Protect Your Research:
Know Your B6 Mouse

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Technical Information Services

The Jackson Laboratory

Leading the search for tomorrow's cures

How well do you know your B6 mice?

Which two are most similar?

A

B

C

How well do you know your B6 mice?

A & B are most similar!

A

B6(Cg)-*Tyr*$^{c-2J}$/J (000058)

B

C57BL/6J (000664)

C

C57BL/6NJ (005304)

- A & B differ by a single allele (*Tyr*$^{c-2J}$)
- B & C differ in multiple alleles
  - Metabolism
  - Neurobiology
  - Immunology
  - Vision & hearing
  - Behavior

Coat Color Mutations

C57BL/6J-*A*$^{w\text{-}J}$/J
(000051)

B6(Cg)-*Tyr*$^{c\text{-}2J}$/J
(000058)

C57BL/6J
(000664)

C57BL/6J-*Lyst*$^{bg\text{-}J}$/J
(000629)

C57BL/6J-*Kit*$^{W\text{-}v}$/J
(000049)

B6J or B6N…

We've Got You Covered!

- C57BL/6J (000664)

- C57BL/6NJ (005304)

- High health status
- Well characterized
- Most published
- Extensive Phenotypic Data
- Consistent Data Reproducibility

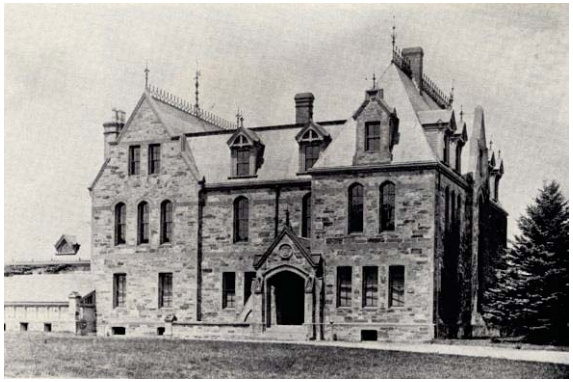
US patents 7592501, 8110721

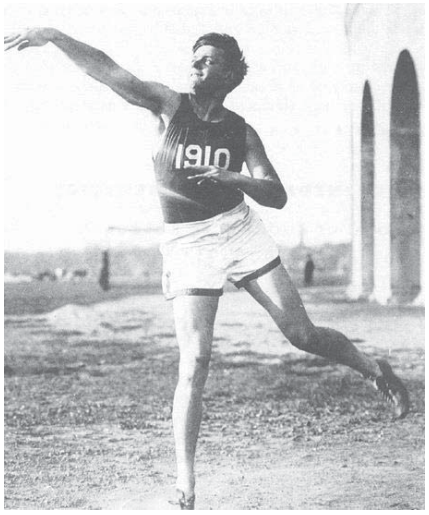
Origins of Inbred Mice

- **1900-1918 Abbie Lathrop, Granby, MA**
  - Mouse fancier, raised and sold mice
  - Provided mice to Bussey Institute, Harvard
- **Mice are ideal for mammalian genetics**
  - Small and easy to maintain
  - Great reproductive performance
  - Anatomy and physiology similar to humans

Origins of Inbred Mice

- 1902 - Dr. William Castle begins using mice, Bussey Institute, Harvard
- 1909 - C.C. Little begins inbreeding mouse stocks as student of Dr. Castle





Origins of C57BL Mice

Miss Abbie Lathrop's “pet shop” stock

C.C. Little (1921) mating of female 57

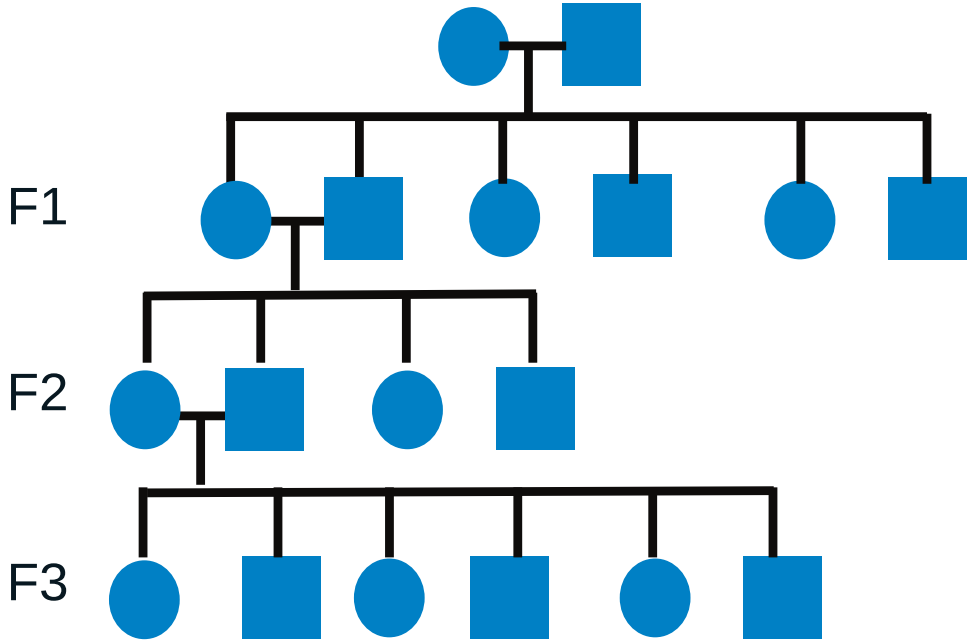
C57BL (BLACK)

C57BR (BROWN)

C57L (LEADEN)

Advantages of Inbred Strains

- Pedigree breeding (brother-sister mating)
  - Inbreds established by 20 generations of brother-sister mating
- Genetic homogeneity
- Statistical reproducibility



Inbred Strain vs. Substrain

Hearing - Avoid Common Research Mistakes

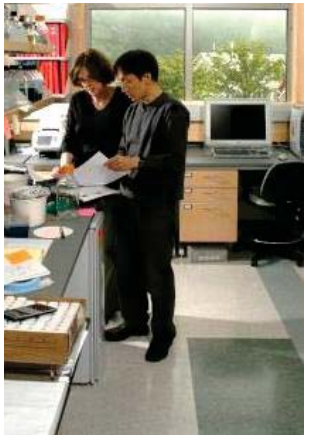
All C57BL/6 substrains (*Cdh23*^{ahl}); consequences of age related hearing loss

- Complication in interpretation of genes influencing diseases, phenotypes & developmental biology of hearing & neurobiology
- Phenotypic analysis of genes implicated in cognitive behavior (fear conditioning in older mice, requires auditory cue)
- Research areas impacted
  - Autism
  - Anxiety & Stress disorders
  - Addiction
  - Cardiovascular function

Inbred strain versus Substrain:

Substrains Develop Quickly

- **Colonies separated by 20 or more generations**
- **Phenotypic or genetic differences are discovered**



C57BL/6
Parent
Colony

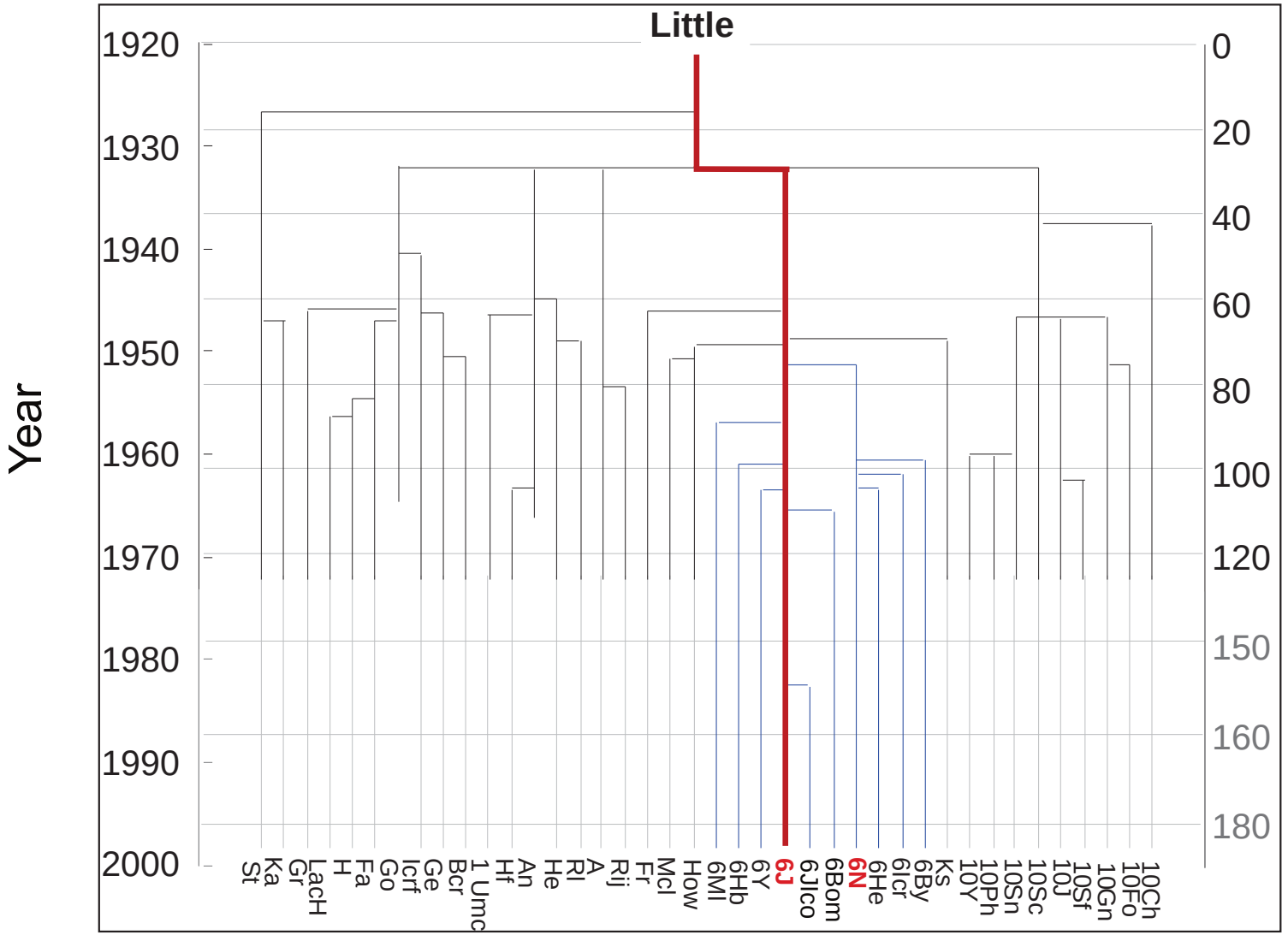
Lab A
Sibling Mating
10 Generations

Generations add up!

Lab B
Sibling Mating
20 Generations

Labs A & B are 30 generations apart!

Many Substrains of C57BL/6 Exist



Adapted from Handbook on Genetically Standardized JAX® Mice, 5th Edition, The Jackson Laboratory, 1997 & Bailey 1982

Know *Your* Substrain:

Use Proper Nomenclature

- C57BL/6J
- C57BL/6NJ
- C57BL/6HaJ
- C57BL/6ByJ
- C57BL/6JEiJ

Parent strain

Substrain designation

- NIH (N)
- Dr. Hauschka (Ha)
- Dr. Bailey (By)
- Dr. Eicher (Ei)

Laboratory maintaining the strain

- Jackson (J)

Institute for Laboratory Animal Research (ILAR) Lab Codes

http://dels-old.nas.edu/ilar_n/ilarhome/search_lc.php

But Aren't All B6 Mice the Same?

C57BL/6 substrains are not the same!

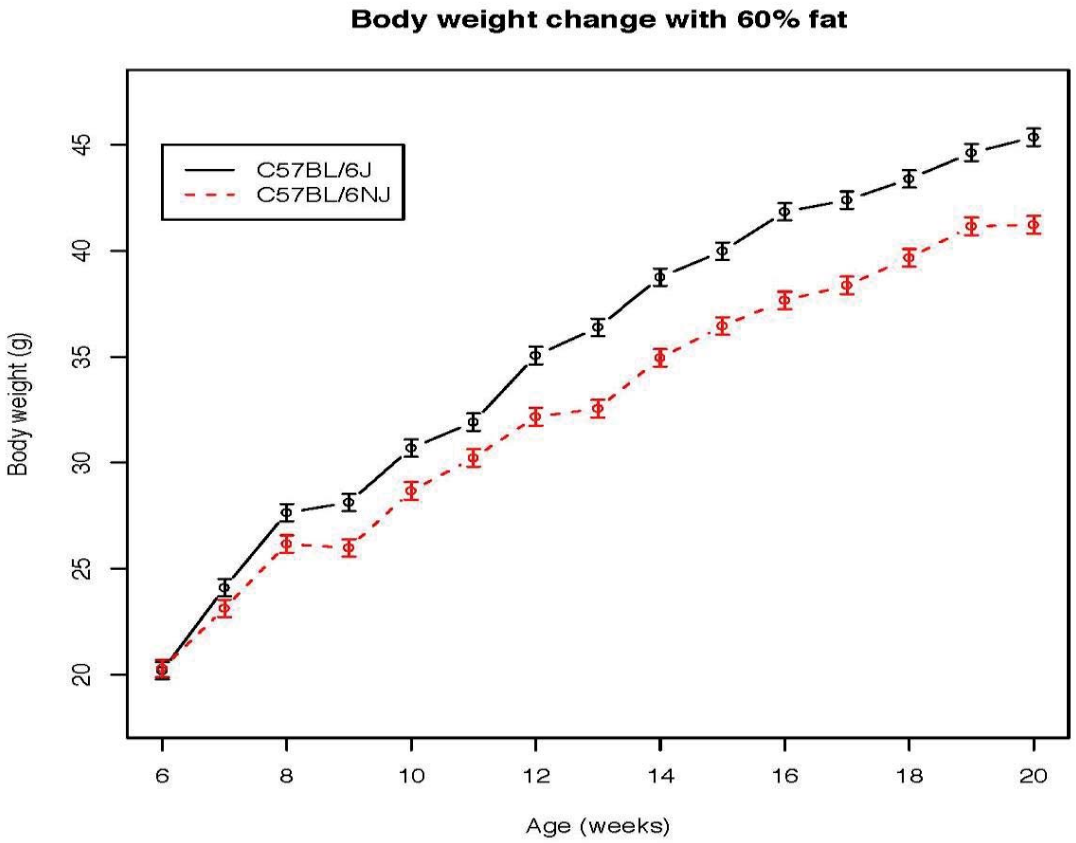
- They differ genetically
  - Single Nucleotide Polymorphisms (SNPs)
  - Insertions & deletions (Indels)
  - Copy number variations (CNVs)
  - Spontaneous mutations

Genetic Differences Translate into Phenotypic Differences

- Metabolism
- Neurobiology
  - Behavior
  - Vision
  - Hearing
- Immunology
- And more…

Metabolic Differences (DIO)

B6J gains more weight than B6NJ on high fat diet (HFD)



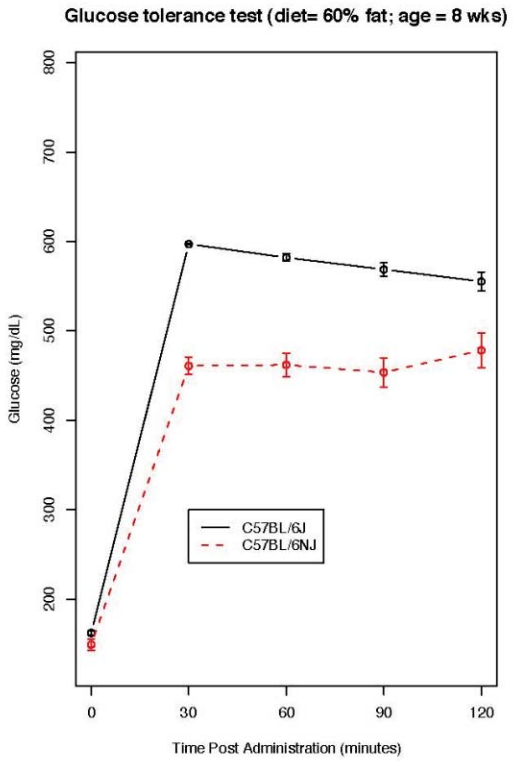
- C57BL/6J (000664) vs C57BL/6NJ (005304
- Mice fed a 60 kcal% high fat diet
  - Beginning at 6 weeks of age

Nicholson, A et al. 2010. *Obesity* 18(10): 1902-1905. PMID: 20057372

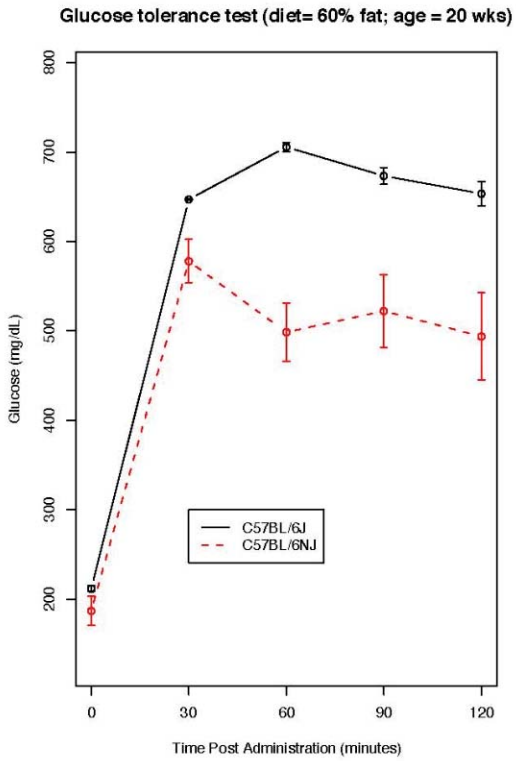
Metabolic Differences (DIO)

B6J more impaired than B6NJ on high fat diet (HFD)

2 wks on HFD



14 wks on HFD



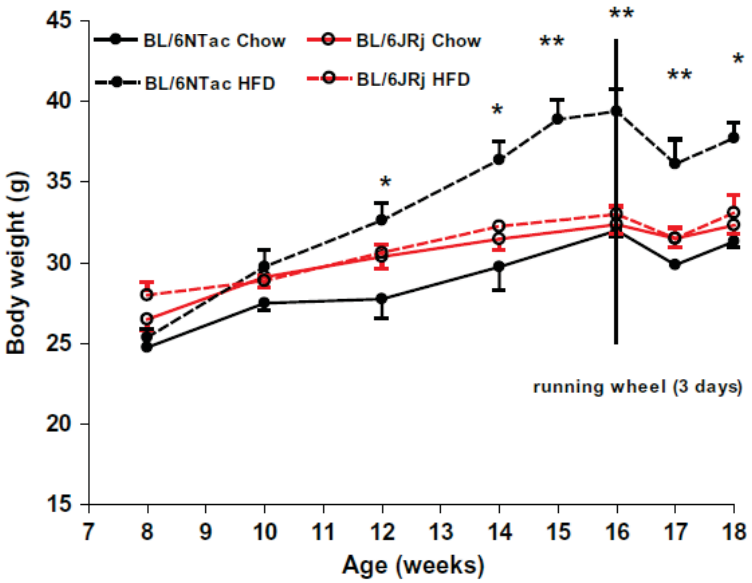
- C57BL/6J (000664) vs C57BL/6NJ (005304
- Glucose Tolerance Test performed (ability to clear glucose from blood)
- Both B6J and B6NJ mice have severely impaired glucose tolerance

Nicholson, A et al. 2010. *Obesity* 18(10): 1902-1905. PMID: 20057372

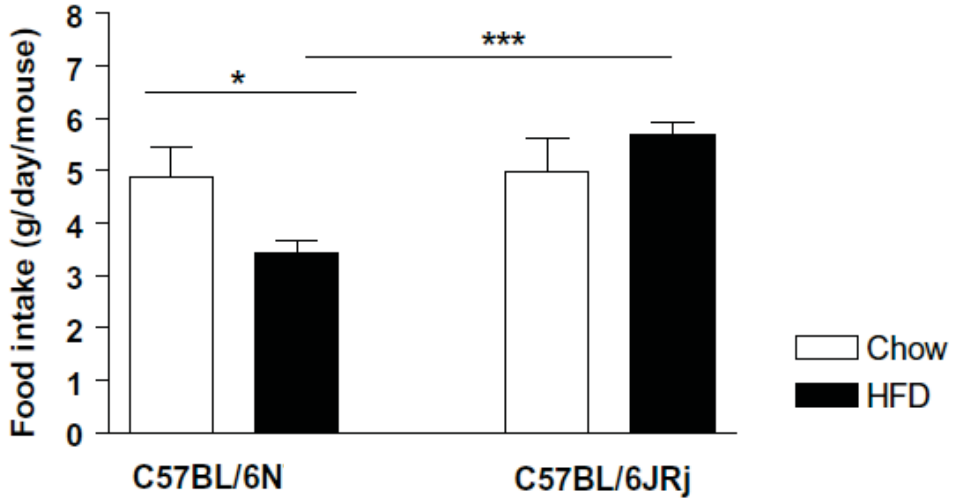
Metabolic Differences (DIO)

C57BL/6JRj mice are DIO resistant

Body Weight



Food Intake



- B6N mice become obese on high fat diet, B6JRj mice do not
- B6JRj mice have greater food intake on high fat diet

Kern, M et al. 2012. *Biochem Biophys Res Comm* 417(2): 717-720. PMID: 22177950

Neurological Differences

Strain	Origin
C57BL/6J	Genomic DNA from JAX
C57BL/6NCrl	Mice from Charles River, Margate, UK
C57BL/6JOlaHsd	Mice from Harlan, Bicester, UK

Specht CG and Schoepfer R. 2001. *BMC Neurosci* 2:11. PMID: 11591219

Neurological Differences

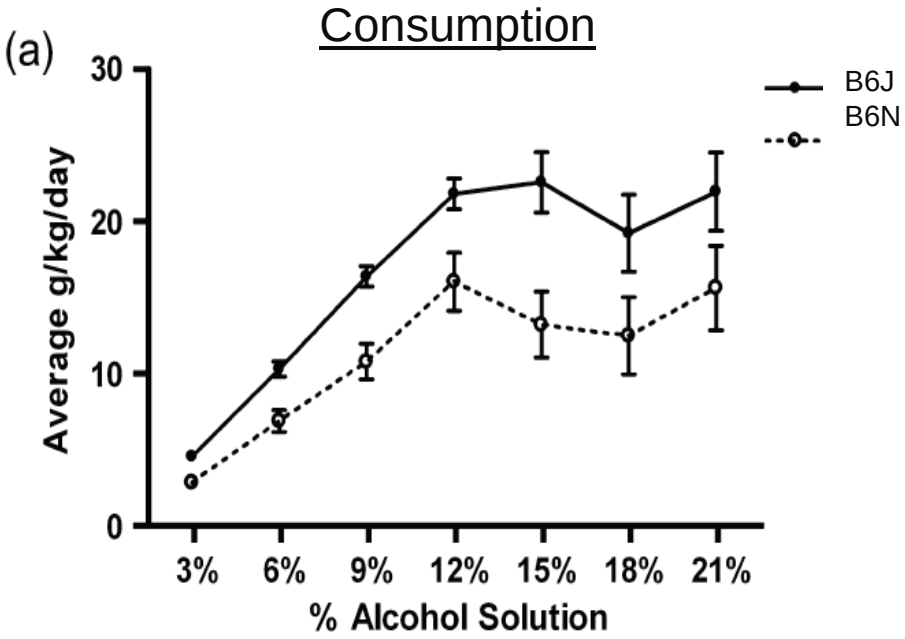
Strain	Origin
C57BL/6J Wild-type *Snca*	Genomic DNA from JAX
C57BL/6NCrl Wild-type *Snca*	Mice from Charles River, Margate, UK
C57BL/6JOlaHsd Deletion of *Snca* – no visible phenotype, but…	Mice from Harlan, Bicester, UK

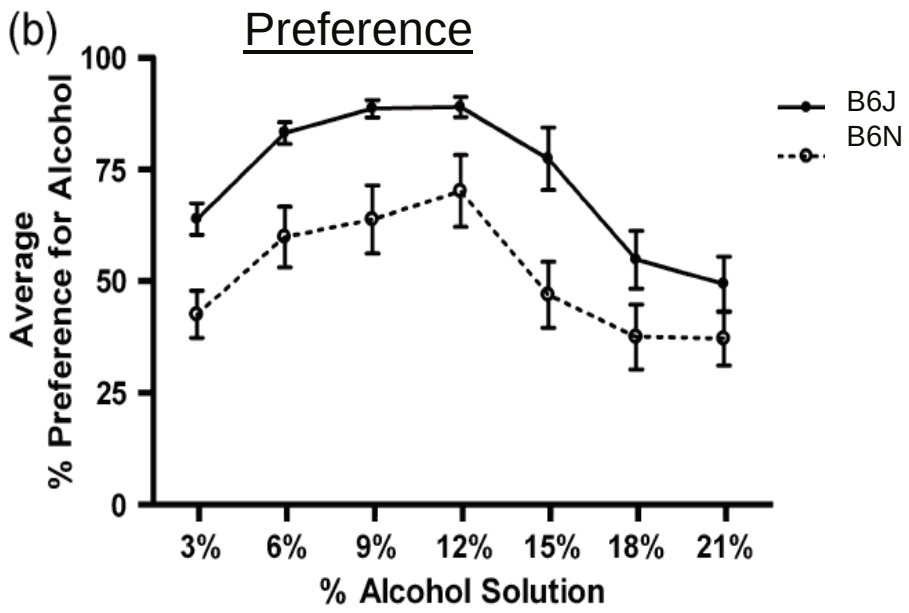
SNCA protein: implicated in a range of neurodegenerative diseases; primary structural component of Lewy bodies found in Parkinson's disease brains

Specht CG and Schoepfer R. 2001. *BMC Neurosci* 2:11. PMID: 11591219

Neurological Differences:

Behavior - B6J Prefers Alcohol More Than B6N





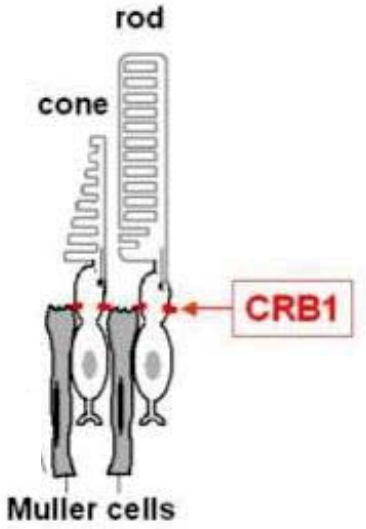
***Also differences in gene expression (not shown)

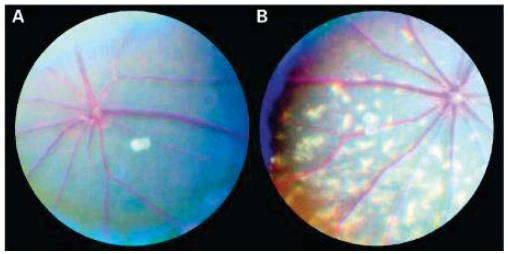
Mulligan, MK et al. 2008. Genes, Brain, and Behavior. 7: 677-689. PMID: 18397380

Neurological Differences

Vision - Substrains Differ In Visual Acuity (*Crb1^{rd8}*)

- Mutations in CRB1 associated with retinal diseases in man:
  - Retinitis pigmentosa
  - Leber congenital amaurosis
- Progressive, spotty retinal degeneration in mice





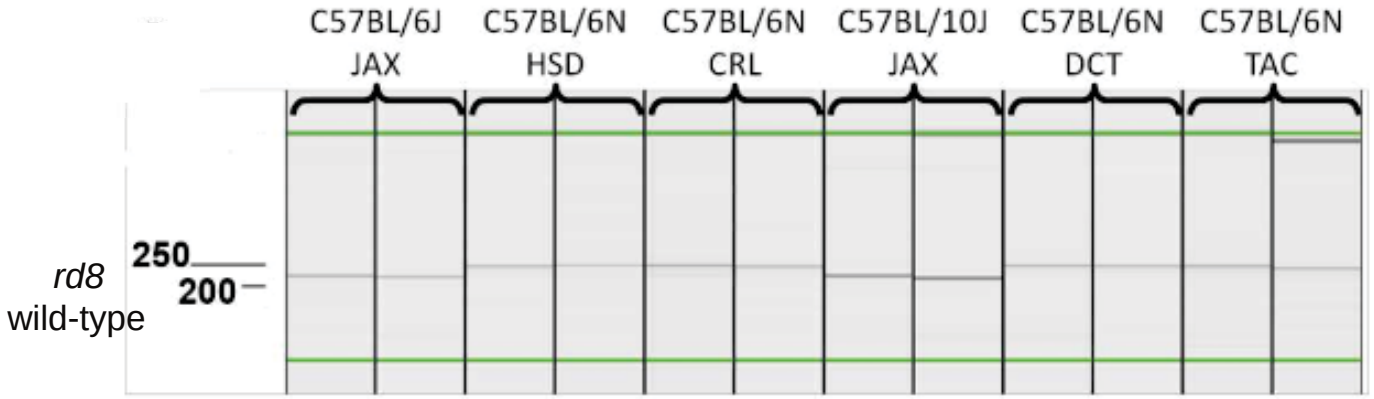
Mehallow AK et al. 2003. *Hum Mol Gen* 12(17): 2179-2189. PMID: 12915475

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

Neurological Differences:

Vision - Substrains Differ In Visual Acuity (*Crb1^{rd8}*)

ALL C57BL/6N substrains are *Crb1^{rd8}/Crb1^{rd8}*



HSD (Harlan)
CRL (Charles River)
DCT (Frederick Nat'l Lab)
TAC (Taconic)

(capillary electrophoresis of PCR products)

C57BL/6J: *Crb1* wild-type (000664)

C57BL/6NJ: *Crb1^{rd8}/Crb1^{rd8}* (005304)

Mehallow AK et al. 2003. *Hum Mol Gen* 12(17): 2179-2189. PMID: 12915475

Neurological Differences

Vision - Avoid Common Research Mistakes

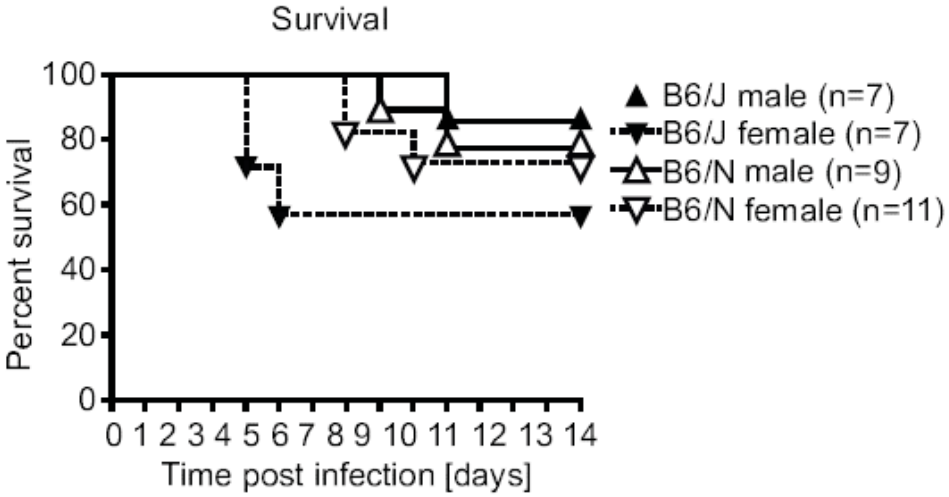
C57BL/6N (*Crb1*^{rd8}); consequences of retinal degeneration

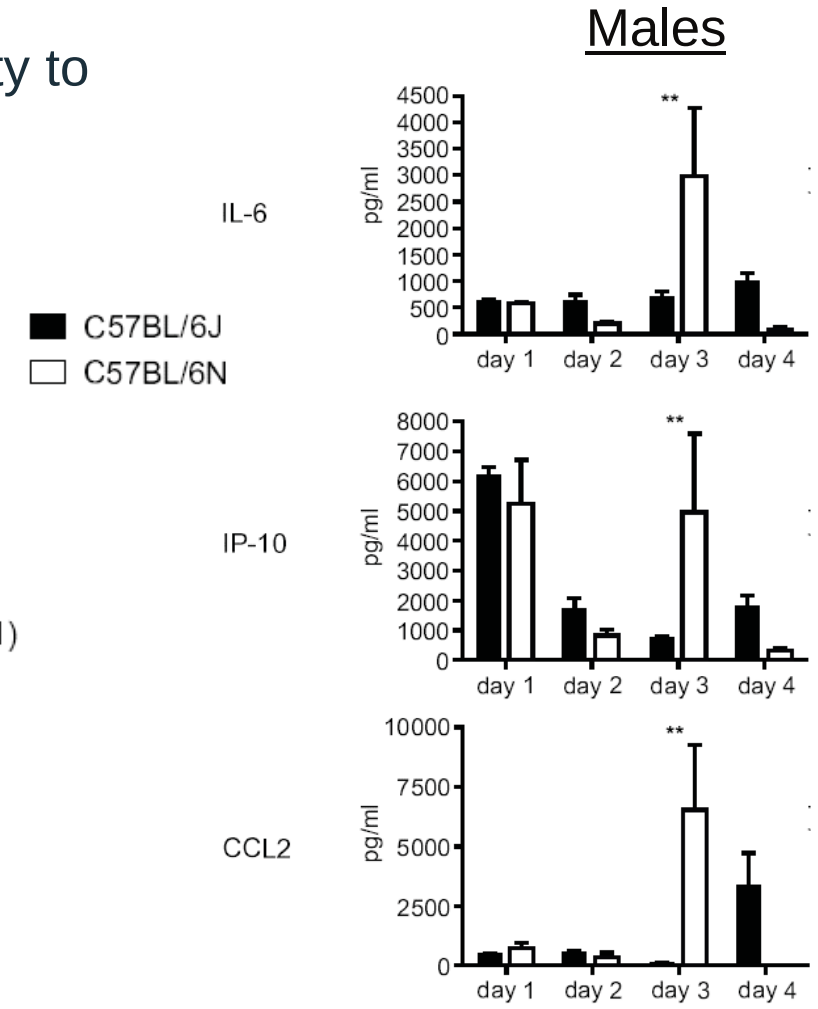
- Complication in interpretation of genes influencing diseases, phenotypes & developmental biology of sight & neurobiology
- Phenotypic analysis of genes implicated in cognitive function (behavioral tests that require visual cues)
- Research areas impacted:
  - Alzheimer's
  - Autism
  - Down Syndrome
  - Rhett Syndrome
  - Neurodegenerative disorders

Immunological Response:

Differential response to *L. monocytogenes infection*

- B6J females show greater susceptibility to *Listeria spp.*
- B6N males show significant pro-inflammatory response on day 3





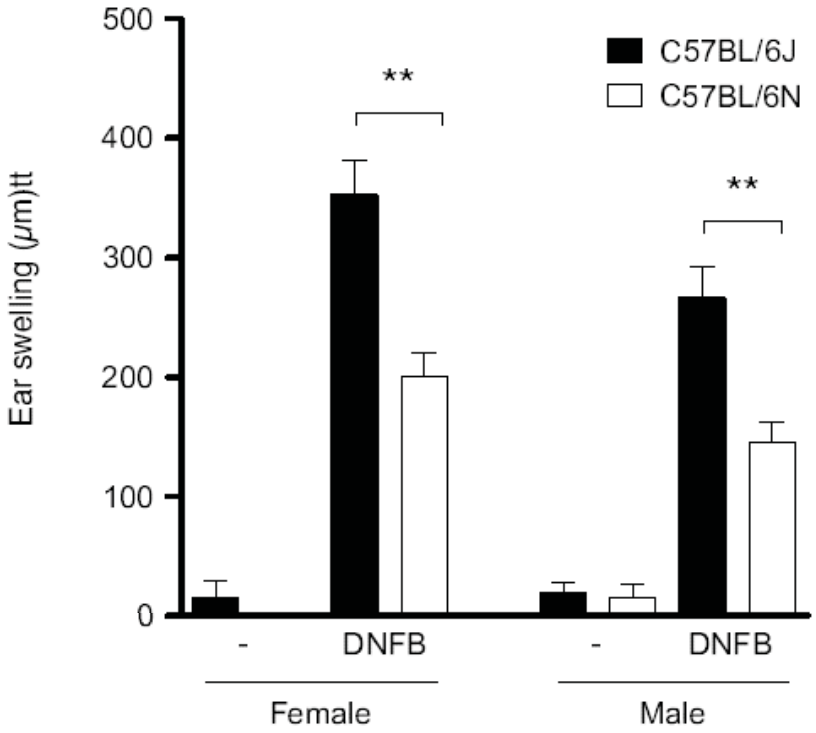
Simon, M. M., et al. (2013). *Genome Biology* 14(7): R82. PMID: 23902802

Immunological Differences

B6J mice show greater DTH Response

Delayed Type Hypersensitivity (DTH) Response

- Sensitization and challenge with dinitrofluorobenzene (DNFB)
- B6J males & females show greater inflammatory response



Genetic Analysis

- Identified multiple SNPs & Indels
- Genomic structural variants

Simon, M. M., et al. (2013). *Genome Biology* 14(7): R82. PMID: 23902802

Distribution of Strains at JAX

Both B6J and B6N genetic backgrounds

Knockout, transgenic, spontaneous, & induced mutants

- ~1,975 strains on the C57BL/6J background
- ~70 strains on the C57BL/6N background
- ~830 strains on the C57BL/6N background going to be created through KOMP^2
- ~200 strains on the C57BL/6N background going to be created through EUCOMM

Considerations for Control Selection

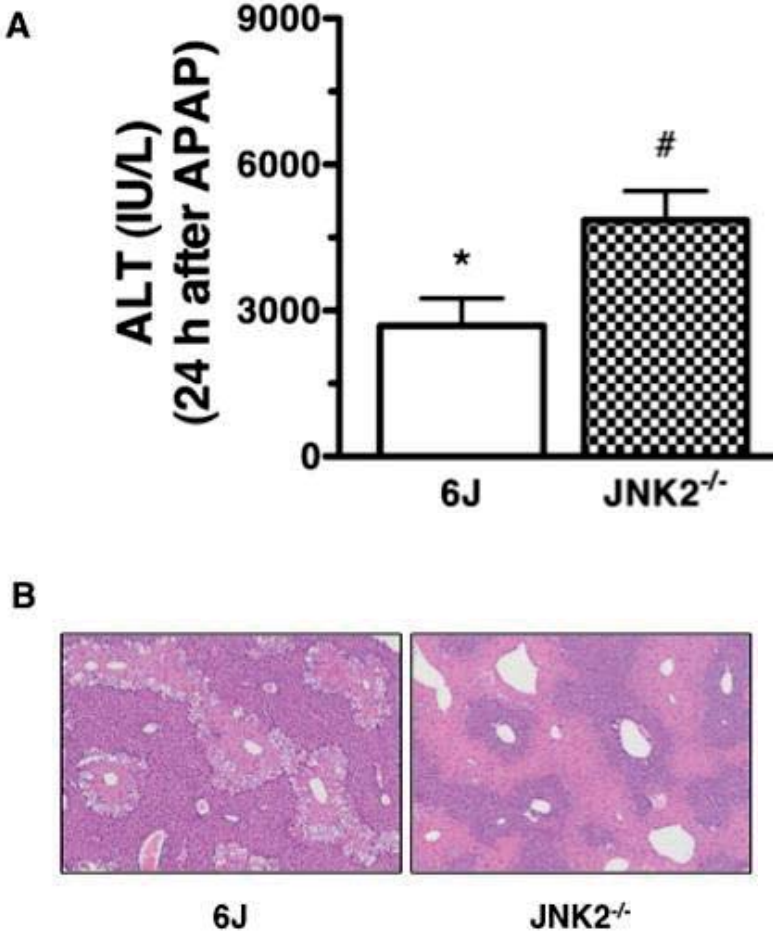
- Congenic Strains*
  - Littermates (het x het, het x wt, or hemi x wt mating scheme)
    - Wild type or heterozygous for mutant gene or allele
    - Non-carriers of transgene
    - Non-littermate controls from the colony
  - Inbred (hom x hom mating)
    - Match background mutant is on (including substrain)
- Mixed Background (B6J and B6N)
  - Littermates
    - Wild type or heterozygous for mutant gene or allele
    - Non-carriers of transgene
    - Can also use non-littermate controls from the colony

* Congenic strains have been crossed more than 10 generations to inbred strain. Acceptable to use inbred as control after N5

Select The Proper C57BL/6 Control

Avoid Common Research Mistakes

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



Bourdi M et al. 2011. *Chem Res Toxicol* 24: 794-6. PMID:21557537

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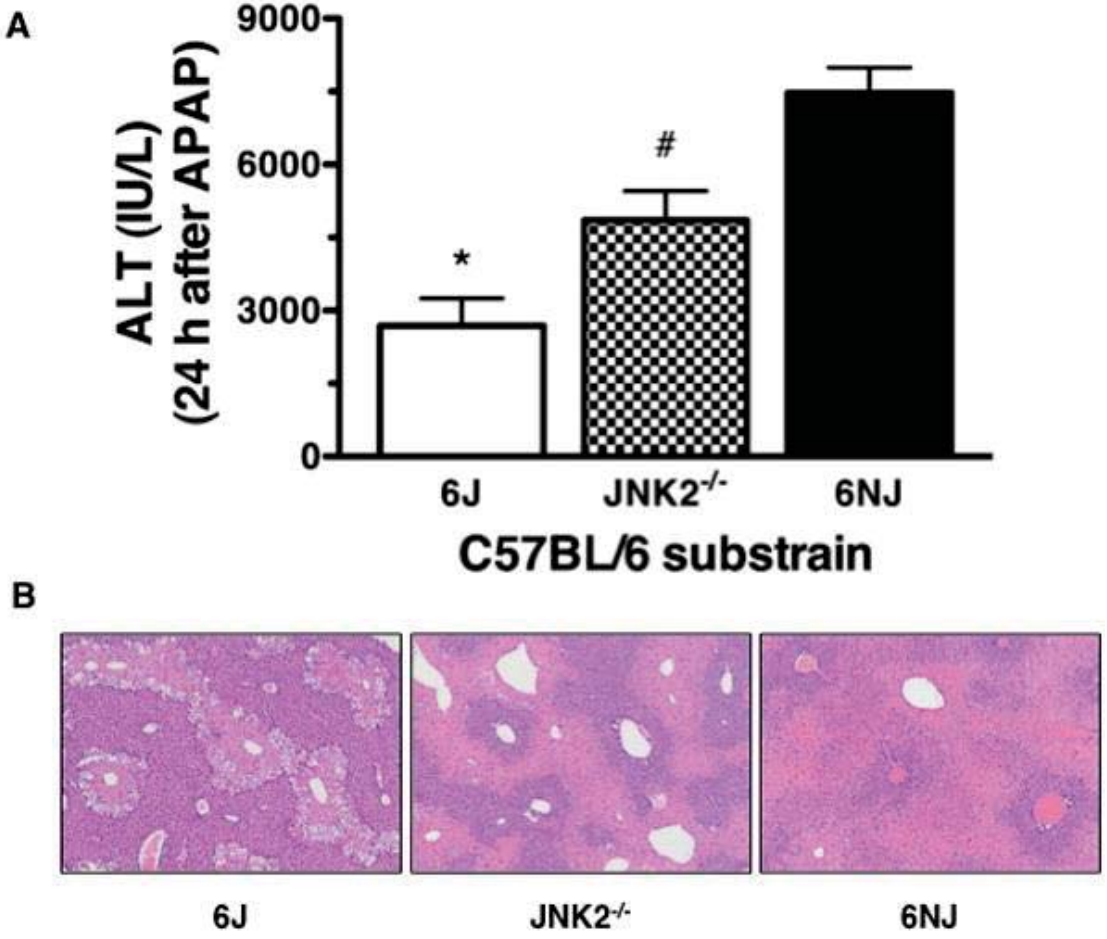

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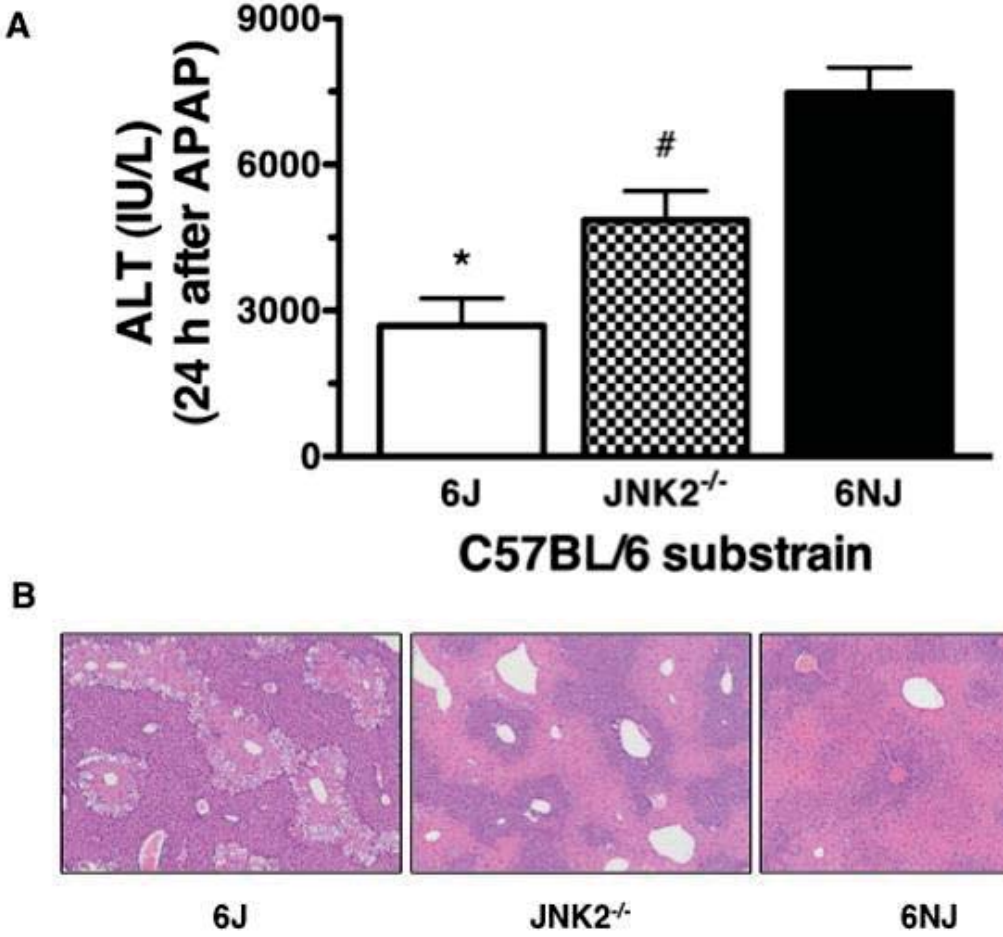

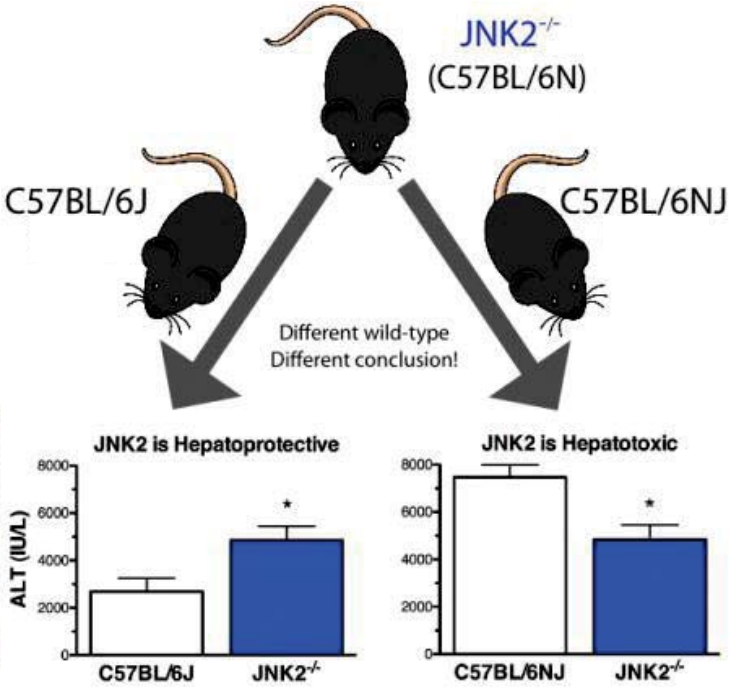

Bourdi M et al. 2011. *Chem Res Toxicol* 24: 794-6. PMID:21557537

Background Strain Information:
Questions to Ask

- What strain was used to develop this stock?
  - Oocyte donor?
  - ES cell line?
- What strains have been introduced through breeding?
  - Cre/FLP
  - Reporters
  - Other mutations
- Current breeding scheme?
- Current generation number?
- Has it been cryopreserved?
  - At what generation?
  - Has the strain been backcrossed to an inbred strain?
- Has the genetic background been verified?

Review Strain Development

Development

A *Pomc* (pro-opiomelanocortin-alpha) bacterial artificial chromosome (RPCI22-372J15) was used to generate mice expressing cre under the control of the mouse *Pomc* promoter. The cre recombinase cDNA was inserted via homologous recombination into the first ATG transcription start site, ablating the first 30bp of the POMC coding sequence. This transgenic vector, created by Dr. Bradford Lowell (see Stock No. 005965) was newly-introduced to FVB-derived embryos, and the line was backcrossed to C57BL/6 (see SNP notes below) for more than 12 generations by the donating laboratory.

A 32 SNP (single nucleotide polymorphism) panel analysis, with 27 markers covering all 19 chromosomes and the X chromosome, as well as 5 markers that distinguish between the C57BL/6J and C57BL/6N substrains, was performed on the rederived living colony at The Jackson Laboratory Repository. While the 27 markers throughout the genome suggested a C57BL/6 genetic background, all 5 markers that determine C57BL/6J from C57BL/6N were found to be segregating. These data suggest the mice sent to The Jackson Laboratory Repository were on a C57BL/6N genetic background.

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International Knockout Mouse Consortium (IKMC)

Mutate all protein-coding genes in C57BL/6N

- Knockout Mouse Project (KOMP) – USA
- European Conditional Mouse Mutagenesis Project (EUCOMM) – Europe
- North American Conditional Mouse Mutagenesis Project (NorCOMM) – Canada
- Texas A&M Institute for Genomic Medicine (TIGM) – USA

http://www.knockoutmouse.org/

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 backgrounds

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

B6J or B6N…

We've Got You Covered!

- C57BL/6J (000664)

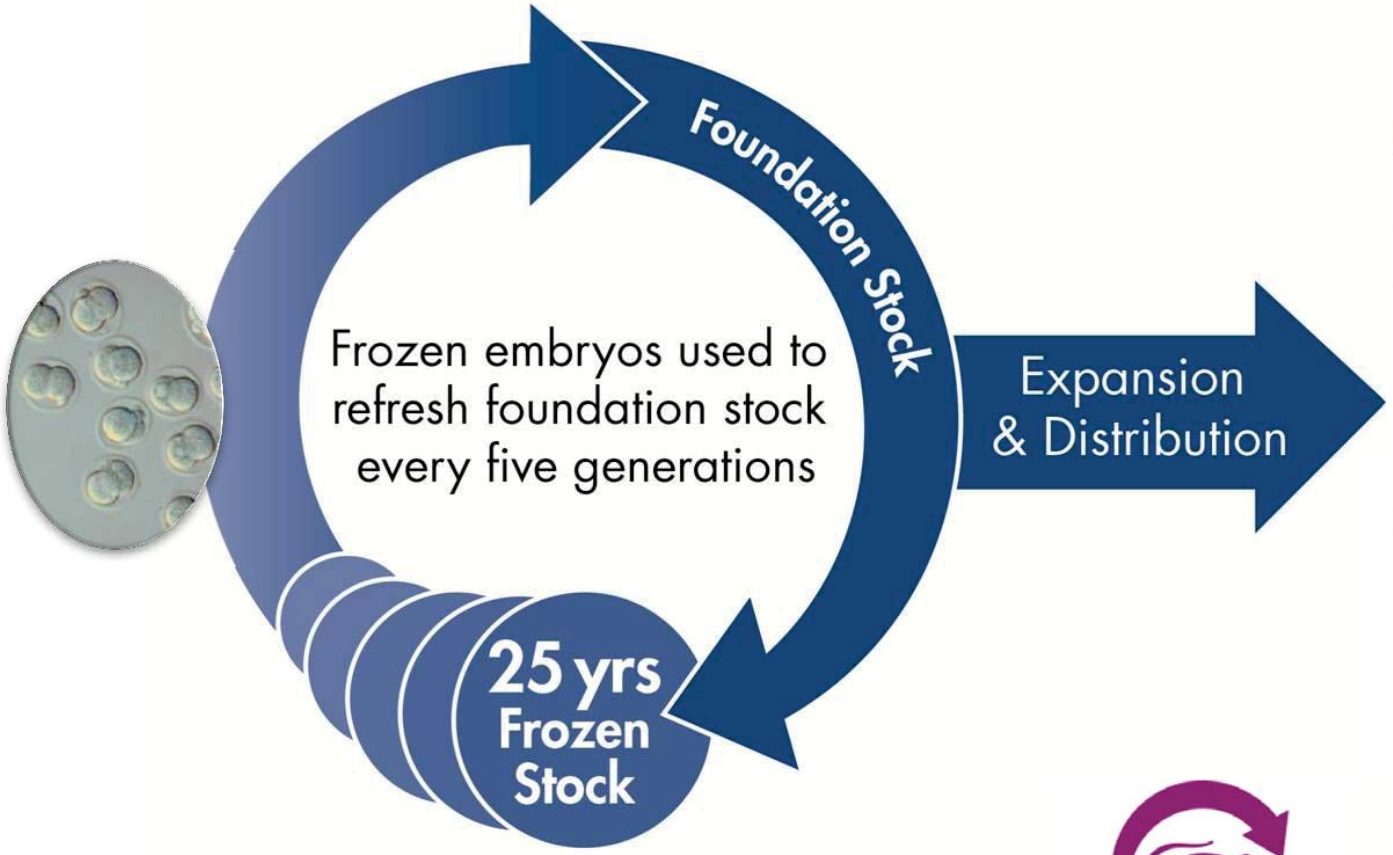
- C57BL/6NJ (005304)

- High health status
- Well characterized
- Most published
- Extensive Phenotypic Data
- Consistent Data Reproducibility

But what about the genetic integrity of these substrains?

Genetic Stability Program (GSP):

Diminish cumulative drift, stabilize phenotype



C57BL/6J (000664)

C57BL/6NJ (005304)

GSP

US patents 7592501, 8110721

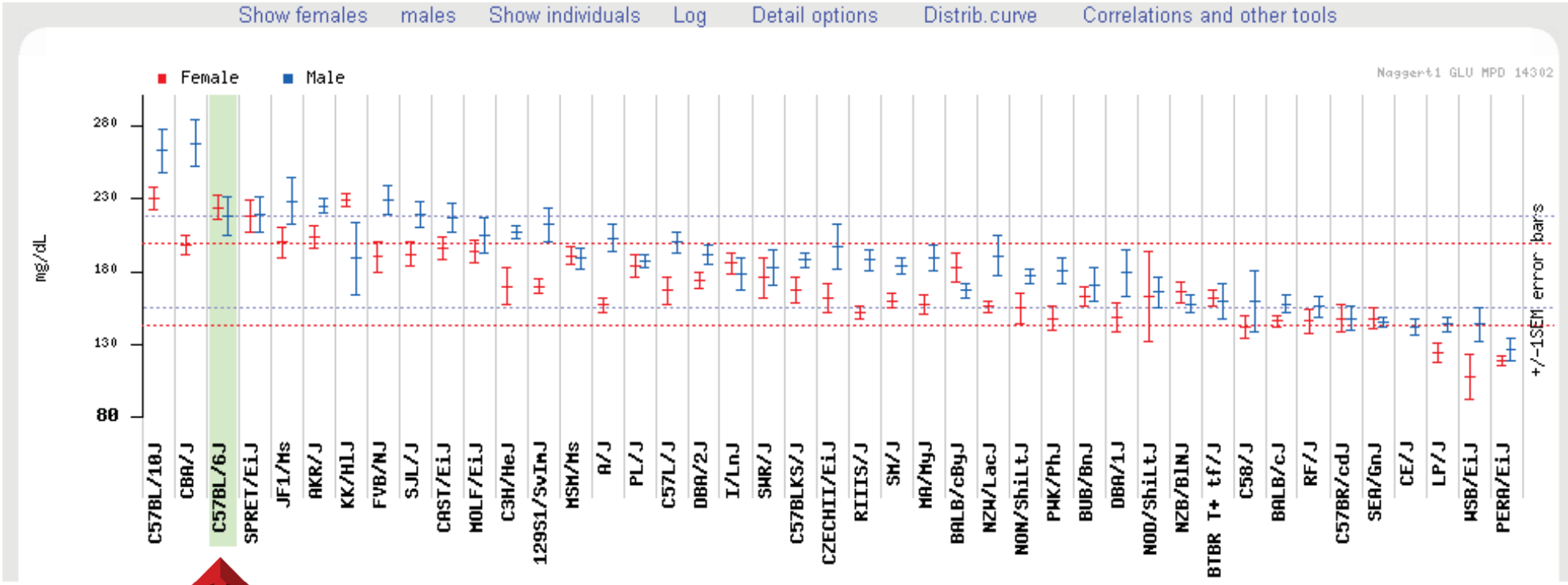
Resources Supporting B6 Mice:

The best characterized & most published strain

- Mouse Phenome Database (MPD) www.phenome.jax.org
  - Over 2700 measurements for C57BL/6J (000664)
- Whole genome sequence data- Sanger Institute
  - Mouse Genomes Project
- Preconditioned mice
  - Streptozotocin (STZ) induced diabetes
  - Diet induced obesity (DIO)
- Inventoried aged mice
  - Custom aging services
- High Health Status at no extra charge!

Choose Wisely….Background Matters: Which strain would you choose?

Mouse Phenome Data (MPD): Baseline Plasma glucose after 4hr fast – 43 strain survey



Access MPD at www.jax.org/phenome

Ensuring Data Validity & Reproducibility

Consider your rodent, your most important reagent!

- Choose your strains wisely
- Use proper nomenclature
- Minimize genetic drift
- Educate and establish a QC culture

Good science results in reduced animal use

Summary

- Multiple genetically and phenotypically unique substrains have developed over time (and continue to do so)
- Knowing and understanding the B6 substrain you are working with is key to proper selection of controls and data interpretation
- Comparison of phenotypes between B6 substrains may allow identification of unique modifier alleles
- At JAX, genetic drift is diminished in C57BL/6J & C57BL/6NJ by GSP to stabilize phenotype over time

Thank you!

Interested in using the B6J vs. B6N characterization panel to verify the genetic background of your mice?

Request a quotation

https://secureweb.jax.org/jaxservices/b6substrain.php

Contact technical support

www.jax.org/jaxmice/support/techsupport-index

JAX® Mice, Clinical & Research Services

1-800-422-6423 • 1-207-288-5845
jaxservices@jax.org • www.jax.org/jaxmice