Genetic Drift: What It Is and Its Impact on Your Research

Technical Information Services

May 11, 2017
The Jackson Laboratory’s Mission

“To discover precise genomic solutions for disease and empower the global biomedical community in the shared quest to improve human health.”

Performing Research
Investigating genetics and biology of human disease

Providing Resources
JAX® Mice Clinical & Research Services, online data resources, technical publications, and more

Educating Scientists
World-class courses, internships, and other programs
JAX® Mice
The *Gold Standard* for Biomedical Research

- NIH-funded resource
- >8,000 strains and growing
  - 2.7 million mice shipped annually
- Unsurpassed genetic quality & animal health
- Best characterized & referenced ~100 new pubs/week
- Common inbred strains (C57BL/6J, BALB/cJ, DBA/2J) support development/collection of specialty strains and other valuable community research resources
Online Resources to Expedite Research

- **JAX® Mice Database**
  www.jax.org/mouse-search

- **Mouse Genome Informatics**
  www.informatics.jax.org

- **Mouse Phenome Database**
  www.jax.org/phenome

- **Others, including:**
  - JAX-Clinical Knowledgebase
  - Mouse Tumor Biology Database
Today’s Learning Goals

- Recognize genetic background of your mouse strain
  - Use proper nomenclature
  - Select appropriate controls

- Implement strategies to reduce genetic drift and increase experimental reproducibility
What is your role?  

What do you hope to learn today?
Genetic Drift...Friend or Foe?

Species Diversity

Phenotypic Diversity

Data Diversity
What is Genetic Drift?

- “the constant tendency of genes to evolve even in the absence of selective forces. Genetic drift is fueled by spontaneous neutral mutations that disappear or become fixed in a population at random”
  - Lee Silver, “Mouse Genetics” Oxford University Press, 1995
    - [www.informatics.jax.org/silverbook/](http://www.informatics.jax.org/silverbook/)

- Single base changes, deletions, duplications, inversions in the DNA
  - Mistakes in meiosis, DNA repair
Genetic Drift and Colony Size
Small colonies are more vulnerable to fix a mutation

For any given mutation, \( \text{\textcolor{blue}{\text{\textbullet}}} \) = heterozygous mutation

Large Colony

Small Colony
Genetic Drift and Colony Size
Small colonies are more vulnerable to fix a mutation
Visible Genetic Drift

Coat Color Mutations

C57BL/6J-\textit{A}^{w-J}/J
\textit{(000051)}

B6(Cg)-\textit{Tyr}^{-2J}/J
\textit{(000058)}

C57BL/6J
\textit{(000664)}

C57BL/6J-\textit{Lyst}^{bg-J}/J
\textit{(000629)}

C57BL/6J-\textit{Kit}^{W-v}/J
\textit{(000049)}
How Rapidly Do Colonies Drift?

“Visible” mutation example

- Using spontaneous mutation rates in coat color genes,
  - Measured $\sim 1.1 \times 10^{-5}$ mutations/locus/gamete/gen.

- Assuming $\sim 25,000$ genes in mice,
  - $(1.1 \times 10^{-5}$ mutations/locus/gamete/gen.)*$(25,000$ loci)$
  - $0.275$ mutations/gamete/gen.
  - $1$ mutations/$3.64$ gametes/gen.

- *1 phenotypic mutation arises every 1.8 generations*
  - Likely underrepresents overall mutation rate due to visibility of mutation

Drake JW et al., 1998, *Genetics* PMID 9560386
How Rapidly Do Colonies Drift?

- Mice have a high rate of spontaneous mutation
- Approx. 25% chance that new mutations will become fixed
- New mutations in coding sequence become fixed every 6-9 generations
  - (Assumptions: inbreeding; small breeding population)
How Rapidly Do Colonies Drift?

“Invisible” mutation example

- Using whole genome sequencing of C57BL/6J,
  - Measured 2 samples separated by 69 filial gens.
- Differences found
  - 669 SNPs (~ 10/gen.)
  - 272/669 SNPs were in genetic coding & non-coding regions
  - 7/272 SNPs altered DNA coding sequence or RNA splicing
- ~1 “impactful” mutation every 10 generations
  - Likely underrepresents overall mutation rate because the analysis did not include non-SNP mutations (deletions, inversions, CNV changes).
Genetic Drift: Substrain Divergence

**Substrains**: Branch of an inbred strain known or suspected to be genetically different from the parent colony.

**Colonies are considered substrains when** . . .

1) Separated from the parent colony for 20+ generations

2) Phenotypic differences with the parent colony are discovered

**Nomenclature**: Strain name “/” Lab code(s)

e.g. **CBA/CaGnLeJ**

<table>
<thead>
<tr>
<th>LAB CODE</th>
<th>ORGANIZATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crl</td>
<td>Charles River Laboratories</td>
</tr>
<tr>
<td>Hsd</td>
<td>Envigo (formerly Harlan Laboratories)</td>
</tr>
<tr>
<td>J</td>
<td>The Jackson Laboratory</td>
</tr>
<tr>
<td>N</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>Rj</td>
<td>Centre D’Elevage R. Janvier</td>
</tr>
<tr>
<td>Tac</td>
<td>Taconic Farms, Inc.</td>
</tr>
</tbody>
</table>

Parent strain

Substrain designations (cumulative)

Lab maintaining strain

Institute for Laboratory Animal Research (ILAR) Lab Codes

**JAX.org** | THE JACKSON LABORATORY
C57BL/6 Substrain Divergence
“Invisible” Genetic Drift
Case Study #1: Sensitivity to infection

C3H/HeJ (000659) +LPS
Endotoxin resistant

C3H/HeOuJ (000635) +LPS
Endotoxin sensitive

Tlr4\(^{Lps-d}\) (1958-1965)

1952

“Invisible” Genetic Drift in C57BL/6
Case Study # 2: Alteration of presynaptic protein α-synuclein (Snca)

- C57BL/6J Genomic DNA from The Jackson Laboratory
  Wild-type Snca

- C57BL/6NCrl Mice from Charles River, Margate, UK
  Wild-type Snca

- C57BL/6JOlaHsd Mice from Harlan, Bicester, UK
  Deletion of Snca – No visible phenotype but…

**SNCA protein:** implicated in a range of neurodegenerative diseases; primary structural component of Lewy bodies found in Parkinson’s disease brains

“Invisible” Genetic Drift in C57BL/6
Case Study # 3: Retinal degeneration in C57BL/6N substrains

**Crb1** (crumbs-like 1)
- Localized to Muller cells and photoreceptor (PC) inner segments
- Mutations in CRB1 associated with retinal diseases in man
  - Retinitis pigmentosa
  - Leber congenital amaurosis

**Crb1^{rd8}**
- Single base deletion
- Shorter PC inner & outer segments as early as two weeks
- Progressive, spotty retinal degeneration

http://crfb.univ-mrs.fr/Crumbs/section/en/CRB1_function/105

“Invisible” Genetic Drift in C57BL/6
Case Study # 3: Retinal degeneration in C57BL/6N substrains

C57BL/6J: Crb1 wild-type

C57BL/6N: Crb1<sup>rd8</sup>/Crb1<sup>rd8</sup>

“Invisible” Genetic Drift
Case Study # 4: Response to high-fat diet in C57BL/6

C57BL/6J Outgains C57BL/6NJ

C57BL/6J: Nnt loss-of-function


Impaired Glucose Tolerance:
C57BL/6J > C57BL/6NJ

The Mice Next Door

- You don’t have enough C57BL/6J mice for your experiment so you got a few from another lab
- The other mice gave really robust responses
- Why do the mice differ in response, even though they are the same strain?
Substrain Development

C57BL/6

Lab A
10 Generations Sibling Matings

Lab B
10 Generations Sibling Matings

20 generations apart
Selecting Proper Controls
Case Study # 5: C57BL/6 control selection

Influence of Mapk9 (Jnk2) on acetaminophen-induced liver injury (AILI)

Selecting Proper Controls
Experimental conclusions may be in opposition

Effects of Mapk9 (Jnk2) on acetaminophen-induced liver injury (AILI)

Selecting Proper Controls
Experimental conclusions may be in opposition

Effects of Mapk9 (Jnk2) on acetaminophen-induced liver injury (AILI)

A Recent High-Profile Example:
Case Study # 6: Copy number variant confounds results

Dock2 copy number variant
duplication of exons 28 and 29) in a commercial C57BL/6 strain

Multiple hematopoietic phenotypes unrelated to the targeted genes

Increased CD8 memory T cells
Loss of marginal zone B cells
More Published Examples

Research report

Further phenotypical characterisation of two substrains of C57BL/6J inbred mice differing by a spontaneous single-gene mutation

Frans Sluyter a, Charlotte C. M. a

a Department of Psychoneuropharmacology, Genétique, Neurogénétique et Comportement, Orléans, Cedex 2, France

---

Research report

Generation and characterization of pilocarpine-sensitive C57BL/6 mice as a model of temporal lobe epilepsy

Marion Bankstahl1,2, Christine J. Müller1,2,3, Esther Wilk1, Klaus Schlattner1,2,3

1 Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine Hannover, Germany
2 Center for Systems Neurosciences, Hannover, Germany
3 Department of Experimental Mouse Genetics, Helmholtz-Centre for Infection Research, Braunschweig, and University of Braunschweig, Germany

---

Genetic Differences among C57BL/6 Substrains

Kazuyuki MEKAKatsumi NAKAO

RIKEN BioResource Research Center

---

The C57BL/6J Mouse Strain Background Modifies the Effect of a Mutation in Bcl212

Stefanie J. Navarro, Tuyen Trinh, Charlotte A. Lucas, Andrea J. Ross, and Grant R. MacGregor

Department of Developmental and Cell Biology, School of Biological Sciences, American University of Beirut, Beirut, Lebanon

---

Spontaneous deletion of epilepsy gene orthologs in a mutant mouse with a low electroconvulsive threshold

Yan Yang1, Barbara J. Beyer1, James F. Otto2, Timothy P. O’Brien2, Verity A. Letts2, H. Steve White2 and Wayne N. Frankel1

1 The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04843, USA and 2 Anticonvulsant Drug Development Program, Department of Pharmacology and Toxicology, University of Ulster, Salt Lake City, Utah, USA

---

Human Molecular Genetics, 2005, Vol. 12, No. 9

DOI: 10.1093/hmg/ddg118
## C57BL/6 Publications

<table>
<thead>
<tr>
<th>SEARCH TERM</th>
<th>PUBMED ENTRIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>37122</td>
</tr>
<tr>
<td>C57BL/6ByJ</td>
<td>112</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>16390</td>
</tr>
<tr>
<td>C57BL/6JOlaHsd</td>
<td>53</td>
</tr>
<tr>
<td>C57BL/6JBomTac</td>
<td>11</td>
</tr>
<tr>
<td>C57BL/6JRj</td>
<td>7</td>
</tr>
<tr>
<td>C57BL/6N</td>
<td>1182</td>
</tr>
<tr>
<td>C57BL/6NCrl</td>
<td>71</td>
</tr>
<tr>
<td>C57BL/6NJ</td>
<td>11</td>
</tr>
<tr>
<td>C57BL/6NHsd</td>
<td>41</td>
</tr>
<tr>
<td>C57BL/6NTac</td>
<td>78</td>
</tr>
</tbody>
</table>

**Complete nomenclature benefits everyone!**

Based on May 1, 2017 PubMed citations search (without limits)
Which Genetic Controls to Use?

- I want to study the effect of GeneX on blood pressure. My GeneX KO has been bred hom x hom for 10 consecutive generations.

- Which wildtype/genetic controls should I use?
  - A. Controls? I don’t need controls!
  - B. C57BL/6 mice
  - C. Outbred mice
  - D. A suitable inbred, F1, or F2 hybrid strain
  - E. A littermate
  - F. Answer not listed
Genetic Drift and Substrains

- Spontaneous mutations can be overt or hidden
  - only apparent by physiological assay

- Substrains can vary significantly genetically and phenotypically

- Know what substrain backgrounds your strains are, and use the proper control
How to Detect Genetic Drift

- Comparing whole genome sequence data
  - Single Nucleotide Polymorphism (SNP) scans won’t do it
- Look for phenotypic differences
Minimizing Genetic Drift

Genetic change can’t be stopped, but it can be slowed down!

- Maintain pedigrees and detailed colony records
Maintaining a Pedigreed Colony

Single Established Colony - any strain type

*sister-brother mating only*

Mutations become fixed more rapidly in sister-brother pedigrees
- More easily identified/more easily removed
Minimizing Genetic Drift

Genetic change can’t be stopped, but it can be slowed down!

- Maintain pedigrees lines and detailed colony records
- Watch for phenotypic changes in mutants and controls
- Refresh breeders frequently (~10 generations)
- Avoid selection pressure
- Verify genetic background with genome scanning
- Cryopreserve unique strains
True or False?

- Large production colonies that breed mice professionally do not experience genetic drift.
The Jackson Laboratory’s Genetic Stability Program (GSP)

Frozen embryos used to refresh foundation stock every five generations

US patents 7592501, 8110721
Genetic Stability Program Works!

Whole genome sequencing on C57BL/6J genomic DNA

- 1984 @ F154 (pre GSP) } 69 gen.
- 2003 @ F223 (GSP “Eve”) } 7 gen.
- 2012 @ F230 (post-GSP)

Evaluated high quality single nucleotide variants (SNPs)

 Constant mutation rate in pre- & post-GSP period
 Mutations accumulate more slowly post-GSP

JAX Genetic Stability Program
Summary

- Spontaneous mutations occur frequently and can be overt or hidden
  - Implement breeding and colony maintenance strategies that minimize genetic drift
- Substrains can vary significantly genetically and phenotypically
  - Know the substrain that you use, and use the proper control
JAX® Mice & Services: Leading Experts in Mouse Modeling

- CRISPR/Cas9 Model Generation
- Common Inbred and Specialty JAX® Mice
- Study-ready, Aged C57BL/6J Mice (25-78 wks)
- Inbred, Outbred and B6J Nude Mice
- Mouse Genome Scanning
- Neurobiology Models and Resources
- NSG™ & NRG Mouse Model Variants
- Cryopreservation and Recovery
- Basic and Complex Mouse Breeding, Speed Congenics, and Rederivation
- Humanized Mice, Patient-Derived Xenograft Preclinical Models and Therapeutic Drug Evaluation

THE JACKSON LABORATORY
Upcoming JAX Webinars™

Subscribe to the monthly webinar announcements email list: https://subscribe.jax.org/

- Research Using Aged B6 Mice: Considerations, Applications, and Best Practices
  - May 18, 2017, 1:00 pm (ET); 5:00 pm (GMT)

- Efficient Mouse Colony Management
  - May 23, 2017, 6:30 am (ET); 10:30 am (GMT); 12:30 pm (CEST); 4:00 pm (IST)

- Modeling HIV, Ebola and Other Infectious Diseases in Mice
  - Jun. 1, 2017, 2017, 1:00 pm (ET); 5:00 pm (GMT)

- CRISPR/Cas: Moving from Founder Mice to Phenotyping
  - Jun. 13, 2017, 9:00 am (ET); 1:00 PM (GMT); 3:00 pm (CEST)

- Predictive Cancer Models Using Patient-derived Xenograft Mice
  - Jun. 22, 2017, 1:00 pm (ET); 5:00 pm (GMT)

www.jax.org/education-and-learning/webinars | THE JACKSON LABORATORY
Thank You!

JAX™ Mice, Clinical & Research Services
www.jax.org/jaxmice

International Suppliers of JAX™ Mice

Looking for a local source of JAX™ Mice in Asia, Australasia, or Europe?

Contact the authorized international JAX™ Mice supplier for your country.