Overview

- Models in laboratory research
- The unique position of the mouse as a model
- Mouse genetics 101
- Genetic engineering for hypothesis testing
- Genetic variation

Why Use Models?

- Allows for controlled experiments
- Environmental variables can be controlled
- Dosage or exposures can be controlled
- Experiments can be replicated
Genes

Phenotypes

Environment

Stochastic Processes

Effects of Genes Can Be Complex

Gene \rightleftharpoons \text{Pleiotropy} \rightarrow \text{Phenotypes}

Phenotype \rightleftharpoons \text{Heterogeneity} \rightarrow \text{Genes}

Unique Position of the Mouse

Genetic Tools

Gene Content

Mutagenesis

Histology

Biologic Tools

Protein Content

Engineering Tools

Transgenics

Knockouts

Knockins

DEFLECTION

Genetics

Mutagenesis

DETECTION

CHARACTERIZATION

VALIDATION
Why Mice As an Experimental Organism?

- Hardy
- Requires little space
- Short life cycle
- Easily bred
- High fecundity
- Mammalian species
- Large amount of phenotypic variation
- Easy to genetically engineer

Evolutionary Relationships

- Humans
- Mice
- Xenopus
- D. melanogaster
- C. elegans

The Mus Species Group

- musculus
- castaneus
- bactrianus
- macedonicus (Greece—Iran—Israel)
- spicilegus (Austria, Ukraine—Bulgaria)
- spretus (Spain/N. Africa)
- caroli (Indonesia)
- coelli (India/S. E. Asia)
- cervicolor (Nepal/S. E. Asia)
- booduga (India/Burma/Pakistan)
- dunni (India/Sumatra)
**Genetic Stocks**

- **Outbred**
  - segregating many alleles
  - ex, Swiss mice, Wistar rats
- **Hybrid**
  - isogenic until bred
  - ex, B6D2, B6SJL
- **Inbred**
  - genetically identical (isogenic)
  - ex, C27BL/6J
- **Mutant/Engineered**
  - specific defects
  - may or may not be ‘genetically clean’

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**Exercise A**

You have developed a compound that you think will help to prevent rejection of transplanted hearts, and you want to test this experimentally.

The experiment will involve heart grafts between a donor and recipient mouse (whose own heart is not removed) with a control group and one treated with the compound.

The following mouse strains are available: outbred ICR and CD1 and inbred C57BL/6J, A/J, and FVB/NJ.

Which strains will you use as donor and recipient? Why?

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**Exercise B**

You also need to test the potential toxicity of the compound, and will want to do a long-term study with control and treated mice. You know it is not acutely toxic.

Being a toxicologist, you reason that in this case since you wish to model humans who are genetically heterogeneous, you decide to use outbred genetically heterogeneous ICR mice, the strategy used by virtually all toxicologists.

Do you decide to go with your initial intuition? Why?
Identify the 'Experimental Unit' 

The unit of randomization 

Two experimental units must be capable of being assigned to different treatments 

Could be:  
a cage of animals  
a single animal  
an animal for a period of time  
a well in a tissue culture dish

The Problem With Genetic Heterogeneity

<table>
<thead>
<tr>
<th>Treated</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td>beagle</td>
<td>chicken</td>
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<tr>
<td>mouse</td>
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<tr>
<td>cat</td>
<td>dog</td>
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<tr>
<td>rabbit</td>
<td>toad</td>
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</table>

A Better Design

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<tr>
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<th>Control</th>
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<tr>
<td>beagle</td>
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<tbody>
<tr>
<td>C57BL/6J</td>
<td>C57BL/6J</td>
</tr>
<tr>
<td>A/J</td>
<td>A/J</td>
</tr>
<tr>
<td>FVB/NJ</td>
<td>FVB/NJ</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>DBA/2J</td>
</tr>
<tr>
<td>SWR/J</td>
<td>SWR/J</td>
</tr>
<tr>
<td>SJL/J</td>
<td>SJL/J</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>BALB/cJ</td>
</tr>
</tbody>
</table>

Variable Results With Heart Transplants

"We transplanted hearts of young ... ICR into ... recipient CD1. An outbred strain was selected since such animals are usually heartier and easier to handle ..."

"We are puzzled by our results ... palpable heart beats were evident in the saline group long after acute rejections ... were expected ... Results in the experimental groups varied considerably ..."

Exercise C: Power Calculations for Sample Size

Barbiturate sleeping time

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c (inbred)</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
<td>ICR (outbred)</td>
<td>40</td>
<td>15</td>
</tr>
</tbody>
</table>

What sample size would be needed to detect a 10% change in mean, with a 90% power and 5% significance level using a 2-sample t-test?

Power Calculations for Sample Size

BALB/c (inbred)  23
ICR (outbred)  297
### Types of Genetic Crosses

<table>
<thead>
<tr>
<th>Cross</th>
<th>Matings</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Backcross</td>
<td>A/a X A/A</td>
<td>Linkage analysis; production of congenic strains</td>
</tr>
<tr>
<td></td>
<td>A/a X a/a</td>
<td></td>
</tr>
<tr>
<td>Inbreed</td>
<td>A/A X A/A</td>
<td>Maintenance of an inbred strain</td>
</tr>
<tr>
<td></td>
<td>a/a X a/a</td>
<td></td>
</tr>
<tr>
<td>Intercross</td>
<td>A/a X A/a</td>
<td>Linkage analysis</td>
</tr>
<tr>
<td>Outcross</td>
<td>A/A X a/a</td>
<td>Initial step in strain production and linkage analysis; production of F1 hybrids</td>
</tr>
<tr>
<td></td>
<td>a/a X a/a</td>
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</tr>
</tbody>
</table>

### Making an Inbred Strain

- **P**
- **F<sub>1**
- **F<sub>2**
- **Inbred**

- Independent Assortment

- % Homozygosity: 0, 50, 100
- 98.7%

### Inbred Strain

- 20 or more generation of brother x sister mating
- Isogenic
- Homozygous
- Phenotypically uniform
- Long-term stability
- Unique strain characteristics
- International distribution
- Easily identifiable

- 'immortal clone of genetically identical individuals'
'The introduction of inbred strains into biology is probably comparable in importance with that of the analytical balance into chemistry'

Gruneberg (1952)

Outbred Stocks

Widely used
Characteristics not widely understood

Advantages
- cheap
- easily available (no alternative for some species)
- breed well
- outbred like humans (?????)

Disadvantages
- unknown genetics (heterozygosity)
- subject to genetic change (inbreeding, drift, selection)
- lack of reliable background information
- genotype not internationally distributed
- not histocompatible
- not easily identifiable

Outbred Mice

CF1
ICR
CD1
MF1
Swiss-Webster

B6D2
Engineered Models

Allows controlled experimental testing of
- specific genes
- specific environmental conditions or exposures

Ideally suited to test specific hypothesis generated from human population studies or other laboratory findings

Engineered Models

Transgenics
- usually used to over-express genes
- can be global or tissue-specific
- can be temporally regulated

Knockouts/knockins
- usually used to inactivate genes
- can be global or tissue-specific
- can be temporally regulated
- can introduce genes into a foreign locus
- can make amino acid modifications

Terminology

Transgenic
(Carries foreign DNA; may or may not be mosaic; two parents)

Mosaic
(May or may not carry foreign DNA; two parents)

Chimeric
(May or may not carry foreign DNA; more than two parents)
Exercise D

You obtain a mouse line from your collaborator that carries a knockout in PPAR-gamma. The collaborator made the mice by gene targeting in 129 strain derived ES cells. He made chimeras with the cells by blastocyst injection into C57BL/6J embryos. After birth, he bred the chimeras to C57BL/6J mice and then intercrossed heterozygous carriers to make the PPAR-gamma knockout homozygous line.

You need wild-type controls for your experiment. What mice do you use for this? Why?

Making a Congenic Strain

Donor Recipient

P  F1  N1  N2  N3  N10  Congenic

Independent Assortment

Residual Heterozygosity
Genetic Variation

Significant extant genetic (and thus phenotypic) variation exists across mouse strains. Can be used to identify a more 'accurate' model of specific human exposures or responses. Phenotypic variation across inbred mouse strains is as great or greater than humans for virtually any trait.

Azoxy methane Induction of Mouse Colorectal Tumors

4 X 1 weekly IP injections (10mg/kg) 22 week latency period

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Tumor</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoxy methane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylazoxymethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyldiazonium (alkylating agent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbonium (methylating agent)</td>
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</table>
Just as one person is not representative of the human population...

AKR/J  SWR/J  A/J

...one mouse strain is not representative of the human species.

Colorectal Cancer Susceptibility

- Swiss strains
- Castle's strains
- Strains from Asia
- Other strains
- C57-related strains
- Wild-derived strains

Mouse Phenome Database

- Search MPD
- Browse, discover, find, download
- Literature, data, and information
- Information for Participants
- Report a Bug
- Contact Us
- Feedback, Comments, and Suggestions
- Search MPD

Future Directions