Mouse as a Genetic Model System for Mammalian Genetics

1. Genetic structures in mice are not found in nature
   - Mapping in inbred strains
   - Recombinant inbred strains
   - Congenic strains/ speed congenics ("marker-assisted breeding ")
   - Haplotype mapping
   - Quantitative trait loci meet genomics

2. Make your own Genotype
   - Transgenesis
   - Gene targeting
   - Chromosome engineering

3. Make your own phenotype
   - Mutagenesis – insertion/ENU
   - Phenome project

4. Comparative genetics
   - Comparative maps/ sequence
   - Biology – comparative phenotypes
     - Functions of a genome
     - Evolution
1. Special Genetic Structures in Mice: Mice can be inbred to homozygosity

“A strain shall be regarded as inbred when it has been mated brother x sister (hereafter called bxs) for twenty or more consecutive generations (F20), and can be traced to a single ancestral breeding pair in the 20th or a subsequent generation. Parent offspring matings may be substituted for bxs matings provided that, in the case of consecutive parent x offspring matings, the mating in each case is to the younger of the two parents. Exceptionally, other breeding systems may be used, provided that the inbreeding coefficient achieved is at least equal to that at F20 (0.99).”

2. Hundreds of inbred strains of mice capture many different types of genetic information

>450 inbred strains

http://www.nature.com/ng/mouse/ng0100_treefinal.pdf

Nomenclature and genetic content

An **intercross** between two parental (P₁) inbred strains produces an F₁ hybrid

\[
\begin{align*}
\text{AABB} & \times \text{aabb} \\
\end{align*}
\]

Strain: C57BL/6J  
(B6)  
Strain: C3H/HeJ  
(C3)

\[
\begin{align*}
\text{AaBb} \\
\end{align*}
\]

\[
\begin{align*}
\text{Strain (B6;C3)F₁} \\
\end{align*}
\]

Nomenclature and genetic content

**An advanced intercross** produces Fₙ hybrids

\[
\begin{align*}
\text{AaBb} & \times \text{AaBb} \\
\end{align*}
\]

\[
\begin{align*}
\text{Strain (B6;C3)F₂} & \\
\text{Strain (B6;C3)F₁} \\
\end{align*}
\]

\[
\begin{align*}
\text{F₂ generation} \\
\text{F₃ generation} \\
\text{F₄ generation} \\
\end{align*}
\]

**A backcross** produces Nₙ generations

\[
\begin{align*}
\text{AABB} & \times \text{AaBb} \\
\end{align*}
\]

\[
\begin{align*}
\text{B₆ is 100% B₆} & \\
\text{F₁ is 50\% B₆} \\
\end{align*}
\]

\[
\begin{align*}
\text{N₂ generation} \\
\text{N₃ generation} \\
\text{N₄ generation} \\
\end{align*}
\]
Mapping in inbred mice
For any polymorphic marker, crosses are completely phase-known

Recombinant inbred mouse strains
resource for genotype and phenotype

(20 generations of brother x sister mating)
Mapping in Recombinant inbred (RI) strains

QTX: software for complex trait analysis
by Jane M. Meer, Robert H. Cudmore, Jr., and Kenneth F. Manly
http://mapmgr.roswellpark.org/mmQTX.html

Strain distribution patterns for Chr 6 in the AKXD RI strain set.

Mouse Genome Informatics:
http://www.informatics.jax.org/searches/riset_form.shtml
Benefits of RI strains:
• "Pre-genome scan" – cumulative information
• Reassay the "same" individual many times (find true mean and deviation for variable traits)
• Highly beneficial for quantitative traits
• Stock of genetic variation (recombinant congenic mice)

Limitations of RIs:
• Small strain sets have limited statistical power.
• Mapping is relatively low resolution on the first pass.

Congenic mice are made by repeated backcrossing while selecting for a specific locus of parental type

10-12 generations of backcrossing/selection
“Speed” congeneric mice are made by repeated backcrossing while selecting for a specific locus of parental type AND screening large sets of progeny with a genome scan to identify those mice which are most inbred

4-5 generations using marker assisted selection to choose the progenitors of the next generation

Chromosome pairs from five different F3 individuals assessed by a genome scan – which should be selected for the next generation?


Overcoming limited statistical power of RI strains

QTX: software for complex trait analysis by Jane M. Meer, Robert H. Cudmore, Jr., and Kenneth F. Manly

Melesic et al., Genomics 62:34-41.
Congenic mice can be used to confirm and refine localizations made with other approaches.

Further, they demonstrate sufficiency of an individual locus to cause a phenotype (e.g. in QTL studies).


Increased resolution from RI mapping

1. RIX - Threadgill

2. Complex Trait Consortium
   - 8-way cross
   - 2048 RI strains (256 on the shelf)
   - www.complextrait.org/Workshop1.pdf
Consomic mice are made by repeated backcrossing to place one chromosome pair from strain “A” onto the genetic background of strain “B”


Haplotype structure in the world of inbred mice

Wiltshire et al., PNAS 100:3380-3385 (2003); www.gnf.org/SNP/
Large haplotype blocks correspond to regions of low SNP density

Wiltshire et al., PNAS 100:3380-3385 (2003); www.gnf.org/SNP/

Mapping with haplotypes: Tyr

Figure 5: Association between a single haplotype and the albinism phenotype caused by a mutation at the tyrasinase locus. Columns show SNPs discovered in ten 500-kb assays with positions (kb) relative to the centre of the genomic segment containing the gene (GenBank accession GI:12865258). The causal mutation (Cys103Ser) is located at +32.6 kb. The association between phenotype and ancestral haplotype for 12 strains would be sufficient to identify a haplotype background and 'critical region' of ~560 kb (including the assays from ~120 kb before Tyr to 200 kb after Tyr) likely to contain the albinism mutation.

Complex traits / polygenic traits / QTL / genetic modifiers

Many traits are influenced by more than one locus, a condition that can't be mapped using standard pedigree analysis. Experiments with maize and *Lycopersicon* demonstrate an approach to this problem that can be used to map such quantitative trait loci (QTLs) using gene dense maps spanning the entire genomes of experimental organisms. This figure demonstrates linkage to three quantitative traits in tomato.

![Graphs showing linkage to three quantitative traits in tomato](image)

**Figure 4.** Likelihood intervals for QTLs mapped in F$_2$, F$_3$, California, and F$_3$Brazil trials. The position of a QTL is shown as the interval over which the LOD score is within 1 or 2 logarithms of the base LOD score, with whiskers (lines extending beyond bars) indicating the region over which the model's probability of giving rise to the data is at least 50 or 100-fold less than at the most likely position. Bars indicate 1-LOD (10-fold) likelihood intervals, with whiskers (lines extending beyond bars) indicating 2-LOD (100-fold) likelihood intervals. Individual QTLs have been named according to trait (MF = mass per fruit; FW = fruit diameter; SI = soluble solids concentration; pH = pH), chromosome (1 to 12), and then sequentially by order of discovery (a, b, etc.). Using this nomenclature, QTL locations have been cross-referenced to the genome map presented in Table 2-4. (Top) Mass per fruit or fruit diameter (left side of chromosome), and soluble solids concentration (right side of chromosome). (Bottom) Fruit pH, with marker names and map distances indicated.
"PURE" positionally cloned QTL in *lypersicum*

Fig. 2. High-resolution mapping of the *fw2.2* QTL. (A) The location of *fw2.2* on tomato chromosome 2 in a cross between *L. esculentum* and a NIL containing a small introgression (gray area) from *L. pennelli* [from (8)]. (B) Contig of the *fw2.2* candidate region, delimited by recombination events at XO31 and XO33 [from (8)]. Arrows represent the four original candidate cDNAs (70, 27, 38, and 44), and heavy horizontal bars are the four cosmids (cos62, 84, 69, and 50) isolated with these cDNAs as probes. The vertical lines are positions of restriction fragment length polymorphism or cleaved amplified polymorphism (CAPs) markers. (C) Sequence analysis of cos50, including the positions of cDNA44, ORFX, the A-T-rich repeat region, and the "rightmost" recombination event, XO33.

Intercross analysis shows that Hpi1 is epistatic to Hpi2.

<table>
<thead>
<tr>
<th>Genotype at Hpi1, Chromosome 5</th>
<th>A/A</th>
<th>A/B</th>
<th>B/B</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype at Hpi2, Chromosome 5</td>
<td>A/A</td>
<td>33.5 ± 4.6</td>
<td>35.6 ± 4.8</td>
<td>35.6 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>A/B</td>
<td>35.6 ± 6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B/B</td>
<td>35.0 ± 3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>35.6 ± 4.8</td>
<td>35.6 ± 6.9</td>
<td>35.0 ± 3.0</td>
</tr>
</tbody>
</table>

| Genotype at Hpi1, Chromosome 13 | A/B | 28.9 ± 5.0 | 35.7 ± 3.0 | 37.8 ± 4.8 | 34.9 ± 2.3 |
|--------------------------------|-----|           |            |            |          |
| B/B                            | 42.5 ± 4.1 | 44.7 ± 5.3 | 69.9 ± 5.5 | 54.8 ± 4.3 |
| Totals                         | 32.0 ± 3.2 | 37.6 ± 2.3 | 49.0 ± 4.3 | 39.5 ± 1.9 |

*a Avg. number of PMN per h.p.f. ± s.e. are given for (n) animals of each genotype class.*

Mouse phenome project

MPD Priority Set of Strains

<table>
<thead>
<tr>
<th>GROUP A</th>
<th>GROUP B</th>
<th>GROUP C</th>
<th>GROUP D</th>
</tr>
</thead>
<tbody>
<tr>
<td>B17/129S1/SvJ</td>
<td>B35 Akr/J</td>
<td>D41 B6/J 8</td>
<td>none BTBR T′ t/tf</td>
</tr>
<tr>
<td>B68 A/J</td>
<td>E4 C57L/J</td>
<td>E9 C57Bl/10J</td>
<td>C57B6/cdJ</td>
</tr>
<tr>
<td>B76 BALB/cByJ</td>
<td>E7 C58/J</td>
<td>E2 C57BKS/J</td>
<td>E5 C57Br/cdJ</td>
</tr>
<tr>
<td>B47 C3H/HeJ</td>
<td>F25 BALB/c</td>
<td>E7 C58/J</td>
<td>B110 CE/J</td>
</tr>
<tr>
<td>E12 C57Bl/J</td>
<td>F25 MOLF/Bl</td>
<td>B55 CBA/J</td>
<td>B29 Fv/J</td>
</tr>
<tr>
<td>F22 CAST/Ei</td>
<td>A14 NOD/Bl</td>
<td>F13 C3H/HeJ</td>
<td>F34 B6F1Ms</td>
</tr>
<tr>
<td>F25 DBA/2J</td>
<td>B112 NZB/Bl</td>
<td>F23 C57J/KJ</td>
<td>E10 MA/MJ</td>
</tr>
<tr>
<td>D74 FVB/J</td>
<td>F43 PERA/Ei</td>
<td>D7 LPP/J</td>
<td>A16 NON/LJ</td>
</tr>
<tr>
<td>A27 PL/J</td>
<td>D75 PL/J</td>
<td>F30 MSR/LJ</td>
<td>B114 NZV/Bl</td>
</tr>
<tr>
<td>A29 SM/J</td>
<td>B37 SWR/J</td>
<td>D100 RII/Bl</td>
<td>F34 PAWek/J</td>
</tr>
<tr>
<td>F34 SPFRET/Ei</td>
<td>B33 SWR/J</td>
<td>F38 WSB/Ei</td>
<td>B36 SEA/GenJ</td>
</tr>
</tbody>
</table>

See explanation of color coding.
BALB/cJ may be used at investigator's discretion.
129S1/SvJ was renamed in Feb '01. Previous name was 129S1/SvJ.
BTBR T′ t/tf was renamed in May '02. Previous name was BTBR+/+t/tf.

www.jax.org/phenome

Transgenesis/ gene-targeting/ chromosome engineering


Micro-injection of DNA into a male pronucleus to create a transgenic mouse.

http://www.med.ic.ac.uk/db/dbbm/tgunit.htm

Embryonic stem cells can be manipulated in culture, then injected into mouse blastocysts to form part of the inner cell mass (ICM). The ICM will form the embryo proper while the surrounding trophoderm cells contribute to extra-embryonic structures (placenta).

Hogan et al., Manipulating the Mouse Embryo, CSHL Press
Chimeras formed from ES cells and host cells.
A chimeras has four parents, but individual cells have genetic information from only one of the two pairs.

“Knock out” mice create null alleles to study loss of function.

Manipulating the germ-line in embryonic stem cells
Conditional knock-outs allow a much greater range of studies for similar investment of effort.

**Diagram:***

- **Bgl II**
- **Apa I, Kpn I**
- **Xba I**

Mate to transgenic mice in which Cre recombinase is controlled by a tissue-specific promoter.

A null allele of *Cbr1* can now be created in a specific tissue type.

**Diagram:***

"Knock-in" mice (mutations, reporters), allow precise temporal and spatial regulation of gene expression.

**Diagram:**

Promoter

Promoter
“Two stage” strategies control gene expression in space and time

a. Cre-recombinase with a cell type-specific promoter plus marker gene

b. Transcriptional activators under pharmaceutical control

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**The temporal requirement for endothelin receptor-B signalling during neural crest development**  
Shin et al., Nature 402:496, 1999
Fig. 8. Targeting lox sites asymmetrically by homologous recombination in ES cells provides target sites to create a reciprocal translocation by expressing Cre recombinase.

Mapping Genome Function: Creating Phenotypes using Mutagenesis

Mutagenesis provides a means of generating new phenotypes in mouse.


1. Sources of mutations
   • Spontaneous, frequency is 10-5/locus/generation, all types of mutations;
   • Radiation, frequency is dose dependent, primarily chromosomal rearrangement;
   • Chemical, ENU gives point mutations at 1/600 gametes per locus at some loci

2. Targets/ mutation types
   • Visible single gene dom. or recessive
   • Allelic series
   • Biochemical pathway
   • Sensitization (Shedlovsky A, McDonald JD, Symula D, Dove WF. Mouse models of human phenylketonuria. Genetics. 1993. 134:1205-10)
Mutagenesis provides a means of generating new phenotypes in mouse.

3. Screens
   - Specific locus test
   - MutaMouse/ Big Blue
   - SHIRPA
   - Special targeted screens
   - Dominant vs. recessive (1st vs. 3rd generation)
   - Mutagenesis in combination with deletion (recessives in first generation)

4. Breeding schemes
   - Recessive over deletion;
   - Modifier (dominant mutation modifies another mutation)
   - Sensitization (recessive mutations in genes that interact in a pathway/ allelic

Large mutagenesis centers, see Trans-NIH Mouse Initiative

- Mouse Genome Center, ENU Mutagenesis Programme, Harwell, http://www.mgu.har.mrc.ac.uk/mutabase/
- German ENU Mutagenesis Center, http://www.gsf.de/isg/groups/enu-mouse.html
Enhancer, gene and promoter trapping

Uncovering recessive mutations with a deletion/ENU mutagenesis screen
ENU mutagenesis in balancer strains
