Animal Source

PSU Animal Resource Program

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Gender Identification in Mice and Rats

Figure 1: Adult male and female mice

Identification of male and female rodents can be challenging, especially with young animals. Here are a few specific characteristics to look for when attempting to determine mouse and rat gender.

1. Anogenital distance is greater in males than females at all ages. A useful method to assist in distinguishing between the sexes, especially in young animals, is to visually compare anogenital distance between animals held next to each other.
2. Female mice and rats have nipples that are visible on the ventral abdomen by 9 days of age in mice and by 8-15 days of age in the rat. Males do not have nipples. Unfortunately, after fur growth is complete, the nipples may not be easily seen on young females, e.g., weanlings.
3. Infant male mice and rats have a larger genital papilla than females and the testes may start to become visible within the scrotal sac by weaning age.

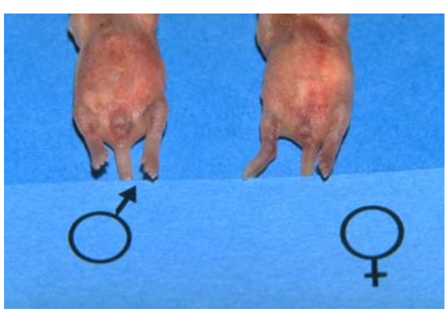

Figure 2: Infant male and female mice

Visit the ARP website at www.research.psu.edu/arp for answers to your animal use questions

Inside this issue:

Breeding Strategies in Mice

Researchers are often required to maintain breeding colonies of mice as part of their experimental investigations. A variety of breeding strategies exist for use in mice, all of which are intended to control the genetic background of the population. What follows is a brief introduction to a few commonly used breeding systems.

The process of breeding closely related individuals for sequential generations is termed inbreeding. Inbreeding will result in increasing genetic homozygosity in a population over time due to the loss and fixation of alleles throughout the genome. A population of mice in which 20 or more generations of brother-sister inbreeding has occurred is called an inbred strain. Members of an inbred strain are essentially genetically identical. Mathematically, mice in the 20th generation would be predicted to have genomes that are 98.7% homozygous. This increases to 99.98% homozygosity by the 40th generation.

Substrains occur when two distinct breeding lines are propagated from the same ancestoral pair for 20 generations of separation. For example, an investigator purchases a breeder pair of C57BL/6 mice from Laboratory A and maintains this line within his or her laboratory through sibling breeding for ten generations, while the colony at Laboratory A also passes through 10 generations. After a total of 20 generations of separation the investigator now possesses a substrain of the original Laboratory A strain. The substrains are genetically divergent due to genetic drift, the process of accumulating mutations through generations of separation. To avoid genetic drift the investigator would need to routinely replace his or her colony with mice from Laboratory A's colony.

The genetic homozygosity of inbred strains leads to a phenomenon called "inbreeding depression". Inbred mice tend to have diminished fecundity, reduced litter size and other deleterious health effects. The opposite of inbreeding depression is heterosis or hybrid vigor. Extensive heterozygosity of the genome results in increased fecundity, larger litters and overall robust health. The offspring resulting from the mating of two distinct inbred strains, termed F1 hybrids, are heterozygous at all loci for which their parental strains differ and exhibit hybid vigor. F1 hybrids are genetically identical but breeding of F1's will result in an F2 generation that will show great variability in genetic composition.

Random breeding involves the use of random number tables or some other mechanism to ensure that the selection of males and females for mating is purely by chance. Theoretically, assuming no selection or mutations occur, random breeding will preserve the gene and genotype frequencies of the population for generations.

In reality, with random breeding, finite populations will eventually experience a decrease in genetic variability (increasing homozygosity) due to fixation and loss of alleles. In addition, it is impossible to completely eliminate the effects of selection, mutation and nongenetic causes of variation. Generally speaking, the smaller the breeding population the more rapidly the decrease in genetic variability will occur.

Various strategies have been devised to preserve the heterozygosity of outbred stocks utilizing breeder rotation systems, such as the Robertson and HAN-rotation systems. These systems use detailed schemes for rotating breeding stock over generations so that the chances of inbreeding are minimized. Even with these systems, the maintenance of an outbred stock requires large numbers of mice and significant resources. This is why most investigators choose to obtain outbred mice directly from commercial vendors. However, investigators should be aware that outbred stocks originating from different sources may differ due to genetic divergence.

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Breeding Strategies, cont. from page 2.

No matter what breeding system an investigator chooses to adopt, some basic facts about mouse reproductive biology should be kept in mind. In general, female mice start breeding at 7-8 weeks of age and will continue to be fertile until 8-12 months of age. With increasing age, female mouse reproductive capacity diminishes resulting in smaller litters and longer intervals between litters. Inbred and genetically manipulated mice may reach this point even earlier. Male reproductive performance will also diminish by 12 months of age. Continuous replacement of breeders with younger stock is necessary to maintain production levels in the colony. Careful record keeping and prompt weaning of litters to allow rebreeding of adults and avoid accidental breeding of young females are also important aspects of colony management.

References:
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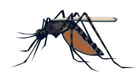
Singleton GR and Krebs CJ. 2007. The Mouse in Biomedical Research. 2nd edition. Volume I: History, Wild Mice and Genetics. Chapter 4: Breeding Systems: Considerations, Genetic Fundamentals, Genetic Background and Strain Types. Amsterdam: Elsevier.



Penn State Scientists in the News:

How to Make Evolution-Proof Insecticides for Malaria Control

Andrew F. Read, Penelope A. Lynch, Matthew B. Thomas

Article Summary:
Insecticides are one of the cheapest, most effective, and best proven methods of controlling malaria, but mosquitoes can rapidly evolve resistance. Such evolution, first seen in the 1950s in areas of widespread DDT use, is a major challenge because attempts to comprehensively control and even eliminate malaria rely heavily on indoor house spraying and insecticide-treated bed nets. Current strategies for dealing with resistance evolution are expensive and open ended, and their sustainability has yet to be demonstrated. Here we show that if insecticides targeted old mosquitoes, and ideally old malaria-infected mosquitoes, they could provide effective malaria control while only weakly selecting for resistance. This alone would greatly enhance the useful life span of an insecticide. However, such weak selection for resistance can easily be overwhelmed if resistance is associated with fitness costs. In that case, late-life–acting insecticides would never be undermined by mosquito evolution. We discuss a number of practical ways to achieve this, including different use of existing chemical insecticides, biopesticides, and novel chemistry. Done right, a one-off investment in a single insecticide would solve the problem of mosquito resistance forever.

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Read the full article at: Read AF, Lynch PA, Thomas MB (2009) How to Make Evolution-Proof Insecticides for Malaria Control. PLoS Biol 7(4): e1000058 doi:10.1371/journal.pbio.1000058

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Reminder:
The ARP Surgery Suite in the CBL is available for use by PSU investigators. Reservations are strongly recommended.
A sign up sheet is located outside the surgery suite.

The Animal Resource Program (ARP) is committed to providing PSU research personnel with high quality animal care services and facilities, to facilitate and improve animal research, and to ensure the health, well-being and humane treatment of all animals at PSU. ARP provides veterinary and diagnostic services, personnel training and expertise in laboratory animal, agricultural and wildlife technology and medicine. ARP veterinarians have specialized training and are available to assist with animal model development, experimental design, budget projections and grant preparation. Participation in collaborative research projects is welcomed.

Animal Illness and Health Alert Card Procedures

ARP has a standard procedure in place to alert research and veterinary staff of possible illness or injury in animals housed in PSU laboratory animal facilities. Animals observed by ARP personnel that appear to be ill or injured will have a green Health Alert Card placed in their cage cardholder indicating that a sick animal report has been filed with the attending veterinarian. A copy of the Health Alert Card will also be hung on the door to the animal room.

The veterinary technician and/or veterinarian will examine the animal(s) and initiate a medical record for that animal. The investigator (or their designated contact) will be contacted and informed of the animal's condition. Animals being treated or observed for clinical illness or injury will continue to be monitored by the veterinary staff with the Health Alert Card remaining in the cage cardholder.

If an investigator chooses to euthanize an animal this should be indicated on the green Health Alert Card. The Card should then be placed on the animal room clipboard so the caretaker may return the Card to the veterinary staff. For animals requiring immediate attention, a reasonable attempt will be made to contact the investigator before medical treatment is started or euthanasia performed. However, the attending veterinarian has the authority to initiate treatment or provide euthanasia without the investigator's consent if indicated. Diagnostic testing may be performed on the animal at the discretion of the veterinarian in order to rule out an infectious process.

Animals found dead in the cage will also have a Health Alert Card filled out. The green Card on the cage of dead animals is intended to alert investigators that a dead animal was found. *The investigator may remove dead animal cards* as soon as they have seen them.