

Genealogies of mouse inbred strains

Jon A. Beck^{1,2}, Sarah Lloyd^{1,2}, Majid Hafezparast^{1,2}, Moyha Lennon-Pierce³, Janan T. Eppig³, Michael F.W. Festing⁴ & Elizabeth M.C. Fisher²

The mouse is a prime organism of choice for modelling human disease. Over 450 inbred strains of mice have been described, providing a wealth of different genotypes and phenotypes for genetic and other studies. As new strains are generated and others become extinct, it is useful to review periodically what strains are available and how they are related to each other, particularly in the light of available DNA polymorphism data from microsatellite and other markers. We describe the origins and relationships of inbred mouse strains, 90 years after the generation of the first inbred strain. Given the large collection of inbred strains available, and that published information on these strains is incomplete, we propose that all genealogical and genetic data on inbred strains be submitted to a common electronic database to ensure this valuable information resource is preserved and used efficiently.

Humans have been interested in the inheritance of traits in mice for at least 3,000 years. The oldest Chinese lexicon written in 1100 BC has a word for spotted mice^{1,2}. Interest gradually spread to the West, via the Imperial courts of Japan, and reports on the inheritance of mouse coat colour were published in Europe in the eighteenth century². The mouse was used as a laboratory animal as early as 1664, when Robert Hooke used one in his studies of the properties of air³. Modern mouse genetics started around 1902 when William Castle began studying inheritance in mice³. Another early contributor was Abbie Lathrop, who generated a number of mouse colonies, including strains that developed tumours. The mice used by Castle and Lathrop are the ancestors of many inbred strains⁴⁻⁶. Castle and his students, especially Clarence C. Little, recognized the value of homozygous mice for studying inheritance and established the first inbred strains by brother×sister matings. The guidelines for generating inbred mouse strains⁷, first published in 1952, state:

“A strain shall be regarded as inbred when it has been mated brother×sister (hereafter called b×s) for twenty or more consecutive generations (F20), and can be traced to a single ancestral breeding pair in the 20th or a subsequent generation. Parent×offspring matings may be substituted for b×s matings provided that, in the case of consecutive parent×offspring matings, the mating in each case is to the younger of the two parents. Exceptionally, other breeding systems may be used, provided that the inbreeding coefficient achieved is at least equal to that at F20 (0.99).”

At 20 generations, on average at least 98.6% of the loci in each mouse are homozygous. Many strains have been bred for more than 150 generations and are essentially homozygous at all loci. Each inbred strain is also isogenic (genetically identical) because all individuals trace back to a common ancestor in the twentieth or a subsequent generation. This feature, shared with F1 hybrids, makes it possible to build up a genetic profile of the strain by typing an individual⁸. The first inbred mouse strain, DBA (which has the coat colour alleles, dilute, *d*, brown, *b*, and

non-agouti, *a*) was started by Little in 1909. Other inbred strains were generated over the next decade, including C57BL, C3H, CBA and BALB/c (ref. 9).

Phenotypic characteristics of inbred strains

Inbred strains have long been used for genetic and immunological studies because of the isogenicity within a strain or F1 hybrid and the genetic heterogeneity between inbred strains. Several Nobel Prizes have been awarded for work which probably could not have been done without inbred strains; examples include Medawar's research of immunological tolerance, Kohler and Milstein's development of monoclonal antibodies, and Doherty and Zinkernagel's studies of major histocompatibility complex (MHC) restriction. The use of inbred strains contributed to the Nobel prize-winning work of George Snell in dissecting the biology of the mouse MHC (ref. 6) and developing the backcrossing methodology, which is now an important tool in genetic mapping studies.

Many inbred strains are bred for specific phenotypes. For example, senescence-accelerated mice (SAM) display characteristics of an increased rate of ageing¹⁰. C57BL/6 has an increased preference for alcohol and narcotics and is used in studies of the genetics of substance preference¹¹. Some inbred strains have features that are advantageous to transgenic and embryonic stem (ES) cell technology: the large pronuclei of FVB mice are useful for gene transfer experiments involving direct DNA injection of the fertilized egg¹², and 129 ES cells are particularly successful in germline transmission. The sensitivity of BALB/c and C3H mice to mutagenesis by ethyl nitrosourea (ENU) has been valuable in mutagenesis programmes (Mouse mutagenesis consortium, <http://www.mgu.har.mrc.ac.uk/mutabase/>; German Human Genome Project, <http://www.gsf.de/isg/groups/enu-mouse.html>). Inbred strains with distinctive behavioural characteristics¹³ are widely used in neuroscience.

Phenotypic differences between inbred strains need to be taken into account when designing experiments. Strains may vary in

¹MRC Prion Unit and ²Department of Neurogenetics, Imperial College School of Medicine (St. Mary's), Norfolk Place, London, UK. ³The Jackson Laboratory, Bar Harbor, Maine, USA. ⁴MRC Toxicology Unit, Hodgkin Building, University of Leicester, Leicester, UK. Correspondence should be addressed to E.M.C.F. (e-mail: e.fisher@ic.ac.uk).

characteristics that may not be relevant to the phenotype being studied, but which may indirectly influence experimental results. For example, C3H mice have a genetic defect that leads to retinal degeneration and are blind from early adulthood, which would affect behavioural analyses requiring sight. It is therefore critical to have comprehensive data on the characteristics of the background strain before assessing the phenotypic consequences of introducing mutations¹⁴.

Practical difficulties may be encountered when trying to breed inbred strains that have very small litter sizes or are incompatible for the generation of fertile progeny. For example, in crosses requiring maximum genetic diversity between parental strains, a *Mus spretus* × laboratory mouse mating may seem ideal because this species is separated from laboratory mice by about one million years of evolution¹⁵. F1 hybrid males, however, are sterile and only hybrid females contribute to the next generation, so one set of genotypes is lost.

The crossing of phenotypically different inbred strains enables the mapping of quantitative or qualitative trait loci¹⁶. Analysis of a complex trait typically involves choosing phenotypically distinct parental strains, but these strains should also be genotypically distinct because genetic mapping depends on the polymorphic differences between parents. But with over 450 different inbred strains, few of which are commonly used, it may not be obvious which potential parental mice should be chosen^{17,18}. A reasonable strategy may be to assess phenotypes and genotypes in mice that are, as far as is known, genetically unrelated to determine which parental traits are sufficiently different to allow meaningful analysis of quantitative trait loci.

The genealogies of inbred mouse strains

The first chart showing the origins and relationships of inbred mouse strains was generated by Joan Staats⁹. We have revised and extended this and more recent charts^{6,19,20} in the poster accompanying this issue (see box). Our chart was compiled by combining available data on the origins of inbred laboratory strains (M.F.W.E., http://www.informatics.jax.org/external/festing/search_form.cgi) with information on the recently collected wild strains¹⁵ and data from other references (see Table 1, <http://genetics.nature.com/mouse/>).

The phenotypic and genotypic similarity between any two related strains depends on the extent to which the parental colony was inbred at the time the strains were separated, and whether other strains contributed to the parentage of either strain. For example, C57BL/6 and C57BL/10 were separated in

1921, at a time when the parental C57BL colony was substantially inbred, and no other strains are known to have contributed to the parentage of either strain.

Knowledge of inbred strain genealogies is critical to the design of experiments that require unrelated parental strains. Many commonly used inbred lines have recently separated and these strains tend to be used in genetic studies, regardless of whether they are the optimal combination. A comprehensive overview of the origins of inbred strains (see box) enables the relationships between strains to be examined and compared with phenotypes which can then be analysed in experimental crosses.

Genetics of inbred strain

An understanding of the genealogy goes hand in hand with the molecular genetic analysis of inbred strains, and is relevant to studying the dispersion of individual alleles. For example, some rare, identical alleles exist in different inbred strains; these may be derived from a single mutation event or they might represent multiple independent events indicating a mutation hot spot. Strain DBA/2 has a rare allele within *Trp53* and a survey of 25 inbred strains showed that the same variant was present in the SM strain. Is this due to common ancestry between DBA/2 and SM, or is it due to the same rare mutation occurring twice? The genealogies suggest the former and show that SM is a strain with a complex ancestry that includes DBA, suggesting that the DBA/2 variant pre-dates the initiation of inbreeding in SM; this can be investigated using molecular genetic techniques and surveying other strains known to be related or unrelated to the SM lineage.

To accompany the genealogical data, there is a need for large-scale molecular genetic surveys and quantifiable scores for genetic variation between inbred lines. Studies of genetic similarity among inbred strains based on biochemical markers^{21–24} and microsatellite variation have been reported (refs 25–34 and <http://www.resgen.com/>). The increase in accurately mapped genetic markers has led to the identification of loci that can be used to distinguish between closely related strains and substrains, such as C57BL/6 and C57BL/10 (ref. 35). With a greater density of typed markers available, it will be possible to define chromosomal regions that differ between strains or show identity by descent, and it may be possible to relate these regions to the inheritance of specific phenotypes.

An electronic resource for genealogical and genetic data on inbred strains

Comprehensive studies of microsatellite variation are available for less than 10% of inbred strains, and little additional information is available in the literature on other DNA polymorphisms such as single-nucleotide polymorphisms (SNPs) or randomly amplified polymorphic DNA markers⁴ (RAPDs). Much data on the genetic variation between strains exists in individual laboratories, but have not been published. Similarly, breeding records containing genealogical data, which are insufficient for publication, are being lost over time. We propose that a comprehensive, curated electronic database—to which researchers can submit small but helpful sets of polymorphic markers, genealogical records and other related data—would ensure ready access to and preservation of information on genealogical and genetic data of inbred mouse strains. The Mouse Genome Data-

A poster illustrating the origins of inbred mouse strains accompanies this report. The genealogies of inbred strains are based on information in Table 1 (see <http://genetics.nature.com/mouse/> and refs 3,15,36–44). The strains are divided into seven categories; these are arbitrary definitions and, although some strains could be in more than one category, we assigned each strain to only one category. The categories are as follows:

- (A) Swiss mice, derived from either albino Swiss mice or wild mice from Switzerland;
- (B) Castle's mice: strain ancestors were originally used in breeding experiments by William Castle (some were also derived from Abbie Lathrop's breeding colonies);
- (C) inbred strains derived from colonies from China and Japan;
- (D) other inbred strains: mice derived from a variety of sometimes unknown sources;
- (E) C57-related mice, derived from an original pair of mice bred by Abbie Lathrop;
- (F) inbred strains derived from species or sub-species of wild mice (Unlike these mice, most inbred strains are a mix of *Mus musculus* sub-species, mainly *M. m. domesticus*⁴⁴);
- (G) mice derived from multiple inbred strains.

This chart is also freely available on the *Nature Genetics* web site (<http://genetics.nature.com/mouse/>) and the Mouse Genome Database (MGD), the Jackson Laboratory (<http://www.informatics.jax.org>). Researchers are invited to submit additional information on genealogical and genetic data of inbred mouse strains to the MGD.

base⁴⁵ (<http://www.informatics.jax.org>) will make the chart of inbred strain genealogies (see box) available on their web site, with hypertext links to data for microsatellite polymorphisms and inbred strain phenotypes, and to the International Mouse Strain Resources database, which lists strains available at repositories (<http://www.jax.org/pub-cgi/imsrlist> or <http://imsr.har.mrc.ac.uk/>). We urge the biomedical research community to contribute updates to these electronic resources to ensure that valuable information on inbred strains is not lost.

A strength of the mouse as a model system lies in the availability of multiple inbred strains. A standardized set of a few mouse strains enables data to be compared between laboratories. But use of these strains alone will not necessarily be optimal for genetic studies. There is a wealth of inbred strains to investigate virtually any phenotype of interest, and taking advantage of this

requires the efficient use of information from these strains and recognizing and maintaining their diversity.

Acknowledgements

We thank all members of the mouse community who supplied information on inbred strains, particularly, J. Staats, P.W. Lane, E.M. Eicher, R. Elliot, J. Forejt, D. Juriloff, E. Leiter, C. Linder, T. Monique, K. Moore, L. Morel, O. Niwa, G. Raisman, D. Tabaczynski and G. Wolff. J.A.B., S.L. and M.H. are supported by the UK Medical Research Council; J.T.E. and M.L.-P. are supported by NIH grant HG00330.

Received 17 December 1998; accepted 1 October 1999.

- Keeler, C.E. *The Laboratory Mouse, its Origin, Heredity, and Culture* (Harvard University Press, Cambridge, 1931).
- Ginsburg, B.E. Muroid roots of behavior genetic research: a retrospective. in *Techniques for the Genetic Analysis of Brain and Behavior* (eds Goldowitz, D., Wahlsten, D. & Wimer, R.E.) 3–14 (Elsevier, Amsterdam, 1992).
- Morse, H.C. *Origins of Inbred Mice* (Academic, New York, 1978).
- Silver, L.M. *Mouse Genetics* (Oxford University Press, Oxford, 1995).
- Staats, J. Nomenclature. in *Biology of the Laboratory Mouse* (ed. Green, E.L.) 45–50 (McGraw-Hill, New York, 1966).
- Klein, J. Biology of the mouse histocompatibility-2 complex. in *Principles of Immunogenetics Applied to a Single System* (Springer-Verlag, Berlin, 1975).
- Davison, M.T. Rules for nomenclature of inbred strains. in *Genetic Variants and Strains of the Laboratory Mouse* (eds Lyon, M.F., Rastan, S. & Brown, S.D.M.) 1532–1536 (Oxford University Press, Oxford, 1996).
- Festing, M.F.W. Inbred strains of mice: a vital resource for biomedical research. *Mouse Genome* **95**, 845–855 (1997).
- Staats, J. The laboratory mouse. in *Biology of the Laboratory Mouse* (ed. Green, E.L.) 1–9 (McGraw-Hill, New York, 1966).
- Takeda, T., Hosokawa, M. & Higuchi, K. Senescence-accelerated mouse (SAM); a novel murine model of senescence. *Exp. Gerontol.* **32**, 105–109 (1997).
- Peirce, J.L., Derr, R., Shendure, J., Kolata, T. & Silver, L.M. A major influence of sex-specific loci on alcohol preference in C57BL/6 and DBA/2 inbred mice. *Mamm. Genome* **9**, 942–948 (1998).
- Taketo, M. et al. FVB/N: an inbred mouse strain preferable for transgenic analyses. *Proc. Natl Acad. Sci. USA* **88**, 2065–2069 (1991).
- Crawley, J.N. et al. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology* **132**, 107–124 (1997).
- Martin, J.E. & Fisher, E.M.C. Phenotypic analysis—making the most of your mouse. *Trends Genet.* **13**, 254–256 (1997).
- Bonhomme, F. & Guenet, J.L. The laboratory mouse and its wild relatives. in *Genetic Variants and Strains of the Laboratory Mouse* (eds Lyon, M.F., Rastan, S. & Brown, S.D.M.) 1577–1596 (Oxford University Press, Oxford, 1996).
- Darvasi, A. Experimental strategies for the genetic dissection of complex traits in animal models. *Nature Genet.* **18**, 19–24 (1998).
- Todd, J.A. From genome to aetiology in a multifactorial disease, type 1 diabetes. *Bioessays* **21**, 164–174 (1999).
- Talbot, C.J. et al. High-resolution mapping of quantitative trait loci in outbred mice. *Nature Genet.* **21**, 305–308 (1999).
- Potter, M. & Klein, J. in *Inbred and Genetically Defined Strains of Laboratory Animals. Vol. 1, Mouse and Rat* (eds Altman, P.L. & Katz, D.D.) 16–17 (Federation of American Societies for Experimental Biology, Bethesda, 1979).
- Festing, M.F.W. *Inbred Strains in Biomedical Research* (Macmillan, London, 1979).
- Festing, M.F.W. & Roderick, T.H. Correlation between genetic distances based on single loci and on skeletal morphology in inbred mice. *Genet. Res.* **53**, 45–55 (1989).
- Hilgers, J. et al. Esterase alleles of inbred mouse strains maintained in the Netherlands. *Genet. Res.* **51**, 29–40 (1988).
- Taylor, B.A. Genetic relationship between inbred strains of mice. *J. Hered.* **63**, 83–86 (1972).
- Atchley, W.R. & Fitch, W. Gene trees and origins of inbred strains of mice. *Science* **254**, 554–558 (1991).
- Fowles, G.A., Adelman, S., Knight, A.M. & Simpson, E. PCR-analyzed microsatellites of the mouse genome—additional polymorphisms among ten inbred mouse strains. *Mamm. Genome* **3**, 192–196 (1992).
- Routman, E.J. & Cheverud, J.M. Polymorphism for PCR-analyzed microsatellites between the inbred mouse strains LG and SM. *Mamm. Genome* **6**, 401–404 (1995).
- Matouk, C., Gosselin, D., Malo, D., Skamene, E. & Radzich, D. PCR-analyzed microsatellites for the inbred mouse strain 129/Sv, the strain most commonly used in gene knockout technology. *Mamm. Genome* **7**, 603–605 (1996).
- Slingsby, J.H., Hogarth, M.B., Simpson, E., Walport, M.J. & Morley, B.J. New microsatellite polymorphisms identified between C57BL/6, C57BL/10, and C57BL/KsJ inbred mouse strains. *Immunogenetics* **43**, 72–75 (1996).
- Neuhaus, I.M., Sommardahl, C.S., Johnson, D.K. & Beier, D.R. Microsatellite DNA variants between the FVB/N and C3HeB/FeJLe and C57BL/6J mouse strains. *Mamm. Genome* **8**, 506–509 (1997).
- Panoutsakopoulou, V. et al. Microsatellite typing of CXB recombinant inbred and parental mouse strains. *Mamm. Genome* **8**, 357–361 (1997).
- Matin, A. et al. Simple sequence length polymorphisms (SSLPs) that distinguish MOLF/Ei and 129/Sv inbred strains of laboratory mice. *Mamm. Genome* **9**, 668–670 (1998).
- Maronpot, R.R., Witschi, H.P., Smith, L.H. & McCoy, J.L. Recent experience with the strain A mouse pulmonary adenoma bioassay. *Environ. Sci. Res.* **27**, 341–349 (1983).
- Festing, M.F.W. A case for using inbred strains of laboratory animals in evaluating the safety of drugs. *Food Cosmet. Toxicol.* **13**, 369–375 (1975).
- Le Voyer, T.E. & Hunter, K.W. Microsatellite DNA variants among the FVB/NJ, C58/J and I/LNj mouse strains. *Mamm. Genome* **10**, 542–543 (1999).
- McClive, P.J., Huang, D. & Morahan, G. C57BL/6 and C57BL/10 inbred mouse strains differ at multiple loci on chromosome 4. *Immunogenetics* **39**, 286–288 (1994).
- Atchley, W.R. & Fitch, W. Genetic affinities of inbred mouse strains of uncertain origin. *Mol. Biol. Evol.* **10**, 1150–1169 (1993).
- Simpson, E.M. et al. Genetic variation among 129 substrains and its importance for targeted mutagenesis in mice. *Nature Genet.* **16**, 19–27 (1997).
- Carlson, G.A. et al. Genetics and polymorphism of the mouse prion gene complex: control of scrapie incubation time. *Mol. Cell. Biol.* **8**, 5528–5540 (1988).
- Fitch, W.M. & Atchley, W.R. Evolution in inbred strains of mice appears to be rapid. *Science* **228**, 1169–1175 (1985).
- Atchley, W.R. & Fitch, W. Gene trees and origins of inbred strains of mice. *Science* **254**, 554–558 (1991).
- Cui, S., Chesson, C. & Hope, R. Genetic variation within and between strains of outbred Swiss mice. *Lab. Anim.* **27**, 116–123 (1993).
- Festing, M.F.W. Origins and characteristics of inbred strains of mice. in *Genetic Variants and Strains of the Laboratory Mouse* (eds Lyon, M.F., Rastan, S. & Brown, S.D.M.) 1537–1576 (Oxford University Press, Oxford, 1996).
- Russell, E.S. A history of mouse genetics. *Annu. Rev. Genet.* **19**, 1–28 (1985).
- Bonhomme, F., Guenet, J.L., Dod, B., Moriwaki, K. & Bulfield, G. The polyphyletic origin of laboratory inbred mice and their rate of evolution. *J. Linn Soc.* **30**, 51–58 (1987).
- Blake, J.A., Richardson, J.E., Davison, M.T. & Eppig, J.T. The Mouse Genome Database (MGD): genetic and genomic information about the laboratory mouse. *Nucleic Acids Res.* **27**, 95–98 (1999).