The Collaborative Cross: What We’ve Learned from Randomized, Structured Populations

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Peracelsus
(Phillippus Aureolus Theophrastus Bombastus von Hohenheim)

1493-1541

Known as the ‘father of toxicology’. The saying “Dosis facit venenum” (The dose makes the poison) is attributed to him. His actual quote translates “All things are poisons, for there is nothing without poisonous qualities...it is only the dose which makes a thing poison.”

therapeutic effect

increasing dose

toxic effect
Claudius Galenus (Galen of Pergamum)
129-217 AD

“But remember throughout that no external cause is efficient without a predisposition of the body itself. Otherwise, external causes which affect one would affect all.”
Classical Model

C57BL/6J — Single genome-based prediction — Genetically Diverse Human Population
Population Model

Genetically Diverse Human Population

Population-based model

Single genome-based model
Outline

Introduction to the Collaborative Cross

Generalized genetic characteristics

Example of ethanol metabolism
Genetic dissection of complex and quantitative traits: from fantasy to reality via a community effort

David W. Threadgill, Kent W. Hunter, Robert W. Williams

Multi-parental recombinant inbred panel

A second resource identified at the CTC organizational meeting is a second-generation recombinant inbred panel. The original RI lines were not developed with complex traits in mind (Bailey 1971). Rather, they were designed to efficiently localize Mendelian traits. With a 1,000-line advanced, multi-parental RI panel, single-gene resolution would be approached if each line archived 100 recombination sites. Such a purposely designed RI panel could serve as a new community mapping resource by combining diverse germ-plasms derived from a variety of inbred strains with the
The Collaborative Cross, a community resource for the genetic analysis of complex traits

Ten Years of the Collaborative Cross

David W. Threadgill*¹ and Gary A. Churchill†
Characteristics of the Collaborative Cross Model

- Genome-wide variation so that all components of systems are interrogated
- Randomization of genetic variation with no minor alleles so causative relationships can be identified
- Sufficiently large to power analysis of modest interactions
- Infinitely reproducible to support data integration and reproduction
Factor Randomization

One of ~250 Independent Collaborative Cross Lines

Collaborative Cross

Nature Genetics, 2004

> 40M SNPs
Power in Numbers

72 lines have been released to public
Founders Equally Represented

Genome Research, 2011; Genetics, 2012; BMC Genomics 2014
Comparison of LD Structures

Extant inbred strains

Chr 1

CC

Chr 19

Genetics, 2012
Initial Proof-of-Concept Experiment

Day 0
45 males from ORNL

Day 1
EtOH metabolism

Day 3
ECG
Urine collection
Blood collection
Feces
MRI

Day 4
Calorimeter

Day 5
Open running wheels

Day 16
ECG
MRI

Day 17
Calorimeter

Day 18
Social novelty
Blood collection
Feces
Tissue collection
DEXA

Influenza susceptibility
Dust mite allergy susceptibility

AJP - Endocrinology and Metabolism, 2011
Initial Proof-of-Concept Experiment

Day 0
45 males from ORNL

Day 1
EtOH metabolism

Day 3
ECG
Urine collection
Blood collection
Feces
MRI

Day 4
Calorimeter

Day 5
Open running wheels

Day 6
Voluntary Exercise

Day 16
ECG
MRI

Day 17
Calorimeter

Day 18
Social novelty
Blood collection
Feces
Tissue collection
DEXA

Influenza susceptibility
Dust mite allergy susceptibility

AJP - Endocrinology and Metabolism, 2011
Video has been removed for posting
OBSERVATION 1: Extreme Transgressive Variation
Average Daily Running Distance

AJP - Endocrinology and Metabolism, 2011
OBSERVATION 2: Disruption of Correlated Traits

Change in Body Fat Composition

Change in Body Fat Percentage

15 km

0.5 km

AJP - Endocrinology and Metabolism, 2011
Initial Proof-of-Concept Experiment

Day 0
45 males from ORNL

Day 1
EtOH metabolism

Day 3
ECG
Urine collection
Blood collection
Feces
MRI

Day 4
Calorimeter

Day 5
Open running wheels

Day 6

Day 7

Day 8

Day 9

Day 10

Day 11

Day 12

Day 13

Day 14

Day 15
Voluntary Exercise

Day 16
ECG
MRI

Day 17
Calorimeter

Day 18
Social novelty
Blood collection
Feces
Tissue collection
DEXA

Influenza susceptibility
Dust mite allergy susceptibility

AJP - Endocrinology and Metabolism, 2011
OBSERVATION 2: Disruption of Correlated Traits

Influenza Infection (A/PR/8)

PLoS Pathogens, 2013
OBSERVATION 3: Emergent Properties

Spontaneous Colitis

The Collaborative Cross as a Resource for Modeling Human Disease: CC011/Unc, a New Mouse Model for Spontaneous Colitis

Allison R. Rogala · Andrew P. Morgan · Alexis M. Christensen · Terry J. Gooch · Timothy A. Bell · Darla R. Miller · Virginia L. Godfrey · Fernando Pardo-Manuel de Villena

Mammalian Genome 25: 95-108, 2014
Host genetic diversity enables Ebola hemorrhagic fever pathogenesis and resistance

Science 346: 987-991, 2014
Initial Proof-of-Concept Experiment

Day 0
45 males from ORNL

Day 1
EIOH metabolism

Day 3
ECG
Urine collection
Blood collection
Feces
MRI

Day 4
Calorimeter

Day 5
Open running wheels

Voluntary Exercise

Day 16
ECG
MRI

Day 17
Calorimeter

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AJP - Endocrinology and Metabolism, 2011
EtOH Clearance Rate

Serum EtOH Clearance Rates (mg/kg/hr)
Genetic Analysis of EtOH Clearance Rate

*Increasing Resolution with Allele Effect Plots*

**Oprd1**

opioid receptor, delta 1
Confirmation of *Oprd1* with Naltrindole

![Box plot showing comparison of Serum EtOH Clearance Rates (mg/kg/hr) between Wild-type and *Oprd1* -/- genotypes. The box plot indicates a significant difference (***) between the two groups.](image-url)
Effect of *Oprd1* on Liver Activity

![Graph A](image)

Graph A: Total Glutathione (nmol/mg protein)

- Wild-type: ~100 nmol/mg protein
- Oprd1 -/-: ~150 nmol/mg protein

** Statistical significance: **

![Graph B](image)

Graph B: Catalase Activity (nmol/min/mg protein)

- Wild-type: ~1000 nmol/min/mg protein
- Oprd1 -/-: ~2500 nmol/min/mg protein

*** Statistical significance: ***
CNS Control of Ethanol Clearance?

1. Gastric ADH

2. Absorption

3. Plasma protein binding

4. Hepatic blood flow

5. Hepatic cell uptake

6. Hepatic cell metabolism

7. Congeners

8. Nutrition

9. Alcoholic liver disease

10. Genetic factors

11. Conjugation

12. Peripheral sites of action

from Clinics in Liver Disease 2, 673 (2005)
Summary: Novel Models

- Extreme transgressive variation
- Disruption of correlated phenotypes
- Emergent disease models
- Models human phenotypic distribution
Why Are Population Models Important for Toxicology?

Variation in dose-response curves

Mouse population-guided resequencing reveals that variants in CD44 contribute to acetaminophen-induced liver injury in humans

Alison H. Harrill, Paul B. Watkins, Stephen Su,
Pamela K. Ross, David E. Harbort, Ioannis M. Stylianou,
Gary A. Boorman, Mark W. Russo, Richard S. Sackler,
Stephen C. Harris, Philip C. Smith, Raymond Tennant,
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Tanupriya Contractor, Timothy Wiltshire, Ivan Rusyn
and David W. Threadgill

Genome Research 19: 1507-1515, 2009
Expanding Uses of the CC

**QTL mapping:** when combined with Diversity Outbred (DO) model derived from CC, fine mapping is now possible at close to single gene resolution. Question of power for highly polygenic traits is still open. Likely only appropriate for oligogenic traits.

**Functional validation/context analysis:** reverse translation of GWAS or other findings to understand function and context.

**Systems integration:** interrogation across phenotypes (molecular and physiological) to understand biological wiring. Requires far fewer lines than QTL mapping since causative drivers of systems are not the goal. More appropriate for polygenic traits.
Data integration over SPACE and TIME by anchoring to a genetic reference population
Acknowledgements

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