Recent Mouse Enrichment and Aging Studies at The Jackson Laboratory

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The Jackson Laboratory
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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

Benefits of enrichment for laboratory mice

- Encourage natural instincts to produce or seek shelter
- Provide a degree of control over their environment
- Can improve brain & neuronal development
- Reduce undesirable traits (stereotypies, barbering)

Toth, et al. 2011 *Comp Med*. 61(4): 314-321
Wurbel. 2001. *Trends Neurosci*. 24(4):207-11

Enrichment breeding study

- Five groups of trio-mated C57BL/6J inbred mice were studied throughout a typical breeding period of 30 weeks
- All groups received same husbandry & autoclaved diet, water, & bedding (aspen chips & shavings) but differed in the type of supplemental enrichment
- Technicians were rotated among groups

Cardboard tunnel

Aspen shavings only

Cardboard hut

Cotton square

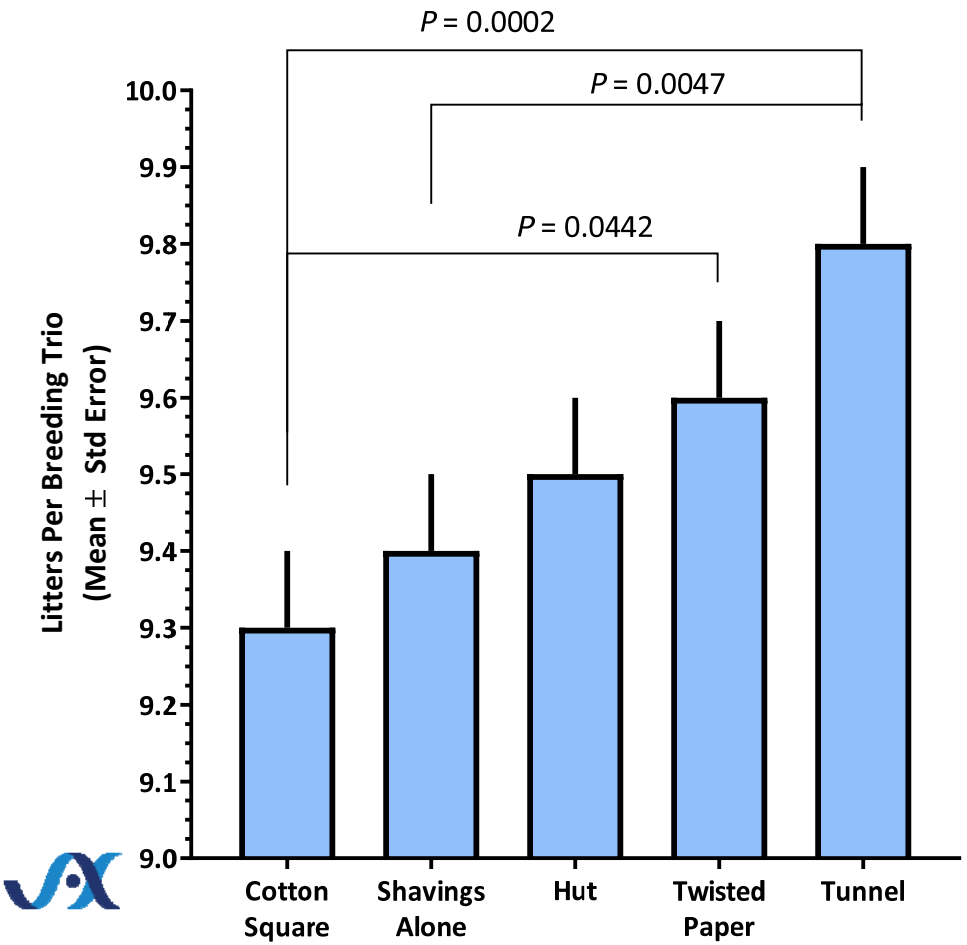
Twisted paper

Enrichment breeding study

- Parameters measured for each cage:
  - Number of litters
  - Pups born
  - Pups weaned
  - Percent survival
  - Pup mortalities
  - Pups euthanized
    - Number of runts
    - Number of barbered pups
  - Number of flooded cages
- Each group contained ~600 cages

Enrichment	Total Cages (% Productive)
Shavings Only	600 (85%)
Twisted Paper	602 (91%)
Hut	598 (89%)
Cotton Square	603 (86%)
Tunnel	598 (91%)

Enrichment had marginal effects on litter counts



A tunnel offered improvements over a cotton square or aspen shavings alone

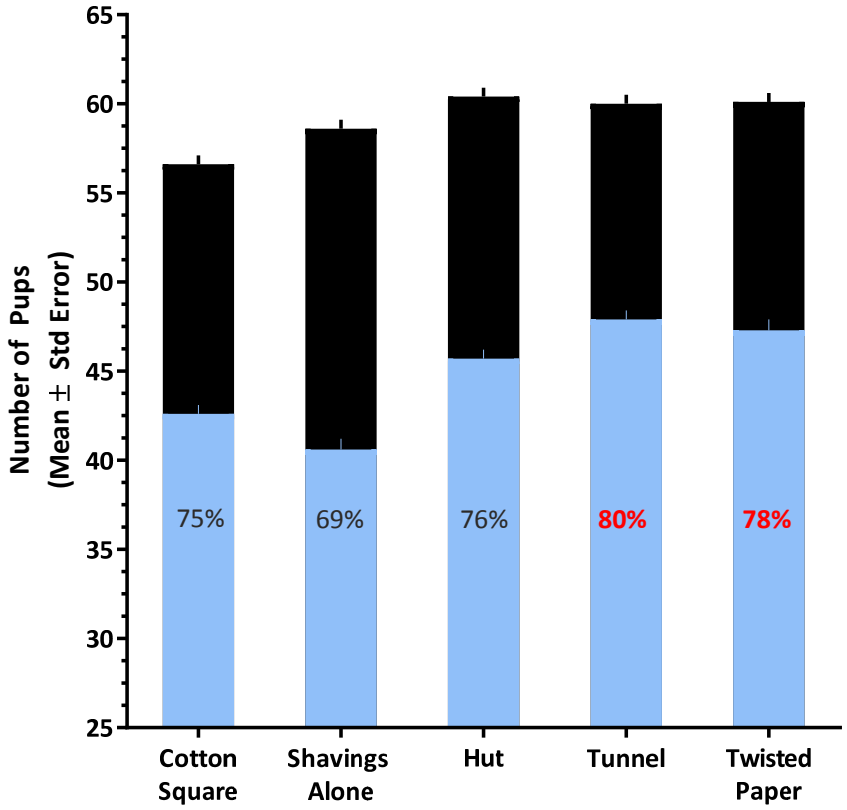
Twisted paper led to an increase over a cotton square

Statistical model: linear regression (ANOVA)

Twisted paper, tunnels improved survival to weaning

Bar graphs are overlaid

% indicates survival to weaning



Significant pairwise differences:

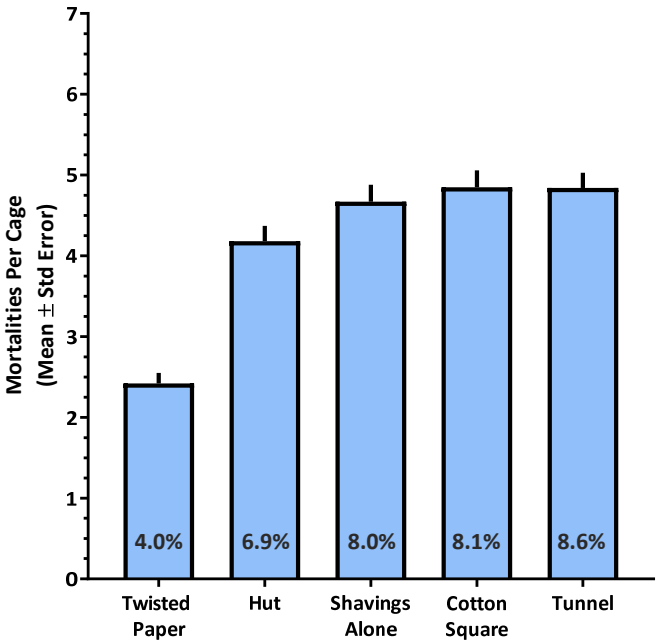
<u>Born</u>
Cotton square vs paper, hut, or tunnel

<u>Weaned</u>
Every pair except twisted paper vs hut or tunnel

<u>Percent Survival</u>
Every pair except twisted paper vs tunnel, hut vs. cotton square

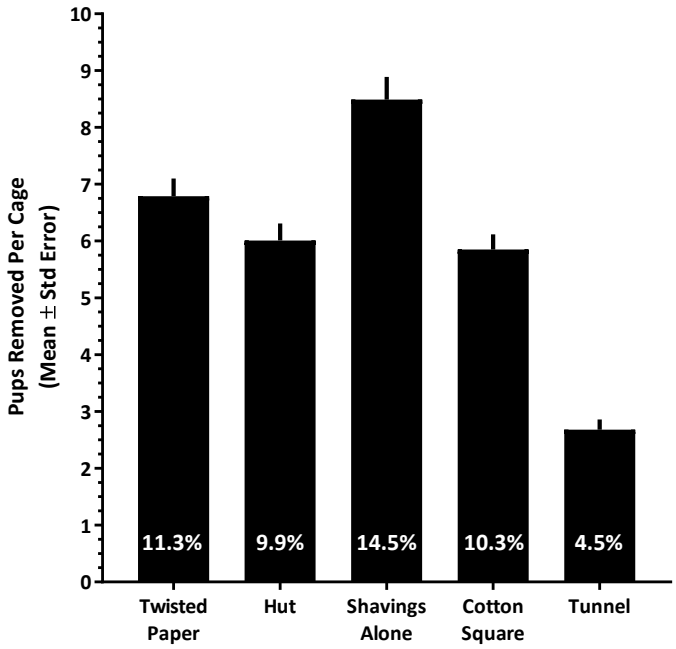
Statistical models: linear regressions (ANOVA)

Twisted paper reduced the number of pup mortalities



Each pairwise difference was significant for twisted paper ($P < 0.0001$)

Tunnels had the fewest pups euthanized for abnormalities

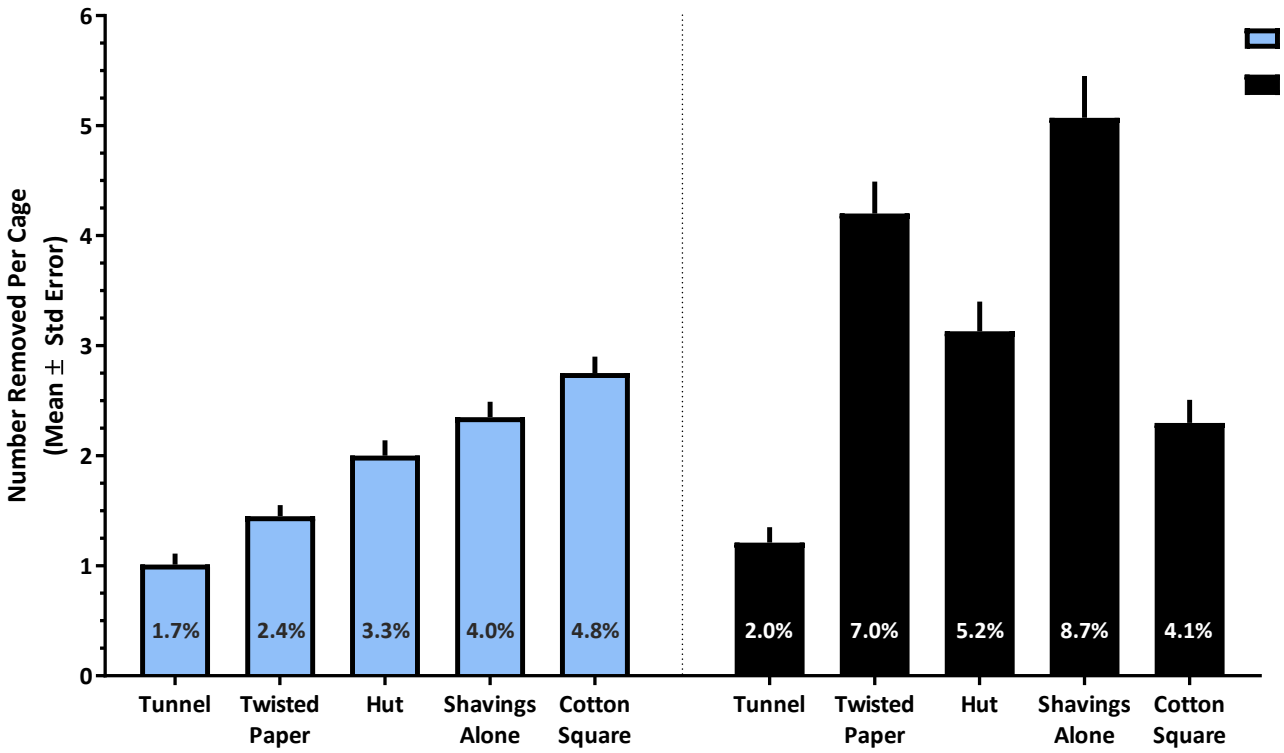


Each pairwise difference was significant for tunnel ($P < 0.0001$)

% indicates the portion of number born

Statistical models: zero-inflated negative binomial regressions

Tunnel cages had fewest runts and barbered pups



Each pairwise different was significant for tunnels

% indicates the portion of number born

Statistical models: zero-inflated negative binomial regressions

The tunnel group had the fewest wet cages

Enrichment	Wet Cages / Total Cages (%)
Tunnel	5/598 (0.8%)[abc]
Hut	16/598 (2.7%)
Shavings Only	21/600 (3.5%)[a]
Cotton Square	24/603 (4.0%)[b]
Twisted Paper	32/602 (5.3%)[c]

[a] $P = 0.0295$, [b] $P = 0.0113$, [c] $P = 0.0009$

Statistical model: binomial (logistic regression)

Breeder Enrichment discussion

- Environmental enrichment improved wellbeing and productivity in a C57BL/6J breeding colony
- Enrichment trials and comparisons help to determine the best type for each strain
- Combinations of cage enrichments should reveal additional benefits

Solving Strain-Specific Challenges Using Enrichment

C57BL/6J

- Most widely used inbred strain
- Prone to eye defects, hair loss hydrocephalus, dermatitis

SJL/J

- Used to make F1s, multiple sclerosis models
- Aggressive

Barbering in C57BL/6J

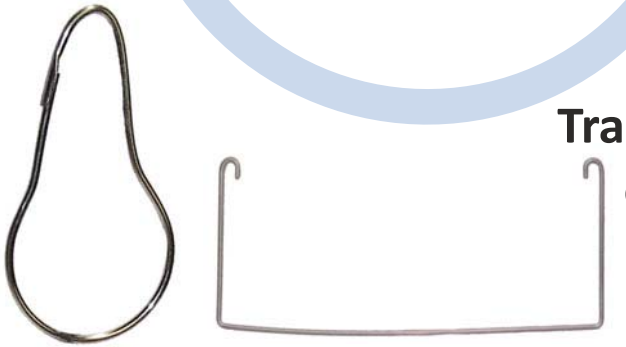
- Female bias
- Often involves whisker-picking, hair loss (alopecia)
- Increases with age, cage density
- Proposed explanations include social dominance, stress responses, obsessive-compulsive disorder

Rotational Enrichment

Cardboard
Tube & Shack

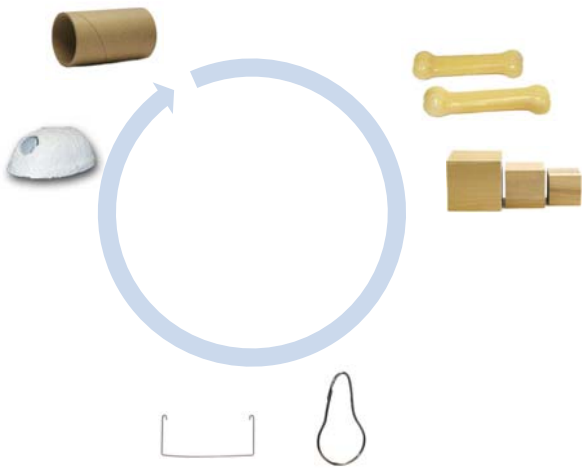
Weekly
Change

Gumabone, Nylabone
& Gnawing Block



Trapeze & shower
curtain rings

Rotational Enrichment Study in B6



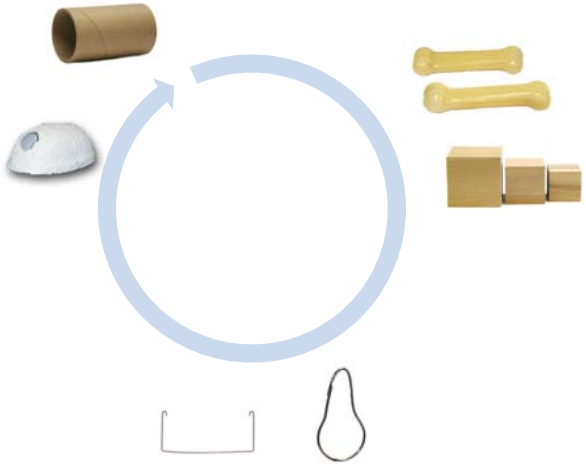
Two sets of 120 female C57BL/6J mice were assigned at weaning to enrichment groups:

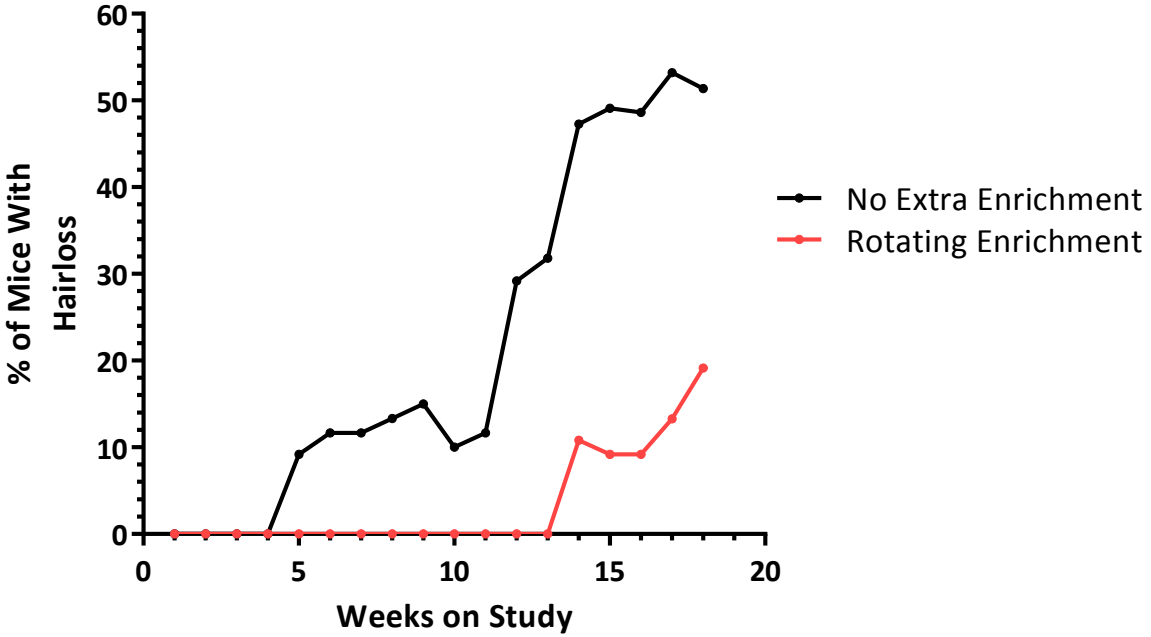
- Rotating enrichment
- No extra enrichment

Typical husbandry practices were followed

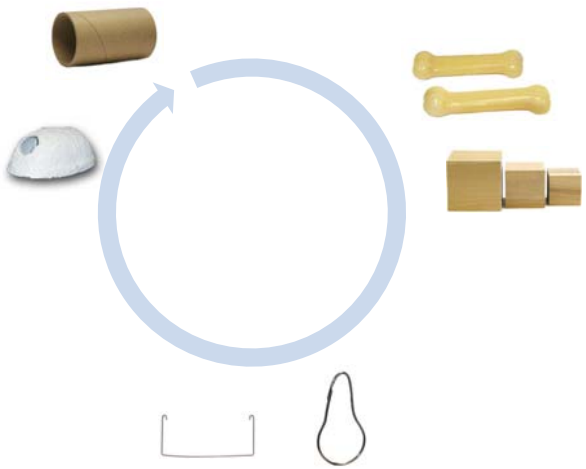
Mice were monitored weekly for hairloss or any other adverse conditions for 18 weeks

Rotational Enrichment Delays & Reduces Hairloss in B6 Females





Rotational Enrichment Delays & Reduces Hairloss in B6 Females



Pros

- Novelty & diversity
- Effective at reducing hairloss

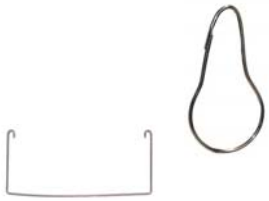
Cons

- Cost
- Operational challenges

Comparing Enrichment: Shelter vs Activity

3 groups were created, each with 1080 female mice:

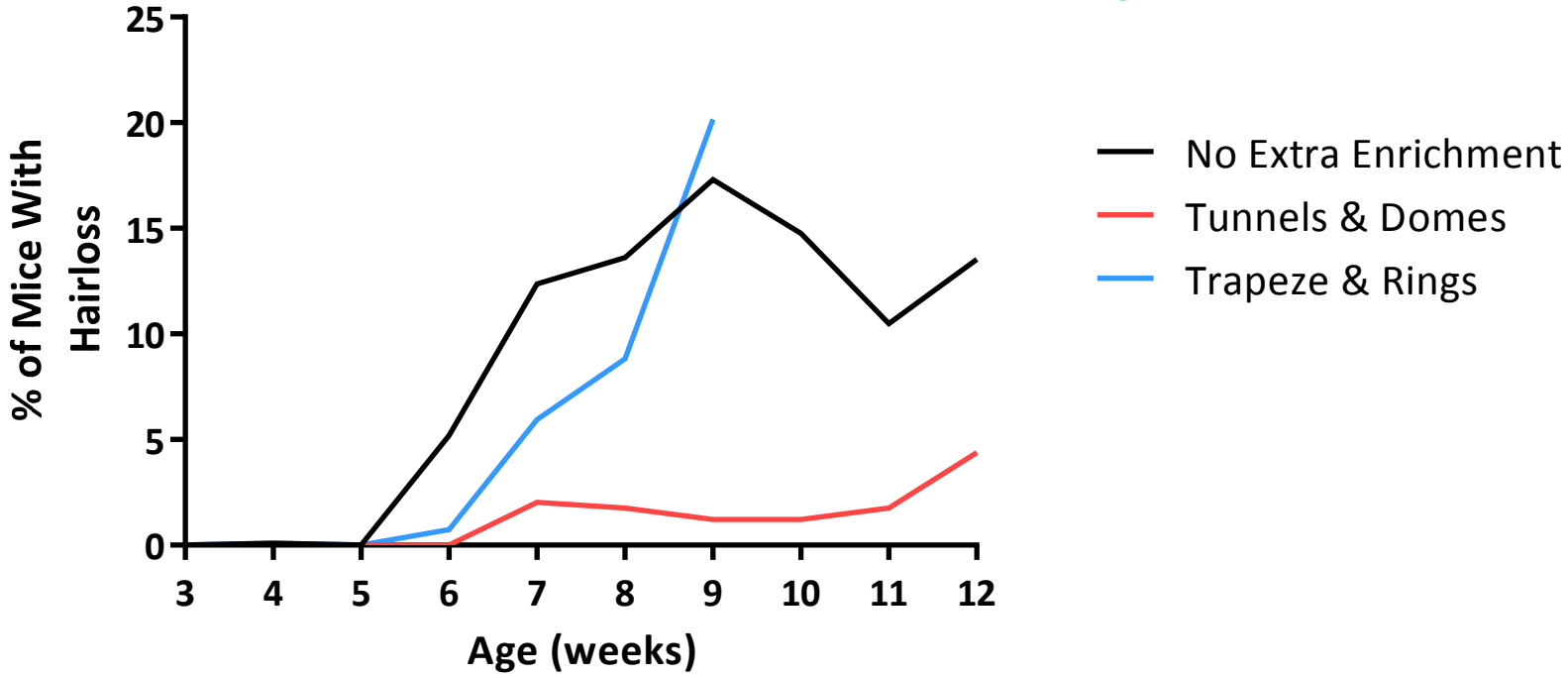
- No extra enrichment
- Cardboard tunnel and dome
- Trapeze and rings



Typical husbandry practices were followed

Mice were monitored between the ages of 3 & 12 weeks for hairloss and other adverse signs

Comparing Shelter & Activity Enrichment in C57BL/6J



C57BL/6J females

Can Different Nesting Materials Reduce Aggressive Tendencies in SJL/J?

- Male bias
- Can affect pups, mates, same-sex cagemates
- Increases with age, cage density

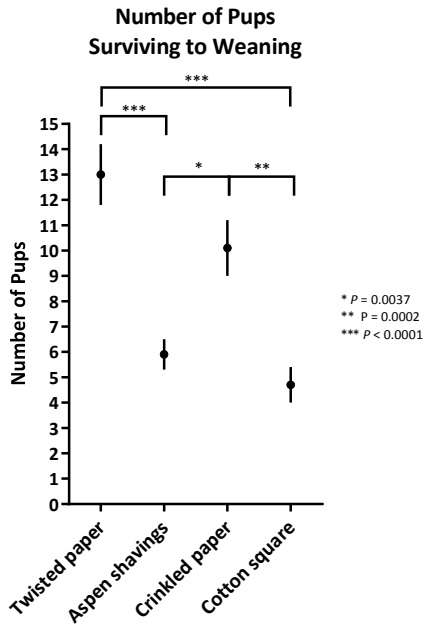
Can Different Nesting Materials Reduce Aggressive Tendencies in SJL/J?

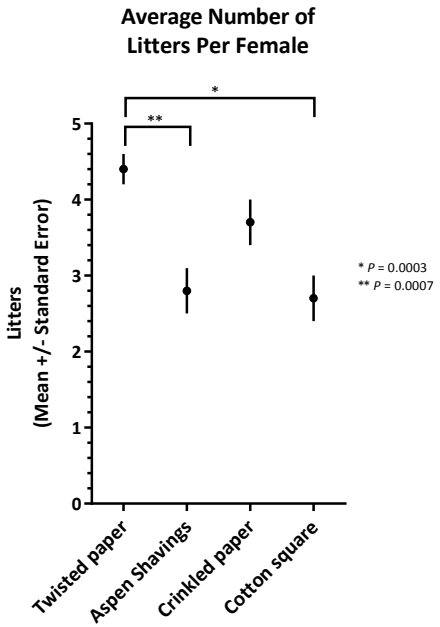
- Supplement aspen chip bedding with one of four different nesting materials
  - Twisted paper
  - Aspen Shavings
  - Crinkled paper
  - Cotton square

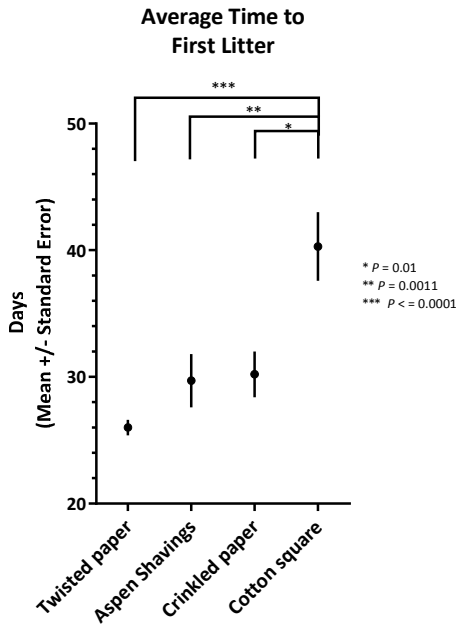
- Productivity, welfare parameters measured throughout a standard breeding period (22 weeks)
- Standard husbandry practices were followed

Impacts of Nesting Material on SJL/J

- Productivity advantages for twisted paper, crinkled paper







Impacts of Nesting Material on SJL/J

- Many factors did not depend on nesting material
  - Born/Wean Ratio
  - Weight of pups at weaning
  - Female breeder mortality
  - Male breeder mortality
  - Pups removed for defects (bite wounds, runts, etc.)
  - Pup mortality

Solving Strain-Specific Challenges Using Enrichment

C57BL/6J

- Hair loss lessened when females were provided extra enrichment
- Shelter enrichment offered similar benefits as rotating enrichment

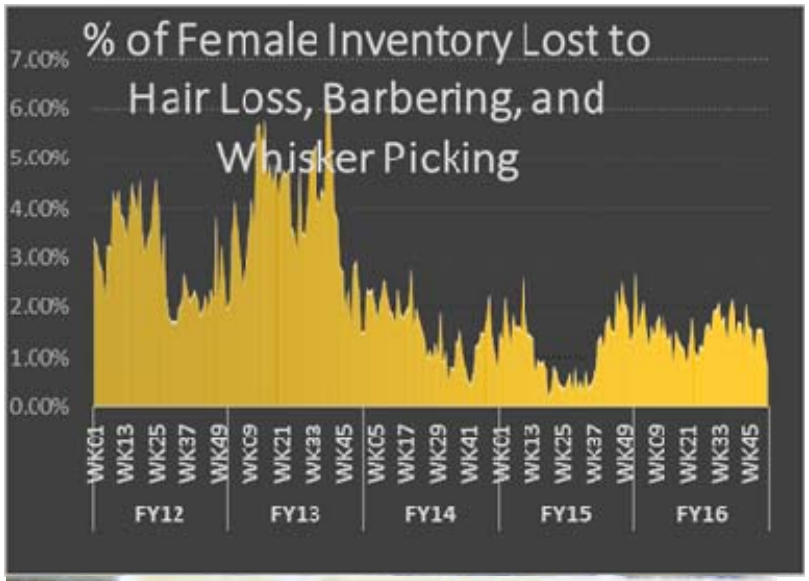
SJL/J

- Twisted paper greatly improved productivity
- No obvious effect on pup survival

Group-Housed Animals

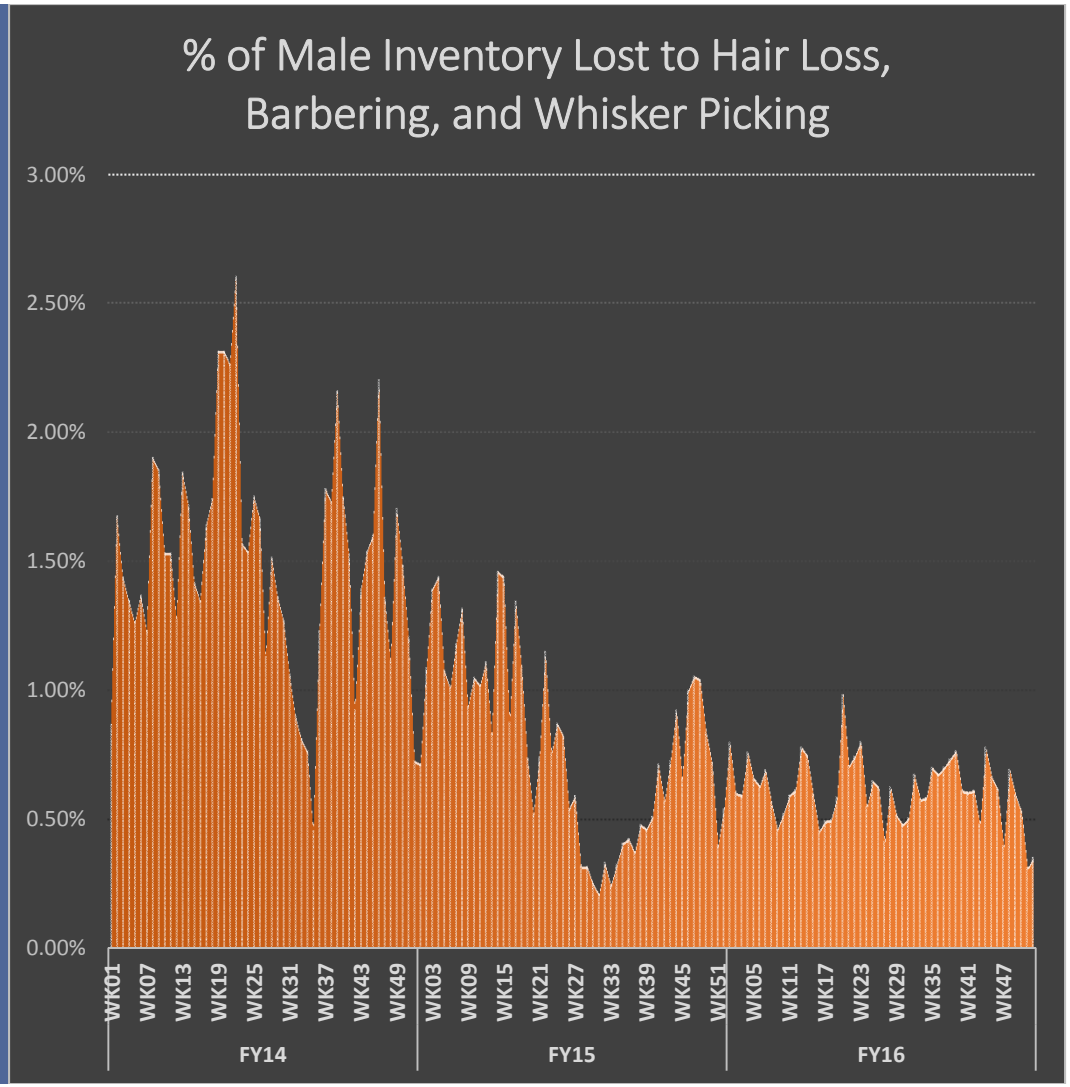
Common reasons for post-wean loss:

- Barbering
  - Hair Loss
  - Whisker Picking
- Aggression
- Eye defects
- Malocclusion



Tunnels & Huts reduced C57BL/6J female post-wean losses

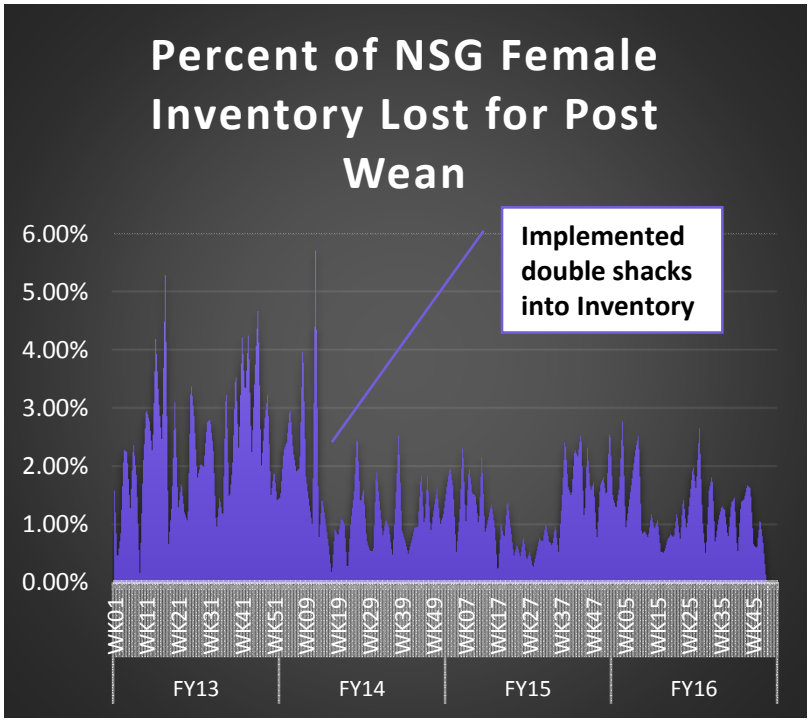
- 50% decrease in loss



Tunnels reduced C57BL/6J male post-wean losses

- 50% decrease in loss

Enrichment in Immunodeficient NSG Mice



NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ

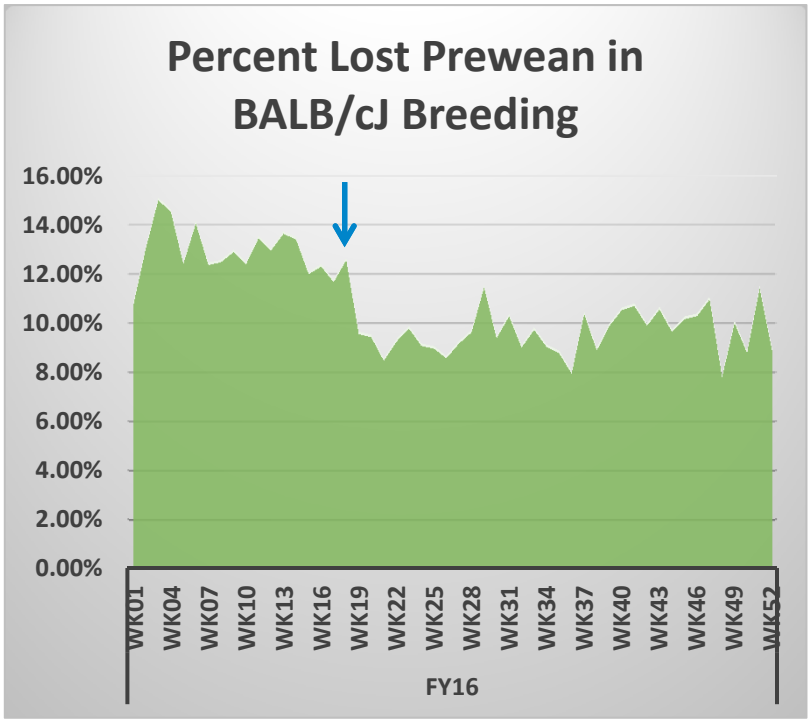
Huts reduced NSG female post-wean losses in inventory

Losses in Breeding Colonies

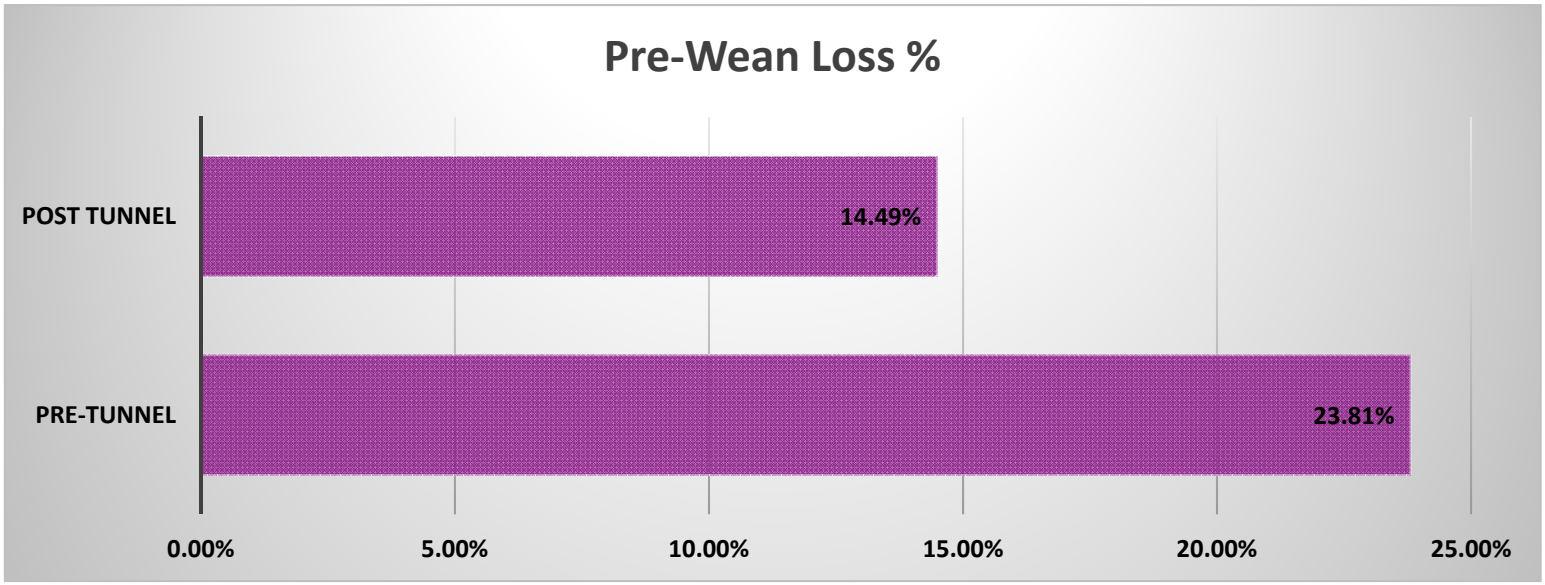
Common reasons for pre-wean loss in breeding cages:

- Runts
- Missing Mice
- Barbering
  - Hair Loss
  - Whisker Picking

Huts Improved BALB/cJ Pup Survival

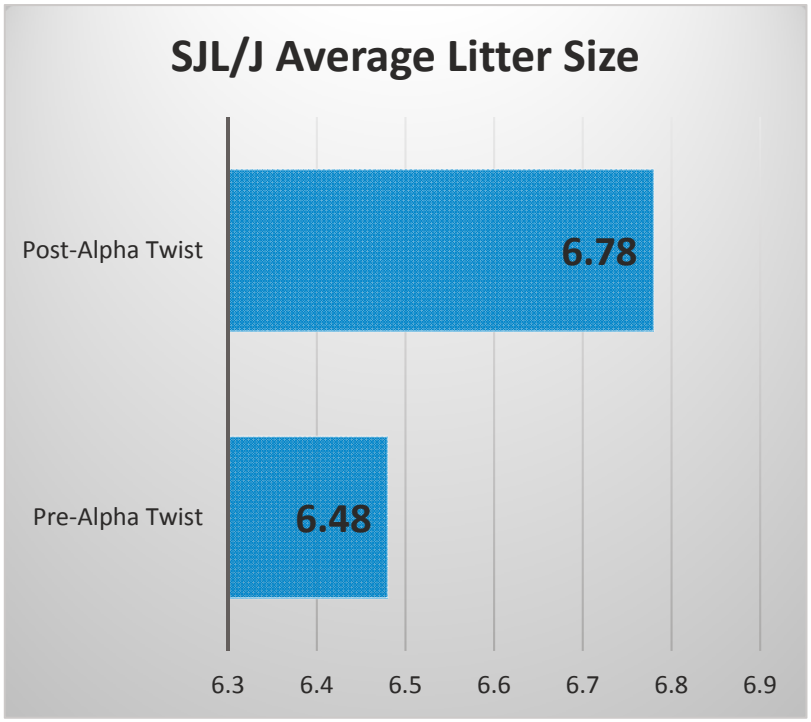


Tunnels Improved C57BLKS/J Pup Survival



Bio Tunnel enrichment supplied to breeders

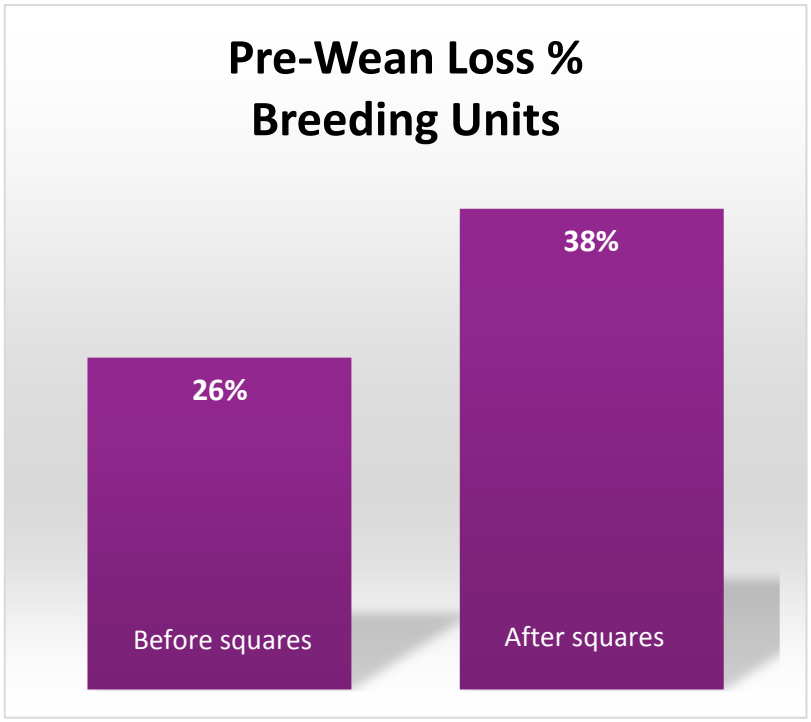
Twisted Paper Enrichment Improves SJL/J Breeding



Twisted paper demonstrated a positive impact on the breeding performance for SJL/J Breeding Colonies.

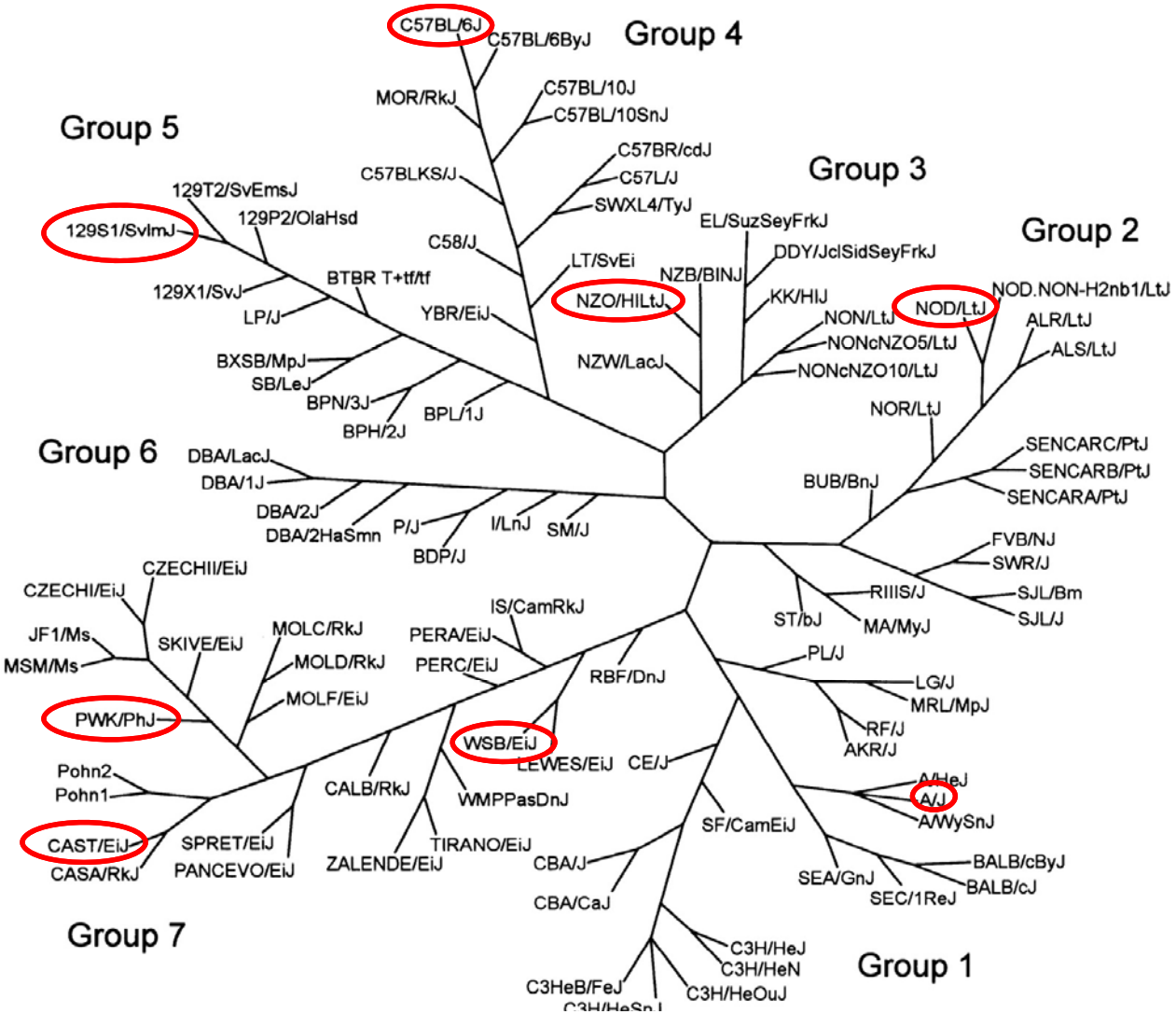
Gains in productivity for both average litter size and wean output.

Cotton Squares Did Not Increase Pup Survival in B6.129S7-*Ldlr*^{tm1Her}/J



- A 12 week trial of cotton squares showed no improvement to breeding or prewean loss.
  - Struggling with runty mice, missing mice, as well as hair loss in pups.

J:DO, A Highly Diverse Population of Mice

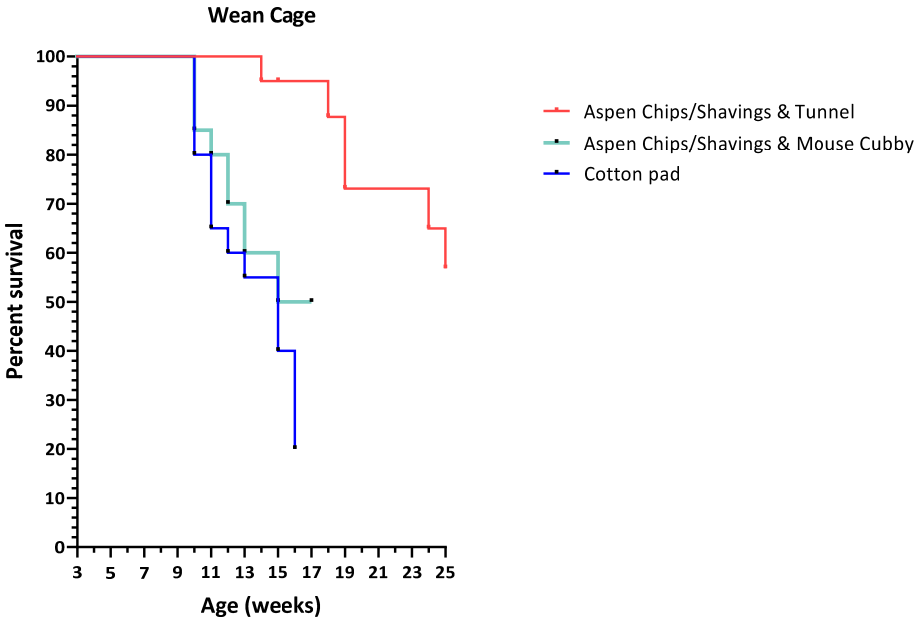

C57BL/6J
C57BL/6ByJ
Group 4
MOR/RkJ
C57BL/10J
C57BL/10SnJ
Group 5
C57BR/cdJ
C57BLKS/J
C57L/J
Group 3
SWXL4/TyJ
129T2/SvEmsJ
EL/SuzSeyFrkJ
129P2/OlaHsd
Group 2
129S1/SvImJ
C58/J
DDY/JclSidSeyFrkJ
LT/SvEi
NZB/BINJ
BTBR T+tf/tf
NOD.NON-H2nb1/LtJ
129X1/SvJ
NZO/HlLtJ
KK/HlJ
NOD/LtJ
LP/J
YBR/EiJ
NON/LtJ
ALR/LtJ
NONcNZO5/LtJ
ALS/LtJ
BXSB/MpJ
NZW/LacJ
NONcNZO10/LtJ
SB/LeJ
NOR/LtJ
BPN/3J
BPL/1J
BPH/2J
SENCARC/PtJ
Group 6
DBA/LacJ
SENCARB/PtJ
BUB/BnJ
DBA/1J
SENCARA/PtJ
DBA/2J
P/J
I/LnJ
SM/J
DBA/2HaSmn
FVB/NJ
BDP/J
SWR/J
CZECHII/EiJ
RIIIS/J
SJL/Bm
CZECHI/EiJ
IS/CamRkJ
ST/bJ
MA/MyJ
SJL/J
JF1/Ms
MOLC/RkJ
PERA/EiJ
SKIVE/EiJ
PL/J
MSM/Ms
MOLD/RkJ
PERC/EiJ
RBF/DnJ
LG/J
MOLF/EiJ
MRL/MpJ
PWK/PhJ
RF/J
WSB/EiJ
AKR/J
LEWES/EiJ
CE/J
Pohn2
A/HeJ
CALB/RkJ
Pohn1
A/J
WMPPasDnJ
A/WySnJ
SF/CamEiJ
CAST/EiJ
SPRET/EiJ
TIRANO/EiJ
BALB/cByJ
CASA/RkJ
PANCEVO/EiJ
ZALENDE/EiJ
CBA/J
SEA/GnJ
BALB/cJ
CBA/CaJ
SEC/1ReJ
Group 7
C3H/HeJ
C3H/HeN
Group 1
C3HeB/FeJ
C3H/HeOuJ

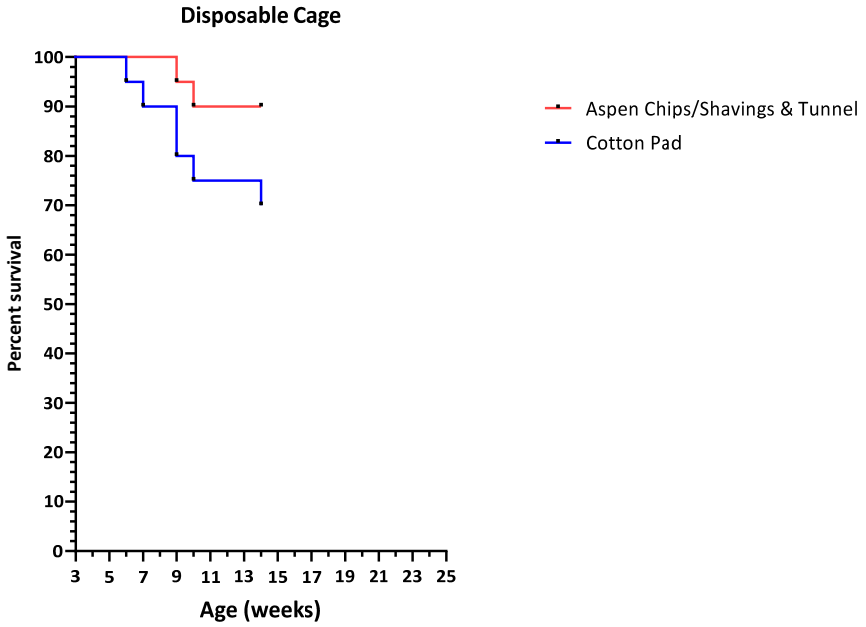
Study Design

To identify conditions leading to reduced aggression, J:DO males were assigned to one of 5 groups at 3 weeks of age:

GROUP	CAGE	BEDDING/ENRICHMENT	MICE/CAGE	NUMBER OF CAGES
A	Disposable	Aspen chips/shavings, tunnel	4	5
B	Disposable	Cotton pad	4	5
C	Wean box	Aspen chips/shavings, tunnel	4	5
D	Wean box	Cotton pad	4	5
E	Wean box	Aspen chips/shavings, cardboard divider	4	5

Aspen Chips/Shavings & Tunnel Group Survived Longest

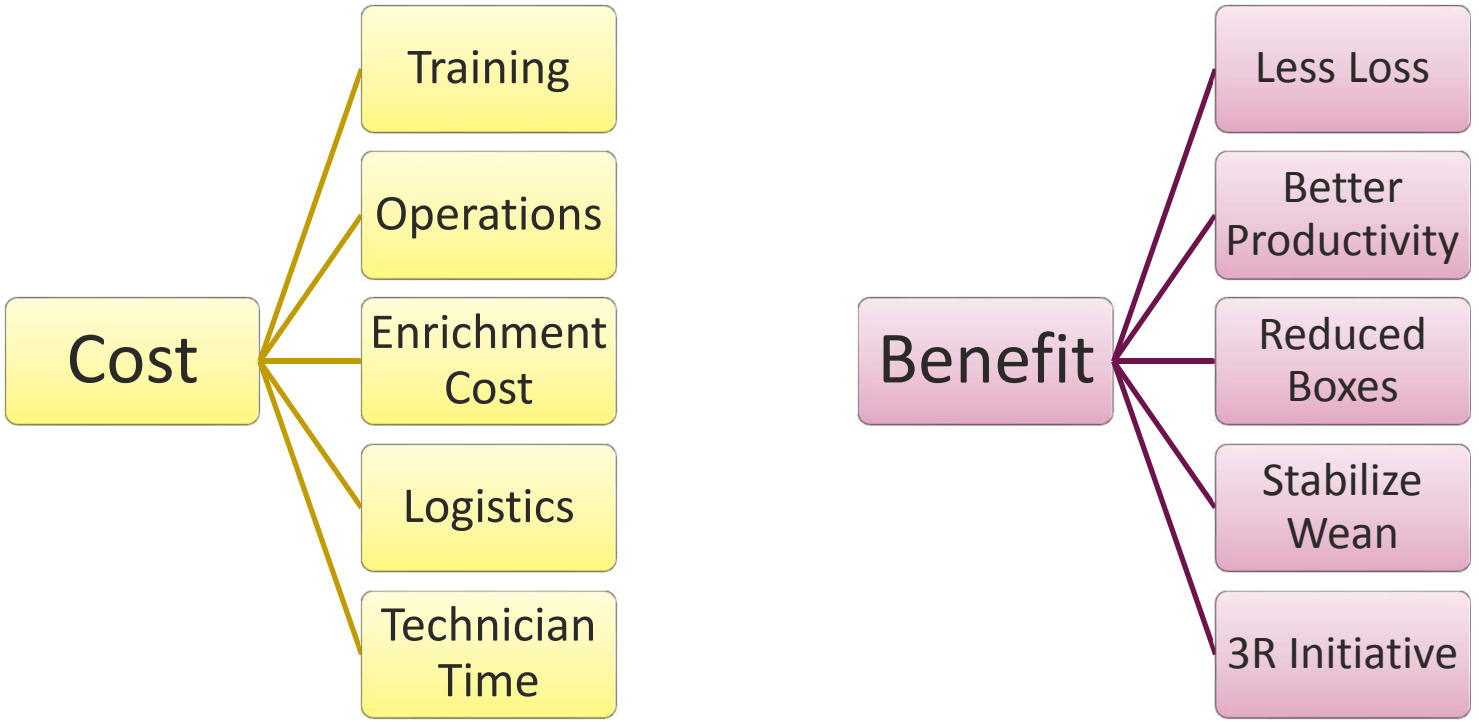




Aspen Chips/Shavings & Tunnel Losses:
- 5 for bite wounds, 1 for barbering

Study discontinued at 14 weeks of age
(chewing on disposable cages)

Cost Versus Benefit



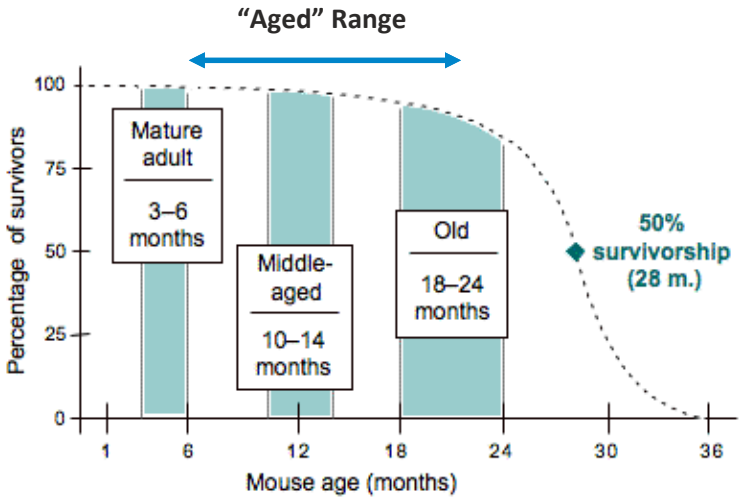
Enrichment Discussion

- Environmental enrichment can improve mouse welfare and breeding productivity
- Benefits depend on the strain
- Enrichment trials and comparisons help to determine the best type for each strain

Aged C57BL/6J Mice

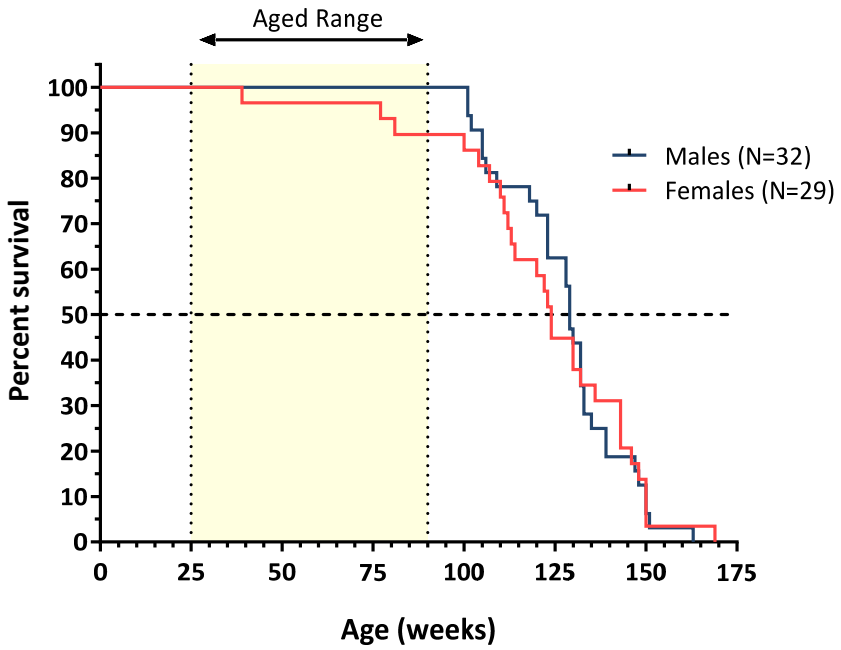
- Most studied inbred mouse strain
- Reference genome for mice
- Prone to eye defects, hair loss, malocclusion, dermatitis
- Males and females are offered up to 90 weeks (~22 months) of age

Human – Mouse Life Stage Equivalencies



Adapted from Figure 20-3: Flurkey, Currer, and Harrison, 2007. 'The mouse in biomedical research.' in James G. Fox (ed.), American College of Laboratory Animal Medicine series (Elsevier, AP: Amsterdam; Boston)

Survival of C57BL/6J



Data from the Nathan Shock Aging Center at The Jackson Laboratory (Yuan2 data set in the Mouse Phenome Database; phenome.jax.org)

Defining Tolerated Conditions for Aged B6 Colonies

Tolerated

- Weight variation
- Coat color changes
- Hair loss

Not Tolerated

- Dermatitis
- Bite wounds
- Malocclusion
- Hernias
- Other adverse clinical conditions

Husbandry & Housing Conditions

Diet: LabDiet 5K52 formulation (6% fat by weight)
Water: Filtered, acidified & autoclaved water in bottles
Change frequency: Weekly (ventilated) or 2X weekly (conventional)
Bedding: Aspen chips & aspen shavings
Extra enrichment: Cardboard shelters

General Observations

Few mice are lost on a typical week

Age Range	Females	Males
6 mo – 1 yr	0.18%	0.16%
1 – 1.5 yrs	0.71%	0.14%
1.5 yrs +	TBD	1.0%

Common reasons for losses

Females

Aged 6 Mo – 1 Yr
Dermatitis (53%)
Bite Wounds (20%)

Aged 1 – 1.5 Yrs
Dermatitis (52%)
Mortality (16%)

Males

Aged 6 Mo – 1 Yr
Mortality (28%)
Dermatitis (18%)

Aged 1 – 1.5 Yrs
Mortality (44%)
Bite Wounds (14%)

Aged >1.5 Yr
Mortality (57%)
Dermatitis (12%)

number indicates % of losses due to the indicated reason

Jan 2017 – Aug 2019 data

Additional Information

- Aged B6 Strain Data Sheet (jax.org/aged-b6)
- Mouse Phenome Database (phenome.jax.org)

The End

Thank you!

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- Richard French