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Optimal breeding strategy for mouse mutant strains

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Summary

Despite the published recommendations after the Banbury Conference in 1997 to maintain a mutation on controlled genetic backgrounds, maintenance of mutant strains and production of experimental groups are still too often hampered by constraints of time, space, and cost.

We propose here a simple and rigorous method that allows not only the efficient production of animals, but especially the precise control of the genetic background and the generation of appropriate control groups, guaranteeing stable and reproducible observations. This method is flexible and allows optimization and adaptation of the production according to the experimental needs, thus reducing the final cost. In addition, the work of the Animal Care technicians is simplified and animal welfare is improved.

Whether a mutation has appeared spontaneously, or was induced pharmacologically or by means of genetic engineering, fixing it onto an inbred genetic background is a *sine qua non* condition not only to generate optimal control groups, but also to ensure the stability and reproducibility of results in space and time.

These theoretical and practical issues related to the impact of genetic background on the phenotypic expression of a mutation are so essential that the scientific community has in the past joined forces to educate researchers, provide guidelines and encourage them to standardize conditions to maintain their mutations of interest and produce experimental animals (Banbury workshop report, 1997).

The first recommendation sustains that the genetic background is described in detail and is easily reproducible. The second recommendation prompts researchers to derive their targeted mutation simultaneously on two standard inbred strains, as shown in Figure 1.

When the mutation of interest is transferred by successive backcrosses simultaneously on two inbred genetic backgrounds, heterozygotes from each congenic strain A^m and B^m are used for the production of experimental animals: a cross between heterozygotes (HT) allows the study of the phenotypic expression of the mutation alternately or simultaneously on inbred (Figure 1b) and hybrid (Figure 1c) genetic backgrounds. Given the complexity of interactions between genes (epistasis), expression of a mutation varies depending on the genetic environment in which it is expressed. The analysis of a mutation simultaneously on two different genetic backgrounds is a powerful tool to identify modifier genes that interact with the mutation and contribute to the complexity of phenotypes (Banbury workshop report 1997; Doetschman 2009). In addition, it allows the phenotypes to be evaluated on an F_1 hybrid back-

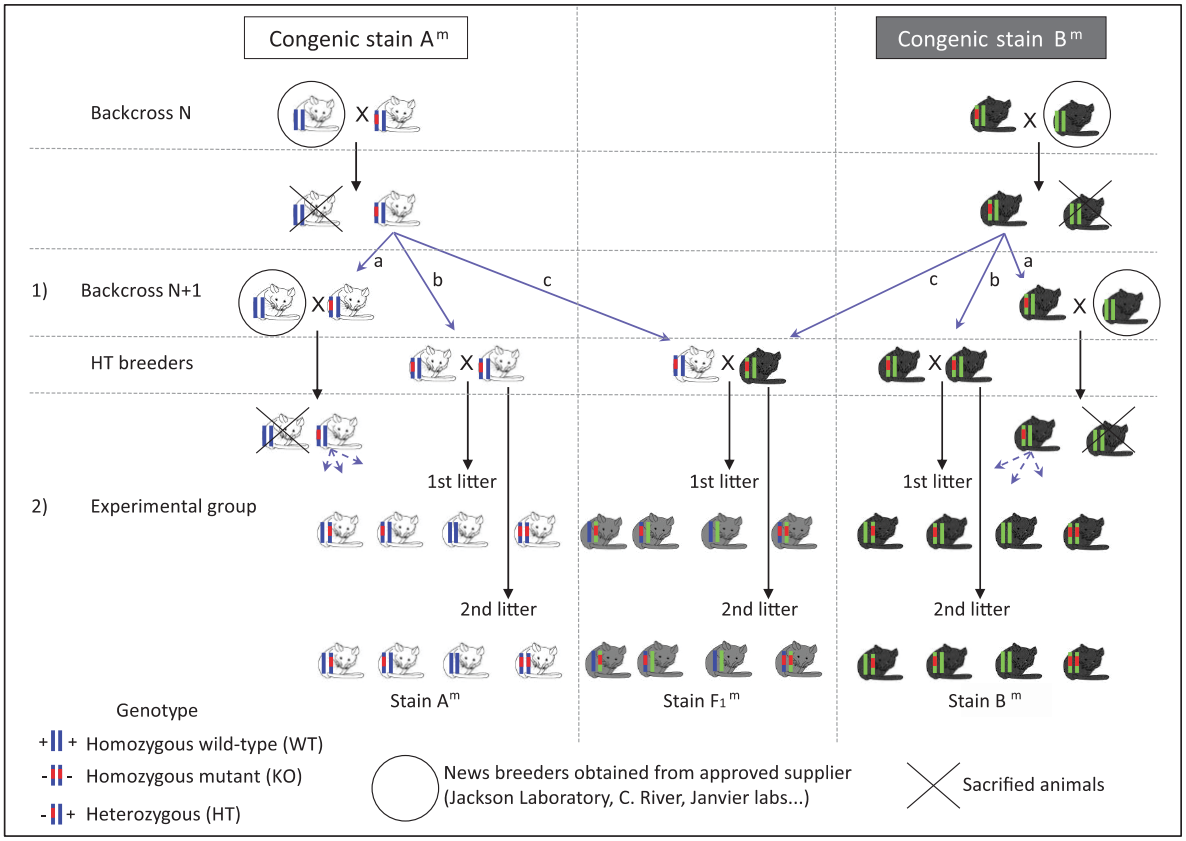

Figure 1: Maintaining a genetic mutation simultaneously on two inbred genetic backgrounds (A and B) allows the production of inbred and / or hybrid (AxB-F_1) experimental animals.

ground that can be very favorable when the homozygous mutation is lethal on an inbred background for example. As shown on Figure 1a, heterozygotes are also used to maintain the congenic strains: consistent backcrosses with identified breeders from approved suppliers are crucial to reduce the risk of genetic drift and thus maintain a stable genetic background along time.

The choice of the optimal background is therefore an important step to maintain a mutation. Since the first DBA inbred strain developed by Little in the 1900s, more than 450 inbred strains have been described, and for the most widely used, many substrains have been derived for so many generations that they have to be considered distinct (Simon et al. 2013; Fontaine and Davis 2016). The choice is even increasing with the ongoing development of new inbred strains (Srivastava et al. 2017) providing a wealth of different genotypes and phenotypes, each having a unique and stable genetic environment that allows phenotypic measures on isogenic individuals (Beck et al. 2000).

How to translate these recommendations specifically in order to organize proper breeding within animal facilities? How to reconcile these requirements with constraints of time, space and budget that all researchers are facing today? To answer these questions, we developed a simple method that adapts to all types of breeding, either reduced to maintain the mutation and produce regular breeders at low returns, for example, or enlarged, for simultaneously generating a large number of experimental animals required for behavioral or pharmacological studies.

The principle:

The method, presented in Figure 2 for four cages, is to use one male for four females (one female per cage) for periodic and controlled coupling. The male is rotated from one cage to another every fortnight (thus staying with each female for 3 to 4 estrous cycles). After four moves, the male is back to the first female. Each female spends two weeks with the male, then one week with another female for company, preferably of a passive strain of a different coat color for easy identification. During the fourth week, the breeding female is left alone for parturition and lactation for four weeks. After weaning, the breeding female recovers for one week with the female companion before the return of the male. Therefore each

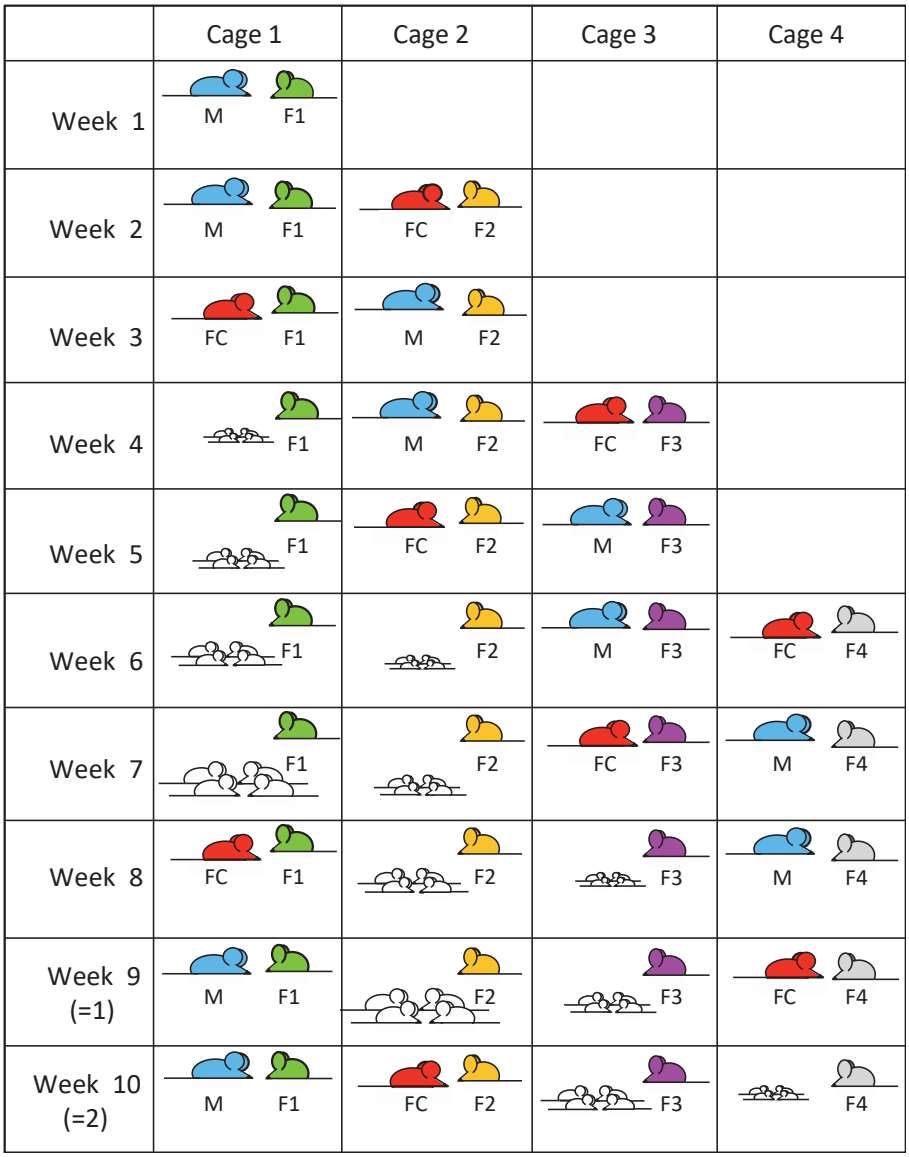

Figure 2. Method for optimized breeding (periodic couples): Example of four cages regime. With four females (F1-F4) per male, each female is successively in the presence of the male for two weeks, a female companion (FC) for one week, and its litter for four weeks before one week of rest with the companion, and then the male again to re-start the cycle. Each female produces a litter every eight weeks and the yield of two litters per month for four cages can be doubled with two breeding females per cage (in this case, the female companion is not needed).

female produces a litter every eight weeks, and the yield is two litters per month for four cages.

This systematic sequence is particularly flexible and therefore suitable for all types of desired production. We give here the most useful variants:

1. With only four cages, the production can be doubled with two breeding females per cage. The female companion is no longer necessary. Both females become quickly synchronized and ready for mating during the first days of the mating. In this option, the yield is two litters every two weeks. The presence of two females optimizes maternal environment (Sayler and Salmon 1969) and for experimental subjects, the pre-weaning environment is homogenized. In case of death of one female, the number of pups can be reduced (for example by choosing one gender) in order to save animals of interest without adoption constraints.
2. To produce larger experimental groups, the number of cages can be doubled. In this option, from 8 cages of two females and two breeding males, 8 litters per month are obtained, and it is still possible to modulate

this production: the two males can be rotated simultaneously for a production of four litters every two weeks, or shifted by one-week to obtain two litters per week.

3. Within the same animal facility, several mutations are often maintained on the same genetic background. The same male can thus serve four cages of breeding females carrying different mutations. This option is particularly suitable to optimize tight spaces.
4. Some protocols require earlier weaning (3 weeks). In this option, the male is left for one week with two breeding females. With 14 females in 7 cages, two litters can be obtained per week.

This method and its variations are suitable for autosomal as well as X-linked mutations and for both the maintenance of the mutant strains (by consistent backcrossing onto defined inbred background) and the production of experimental groups (Figure 1). For autosomal mutations, production with both breeders heterozygous for the mutation gives rise to wild-type (WT), knockout (KO) and heterozygous (HT) pups in the Mendelian' proportions 1/4, 1/4 and 1/2, respectively. Note that the WT littermates are the optimal control group, sharing with the experimental KO and HT subjects not only the same genetic background, but also the same pre-and post-natal maternal environments. In the case of X-linked mutations, conventional backcross of a heterozygous X^+/X^- female with a WT male serves simultaneously the maintenance of the genetic background and the production of experimental groups of males (X^+/Y and X^-/Y) and females (X^+/X^+ and X^+/X^-).

In terms of husbandry procedures, this systematic sequence has many advantages to enhance the welfare of laboratory mice. We particularly emphasize the absence of the male during parturition and lactation, which is essential for optimized breeding quality. In most laboratories, mating during a postpartum oestrus is often used in order to shorten inter litter interval and increase the yield (Mantalenakis and Ketchel 1966). Continuous mating, however, implies simultaneous pregnancy and lactation that increased cost for the dam: since laboratory mice have similar gestation and lactation lengths, the peak demand of pregnancy overlaps with the peak demand of lactation, thus increasing the energy burden experienced (Johnson, Thomson and Speakman, 2001). Classical mouse genetic literature has shown that concurrent lactation was responsible for delayed implantation, increased post-implantation mortality, and potential morbid effects on the pups subsequently born in the second litter, including long-lasting detrimental effects (McCarthy 1965; Eisen and Saxton 1984; Fone and Porkess 2008; Lerch et al. 2015). For postpartum fertilization, the duration of pregnancy is increased, and the extent of the delay in implantation is correlated with the number of suckling pups, which may directly impact developmental studies (Mantalenakis and Ketchel 1966; Bindon 1969; Norris and Adams 1981). In addition, the immune status of pregnant females during lactation is also affected, which influences the descendants and weakens the health of the entire colony (Lloyd 1983). In the present sequence, one week of post-weaning "reset" for breeding females preserves their hormonal and immune states.

Note that a few exceptional strains - wild inbred for example - may only tolerate permanent couples. In these cases, females are not receptive to the male throughout lactation (Guenet JL, personal communication). But for most standard inbred strains, the absence of the male during lactation is an essential factor that maintains efficient litter sizes and the health quality of animal facilities and contributes to optimal breeding.

We also insist on the fact that in such breeding sequences, animals are never isolated, limiting stress (Valzelli 1973; van Loo et al. 2003; Varty et al. 2006; Fone and Porkess 2008). Finally, by setting the weaning age at one month, the sequence is not disturbed even in case of late fertilization and it adapts to most strains, including when the mutation induces a pre-weaning developmental delay.

The Figure 3 presents the reproductive performance that we obtained in our animal facilities to maintain a mutation simultaneously on two inbred C57BL/6NCrl (B6) and DBA/2J (D2) genetic backgrounds by regular backcrosses. This retrospective analysis shows that, breeding within our facilities with periodic couples, compared to permanent couples, improved the litter size at birth, but only significantly in the B6 strain ($F(1,75) = 21.7$, $P = 0.03$) and this effect was observed from the first litter. In addition, a 4-fold reduction of pre-weaning deaths was observed in both B6 and D2 strains.

It is worth noting that this systematic sequence has also advantages for the animal care staff. Periodic couples facilitate the monitoring of the breeding colonies, with programmed production and constant rates. The work of the Animal Care technicians is simplified: a weekly visit on a fixed day is enough to handle the breeding program, which consists succes-

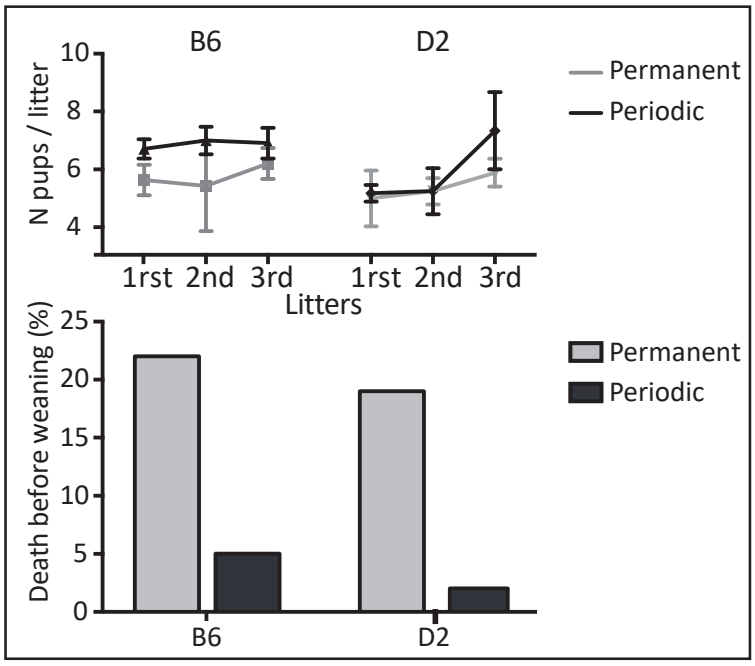

Figure 3: Retrospective analysis of reproductive performance of permanent compared to periodic couples.
A mutation was maintained simultaneously on the B6 and D2 inbred strains by regular backcrosses using one male for two females, in either permanent or periodic (males moved every two weeks, Figure 2) couples. Breeding performances are expressed in term of litter size according to litter parity (top) and numbers of death before weaning (bottom). Numbers of females in permanent couples were N = 22 B6 and N = 16 D2, and in periodic couples N = 42 B6 and N = 34 D2. Total number of pups born for permanent couples: B6 = 330 and D2 = 281, and periodic couples: B6 = 414 and D2 = 167.

sively of changing the company female, the male, or weaning the litters.

In conclusion, this method provides birth control that reduces the number of animals produced, with a higher survival rate, in fitting with the 3Rs (reduce, refine, replace), established principles in operation worldwide (Richmond 2000).

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References

Banbury Workshop Report, (1997). Mutant mice and neuroscience: recommendations concerninggenetic background. Banbury Conference on genetic background in mice. *Neuron*. **19**, 755-759.

Beck, J.A., Lloyd, S., Hafezparast, M., Lennon-pierce, M., Eppig, J.T., Festing, M. F., Fisher, E. M., (2000). Genealogies of mouse inbred strains. *Nature Genetics*. **24**, 23-25.

Bindon, B.M., (1969). Mechanism of the inhibition of implantation in suckling mice. *Journal of Endocrinology*. **44**, 357-362.

Doetschman, T., (2009). Influence of genetic background on genetically engineered mouse phenotypes. *Methods in Molecular Biology*. **530,** 423-433.

Eisen, E.J., Saxton, A.M., (1984). Effects of concurrent lactation and postpartum mating on reproductive performance in mice selected for large litter size. *Journal of Animal Science*. **59**, 1224-1238.

Fone, K.C., Porkess, M.V., (2008). Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. *Neuroscience & Biobehavioral Reviews*. **32**, 1087-1102.

Fontaine, D.A., Davis, D.B. (2016). Attention to Background Strain Is Essential for Metabolic Research: C57BL/6 and the International Knockout Mouse Consortium. *Diabetes*. **65**, 25-33.

Johnson, M.S., Thomson, S.C., Speakman, J.R. (2001). Limits to sustained energy intake. III. Effects of concurrent pregnancy and lactation in Mus musculus. *Journal of Experimental Biology*. **204**, 1947-1956.

Lerch, S., Brandwein, C., Dormann, C., Gass, P., Chourbaji, S., (2015). Bred to breed?! Implications of continuous mating on the emotional status of mouse offspring. *Behavioural Brain Research*. **279**, 155-165.

Lloyd, S., (1983). Effect of pregnancy and lactation upon infection. *Veterinary Immunology and Immunopathology*. **4**, 153-176.

Mantalenakis, S.J., Ketchel, M.M., (1966). Frequency and extent of delayed implantation in lactating rats and mice. *Journal of Reproduction and Fertility*. **12**, 391-394.

Mccarthy, J.C., (1965). Effects of Concurrent Lactation of Litter Size and Prenatal Mortality in an Inbred Strain of Mice. *Journal of Reproduction and Fertility*. **9**, 29-39.

Norris, M. L., Adams, C.E., (1981). Concurrent lactation and reproductive performance in CFLP mice mated post partum. *Laboratory Animals*. **15**, 273-275.

Richmond, J., (2000). The 3Rs - Past, present and future. *Scandinavian Journal of Laboratory Animal Science*. **27**. 84-92.

Sayler, A., Salmon, M., (1969). Communal nursing in mice: influence of multiple mothers on the growth of the young. *Science*. **164**, 1309-1310.

Simon, M.M., Greenaway, S., White, J.K., Fuchs, H., Gailus-Durner, V., Wells, S., Sorg, T., Wong, K., Bedu, E., Cartwright, E.J., Dacquin, R., Djebali, S., Estabel, J., Graw, J., Ingham, N.J., Jackson, I.J., Lengeling, A., Mandillo, S., Marvel, J., Meziane, H., Preitner, F., Puk, O., Roux, M., Adams, D.J., Atkins, S., Ayadi, A., Becker, L., Blake, A., Brooker, D., Cater, H., Champy, M.F., Combe, R., Danecek, P., di Fenza, A., Gates, H., Gerdin, A. K., Golini, E., HancocK, J.M., Hans, W., Holter, S. M., Hough, T., Jurdic, P., Keane, T.M., Morgan, H., Muller, W., Neff, F., Nicholson, G., Pasche, B., Roberson, L.A., Rozman, J., Sanderson, M., Santos, L., Selloum, M., Shannon, C., Southwell, A., Tocchini-Valentini, G.P., Vancollie, V.E., Westerberg, H., Wurst, W., Zi, M., Yalcin, B., Ramirez-Solis, R., Steel, K.P., Mallon, A.M., De Angelis, M.H., Herault, Y., Brown, S.D., (2013). A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains. *Genome Biology*. **14**, R82.

Srivastava, A., Morgan, A.P., Najarian, M.L., Sarsani, V.K., Sigmon, J.S., Shorter, J.R., Kashfeen, A., McMullan, R.C., Williams, L.H., Giusti-Rodriguez, P., Ferris, M.T., Sullivan, P., Hock, P., Miller, D.R., Bell, T.A., McMillan, L., Churchill, G.A. & De Villena, F.P., (2017). Genomes of the Mouse Collaborative Cross. *Genetics*. **206**, 537-556.

Valzelli, L., (1973). The «isolation syndrome» in mice. *Psychopharmacologia*. **31**, 305-320.

van Loo, P.L., van Zutphen, L.F., Baumans, V., (2003). Male management: Coping with aggression problems in male laboratory mice. *Laboratory Animals*. **37**, 300-313.

Varty, G.B., Powell, S.B., Lehmann-Masten, V., Buell, M.R. & Geyer, M.A. (2006). Isolation rearing of mice induces deficits in prepulse inhibition of the startle response. *Behavioural Brain Research*. **169**, 162-167.