Upcoming JAX Webinars™


- July 23, 2015, 1pm ET USA
  Reproductive Biology of the Laboratory Mouse

- August 6, 2015, 1pm ET USA
  Mouse Phenome Database: Knowledge to Drive Discovery

- August 13, 2015, 1pm ET USA
  Cutting Edge Human Disease Modeling Using the NSG Mouse
Dear JAXY: How Rapid is Genetic Drift in Mouse Colonies?

By Jim Yeaston, Ph.D.

Genetic drift is a mouse geneticist’s nightmare. It is a subtle, insidious force that causes separated populations of a mouse strain to diverge genetically, and is the basis for substrain development. From a practical standpoint, it can confound our experimental results, leading to false conclusions and irreproducible results. Recently, while preparing materials for a webinar on protecting mouse colonies from genetic drift, I asked myself the question, “How rapidly do colonies drift?” What I discovered surprised me, and, I expect, may surprise other researchers, too.

Mice have a high mutation rate.

In order to understand how rapidly drift occurs in mice, we first need an estimate for the rate at which mutations arise spontaneously in mice. Spontaneous mutation rates in mice have been estimated using various methods. Some studies have estimated spontaneous mutation rates in mice based on genetic divergence between mice and other species over evolutionary time (see, for example Kumar and Subramanian (2002)).
The Jackson Laboratory’s Mission

Performing Research
Investigating genetics and biology of human disease

Providing Resources
JAX® Mice Clinical & Research Services, bioinformatics data, technical publications and more…

Educating Scientists
World-class courses, internships and other programs

www.jax.org/courses
JAX® Mice
The Gold Standard for Biomedical Research

- NIH funded resource
- >7,000 - 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  
  [jaxmice.jax.org/](jaxmice.jax.org/)

- Mouse Genome Informatics  
  [informatics.jax.org](informatics.jax.org)

- Mouse Phenome Database  
  [phenome.jax.org/](phenome.jax.org/)

- And many more unique resources

[www.jax.org/jaxmice/support/techsupport-index](www.jax.org/jaxmice/support/techsupport-index)  |  THE JACKSON LABORATORY
Threats to Mouse Resources

- Natural disasters
- Disease outbreaks
- Breeding errors or breeding cessation
- Facility equipment failures
- Genetic contamination
- Genetic drift
The Dynamic Genome and Genetic Drift

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.”
- from Lee Silver’s “Mouse Genetics” Oxford University Press (1995)

What are spontaneous mutations and how do they arise?
- single base changes, deletions, duplications, inversions
- mistakes during DNA replication (meiosis)
- DNA damage: mistakes during DNA repair

www.informatics.jax.org/silverbook/
Genetic Drift... Friend or Foe?

Species Diversity

Phenotypic Diversity

Lama2^{dy-2J} muscular dystrophy

Data Diversity
Genetic Drift and Mouse Colonies

Small colonies are particularly vulnerable to genetic drift

Small populations are subject to more drift than large ones because any departure from the norm (i.e. mutation) in one individual causes a disproportionately greater deviation from the norm in the overall population.
Genetic Drift and Mouse Colonies

1 in 100 with mutation

1 in 10 with mutation

= het.
Genetic Drift and Mouse Colonies

~ 25% chance that new mutations will become fixed

= het.
= hom.
How Rapidly Do Colonies Drift?

1. Based on spontaneous mutation rates in coat color genes:
   \[ \sim 1.1 \times 10^{-5} \text{ mutations/locus/gamete/generation} \]

Assuming \( \sim 25,000 \) genes in mice:

\[
(1.1 \times 10^{-5} \text{ mutations/locus/gamete/generation}) \times (25,000 \text{ loci}) \\
= 0.275 \text{ mutations/gamete/generation} \\
= 1 \text{ mutation/3.64 gamete/generation}
\]

*This number likely underrepresents the overall mutation rate because mutations with inconspicuous effects were not included in the estimate.*

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?

2) Based on whole genome sequencing of C57BL/6J:

Two samples separated by 69 generations (F69)

Found:

- 669 single-nucleotide polymorphisms (SNPs) (~ 10/generation)
- 272/669 SNPs in genes (coding & non-coding segments)
- 7/272 SNP altered DNA coding sequence or splicing

*This number likely underrepresents the 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

- Parent strain
- Substrain designations (cumulative)
- Lab maintaining strain

*Institute for Laboratory Animal Research (ILAR) Lab Codes*
Substrain Development

C57BL/6 Vendor

Lab A
24 Generations
Sibling Matings

Lab B
14 Generations
Sibling Matings

38 generations apart

JAX® Mice | THE JACKSON LABORATORY
C57BL/6 Substrain Divergence
Visible Genetic Drift: Coat Color Mutations

- C57BL/6J-A^w^-J/J (000051)
- B6(Cg)-Tyr^c^-2J/J (000058)
- C57BL/6J (000664)
- C57BL/6J-Lyst^{bg}-J/J (000629)
- C57BL/6J-Kit^{W-v}/J (000049)
“Invisible” Genetic Drift
Case Study # 1. C3H/HeJ (000659)

C3H/HeJ (000659)
+LPS
Endotoxin resistant
Tlr4<sup>Lps-d</sup> (1958-1965)

C3H/HeOuJ (000635)
+LPS
Endotoxin sensitive
Tlr4 wild-type

“Invisible” Genetic Drift
Case Study # 1. C3H/HeJ (000659)

C3H/HeJ
(000659)

C3H/HeOuJ
(000635)

And another thing….

**In(6)1J**

homozygotes

- Inversion of 20% of chromosome 6
- Affects mapping crosses on chromosome 6
- Not known to affect physiology

C57BL/6 Alteration of pre-synaptic protein $\alpha$-synuclein ($Snca$)

Case Study # 2

- **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

C57BL/6 Retinal degeneration in C57BL/6N substrains

Case Study # 3

*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*<sup>rd8</sup>

- 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
C57BL/6 Retinal degeneration in C57BL/6N substrains

Case Study # 3

C57BL/6J: *Crb1* wild-type  
C57BL/6N: *Crb1*\textsuperscript{rd8}/*Crb1*\textsuperscript{rd8}

C57BL/6 substrains respond differently to DIO

**Case Study # 4**

B6/J more severely glucose intolerant than B6/NJ (not shown)


C57BL/6 Select the proper control

Case Study # 5

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

More Examples

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

Frans Slijter, Charlotte M., Orléans, Cedex 2, France

Research report

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

Marion Bankstahl, Christine J. Müller, Esther Wilk, Klaus Sch"uck, Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine Hannover, Germany

Genetic Differences among C57BL/6 Substrains

Kazuyuki MEKADA, Hatsumi NAKATA, RIKEN BioResource Center

The C57BL/6J Mouse Strain Background Modifies the Effect of a Mutation in Bcl2I2

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

Human Molecular Genetics, 2018, Vol. 22, No. 9

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

Yan Yang, Barbara J. Beyer, James F. Otto, Timothy P. O’Brien, Verity A. Letts, H. Steve White and Wayne N. Frankel

JAX® Mice | THE JACKSON LABORATORY
C57BL/6 Publications

Total **28,629** PubMed publications using C57BL/6 mice

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**Complete nomenclature benefits everyone!**

Based on August 28, 2013 PubMed citations search (without limits)

[www.jax.org/jaxmice/support/nomenclature](http://www.jax.org/jaxmice/support/nomenclature)
Genetic Drift and Substrains

- Spontaneous mutations can be overt or hidden
  - Only apparent by physiological/metabolic assay

- Substrains can vary significantly genetically and phenotypically

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

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

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

- Maintain pedigrees lines and detailed colony records
Single Established Colony - any strain type

*sister-brother mating only!

Pedigree 1

F1

F2

F3

Pedigree 2

Mutations become fixed more rapidly in sister-brother pedigrees

• More easily identified/more easily removed
Maintaining a Pedigreed Colony

Single Established Colony - any strain type

*sister-brother mating only!

Pedigree 1

F1

F2

F3

Pedigree 2

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
- Avoid selection pressure
- Watch for phenotypic changes in mutants and controls
- Refresh breeders frequently (~10 generations)
- Cryopreservation
Cryopreservation

- Protects your investment from genetic drift, genetic contamination, disease outbreaks, disasters, etc.
  - each strain takes 2-3 years and more than $100,000 to create
- Cost savings – cheaper to freeze and recover than maintain?

Can you afford not to preserve your strains?

Cryopreservation & Recovery Services
The Jackson Laboratory’s Genetic Stability Program (GSP)

Frozen embryos used to refresh foundation stock every five generations

www.jax.org/jaxmice/genetichealth/stability

US patents 7592501, 8110721
GSP Works!

Evaluated high quality single nucleotide variants (SNPs)

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

Whole genome sequencing on C57BL/6J genomic DNA

- Constant mutation rate in pre- & post-GSP period
- Mutations accumulate more slowly post-GSP
Are Your Mice What You *Think* They Are?

- What do you do if you suspect genetic contamination?
- How can you verify the backgrounds of collaborators’ mice?
 Genome Scanning Service

- Uses our validated panels of > 2K SNP markers (~ 150 SNPs/panel)
  - Each marker is informative

- Capabilities:
  - Verify genetic background
  - Evaluating congenicity (one-time scan)
  - Detecting recent genetic contamination
  - Marker-assisted backcrossing to create new congenic or consomic strains (multi-generational)

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C57BL/6J vs C57BL/6N
Substrain Characterization Panel

Can you tell the B6 difference?...

We can!

Our new SNP panel distinguishes between C57BL/6J and C57BL/6N

Contact JAX® Genome Scanning Services for more information.
jaxservices@jax.org
1-(800) 422-6423

Genome Science Services | THE JACKSON LABORATORY
Don’t Let Genetic Drift Derail Your Research

JAX® Patented Genetic Stability Program

Consistent performance and data reproducibility
JAX® Mice GSP is the only program that effectively limits cumulative genetic drift. Through rigorous refreshing of foundation stocks every five generations from a 25 year supply of cryopreserved embryos, GSP ensures your data lasts for generations to come.

JAX patents 7,692,501 and 8,110,721

- Common inbred & specialty JAX® Mice strains
- Basic, custom & complex breeding capabilities and speed congenics
- Genome scanning
- Cryopreservation & recovery
- Compound efficacy testing
Thank you!

In need of mouse breeding and colony management expertise to advance your research?

Contact your regional representative today
http://jaxmice.jax.org/support/regionalcontacts

Contact technical support
http://jaxmice.jax.org/support/techsupport-index

JAX® Mice, Clinical & Research Services
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jaxservices@jax.org • http://jaxmice.jax.org/