ENVIGO

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Genetic variability in inbred mice and strategies to reduce genetic drift

Genetic services

Introduction

Having genetically well-characterized inbred mice is a key factor in generating reliable and reproducible experimental data. Reproducibility of preclinical animal data has become an important topic in recent years, including a call for action by the National Institutes of Health (NIH) to raise the standards of preclinical research to better ensure that data are even more predictive of human biology.[1] However, the constant renewal of inbred mouse populations makes it challenging to stabilize their genetics, given that genetic variation is an inevitable consequence of breeding in all biological organisms, including inbred mice. As the number of mouse models available to researchers continues to increase, it is of utmost importance that scientists understand the genetic background of their inbred mice, as well as how to monitor and maintain mouse colonies, to minimize the genetic variation that can be introduced via genetic contamination and drift.[2,3]

Neglecting the effects of genetic variability can lead to confounding experimental results, in addition to wasted time, money, animals and damaged scientific reputations.[4,5] With an increasing appreciation for the importance of the genetic background of inbred mice, the intention of this article is to provide an overview of the latest scientific thinking on the topic of genetic changes or drifts in inbred mouse colonies and how to minimize and detect these changes.

As an example, we discuss some of the genetic and phenotypic changes in C57BL/6 substrains, since these are some of the most heavily used models in biomedical research.

Overall, continually seeking to strengthen the genetic quality standards for inbred mice will have many benefits, including the generation of robust and reproducible scientific data, which is an integral part of the scientific process.

As the number of mouse models available to researchers continues to increase, it is of utmost importance that scientists understand the genetic background of their inbred mice, as well as how to monitor and maintain mouse colonies

Inbred mice: defined

Relative to outbred stocks of mice, which are often referred to as being genetically undefined and bred to maintain maximum heterozygosity,[6] inbred strains are genetically homogenous and provide more uniform, more reproducible, and better-defined models[5] (although the heterogeneity of outbred stocks are certainly useful in the study of genetics, toxicology, and pharmacology).[6]

The commonly cited definition for inbred strains of mice was originated in 1952 by *The Committee on Standardized Nomenclature for Inbred Strains of Mice:*[7]

A strain shall be regarded as inbred when it has been mated brother x sister for twenty or more consecutive generations.

Parent x offspring matings may be substituted for brother x sister matings, provided that in the case of consecutive parent x offspring matings the mating in each case is to the younger of the two parents.[7]

Based upon these criteria at 20 generations, on average at least 98.6% of the loci in each mouse are homozygous, which in fact, highlights the notion that inbred mice are not isogenic, and this definition actually integrates an allowance for a small level of genetic variability.[8-10]

Most scientists working with inbred mice have likely encountered substrains of these animals. Substrains are branches of the inbred strain that have diverged from the original founding strain.[7,11] Genetic contamination and genetic drift are only two ways in which substrains may arise, others include incomplete inbreeding (residual heterozygosity), and intentional outcrossing of strains.[12-14] Phenotypic variations in substrains have been reported, despite high genetic concordance among the substrains. To illustrate, Table 1 shows that there is high genetic concordance among C57BL/6 substrains, based on a 560 single nucleotide polymorphism (SNP) panel;[1] an observation that has also been reported by various independent research groups.[15-18] Despite this high genetic concordance, some phenotypic variations among C57BL/6 substrains are known,[19-21] which further highlights the importance for researchers to have a clear understanding of the genetic background of their inbred mice.

In the following sections we provide a brief discussion of the pathways that lead to genetic variation, highlight some common genetic and phenotypic differences across C57BL/6 substrains, as well as strategies that can help reduce genetic variation in inbred colonies.

Pathways to genetic variation

The genetic stability of an inbred mouse strain can be influenced by several factors, including, but not limited to, accidental genetic contamination and genetic or epigenetic drift,[22,23] caused by spontaneous mutations and epigenetic alterations, respectively, within the inbred line.

Accidental genetic contamination, primarily due to human error, occurs by the inadvertent mixing of two mouse strains.[2] Substantial investments in infrastructure and personnel training, together with optimal mouse husbandry, breeding practices and genetic quality control programs have greatly reduced the potential for accidental genetic contamination for most commercial vendors of rodent models.[2,24,25] However, the risk for genetic contamination remains high in research facilities where animals are moved between locations and co-housed with other strains having the same coat color and/or different genotypes. In these types of facilities, adequate personnel training and the application of traditional strategies for maintaining inbred mice can help to minimize the opportunities for genetic contamination to occur (see later section: Strategies to Reduce Genetic Variation).

For commercial rodent breeders, genetic drift is a difficult challenge because it is driven by spontaneous mutations, a naturally occurring process which cannot be completely prevented. Spontaneous mutations can arise by different mechanisms, such as DNA replication errors, DNA repair deficiencies, and transposable genetic elements.[2,26]

The relatively low rate of spontaneous mutations in mammals combined with the redundancy of the genetic code and industry standard breeding practices for inbred mice means that mutations that arise in inbred strains typically do not result in a fixed phenotypic change.[14,51] Nevertheless, the introduction of these rare spontaneous mutations can occasionally have unpredictable consequences at the phenotypic level. Among the mutations that affect a typical gene, different kinds produce different impacts,[26-28] whereby the impact of mutation varies depending on the nature and location of the DNA sequence it has changed.

Most spontaneous mutations are SNPs that are in the non-coding region of the DNA,[29] and thus, most of these genetic changes are phenotypically insignificant. Many newly arisen mutations in functional genes are thought to be deleterious and will be negatively selected, but some protein evolution results from favorable mutations.[26-28] Overall, spontaneous mutations result in phenotypic changes that can be categorized as 1) synonymous (having no effect on the protein product produced), 2) missense (producing a single amino acid change in the protein), or 3) nonsense (producing a truncated protein product).

Other phenotypic variation can be driven by metastable epialleles, whereby the epigenetic state of these alleles directly correlates with phenotype variability.[30] A number of mouse metastable epialleles have been described in the literature, including the *agouti viable yellow* (A^{vy}),[22,31,32] and *Cabp* (CDK5 activator binding protein)[33] alleles, among others.[34]

[1] A SNP is a variation at a single nucleotide position in a DNA sequence among individuals

Pathways to genetic variation

The A^{vy} mouse has long been used as a model of epigenetic metastability. The coat color of these animals has been shown to inversely correlate with the DNA methylation state of long terminal repeat of the intracisternal A particle (IAP) retrotransposon, which, at some point in the past, integrated upstream of the *agouti* gene.[35]

While some mutations and epigenetic changes will result in an obvious phenotype, such as the early instances of unexpected coat color change in mouse colonies,[22,36-42] others may be silent or result in a non-apparent phenotype and can go undetected for years.[2,28] In the instance where an obvious phenotypic change is detected, these animals can be quickly separated from the breeding colony, effectively removing the spontaneous mutant from the gene pool. However, there have been many instances where an undetected mutation has become fixed in an inbred colony, primarily because the mutation produced no obvious phenotypic effect, and its effects only became apparent under specific experimental conditions.

Furthermore, detecting de novo mutations on a large scale is a nearly impossible endeavor for commercial rodent breeders. Thus, it is often the case that spontaneous mutations go undetected for years or decades, which provides a suitable environment for novel mutations to become fixed in a population of inbred mice.[28]

Notably, since genetic drift is a common feature of all biological organisms, it is not surprising that the genomes of inbred strains of mice have a mosaic structure of variation[43,44] and that spontaneous mutations have become fixed and reported for a number inbred strains of mice, including (not a comprehensive list), the 129,[45] BALB/c,[46,47] and C57BL/6[48-50] strains.

To better define the importance of spontaneous mutations, researchers have attempted to measure the rates of mutation in various mouse lines. Schlager and Dickie[51] reported that the mutation of dominant to recessive coat color alleles was at a rate of 8.9×10^{-6} per locus per gamete. Anticipating that different genes might have different mutation rates, Schlager and Dickie[52] did another study involving 26 different loci and 28 different recessive mutations. They found an overall rate of mutation of 6.7×10^{-7} per locus per gamete.

Overall, a commonly cited range for the estimated rate of spontaneous mutations in mammals is 10^{-5} to 10^{-7} per locus per gamete based on breeding and specific loci testing[14,52] and even lower based on sequencing data in humans.[53] Therefore, across the genome, spontaneous mutation rates are low.

The example of C57BL/6:
substrain genetic and phenotypic differences

In this section, we use the C57BL/6 model to highlight some genetic and phenotypic differences that have arisen across its substrains over several decades of breeding. It is important to emphasize that phenotypically significant genetic drift has been exceptionally rare in C57BL/6 strains and substrains, and this has been objectively validated by numerous research groups as described later.

Origins of C57BL/6 mice

Inbred strains of mice, including C57BL/6, were developed in the early 20th century for genetic studies. Beginning in 1902, William Castle at Harvard University and Abbie Lathrop carried out studies indicating that brother-sister matings of mouse stocks could reduce the degree of genetic heterozygosity in the offspring.[54]

A student of Castle's, Clarence Cook Little (commonly referred to as C. C. Little), found that brother-sister inbreeding for more than 20 generations resulted in essentially complete homozygosity (but not isogenicity) of the genetic loci of the offspring.[55] While inbreeding of other species often resulted in decreased fertility or decreased resistance to disease, mice seemed to adapt well to the process of inbreeding.[54]

Origins of C57BL/6 mice

In 1909, using this repeated brother-sister inbreeding, C. C. Little developed the first inbred strain of mice, known as DBA (with coat color alleles dilute (d), brown (b), and non-agouti (a)).[54,56] Later, while at the Carnegie Institute (Cold Spring Harbor, NY) in 1921, C.C. Little obtained some black strains of mice from a breeder named Abbie Lathrop and developed another inbred mouse strain. He bred a female mouse (#57) from Lathrop's C stock with a male (#52) from the same stock. The female mouse offspring were then bred using brother-sister inbreeding for more than 20 generations, which led to the generation of the stable C57BL line.[55] After being bred successfully for a number of years, the C57BL line was separated into two substrains (C57BL/6 and C57BL/10) at some point prior to 1937, and these were then maintained as inbred lines at the Jackson Laboratory up until the 1950s.[55]

In recent studies, these two substrains have been shown to possess allelic differences at two loci on chromosome 12 and at six loci on chromosome 4.[57] These differences have been shown not to be due to genetic contamination from other strains.[58] In 1951, C57BL/6 mice were shipped from the Jackson Lab to the NIH in Washington, DC, to establish a second colony.[15]

This major bifurcation of the C57BL/6 line resulted in mice referred to as C57BL/6N (for NIH) and C57BL/6J (for Jackson Laboratory) (**Figure 1**). Data collected by the International Knockout Mouse Consortium (IKMC) indicate that several phenotypic differences exist between these substrains.[59] From these two major substrains, other substrains have been established by transferring the 6N and 6J strains to different commercial mouse breeders (**Figure 1**).

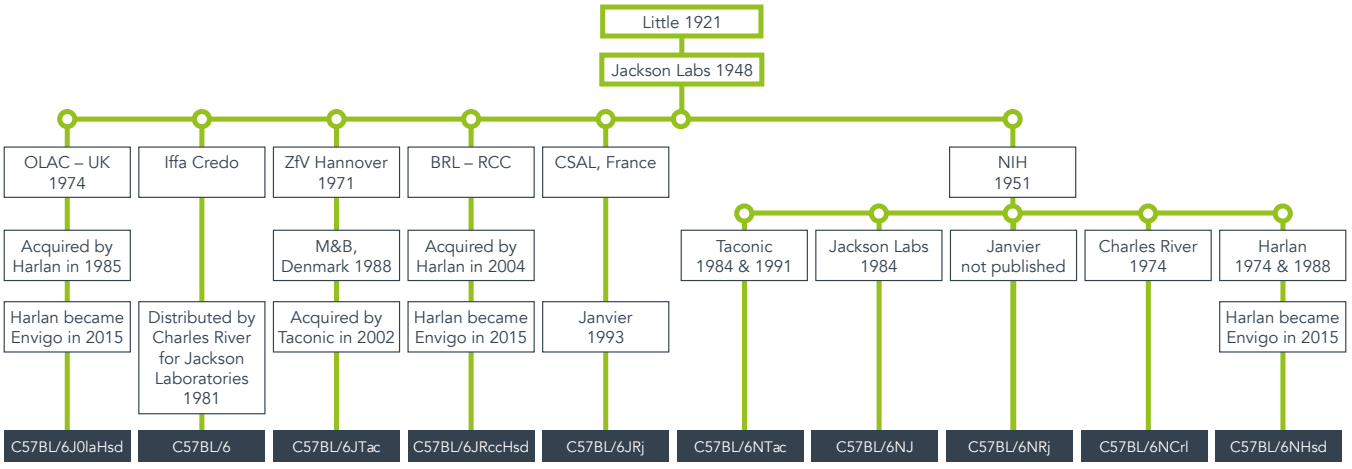

Figure 1 – C57BL/6 Substrain genealogy

Origins of C57BL/6 mice

In 2002, the complete genetic sequence of the C57BL/6J mouse strain was published,[60] followed by additional strains sequenced by the National Institute of Environmental Health Sciences Resequencing Project.[61] Notably, the C57BL/6J inbred strain remains the best genetically characterized of the inbred lines, and this detailed genetic information has significantly increased the ability to conduct SNP analyses in the various C57BL/6 sublines to evaluate genetic divergence of the strains. The C57BL/6 inbred line has been maintained by a number of institutions worldwide with the appropriate nomenclature for the distinct sublines. The next section will describe some of the known differences across these substrains.

The C57BL/6J and C57BL/6N substrains have been extensively examined for SNPs that indicate spontaneous mutations leading to divergence of the two lines over more than 60 years of separated breeding. For instance, the analyses of Zurita et al.18 and Mekada et al.[15] independently showed only 12 differences out of 1,449 possible SNPs, while Pettitt et al.[17] showed 102 differences out of 139,561 SNPs. Other studies by Bothe et al.[62] and Simon et al.[59] revealed 12 microsatellite marker differences from a total of 342, and 34 coding SNPs leading to amino acid substitutions, two coding indels (insertions or deletions), 146 noncoding SNPs, and 54 noncoding indels, respectively.

Most recently, Mekada et al.[16] identified new SNPs specific to the C57BL/6NJ strain that is being used as the genetic background for producing knockout mice in large projects such as the IKMC (members of the IKMC are working to mutate all protein-coding genes in the mouse using C57BL/6NJ mouse embryonic stem (ES) cells). Overall, a total of 1,361 candidate SNPs from the SNP database distinguished the C57BL/6NJ strain from 12 other inbred strains, and 277 C57BL/6NJ-specific SNPs were confirmed by these researchers.

Remarkably, these data demonstrate that there has been a limited degree of genetic drift during the last approximately 60 years across the C57BL/6 substrains, although there are examples of genetic differences affecting phenotypes between the 6N and the 6J substrains. Samplings of some of the more common polymorphisms that have been observed between commercial supplier-specific substrains are indicated in Figure 2.

Origins of C57BL/6 mice

Figure 2 – Substrain gene mutations

Strain	Supplier	Deletion			
		Nnt	Scna	Mnm1	Rd8
C57BL/6JOlaHsd	Envigo	No	Yes	Yes	No
C57BL/6JRccHsd	Envigo	No	No	No	No
C57BL/6NHsd	Envigo	No	No	No	Yes
C57BL/6J	Jackson Laboratory	Yes	No	No	No
C57BL/6ByJ	Jackson Laboratory	No	No	No	No
C57BL/6J	Charles River	Yes	No	No	No
C57BL/6JCrl	Charles River	Yes	No	No	No
C57BL/6NCrl	Charles River	No	No	No	Yes
C57BL/6JBomTac	Taconic	No	No	No	No
C57BL/6NTac	Taconic	No	No	No	Yes
C57BL/6JRj	Janvier	Yes	No	Yes	No
C57BL/6NRj	Janvier	No	No	No	Yes

Figure 2, Table 1 – Examples of mutations present in various C57BL substrains from numerous suppliers

	C57BL/6J	C57BL/6JRccHsd	C57BL/6JOlaHsd	C57BL/6NHsd	C57BL/6NTac	C57BL/6NCrl
C57BL/6J	--	98.5%	98.5%	97.8%	97.8%	97.8%
C57BL/6JRccHsd	98.5%	--	100%	99.3%	99.3%	99.3%
C57BL/6JOlaHsd	98.5%	100%	--	99.3%	99.3%	99.3%
C57BL/6NHsd	97.8%	99.3%	99.3%	--	100%	100%
C57BL/6NTac	97.8%	99.3%	99.3%	100%	--	100%
C57BL/6NCrl	97.8%	99.3%	99.3%	100%	100%	--

Table 1: Genetic concordance among C57BL/6 substrains. Most random mutations in a population are single nucleotide polymorphisms and do not affect the phenotype due to the redundancy of the genetic code. The SNP panel for the above concordance table contained 560 SNPs (Envigo).

Origins of C57BL/6 mice

The most well-known of these is in the nicotinamide nucleotide transhydrogenase (*Nnt*) gene. In the 6J substrain, a spontaneous in-frame five-exon deletion in the *Nnt* gene results in the complete absence of the *Nnt* protein.[48] Interestingly, the *Nnt* gene is observed to be deleted in only some of the 6J substrains available from different suppliers (i.e., C57BL/6J and C57BL/6JCrl) and not others (C57BL/6JOlaHsd, C57BL/6JRccHsd, and C57BL/6JTac). Thus, it appears that the *Nnt* mutation occurred at the Jackson Laboratory prior to the transfer of mice to Charles River in 1986 but after the transfer of C57BL/6J to other labs in the early 1970s.[48,63] In the study by Simon et al.,[59] the observed genetic changes discussed earlier resulted in 43 structural variations in proteins that included *Nnt*, Vmn2r65 (vomeronasal 2, receptor 65), and Cyp2a22 (cytochrome P450 family 2, subfamily a, polypeptide 22). Other genes that were affected in the 6J line included Rptor (coding for Raptor, a key protein in the mTORC1 pathway), *Plk1* (a cell cycle regulatory gene), *Herpud2* (related to endoplasmic reticulum protein processing), *Crb1* (related to diabetic retinopathy), and *Cyfip2*^{M1N} (related to actin polymerization in neurons). Phenotypic differences included metabolic, physiologic, behavioral, and immunologic characteristics.[59] Under high-fat diet conditions, both 6J and 6N were shown to develop glucose intolerance, but for the 6J mice, the levels of blood glucose were comparatively higher.[64] This also corresponded to a lower level of insulin secretion in the 6J mice, which also have a greater tendency toward obesity.[64] Simon et al.[59] reported a lower metabolic rate (i.e., O2 consumption, CO2 production, and heat production) in the 6J mice compared to the 6N mice.

Neurologically, other differences included reduced vision for the 6N compared to the 6J59 (probably related to the *rd8* mutation in the *Crb1* gene in the 6N mice[65]).

The 6N male mice showed significantly reduced performance in the Morris water maze test compared with the 6J male mice.[59] Immunologically, the 6J mice showed a greater dinitrofluorobenzene (DNFB)-induced contact hypersensitivity response compared with that seen in the 6N mice.[59] According to the authors, this increased response may be related to a larger fraction of natural killer (NK) cells that were activated by interleukin-12 (IL-12) in the 6J mice compared to the fraction activated in the 6N mice.[59]

Both the *Scna* (alpha-synuclein) and *Mnm1* (multimerin1) gene mutations appeared only in the C57BL/6JOlaHsd substrain after it was transferred to Harlan Olac (UK) in 1974. Despite the many described functions for alpha-synuclein,[66,67] loss-of-function mutants appear phenotypically normal.[49,68] The defect in multimerin 1 results in impaired platelet adhesion and impaired thrombus formation.[69] The *Rd8* (*Crb1* gene) mutation appears only in 6N substrains and arose after the transfer of C57BL/6 to the NIH in 1951 but before the transfer to other suppliers starting in 1974. The phenotype of this mutation involves distinct retinal degeneration.[65,70] An instance of copy number variation (CNV) for the gene Ide (coding for insulin degrading enzyme) is another example of spontaneous mutation in the C57BL/6J substrain.[71]

Origins of C57BL/6 mice

An analysis showed that 64% of the C57BL/6J mice from the Jackson Laboratory colony were heterozygous for the Ide gene and that this heterozygosity likely arose in the early 1990s.

It is also important to note that genetic drift in inbred mouse strains has been of great value in some instances, as they have been a rich source of new phenotypes that may be relevant to the study of mouse pathology and corresponding human disease.[72] In 1978, the Mouse Mutant Resource Center (MMRC) was established to manage and characterize spontaneous mutants derived from inbred strains. Currently, the collection includes more than 700 mutant mouse strains, most of which are cryopreserved. Some of the most well-known mutant strains include Snell's Dwarf (*Pou1f1*^{dw}), obese (*Lep*^{ob}), diabetic (*Lep*^{db}), and Duchenne muscular dystrophy (*Dmd*^{mdx}).[73] The study of mouse mutant phenotypes has also led to the discovery of novel gene functions and related human pathologies. For instance, Seymour et al.[74] showed that a mouse chronic proliferative dermatitis (*Cpdm*) mutant phenotype led to the identification of the mouse Sharpin gene (SHANK-associated RH domain interacting protein). These studies pointed to the presence of a similar gene mutation in humans causing hyper-eosinophilia disorders.

Odgren et al.[75] identified a disheveled hair and small ear phenotype that turned out to be a mutation of laminin A (*Lmna*^{Dhe}), an intermediate filament protein of the nuclear membrane. This gene was shown to be important in craniofacial development. The human homologue of this gene was also shown to be involved in human laminopathies and in Hutchinson-Gilford progeria syndrome.

Recently, Fairfield et al.[76] used high-throughput exome sequencing to identify the causative mutations for 172 distinct, spontaneously arising mouse models of Mendelian disorders (maintained by the MMRC). Notwithstanding the use of newer genomic technologies, putative pathogenic mutations were identified for only approximately half of the models, indicating that there remains much to be learned regarding the relationship between spontaneous mutation and phenotypic manifestation.

Origins of C57BL/6 mice

These examples illustrate the possibility of genetic drift developing in an inbred mouse strain through spontaneous mutation during long-term breeding. The fixation of spontaneous mutations in a colony can have a variable effect on a phenotype, depending on the nature and location of the mutation in the genome. These examples also confirm the importance of proper methodology in the maintenance of inbred mouse strains. Researchers must have a solid understanding of the genetic background of the C57BL/6 inbred mice they use, since some genetic and phenotypic differences exist across substrains, which can impact experimental results. Careful consideration should also be given when comparing experimental results generated from different C57BL/6 substrains. The importance of this point was recently substantiated in an article by Fontaine and Davis.[11] The authors published an article in the journal *Diabetes* reporting on a survey of 616 publications during 2010–2014 in which genetically modified inbred mice were utilized.

In 58.5% of the cases, incomplete information was provided on the substrain information. When C57BL/6 mice were used, in 63% of the cases, incomplete information on substrain information was included. These data suggest a lack of awareness on the part of many scientists concerning the specific genetic backgrounds of the mice that they use. Enhanced attention to the particular substrains of mice used in experiments will lead to more reliable conclusions. Below we discuss various strategies that can help reduce genetic variability in inbred mice.

These examples illustrate the possibility of genetic drift developing in an inbred mouse strain through spontaneous mutation during long-term breeding.

Strategies to reduce genetic variation

The process of maintaining and propagating inbred mice has certainly improved over the last few decades due primarily to the development of biological and molecular techniques to monitor the phenotypic and genetic integrity of the animals. However, knowledge of the genetic integrity of inbred mice is not always fully considered by researchers. Here we provide some practical guidelines that can help minimize genetic variability in an inbred colony.

As described earlier, the risk of accidental genetic contamination has been greatly reduced in the context of commercial rodent breeders, but it is not completely negligible. Other environments (e.g., academic research facilities) pose substantial risk of accidental genetic contamination. In such cases, adequate personnel training programs and careful attention to traditional best practices in animal husbandry can minimize (but not eliminate) the occurrence of errors that can cause genetic contamination. At a minimum, considerations should include familiarity with breeding methods, nomenclature, typical strain characteristics (so that the unexpected phenotypes can be quickly identified), and precise record-keeping.[13,24,25]

Methodologies to objectively determine strain identity and/or monitor genetic integrity in inbred strains of mice include biochemical and immunological markers, skin grafting, test-matings, morphological measurements, routine gross pathological assessments, and genetic techniques.[24,77-79] Most of these practices rely on the obvious phenotypic manifestation of a genetic change. However, as described earlier genetic changes do not always result in overt phenotypes, and in fact, most genetic changes are actually silent.[28] Thus, a genetic monitoring program, for example based on SNP analysis, is the gold-standard when it comes to a genetic quality control program. Notably, this type of genetic monitoring is largely meant to guard against contamination by other strains, and is generally not intended as a foolproof mechanism for identifying spontaneous mutations due to its inherent limitations in detecting rare changes across the genome. When considering a choice in commercial rodent vendors, one must give careful consideration to the robustness of the routine genetic monitoring program in place. A successful program to prevent and detect genetic contamination in inbred strains includes the appropriate number of animals being tested in each colony, frequent testing of newly mated cages, a high number of SNP markers that can distinguish between all major inbred models being bred by the vendor, and transparency of the program and results.

Strategies to reduce genetic variation

Another approach that has been taken to resolve the problem of genetic drift has been the re-derivation of the original inbred line by the introduction of the lost gene or the repair of a mutated gene. Approaches to such gene correction include the use of mouse transgenic and knock-in technologies.[28] Alternatively, a wild type gene can be bred into an inbred strain lacking the specific locus to generate a congenic strain with a repaired genotype. For example, Banks et al.[80] utilized this approach using the C3H inbred strain which carries a mutation in the *Pde6b* gene, resulting in retinal degeneration and blindness by 1 month of age. These animals were bred with wild-type *Pde6b*-containing BALB/c mice, which resulted in a new substrain that removed the *Pde6b* mutation. Using this new mouse, which is genetically similar to the C3H inbred strain, behavioral studies requiring normal eyesight in the C3H background could now be conducted.

While this approach has been successful in some instances, it is not a commercially or practicable method for vendors of rodent models with large colonies of the same inbred model at multiple locations across the globe. In addition, for numerous reasons, many researchers would likely express concern if a vendor altered a model that they have historically relied upon for their research only because a spontaneous mutation had recently been detected. Undoubtedly, inbred mouse strains, such as C57BL/6, will continue to play a major role in pushing new scientific frontiers. By employing strategies that help strengthen the genetic quality standards of inbred mice, the long-term genetic stability of these lines will be ensured. SNP analysis and other advanced genomic technologies will lead to better genetic characterization and monitoring of inbred lines which can help to identify genetic variation as swiftly as possible.

Conclusions

A clear understanding of the genetic characterization of inbred mice can help scientists in their quest to generate reliable and reproducible experimental data. However, genetic variation is inexorable in all biological organisms, including inbred mice. An awareness of the genetic background of inbred mice, as well as the ability to monitor and maintain the genetic background, can help decrease the risks associated with genetic variation that can be introduced via genetic contamination and genetic drift.

Using the C57BL/6 strain as an example, we demonstrate that there has been little phenotypically significant genetic drift in C57BL/6 strains and substrains. Specifically, the C57BL/6J and C57BL/6N substrains have been extensively examined for SNPs, and the data indicate spontaneous mutations have led to the divergence of the two lines over more than 60 years of separated breeding. Indeed, some of the genetic differences have led to phenotypic changes which could affect experimental results. These findings highlight the need for careful consideration when embarking on the selection of an inbred mouse model for specific types of testing.

In addition to an increased awareness of the genetic background of inbred mice, following best practices in animal husbandry can help reduce the risk of accidental genetic contamination. Further, a genetic quality monitoring program can also help decrease the possibility for undetected genetic variability negatively affecting your results and scientific reputations. By continually strengthening the genetic quality standards for inbred mice, this will have many tangible benefits, including the generation of more reproducible scientific data, which is an important feature of the scientific process, and has been identified by the NIH as an area to be strengthened.

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