PART 1

History, Development and Genetics of the Mouse as a Laboratory Model

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Introduction

Based on paleontological data it seems that men and mice have been in contact since the early Pleistocene (Berry, 1970), which means for over a million years (Myrs), and numerous historical records (Keeler, 1931; Staats, 1966; Morse, 1978; Berry, 1987; Moriwaki et al., 1994) indicate that mice were already bred as pets 3 millennia ago: it was then logical that these small mammals, as well as the rat and some small sized pet-birds, be used by scientists of the early days for performing their experiments. However, if this choice was more opportunistic rather than based on scientific considerations, it nevertheless appears to be an excellent one in the context of modern biomedical research where the house mouse has become a model of predilection.

Mice are easy to keep. Because they are rodents, they eat a rather large quantity of food but do not have very specific or expensive nutritional requirements. They breed all year round, with a short generation time; they deliver relatively large progenies and tolerate inbreeding rather well compared to other mammalian species. With the passing years, hundreds of mutations, most of them with deleterious alleles, have been collected that all have contributed and still contribute to the identification of genes by their function(s), and several programs of intensive mutagenesis have been developed worldwide to increase further this invaluable resource. Another very important advantage to be credited to the mouse is that it seems to be one of the rare, maybe the only species, where it is possible to grow totipotent embryonic stem (ES) cells in vitro, which can be genetically engineered in a number of ways and still retain the capacity to participate in
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the formation of the germ line once re-injected into a developing embryo. Finally, and this is not the slightest of the advantages, the complete sequence of the mouse genome is now available (Waterston et al., 2002), which will allow comparisons with other mammalian genomes and annotations concerning the function of the genes to be made. In short, the mouse is the only mammalian species whose genomic sequence is known and for which technical procedures exist for the generation of a virtually unlimited number of genetic alterations.

In this chapter we will describe the origins of laboratory mice, starting with their phylogenetic relationships with the other mammalian species. We will also discuss the advantage of strains established from recently trapped wild specimens as a source of polymorphisms for scientific research.

The phylogenetic relationships of the house mouse

The position of rodents among mammalian species

Mice are rodents. They belong to the most abundant (around 40%) and diversified order of living placental mammals, with slightly over 2000 species grouped in 28 families (Huchon et al., 2002). Because of their great diversity, the phylogenetic relationships between the different species of this order has been a matter of controversy for many years, especially when morphological markers were the only criteria available for the establishment of phylogeny. Nowadays, with the use of various molecular (mostly DNA) markers and possible references to the complete genomic sequence of numerous orthologous genes, the situation is much clarified and Figure 1.1 represents the most likely phylogenetic tree for a sample of 40 different eutherian mammals. Based on comparisons at the level of nuclear DNA sequences, the divergence between man and murid rodents (Mus or Rattus genus) has been set somewhere between 65 and 75 Myrs ago (Waterston et al., 2002).

Mice among rodents

The rodent family of Muridae encompasses at least 1326 species grouped in 281 genera (Musser and Carleton, 1993). The establishment of the evolutionary systematics in this group has also been disputed but, this time, it was because many mammals in this family are very similar in size and shape. Here again studies making use of DNA sequences of various types (Michaux et al., 2001; Lundrigan et al., 2002) have greatly contributed to clarify the situation and Figure 1.2 represents the evolutionary relationships among a sample of 21 rodent species anchored into the broader phylogeny of eutherian mammals. The divergence between the Mus and Rattus genus has been estimated at around 10–15 Myrs ago (Jaeger et al., 1986; Murphy et al., 2001), while the divergence of these two genera with Peromyscus maniculatus, the deer mouse (subfamily Sigmodontinae), occurred at around 25 Myrs ago. This is to be remembered because deer mice, which are abundantly used as laboratory models, are often considered close relatives of the laboratory mice while, in fact, they are no more related to them than hamsters.

Systematics in the genus Mus

Figure 1.3 (Guénet and Bonhomme, 2003 and references therein) summarizes the phylogenetic relationships within the genus Mus (subfamily Murinae). The individualization of the subgenus Mus sensu stricto occurred around 5 Myrs ago with the split of three other different subgenera, the African Nannomys and the Asian Coelomys and Pyromys.

The subgenus Mus comprises several species that are extremely similar in size and shape but never hybridize in the wild. Among the Asian species are Mus cervicolor, Mus cookii, and Mus caroli as well as the group of Indian pigmy mice related to Mus dunnii. Mus famulus from India should also be cited as well as the recently discovered species Mus fragilicauda (Auffray et al., 2003) from Thailand.

Mus spicilegus and Mus macedonicus are short tailed mice that are found in central Europe and the eastern Mediterranean, respectively, while mice belonging to the species Mus spretus are common in the western Mediterranean regions (south east France, Spain, Portugal and North Africa).

Mice of the Mus musculus complex are closely related. They have their evolutionary origins in the Indian subcontinent (Bonhomme et al., 1994) but are now spread over the five continents. The best known representatives of the complex are the three Mus musculus subspecies: Mus m. domesticus, common in western Europe, Africa, the near-East, and transported by man to the Americas and Australia; Mus m. musculus, whose habitat spans from eastern Europe to Japan, across
Russia, and northern China, and Mus m. castaneus, which is found from Sri Lanka to south east Asia including the Indo-Malayan archipelago. Various molecular criteria discriminate easily between these different species (Figure 1.4; Boursot et al., 1993; Moriwaki et al., 1994)

Mouse interspecific hybridization

Hybrids between mice of the genus Mus and mice of the subgenera Nannomys, Coelomys or Pyromys have never been reported and probably never occur. Hybrids between wild mice of the species Mus cervicolor, Mus caroli, Mus dunni and mice of the Mus musculus complex have never been found in the wild but hybrids between the former three wild species and laboratory mice have been produced by artificial insemination (West et al., 1977). In these experiments, hybrids generated by insemination of female laboratory mice with Mus cervicolor sperms failed to complete more than a few cleavage divisions. Hybrids generated from Mus dunni sperms and laboratory female oocytes implanted but died in utero at a very
Figure 1.2  Phylogenetic relationships between 32 species of rodents representing 14 subfamilies of Muridae (redrawn from Michaux et al. (2001). Mol. Biol. Evol. 18, 2017–2031).

Figure 1.3  Evolutionary tree of the genus Mus (the time scale is in Myrs). The exact branching for Mus dunni is not precisely known. The four species at the origin of the classical laboratory strains are highlighted in bold (from Guénet, J.L. and Bonhomme, F. (2003). Trends Genet. 19, 24–31).
early developmental stage. Hybrids generated from the same laboratory females and sperm from *Mus caroli* completed fetal development and a very low percentage of them even survived to maturity but none reproduced.

Although they share the same range (they are sympatric) with some *Mus musculus* subspecies, the short tailed species *Mus spretus*, *Mus spicilegus* and *Mus macedonicus* rarely produce hybrids in nature. However, evidence from studies on mtDNA (Orth et al., 2002) and LINE transposable elements (Greene-Till et al., 2000) indicate that exchanges can occur sporadically that would allow alleles with a selective advantage to circulate outside the species in which they originated. The three species mentioned above produce viable offspring with laboratory mice but male offspring of these crosses are sterile in compliance with Haldane’s rule. Male hybrids born from a *Mus musculus* × *Mus spretus* cross, for example, are invariably sterile regardless of the direction of the cross. This sterility is controlled by a relatively small number of genes since fertile males are frequently observed in the backcross progeny of F1 females with a male of either of the parental species (Guénet et al., 1990; Forejt, 1996; Pilder et al., 1997; Elliott et al., 2001).

Mice of the *Mus musculus* complex are not genetically isolated and, in those locations where they meet, there is evidence of gene exchanges ranging from limited introgression to complete blending (Boursot et al., 1993). The best-documented cases of such gene exchanges are those occurring between *M. m. musculus* and *M. m. domesticus* in Europe, along a narrow hybrid zone, and between *M. m. musculus* and *Mus m. castaneus* in Japan. In this archipelago, the two subspecies have hybridized extensively, giving rise to a unique population often referred to as *Mus m. molossinus* (Yonekawa et al., 1988). These gene exchanges, which indicate that the speciation process is in progress but not yet completed, explain the use of Latin trinomens for the designation of the different subspecies in the *M. musculus* complex.

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**Figure 1.4** Geographical distribution of the different species of the genus *Mus* and routes of colonization. Mice of the American and Australian continents were imported by man during colonization (from Guénet, J.L. and Bonhomme, F. (2003). *Trends Genet.* 19, 24–31).
The house mouse as a laboratory model: a historical perspective

Mice, rats and other small vertebrates have been used in biomedical research since the middle of the sixteenth century when biology progressively shifted from a descriptive to an experimental science. Morse (1981), reported that William Harvey used mice for his fundamental studies on reproduction and blood circulation and, according to Berry (1987), the earliest record of the use of mice in scientific research seem to have been in England, in 1664, when Robert Hooke used mice to study the biological consequences of an increase in air pressure. Much later, Joseph Priestly (1733–1804) and his intellectual successor, Antoine Lavoisier (1743–94), both used mice repeatedly in their experiments on respiration.

During the nineteenth century several fanciers in Europe and the United States were breeding and exchanging pet mice segregating for a variety of coat color or behavioral mutations. According to Grüneberg (1957), one of these fanciers, M. Coladon, who was established as a pharmacist in Geneva, reported results from his breeding experiments that were in perfect agreement with the Mendelian expectations ... but this was 36 years before the publication of Mendel's own results on peas. As mentioned by Paigen (2003a,b) in his notes about a century of mouse genetics, it seems that Mendel's first experiments on the transmission of characters were made using mice segregating for coat color markers but Mendel was rapidly asked by his ecclesiastical superior to stop breeding in his cellule awfully smelly creatures that, in addition, had sex and copulated. Mendel changed his experimental material for peas and published in 1866 his observations in a botanical journal where they had a much lower impact and remained virtually ignored until the very beginning of the twentieth century. Once rediscovered by De Vries, Correns and von Tschermak, the three of them working independently with plants, it was really tempting to check whether the so-called Mendel's laws were also valid for animals and experiments were published in 1902 by L. Cuénot (1902), indicating that this was indeed the case. Cuénot's observations were shortly confirmed and extended to other species as well as for other genetic traits by G. Bateson, E.R. Saunders, A. Garrod, W.E. Castle and C.C. Little (Paigen, 2003a).

Even if mice have been extensively used during the twentieth century in most areas of biomedical research, animals of this species have played an instrumental role in research in immunology, oncology, and genetics because the breeding systems which are used to produce them allow the establishment of highly standardized strains whose characteristics are precisely known and monitored generation after generation. Most laboratory strains have their origins from a few pet dealers who progressively became suppliers of 'laboratory' mice. For many years, most of the albino strains used in laboratories were collectively designated 'Swiss' mice to recapitulate their Helvetian origin.

Strain DBA/2 (formerly dba, then DBA) is the most ancient of all inbred strains since it was established by C.C. Little in 1909 (Russell, 1978), by intercrossing mice homozygous for the coat color markers non agouti (a), brown (formerly b now Tyrp1), and dilute (formerly d and now Myo5a). About 10 years later, strain C57BL/6 was established by Miss Lathrop (Granby, Massachusetts, USA) intercrossing the 'black' offspring of her female 57, while strains C3H, CBA, and A were created by L.C. Strong, a cancer geneticist established at Cold Spring Harbor Laboratory (Strong, 1978). At this point, it is interesting to note that, among the strains established by L.C. Strong, strains CBA and C3H stemmed from the offspring of an outcross with a few wild specimens trapped in a pigeon coop in Cold Spring Harbor. This explains how the wild allele at the agouti locus (A) was re-introduced in laboratory strains.

With a few exceptions, historical records concerning the genealogy of most laboratory inbred strains are well documented and several interesting reviews on this subject are available (Morse, 1978; Festing, 1979). A chart concerning the genealogy of these strains, including the recently established ones, has been published (Beck et al., 2000) and regularly up-dated information is available at the website <http://www.informatics.jax.org>.

In addition to its contribution to the re-discovery of Mendel's laws, the mouse has been closely associated with many important discoveries in biology during the twentieth century. To cite just a few among the most

\[\text{3Among the genetically standardized strains, inbred strains are the most widely used. They result from the systematic and uninterrupted mating of brothers to their sisters, which leads to complete homozygosity for the same allele at all loci.}\]
important, we could say that our understanding of the genetic determinism underlying the success or failure of tissue transplantations is a consequence of the many experiments performed with inbred mouse strains by P.A. Gorer (Gorer et al., 1948), then by G.D. Snell and co-workers (Snell, 1978) who developed a series of congenic resistant strains, which were all genetically identical to the C57BL/10Sn background strain, with the exception of single short-sized chromosomal regions determining graft rejection. The discovery and genetic interpretation of the phenomenon of X-inactivation in female mammals, by M.F. Lyon (Lyon, 1961), has been facilitated by the existence and use of several X-linked mutations in the mouse and the observation of variation in the coat color. The first chimeric organisms produced by A.K. Tarkowski, in Warsaw (Tarkowski, 1961), and B. Mintz, in Philadelphia (Mintz, 1962), were mice. The observation of a particularly high frequency of testicular terato-carcinomas in strain 129 (Stevens and Little, 1954; Stevens, 1970) and the in vitro culture of cell lines derived from these tumors, which represented for almost a decade a material of choice for investigating the processes at work in tissue differentiation (Jacob, 1983), undoubtedly opened the way to the establishment of the so-called embryonic stem (ES) cells, by G.R. Martin (1981) and, simultaneously, by M.J. Evans and M.H. Kaufman (1981). The discovery of parental imprinting of some chromosomal regions has been a consequence of experiments performed by J. McGrath and D. Solter (1984) and M.A. Surani and co-workers (1984), who demonstrated that a normal mouse embryo can only result from the fusion of a male and a female pronucleus, while B.M. Cattanach and M. Kirk (1985) demonstrated that the parental origin for the two elements of a given chromosome pair was sometimes not genetically equivalent. The first transgenic mammal created by pronuclear injection of a cloned DNA, was a mouse (Gordon et al., 1980; Brinster et al., 1981; Costantini and Lacy, 1981; Gordon and Ruddle, 1981; Harbers et al., 1981; Wagner et al., 1981a,b) and the first in vitro genetically engineered mammalian organism was also a mouse (Kuehn et al., 1987). Only the first cloned mammal was not a mouse although this type of uniparental procreation has been achieved also in the mouse (Wakayama and Yanagimachi, 1999).

Information concerning many aspects of the biology of the mouse considered as a laboratory model, in particular about its genetics, has been published in the 95 issues of the Mouse News Letters. First issued in 1949 and published regularly every semester till 1997, this informal publication, edited by the scientists from the MRC at Harwell and distributed at low cost, has been for several decades the major medium for the dissemination of information among the community of mouse geneticists. In this sense, the Mouse News Letters will for ever remain the best place to get information about the history of mouse genetics, and in particular, about the history and location of most inbred strains, the progressive development and refinement of the linkage map and the discovery of hundreds of spontaneous mutations.

The Jackson Laboratory (the JAX-Lab), which was founded in Bar Harbor (Maine, USA), in 1929, by C.C. Little, has played a pivotal role in the promotion of the mouse as a laboratory model and still is a unique center for mouse genetics. The Jax-Lab, a non-profit organization entirely dedicated to basic research on the genetics of mammals, is nowadays almost exclusively dedicated to the mouse. It is, at the same time, a top ranked research institution, a meeting place where courses and conferences are organized on the various aspects of mouse genetics, and in particular, about mouse genetics and biological samples of all kind are stored, under the form of frozen embryos or sperm cells, for distribution to the community. Several other Institutions, like the

The name Mouse News Letter was changed for Mouse Genome in 1990 when this publication became a peer reviewed journal. From 1998, Mouse Genome and Mammalian Genome have merged in one and a single journal.

Figure 1.5 Mice of the Mus spretus species (left, with an agouti coat color) and C57BL/6 (right, with a non agouti or solid black coat color). In spite of their great similarity in size and body shape these mice are distantly related species but can still produce viable and fertile hybrids (female only). Mice of the Mus spretus species have been extensively used for the development of the mouse genetic map. (See also Color Plate 1.)
The house mouse and its wild relatives

As discussed above, the classical laboratory inbred strains of mouse have many advantages that are related to their great genetic homogeneity. After all, a population of F1 hybrid mice, born from an intercross set between two highly inbred strains, can be considered identical from the genetic point of view to a population of cloned mice. Unfortunately, the coin has another side and these inbred strains, because they are derived from a relatively small pool of ancestors do not exhibit a greater variety of genetic polymorphisms of natural origin. This relative genetic homogeneity is well reflected in the fact that most of the classical strains possess the same maternally inherited molecule of mitochondrial DNA (Yonekawa, 1980; Ferris et al., 1982) and relatively reduced polymorphisms for the Y chromosome (Bishop et al., 1985; Tucker et al., 1992). Aside from this relative genetic homogeneity, a careful analysis of the genetic polymorphism also indicates that laboratory strains have a mosaic genome derived from more than one species (Bonhomme et al., 1987; Wade et al., 2002) and today’s classical laboratory strains should be regarded as interspecific recombinant strains derived (in unequal percentages) from three parental components: Mus m. domesticus, Mus m. musculus and Mus m. castaneus. For this reason, and to point to a relatively unnatural genetic constitution, it would probably be more appropriate to designate them as Mus m. ‘laboratorius’!

Over the last 20 years a variety of strains, derived from small breeding nuclei of wild specimens, trapped in well-defined geographical areas and belonging to well characterized species, have been established in various laboratories. A list of the strains that are completely inbred, i.e. that have been propagated by strictly unrelaxed brother × sister matings for more than 20 generations, is given in Bonhomme and Guénet (1996). Other useful stocks of wild mice are also maintained in various laboratories and a complete description of these stocks has been published by Potter (1986). These ‘new’ inbred strains have been extremely important over these last years because they represent a virtually unlimited reservoir of genetic polymorphisms. Wild mice have been useful in providing geneticists with polymorphisms such as electrophoretic variants, restriction fragment length polymorphisms (RFLPs), or more generally single nucleotide polymorphisms (SNPs) that were much less numerous in standard inbred strains. With the introduction of strains derived from wild progenitors, in particular, from Mus spretus, the genetic map of mouse has dramatically increased its resolution (Guénet, 1986). Comparisons of non-coding orthologous regions at the sequence level indicate that any inbred strain derived from Mus spretus exhibits, on average, one SNP at every 80–100 bp when compared with any of the classical laboratory strains. This means that virtually any DNA sequence of 100–200 bp, can be used as a molecular marker in assays such as denaturing gradient gel electrophoresis (DGGE) or single strand confirmation polymorphism (SSCP). This high density of polymorphisms represents a considerable advantage in experiments when the aim is positional cloning of a gene identified only by phenotype because it allows an accurate delineation of the targeted locus. In fact, one can consider that there is no upper limit to the density in molecular markers when a genetic map is established from an interspecific or intersubspecific cross (Breen et al., 1994). The high density of polymorphisms turns out to be an even greater advantage when quantitative traits are mapped, because every animal with a relevant phenotype can be genotyped for a very large number of markers. In this respect, the mouse is unique since the frequency of SNPs between humans is roughly one order of magnitude lower than that of Mus spretus compared to laboratory strains (Flint and Mott, 2001; Matin and Nadeau, 2001). The high frequency of SNPs in coding regions means that the genome of Mus spretus or Mus m. musculus is full of ready-made ‘quantitative trait loci’ (QTL)-point mutations’ waiting for functional genomic studies!

Wild mice have also been invaluable in providing cytogeneticists with a large collection of Robertsonian translocations (or centric fusions) recovered from the
many populations of *Mus m. domesticus* where they occur in homozygous conditions (Gropp and Winking, 1981). These translocations are characterized by the fusion of two acrocentric chromosomes by their centromeres and they often interfere with the normal process of meiosis resulting in the production of gametes with an aneuploid (unbalanced) complement. Using carefully designed crosses involving these centric fusions, it has been possible to produce and study trisomies and monosomies for all mouse chromosomes (Epstein, 1986).

In addition to their homogeneity in terms of chromosome morphology, laboratory strains have only long telomeres while, for instance, strains of *Mus spretus* origins, have both long and short telomeres (Coviello-McLaughlin and Prowse, 1997; Zhu et al., 1998). This peculiarity might be helpful for investigating the significance of the still mysterious variations in telomere size found in mammalian cells.

When infectious agents of various kinds are injected into mice it is common to observe that some strains are more susceptible than others and that wild derived strains are in general more resistant than classical laboratory strains. A commonly accepted explanation, although not demonstrated, is that some alleles of laboratory strains, which are essential for determining innate or acquired mechanisms of defense, have been by chance replaced by a defective mutant allele without any consequences for the mice because these animals are kept in protected environments. Even if in most cases the level of susceptibility or resistance is controlled by several genes interacting together (QTLs) or having an additive effect, the situation is sometimes under the control of a single gene making its analysis relatively simple. This is the case, for example, when mice of most laboratory strains die after an injection with an appropriate dose of orthomyxoviruses while most wild strains are resistant (Haller et al., 1998). This phenotype is controlled by a single gene (*Mx1* - chromosome 16) with two alleles: *Mx1* (resistant, dominant) and *Mx1* (susceptible, recessive) and the discrepancy between wild mice and laboratory mice in terms of susceptibility indicates that the mutated allele of *Mx1* is over-represented in laboratory strains, probably due to a sampling effect. A similar example exists with experimental flavivirus infections where all laboratory inbred strains, except strain PL/J, are susceptible while most wild derived inbred strains are resistant (Sangster et al., 1998). Here again, it seems that *Flv*, the allele responsible for susceptibility at the *Flv* locus (chromosome 5), has been fortuitously selected in laboratory strains while it is rare or absent in wild mice.

Similar phenotypes of resistance/susceptibility have also been reported for a variety of pathogens (Sebastiani et al., 2000; Lengeling et al., 2001) and, even if in most instances genetic differences have been observed among classical laboratory strains, these differences also exist between laboratory and wild derived strains making the genetic analysis much easier. Wild animals also proved particularly useful for investigating the biology of murine leukemia viruses and both new *Flv* loci and new alleles at the *Flv1* and *Flv2* loci have been discovered in wild mice (Gardner et al., 1991; Qi et al., 1998).

The comments addressed concerning the susceptibility of mice to infectious diseases also apply to carcinogenesis and comparisons between classical laboratory strains allowed the complex influences of genetic background on tumor susceptibility to be unraveled and several genes modifying tumor susceptibility have been identified. However, while the phenotype of F1 hybrids between any two classical laboratory strains is generally intermediate between the two parental strains, it is often identical to the phenotype of the wild parent when crosses are performed with wild inbred strains, indicating dominance of the wild derived allele. (Nagase et al., 2001).

Besides their use in mapping, interspecific crosses also offer an opportunity for analyzing the effects of bringing together the products of genes separated by divergent evolution in the cells of an offspring. This can help identify the genetic functions that are subject to rapid divergence and to pinpoint the functions that eventually promote speciation. Those functions that are mostly unaffected during the evolution of the taxa are most likely to be basic functions that are under more constraint. This last point will be important in the comparison of orthologous regions between human and mouse genomes now that sequencing is completed for both species.

Questions concerning epistatic interactions can also be addressed by investigating offspring of interspecific crosses at the genomic level. So far, we have no clear answers to this question but data exist indicating that some combinations of alleles are strongly counter-selected in the offspring of some interspecific crosses (Montagutelli et al., 1996).

A less dramatic but still very interesting situation is frequently observed when wild mice are used for the mapping of mutations with deleterious phenotypic effects. In this case, the interspecific offspring, homozygous for the mutant allele, often exhibit a wide range of variations in the degree of severity of their phenotypes with severe forms and weaker ones. In these cases,
genes or loci with a modifying effect can be identified, mapped and eventually cloned (Upadhya et al., 1999; Sawamura et al., 2000). Genes of this kind, which are of potentially great value cannot be recognized in an animal with a normal genotype.

Because many different inbred strains belonging to several more or less related taxa of the genus Mus are now easily available, it is possible to address questions aimed at a better understanding of genome structure and functions. For example: are all the genes present in one strain also present in the others, or are there differences and/or variations in the copy number? If the answer is that a particular gene exists in one strain and not in a closely related one, then what use is the gene in question? Examples of that kind have already been reported (Ye et al., 2001) and have allowed fundamental questions to be answered in a very elegant way.

It would also be interesting to study certain categories of orthologous genes in closely related species to see how their pattern of spatio-temporal expression evolves and in what sort of sequence variation this evolution is involved. This can be particularly interesting when adaptive traits are concerned.

Investigations at the genomic level using inbred strains derived from wild mice are bound to become very popular in the near future because they can be achieved with a high level of refinement and can be correlated in a very reliable way to the phenotype of the living animal. At this point it is no exaggeration to say that this new type of mouse strain is bound to be of expanding interest when adaptive traits are concerned. It would also be interesting to study certain categories of orthologous genes in closely related species to see how their pattern of spatio-temporal expression evolves and in what sort of sequence variation this evolution is involved. This can be particularly interesting when adaptive traits are concerned.

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