The Use of Randomly Bred and Genetically Defined Animals in Biomedical Research

Thomas J. Gill, MD

The rational selection of animals for experimental purposes is a very important part of the experimental design. The information obtained is neither as accurate and precise as it could be nor is it generally applicable to the body of knowledge in its field unless it utilizes the proper animal model. There are four major types of animals available for use in biomedical research, and each has its specific applicability. First, randomly bred animals can be derived either from colonies or from wild populations. They are particularly useful for first-level chemical screening, as a source of mutants, and as the starting material for developing inbred lines. Randomly bred animals from wild populations are also useful for studying the dynamics of genes in natural populations. Second, specifically structured outbred populations provide a stablilized gene pool that is useful for first- and second-level screening procedures. Third, inbred strains and F1 hybrids are useful for studying individual traits in a population, for answering specific experimental questions, for comparing results over a long period of time, and for detailed genetic analyses. Fourth, congenic strains are useful for studying the effects of specific genes and their alleles against a common inbred background. Detailed knowledge of the properties of these different types of animals and of the cost-effectiveness of their use provides an important basis for the appropriate choice of animal models for biomedical research.

THE MAJOR USES of animal models in biomedical research are to elucidate host defense mechanisms and disease processes, to point the way for subsequent studies in humans, and to screen various substances, such as drugs and environmental pollutants, for their effectiveness or toxicity. Examples of the way in which information has been transferred from a basic to a clinical setting are studies on the role that the genes in the major histocompatibility complex play in governing immune responsiveness, disease susceptibility, and the success of tissue transplantation and in developing vaccines and immunization techniques that are subsequently used in human medicine. This approach forms an important cornerstone of biomedical research that cannot be replaced by *in vitro* tests, despite the utility of the latter in certain restricted contexts. The types of experiments in which animal models are used can be cast into four broad categories. First, large numbers of animals are used in bioassays. Ran-

---

From Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Supported by Grants CA 18659, HD 08662, HD 09880 and GM 07349 from the National Institutes of Health and by grants from the Beaver County Cancer Society and the Tim Caracio Memorial Cancer Fund.

Presented at the Workshop on Needs for New Animal Models of Human Disease, sponsored by the Registry of Comparative Pathology of the Armed Forces Institute of Pathology and the Universities Associated for Research and Education in Pathology, Inc., April 28-29, 1980, Washington, DC.

Address correspondence to Thomas J. Gill, MD, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

0002-9440/80/1209-0S21$01.00

domly bred, outbred, and inbred animals are useful, and the choice depends upon the information that is required. Second, studies focusing on a specific animal or species in order to develop broad biologic concepts are an important source of fundamental knowledge. In these types of studies, inbred strains are probably the animals of choice. Third, animals can be used as environmental monitors—for example, fish for water pollution. This area has not been widely exploited, although it has great possibilities if genetically characterized animals specifically susceptible to certain chemicals can be developed. Finally, the development of models for human disease processes and for the effects of therapy on these disease processes is an important theoretical and practical area.

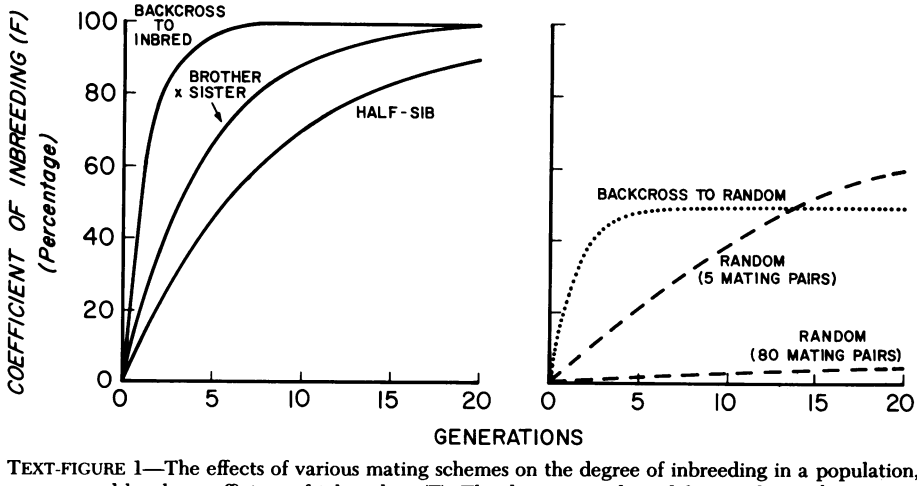
In choosing among the major classes of animal models—randomly bred, outbred, inbred, and congenic—careful selection must be made for the characteristics necessary for the experiments contemplated. This choice is critical because a great deal of important information can be lost or great cost and effort expended for no useful information if the inappropriate animal model is chosen. Since the major focus of this conference is on the use of animal models for human disease, a word is in order on how one can select an animal model to best "approximate a human population." Conventional wisdom suggests that a randomly breeding group of animals would be the best choice, since humans are "a randomly breeding" population. However, closer examination of this proposition shows that it is not completely tenable. Human populations do not breed randomly in a strict sense, since they are segregated by geographical, ethnic, and sociological factors that limit mating behavior and that tend to concentrate certain genes in specific populations. The randomly bred animal populations that are often used to approximate human populations show some degree of genetic restriction also, since the colonies from which they come can engender a variable amount of inbreeding over a period of time, and the amount depends upon the colony size (Text-figure 1). Hence, comparing the human and the randomly breeding animal may have the dual disadvantage of a variable level of selection in each species and the possibility of the selection's being in directions that would affect the characteristic being studied in an opposite manner. This situation would be particularly deleterious if quantitative variables were being studied, because the variance in the measurements would most likely render the comparisons meaningless. Many wild populations are also restricted in their breeding patterns because of the social structure of the species; therefore populations from different locales should be examined when this type of animal is used. Such studies are, however, very expensive and difficult to do. In summary, it is difficult to say *a priori* which type of animal

population, if any, is the best one to mimic a particular disease or an effect of therapy in a human population. Questions must be asked more perceptively, and the animal model must be chosen to answer them specifically.

Random Mating

There are two basic types of randomly mating populations: those that are colony bred and those that are found in the wild. The term "random" is somewhat misleading, however, since in each case the population can show genetic bias as a result of partial inbreeding. Text-figure 1 shows that a considerable amount of inbreeding occurs in a randomly mating colony that is maintained by only a small number of mating pairs. As the number of mating pairs increases, the amount of inbreeding in the colony decreases. In wild populations the genetic constitution of the animals can be selected by natural forces and by the social structure of the species. As noted above, it is very difficult to mimic the characteristics of human genetic variability, and animal models must be chosen to answer explicitly specific questions.

Randomly breeding populations are useful for developing studies relevant to human disease because they can generate mutants that mimic a disease process and because some of the animals may have specific response characteristics that make them particularly susceptible to disease agents. These characteristics can then be isolated and bred into stable lines. Randomly breeding animals are also useful as the first approxima-



TEXT-FIGURE 1—The effects of various mating schemes on the degree of inbreeding in a population, as measured by the coefficient of inbreeding (F). The data were adapted from Falconer [1] and from Festing.[2]

tion for testing the effects of drugs or for testing chemicals that may be toxic.

Outbred Populations

Outbred populations differ from randomly bred ones in that they are systematically bred to maintain the maximal genetic heterogeneity. The way in which this is done depends upon the number of breeding pairs used.[3] With 10–25 breeding pairs, a rather complicated system called "maximal avoidance of inbreeding" is used. When an intermediate number of breeding pairs (26–100) is used, specific rotation of the breeding pairs is done in order to keep the genetic heterogeneity maximal. With over a 100 breeding pairs, random mating can maintain heterogeneity of the population by chance alone. There are three basic rules for developing and maintaining such a population. First, the population must be closed for at least four generations. Second, there should be less than 1% inbreeding per generation. The amount of inbreeding can be calculated by the techniques of quantitative genetics, but it is rather complicated. The general rules about colony size and breeding schemes stated above are usually adequate to minimize inbreeding. Third, there should be no artificial directional selection for any character except reproduction.

The outbred population can be established starting either with inbred animals or with genetically undefined animals. It must be maintained by rigid criteria, and the animals that are utilized must be selected in a standard way. For example, an outbred population using a rotational mating pattern could be established in the following manner. Each of the initial mating combinations could be established by forming F1 hybrids between different inbred strains, which would provide the genetic constitution of the population. Each of the mating combinations would be labeled; they would then be systematically rotated so that the offspring in Population 1 would be selectively mated with the offspring from Population 2, for example, by a scheme such as taking 6 males from Population 1 and mating them with 6 females from Population 2, and so forth. This procedure would continue for at least four complete rotations before the population was utilized, and all of the animals used for experimental study would be drawn from one population, usually the last one in the sequence. The production of an outbred colony requires careful breeding and a considerable amount of work, but it provides animals with a broad genetic constitution, which is stable, and with good reproductive characteristics. For those engaged in large-scale testing procedures, this type of population is well worth the investment required to establish it.

Inbred Strains

There are three types of animals in this category: inbred strains, recombinant inbred strains, and F1 hybrids. The latter animal is made from inbred strains each time that it is used.

Inbred strains are difficult to develop, and approximately 10–20% of the lines that are started are successfully established as inbred strains. Implicit in the derivation of all inbred strains is selection for reproductive capacity in addition to that specific trait for which selection is being done. The definition of a strain's being inbred is that it is the product of 20 generations of brother × sister mating. After this time 98.6% of the loci should be fixed in any given individual.[2,3] In the process of developing these strains, inbreeding depression, which is the opposite of heterosis or hybrid vigor, occurs after approximately 5–7 generations of inbreeding: Reproductive capacity greatly decreases, and most strains are lost at this point. There can also be lesser degrees of inbreeding depression at generations around 14 and 20. The inbred strains are isogenic and not homozygous or "genetically inbred" at all loci. There is always a 1–2% residual heterozygosity because of the statistical probabilities of inbreeding and because of new mutations in the line.

In the derivation of inbred strains or in their maintenance in different laboratories, there is the recurring problem of subline formation due to genetic drift. When an inbred strain has been separated from its primary source for 8 or more generations, it should be identified as a subline by giving it a laboratory designation following the strain name.

All inbred colonies should have a specific structure, detailed documentation of breeding, and a quality control program.[2,3] The general plan of an inbred colony is to establish a foundation colony that is maintained by brother × sister mating and pedigreed. Animals from this colony are then used to form an expansion colony that is also maintained by brother × sister breeding and pedigreed. This colony is usually larger and serves experimental purposes or as the base for a production colony. The expansion colony should follow the minimal generation rule; that is, it should not be separated by more than 3–7 generations from the foundation colony. The production colony, which is derived from animals in the expansion colony, is maintained by mating animals at random, and it serves to generate animals for experimental or commercial purposes.

Documentation of the colony structure is very important, and this entails the use of cage cards with specific breeding information and pedigree charts. In any generation of the foundation colony, all animals should trace back to a common ancestor within 5–7 generations. In this way

there is minimal divergence of the developing strain, and subline formation within a colony is avoided.

Finally, quality control is absolutely essential, especially during the establishment of the colony. Periodic testing thereafter will serve as a check on the accuracy of the pedigree records. A number of different markers can be used for quality control, and the emphasis should be placed on those markers that reflect the major use of the colony. The easiest and most accurate way to check the colony is by serologic testing for the antigens of the major histocompatibility complex. Skin grafting may be quite useful, but it is more complicated, costly, and time-consuming. In broad screening and in the comparison of many stocks and strains, examination of skeletal morphology—for example, the measurement of mandible shape—is sensitive, and even closely related sublines may be differentiated. A major disadvantage, however, is that this approach must be interpreted in terms of statistical probability rather than in absolute terms. Other parameters, such as biochemical and enzymatic markers and coat color, can also be used, but they are less comprehensive and discriminating because of overlapping phenotypes in many strains.

A variety of strains of mice and rats are available for genetic studies, whereas the guinea pig, chicken, rabbit, and hamster have only a few inbred or genetically defined lines. These species and their characteristics have been extensively documented in current handbooks,[2,4-9] and recent developments are documented in newsletters [10,12] and in journal articles.[13,14]

Some of the salient characteristics that make inbred strains useful are their minimal genetic variance (homozygosity at nearly all loci), isogenicity, and genetic stability. They allow experimental results obtained in inbred strains to be compared over a long period of time and in different laboratories. This reproducibility is essential for many problems in biomedical research. The two significant disadvantages of inbred strains are that each strain represents only a narrow spectrum of the genetic constitution of the species and that they are more susceptible to phenotypic variance caused by environmental changes than are other types of animals with a less narrowly defined genetic constitution. The major uses of inbred strains can be organized into four categories. First, they are most useful in answering specific questions, for example, the effect of a drug on a tumor or a leukemia that is unique to an inbred strain. Second, they are used to fix specific traits that are desirable for study in greater detail on a defined genetic background. Third, pathologic processes and the effectiveness of host defense mechanisms can be compared in different populations (strain distribution pattern); then the right combinations can be selected for de-

tailed genetic studies. Finally, a very common and important use of inbred strains is in detailed genetic analysis. A variety of different approaches can be taken, and the major types of matings that can be used in genetic analyses are summarized in Table 1.

A unique type of inbred animal, called recombinant inbred (RI) strains, has been developed by Bailey.[16] In the derivation of these strains, two inbred progenitor strains are mated to form F1 hybrids, which are subsequently mated to form F2 hybrids. Males and females are selected randomly from the F2 hybrids and then inbred to form multiple lines. No selection is applied in the process, so that the reassortment and fixation of the genes originally present in the progenitor strains occur in a random fashion in the recombinant inbred strains. Each of the recombinant inbred

Table 1—Mating Schemes Most Commonly Employed

Type of mating	Purpose	Generations to attain 95% incrosses at a locus distant from the reference locus by	
		50 cM	10 cM
Random	Preserve genetic variability in a large population	Not applicable	
Outbred	Maintain fixed diversity of the gene pool in a given size population by systematically varying the mating pairs	Not applicable	
Brother × sister	Reduce genetic variability at all loci to develop isogenic strains.	16 (all loci)	
Backcross	Place a specific gene, especially a dominant one, on a standard inbred background	6	29
Cross–intercross	Place a specific recessive viable gene on a standard inbred background when the recessive gene is readily detected	12	62
Cross–backcross–intercross	Same when recessive gene is not easily detected—also more efficient	9	48
Brother × sister backcross	Place a recessive viable gene and its normal allele or a semidominant lethal gene and its normal allele on a common inbred background	15	26
Brother × sister intercross	Place a recessive lethal gene and its normal allele or a semidominant lethal gene and its normal allele on a common inbred background	15	22

Adapted from Green and Doolittle.[15]

Table 2—Uses of Different Types of Animal Populations

Type of animal	Advantages	Disadvantages	Major use
Random			
Colony bred	Inexpensive	Genetically ill-defined	First level of chemical screening
	Good reproductive performance	Variable amount of inbreeding depending upon colony size and mating scheme	Source of mutants, eg, for disease models
			Source of specific genes for development of inbred lines
Wild	Genetic constitution of population selected by natural forces	Difficult to obtain	Study dynamics of genes in natural population
		Gene pool and reproductive pattern biased by social structure of the species	
Outbred	Fixed and highly stable gene pool	Considerable effort to establish and maintain a large enough colony	First and second levels of chemical screening
	Good reproductive performance		Stabilize diverse gene pool over a long period of time
Inbred			
Strain	Isogenic (homozygous at almost all loci)	Difficult to establish (about 2/10 survive)	Compare specific traits and answer specific questions in genetically defined populations
	Information comparable and reproducible over a long period of time and in different laboratories	Not representative of the genetic structure of the species (loss of genetic variability)	Fix specific trait, e.g., a mutation, in a genetically defined strain
		Susceptible to environmental influences	Detailed genetic analysis of a trait
		Variable reproductive performance among strains	Linkage studies
		Genetic drift and subline formation possible	

Table 2—Continued

Type of animal	Advantages	Disadvantages	Major use
Recombinant inbred	Well-defined and stable variety of gene combinations	Same as inbred strains Effort and expense to make adequate number of lines	Linkage studies
FI hybrid	Isogenic (heterozygous at all loci) Less susceptible to environmental influences Easy to produce (hybrid vigor) Many different combinations available	Reflects a relatively narrow spectrum of the genes in a species	Structured and defined population for selected types of investigation
Congenic	Establishes alleles of a small and well-defined set of loci on a common genetic background	Same as inbred strains Effort to develop and maintain Eventual genetic drift from inbred partner	Study effects of the alleles of a specific gene on a common inbred background
Coisogenic	Differs from inbred partner at one locus only	Arises only by chance	Study effects of one specific gene

strains must then be typed by all available genetic methods to determine which of the progenitor alleles is fixed at each locus. The major use of these strains is in linkage studies where the strain distribution pattern (SDP) of alleles indicates which ones are most likely to be linked because they occur together. When a new trait is being analyzed, recombinant inbred strains can be studied to see if the trait segregates with loci already

defined. If it does, there is a high probability that it is linked, and this hypothesis can be tested further in a formal linkage study. The linkage information becomes cumulative in RI strains because they are stable and well-defined; hence they can form a library of linkage information. Since RI strains are derived from two inbred strains, they reproduce well and are less sensitive to environmental influences than are the parent inbred strains. Constructing RI strains does not entail any great technical difficulties, but large numbers of RI strains are necessary to eliminate chance variation, and an enormous investment must be made in their development. Thus, the use of RI strains is not generally practical, and only a few highly specialized laboratories have such strains available.

The F1 hybrid of two inbred strains can be a useful animal for many purposes. It is isogenic, but heterozygous at all loci, easy to produce (hybrid vigor), and less susceptible to environmental influences than the parent inbred strains. It provides a structured and well-defined population, albeit one with quite limited genetic diversity. This type of animal has not been widely used in research or screening programs, and it represents a potentially important area of development.

Congenic Strains

Congenic strains were developed by Snell [4] in order to study specific genes, especially those of the major histocompatibility complex, on common inbred backgrounds, and they exist only in mice and rats. The choice of breeding system depends upon the trait selected (Table 1), but the most common technique is backcrossing. The F1 hybrid carrying the trait to be selected is backcrossed to the background strain for 10–12 generations, and the line is maintained in a homozygous state by brother × sister mating. In congenic strains not only the trait being selected is placed on the inbred background, but a portion of the chromosome adjacent to the genes controlling the selected trait is also transferred. The size of this chromosomal segment depends upon the number of generations of backcrossing used in deriving the congenic strain.[9] The gene controlling the trait selected is called the differential locus, and the genes that accompany it are called passenger loci. In the derivation of congenic strains, adequate backcrossing must be done to reduce the segment around the differential locus to as small a size as possible, and very accurate records must be kept to document the derivation of the congenic strain. The size of the chromosomal segment around the differential locus can be calculated by the techniques of quantitative genetics, but as yet no body of information documenting the size of the chromosomal segment has been developed because there are not enough appropriate markers currently available.

Congenic strains also show genetic drift, so that in the long-term maintenance of these strains they should be periodically backcrossed into their inbred partners in order to prevent divergence from their backgrounds.

The major use of congenic strains is to compare the effects of the alleles of a specific gene against a common inbred background in order to eliminate the interference due to background genes. Another important use of congenic strains is to determine linkage by ascertaining which passenger loci travel with the differential locus.

Occasionally a mutation arises in an inbred strain, and the mutant is then truly different from the inbred strain at only one locus: These animals are called coisogenic. Since mutations, especially those that are detectable, arise by chance, such strains are quite rare. The congenic strains were developed to approximate coisogenic animals, and they are the only practical approach to studying the effects of the alleles of a genetic locus against a common inbred background.

Summary

The major types of animals available for biomedical research and their advantages, disadvantages, and major uses are summarized in Table 2. It is very important to select the appropriate animal for a particular experiment in order to obtain the maximal amount of information and to have the findings fit into the body of knowledge in the field. In addition, judicious selection of animals is cost-effective, and this is particularly important in large studies. It is not experimentally justified or cost-effective to use expensive and relatively rare animals for screening studies, and it is not rational to draw genetic conclusions from a genetically poorly defined population.

References

1. Falconer DS: Introduction to Quantitative Genetics. New York, Ronald Press, 1970 (reprinted)
2. Festing MFW: Inbred Strains in Biomedical Research. New York, Oxford University Press, 1979
3. Laboratory animal management: Genetics. ILAR News 1979, 23:A1–A16
4. Green EL (ed): Biology of the Laboratory Mouse. 2nd edition. New York, McGraw–Hill, 1966
5. Altman PL, Katz DD (eds): Inbred and Genetically Defined Strains of Laboratory Animals. Part I, Mouse and Rat. Part II, Hamster, Guinea Pig, Rabbit and Chicken. Bethesda, Federation of American Societies for Experimental Biology, 1979
6. Morse HC III (ed): Origins of Inbred Mice. New York, Academic Press, 1978
7. Silvers WK: The Coat Colors of Mice. New York, Springer-Verlag, 1979
8. Baker HJ, Lindsey JR, Weisbroth SH (eds): The Laboratory Rat. Vol I, Biology and Diseases. Vol II, Research Applications. New York, Academic Press, Vol I, 1979; Vol II, 1980

9. Klein J: Biology of the Mouse Histocompatibility-2 Complex. New York, Springer–Verlag, 1975
10. Mouse News Letter. Edited by J Staats. Bar Harbor, Maine, Jackson Laboratory
11. Rat News Letter. Edited by MFW Festing. Carshalton, Surrey, UK, MRC Laboratory Animals Centre
12. Lyon M: New rules for nomenclature of genes, chromosome anomalies and inbred strains. Mouse News Letter 1979, 61:4–16
13. Staats J: Standardized nomenclature for inbred strains of mice: Sixth listing. Cancer Res 1976, 36:4333–4377
14. Gill TJ III, Kunz HW, Hansen CT: Litter sizes in inbred strains of rats (Rattus norvegicus). J Immunogenet 1979, 6:461–463
15. Green EL, Doolittle DP: Systems of mating used in mammalian genetics, Methodology in Mammalian Genetics. Edited by WJ Burdette. San Francisco, Holden–Day, 1963, pp 3–55
16. Bailey DW: Recombinant inbred strains: An aid to finding indentity linkage and function of histocompatibility and other genes. Transplantation 1971, 11:325–327