



Systems genetics for identification of addiction-related genes using high-throughput behavioral phenotyping

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Systems genetics

- Systems Genetics is the application of systems biology in the context of genetic variation.
- Genetic variation
 - influences variability in behavior
 - enables correlation of behaviors across individuals
 - enables correlation of behaviors to molecular mechanisms of disease
- Systems genetics enables a simultaneous genome-wide search for genetic variation and molecular mechanisms of behavior.

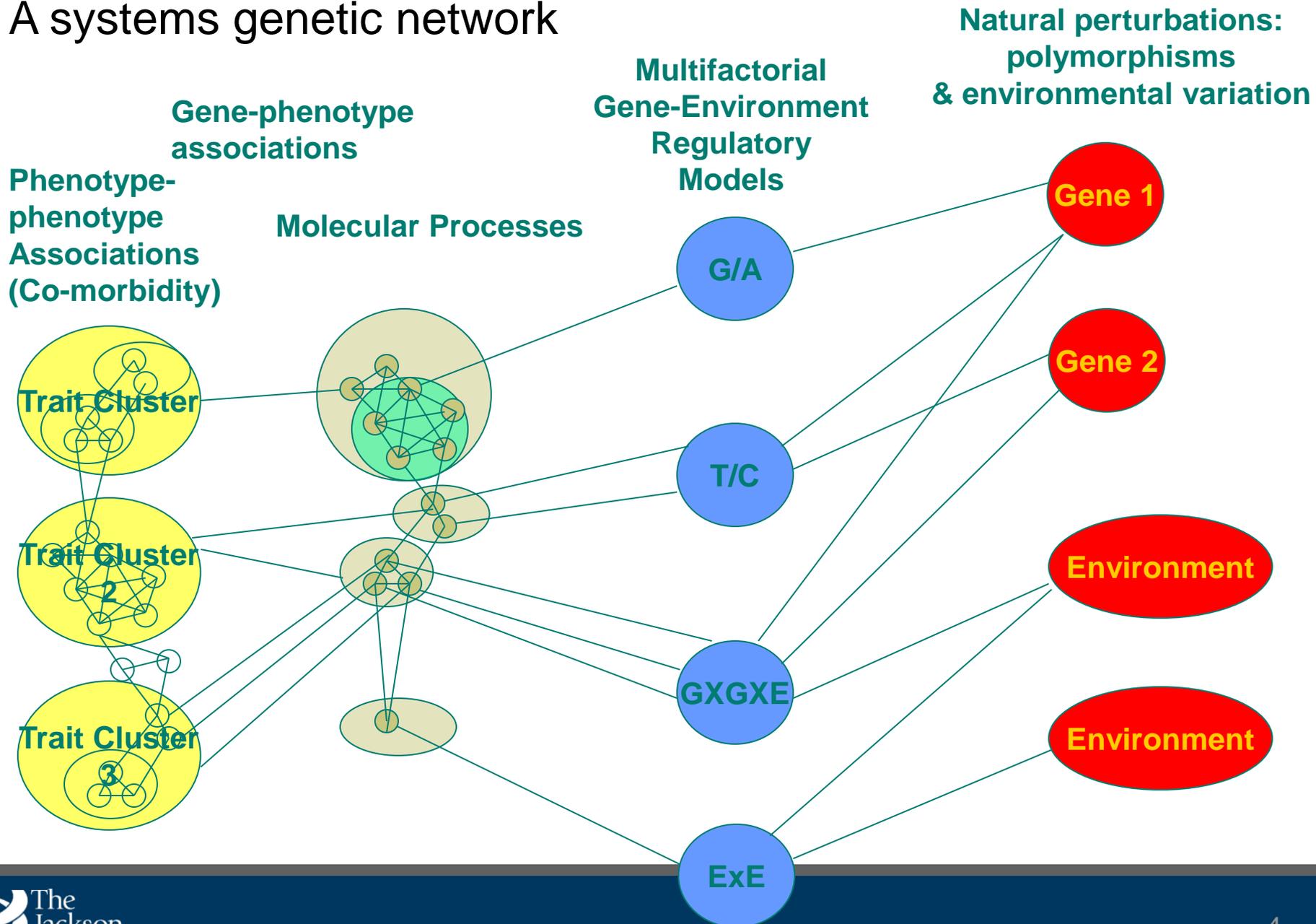
Systems genetics of behavior in laboratory mice

Global objectives are to:

- identify the sources of genetic variation underlying related behavioral phenotypes
- exploit variation to define categories of related and distinct behavioral traits
- classify behaviors based on associated molecular networks, rather than overt manifestations

Together, these facilitate individualized therapeutics by finding genes that are predicted to cause behavioral heterogeneity in people.

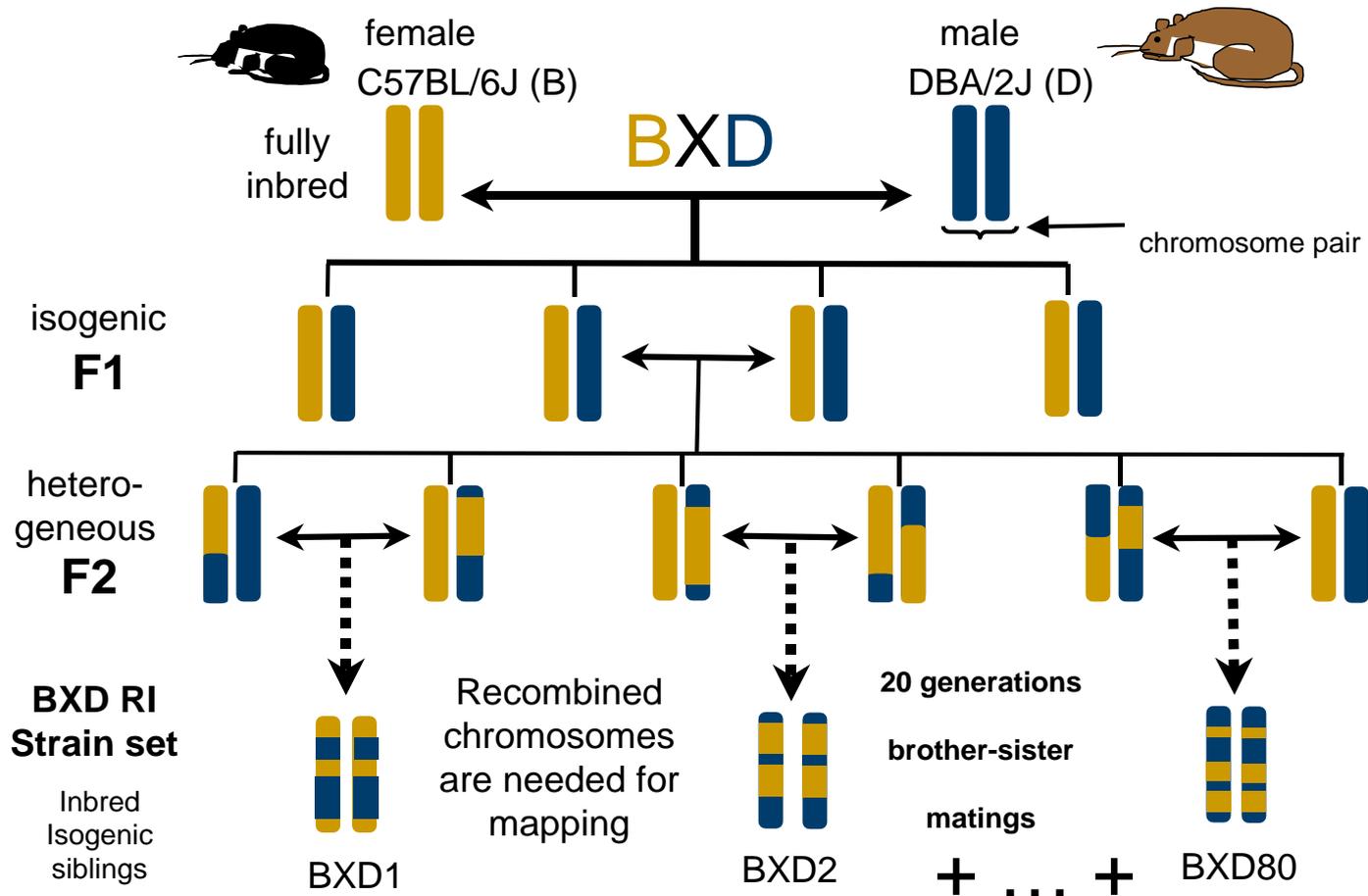
A systems genetic network



Reference Populations Enable Integrative Systems Genetics

RI LINE	GENOTYPES					GENE EXPRESSION					CLASSICAL PHENOTYPES											
1	B	B	D	D	D	9.8882702	7.8823091	6.8766717	8.1543413	9.0514759	115.52478	130.04168	859.05468	827.4036	304.99199							
2	D	B	B	B	B	6.4572105	9.4718	GENOTYPES					GENE EXPRESSION					CLASSICAL PHENOTYPES				
3	B	B	D	D	D	5.9429958	7.0423	GENOTYPES	Linkage Map Construction	QTL MAPPING Expression QTLs	QTL MAPPING Classical QTLs											
4	D	D	B	B	D	8.481914	9.1225															
5	D	D	D	B	B	9.220692	5.5148															
6	B	D	D	D	B	8.1342232	7.2750															
7	D	D	D	B	D	7.8069325	12.646															
8	B	D	D	D	D	5.1141369	8.5804															
9	D	B	B	B	B	5.0737463	8.0057															
10	B	D	D	D	D	8.195148	10.136															
11	D	B	B	D	D	7.5016105	10.385															
12	B	D	D	D	B	7.3512233	7.2140															
13	D	B	B	B	B	9.024714	6.524															
14	B	B	B	D	D	8.7960284	10.283															
	GENE EXPRESSION											CO-EXPRESSION NETWORKS	GENE EXPRESSION RELATIONSHIP TO COMPLEX PHENOTYPES									
	CLASSICAL PHENOTYPES													GENETIC CORRELATION OF COMPLEX PHENOTYPES								

A Genetic Reference Population: Recombinant Inbred Strains





Behavioral Phenotyping In BXD RI mice

Baseline behaviors and effects of Morphine,
Cocaine, Ethanol, Stress, MDMA

Withdrawal	Self-administration
Activity	Anxiety
Neurogenesis	Nociception
Fear Conditioning	Conditioned place preference
Pain sensitivity	Sensorimotor Gating
Locomotor sensitization and activation	

Philip VM, Duvvuru S, Gomero B, Ansah TA, Blaha CD, Cook MN, Hamre KM, Lariviere WR, Matthews DB, Mittleman G, Goldowitz D, Chesler EJ. High-throughput behavioral phenotyping in the expanded panel of BXD recombinant inbred strains. *Genes Brain Behav.* 2009 Sep 22.

Tennessee Mouse Genome Consortium BXD Behavioral Phenotyping Project

Deep replication: 10 males, 10 females per strain

Broad profiling: Approximately 58 strains per phenotype

255 phenotypic measures from ~35 assays

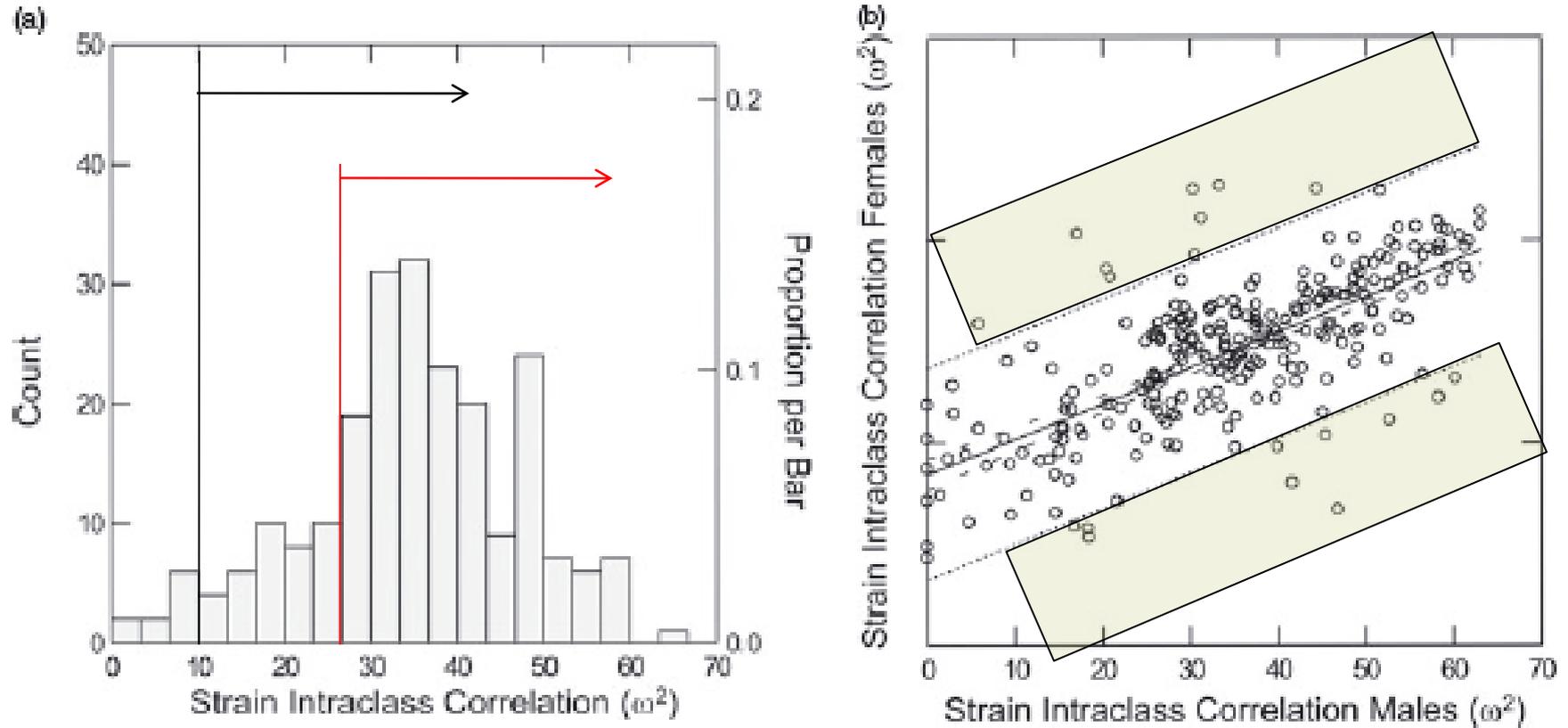
All data are publicly available in GeneNetwork.org and
[Ontological Discovery.org](http://OntologicalDiscovery.org)

Complement microarray measures of gene expression in
multiple brain regions

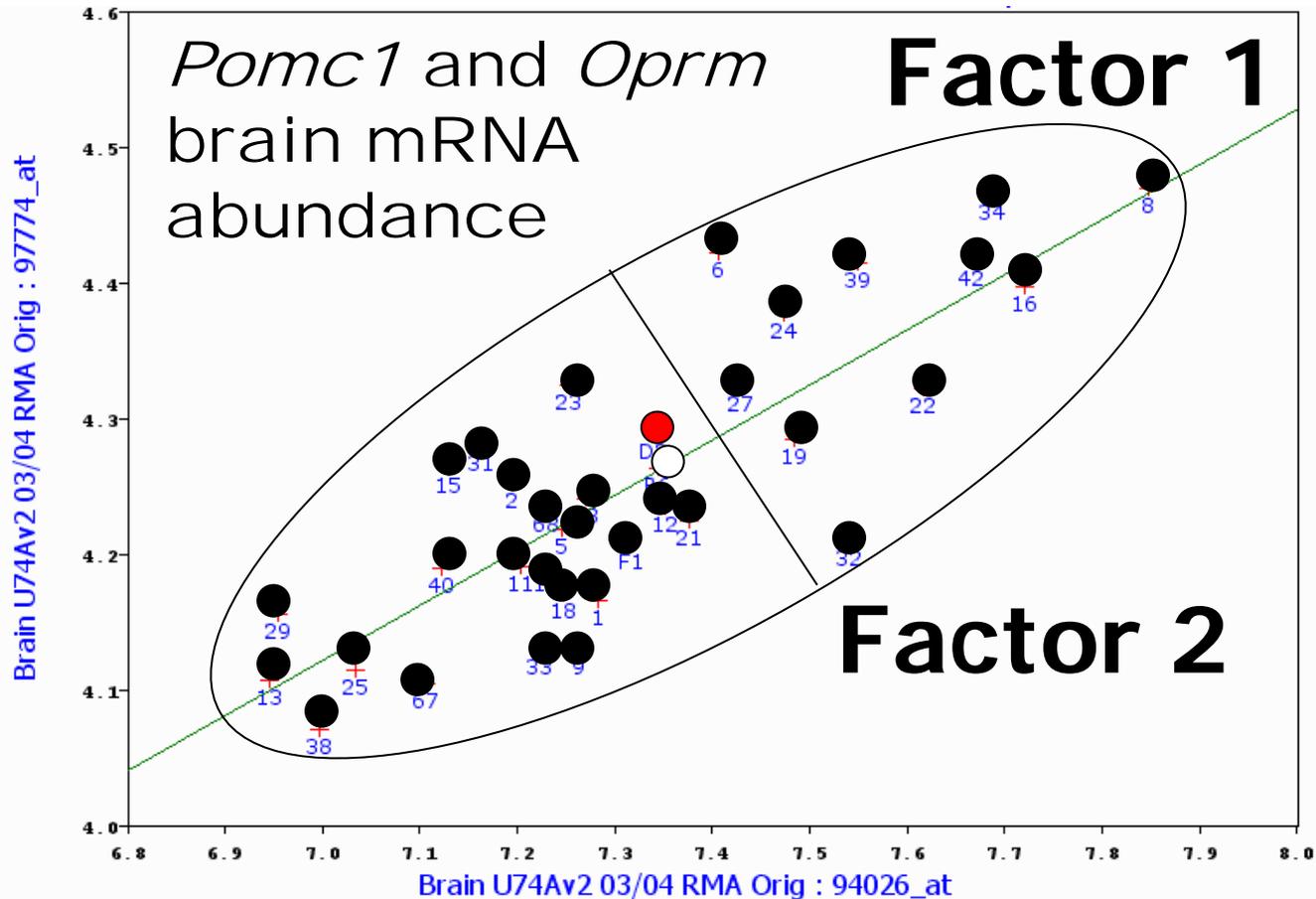
Complement decades of characterization of the BXD lines



Identifying the heritable phenotypes



Genetic Correlations Reveal Co-Regulation by Genetic Factors



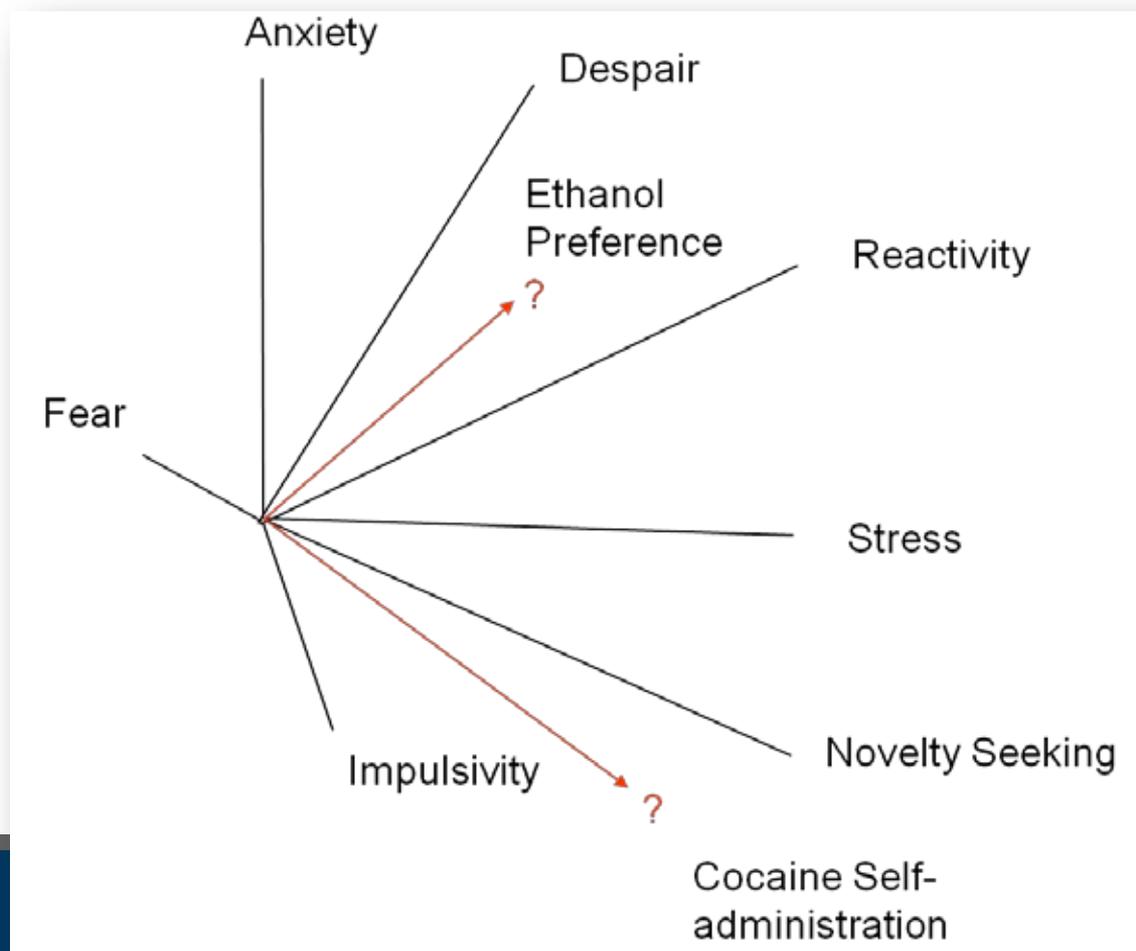
Factors underlying 255 behavioral measures obtained in 58 strains of BXD RI mice

Factor	Interpretation	Highlights:
1	Locomotor (injection stress induced)	Activity after saline or cocaine
2	Morphine Withdrawal	Salivation, urination, defecation
3	Morphine Activity	Initial response to morphine
4	Anxiety/reactivity	OFA, nociception and startle
5	Locomotor response to a novel environment	Early locomotor time
6	Activity (conflict avoidance)	Zero Maze Anxiety
7	Morphine Duration	Activity in later time points
8	Cocaine Sensitization/Activation	locomotor activity following 2nd cocaine
9	Stress	Adrenal weights, nociception
10	Vertical Activity (exploration)	Rearing, LD transitions

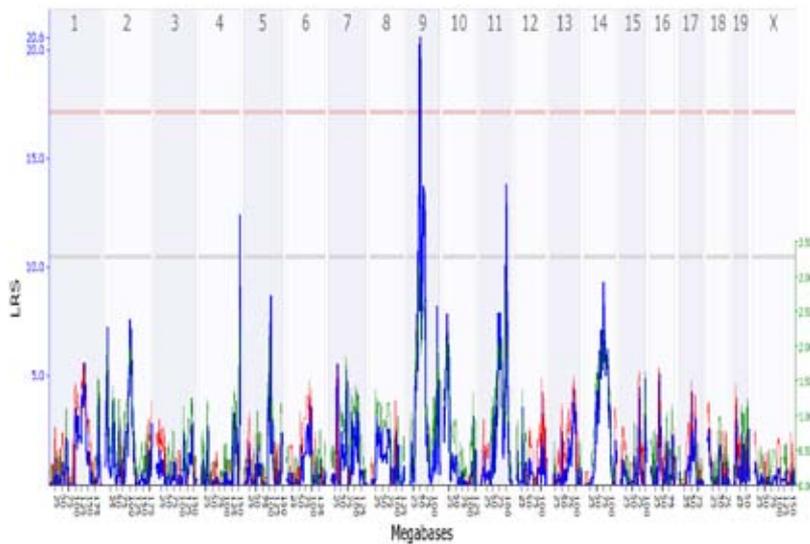


Multi-dimensional behavioral trait analysis

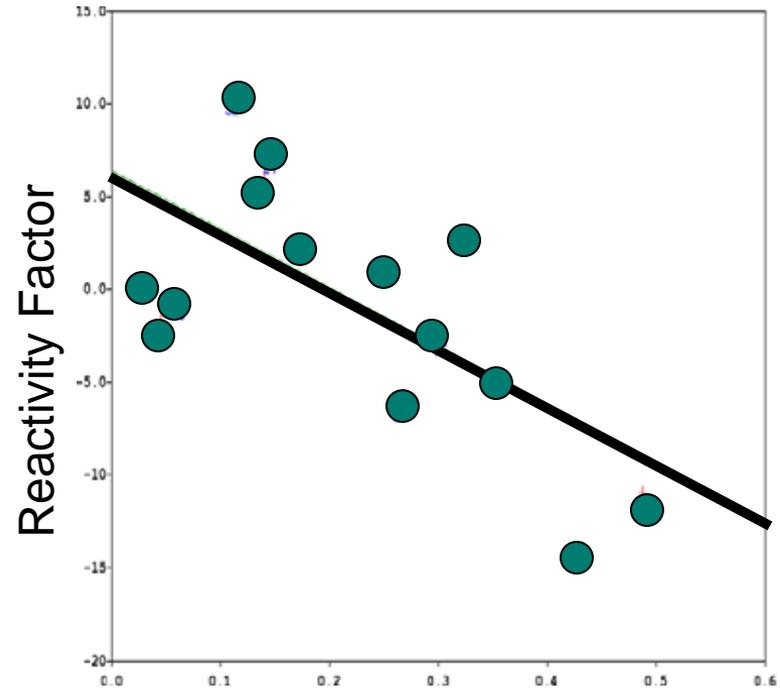
Allows analysis of the correlation between drug and alcohol self-administration to behavioral traits.



Correlating behavioral factors to alcohol preference.



QTL analysis for 'reactivity'

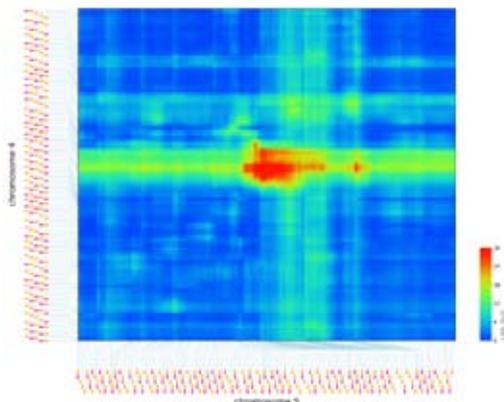


Preference for 10% ethanol (g/kg) vs. tap water by *Phillips TJ et al., ACER, 1994*

Finding Targets: QTL analysis of cocaine sensitization

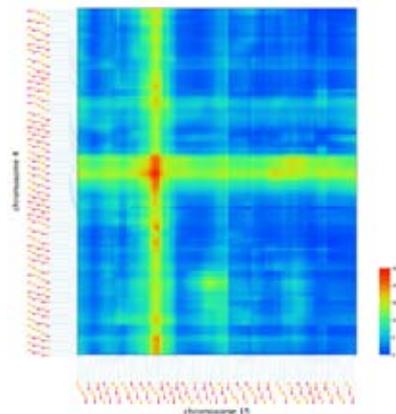
Locomotor response to 2nd dose of 10 mg/kg i. p. cocaine

Chr 4 x Chr 5

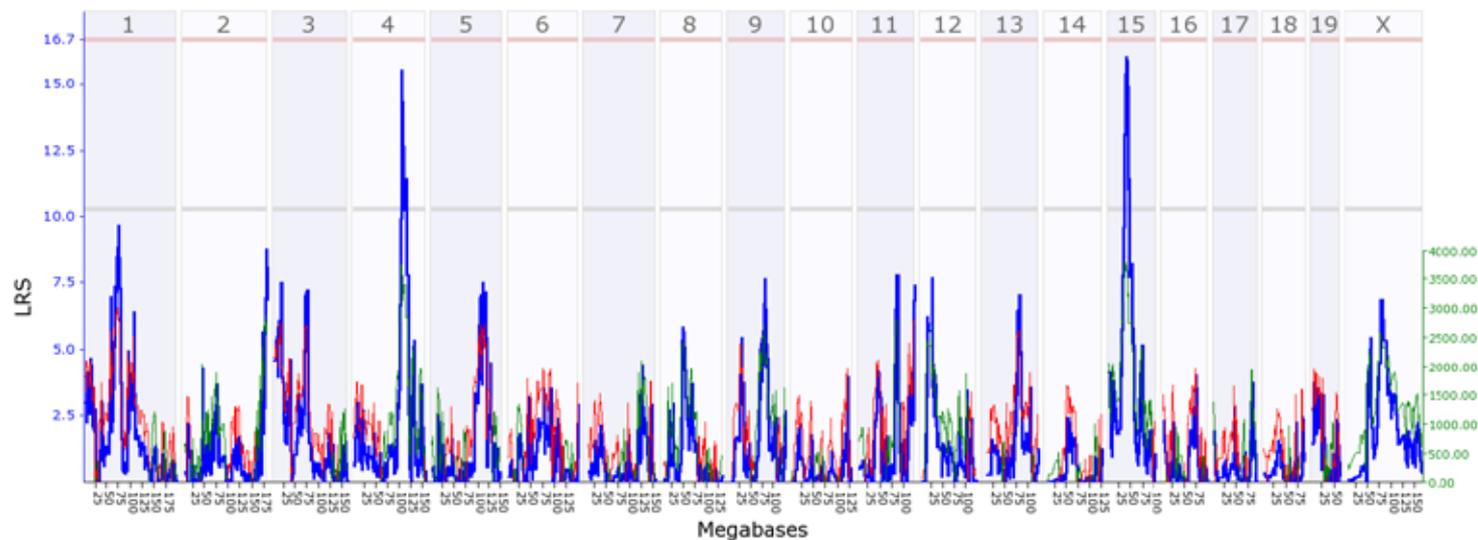


Interacting
 $p < .05$

Chr 4 x Chr 15



Additive



Positional candidate genes in the QTL

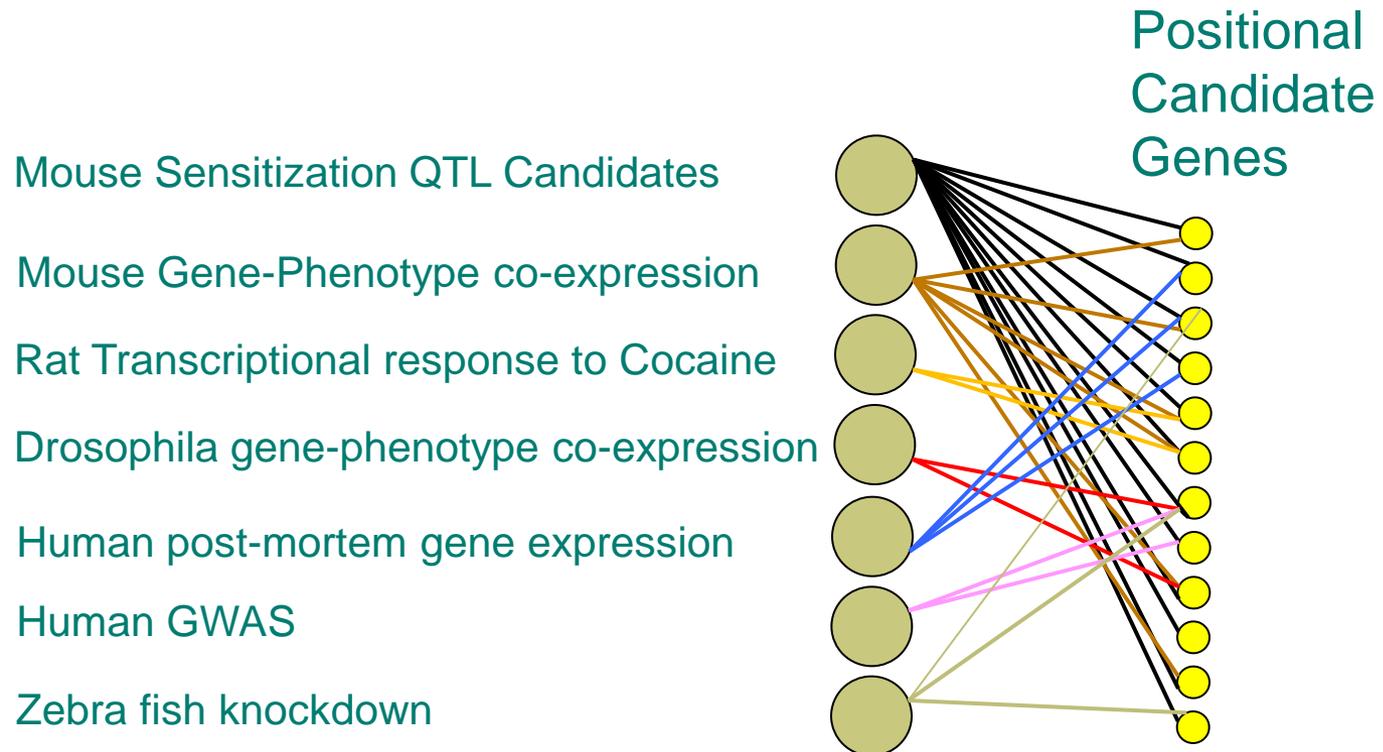
Interval Analyst : Chr 4 from 100.000000 to 117.000000 Mb [Customize](#)

GeneNetwork

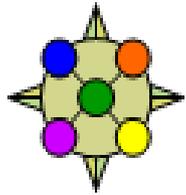
	Gene Symbol	Mb Start (mm9)	Gene Length (Kb)	SNP Count	SNP Density (SNP/Kb)	Avg. Expr. Value	Human Chr	Mb Start (hg19)	Gene Description
1	Ror1	99.768395	346.755	15	0.043258		1	63.951734	receptor tyrosine kinase-l...
2	Ube2u	100.151471	71.279	3	0.042088		1	64.381510	ubiquitin-conjugating enzy...
3	Cachd1	100.449283	227.068	8	0.035232				cache domain containing 1
4	Raver2	100.741642	83.333	2	0.024000				ribonucleoprotein, PTB-bin...
5	Jak1	100.824578	113.309	4	0.035302		1	65.011778	Janus kinase 1
6	E130102H24Rik	101.0191:							
7	O610043K17Rik	101.0263:							
8	Ak3l1	101.0923:							
9	Dnajc6	101.2231:							
10	Leprot	101.3203:							
11	C130073F10Rik	101.3879:							
12	Lepr	101.3900:							
	8823-149D11.4	101.4083:							



Integration of convergent data to further implicate positional candidates



Integrative Genomics in the Ontological Discovery Environment



ONTOLOGICAL DISCOVERY ENVIRONMENT
www.ontologicaldiscovery.org

- Data integration across diverse genomic experiments in six species
Mus musculus Rattus norvegicus
Homo sapiens Drosophila melanogaster
Danio rerio Macaca mulatta
- Uses combinatorial algorithms for data integration
- Designed with behavioral neuroscientists in mind.

Baker EJ, Jay JJ, Philip VM, Zhang Y, Li Z, Kirova R, Langston MA, Chesler EJ. Ontological discovery environment: a system for integrating gene-phenotype associations. *Genomics*. 2009 Dec;94(6):377-87.

Baker and Chesler. The importance of Open-source integrative genomics for drug discovery. *CODD* (2010)

Applications: Identification of high priority targets for addiction biology

- The NeuroSNP project (Saccone et al, 2009)
 - Highly connected genes are used to aid prioritization of human SNPs in homologous genes for genotyping assays
- The knock-out mouse project
 - Highly connected genes can be compared to extant mutant mouse resources and prioritized gene for mouse phenotyping
- Deep sequencing
 - Identify rare gene variants in affected populations

Conclusions

- Constructing and analyzing gene-phenotype networks enables data-driven genome-wide discovery
- The systems genetic approach facilitates understanding of the relations among behavioral phenotypes
- Systems genetics methods enable identification of both causal polymorphic genes and biomolecular processes amenable to therapeutic targeting

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