HHS Public Access

Author manuscript

Ann N Y Acad Sci. Author manuscript; available in PMC 2016 July 14.

Published in final edited form as:

Ann N Y Acad Sci. 2011 December ; 1245: 31–33. doi:10.1111/j.1749-6632.2011.06325.x.

The blessings and curses of C57BL/6 substrains in mouse genetic studies

Camron D. Bryant

Department of Human Genetics, University of Chicago

Abstract

Phenotypic and genetic differences among C57BL/6 substrains are accumulating. Investigators must address these differences to improve the quality of their studies.

The C57BL/6 (B6) mouse strain is the most widely used strain in biomedical research, with nearly 25,000 articles on Pubmed documenting its use. Nearly half of these articles cite the use of C57BL/6J (B6/J), the original B6 strain from the Jackson Laboratory (JAX) from which all other B6 substrains were derived. In 1951, the first B6 substrain, C57BL/6N (B6/N), was created after breeders were shipped to the National Institutes of Health. Hundreds of generations later, a number of genetic and phenotypic differences have been reported between B6/J and B6/N. This paper discusses how these differences arose, the problems of treating B6 substrains as equals, and our present state of knowledge regarding these differences. I will also outline specific action items for dealing with the unavoidable use of multiple B6 substrains in genetic engineering studies and opportunities that B6 substrains offer for finding novel genes contributing to complex traits.

Theoretically, the essence of an inbred strain is that each individual shares the same homozygous allele for every DNA sequence in the genome and thus, is genetically identical. Furthermore, a common assumption is that this fixation is genetically stable across time. In reality, a very small amount of the genome between any two individuals will always differ, owing in part to unique residual heterozygosity that averted fixation during inbreeding and spontaneous mutations that introduce *de novo* heterozygosity. These genomic impurities can eventually become fixed and lead to the formation of a new substrain. This fixation occurs more rapidly when a small number of founders are used to establish a new B6 colony and could quickly contribute to deviation in one's favorite phenotype and, thus, to the creation of a new substrain.

The B6 inbred strain is a popular choice for researchers conducting behavioral studies because it is physically active, capable of learning a variety of tasks, and breeds frequently. Furthermore, phenotypic differences among B6 substrains (sometimes very large differences) can offer flexibility in studying many behaviors. Behavioral differences between B6/J and B6/N in ethanol consumption and preference were noted in the early 1980s and

Conflicts of interest

The author declares no conflicts of interest.

have since been replicated in at least two laboratories (reviewed in Bryant *et al.*[1]). Other examples of large, replicable phenotypic differences between B6/J and B6/N include fear learning and anxiety that is greater in B6/N than in B6/J, whereas pain sensitivity and rotarod performance are greater in B6/J than in B6/N.[1,2] These differences allow investigators to choose the most appropriate B6 substrain for their experiments. For example, because the B6/J strain readily drinks ethanol, this strain is appropriate for examining manipulations that are hypothesized to decrease ethanol consumption. In addition, because the B6/N strain shows a large degree of fear learning, this strain is the most appropriate choice for studying manipulations expected to decrease fear. The advantage of choosing among B6 substrains as opposed to other inbred strains is that the results might be more applicable for reverse genetic studies (e.g., knockouts and transgenics), which overwhelmingly use B6 mice. However, investigators do not always report the specific substrain employed, making it difficult to know which one is appropriate for a particular phenotype.

The Knockout Mouse Project (KOMP) is an international effort to create mice harboring null mutations for each protein-coding gene in the mouse genome.[3] The B6/N strain was employed as the choice of embryonic stem (ES) cell line for harboring these mutations, likely because of its technical superiority over B6/J.[4] However, the specific B6/N substrain used for KOMP is not entirely clear. Before the advent of KOMP, a majority of genetic engineering studies used ES cells from a substrain of 129 origin to harbor the mutation, mainly because of the high success rate of germline transmission following blastocyst injection. The use of B6/N offers two perceived advantages. First, there is no longer any need to backcross mutant mice to B6 to create a congenic mouse with an isogenic background—this is both expensive and time consuming. Second, the criticism that polymorphisms in the congenic region that flanked the mutation could cause the phenotype[5] is no longer valid. However, unless the exact same B6 substrain is used to introduce the mutation, and to backcross, there still is cause for concern that a mixed background or the congenic region could account for the results.

In examining a recent large dataset providing SNPs among B6 substrains, there are approximately 150 SNPs with homozygous calls that distinguish B6/J from B6/N, depending on the specific substrain comparison. In contrast, the N substrains seem to be much more similar to each other, differing at only 10–20 homozygous SNPs out of several hundred thousand.[6] Recently published next-generation sequencing data of C57BL/6J and C57BL/6NJ (an N substrain that is now bred at JAX) from the Wellcome Trust Center at the Sanger Institute reveal much more potential genetic variation.[7,8] Even when just considering nonsynonymous coding SNPs, there are more than 80 high-confidence SNP calls and over 400 putative ones. In addition, there are thousands of other SNPs that could affect transcript and splice variant levels and structural or copy number variants. A query for this dataset is provided by the Wellcome Trust at http://www.sanger.ac.uk/cgi-bin/modelorgs/mousegenomes/snps.pl. It is clear that the genetic differences between B6/J and B6/N are quite extensive and most likely contribute to phenotypic variation. Thus, if a KOMP-generated mutation (B6/N derived) is placed on a B6/J background, the same problems that were thought to be overcome with B6/N ES cells still exist: the phenotypic effect of the KOMP mutation could depend on the mixed B6/J and B6/N backgrounds or the effect

thought to be caused by the KOMP mutation could actually be caused by an N/J genetic variant that is in linkage disequilibrium with the null mutation on a congenic background.

As the list of variants distinguishing B6 substrains continues to grow, what action should investigators take to address the potential problems that can be anticipated from using a B6 background strain that is different from the KOMP B6/N strain? First and foremost, there is a need to carefully document which substrains are used for ES cell generation and backcrossing and to treat these substrains as different strains, not as equal ones. Second, it would be extremely helpful for those investigators who suspect that their previous findings might be explained by B6 substrain differences to address this possibility and to report any revised conclusions.[9] Furthermore, the choice of B6 background strain for a genetic engineering study should be tailored to the specific phenotype. If a B6/J strain must be used as the background, sequencing the congenic boundary flanking the transgene and comparing these results with the latest sequencing data will define how many polymorphic genes within the congenic region could potentially affect the phenotype.

Although genetic differences among B6 substrains present problems for reverse genetic studies, these same differences offer opportunities for forward genetic studies, which thrive on genetic and phenotypic variation. The identification of genomic regions harboring B6 variants associated with variance in a trait (quantitative trait loci [QTL]) could rapidly lead to the identification of genes harboring the genetic variants. Because the genetic backgrounds between any two B6 substrains are nearly identical, the majority of the genome can be eliminated in considering which genes underlie the QTLs. The usefulness of this approach for B6 substrains has yet to be tested and will depend on both the amount and distribution of genetic variation that underlies a QTL. If the SNPs are highly abundant and widely distributed across most of the genes, then the typical problems of F2 studies will still exist: low resolution and hundreds of genes to parse among. If however, the SNPs are limited to a finite number of genes, then it might be possible to narrow the gene list to a sizeable number of candidates. A recent study using C57BL/6J and the closely related C57L/J and C58/J strains suggest that this approach will be useful.[10]

To summarize, researchers must beware of the differences among B6 substrains if their contribution to forward and reverse genetic approaches to complex traits is to be fully realized. If researchers are prepared to address these differences, they can minimize their potential confounding effects and, at the same time, maximize the chance for novel gene discovery. It will be important to sequence the genomes of other substrains of B6/J and B6/N because behavioral and genetic differences exist even within strains derived from each of these two core substrains.[1] Finally, it is important to consider that environmental differences may also play an important role in phenotypic variation among B6 substrains and, thus, this question can be addressed by cross-fostering studies and other approaches that attempt to control for the substrain environment.

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