approach. We will use the resulting data to perform a genome-wide association study. This will allow us to identify genes that underlie the individual variability in PCA observed among outbred Sprague Dawley rats. This project has the potential to open up completely new molecular avenues for exploring the role of cues in shaping behavior. The sample size is larger than any similar mouse or rat study that we are aware of and thus provides outstanding power.
Genome-wide association study of behavior in outbred mice

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Understanding the genetic basis of behavior remains a major challenge. We have taken advantage of an extant outbred population that has been maintained using an outbred breeding scheme for more than 100 generations. We have carefully phenotyped more than 1,000 male CFW mice for a battery of behavioral and physiological traits including methamphetamine sensitivity, fear conditioning, prepulse inhibition, fasting glucose, and body weight. We have genotyped these mice at ~100,000 SNPs using a genotyping-by-sequencing approach. We are using these data to perform a genome-wide association study for each trait. In addition, we have performed RNASeq on three brain regions (prefrontal cortex, hippocampus, and striatum), which allows us to search for behavioral and expression QTLs that co-map to the same intervals.
Genetic Control Of Obesity In Response To High-Fat/High-Sucrose Feeding: A Systems Genetics Study In The Mouse

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Consumption of a high-fat diet rich in refined carbohydrates is a key environmental factor driving the worldwide obesity epidemic. To understand the genetic and biological pathways contributing to obesity we employed a powerful systems genetics approach in the mouse capable of high-resolution genome-wide association mapping and integration of traits across multiple scales of biology (DNA, RNA, Protein, and Metabolite). We fed more than 100 unique inbred strains of male and female mice a high-fat/high-sucrose (HF/HS) diet. Mice were fed HF/HS diet for 8 weeks and body composition was measured every two weeks using magnetic resonance imaging (MRI). The results show remarkable variation in response to HF/HS feeding from no change to more than a 600 percent change in body fat percentage after 8 weeks. Changes in body fat were highly heritable (>70%) and genetic mapping identified over a dozen genome-wide significant loci associated with changes in body fat after HF/HS feeding. A number of loci contained genes with previously described roles in obesity and genes identified in human GWAS studies for obesity, such as Npc1, Negr1, and Lyplal1. After HF/HS feeding several strains demonstrated signs of metabolic disease, such as hyperinsulinemia, hyperglycemia, and fatty liver disease, suggesting some strains may be prone to metabolic diseases after HF/HS feeding. Ongoing, integration of multiple high-throughput data sets, such as transcriptomic and metabolomic will allow for comprehensive analysis of dietary interactions contributing to obesity across multiple scales of biology and has the potential to greatly enhance our understanding of obesity and associated diseases.
Identification of QTL for Hematological Traits in Diversity Outbred Mice

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The Jackson Laboratory

Studies in multiple species reveal that a significant genetic component underlies baseline peripheral blood traits including hemoglobin (Hgb); hematocrit (Hct); red cell indices; total red cell, white cell, and platelet counts; and cell volumes. Baseline peripheral blood traits are independent risk factors for complex human diseases with considerable morbidity and mortality. Previously, we identified multiple QTL for these and other peripheral blood parameters using twelve traditional 2-progenitor crosses. However, with rare exception, confidence intervals were exceedingly large. Here, we used 647 Diversity Outbred mice (G4-G7) to map peripheral blood traits obtained using an Advia whole blood analyzer equipped with mouse-specific software. Data were analyzed using QTL/REL with modifications to accommodate the 8-founder origin of DO mice. Significance levels were determined by permutation. There was a high concordance of QTL identified in DO and F2 mice. For example, of 22 loci related to erythroid traits detected in DO mice, 16 were previously detected in F2 intercrosses, including the large effect Chr 7 cell hemoglobin concentration mean (CHCM, analogous the MCHC) QTL, Chcmq3. Confidence intervals, which on average were many cM in conventional crosses, were much smaller in the DO population. For example, the smallest interval for Chcmq3 obtained in conventional crosses was 11 Mb. In the DO population, it was 0.9 Mb. Notably, for both erythroid and white blood cell traits, highly correlated measures (e.g., Hgb, Hct) mapped to the same loci, which allows for combined analyses. Candidate genes for these loci are currently being assessed.
Identifying transcriptional signatures of brain region-specific volume from whole brain RNA-Seq data

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For many heritable complex neurological phenotypes, such as substance use disorders (SUD), no single brain region or cell type is solely responsible for its etiology and it is clear that the brain operates as a network of functionally linked cells and regions. Often in such situations of ambiguity, whole brain tissue is used for initial transcriptome analyses. However, transcriptional associations in brain can be confounded by genetic variation in proportions of brain regions and cell types, and these differences can create confusion about the source of transcriptional variances (e.g., transcriptional differences within cells or differences in the proportion of cells). We evaluated weighted gene co-expression network analysis (WGCNA) and independent component analysis (ICA) based on their ability to extract signals related to brain region-specific volume from whole brain transcriptome data from the BXD recombinant inbred panel (http://www.genenetwork.org). These signals allow volume to be accounted for in associations between brain transcription levels and phenotypes. Over 8,000 highly expressed transcripts were identified and quantified using RNA-Seq data from 31 RI strains. WGCNA and ICA identified eigengenes and source signals, respectively, that were associated with differences in proportional brain region volumes. ICA had the additional benefit of relating the different sources back to individual transcripts that were expressed in more than one brain region. ICA is useful for extracting expression signals from multiple sources and including these signals in transcriptional analyses of complex traits will increase interpretability of genetic associations. Supported by NIAAA (AA013162, AA013162-08S1, AA016662, AA013499), the NFPCDD, and the Banbury Fund.
Fine-mapping diabetes-related traits within rat chromosome 1 in heterogeneous stock rats

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Type 2 diabetes (T2D) is a disease of relative insulin deficiency resulting from both insulin resistance and beta cell failure. We have previously used heterogeneous stock (HS) rats to fine-map a locus for glucose tolerance. We show here that glucose intolerance in the founder strains of the HS colony is mediated by different mechanisms: insulin resistance in WKY and an insulin secretion defect in ACI, and we demonstrate a high degree of variability for these measures in the HS rats. As such, our goal was to use HS rats to fine-map several diabetes-related traits within a region on rat chromosome 1. We measured blood glucose and plasma insulin levels after a glucose tolerance test in 782 male HS rats. Using 97 SSLP markers, we genotyped a 68 Mb region on rat chromosome 1 previously implicated in glucose and insulin regulation. We used linkage disequilibrium mapping by mixed model regression with inferred descent to identify a region from 198.85 – 205.9 that contains one or more quantitative trait loci (QTL) for fasting insulin and a measure of insulin resistance, the quantitative insulin sensitivity check (QUICKI). This region also encompasses smaller loci identified for fasting glucose and Insulin_AUC (Area Under the Curve). Using a novel penalized regression method we then estimated effects of alternative haplotype pairings under each locus. Preliminary results using expression analysis and founder sequence indicate a potential candidate gene within this region. These studies highlight the utility of HS rats for fine-mapping genetic loci involved in the underlying causes of T2D.
Sources of Natural Selection on Body Size in Island Populations of Wild Mice

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House mice (Mus musculus) have a virtually cosmopolitan distribution across the planet. Because of their commensal relationship with humans, they have inadvertently been introduced to both human inhabited and uninhabited islands. This wide distribution of mice on islands with variable biotic and abiotic environments creates a valuable resource to understand how natural selection shapes phenotypic variation. The “Island Rule” states that small mammals will grow larger body sizes on islands whereas large mammals will grow smaller. As mouse body size is a canonical complex genetic trait, it is of interest to examine potential determinants of this trend within house mice. In this investigation, we compare published body masses for island house mice to a series of environmental factors, including geographic location, island area, island perimeter, temperature, precipitation, solar power, human population, number of predators (mammal and avian), and number of competitors. Regression analyses identify multiple biotic and abiotic variables that collectively explain a substantial percentage of the variance in body mass. We compare body mass variation in island mice to that observed across laboratory strains to place our findings in the context of mouse genetics. This research highlights the power of wild house mouse populations for understanding the evolution of biomedically relevant phenotypes.
Evolution of Extreme Body Size in a Natural Population of House Mouse.

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Laboratory house mice (Mus musculus) are the leading mammalian model system for understanding genetic variation in complex phenotypes. Body size has been especially well studied, mostly due to its pervasive correlations with morphological, physiological, and life-history traits, and the underlying pleiotropy of the genes regulating body size. For example, many of the same genetic factors that contribute to variation in body size have been implicated in human diseases, ranging from coronary disease to obesity and cancer. Although most research on the genetic basis of body size in house mice has focused on the classical inbred strains or their derivatives, wild house mice also show substantial variation in body size across a wide range of habitats. Studying wild populations can connect phenotypic variation to the natural environment in which it evolved. This combination of genetic tools and natural variation provides a practical platform for dissecting the genetic underpinnings of complex trait variation. House mice from Gough Island provide an especially dramatic example of extreme body size evolution: they are larger than any other wild population and most inbred strains. Here, we present body mass data from our study which aims to identify the genomic regions responsible for large body size in Gough Island mice. Analyses are reported for weekly weights, growth curves, and growth rate variation within and between the four crosses and two sexes. Gough Island mice are born heavier and grow faster at many ages compared to a wild-derived inbred strain (WSB) from the same subspecies. F2 body weights from intercrosses between semi-inbred Gough Island mice and WSB are normally distributed. Our results suggest that multiple loci contribute to extreme body size in Gough Island mice and motivate genetic mapping to identify these loci.
Detecting Epistasis Through Analyses of Genomic Ancestry Tracts in Admixed Populations

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Studying genomic variation in natural populations has great potential for revealing the genetic bases of complex traits. However, despite the progress made so far our understanding of how multiple evolutionary forces shape variation among genomes is still lacking. We are integrating population genomic simulations and analyses of genomic data from a hybrid zone between two nascent species of mice (Mus musculus musculus and M. m. domesticus) to identify deleterious epistatic interactions (Dobzhansky-Muller Incompatibilities) that block gene flow between species. The simulation engine models haplotypes as tracts of ancestry (identity-by-descent) and tracks junctions (recombination events between ancestry states in the genome). This allows us to quickly and efficiently simulate the evolution of entire genomes at arbitrarily fine resolutions. Furthermore, the simulation engine incorporates selection, drift, migration, and recombination allowing us to examine how each of these forces shapes haplotype variation during population admixture. We present results from these simulations that show detectable signatures in the frequency of junctions in genomic regions that harbor alleles under different selection regimes. These results show that junction frequencies have potential use in genomic scans for selection that, notably, can detect epistatic interactions. Thus, junctions may be useful for mapping Dobzhansky-Muller incompatibilities between species and detecting epistatic interactions in admixing populations. Similarly, junction based analyses may be useful in detecting epistasis in the mouse collaborative cross.
Polymorphism for hybrid male sterility during the early stages of speciation in house mice

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House mice provide a powerful system for dissecting the genetic basis of phenotypes that contribute to reproductive isolation during the early stages of speciation. There are often multiple intrinsic barriers that isolate closely related species, and during the early stages of speciation these barriers may vary both within and among populations. Understanding the nature of this variation provides insight into speciation and the evolutionary forces driving divergence. One of the best examples of polymorphic reproductive isolation is the genetic variation for hybrid male sterility observed in natural populations of house mice. We are combining genome-wide surveys of gene expression in testis with quantitative genetic crosses to dissect the basis of polymorphic hybrid male sterility between two subspecies of house mice, Mus musculus musculus and M. musculus domesticus. Our data suggest that multiple hybrid incompatibilities remain polymorphic within these subspecies, including one or more sterility factors that are independent of previously described genes underlying sterility in mice.
The evolution of skeletal variation in an island population of house mouse exhibiting extreme body size

Michelle Parmenter, Melissa Gray, Peter Ryan, Bret Payseur

Rapid changes in body size and shape are known to occur in mammals after island colonization, and provide an excellent opportunity to study mechanisms underlying morphological evolution. The evolution of extreme changes in morphology is known to occur frequently in insular rodents. A population of wild house mice, Mus musculus domesticus, found on Gough Island have undergone a dramatic, rapid change in body size since its recent colonization from the mainland, becoming the largest population of house mice in the world. The goal of this study is to determine the genetic basis underlying the extreme morphological changes that occurred in the Gough island mouse population, with a focus on functional structures. Morphological analysis includes bone measurements from X-Ray images and CT-scans, and allometric analysis of functional morphology within and between skeletal features. Although the genetic basis of skeletal differences has been studied in laboratory strains of mice, natural variation in these traits has rarely been examined. By performing an F2 intercross between partially inbred Gough mice and WSB, a small wild-derived inbred strain, QTL analysis can be used alongside the powerful genetic resources of M. musculus domesticus to identify loci driving these morphological changes within this natural population.
Variation in meiotic recombination rate between WSB and Gough mice

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Meiotic recombination is a fundamental genetic process involving the reciprocal exchange of genetic material and the formation of chiasmata between homologous chromosomes during gametogenesis. This process not only creates new haplotypes, but is necessary for the proper segregation of chromosomes in most eukaryotes. The rate at which recombination occurs across the genome is a quantitative trait that can vary dramatically between species, populations, and even individuals. One of the ways in which this trait can be measured is through immunohistochemistry; meiotic cells from an individual are isolated and stained for proteins associated with a recombination breakpoint. The number of recombination events in each cell, and the average for an individual, can be directly observed under a microscope. Here, we present preliminary data using this technique on mice from a unique F2 cross between the WSB strain and the Gough Island mouse. The Gough mice are derived from a wild, invasive population of mice from a remote island in the south Atlantic. In addition to distinct differences in body size and feeding behavior, the Gough mice have an elevated level of recombination relative to the WSB strain. We will use our data from this F2 cross to map the genetic loci underlying the difference in recombination rates.
Plague is caused by the Gram-negative bacterium Yersinia pestis. Laboratory mice are susceptible to plague. We have recently described that wild-derived Mus spretus SEG/Pas mice were exceptionally resistant (90%) to the virulent CO92 wild-type strain of Y. pestis in an experimental model of bubonic plague. We screened other Mus spretus derived strains and identified the STF/Pas strain as susceptible. QTL mapping in an intercross between SEG resistant and STF susceptible strains led to the identification of two genomic intervals on chromosome 8. These QTLs are distinct from the three previously identified in a cross between SEG and C57BL/6J strains (Blanchet et al., 2011). Incipient-congenic (N5-N7) females carrying a heterozygous Chr8-SEG/STF fragment showed statistically higher survival rate after infection than homozygous Chr8-STF/STF female littermates. Macrophages are well recognized as being at the forefront of innate immune response to Y. pestis. We extracted peritoneal macrophages from both parental Mus spretus strains and incubated them ex vivo for 3 hours with Y. pestis. We also performed exome sequencing of both strains. Preliminary transcriptomic and exomic differences will be presented. Combination of QTL, exomic and transcriptomic datas should help in unravelling some of the mechanisms involved in resistance to plague.
Role of ECE1 in Mediating Maternal Cardiovascular Adaptation to Pregnancy

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Preeclampsia (~8% of all pregnancies), fetal loss (~15% of all pregnancies) and intraterine growth restriction (~10% of newborns) are common pregnancy complications. Our laboratory has isolated a pleiotropic quantitative trait locus (QTL) in mouse chromosome 4 in recombinant congenic mice (HcB-8 and HcB-23) that harbors differentially expressed Ece1 (the gene encoding ECE1) alleles resulting in differences in reproductive performance. Recent data in 2 newly created congenic strains in which the QTL has been isolated are consistent with those obtained in HcB-8 and HcB-23. Long Chromosome 4 congenic (C4C-L) 17.5 dpc females have smaller litter sizes than C3H females (5 ± 1.5 v 7 ± 1.5, respectively, p = 0.034), but heavier placental weight relative to C3H 17.5 dpc females (0.126 ± 0.117 v 0.108 ± 0.103, respectively, p = 0.009) with no significant difference in 17.5 dpc embryos weight. These findings suggest the possible differences in placental efficiency between C4C-L and C3H mice. There are no significant differences observed between short chromosome 4 congenic (C4C) and C3H females. Doppler ultrasonography embryonic measurements of 17.5 dpc females show that C4C-L embryos have slower umbilical vein velocity than C3H embryos (145.6 ± 29.7 v 219.9 ± 40.3, respectively, p = 0.025). The embryonic heart rate is also slower in both C4C and C4C-L embryos relative to the C3H embryos (169 ± 33, 204 ± 41.8 v 315.8 ± 64.5, respectively, p = 0.016 (C4C v C3H), p = 0.027 (C4C-L v C3H). These data suggest that the chromosome 4 QTL mediates important differences in reproductive performance.
Splicing Landscape of 8 Founder Strains

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Inbred laboratory mouse strains are an invaluable research tool. In an effort to understand the functional differences among different strains, we have analyzed the splicing landscape of eight inbred mouse strains. The strains include those of classical laboratory strains (129, A/J, C57, NOD, and NZO) and 3 wild derived inbred strains (CAST, PWK and WSB). Using RNA-seq data mapped to the C57BL/6J mouse reference genome, we found that much of the splicing landscape across the strains are shared with ~70% of all spliced junctions being shared between at least 2 strains and 24% of the junctions being shared among all the strains. However ~10-16% of junctions were found to be strain specific, with PWK and CAST having the highest percentages. A striking majority (~96%) of the strain specific junctions were found to be novel unannotated junctions. Furthermore among the high confidence strain specific junctions (> read coverage of 10) we found that only ~10% of them resided within annotated genes with ~94% of the genes being unique to an individual strain. These findings suggest that key splicing differences may help to further define the functional differences between the strains. The resulting strain specific junctions (genomic coordinates and read coverage information) is freely available to the research community. This work was supported by grants AA010760, AA011034, MH051372, and AA013484.
Tools for QTL Analysis in the Mouse Genome Informatics (www.informatics.jax.org) Resource

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Analysis of the genetic basis of continuously varying and quantitative phenotypes including obesity, atherosclerosis, autoimmunity and susceptibility to infection, preferences for alcohol and drugs, and behavioral responses, is a continuing challenge. Quantitative trait loci (QTL) are identified by looking for genomic regions that contribute to phenotypic variation, frequently using populations of F2 crosses or recombinant inbred strains. The expanded BXD recombinant inbred strain set, Diversity Outcross (DO), and Collaborative Cross (CC) populations provide new resources to identify QTL and more exactly localize and identify these variants in the genome. The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org) has cataloged nearly 5000 QTL and curated mapping and phenotypic trait information on most of these. 8613 QTL variants distinguishing strain-specific locus differences have been integrated in MGI with data on genome sequence, gene expression, genetic polymorphisms and phenotypic consequences. Two graphical QTL browsers are implemented in MGI. The Mouse Genome Browser (Mouse GBrowse) provides a customizable graphic representation of those QTL that can be defined in the context of known genetic markers on a sequence-based map. This representation permits exploration of causative candidate genes and prioritization of these candidates for experimental analysis. Mouse GBrowse displays may suggest new strategies to help refine the region in which a particular QTL is located. A new Cancer QTL Viewer is available through the Mouse Tumor Database (MTB) and will be adapted for use in MGI as a whole. Examples of both Mouse GBrowse and the Cancer QTL viewer will be shown.

Supported by NIH grant HG000330.
Independent Studies in Computational Biology: Engaging Talented Youth in Authentic Research

Sue McClatchy and Gary Churchill
The Jackson Laboratory

Independent Studies in Computational Biology delivers an award-winning* form of education that partners high school students and teachers with investigators who generate and analyze large-scale data (genomedynamics.org/education/iscb.shtml). Students engage in a hypothesis-driven approach to large-scale data analysis under the guidance of the investigator. They acquire skills and knowledge in quantitative trait locus (QTL) analysis, R programming, genetics, statistics, and the art of scientific communication. Students are active partners in our group’s research and some have published in peer-reviewed journals.

We engage talented high school students in research and expect graduate-level performance from them. We deliver background information, offer guidance, and assess scientific process and communication skills during weekly web conferences to student research teams located along the length of the East Coast. On-site educators support research teams by reinforcing information that we present, directing students to web tools and resources for advancing their research, debugging R code, and reviewing student presentations prior to delivery.

Large-scale data analysis requires many hands, and we welcome investigators and educators to join us during weekly web conferences to learn how to implement our model of distributed research and education. Our delivery method employs hybrid online learning with in-class support from educators and any-time access to online resources. It is unique and different, though, in that students have real-time guidance from a principal investigator at a distance.

* To be announced in the May 31 edition of Science magazine
Reading the Whole Transcriptome

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In order to utilize the full power of RNAseq, these two aspects of transcriptomics that need to be addressed: 1) assigning reads to the approximately 10% of overlapping genes and 2) developing a full annotation of reads aligning to non-exonic regions. We have developed a framework that allows investigation of both the complex regions of genic overlap and annotation of reads that align to unannotated regions of the genome. We used stranded libraries of polyA RNA or riboZero treated RNA in the development and testing of this framework. Scenarios when each of these protocols would be appropriate are discussed to guide discovery. In the resulting annotation files, the genome is portioned into categories according to strand, exon and intron overlaps and intergenic regions. This is summarized at the gene, exon, transcript and intron/intergenic regions. Complex regions of overlapping genes were further characterized. To help define regions where reads align to nongenic areas of the genome, Non-Code annotation and other genomic features such as histone marks and DHS can also be integrated. This is highly extensible for any RNA-seq pipeline and freely available to the research community. This work was supported by grants AA010760, AA011034, MH051372, and AA013484.
Deciphering Genetic Complexity with the Combined Analysis of Pleiotropy and Epistasis

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Recent advances in mouse genetic resources, high-resolution genotyping, and multidimensional phenotyping are designed to enable precise genetic modeling of complex biological systems. The success of this approach is contingent upon the continued development of computational methods to dissect genetic complexity. Here we present an analytical strategy to infer models of how multiple genetic variants interact to influence multiple phenotypes. The method combines signals of epistasis between partially pleiotropic genes across multiple phenotypes to derive specific models of how each genetic variant enhances or suppresses the effects of other variants, and, in turn, affects each phenotype. The resulting network model interprets statistical epistasis in terms of more specific, directional hypotheses of variant-to-variant action. The method is designed to be flexible and scalable for application to populations with extensive genetic diversity. We present examples in yeast, fly, and mouse model systems, demonstrating the ability of the approach to both map large-scale genetic architecture and generate specific pair-wise genetic hypotheses.
Mapping quantitative trait loci underlying function-valued phenotypes.

Il-Youp Kwak and Karl W. Broman

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Most statistical methods for QTL mapping focus on a single phenotype. However, multiple phenotypes are commonly measured, and recent technological advances have greatly simplified the automated acquisition of numerous phenotypes, including function-valued phenotypes, such as height measured over time. While there exist methods for QTL mapping with function-valued phenotypes, they are generally computationally intensive and focus on single-QTL models. We propose two simple fast methods that maintain high power and precision and are amenable to extensions with multiple-QTL models using the penalized likelihood approach of Broman and Speed (2002). After identifying multiple QTL by these approaches, we can view the function-valued QTL effects to provide a deeper understanding of the underlying processes. Our methods have been implemented as a package for R.
Haplotype Association Mapping in Multiple Founder Crosses with LASSO-based Resample Model Averaging

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Highly recombinant populations derived from multiple founder crosses, such as heterogeneous stocks, can be used to map loci far more accurately than possible with standard intercrosses. However, the varying degree of relatedness that exists between individuals complicates the analysis. Incorporating polygenic effects based on known or inferred kinship can provide an efficient solution for significance testing at single loci. But several groups have shown that this can be improved in several ways: by using kinship informed by previous model selection, and by explicitly modeling multiple loci. Moreover, the recent consensus in the animal breeding community, and elsewhere, suggests explicit model selection even in the absence of kinship-like modeling may be beneficial. We add to this literature by exploring the use of model selection and model averaging techniques applied over the entire genome, comparing these with some popular alternatives. In our method, LLARRMA-haplo, we select multiple haplotype locus association models using a least absolute shrinkage and selection operator (LASSO) based regression applied to resampled data sets. We provide resample model averaging statistics about the probability of haplotype regions being included under model selection, providing a highly generalizable frequentist alternative to Bayesian model selection. This often leads to more accurate identification of haplotype regions than by single-locus mapping with polygenic effects. The generality of our approach means it can potentially be applied to any multiple founder population of unknown structure.
Genetics of response to drug in the diallel: heritable architecture of adverse reactions to haloperidol a mouse model


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Haloperidol is an efficacious antipsychotic drug that has serious, unpredictable motor side effects that limit its utility and cause non-compliance in many patients. We describe a diallel study to characterize the genome level genetic architecture of haloperidol response among eight mouse strains – specifically, the founder strains of the Collaborative Cross. Treating at males and females with haloperidol and placebo, we measured changes in open field activity, inclined screen rigidity, orofacial movements, prepulse inhibition of the acoustic startle response, as well as plasma and brain drug level measurements, and body weight. To understand the genetic architecture of haloperidol response we developed a new statistical model linking heritable variation with causal treatment effects. Specifically, we adapt our existing Bayesian hierarchy for the diallel (Lenarcic et al, 2012) to measure genetic effects of treatment response, using counterfactual arguments (after Rubin, 2005). In doing so we decompose the effects of genetic background on haloperidol response into additive, inbred-specific, parent of origin, and epistatic effects, as well as sex-specific versions thereof. Our results provide the first quantitative description of the genetic architecture of haloperidol response in mice. The fact we also replicate estimates of genetic effects on body weight previously reported in an independent diallel experiment, strongly supports the robustness of our findings.
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