Improvement of the Breeding Performance of Wild-Derived Mice

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As the use of laboratory mice in scientific research has dramatically increased, animal facilities have had to adjust to greater husbandry and space demands. Ventilated caging and automatic watering systems allow for more caging per rack and less demand on personnel by allowing less frequent cage changes and no water bottles to fill. As systems become more streamlined this may benefit the day-to-day operation of the facility but may also cause problems for finicky mouse strains with more husbandry requirements.

Wild-derived mouse strains tend to be more hyperactive and sensitive to environmental stress than conventional inbred laboratory strains. They often require special handling and are more difficult to breed. They are useful as mapping tools when crossed with inbred laboratory mice. Improving the breeding performance of wild-derived strains would lessen the number of breeder mice needed to produce a desired experimental colony. Environmental conditions can influence breeding performance. However, having a totally separate housing system for these strains would not be cost effective or efficient time management. It would be better to use current facility equipment with the control of specific variables to generate the best breeding conditions possible.

The goal of this project was to find the best method for breeding success, using a ventilated caging system with automatic watering, for the wild-derived mouse strain *Mus musculus castaneus.* We found that breeding performance was affected by rack location; the mice that were in a quieter corner of the room bred better than those in a rack in the center of the room. In addition, the upper rows of the centrally located rack had a similar good breeding performance as the cages on the corner rack. Thus we conclude that the cage position both on a rack and in the room can affect breeding performance, with the best results being at the tops of racks and in the quieter areas of the room.

Materials and Methods

To determine optimal conditions for breeding we studied several variables including the position of the rack in the room, location of cage on the rack, addition of two different enrichment devices and a food supplement given as a treat. Mice were housed on ventilated racks with automatic watering (Allentown Caging). Forty-two four-week-old male and female *Mus musculus castaneus* were purchased from The Jackson Laboratory. They were received in two shipments, and were allowed to acclimate in the facility for 4-7 days prior to being set up in breeding pairs. Due to some initial fighting, some replacements were ordered and received in a third shipment. The first pairs were set up on 6/18/04 and the study was terminated on 10/15/04. Mice were randomly set up in breeding pairs. Cage numbers and rack were then assigned randomly. Cages 1-42 were assigned to Rack 9 (center of room) and cages 43-84 were assigned to Rack 5 (corner of room) (fig. 1A). Cages were placed in a rack position by drawing a slip of paper with the cage position (ex. row 4 position D). There were three rack groups: top (rows 1 and 2), middle (rows 5 and 6) and bottom (rows 9 and 10). After all the cages on

one rack were assigned, the same positions were used to assign cages on the other rack, so they would have the same configuration (figure 1B).

Once cage assignments were completed, a specific enrichment device was assigned to each cage. Two types of devices were used, mouse igloos and mouse huts, both in red (Bio-Serv). Alternate cages were assigned an igloo or hut, to give approximately the same number of each per row. All cages were given nestlets, as per facility protocol. Supreme Mini-Treats, Very Berry flavor (Bio-Serv) were used as a treat because it is nutritionally complete. Which cage would receive treats was determined randomly by drawing cage ID's based on rack group keeping the number of cages with huts and igloos equal. Treats were given approximately every week, and 10 pellets were given at a time. Cage position, enrichment device and treat assignments are shown in figure 1B.

Male mice were weighed approximately once per month. The ease of catching the mice and general behavior of the mice at time of weighing was noted. (i.e. were the mice very excitable and difficult to catch, trying to escape the cage or calm). Observations regarding the usage of igloo/hut were made without disturbing the cage when possible. Treat consumption was observed a half hour after treat was given and how many treats were left the next day. Cages were checked for new litters a minimum of twice per week. The litter birth date, any litters found dead prior to weaning and the number of pups surviving to weaning was recorded. Weaning age was 4 weeks.

Results and Discussion

There was no significant difference in body weight of the males in any cage position (data not shown). The mice were very jumpy initially, but did calm down once cages were established. The litter totals for each rack are shown in figure 2A. Rack 5 had 46 total litters born, while rack 9 had 34 total litters. Some litters were lost bringing the net litter total for rack 5 to 36 and a net total for rack 9 to 31. Two cages with litters were lost due to flooding of the water system in the cage. These are marked with asterisks next to the "number of litters found dead" and the "net number of litters" categories for rack 5. The total numbers are used in our analysis because both litters lost due to flooding were healthy and would likely have survived to weaning. The number of pups in these two litters is not included elsewhere, because litter size was measured as litter size surviving to weaning. The average litter size for both racks is similar, 4.5 vs. 4.8 pups per litter. Since all of the cages on rack 5 bred better than those on rack 9 we conclude that the corner location is better for breeding these wild derived mice.

The result for the litter size for different cage locations on a rack is shown in figure 2B. Comparing the total and net numbers of litters for rack 5 shows no difference between any cage position. The top rows have more total weanlings, with 62 vs. 45 (middle) and 47 (bottom). The top rows of rack 9 had a much higher number of litters than either the middle or lower rows. As was seen for the rack location, the location of cages on a rack also affects breeding. The cages that were at the top of the racks, where there is less activity, were better at producing litters. Again this supports the conclusion that areas of a mouse facility with less activity are better for breeding *Mus musculus castaneus* mice.

The effect of enrichment device is shown in figure 2C. Rack 5 seemed to prefer the mouse huts, with 22 net litters and 98 total weanlings vs. 12 net litters and 56 total

weanlings for the igloos. The reverse was true for rack 9, where igloos were preferred, with 22 net litters and 110 total weanlings vs. 9 net litters and 38 total weanlings for the huts. It is interesting that the rack in the active part of the room preferred the igloo, which gives more concealment than the hut. Most, but not all, pairs built their nests inside the device. Some pairs preferred to build their nest in a different area of the cage. However, all pairs used the device in some way (climbing or hiding) even if they did not use it for nesting. While mice receiving treats appeared to enjoy them, often consuming them immediately, there was no significant difference between the groups given a treat or not (figure 2D).

The Jackson Laboratory houses their *Mus musculus castaneus* colony in conventional caging, not ventilated racks (personal communication, micetech@jax.org). Our results using ventilated racks compare favorably with their litter data. They report that the average litter size is 4-5; our overall average is 4.6. They keep their breeding pairs together for 8 months, seeing 4-5 litters per female, with a high number of non-productive matings. Our pairs were kept together for 4 months, and out of 75 pairs we had 42 producing litters, 28 of which had 2-3 litters (data not shown). There were two issues associated with using ventilated racks. One involved weaning age. All mice were weaned at 4 weeks, due to recommendations using automatic watering. Occasionally a subsequent litter was born prior to the previous litter being weaned. This may have accounted for some of the litters found dead. A possible solution to this problem would be to wean litters at 3 weeks and provide a water bottle in the weanling cages for a week or two. The second issue involved the aggressiveness of these mice. This particular strain is very aggressive. While this parameter was not followed in this study, it appeared that the onset of fighting between cagemates occurred at an earlier age than in standard microisolator caging. This was noted with new arrivals from Jackson Laboratory and with weanlings. Careful monitoring and separation of cagemates once signs of fighting are observed will correct this problem.

Overall, it is possible to successfully breed *Mus musculus castaneus* in ventilated caging. While a quiet location, such as a corner of the room, provides the most success, using the top cages on the rack and an appropriate housing device will help breeding performance. This should be true for any strain that is susceptible to environmental stress. Many transgenic strains are also troublesome breeders. Placing breeding cages in the corner (quietest) racks in the room, or, if that is not possible, place them on the upper rows of the rack and adding an enrichment device allow the mice a place to nest/hide should improve their breeding performance as well.

Generating the best environment for breeding decreases the number of breeding animals required to produce an experimental colony and allows for more accurate mouse number calculations required for grant and animal care and use committee reporting. Also, since many studies require a group of mice that are the same age, a reliable productivity level is necessary to generate a group of animals of the same age at one time, increasing experimental accuracy and decreasing the number of unwanted animals.

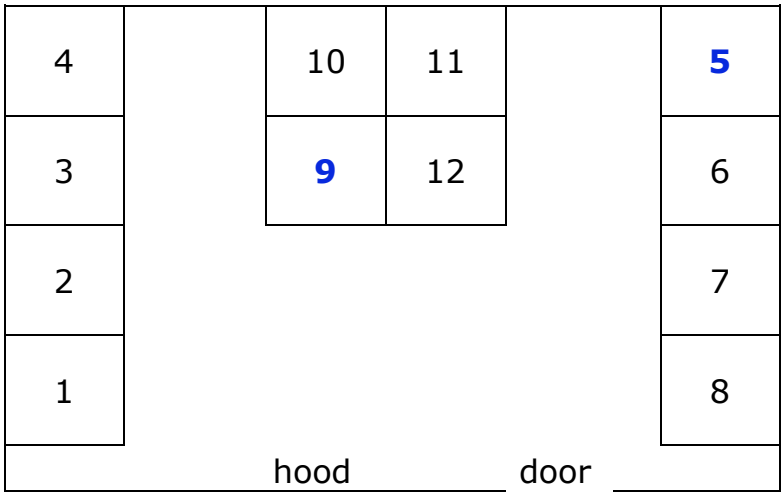

Figure 1A – Rack position in the room. Drawing not to scale. Racks 5 and 9 were used in this study. "hood" indicates the location of the portable hood in the room when not being used for cage changing. Rack 9 was chosen because it was centrally located in the room, where there would be the most activity. Rack 5 was chosen because it is in a quieter corner of the room.

RACK 5	A	B	C	D	E	F	G
1	**74** igloo T	**51** hut T	**72** igloo	**78** hut		**57** hut	**60** igloo T
2	**55** hut T	**67** igloo T		**44** igloo	**76** hut T	**50** igloo	**47** hut T
3	BLANK	ROW					
4	BLANK	ROW					
5	**61** hut	**75** igloo	**66** hut T	**54** igloo T	**79** hut T	**53** igloo T	**71** hut T
6	**56** igloo	**68** hut		**77** hut	**62** igloo	**58** hut	**64** igloo T
7	BLANK	ROW					
8	BLANK	ROW					
9	**45** igloo	**43** hut	**70** igloo T	**46** hut T	**65** igloo	**73** hut T	**49** igloo
10	**59** hut T		**82** hut	**69** igloo T	**63** hut	**48** igloo T	**Sentinel**

RACK 9	A	B	C	D	E	F	G
1	**18** igloo T	**23** hut T	**6** igloo	**8** hut	**3** igloo	**26** hut	**16** igloo T
2	**2** hut T	**32** igloo T		**34** igloo	**15** hut T	**35** igloo	**38** hut T
3	BLANK	ROW					
4	BLANK	ROW					
5	**28** hut	**33** igloo	**37** hut T	**27** igloo T	**29** hut T	**22** igloo T	**14** hut T
6	**9** igloo	**30** hut		**12** hut	**24** igloo	**10** hut	**7** igloo T
7	BLANK	ROW					
8	BLANK	ROW					
9	**25** igloo	**20** hut	**1** igloo T	**31** hut T	**5** igloo	**36** hut T	**21** igloo
10	**39** hut T		**17** hut	**13** igloo T	**11** hut	**4** igloo T	**Sentinel**

Figure 1B – Cage position on each rack. Numbers in bold are the cage numbers. Numbers 1-10 indicate row on the rack, letters A-G indicate column. Enrichment device (hut or igloo) as indicated. T= cage received treats. Sentinel – location of sentinel cage on the rack.

	Rack 5	Rack 9	Total
Total number of litters	46	34	80
Number of litters found dead	12 (10)*	3	15 (13)*
Net number of litters	34 (36)*	31	65 (67)*
Total number of weanlings	154	148	302
Average litter size	4.5	4.8	4.6

Figure 2A – Litter data for rack position. The number of pups that survived to weaning determined litter size. * Indicates data where litters were lost due to cage flooding. The numbers in parentheses are considered the more indicative data because these two litters would likely have survived to weaning otherwise.

Location	Litter Data	Rack 5	Rack 9	Total
Top Rows	Total number of litters	16	18	34
	Number of litters found dead	3	3	6
	Net number of litters	13	15	28
	Total number of weanlings	62	73	135
	Average litter size	4.8	4.9	4.8
Middle Rows	Total number of litters	15	7	22
	Number of litters found dead	4	0	4
	Net number of litters	11	7	18
	Total number of weanlings	45	36	81
	Average litter size	4.1	5.1	4.5
Bottom Rows	Total number of litters	15	9	24
	Number of litters found dead	5	0	5
	Net number of litters	10	9	19
	Total number of weanlings	47	39	86
	Average litter size	4.7	4.3	4.5

Figure 2B - Litter data for cage position. The top rows of Rack 9 had a greater number of litters than the middle and bottom rows of the same rack. Overall the top rows generated a greater number of weanlings.

Enrichment Device	Litter Data	Rack 5	Rack 9	Total
Igloo	Total number of litters	15	23	38
	Number of litters found dead	3	1	4
	Net number of litters	12	22	34
	Total number of weanlings	56	110	166
	Average litter size	4.7	5	4.9
Hut	Total number of litters	31	11	42
	Number of litters found dead	9	2	11
	Net number of litters	22	9	31
	Total number of weanlings	98	38	136
	Average litter size	4.5	4.2	4.4

Figure 2C – Litter data for enrichment device. The breeding pairs on Rack 5 preferred huts while the pairs on Rack 9 preferred igloos.

Enrichment Device	Litter Data	Rack 5	Rack 9	Total
Treat	Total number of litters	20	16	36
	Number of litters found dead	4	2	6
	Net number of litters	16	14	30
	Total number of weanlings	70	67	137
	Average litter size	4.4	4.8	4.6
No Treat	Total number of litters	26	18	44
	Number of litters found dead	8	1	9
	Net number of litters	18	17	35
	Total number of weanlings	84	81	165
	Average litter size	4.7	4.8	4.7

Figure 2D – Litter data for treat supplement. The addition of this treat did not affect the litter data significantly.