Laboratory Animal Science and Medicine 2

Fernando Benavides
Axel Kornerup Hansen *Editors*

Rodent Quality Control: Genes and Bugs

Monitoring Health and Genetics of Laboratory Animals

Laboratory Animal Science and Medicine

Volume 2

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Editors

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Preface

As a part of the *Laboratory Animal Science and Medicine* series, endorsed by the European Society for Laboratory Animal Veterinarians (ESLAV), this book aims to offer a comprehensive and easily accessible toolbox for researchers, veterinarians, and technicians working with laboratory rodents, specifically mice and rats. This book thoroughly describes quality assurance programs for laboratory animal facilities, focusing on microbiological and genetic monitoring. It encompasses common protocols aimed at ensuring the desired level of microbiological and genetic quality controls. Additionally, the content includes sections detailing standardized strains and genetically altered rodents, with recommended quality controls, standardized nomenclature, and cryopreservation. Health monitoring programs are addressed with details on how to do viral, bacterial, and parasitological diagnostics, accompanied by considerations for statistical analysis. The quality controls presented in this book play a crucial role as an essential component of the 3Rs (replacement, reduction, and refinement), contributing to the refinement and reduction in the use of laboratory mice and rats. It is our hope that this book will be helpful for laboratory animal staff involved in the quality assurance of laboratory rodents.

Houston, TX, USA — Fernando Benavides
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Axel Kornerup Hansen Axel Kornerup Hansen graduated in 1985 as DVM from the Royal Veterinary and Agricultural University in Copenhagen, Denmark. After 2 years in a small animal hospital, he became head of laboratories at Møllegaard Breeding Center, today a part of Taconic Ltd. In 1993, he became Associate Director at the Department of Experimental Medicine at the Faculty of Health and Medical Sciences at the University of Copenhagen. In 1996, he was awarded the degree of Dr. Vet. Sci. based upon his thesis on the impact of bacteria in laboratory rats. From 1997, he has been Professor in laboratory animal science and welfare at the Royal Veterinary and Agricultural University, which in 2007 became a part of University of Copenhagen. From 2007 to 2022, he was head of the Section of Experimental Animal Models. In 2000, he together with six others founded the European College of Laboratory Animal Medicine. His research area has primarily been on how infections

and the microbiota have an impact on laboratory animal models for human diseases and how this works in conjunction with the diet, but he has also published work on other issues in laboratory animal welfare. He has published 251 peer-reviewed papers in scientific journals and two full textbooks, i.e., *The Laboratory Swine* and *Handbook of Laboratory Animal Bacteriology*. He is chairing the board of the Danish National Center for the 3Rs and Denmark's National Committee for Animal Experimentation and Alternatives.

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Abbreviations

AALAS	American Association for Laboratory Animal Science
AAV	Adeno-associated virus
API	Analytical profile index
ARRIVE	Animal Research Reporting of In Vivo Experiments
ART	Assisted reproductive technology
ATP	Adenosine triphosphate
CAR	*Filobacterium rodentium* (cilia-associated respiratory bacillus)
CF	Complement fixation test
CLSI	Clinical and Laboratory Standards Institute
CPA	Cryoprotective agent
Cre	Cre recombinase
CRISPR	Clustered regularly interspaced short palindromic repeats
CTAB	Cetyltrimethylammonium bromide
DBS	Dried blood spots
DNA	Deoxyribonucleic acid
DSB	Double-strand break
EAD	Exhaust air dust
ECTV	Ectromelia virus
EDIM	Mouse rotavirus (epizootic diarrhea of infant mice)
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ES	Embryonic stem
F1, F2, etc.	Filial generations (first, second, etc.)
FEC	Fecal egg count
FELASA	Federation of European Laboratory Animal Science Associations
GEM	Genetically engineered mouse
GMS	Grocott’s methenamine silver reaction
GP	Guinea pig
GV-SOLAS	Gesellschaft für Versuchstierkunde-Society of Laboratory Animal Science
GWAS	Genome-Wide Association Study
H	Hamster
HDR	Homology-directed repair
HE	Hematoxylin and eosin

HET	Heterozygous
HI	Hemagglutination inhibition test
HOM	Homozygous
HR	Homologous recombination
ICLAS	International Council for Laboratory Animal Science
ICSI	Intracytoplasmic sperm injection
IDIR	Infectious diarrhea of infant rats
IFA	Immunofluorescence assay
ITS	Internal transcribed spacer
IVC	Individually ventilated cages
IVF	In vitro fertilization
KI	Knockin
KO	Knockout
LABA	Laboratory Animal Breeders Association
LAMP	Loop-mediated isothermal amplification
LASA	British Laboratory Animal Science Association
LCMV	Lymphocytic choriomeningitis virus
LDV	Gammaarterivirus lacdeh (lactate dehydrogenase elevating virus)
LOF	Loss of function
LOXP	Locus of X-over P1
LPSN	List of Prokaryotic Names with Standing in Nomenclature (https://lpsn.dsmz.de/)
M	Mouse
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
MAP	Mouse antibody production test
MCMV-1	Mouse cytomegalovirus 1
MDA	Multiple displacement amplification
MFIA	Multiplex fluorescence immunoassay
MHV	Mouse hepatitis virus
MIC	Minimal inhibitory concentration
MKPV	Mouse kidney parvovirus
MLST	Multilocus sequence typing
MNV	Mouse norovirus
MPF	Murine pathogen free
MPtV	Murine pneumotropic virus
MPV	Mouse parvovirus
MPyV	Murine polyomavirus
mRNA	Messenger RNA
MuAstV	Murine astrovirus
MVM	Minute virus of mice
NAD	Nicotinamide adenine dinucleotide
NGS	Next-generation sequencing
NHEJ	Non-homologous end joining
OHC	Optimal hygienic conditions
ORF	Open reading frame
PAM	Protospacer adjacent motif
PAS	Periodic acid-Schiff

PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
PTAH	Phosphotungstic acid hematoxylin
PVM	Pneumonia virus of mice
qPCR	Quantitative PCR
QTL	Quantitative trait locus
R	Rat
RAP	Rat antibody production test
RatPyV2	Rat polyomavirus
RFLP	Restriction fragment length polymorphism
RIA	Radioimmune assay
RMV	Rat minute virus
RNA	Ribonucleic acid
RPV	Rat parvovirus
RT-PCR	Real-time PCR
RV	(Kilham) rat virus
SCID	Severe combined immunodeficiency
SFB	Segmented filamentous bacteria
sgRNA	Single-guide RNA
SNP	Single-nucleotide polymorphism
SNV	Single-nucleotide variant
SOPF	Specific opportunistic pathogen free
SPF	Specific pathogen free
TALEN	Transcription activator-like effector nuclease
TG	Transgenic
TMEV	Theiler's murine encephalomyelitis virus
TYGS	Type Strain Genome Server
UTR	Untranslated region
VAF	Virus antibody free
VLP	Viral-like particle
WGS	Whole genomic sequencing
WHO	World Health Organization
WT	Wild type
ZFN	Zinc finger nuclease

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Genetically Standardized Laboratory Rodents

1

Fernando Benavides and Jean Jaubert

Abstract

This chapter provides a comprehensive overview of the current and expanding knowledge base concerning standardized laboratory strains of rodents, with a specific focus on the mouse (*Mus musculus*) and the rat (*Rattus norvegicus*), the predominant species utilized in biomedical research. We include fundamental information on various genetically standardized strains, with a primary emphasis on inbred strains and their derivatives. The production and maintenance processes of these strains are explained, accompanied by a historical context and an exploration of their principal applications. Addressed to veterinarians, animal facility technicians, researchers, and students employing mouse and rat models, this chapter provides an up-to-date account of the different genetically defined stains associated with these widely used laboratory rodents.

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Keywords

Rodents · Inbreeding · Inbred strains · Outbred stocks · Congenics

1.1 Introduction

At the beginning of the twentieth century, when genetics emerged as an experimental science, laboratory rodents were extremely limited. Three rodent species were the first to be used to test the recently rediscovered Mendel's laws and to prove that it was possible to develop genetically pure strains. These attempts began in the first decade of the 1900s in the USA and involved the use of the guinea pig by Sewall Wright and George Rommel, the rat by Helen Dean King, and the mouse by Clarence C. Little and Abbie Lathrop. In France, biologist Lucien Cuénot conducted pioneering genetic experiments with mice, specifically focusing on coat color inheritance, providing crucial insights into the principles of Mendelian inheritance in mammals.

Wright and Rommel were the first to start inbreeding experiments in 1906 with guinea pigs at the United States Department of Agriculture. Guinea pig strains 2 and 13 originated after these experiments and are still in use today (2/N and 13/N strains). Clarence Little, while at Harvard University, was the first to try to develop pure mouse lines by inbreeding. The first mouse

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inbred strain, "dba," was started in 1909 by Little through inbreeding mice homozygous for three recessive coat color alleles (named at the time as *a*, *b*, and *d*). Simultaneously, Helen King worked toward developing the first inbred rat lines at the Wistar Institute in Philadelphia, eventually creating the Wistar King Albino (WKA) inbred rat strain. A mutant rat in one of her colonies is also the origin of the Brown Norway inbred strain.

1.2 Inbred Strains

According to the definition by the International Committee on Standardized Nomenclature for mice, "Strains can be termed *inbred* if they have been mated brother x sister for 20 or more consecutive generations, and individuals of the strain can be traced to a single ancestral pair at the 20th or subsequent generation." At this stage, the genomes of the animals will typically exhibit only 1% residual heterozygosity on average. Consequently, they can be considered genetically identical for most practical purposes. The breeding protocol commonly used to produce an inbred strain is called brother x sister (bxs) or sibling x sibling (sxs) and consists of mating a male and a female from the same litter in successive generations [44] (Fig. 1.1).

In practice, most of the mouse and rat strains that are commonly used in research laboratories nowadays have undergone several tens of generations of bxs matings (indicated with an "F," for filial), and some among the most ancient have passed 200 generations (DBA/2, for example, is over F224). It is important to provide some clarifications on the definition of an inbred strain. Generally, animals within the same inbred strain are genetically almost identical, except for sex-linked traits and de novo mutations. To describe this important characteristic, geneticists say that the mice in question are *isogenic*. This is due to the rigorous inbreeding process that results in all individuals within a given strain becoming homozygous for all loci that were segregating in the founder ancestors (i.e., the original or ancestral breeding pair). As a result, they all carry the same alleles on both maternal and paternal chromosomes. This is also known as *autozygosity* because the two alleles are copies of the same ancestral allele.

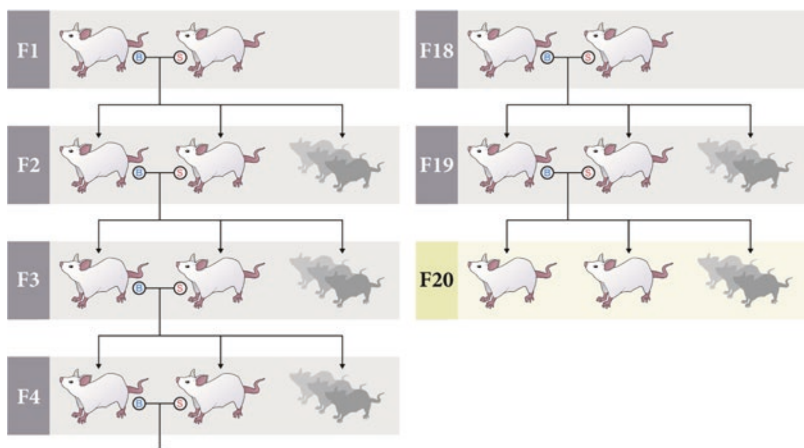


Fig. 1.1 *Inbreeding crosses.* This drawing schematically represents the breeding protocol commonly used to produce an inbred strain: mating a male and a female from the same litter (brother x sister) in successive generations. The uppercase letter F followed by a number represents the number of inbreeding generations. When this number is not known, a question mark is used: F? + 22, for example, would indicate that the number of brother x sister matings was not known when the strain was imported, but 22 generations of unrelaxed inbreeding have been added since this time. Strains can be termed *inbred* if they have been mated brother x sister for 20 or more consecutive generations

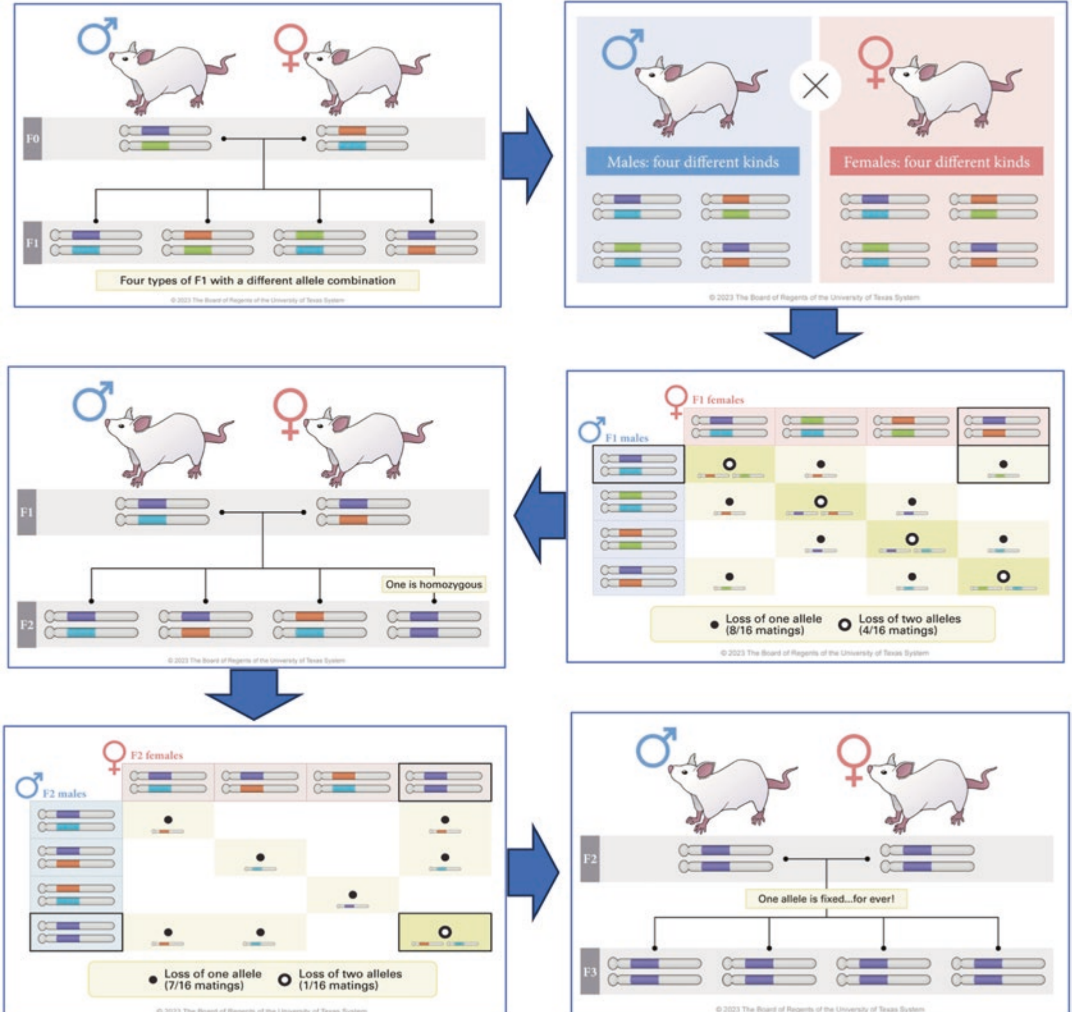


Fig. 1.2 *Alleles are fixed during inbreeding.* The brother x sister mating system results in the fixation or loss of certain alleles in subsequent generations. Using only one pair of chromosomes as an example, and four different colors (blue, green, orange, and violet) representing four alleles, these figures show the probability (percentage of matings) of losing alleles on the initial generations. In the example, the random F1 mice crossed already lost one allele (green) (8/16 probability), later the randomly crossed F2 mice lost two alleles (blue and orange) (1/16 probability). At the F3 generation, one allele (violet) becomes permanently fixed within this inbred colony

The process of homozygosity through progressive allele loss, or fixation, is easy to understand. When an allele present in generation Fn is not transmitted to at least one member of the breeding pair at generation Fn + 1, it is permanently lost (Fig. 1.2). As inbreeding continues, alleles are consistently lost and none are introduced, except for rare de novo mutations. This process leads to both homozygosity and isogenicity. The categorization of alleles that are lost or retained at each generation largely depends on chance. If the inbreeding protocol were reset using the same founder animals, it would result in a strain with a different genetic makeup after undergoing the 20 generations. Thus, an inbred strain represents a unique and random assortment of alleles.

During the process of inbreeding, the progression toward homozygosity is not linear. It occurs relatively quickly during the first few genera-

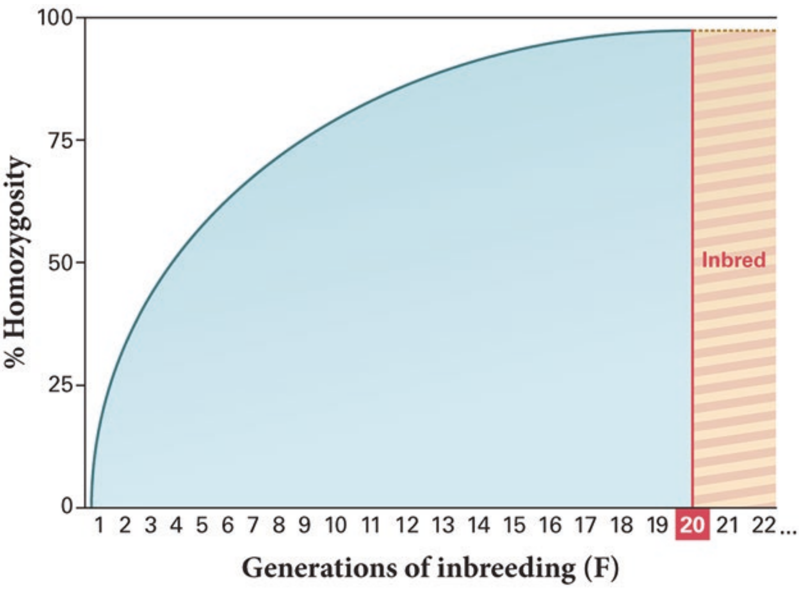


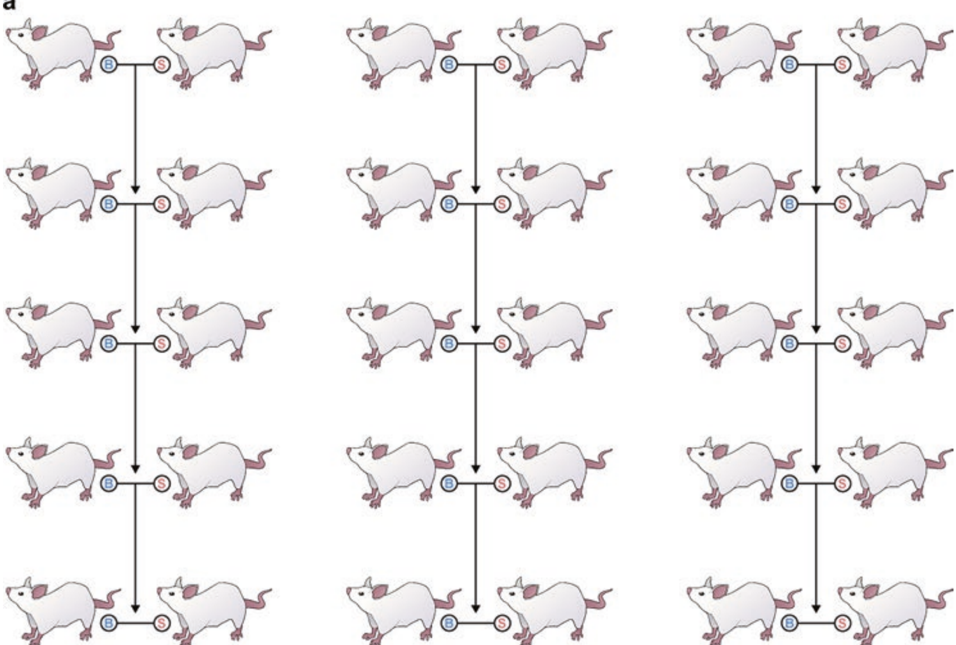
Fig. 1.3 *Progression of inbreeding*. The curve was drawn based of the Fibonacci series and represents relatively faithfully the cumulated percentage of genes that have become fixed in the homozygous state as inbreeding progresses. From generation F5 onward, this percentage is incremented by ~19.6% at each generation

tions, as many genes become homozygous. However, the process slows down over time. After 20 generations of inbreeding, not more than 1–2% of the loci that were heterozygous in the ancestors are still segregating. A mathematical series, based on Fibonacci's numbers, is traditionally used to model the progression toward homozygosity as the number of brother x sister matings increases. Although this curve is only an approximation, it accurately represents the evolution of heterozygosity over time (Fig. 1.3).

When breeding inbred individuals, it is crucial to prevent the formation of independent lines within the colony. These lines may undergo progressive divergence from each other as a result of genetic drift (Fig. 1.4a). Additionally, if a strain stops breeding (a common occurrence in practice), it is permanently lost. The example depicted in Fig. 1.4b, which involves establishing three new pairs of inbred mice from generation N to breed mice of generation N + 1, is considered the best option. However, when the progenies are small (another common occurrence), it may not always be feasible to establish three new pairs of siblings. Therefore, the substitute system depicted in Fig. 1.4c is common practice.

The progression toward full homozygosity during inbreeding involves blocks of chromosomes of variable sizes rather than individual genes. This explains why independent inbred strains carrying the same allele at a given locus have a great chance of sharing the same short segment of neighboring DNA (haplotype) on both sides of the allele in question. For example, mouse strains homozygous for the albino (Tyr^c) allele (e.g., A; AKR; BALB/c; and SJL) are probably homozygous for the same short segment of chromosome 7 flanking the albino mutation (Tyr^c), because the mutation shared by these strains results from the same mutational event that occurred well before the creation of these strains (i.e., identical by descent or IBD). It is important to keep in mind this unique characteristic of inbred strains, as it can have advantages or disadvantages when designing an experimental protocol. We will revisit this point in the section on congenic strains.

In most mammalian species, inbreeding of a natural population often has deleterious effects of variable intensity and phenotypic expression. In some cases, newborns exhibit growth retardation and finally die. In other instances, there is a



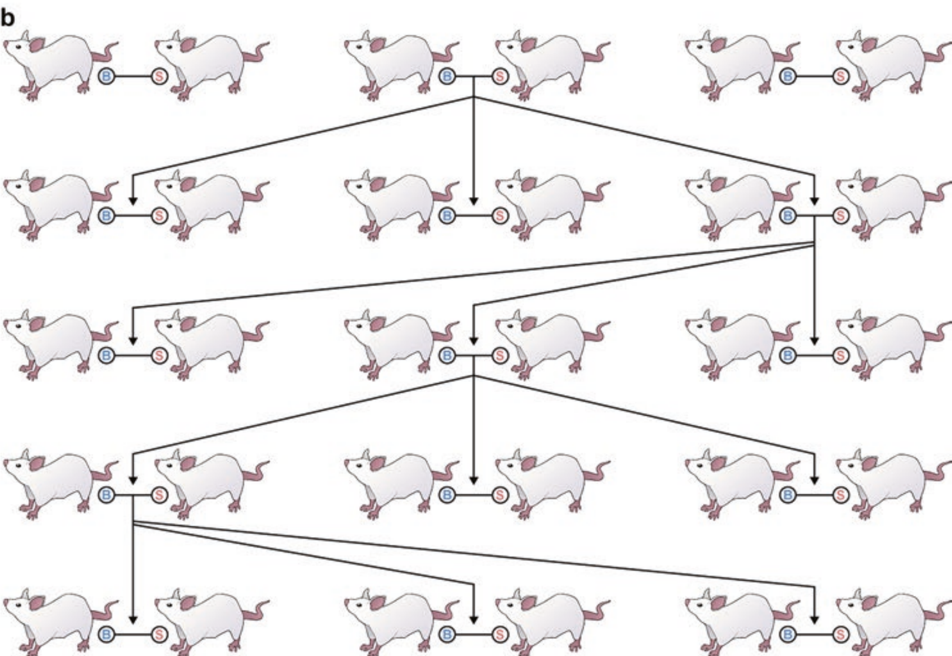


Fig. 1.4 *Breeding inbred mice*. (**a**) The system represented here is not recommended because it will lead to the establishment of three (and not one) independent inbred strains, which are progressively divergent from one another due to genetic drift. In addition, if a strain stops breeding (a common situation in practice), it is then permanently lost. (**b**) The system represented is certainly the best one, since, at each generation, three new pairs are established from a single cage at generation N to breed mice of generation N + 1. However, when the progenies are very small in size (a situation that is also common), it is not always possible to set the three new pairs of brothers and sisters. (**c**) Finally, the modified system represented here is the one that is generally used in practice