



Guide to Genetic Standards for Laboratory Animals (1969)

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A Guide To Genetic Standards For Laboratory Animals

**A Report of the
Subcommittee on Genetic Standards
Committee on Standards
Institute of Laboratory Animal Resources
National Research Council**

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DEDICATION

To the memory of our respected friend
and colleague, Margaret M. Dickie, who,
until her untimely death, lent her talents
to this and other causes with unfailing
generosity.

**INSTITUTE OF
LABORATORY ANIMAL RESOURCES**

The Institute of Laboratory Animal Resources (ILAR) was founded in 1952 as a subsidiary of the National Academy of Sciences - National Research Council. Established as a coordinating agency, the institute disseminates information, surveys existing and required resources, establishes standards, and promotes education in the field of laboratory animal resources so that needed information and quality animal stock will be available to research workers. In this effort, ILAR works to enlighten the research animal scientist, veterinarian, technician, and supplier by furnishing them with information and guidelines developed through the participation of authorities in the field.

PREFACE

The upgrading of standards for production and use of laboratory animals has led to an increased awareness of the need for a concise statement of concepts and practices derived from genetic studies. This guide is designed for nongeneticists, and it is restricted to a number of genetic considerations that can be applied to more efficient and economical production and maintenance of genetically defined laboratory animals for research. Thus the reader is not expected to become an "instant" expert in genetics or in the genetic management of an animal colony. More detailed expositions from the literature on theory and practice are cited in the text and listed as references. A selected bibliography is also included.

Because of their research experience, the subcommittee members draw heavily on the laboratory house mouse (Mus musculus) as a model for illustrative examples. The same principles and practices can be adapted to other species.

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PRINCIPLES OF CONTROL OF GENETIC RELATIONSHIPS

The variety of goals of laboratory animal research programs is almost infinite, but the common purpose of all experimental research is to obtain the most accurate results possible. Environmental and genetic variability among the subjects of an experiment results in inaccuracies that often invalidate the research findings. Therefore, uniformity of laboratory animals is the ideal toward which animal breeders and investigators must strive.

Because variability arises from both environmental and genetic sources, its reduction must be approached through control of both. Environmental control can best be attained by keeping nutritional, disease-prevention, and atmospheric factors as constant and optimal as practicable. Control of heredity, however, is much more complex because it involves genetic selection based on the factors outlined in the following paragraphs.

The nuclei of the tissue cells of every individual contain pairs of thread-like structures called chromosomes. Along the length of these structures many different genes are arranged in characteristic positions. Each member of a chromosome pair contains genes that are paired with the genes located on the other member of that chromosome pair. One can refer to a gene by specifying one small segment, or locus, of a given chromosome. In the case of man, the 23 pairs of chromosomes contain many thousands of paired gene loci. Each gene

can exist in a variety of forms, or alleles, which are recognized by their different effects on the individual's traits. When the alleles of a gene pair are alike, the individual is described as homozygous; when the alleles are different, he is described as heterozygous.

The sex cells that are transmitted through mating from parents to offspring are produced by a special process of cell division. When this takes place, the newly created cells normally contain only one parental chromosome of each pair and, therefore, only one parental gene of each pair. At conception, the maternal cell (ovum) and the paternal cell (sperm) join to form one cell in which the chromosomes (and genes) are again paired.

Principles of Inbreeding

The breeding method used to control heredity is inbreeding, the mating of individuals that have one or more common ancestors. An offspring of such a consanguineous mating will be more likely to have identical alleles at any locus because of the single ancestral source of its alleles. Therefore, the essential effect of inbreeding is the production of genetically pure, or homozygous, individuals.

Even in a random-mating population there will be some consanguineous matings, the average number depending on population size; the smaller the population, the greater the chance of a consanguineous mating. In a random-mating population, consanguineous matings would cause, on

the average, $1/8\bar{m} + 1/8\bar{f}$ of the remaining heterozygous loci in a generation to become homozygous in the succeeding generation, where \bar{m} = number of effective breeding males and \bar{f} = number of effective breeding females in each generation (after Wright, 1921).

Thus, the intensity of inbreeding can be deliberately increased by reducing the size of the breeding population. However, a more systematic procedure with the same effect is the mating of specified relatives through succeeding generations. If the system is strictly followed, the number of common ancestors progressively increases through the generations, which results in rapid accumulation of homozygous loci, with concomitant genetic uniformity.

The rate at which genetic uniformity is attained by inbreeding is dependent on the closeness of the relatives mated. The most rapid and most commonly used system for laboratory animals is brother X sister, or full-sib mating. Equally rapid is the mating of the younger parent with its offspring, but the age differential in mates makes this cumbersome so it is used only rarely.

The ultimate result of inbreeding is genetic fixation, the state in which both parents are homozygous for the same allele at a particular gene locus. Barring mutation or laxity in adherence to the strict mating regime, a genetically caused diversity cannot arise from a locus after it is genetically fixed.

The percentage of loci that have attained genetic fixation through each generation can be calculated on theoretical grounds. Values for a brother X sister mating system follow.

GENERATION (F):	1	2	3	4	5	6	7	8	9	10	11	12	.	16	20	24
PERCENT FIXED:	0	12	28	41	52	62	69	75	80	84	87	89	.	95	98	99*

Note that genetic fixation is a more meaningful measure of inbreeding achievement than is "homozygosis," which includes, in addition to genetically fixed loci, those loci at which different individuals are homozygous, but for different alleles.

Genetic uniformity in an inbred population in such traits as coat color is made obvious by the uniform appearance of the individuals. Such traits, however, are determined by one or a very few genes. The effects of inbreeding are usually less apparent in polygenic traits (traits determined by many genes) such as body size, but they become apparent in the data where there is usually a pronounced reduction in the variability or spread of the measurements about the average value, or mean. Since the variability originates from environmental as well as genetic sources, it can never be completely eliminated by inbreeding. There will always be some variation about the mean.

Since it is a matter of chance which alleles become fixed during the process of inbreeding, the inbred animal in some cases might have become fixed for genes that result in a greater sensitivity to the

*After Green and Dolittle (1963).

environment. This results in an increased variability -- just the reverse of what the breeder set out to accomplish. The investigator must be alert to such an effect in new traits to be studied and might remedy it by selecting another strain (or F₁ hybrid) for the experiment.

Inbred animals, as compared with random-bred or minimally inbred animals, not only generally display greater uniformity, but also often display greater constancy of traits over time. By the process of genetic fixation, alternate genes, or alleles, have been eliminated from the population, thereby preventing chance drifting of gene frequencies and the changing of traits. However, constancy of traits cannot be absolute because new mutations accumulate and become genetically fixed in time. Nevertheless, an investigator has greater assurance of repeating results of an earlier experiment if he uses animals of the same inbred strain.

It is important to note that an inbred animal is not always the animal of choice in a given experiment. Sometimes the F₁ hybrid offspring of a mating between different inbred strains exhibit more vigor and greater uniformity in those traits of particular interest to the investigator.

Inbred Strains, F_1 Hybrid Animals, and Substrains

Introduction

Inbred strains are as genetically pure and homogeneous, or homozygous, as living biological material can be, because they are the product of at least 20 consecutive generations of brother X sister matings, which necessarily causes genetic fixation of many kinds of characteristics. A by-product of inbreeding that often occurs is "inbreeding degeneration," a decline in fertility and litter size.

F_1 hybrid animals are produced by crossing two strains of inbred animals. Reciprocal F_1 hybrids are produced by reversing the strains of the sire and dam. This method of breeding produces two litters of contrasting types of F_1 hybrid animals. One litter is the offspring of a male from one strain and a female from another; the other litter is the offspring of a male from the second strain and a female from the first strain. Any two inbred strains can be used, but F_1 hybrids do not "breed true" and, therefore, can be produced only by crossing between strains.

F_1 hybrid animals are heterozygous at all loci at which the parent inbred strains differ. They are often more vigorous and survive longer than animals of the parental strains. Moreover, characteristics of F_1 hybrids are usually intermediate between those of the parental strains, but occasionally, characteristics or conditions more extreme

than those found in either parental strain can be observed. This is one of the effects of hybridization caused by a new combination of genes. Reciprocal F_1 hybrid animals are useful when different maternal environments have differing effects on the young. For example, in mice, strain C3H/He female x C57BL/6 male will produce female offspring with a high incidence of mammary tumors, but the reciprocal C57BL/6 female x C3H/He male mating will produce F_1 females with a low mammary tumor incidence, because the mammary tumor agent (MTA) which is present in the milk of the C3H/He females is absent in C57BL/6 females. *

Except for some possible differences involving genes on the X (sex) chromosomes of the different strains, the genetic composition of the reciprocal hybrids is the same. Finally, F_1 hybrids are often more uniform in their responses to treatments than many inbred strains because inbreeding may increase the variability in the response of a particular inbred strain to specific treatment.

A substrain is produced when a portion of an inbred strain is separated from the parent strain after 8 to 19 generations of

*Strains of mice are conventionally designated by combinations of uppercase letters and numbers, or by letters alone.

brother x sister inbreeding and then maintained in the same colony without intercrossing for an additional 12 or more consecutive generations of brother x sister matings. A substrain designation includes the name of the parent strain followed by a slant line and a substrain symbol, such as an abbreviated name, a number, or a lower case letter. Two examples are: (1) A/He, representing the substrain of strain A maintained by Heston, and (2) DBA/1, the first substrain of ~~DBA~~ mice.

Nomenclature Conventions and Symbols

As the use of inbred strains, F₁ hybrids, and special stocks has increased over the past few decades, the naming (and misnaming) of strains has led to confusion and to misunderstandings that are now in the literature. With regard to mice, for example, there are over 200 inbred strains throughout the world. Since 1949 the Committee on Standardized Genetic Nomenclature for Mice has been devising rules for orderly naming of inbred strains and hybrids. The full listing of these rules can be found in Cancer Research 28:391-420, 1968.* This listing is updated every 4 years, and it includes a complete list of inbred strains; their known genetic

*Reprints available from The Librarian, The Jackson Laboratory, Bar Harbor, Maine 04609.

constitutions, origins, and characteristics; and the places where they are maintained. A companion report to Mouse News Letter, titled Inbred Strains of Mice, is produced every two years. This listing gives more details about the many sublimes maintained in various laboratories around the world. Also, an extensive list of strains or stocks of a number of species has been prepared by Jay 1963. Additional information about nomenclature conventions, as well as examples of strains, their histories, and their locations, is contained in Standardized Nomenclature for Inbred Strains of Mice, Mouse News Letter, and Inbred Strains of Mice (see Bibliography).

The user should be aware of the genetic differences between strains, and he should know that the total environment, including methods of husbandry, may introduce uncontrolled variables into an experiment. He should determine the demands imposed by the experimental design with regard to the level of genetic purity required, because this will determine the source from which he obtains his animals. At the same time, the breeder or supplier should be aware of the variety of specifications that users may demand, and he should determine within what limits he feels he can operate.

Both the user and the supplier should know, understand, and abide by the rules and symbols devised by consensus of experts; otherwise confusion, unnecessarily contradictory results, and misunderstandings will clutter scientific communications. The shorthand used within a laboratory all too often finds its way into the literature and becomes a questionable piece of information because of misuse of strain names, hybrid names, and other names for various stocks.

Characteristics

The usefulness of inbred strains depends not only upon their genetic uniformity, but upon the characteristics and responses of these strains to their environments and to experimental treatments. These characteristics include reproductive performance, life span, and tumor incidence, as well as responses to radiation treatment, hormonal administration, infectious disease, and other environmental and manipulative alterations.

The user may particularly be concerned with the life span, types of pathology, tumor incidence, radiation sensitivity, drug response, disease susceptibility, gestational survival, and congenital defects of the animals. The breeder, on the other hand, is more interested in their reproductive performance and their responses to environmental and nutritional changes and to stress.

It is not possible to go into detail about the characteristics mentioned, but the following brief examples indicate the scope of these concerns.

Maintenance of strains, particularly those that develop tumors, is difficult. Many investigators, for example, work primarily with strains characterized by a high frequency of mammary tumors and depend on the continued production of animals that spontaneously develop these tumors at a specific age. It is known that the mammary tumor agent (MTA) in C3H mice is different from the MTA in strain DBA and substrain A/He. If in continuing the inbreeding production of DBA or A/He mice, no effort is made to ensure that the main line comes from a female that developed a tumor, the strain will lose its high tumor incidence. This is true less often in strain C3H and its high tumor sublines. If a strain loses its high tumor incidence, research that is dependent on this characteristic will be adversely affected.

If strains are studied carefully, it becomes apparent that there are important strain differences in time of first litter, interval between litters, litter size, and sex ratio within litters. Another variable is the litter size of pair matings versus that of trio matings (2 females x 1 male sib, or any other ratio of females to males). Some strains produce fewer offspring per female from trio matings than from pair matings, while other strains produce more offspring (but not twice as many) from trio matings than from pair matings.

It should be noted that, for reasons that remain obscure, not all matings to produce hybrids are equally productive. For example, in one set of experiments using DBA/2J and C3H/HeJ mice it was found that 4 times as many offspring were produced in the C3D2F₁ generation as in the reciprocal D2C3F₁. Many of the DBA/2J females had repeated false pregnancies, a few vaginal plugs, and some indication that there was a deleterious reaction in the uterus and oviduct after insemination with C3H/HeJ sperm. In another instance with reciprocal crosses of CE female x C57BL/10 male and C57BL/10 female x CE male, the latter cross was extremely productive, while the former was practically sterile. This difference in reproductive performance between the reciprocal crosses was not apparent in matings of strain CE with a variety of other strains. These examples indicate some characteristics and responses of inbred strains that should be kept in mind in the maintenance of any colony.

Production

A brother x sister mating system is most useful in developing or maintaining an inbred strain. However, its effectiveness depends on the numbers of breeding animals involved. If the system is used in a colony where all mating pairs in any one family line can be traced to a common ancestor within three to five generations, and if the number of family lines is limited, then the maximum homozygosity for the entire strain can be realized and maintained. However, if a

brother x sister mating system is used in a colony and no effort is made to limit the number of family lines in the colony, within ten to twelve generations such a colony will contain many family lines. Each of these lines may be closely inbred, but the differences that have accumulated between lines might be enough that overall variation for the strain may be considerably greater than if the number of family lines were limited. Thus, the amount of persistent variation in a strain will depend on how the brother x sister mating system is used.

It should be noted that establishing inbred strains from originally wild, or at least noninbred, genetically varying mates is not an easy matter. Perhaps the majority of parallel family lines produced by inbreeding the descendants of different ancestral pair matings could become extinct, because genetic fixation results in homozygosity for different recessive deleterious alleles that are normally sheltered in ancestral heterozygotes.

The breeder, then, must determine what animals it will be economically feasible to produce and, consequently, what types of animals he will supply. The demands of users vary greatly, but these requirements will play a role in determining how the supplier organizes and maintains his colonies. Users should have access to information about the breeder's colony-maintenance methods.

Various methods can be used to produce the numbers of animals needed. Most commercial breeders have adapted selection and mating systems to their operations by dividing the colony into a foundation stock (FS) and a production stock (PS). In most cases, all matings for both colonies are produced by the foundation stock. Only those litters meeting or exceeding an array of selection criteria are used for establishing matings in the foundation stocks.

At the Jackson Laboratory the foundation stocks consist of 10 to 40 pairs of each inbred mouse strain. All matings are descended from one to three sib pairs (depending on colony size) in each generation, so that there is no possibility that sublines will be produced. All pairs not used for FS propagation are sent to the pedigreed expansion stocks (PES). The pairs sent to PES from FS are bred for up to four generations in PES, and their offspring provide a colony that can (1) maintain itself with regular additions from FS and (2) provide all matings necessary for the PS. The PS are of the sizes calculated to supply the demand for the strains. All offspring from PS are used for distribution.

Using this system, the offspring from PS are never more than three to five generations removed from a common ancestor, and FS is the ultimate source of all the PS. Since all the strains in FS are highly inbred, up to F₁₄₀ (140 consecutive sib-pair matings, or more

in some instances), genetic selection is unnecessary. The changes that occur are usually the result of environmental changes, seasonal changes, genetic fixation following mutations, and cyclic performance of the strains themselves.

In the FS the criteria for selection of the base pairs for propagating the next generation differ according to the strain. Generally, first litters of at least two pairs, from dams that have either produced the next litter or are pregnant, are used for FS colony maintenance. Pairs can be added to the colony through the fourth or fifth litter. If selection of the base pair for the next generation were based on a larger litter size, it would not be possible to propagate some strains. It is important in high mammary tumor lines to be sure that at least the granddam had a mammary tumor. All pairs in excess of those needed for FS maintenance are used in PES.

One systematic feature of propagation in FS, PES, and PS should be that a specified number of new breeding units are introduced into each strain colony every month and a like number of the oldest units are removed. In FS at least two to eight pairs (depending on the size of the colony of this strain) are added monthly. This ensures a continuous flow of animals rather than high production during some months and low yields in other months. In PES and PS the numbers of breeding units added each month are determined by the demand for the

strain. If there is a moderate or small demand for a particular strain, only two colonies may be needed, FS and PES.

If the demand warrants, it is possible to expand the breeding colonies in PS with a final nonsib mating generation. Offspring of such matings should be kept separate from those of sibmatings, and these animals should be termed inbred-derived. Such animals are useful for many purposes but must not be used for propagation.

F₁ hybrid production is part of the Production Stocks operation. Every month the appropriate numbers of females and males are procured from PES and PS for such matings.

Minimally Inbred Animals

Theory of Minimal Inbreeding

"Minimal Inbreeding" is defined by Falconer (1967) as "planned breeding designed to give the lowest possible rate of inbreeding with a given number of parents." This system is useful in maintaining maximum genetic variability, and thereby in providing animals of different and undefined genotypes.

For breeding colonies that produce noninbred laboratory animals, minimal inbreeding is greatly preferred over so-called random breeding for several reasons. In practice, random breeding is illusory because selection is consciously or unconsciously practiced in every breeding colony. Thus, families weaning the greatest number of healthy and rapidly growing offspring usually contribute more breeding stock to the next generation than other families. This increases the rate of inbreeding and thus negates the illusion that so-called random-bred animals are truly "minimally inbred!" Even true random breeding, if it were practicable, would not reduce the rate of inbreeding to a minimum, because animals comprising the breeding stock for the following generation are picked at random from all available animals of about the same age. By chance, this will result in some families' contributing more offspring to the next generation than other families, which constitutes nonminimal inbreeding.

From a practical standpoint, the most important reason for using a system of minimal inbreeding in colonies producing noninbred animals is that it retards inbreeding to the maximum possible extent, and thus maintains the greatest possible genetic diversity in the breeding colony. The rate of inbreeding obtained under random mating is reduced by 50 percent by minimal inbreeding, which also permits selection for high productivity and vigor. A breeding system designed for minimal inbreeding and based on population-genetics theory will give the breeding colony manager maximum genetic control and thus will assure the production of laboratory animals with known inbreeding coefficients. Thus, the theoretical rate of inbreeding per generation of a particular colony at any time can be a matter of knowledge rather than speculation. This rate is approximately equal to $1/4N$, where N is the number of parents ($1/8m + 1/8f$) (Falconer, 1967).

Practice

In order to achieve the theoretically minimal rate of inbreeding possible with a given number of breeding stock animals, it is necessary to use a mating system like Robertson's System of Minimal Inbreeding (Falconer, 1967), which is illustrated in Table 1 in connection with the following basic requirements:

1. Mating units in numbers that are a power of 2
2. Contribution of equal numbers of offspring by all mating units to the subsequent generation of breeding stock
3. Selection of breeding stock from later litters of the parents

The first two items are necessary to reduce the rate of inbreeding. The third item, while it has no effect on the rate of inbreeding per generation, reduces the total amount of inbreeding within a given period of time. This last rule, as well as the first two rules, while simple in principle, becomes difficult to implement in large colonies.

Mating units, as used in this system, can be either single matings or groups of matings and must be established in numbers that are a power of 2 (4, 8, 16, 32, 64, etc.) (see Table 2). Within each group of matings, selection for productivity can be practiced. However, it is important that each such group of matings contribute the same total number of offspring to the subsequent generation of breeding stock as every other group. If each breeding cage houses one male and more than one female, the system of minimal inbreeding can still be applied. In this case, all the offspring from each cage should be dealt with as if they were produced by one pair of parents.

Table 1 Robertson's System of Minimal Inbreeding^a

Male from Mating Unit Number		Female from Mating Unit Number		Mating Unit Number (next generation)
1	+	2	→	1
3	+	4	→	2
5	+	6	→	3
7	+	8	→	4
9	+	10	→	5
11	+	12	→	6
13	+	14	→	7
15	+	16	→	8
2	+	1	→	9
4	+	3	→	10
6	+	5	→	11
8	+	7	→	12
10	+	9	→	13
12	+	11	→	14
14	+	13	→	15
16	+	15	→	16

^aThis illustration is limited to sixteen mating units. However, the system can be used for any number of mating units that are a power of 2 (4, 8, 16, 32, 64, etc.).

Source: Falconer (1967)

Table 2 Rate of Inbreeding per Generation under Single-Pair
Mating System for Minimal Inbreeding

Number of Pairs of Parents^a	Rate of Inbreeding per Generation (%)
4	3.571
8	1.695
16	0.820
32	0.402
64	0.198
128	0.100
256	0.049
512	0.025
1,024	0.012

^aThis is the number of pairs contributing breeding stock to the next generation, i.e., the effective breeding population. It is not the total number of pairs in the breeding colony.

Source: Falconer (1967)

GENETIC CONTROL: TESTS OF GENETIC CONSTANCY

Inbred Animals

Nothing substitutes for prescribed systems of mating and the keeping of accurate and complete records in the animal colony to ensure that inbred strains, F₁ hybrids, and special stocks are genetically what they are purported to be. Certain precautions can be taken to reduce errors that may occur in mating or in transfer of animals from one cage to another. For example, it is common practice to separate strains that look alike; an albino strain is not placed next to another albino strain, and substrains with similar strain names are not placed next to one another. Neither are stray animals put into cages from which it is assumed they have escaped.

Mechanical errors are not the only cause for concern in an animal colony. Spontaneous hereditary changes, or gene mutations, may occur that perceptibly change the behavior or the morphological or physiological characteristics of an inbred strain. Such mutations can become incorporated into the strain's genetic constitution, or genotype, by chance (genetic fixation) in a few generations. It should be understood that, in time, any closed inbred colony will diverge from sister strains that are maintained in parallel. A majority of the mutations that occur will not be observed. It is only the more obvious and viable mutations that can be perceived without the use of special tests.

Even the uniform appearance of animals may lead to a false sense of security. For example, a mutation at a gene locus affecting coat

color, such as black to brown, can occur in an albino strain and the animals will remain albino.

Therefore, we must expect that some of the characteristics (and genotypes) of inbred strains will, in time, change. Genetic constancy of locally bred strains is maintained within the limits imposed by the needs of the investigator and by the ability to identify the type and extent of genetic divergence from the strain of origin. To keep the strain like its foundation line, it is necessary periodically to replace the local inbred population with animals of the accepted standard foundation line.

There are several tests that can be used to check genetic purity at a specified locus or set of loci. These include outcrossing, transplants and anatomical and pathological examinations.

Outcrossing

In the case of an albino strain, named loci affecting pigmentation can be checked by outcrossing to a pigmented strain that is homozygous for a number of recessive genes affecting pigment. Deviations from expected traits for these loci will be revealed in the F_1 generation. Similar tests reveal the genetic situation obtaining at other gene loci.

Transplantation Tests

In mice, as an example, inbred strains potentially differ at 20 or more histocompatibility loci. Similar variations occur in

other laboratory animals. The divergence of strains can be detected by grafts of tissue between animals of the same sex of a given strain or between like-sexed animals of a given strain and its foundation line. There are several methods of grafting that may be used, but skin and tumor grafting are the most common. If reciprocal grafts are accepted, the animals are said to be histocompatible; if they are rejected, these animals are said to be histoincompatible, and they are assumed to differ at one or more histocompatibility loci. If the animals have been properly preimmunized, this method offers a good way of checking suspected divergence at histocompatibility loci. Finally, an additional immunological test involves the method of red blood cell typing. Tests should be made only by qualified personnel who are competent to interpret results.

Anatomical and Pathological Examinations

Bone and Tooth Features. Dentition pattern and skeletal structure may be used to check genetic purity of a strain. These are quantitative characters determined by genes at many loci (polygenic), acting in an additive way. Although changes in a few loci are usually not obvious, such characters constitute a sensitive index of genetic divergence in time.

Tissue Examinations. Frequently, animals of an inbred strain or closed colony are necropsied for incidence of such pathologic changes as spontaneous mammary tumors, spontaneous lymphocytic

leukemia, amyloid degeneration of the kidneys, and several other changes that may be characteristic of the strain.

Because of their highly technical nature, the implementation and interpretation of the above experimental checks on genetic purity should be carried out under the supervision of a qualified geneticist. It should be emphasized again, however, that these tests help only to impose limits on the range of the inevitable genetic variation occurring in time. Such tests may not disclose more subtle differences that could arise from mutation and genetic fixation.

Minimally Inbred Colonies: Monitoring of Genetic Characteristics

A recessive allele is recognized only in the homozygous condition, while a dominant allele is recognized in either homozygous or heterozygous individuals.

Minimally inbred populations can be defined genetically and differentiated from each other by the pattern of gene frequencies at all heterozygous loci. For practical purposes it is possible to determine this pattern only at a limited number of loci. However, if any of the loci that determine visible traits, such as coat color or coat-color pattern, are heterozygous in the population, it is relatively easy to obtain estimates of the frequency of each allele at each locus by mating every breeding male to a female that is homozygous recessive at each of these loci. Thus, if B represents the dominant allele for black hair pigmentation, and b stands for the recessive allele for brown hair pigmentation; then only the homozygote bb has brown hair, while both the homozygote BB and the heterozygote Bb have black hair. With the aid of a multiple-recessive stock, not only can the genotype and gene frequency among breeding males be determined, but at the same time, spontaneous mutations at these loci can be detected. The genotype and gene frequencies at other heterozygous loci, which control such characteristics as histocompatibility or isozyme differences, can be determined in the same manner, except that techniques other than visual examination of the animals must

be used. Detection of spontaneous mutations at such loci can, of course, be accomplished in the same manner used to determine coat-color mutations.

The maintenance of constancy of quantitative traits such as rate of weight gain, and average number of young born and weaned must be checked empirically because many genes with individually small effects contribute to their genetic determination. Mutations at such loci are almost impossible to detect unless they have very marked effects. Consequently, for practical purposes, it is necessary to maintain a constant check of these characteristics by the use of adequate production records. These will provide continuing quality control by enabling the breeder to check the constancy of individual characteristics at regular intervals. Characteristics such as rate of weight gain can be checked by use of a randomly selected sample of males and females of appropriate age. This should be done at least once a year. The sample should be as large as conveniently possible and should consist of not less than 25 males and 25 females.

SPECIAL ANIMALS

Animals Produced by Manipulating the Microbial Environment

Research in many fields today demands that animals meet certain health standards. In radiation studies, for instance, it is known that mice carrying Pseudomonas react very differently to different doses of radiation than those without Pseudomonas. Similarly, those carrying Salmonella respond to experimental procedures differently from those without these bacteria. Basic sanitation procedures and management should ensure that colonies are free of such harmful bacteria and also free of ectoparasites and endoparasites. Certain strains known as specific-pathogen-free (SPF) have been developed. The term SPF has come to be used rather loosely, but in general it indicates that at the source the colony is free of certain pathogenic bacteria. Other colonies, also called SPF, have been raised in an even more germ-free environment, have been tested, and are shown to be free of various other pathogenic bacteria. It is much more difficult to be certain of the absence of viruses.

Another class of animals, known as germ-free, or axenic, are raised in a completely sterile environment and are presumably free of all bacteriological agents. Gnotobiotic animals contain controlled and specified flora. Maintenance of such colonies is highly specialized.

It seems likely that SPF animals or some modification of this type will be the generally acceptable animal for research in the

future. This means that all suppliers will be required to meet certain sanitation standards and tests to assure that users are receiving animals that are clinically healthy.

Stocks Carrying Mutations and Produced by Genetic Selection

Animals that are congenic with an inbred strain, or those in which a mutant gene is maintained on an inbred background, are becoming more useful in biomedical research.

The techniques for production and maintenance of congenic animals differ sharply from practices employed for inbred strains and F_1 hybrids. However, many mutant types, including muscular dystrophy, various anemias, neurological mutants, and such physiological mutants as diabetes, are maintained in inbred strains and produced in quantity. The advantage of producing congenic lines is that each mutant type does not need a littermate control. The investigator can use animals from the regular inbred population as controls. Also, a number of mutants on the same genetic background are easily compared, and their differences are easily observed.

Because mutations are usually somewhat deleterious and difficult to maintain, the propagation of many of them can be undertaken only if there is experienced genetic supervision. Techniques such as ovary transplantation or artificial insemination may be necessary to aid in propagation of some of the mutant strains.

PRINCIPLES OF RECORD-KEEPING

No matter what kind of colony is maintained, it is essential to keep some information with each breeding cage. The amount of information needed is determined by the kind of colony being developed.

Many detailed procedures and examples have been published on exactly how to keep records, but it seems appropriate here to point out the information that may be essential, rather than to give details on one system.

The basic requirement of record-keeping for an inbred line, or any other breeding colony, is to be able to trace with ease the collateral relatives, ancestors, and descendants of any animal. In addition to this primary role, records should also be able to provide information about birth, mating, death, offspring produced, location and other informative statistics. The requirements may range from the simple to the extremely elaborate, and may include computer systems of record-keeping.

In some colonies a numbering system has been streamlined to incorporate safeguards against mixing or mislabeling strains. For example, the pair rather than the individual is considered the unit for identification. This means that sib pairs cannot be exchanged, that there are no isolated pregnant females that can be inadvertently returned to the wrong cage, and that the identification system does

not require a lengthy search to produce a pedigree chart. Additional details are presented by Dickie (1966) in the chapter "Keeping Records," in Biology of the Laboratory Mouse. Moreover, the chapter "Practical Mating Systems and Record-Keeping in a Breeding Colony," by Wolff (1967) in The UFAW Handbook is also recommended.

The method used to propagate the colonies will determine to some extent the kind and amount of record-keeping needed, but the principle that the basis of record-keeping is to obtain information about reproductive performance and, above all, to trace all relatives of the animal in question should be kept in mind.

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