Tips for Successfully Breeding Your Mice

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Breeding Objectives

- Maximize pup yield
  - Meet PI research goals
- Maximize quality
  - Produce healthy offspring
  - Desired genotype
- Minimize costs (personnel, per diems)
  - Avoid excess pups
  - Recognize poor producers

Meeting Objectives

- Know your goals
- How many breeders
- How many offspring
- How long will it take to produce #’s needed

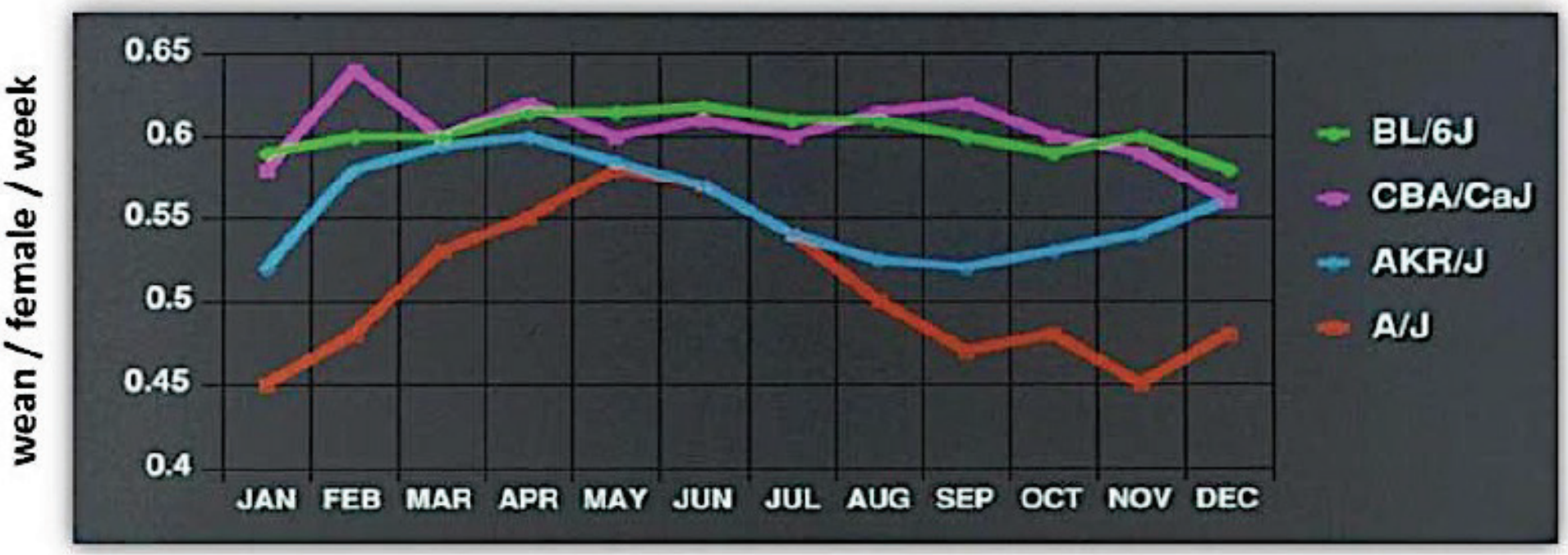
Reducing Costs

- Size your colony to meet your needs
- Use both sexes when possible
- Use most productive age range for your strain (e.g., 4-8 weeks of age)
- Rotate breeders on strict schedule
- Replace poor breeders ASAP
- Hire a productive colony manager
- Cryopreserve unique & low-use strains

Observing the 3 R's

- Reduce – Minimize # of animals used through sound breeding management practices
- Refine – Minimize pain/distress through sound breeding practices
- Replace – Replacement of animals with non-animal models when possible

Seasonal Variation



4 Life Stages of the Mouse

- Neonate
  - Birth to weaning at 21 to 28 da (3 - 4 wk)
- Sexual maturity
  - 40 to 60 days (5.5 - 8.5 weeks) – M a bit later than F
- Adult size
  - 8 to 10 weeks
- Geriatric
  - 18 Mo+

Reproductive Data

Life span (y)	1–3
Puberty age	male 5–7 wk female 4–5 wk
Optimum reproductive age span	male 2–10 mo female 2–10 mo
Sexual cycle type	Polyestrous
Sexual (estrous) cycle duration	4–5 d
Estrus duration	12–14 h
Postpartum estrus	Fertile
Gestation period	19–21 d
Litter size/# of young	4–12
Age at weaning	21–28 d

Reproductive Data of Mice

Stage	Age
Sexual maturity	40–60 days
Estrous cycle	4–5 days
Postpartum estrus	Within 24 hr
Gestation	18–21 days
Weaning age	21–28 days
Puberty	F: 4–5 wk; M: 5–7 wk
Reproductive life	±8 months
Life span	1–3 years
Pups begin eating solids	12–14 days

Sexual cycle type: Non seasonal polyestrus

Breeding Facts

- Fecundity (ability to produce offspring rapidly and in large numbers)
  - Outbred greater
  - Inbred diminished

Estrous Cycles

- 4 to 5 da
- Receptivity
  - 10 hrs
  - Vulvar swelling or redness

Receptivity Concepts

Lee-Boot Effect

- Pseudo pregnancy in F's housed together (no male in cage)
- Unknown factor(s) related to all-female housing
  - F pheromones influence???
  - Lack of M pheromones???
- To induce estrus
  - Separate F's
  - Add bedding from M cage to expose to M pheromones

Whitten Effect

- Isolated F's (no male in cage)
- M is introduced to F's
- Estrus synchronization - cycling starts in unison within 72 hrs & conceive together (due to M pheromones)
- Used for timed mating

Bruce Effect

- Pregnant F's (no male in cage)
- Strange M introduced within 24 hr of mating
- Prevents embryo implantation
- Estrus occurs in ~4 da
- Occurs in wild, outbred & some inbred strains

UTD Recognized Breeding Schemes

- Monogamous Pairs
  - 1 Male/1 Female in same cage
- Harem Mating
  - 1 Male/2-3 Females in same cage

Monogamous Pairs

- 1 M/1 F
- Pair remains together when F pregnant or delivers
- 3-wk old litter needs separated < next litter born
- M may need removed during post-partum estrus to prevent impregnating lactating F

Monogamous Pairs

- Advantages
  - Takes advantage of 24-hr post-partum estrus
  - Easy to track/greater control over production
  - Reduces risk of fighting bet aggressive F's
- Disadvantages
  - Costs – requires more cages
  - Space – more cages = more space
  - Older litter has to be weaned before next litter is born. May be problem with weak strains
  - Production decreased (vs. harem)

Harem Mating

- 1 M/2-3 F
- Detected preg F is removed to new cage
- Avoiding overcrowding - alternatives
  - Only 1 nursing F with litter in cage
    - May return F to harem > her pups weaned
  - 1 or more F's noticed pregnant, M removed & housed singly
    - Remaining F's may deliver in same cage – after pups weaned, M may be returned to the harem cage

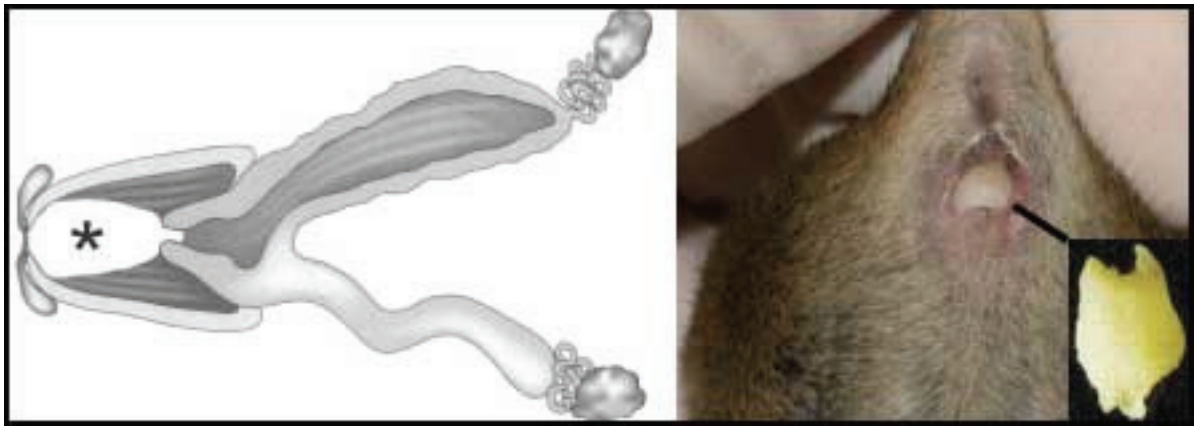
Harem Mating

- Advantages
  - Other adults may help raise progeny
  - Maximizes ave # of litters/F & minimizes cost
  - M can't kill newborns
- Disadvantages
  - Misses 24-hr post-partum estrus
  - More difficult to track progeny – increased logistics
  - Easier to lead to overcrowding – if pregnancy missed when another litter in cage

Detecting Mating

Plug

- Plug = Coagulated spermatic fluid
- Tip protrudes from vagina – Falls w/in 12 hr
- If not visible, use a probe
- Most mice breed at night



Plugged or Not Plugged

Why Check Plugs

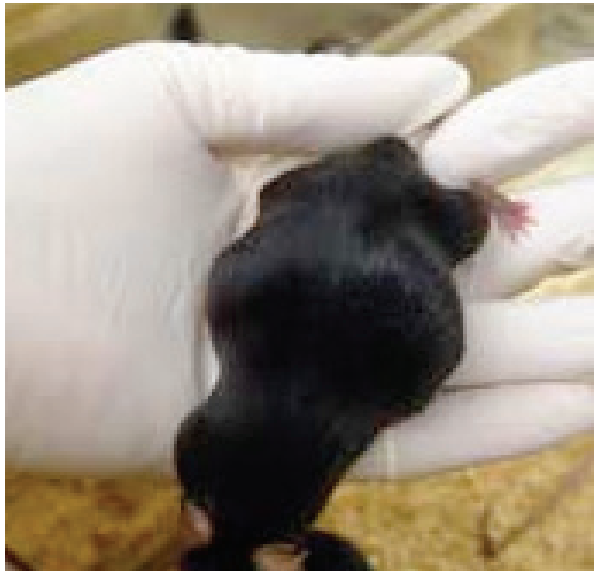
- Used for time mating
- Provides exact date of gestation to
  - collect embryos
  - plan delivery date for experiments

Checking Plugs

- Take F to M's cage late afternoon
  - Most mating occur during dark cycle
- Check plugs early next day
  - Before they melt away
- Plug detection = 1st day of pregnancy
  - However, plug ≠ pregnant &
  - No plug ≠ not pregnant

Identifying Pregnancy

- Observable at 10 da
- Clearly observable at 12 da
- Palpable at 14 da – '*string of pearls*'
- Small litters later detection
- Check for pregnancy to
  - provide nesting material
  - separate animals if needed/required
  - Identify any breeding problems

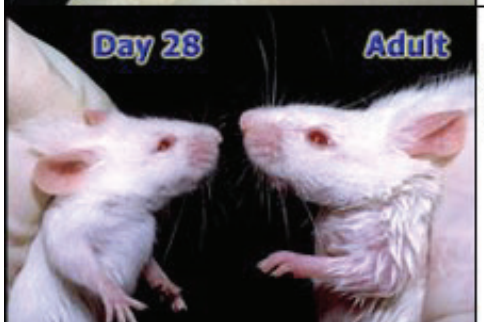


Parturition

- Disturbing cage ~2-3 da pre partum, 3-5 da post partum may lead to cannibalism or pup rejection
- Enrichment/nesting material paramount
- Role of male
  - C57BL/6 male often sits on pups to keep pups warm. Female with companion less likely to neglect pups
  - 129 males don't help & may distract female

	The ears and eyes are closed. If the pups are nursing, milk can easily be seen in the stomach and is referred to as a milk spot.
	The pups are less red, more pink. Milk can still be seen in the stomach.
	The ear flaps begin to open. If the pups are black or agouti, the skin pigment begins to develop.
	The ears are erect, and milk is no longer visible in the stomach.
	The pups begin to develop light, fuzzy dorsal fur.

	The fur is thicker across the shoulders, and the coat color may be evident.
	The back of the pups is now completely covered in fur.
	The belly begins to show fur.
	The fur is now thicker. Females may show nipples (there are five pairs of mammaries).
	The fur growth is complete. The pups are more active.

	The teeth are beginning to erupt, and the eyes start to open
	At days 12-14, the eyes are open, and the pups begin to nibble solid food. By 3 weeks of age, the pups become very active (popcorn stage).
	Pups are weaned at 21 days.
	Compare the size and shape of the head of a 28-day old (L) vs an adult mouse (R).

Pup Age Day 1-13 Summary

Postnatal Day 0: Blood red; possible milk spot

1: Lighter color red; milk spot present

2: Ears appear as nubs; pigment may start to appear in some strains

3: Ear flap starting to come away from head (one or both)

4: Ears fully developed; completely off head; ears may start to migrate towards back

5: Ears all back; skin appears much thicker with more color density to skin

6: Milk spot disappearing or gone; skin still shows; colored fuzz appears behind ears or on neck

7: Colored fuzz starts to cover pup; skin does not show

8: Belly starts to show fur; lower incisors show but not erupted

9: Fur now thicker; females start to show inguinal nipples (there are 5 pairs of nipples)

10: Lower incisors erupted

11: Upper incisors erupted

12: Eyes still closed

Record Keeping

- Identify ancestors, siblings & descendants
- Monitor mating activity and litters born
- Identify mice for experiments
- Select next generation of breeders
- Track DNA samples

Founder mice	~Copy number	Transmission	Liver Expression (See example 5)
D/1 female	40	+	-
D/4 male	4	+	+
D/13 female	39	+	-
D/18 male	50	-	+
D/34 female*	90*	+	-
" " " *	4*	+	+
D/63 female	30	+	-

*two transgene locl identified in this founder. These segregate in the next generation to generate a high copy (~90) and a low copy (~4) line. Note that founder D/18 did not transmit the transgene sequences.

Suggested Records

- Strain data – nomenclature, genetic background, history
- Animal numbers
- Pedigree records
- Birth dates
- Mating pairs (dam x sire) & date mated
  - Litters & pups born
  - Weaned pups – number, gender
- Genotypes
- Generation #

Suggested Cage Card Coloring System

	**Pregnant females**
	**Litter born**
	**Pups 1 week or less from weaning**

Breeding Card Sample

ID:					Source:			
					Species:			
male	female(s)	1	e.t.		Generation #	N#	F#	
1	1	2	e.t.		strain			
e.t.	2							
mate date	DOB	# NB	females	males	wean date	generation	Comments	

Breeding Card Sample

ID:					Strain:			
					DOB	Parent #	Breeder #	Genotype
Generation #		N#		F#			F#	
Set Up Date:							F#	
Genotyping sample(s)		Ear	Blood	Tail			M#	
Genes of interest:					Backcross?	No	Yes	To line #
DOB	# NB	F wnd.	M wnd.	Total wnd.	Wean Date	Dead	Comments	

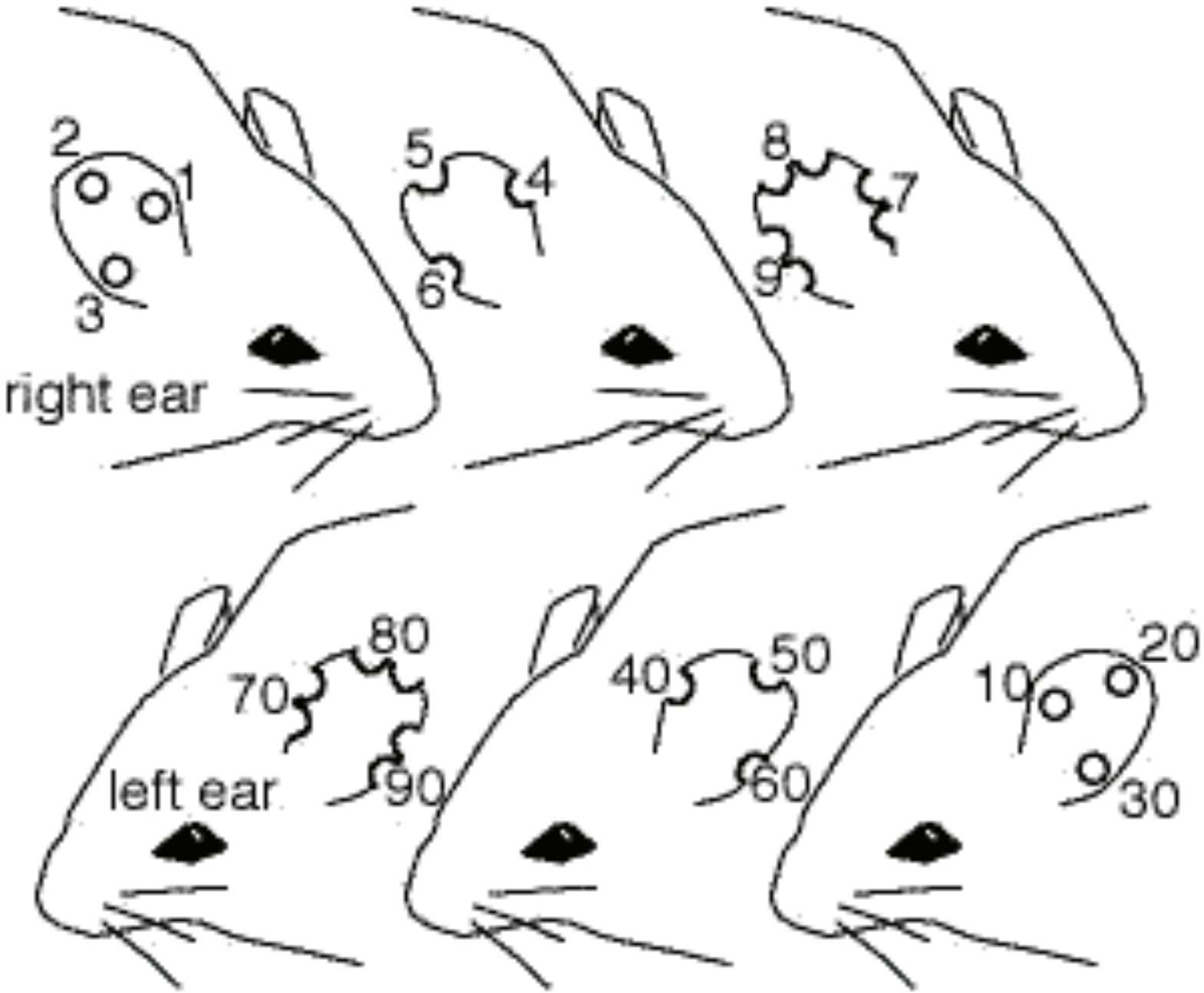
Weaning Card Sample

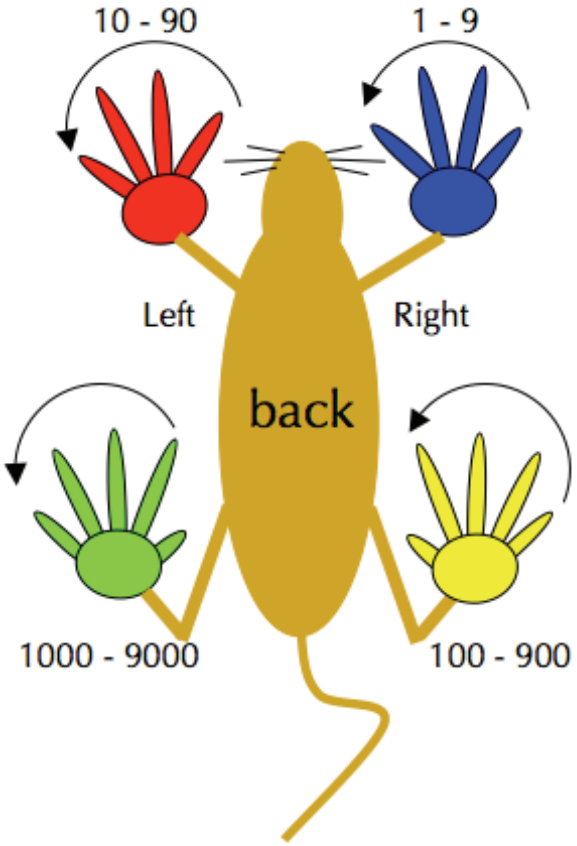
PI:	Protocol	# of mice
Strain:		sex
		DOB
		Wean Date
Sire: Dam:		Generation
ID# / Experiment		

weaning card

Pup Identification

- Ear punching or notching
  - Correspond to a numbering system
- Metal ear tag
  - Metal clip stamped with a number
- Toe clipping
- Tattooing
- Microchip
  - SC implant read remotely


2
1
3
right ear
5
4
6
8
7
9
80
70
90
left ear
40
50
60
10
20
30



Right front foot

0 1 2 3 4 5 6 7 8 9

Left front foot

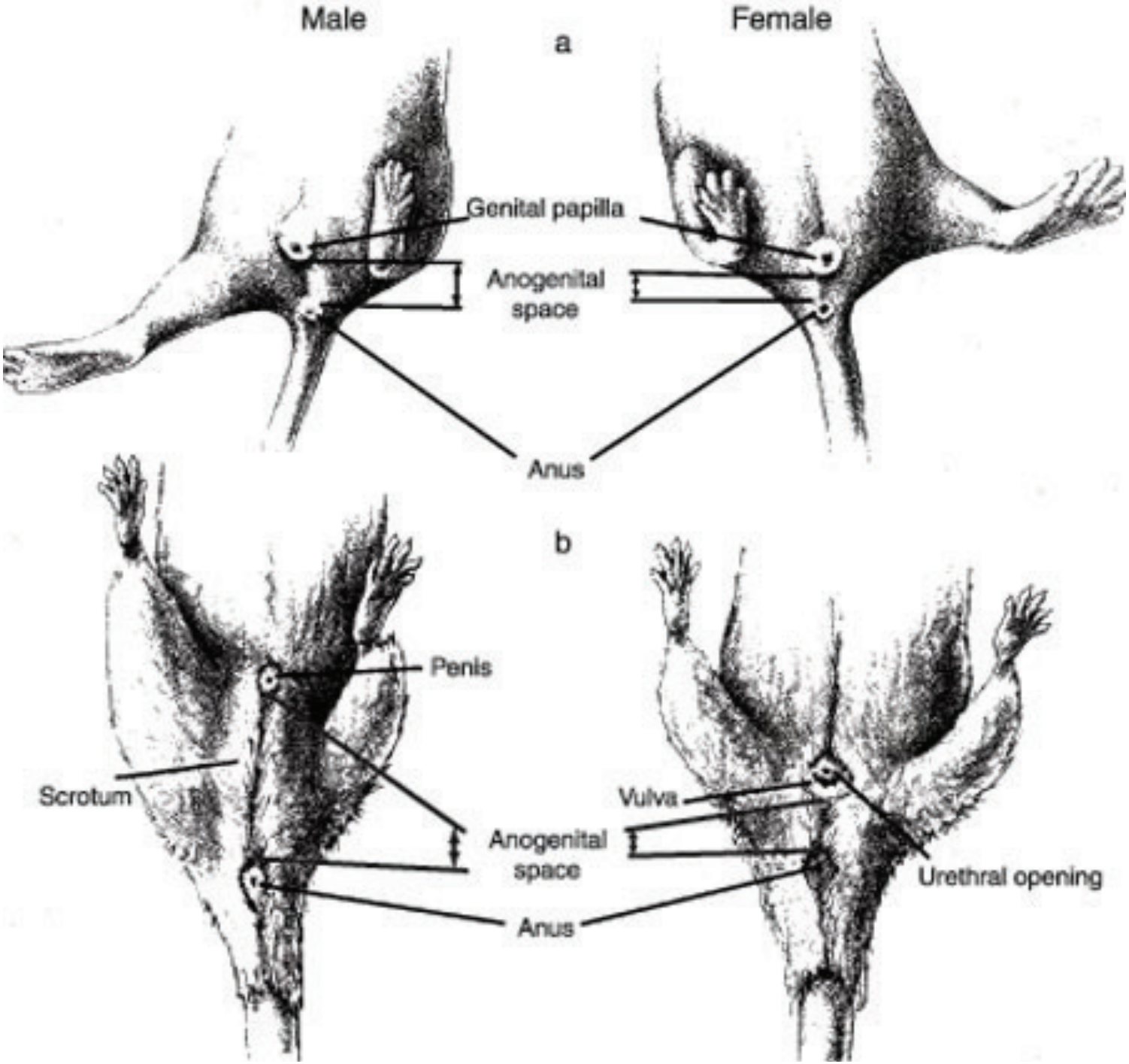
0 10 20 30 40 50 60 70 80 90

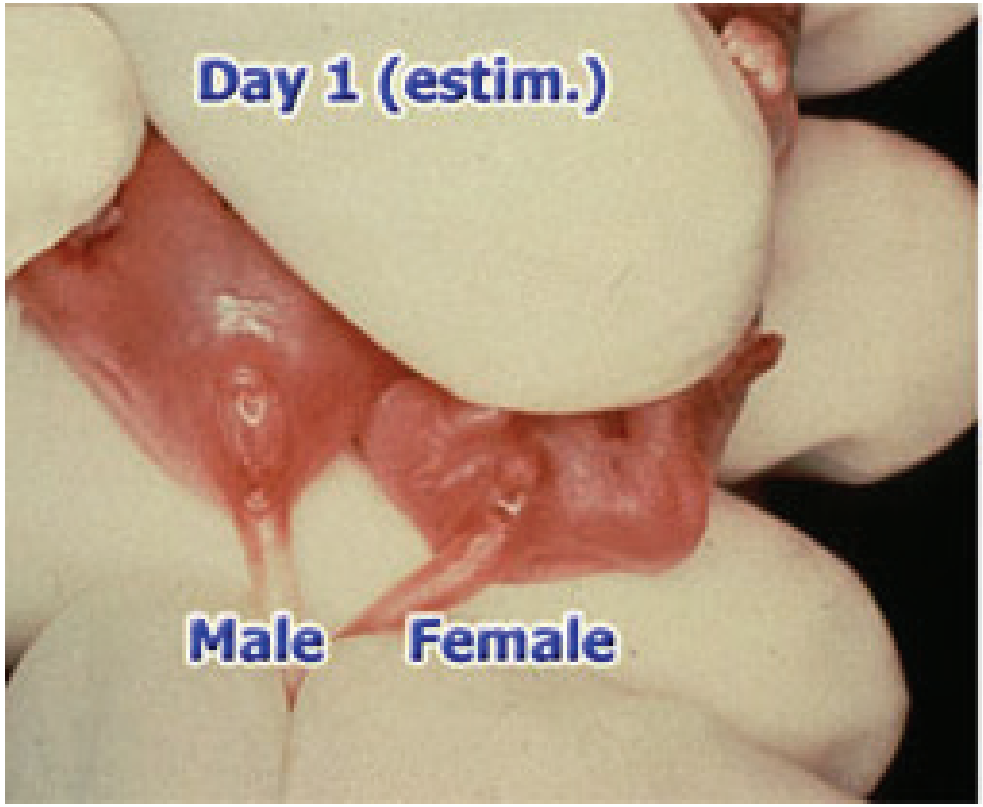
Right rear foot

0 100 200 300 400 500 600 700 800 900

Left rear foot

0 1000 2000 3000 4000 5000 6000 7000 8000 9000


Male
Female
a
Genital papilla
Anogenital space
Anus
b
Penis
Scrotum
Vulva
Anogenital space
Urethral opening
Anus


Day 1 (estim.)
Male
Female


Day 9 (estim.)
Female
Male

Method	Pros	Cons
Ear punching/ notching	Simple, inexpensive, easy to read	Subject to tearing and healing, limited numbers
Metal ear tags	Unique numbering, relatively inexpensive	Loss of tags, infections, hard to read
Toe clipping	Simple, inexpensive, permanent	Controversial, must be done at early age
Tattooing	Relatively permanent, easy to read, may be done on newborns	More difficult and time-consuming, may fade with time if done improperly
Microchips	Permanent, virtually unlimited numbers, can provide physiologic data	High cost per mouse, requires costly reader

Factors Affecting Breeding

Widespread & Isolated Problems

- Widespread
  - Environmental influence
  - Location of cages
  - Noise/vibration
  - Temp/humidity
  - Diet
- Isolated – When very few cages affected

Environmental Stressors

- Limit
  - Vibration
  - Noise
  - Light intensity
  - Traffic
  - Other stressors
- Falling barometric pressure makes some strains hyperactive & decrease breeding performance
- Environmental enrichment very important!!!

Limit Vibration

- Construction
  - Same building
  - Adjacent building
- Moving equipment
- Cage wash too close to animal rooms
- Minimize with vibration attenuation pads under racks

Limit Noise

- Speak softly
- Avoid extraneous sounds
- Avoid loud equipment
- Avoid music, bring vol down, use ear buds
- Close doors quietly
- Avoid sound fire alarms – replace with strobe light or low amplitude sound fire alarms

Limit Light

- Attenuate light &/or move to darkest location in room
- Ensure dark cycle is maintained once the lights are off
- Consider 14:10 (light:dark) cycle
- Continuous light halts all breeding in 3 days

Limit Light

Anecdotal story

- All breeding stopped for 3 mos
- No one thought of checking light:dark cycle for 3 mos
- Lo & behold!… lights continuously on
- Breeding resumed once light:dark cycle reestablished
- Precious research & publication time lost

Limit Traffic

- Limit room activity
- Use same staff consistency
- Move breeding racks away from entrance door
- Resist the temptation to check on mice too often

Temperature & Humidity

- Too high often the problem
  - Temp above thermal neutral zone (TMZ)
  - High humidity
  - Ask facility management if they've been getting high temp/humidity alarms

Diet

- Increased or decrease caloric needs
- Fat content in diets ranges from 4% to 11%
- Diets with a higher (or lower) fat or protein, compared to standard diet, may improve productivity of challenging strain

Diet

Increased fat helps some weak strains improve milk production

- Overweight males may stop breeding
- Sometimes 50:50 breeder's:standard diet improves breeding w/o overweight issues
- Consider softened diet such as Clearh2o's *DietBoost* Gel or wetted pellets on cage floor

Limit Other Stressors

- Extraneous odors – prohibit use of perfume
- Limit cage change intervals especially individually ventilated caging
  - Rapid air movement within cage readily removea NH3, CO_2 & dries cage allowing less frequent changes
- Consider bedding – Some strains may prefer a particular bedding
- The overly worried PI

The Overly Worried PI

Anecdotal story

- PI reports chronic pup cannibalism
- While working in room I observe PI opening cage and handling newborns (by the way, with bare hands)
- What's the problem?

Environmental Enrichment

- A powerful tool to diminish stress & improve breeding
- Nestlets, Enviro-dri, huts, food treats, Kimwipes
- Chewing toys

Environmental Enrichment

Anecdotal story

- UTSA implements red huts in all breeder cages
- 2 months later PI emails LARC
  Ever since implementation of the red huts, my animals have gone into a breeding frenzy. My rats have doubled their pup yield

Environmental Enrichment

Anecdotal story

- Texas Tech HSC, El Paso
- Hypertensive Zucker rats implanted with blood pressure telemetry in abdominal aorta
- Days > recovery, added Enviro-Dri
- *Mean BP drops 10-20 points*

Disselhorst D, Long L, Baran S, Johnson E, Terreros D, Perret-Gentil M. *Effects of Environmental Enrichment During the Post-Surgery Recovery Period of Rodents*. Poster. 48th Texas Branch of AALAS, San Marcos, TX, April 2010

Breeding Problems

Common Complaints

- Moms not getting pregnant
- Moms killing pups
- Moms neglecting pups
- Mom died. What do I do with pups?
- Litters too small
- Pup yield genotype/phenotype not what was expected

Female Not Getting Pregnant

- Too young/too old? Time to replace?
- Mate early. 6-8 wk-old mice breed better than when started older
- Environmental issues?
- Is there a male in cage? (wrong sexing)
- Sterile male? Have you done a sperm count?
- Same male? Consider rotating males
- Inexperienced male? Experienced males often get the job done
- Did you observe plugs?
- Checked if vaginal septum?

Pups Cannibalized or Neglected

- Are cages being disturbed (too freq observations)
- Room too hot?
- 1st litter? Many 1st litter moms do well with 2nd litter
- Is male eating pups?
- Nesting material in cage?
- Another female or male in cage may help raise young
- Should companion female be removed?
- Older litter with new litter?

Dystocia

- Malpositioned or too large pups?
  - C-section & cross fostering may be needed

Cross Fostering

Why Foster

- Death of mother
- Maternal neglect, inability to care for young or cannibalism
- Strain known to be poor moms
- Poor milk production
- Litter too large & want to keep every pup
- Eliminate certain pathogens (e.g., *Helicobacter*)

Selecting Foster Mothers

- Proven mother (2-3 successful litters)
- Mother should have delivered ±48 hrs older or younger than pups to be fostered
- Use different color than fostered pups to allow identification at weaning
- Outbred strains (CD-1, ICR, or Swiss) make great mothers

Important Conditions

- Total litter size should remain about the same
  - Implies removing some of foster mother's own pups
  - Foster mother may have enough milk for 1-2 more extra pups
- Foster & natural pups within 48 hrs of age of each other
  - Milk production related to age of the mother's own pups

Introducing Foster Pups

1. Use clean gloves
2. Place foster mom in different cage
3. Have mother urinate on gloves
4. Rub or roll soiled litter on foster pups
5. Place foster pups in cage among natural pups
6. Wait 15 minutes
7. Return foster mom (and dad if he was housed with the litter) to pups cage

Improving Fostering Success

- Distract foster mother when introducing to foster pups
  - A whiff of a drop of wintergreen, alcohol or Betadine
  - Food treats on the floor of the cage (e.g., Bacon Softies)
- Do not disturb cage for several hrs or observe from a distance
- A good foster mom will begin nursing & clean pups w/in 1 hr

Unsuccessful Fostering

- After fostering complete – Do not disturb!
- Place cage on a surface where animals can be observed from a distance or at end of a rack
  - Find another foster mom if no nursing or cleaning within 6 hrs

When Disaster Strikes

- Replenishing founders or difficult transgenic lines?
  - Semen or embryo storage?
  - Are these lines available elsewhere?
  - If not, have you cryopreserved semen or embryos?

Benefits of Cryopreservation

- Saves space - especially when a strain is used infrequently
- Saves money - initial cost may seem high, but follow-up cost of maintenance in a cryopreserved state low compared to maintaining live colony for same amount of time
- Insurance against catastrophic loss - fire, flood, earthquakes, disease…

Benefits of Cryopreservation

- Provides a method for eliminating pathogens from a mouse colony
- Reduces risk of a strain contamination due to breeding errors
- Prevents spontaneous loss of phenotype
- Slows the rate of genetic drift dramatically
- Diminishes possibility that the transgene copy number will change
- Peace of mind: Priceless