Psych 3102
Introduction to Behavior Genetics
Lecture 9
Methodology in Behavior Genetics
Animal studies
HEREDITY

nature (genotype)

quantitative genetics

ENVIRONMENT

nurture

molecular genetics

BEHAVIORAL TRAIT

phenotype

presence & nature of genetic influences

locating and identifying specific genes in the genome
Animal Breeding Studies
Simple genetic breeding experiments used to demonstrate presence of genetic influence on behavior:

1. **selection studies** - animals bred together to try to influence level of a trait (artificial selection not natural selection)
   
a successful selection study proves genetic influence on the trait

2. **inbred line studies** - develop inbred lines (strains) test the lines to see if they differ (by chance) for level of the trait of interest
   
existence of differences between inbred lines for a trait proves genetic influence on that trait
DOGS

Canis familiaris sub-species = breeds

- chow chow
- poodle
- schnauzer
- bulldog
- greyhound
- dalmatian
- collie
- German shepherd
- Great Dane
- Saint Bernard
All breeds of dogs have been produced by artificial selection, a demonstration of the power of this method for bringing about change.

Wolf-like ancestor domesticated ~50,000 ya, guarded camps, removed vermin

Ancestral dog selective breeding began ~ 14,000 ya
• original animals subjected to thousands of years of selective breeding by humans to produce present-day breeds, then inbred within the breed (breed = inbred line)

• breeds differ for many traits:
  - physical appearance
  - size
  - intelligence
  - temperament
  - emotionality
  - activity level
  - aggressiveness

all of these traits must show some genetic influence for them since they breed-true, even after cross-fostering and varying environments

  genetic component of variance
Research by Scott & Fuller (1965)

- 20 years of study into dog behavior

5 breeds of dog:
- fox terrier
- cocker spaniel
- basenji
- beagle
- Shetland sheepdog

variance between breeds much greater than variance within breeds
Methods of study use the following:

- variance within breeds  what does this tell us?
- comparisons between breeds  what can this tell us?
- crosses between breeds to form F$_1$ hybrids  what can this tell us?  would we expect the F1 animals to vary much?  what about F2 animals?
- cross-fostering to test for maternal effects on behavior (an environmental effect)
- behavioral traits:  emotionality, trainability, problem-solving, sexual behavior, sociability

For each measured trait:

 variance within breeds  = a measure of environmental variance
 variance between breeds  
   = a measure of genetic + environmental variance
 variance between – variance within  = measure of genetic variance

ANOVA  analysis of variance
Detour test
Selection studies

- successful selection for a trait provides evidence the trait is heritable = under the influence of genes

  high line: bred for high level of expression of the trait
  low line: bred for low level of expression
  control line: unselected animals, randomly mated within the line (animals originally from same population as selected lines)

Examples

response to alcohol: ethanol sleep time
learning: maze-learning ability
emotionality (anxiety) level: open field behavior
Response to selection for maze-learning in rats

Tolman & Tryon

Selection began 1920's
Selection for ethanol sleep time

Began in 1960's
Open field behavior in mice

• mouse placed in brightly-lit box for 6-minute trials

Fearful mouse
  freezes or shows low activity levels
  defecates a lot       urinates a lot
  stays close to sides of box (+ve thigmotaxis)

Non-fearful mouse
  explores, shows higher activity levels
  defecates less       urinates less
  ventures into middle of arena
Selection for activity level
high line: mate together highest scoring mice for activity in the open field each generation
low line: mate together lowest scoring mice

Selection for defecation level
  high line: mate together highest defecators
  low line: mate together lowest defecators

Results
after 30 generations of selection (separately for each trait)
for activity: 30-fold difference between high and low lines
for defecation: 7-fold difference between high and low line
no overlap in scores between high and low line
Response to selection for open field activity in mice
DeFries (1978)
What else can a successful selection study tell us?

• by analyzing response to selection over the generations:
  1. can get an estimate of number of genes influencing the trait
  2. can get idea about nature of gene action

Examples:
large initial difference between lines in a few generations then leveling off of response indicates fewer genes, possibly non-additive gene effects

smaller differences between lines continuing for many generations indicates more genes, additive gene effects
Selective breeding of house mice has been used to study the evolution of locomotor behavior.

4 replicate lines selectively bred for high voluntary wheel running (High-Runner)
4 bred randomly (Control).

Major changes in High-Runner lines appear to have taken place in the brain rather than in capacities for exercise. Neurobiological profile resembles human attention deficit hyperactivity disorder (ADHD) and is also consistent with high motivation for exercise as a natural reward. Both ADHD and motivation for natural rewards (such as food and sex), as well as drugs of abuse, have been associated with alterations in function of the neuromodulator dopamine, and High-Runner mice respond differently to dopamine drugs. In particular, drugs that block the dopamine transporter protein (such as Ritalin and cocaine) reduce the high-intensity running of High-Runner mice but have little effect on Control mice. In preliminary studies of mice exercised on a treadmill, brain dopamine concentrations did not differ, suggesting that changes in the dopamine system may have occurred downstream of dopamine production (e.g., receptor expression). Brain imaging by immunohistochemical detection of c-Fos identified several key regions (prefrontal cortex, nucleus accumbens, caudate, lateral hypothalamus) that appear to play a role in the differential response to Ritalin and increased motivation for running in High-Runner mice. The activation of other brain regions, such as the hippocampus, was closely associated with the running itself. Running also increased the production of new neurons to apparently maximal levels in the hippocampus, but impaired learning in High-Runner mice.
INBRED STRAINS

Strain A

Strain B

Strain C
Inbred strain studies

Inbred strain (line)
- a population of animals produced by mating related individuals together for many generations
  in mice: 20 generations (5 years) brother/sister, parent/offspring

- animals within the strain are homozygous for most segregating loci and are genetically very similar to each other

Different inbred strains may have different alleles fixed at the loci influencing a behavioral trait

Differences in phenotype between strains will reflect these genetic differences between strains if the trait is influenced by genes

Note: alleles (and hence, phenotypes) are fixed at random, there is NO selection involved in producing inbred strains
Inbred Strain Characteristics

PKC = protein kinase C (signal transduction)  LPS = lipopolysaccharide (elicits immune response)
What can studies of inbred strains tell us?

1. strain differences in phenotype prove genetic influence on the phenotype

2. genetic and environmental variance can be estimated how?

3. crosses between inbred strains will tell us the nature of gene action how?

Examples of traits shown to differ between inbred lines:
- avoidance learning
- maze learning
- open field activity and defecation
Figure 3.5
Mouse in an open field employed by DeFries et al. (1966). The holes near the floor transmit light beams that electronically record an animal’s activity. (Courtesy of E. A. Thomas.)

Figure 10.4
Shuttle box used to study avoidance learning in mice. (From The Experimental Analysis of Behavior by Edmund Fantino and Cheryl A. Logan. W. H. Freeman and Company. Copyright © 1979.)
Diallel design maximizes information from inbred strain studies
- systematic cross-mating of several inbred strains, with reciprocals
- reciprocal cross differences indicate sex-linkage, prenatal maternal influences

Cross-fostering can separate pre- and post-natal maternal effects
Environment can be manipulated to study environmental effects
Use of rat and mouse consomic strains to identify genes

- **consomic strain** = inbred strain that has some portion of its genome replaced by homologous genetic material from another inbred strain via a series of marker-assisted backcrosses

- by introducing genetic material in a systematic way, genes that influence a behavior can be discovered
Series of 4 consomic strains, flanked by parental strains A and B (organism has 4 pairs of chromosomes)
Example
Strain A shows high deficit for long-term fear memory
Strain B shows low deficit for long term fear memory

- make a series of consomic strains that introduce 1 chromosome from Strain B at a time into Strain A animals,
- see which chromosome rescues the deficit
- narrow down the search by introducing smaller & smaller pieces of chromosome
Systematic analysis of emotionality in consomic mouse strains established from C57BL/6J and wild-derived MSM/Ms

A. Takahashi1,2, A. Nishi1,2, A. Ishii1,2, T. Shiroishi1,2 and T. Koide1,2

1Mouse Genomics Resource Laboratory, National Institute of Genetics, Mishima, Shizuoka, *Department of Genetics, The Graduate University for Advanced Studies (SOKENDAI), Hayama, and *Mammalian Genetics Laboratory, National Institute of Genetics, Mishima, Shizuoka, Japan

*Corresponding author: T. Koide, Mouse Genomics Resource Laboratory, National Institute of Genetics, 1111 Yata, Mishima, Shizuoka-ken 411-8540, Japan. E-mail: tkoide@lab.nig.ac.jp

Consomic strains have recently attracted attention as an advantageous method to screen for genes related to developmental, physiological, and behavioral phenotypes. Recently, a new set of consomic strains was established from the Japanese wild-derived mouse strain MSM/Ms and C57BL/6Jcd. By analyzing the entire consomic panel, we were able to identify a number of chromosomes associated with anxiety-like behaviors in the open-field (OF) test, a light-dark box and an elevated plus maze. Detailed observation of the OF behavior allowed us to identify chromosomes associated with those ethological traits, such as stretch attend, rearing, and jumping. Repeated OF test trials have different meanings for animals, and we found that some chromosomes responded to only the first or second trial, while others were consistent across both trials. By examining both male and female mice, sex-dependent effects were found in several measurements. Principal component analysis of anxiety-like behaviors extracted five factors: "general locomotor activity", "thigmotaxis", "risk assessment", "open-arm exploration" and "autonomic emotionality". We mapped chromosomal associations with these five factors of emotionality.

Keywords: Emotionality, consomic mouse strains, wild-derived MSM, genetic mapping, QTL

Received 23 January 2008, revised 23 June 2008, accepted for publication 25 June 2008

Individual differences in most behaviors derive from polygenic influences, rather than Mendelian mutations with large effects (Plomin et al. 2001). To date, a vast number of quantitative trait loci (QTL) related to anxiety-like behaviors have been reported in mice and rats by using F2 intercross, N2 backcross, recombinant inbred strains, and heterogeneous stocks (Fint 2002; Plomin et al. 2001; Valdar et al. 2008). Fint et al. (2005) reviewed several QTL studies and found that most QTL have just a small effect size, contributing approximately 6% of the total phenotypic variance for behavioral and physiological phenotypes. Also, extensive genome-wide high-resolution mapping using heterogeneous stock mice revealed 843 QTL for a variety of phenotypes, including behavior, and found that only 10 QTL had effect sizes of greater than 5%, while 109 QTL had less than 2% (Valdar et al. 2008). Because of this small effect of each QTL, an enormous amount of effort is required to identify quantitative trait genes (QTLs) for behavior.

Consomic strains, also known as chromosome substitution strains, are a favorable resource for investigating QTL; genotyping to map the chromosome is unnecessary, results are reproducible, QTL detection is statistically significant, and making congenic strains is rapid (Belknap 2003; Nadeau et al. 2000). Analysis of consomic strains established from C57BL/6J (B6) and A/J has successfully shown the chromosomes affecting several phenotypes including anxiety-related behaviors (Laarakkers et al. 2006; Ponder et al. 2007; Singe et al. 2004, 2005). Recently, a new set of consomic strains was established, dubbed B6-ChrNMSM consomic panel mice, using a different subspecies group of mouse strain MSM/Ms (MSM) (Takada et al. 2008). In this panel, each of the B6 chromosomes was introduced into the B6 background to encompass the whole genome. MSM was derived from Japanese wild mice (Mus musculus molossinus), and they had not undergone a strong selection history for domestication during breeding. Thus, it was expected that they would retain several behavioral characteristics of wild mice. It is known that several behavioral responses have been changed or sometimes attenuated in standard laboratory strains (Blanchard et al. 1998; Fernandes et al. 2004; Holmes et al. 2000; Koide et al. 2003), and thus, wild-derived mouse strains may offer interesting alternatives for behavioral analysis. We previously showed that MSM exhibited higher spontaneous activity in the home cage, reduced novelty-induced activity and increased freezing and grooming, in a novel situation, difficulty in habituation to novelty, and reduced pain sensitivity compared to B6 (Koide et al. 2000; Takahashi et al. 2006). Consomic strains derived from MSM are expected to be useful for identifying genetic loci associated with the widely diverse phenotypes, some of which may have been lost in the laboratory strains. To date,