Mouse Reproductive Biology for Strain Rederivation

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Reproductive Characteristics

- **Sexual maturity**
  - ≥ 3 - 4 wks (females, varies with strain)
  - ≥ 8 wks (males, varies with strain)

- **Estrous cycle** - every 4-5 days

- **Gestation** - 18 - 21 days (varies strain)

- **Weaning age** - ~ 3 weeks (varies with strain)

- **Age when mice most productive** (varies with strain)
  - Females: 6 wks - 6 mo
  - Males: 8 wks - 8 mo
Not all strains created equal! Know your strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Productive matings</th>
<th>Weeks at first mating</th>
<th>Litter size</th>
<th>No. of litters</th>
<th>Relative fecundity</th>
<th>Response to superovulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>129/SvJ</td>
<td>75%</td>
<td>7.9</td>
<td>5.9</td>
<td>4.1</td>
<td>18.1</td>
<td>High</td>
</tr>
<tr>
<td>A/J</td>
<td>65%</td>
<td>7.6</td>
<td>6.3</td>
<td>2.9</td>
<td>11.9</td>
<td>Low</td>
</tr>
<tr>
<td>AKR/J</td>
<td>84%</td>
<td>6.6</td>
<td>6.1</td>
<td>2.2</td>
<td>11.3</td>
<td>Low</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>47%</td>
<td>8.0</td>
<td>5.2</td>
<td>3.8</td>
<td>9.3</td>
<td>Low</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>86%</td>
<td>6.7</td>
<td>5.7</td>
<td>2.9</td>
<td>14.2</td>
<td>Low</td>
</tr>
<tr>
<td>C3H/HeOuJ</td>
<td>99%</td>
<td>5.9</td>
<td>6.4</td>
<td>3.7</td>
<td>23.4</td>
<td>-</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>84%</td>
<td>6.8</td>
<td>7.0</td>
<td>4.0</td>
<td>23.5</td>
<td>High</td>
</tr>
<tr>
<td>C57BL/10SnJ</td>
<td>67%</td>
<td>7.7</td>
<td>6.3</td>
<td>2.8</td>
<td>11.8</td>
<td>Low</td>
</tr>
<tr>
<td>CBA/CaJ</td>
<td>96%</td>
<td>6.4</td>
<td>6.9</td>
<td>2.7</td>
<td>17.9</td>
<td>High</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>75%</td>
<td>7.4</td>
<td>5.4</td>
<td>3.9</td>
<td>15.8</td>
<td>Low</td>
</tr>
<tr>
<td>FVB/N</td>
<td>&gt;90%</td>
<td>-</td>
<td>9.5</td>
<td>4.8</td>
<td>41.0</td>
<td>Moderate</td>
</tr>
<tr>
<td>SJL/J</td>
<td>72%</td>
<td>7.4</td>
<td>6.0</td>
<td>3.1</td>
<td>13.4</td>
<td>High</td>
</tr>
</tbody>
</table>

Note differences between BALB/c and C57BL/6 with regard to “Productive Matings” (pups born) and females’ response to superovulation.

Oxford University Press. [wwwinformatics.jax.org/silver/](http://wwwinformatics.jax.org/silver/)
Mating Cages

• Avoid top shelf *(too much light).*

• Avoid noise, vibrations, or the front of rack *(too much traffic /disturbances).*

• Avoid strong odors *(wearing strong perfumes).*

• Provide paper tube *(gives females a place to hide from male) and nestlets.*

• No breeder diet for mating cages *(males get fat).*

• Handle cages gently and slowly!
Pregnant Females

- Place (2) pregnant females into a clean cage with paper tube + nestlet + breeder chow. Place a pink special request card stating “DO NOT DISTURB - LAB WILL CHANGE CAGE”

- (2) pregnant females per cage takes advantage of “aunting.”

- Cages should be placed in a quiet area and isolated from mating cages or cages with males.

- Check daily. Change cage if dirty but never if P0-P3 pups present → mother may kill the pups.
How old are the pups?

P0 - bright red, do NOT disturb!

P1 - pink, do NOT disturb!
P3 - ear flaps 90° from head, males of black strains have scrotal spot, do NOT disturb!

P4 - ears in normal position; all toes separated; can change cage.

P6-7 - hair on back but not on belly.

P9-10 - hair on belly; nipples obvious in females; males do not have nipples

Change cage at P4. If the litter is large and you can identify males using scrotal spot, cull excess males. If can’t sex at P4, sex and cull at P10.
P11-13 - eyes start to open; appear as teary “slits”; teeth start to erupt on P11

P21 - wean (“popcorn stage” - very active)
Sexing Pups Prior to Weaning

- Why important - can cull males as early as possible \(\rightarrow\) avoids overcrowding; allows pooling and fostering of female pups (4-5 per dam) \(\rightarrow\) less cages; growth advantage of female pups (more milk).

  - **Anogenital distance**
  - **Scrotal spot**
  - **Nipples**
  - **Hairless Line**
  - **Combination**
Anogenital Distance (hardest)

- **P0** (albino strain)
  - Male
  - Female
  - Distance in males is ~2X females
  - Not recommended as stand alone; takes practice; but only way to sex albino strains from P0 - P8.

- **P5** (albino strain)
**Scrotal Spot** (P0 - P8, pigmented strains)

- Scrotal spot: easy but pigmented strains only (males only)
- Nipples: ≥ P8 (all strains; females only; easiest at P10)
- **Nipples**: P8 - P10 (white hairs in black mice; bare spots in albino or agouti mice; easiest at P10; can become difficult with age in agouti mice)

- **Hairless Line** - between vulva and anus (all strains; ≥P10; females)
P21 (weaning)

- Hairless line between vulva & anus (blue arrow)
- Nipples (red arrow) - not obvious in the agouti female

Male

Female

Male

Female

- Anogenital distance
Lee-Boot + Whitten Effects

- Females crowded together (5 mice/cage) **without a male** $\rightarrow$ estrous cycle stops (Lee-Boot Effect).

- Non-cycling females re-initiate estrous after exposure to a male (Whitten Effect).

- Why important - synchronizes estrus. Adding a stud male after 2 weeks $\rightarrow$ all females enter estrus $\rightarrow$ all females get pregnant and give birth at about the same time $\rightarrow$ many female pups at about the same age (cull male pups at P10 or earlier to avoid over-crowding and enhance growth of female pups)
Help is Available!

- If you do not have experience breeding large numbers of mice, consult with Dr. Claude Nagamine (cnagamin@stanford.edu) before starting the breeding.

- Once you’ve gotten several litters and identified $\geq 10$ female pups, contact the Transgenic Core to schedule a rederivation date.

- Your breeding males can be used for studs or IVF.