TITLE: Putting a strain on diversity

SUBTITLE: The relevance of mouse model genetic background for ageing research

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Human life expectancy is increasing on a global scale, but healthspan – the period of life free from age-associated ill health – is not improving at a comparable rate. This disconnect means that a greater proportion of the general population will spend a longer period of their life suffering from one or more debilitating age-associated disease, such as cardiovascular disease, Alzheimer's disease, osteoporosis, sarcopenia and various cancers. Understanding the processes underlying ageing and age-related diseases is therefore a major and pressing research challenge in biomedical research.

Much of what we know about the ageing process has come from research on highly tractable model organisms, specifically yeast *Saccromyces cerevisiae*, the roundworm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* and the house mouse *Mus musculus*. These models have enabled researchers to identify a number of molecular processes that underlie ageing and that appear to be shared across evolutionary distances. Moreover, there is a growing body of evidence that the ageing process can be experimentally modulated through dietary, genetic and pharmacological means, which may also elicit beneficial effects in humans.

In mice, most of what we know about the biology of ageing has been generated from one inbred strain, the C57BL/6, and studies have disproportionately focused on a single sex

(males) and sub-strain (C57BL/6J). This reliance on a single inbred strain, whilst patently successful, does raise the potentially heretical viewpoint that observations may be specific to this strain alone and not common to other mouse strains, let alone humans. This undercuts the principle behind using a representative model, suggesting that other approaches are needed to more broadly understand ageing mechanisms and, ultimately, to translate findings to humans.

The aim of this Commentary is to discuss factors contributing to differences in lifespan and healthspan in mice of different genetic backgrounds, and to highlight benefits that might be gained from diversifying mouse strains in ageing research: there are numerous commercially strains available, which differ in their lifespan, metabolic phenotype, fecundity, disease susceptibility and cause of death. To motivate this transition, we highlight several factors that subject laboratory mice to evolutionary forces that are weak or altogether absent in humans, thereby enhancing the mouse-human "translation gap" (Table 1). We propose that the choice of strain in any one study should not depend upon convenience or tradition, but rather depend on the research question and an understanding of mouse genetic diversity. We believe there is tremendous untapped potential for using mice as a tool in ageing research, but utilizing this potential requires nuanced research paradigms to better exploit the full breadth of mouse genetic diversity.

Inbred C57BL/6 mice as the default strain in biomedical research

The C57BL/6 mouse is the most common mouse strain, used in around 90% of biomedical research. It was initially bred in what was to become the Jackson Laboratory in Bar Harbor, USA, in the 1920s by Clarence Cook Little and colleagues from a founding population of pet or 'fancy' mice. The idea that highly inbred animals can provide models for understanding the physiology of outbreeding species such as humans has since been widely adopted. This is partly because inbred strains are genetically uniform, which enables repeatability of laboratory experiments. Notably, however, the protocol for generating inbred mice requires a population structure without any analogue among free-living mammals, which is expected to have pervasive genetic and phenotypic effects (Table 1). One outcome of inbreeding over 100s of generations, for example, is that it will potentially purge deleterious recessive alleles or randomly fixate alleles by genetic drift. Moreover, despite the assumption that long-term inbreeding of the C57BL/6 strain has resulted in mice that are comparable across time and between labs, this is not completely correct as the genome of any one inbred strain continues

to accumulate spontaneous mutations. Moreover, since lab populations are maintained at small size, dynamics of these mutations will increasingly be determined by random genetic drift rather than selection, leading to accumulation of deleterious mutations that would otherwise be eliminated in larger populations (Table 1).

This genetic drift is further augmented by founder effects whenever new colonies are established. This has been common with the C57BL/6J genotype as sub-strains have been generated from the original founder line, including a well described line that was originally moved to the NIH in the 1950s: C57BL/6N. The 6J and 6N sub-strains have now been separated for hundreds of generations and distinct genetic and phenotypic differences exist, such as metabolic rate, activity, food intake, glucose tolerance and behaviour. In addition, a number of single nucleotide polymorphisms (SNPs) have also been found to differ between these sub-strains, and epigenetic effects may underlie some of the phenotypic differences observed. Of note, many of these phenotypic differences may directly, or indirectly, impact lifespan and healthspan.

The response of C57BL/6 mice to dietary restriction (DR) or a high-fat diet on the ageing process is also variable, likely owing to unrecognized genetic differences among sub-strains. A previous meta-analysis across 22 studies using C57BL/6 mice found that the effect of DR on median lifespan ranged from a 26.8% increase to a 32.8% decrease (average increase 6.7%) relative to *ad libitum* (AL) controls (Swindell, 2012). Of course, the impact on lifespan is affected by age at which DR is initiated, level of DR, duration and other factors, such as husbandry and sex. To some degree, however, the responses may also be due to sub-strain differences that developed over time as distinct populations have been maintained in the laboratory. However, comparative studies using different C57BL/6 sub-strains under the same experimental conditions (e.g. DR) have not been performed to date. Even meta-analysis approaches are problematic, because the particular sub-strain used is often not made explicit within materials and methods of publications.

Additional inbred mouse strains

Although the C57BL/6 mouse has maintained its stronghold on the biomedical research community, other inbred lab-adapted strains have also been investigated to a lesser degree: BALB/cJ, DBA/2J, 129X1/SvJ and others. The diversity of phenotypes among these strains

is striking, with each having particular “selling points” as models for certain human disease processes.

It is important to recognize that lifespan and other age-related traits may be especially variable among inbred mouse strains. When inbred lines are derived from a genetically heterogenous source, phenotypic variance for a neutral trait redistributes among lineages due to genetic drift, with the rate of increase inversely proportional to the effective population size of the lineage. Thereafter, spontaneous mutations will accumulate, further increasing the genetic distance among lineages. Curtailing the lifespan of laboratory-maintained mice will also mean that lifespan and other age-related phenotypes may be effectively neutral with respect to fitness, further facilitating mutation accumulation and enhancing the role of genetic drift relative to selection. Each strain is thus expected to exhibit idiosyncratic characteristics, with varying levels of inbreeding depression, mutation accumulation and laboratory adaptation. These processes enhance variation and may contribute to divergent responses between strains for the same putative anti-ageing intervention. On the other hand, the collective diversity among strains provides a resource that can be used to identify genetic, hormonal and phenotypic correlates of intra-species longevity.

In a comprehensive study, Paigen and colleagues from The Jackson Laboratory assayed lifespan in 1913 male and female mice from 31 genetically-diverse inbred strains under AL feeding (Yuan, Tsaih et al., 2009). They showed significant variation: the shortest living AKR/J strain had a median lifespan of 251 days for females and 228 days for males, while the longest-lived female strain was the wild-derived WSB/EiJ strain (median lifespan 964 days) and the longest lived male strain was the C57CBL/6J (median lifespan 901 days). These more than 3-fold differences in lifespan far exceed the typical changes observed from postulated anti-ageing interventions in mice (more typically <30%). Much of the information from this study, along with information on phenotypic parameters, is freely available to researchers within the Mouse Phenome Database (https://phenome.jax.org/).

The question of whether such differences correspond to differential responses to DR has not been fully addressed, although some investigators have taken on the challenging task of comparing DR responses in multiple mouse strains. This has increased awareness of the fact that favourable effects of DR on lifespan are not universal as frequently stated in the literature. Turturro and colleagues determined lifespan under AL and 40% DR in both sexes

from two inbred and two hybrid mouse strains (Turturro, Witt et al., 1999) and showed that DR extends lifespan in every cohort, although it was less pronounced in some strains, most notably inbred male DBA/2 mice. However, several other studies reported that DR had no effect or actually shortened DBA/2 mouse lifespan, with well-characterised differences for a range of metabolic parameters between DBA/2 and C57BL/6 mice under AL and DR (see (Mulvey, Sinclair et al., 2014)).

The apparent lack of response to DR in DBA/2 mice led Forster, Sohal and colleagues to advocate DBA/2 mice as negative controls (Forster, Morris et al., 2003). Another recent study re-visited the impact of DR on lifespan and healthspan in male and female C57BL/6J and DBA/2 mice (Mitchell, Madrigal-Matute et al., 2016). In agreement with Turturro et al, DBA/2 mice showed lifespan extension under DR but this was again less pronounced than in C57BL/6J mice. Surprisingly 40% DR did not extend lifespan significantly in any group beyond that seen at 20% DR, and 40% actually reduced lifespan in C57BL/6J females back to that of AL controls. The complexity of strain- (and sex-) specific responses to DR was further emphasized by increased mortality in male DBA/2 male following initiation of 40% DR, in agreement with the earlier study by Forster *et al*.

Recombinant inbred mice

In an attempt to broaden the scope of genetic variation, several groups have investigated ageing interventions in recombinant inbred mice, most notably the ILSXISS strains. These were derived from a genetically heterogeneous ancestral population by an 8-way cross among inbred mouse strains (BALB/c, C3H/2, C57BL, DBA/2, Is/Bi, and RIII), followed by differential selection for ethanol sensitivity leading to Inbred Long-Sleep (ILS) and Inbred Short-Sleep (ISS) lineages, reciprocal crossing of the ILS and ISS lineages, and 30 or so successive generations of inbreeding to obtain 70 genetically homogenous ILSXISS lines. These strains provide an excellent tool for genetic analysis. From the standpoint of studying the physiology of ageing, however, each individual strain can be said to suffer from drawbacks similar to C57BL/6 and other inbred genotypes, with idiosyncratic oddities owing to cumulative effects of genetic drift, founder effects, inbreeding depression, purging of deleterious recessive alleles, laboratory adaptation and accumulation of mutations (Table 1). Consequently, it is difficult to state with confidence that these models are more ‘human-analogous’ and more suitable for translation. However, despite this limitation, they have been

valuable for evaluating how interventions impact lifespan and healthspan in different inbred genotypes in addition to the commonly used lines.

The ILSXISS strains have been instrumental in bringing about a "paradigm shift" in DR research by challenging the idea that DR acts universally across all organisms to extend lifespan. This work examined the impact of 40% DR on male and female lifespan in ~40 strains of ILSXISS mice (for discussion see (Mulvey et al., 2014)). The major finding was that more ILSXISS lines showed no effect or a shortening of lifespan following 40% DR compared to those lines exhibiting the predicted lifespan extension. Despite some subsequent criticisms, most notably regarding the DR protocols employed, study-specific differences in the age at which DR was initiated, the specific DR regime used (daily feeding vs every second/third day feeding), the use of a fixed vs flexible DR food intake relative to AL mice over time, and the relatively small sample sizes, the original studies demonstrate that differences exists in the lifespan of ILSXISS mice under both AL and DR conditions. The difference in the husbandry conditions between studies may also help explain the general lack of correlation in lifespan within a particular strain (for females only) between both studies.

At this point there is a need to revisit the DR-responsiveness in ILSXISS strains using larger sample sizes and to examine the effect on lifespan at different levels of DR. For all mouse strains, there will likely be a 'sweet-spot' for the percentage of calorie (or macro- or micronutrient) reduction that maximises lifespan, and 40% DR may be too extreme for many strains, especially if they have recessive genetic disease due to inbreeding. This DR dose-response approach has been used extensively in invertebrate model systems, but to do this for rodents, even at two levels of DR is a major undertaking in terms of time and expense. However, we argue that this is necessary, and could potentially identify ILSXISS lines that show reproducable effects at particular levels of DR. Clearly, strain and sex-specific responses to DR-induced longevity exist, and the recent comparison between C57BL/6J and DBA/2 mice suggests that 20% DR rather than 40% may maximise lifespan in certain strains (Mitchell et al., 2016). The inherent genetic variability of ILSXISS strains makes them useful and unbiased tools to better understand mechanisms underlying particular interventions.

Hybrid mice generated by crossing inbred lines

In 2003, the US National Institute on Aging initiated the Interventions Testing Program (ITP) to evaluate a number of compounds suggested by the scientific community as candidates to delay ageing in mice. From its conception, this initiative has used both male and female mice derived from a genetically heterogeneous four-way cross, generating genetically distinct full sib progeny (HET3). Lifespan and aspects of healthspan are measured across three independent test sites (the Jackson Laboratory, Univ. Texas, Univ. Michigan) to ensure repeatability of outcomes. In contrast to the inbred strains discussed above, HET3 mice are outbred and thus do not exhibit inbreeding depression for lifespan or other age-related traits (Table 1). Additionally, a central argument for HET3 mice has been that their use avoids the risks and quirks of studying single inbred lines, while their heterogeneity permits genetic association studies for age-related traits.

While it is likely that HET3 mice offer advantages over inbred strains, the historical population structure of HET3 mice remains unusual compared to primate species, with an extreme level of historical inbreeding and laboratory maintenance protocols (Table 1). Additionally, for HET3 and all other hybrid strains, crosses between distantly related lineages can lead to either heterosis or outbreeding depression (Table 1), which would be absent in inbred lines or large unstructured populations, but may influence age-related phenotypes of hybrid strains in ways that are difficult to predict. Prior meta-analysis of DR studies has indeed demonstrated that the response of hybrid strains differs from inbreds, with the average DR-induced lifespan extension approximately 2-3X greater in hybrids (Swindell, 2012).

Similarly, compared to inbred strains, hybrid mice appear to have greater increases in lifespan following rapamycin treatment (Swindell, 2017). One possible explanation is that hybrid strains are physiologically more robust owing to alleviation of inbreeding depression with outcrossing. Consequently, hybrids may be better able to tolerate severe regimes of 30-40% DR and show an increase in overall median lifespan with DR. Alternatively, heterosis induced by outcrossing has been known to increase growth rates when breeding plant and animal strains; in mammals such increased growth may involve early or heightened activation of the GH/IGF-1 axis. Consequently, any intervention that increases lifespan by inhibiting systemic GH/IGF-1 levels, such as DR and rapamycin, may tend to have stronger and more favorable effects in hybrids compared to inbreds. This “hybrid GH/IGF-1 activation” hypothesis requires further empirical evaluation. It is notable, however, that the average serum IGF-1 level of HET3 mice was previously reported to be 718 ng/mL (n = 961 mice;

(Hanlon, Lorenz et al., 2006)), which is far above the range of average serum IGF-1 levels reported in 31 inbred mouse strains (159 – 468 ng/mL; 6 months of age; Table 3 from (Yuan et al., 2009)).

Notwithstanding these caveats, HET3 mice show robust lifespan extension under ~30% DR, and the ITP program has demonstrated that several compounds including rapamycin, aspirin, acarbose and 17α-estradiol can increase lifespan and healthspan in HET3 hybrid genotypes (https://www.nia.nih.gov/research/dab/interventions-testing-program-itp). In addition, the ITP program has further identified a number of compounds with sexual-dimorphic effects; findings that have obvious implications for human translation. We believe HET3 hybrid mice provide a superior model for identifying robust pro-longevity interventions with translation potential to humans. However, we caution that HET3 mice are not a panacea free from potential artifacts of laboratory breeding (Table 1), and further study is needed to determine the degree to which inbreeding and/or outbreeding depression may influence translation of ITP findings to humans and other species.

Outbred wild mice as models to avoid artifacts of laboratory breeding in ageing research

All models described above have been selectively bred over many generations for rapid growth, increased food intake and high fecundity under laboratory settings, resulting in significant genetic and phenotypic differences between inbred mouse strains and their wild ancestors (Table 1). To avoid these complicating factors, an alternative strategy is to capture wild mice directly from nature, and use their outbred offspring or grand-offspring in ageing studies. This approach differs from the use of so-called "wild-derived" inbred mice (e.g., MOLF/Ei and CAST/Ei), which are lineages initiated from wild-captured individuals, but nonetheless highly inbred and likely adapted to laboratory conditions. In contrast, wild mouse populations have recently been introduced to the laboratory, and have not yet undergone inbreeding or laboratory adaptation. Despite the possible advantage of this approach in terms of translation to humans, few groups have investigated efficacy of ageing interventions in such populations, most likely due to the challenges of initiating, breeding and maintaining wild mouse cohorts in captivity. Despite the practical challenges, however, this may be the only approach that allows the mouse model system to be used while avoiding nearly all potential artefacts and pitfalls (Table 1).

The potential importance of this type of approach was demonstrated by seminal research nearly two decades ago, which showed that mice derived from wild-trapped progenitors were long-lived, smaller, lighter, typically had lower plasma leptin and IGF-1 levels and the females were slower to reach sexual maturity compared to HET3 mice (Miller, Harper et al., 2002). Indeed, the longest-lived wild-derived mouse in this study reached an impressive 1450 days of age while on an AL diet. This maximum lifespan, albeit of a single individual, is comparable with the longevity reported for many long-lived genetic mutant and DR studies.

Only one study to date has investigated the impact of DR on lifespan in wild-derived outbred mice (Harper, Leathers et al., 2006). Grand-offspring from wild-caught house mice were maintained from 4 months of age onwards with 40% DR. No difference in mean lifespan was observed between the AL and DR groups, although in the DR cohort there was evidence of reduced survival early in life (<600 days) but increased survival later in life. Of note, DR in these mice reduced tumour incidence relative to AL mice. Again both AL and DR cohorts displayed relative longevity compared to that typically reported for inbred strains such as C57BL/6, with the oldest DR mouse reaching 1601 days of age. The authors provided three potential interpretations to explain the lack of DR effect: (1) that selection under captivity is required for the CR effect; (2) that the DR regime at 40% was too severe for these animals to see any beneficial effect; or (3) that significant variation in the DR response exists within wild populations. We believe that all 3 explanations have merit. The second possibility is supported by the observation that short-lived CR-fed mice in this study exhibited more weight loss compared to long-lived CR-fed mice (Harper et al. 2006), and is further consistent with data showing that ILSXISS strains with greater fat loss are less likely to benefit from a 40% DR diet. It is thus possible that wild mice lack the metabolic reserve and adiposity resulting from decades of laboratory adaptation (explanation 1), and, like all traits, this metabolic response to DR would be expected to vary more in genetically diverse wild mouse populations (explanation 3).

Long-lived genetic mouse models and genetic background

During the past two decades, a large number of long-lived genetically modified mice have been described. Almost exclusively these mice have been maintained on a C57BL/6J or mixed genetic background, and many of these mutations have targeted the growth hormone

(GH)/IGF-1 pathway. Much of this work has been instrumental in demonstrating the relevance of GH/IGF-1 to age-related traits and lifespan. Very few examples exist in which lifespan has been determined in the same mutant maintained on different genetic backgrounds (Mulvey et al., 2014). The role of genetic background in this context is important, however, partly because circulating IGF-1 levels vary greatly among inbred mouse strains, and because reduced IGF-1 in wild mice suggests that laboratory breeding has artificially elevated GH/IGF-1 levels in laboratory-adapted strains. The pessimistic viewpoint may indeed suggest that mutations increasing lifespan by disrupting GH/IGF-1 are only correcting a breeding artefact. A corollary of this idea is that mutations disrupting GH/IGF-1 would have differing effects on lifespan in strains with low as compared to high IGF-1; in principle, such mutations may lack any positive effect on lifespan of wild mice not previously subjected to laboratory breeding. Secondly, mutations that inhibit GH/IGF-1 may not have beneficial effects in primates or humans, for which physiological GH/IGF-1 activation may be non-pathological or potentially even beneficial. At present, we lack definitive data to address these possibilities.

Even for studying mutations not specifically targeting the GH/IGF-1 pathway, the choice of genetic background is an integral aspect of experimental design, which probably has received insufficient attention in ageing research and other fields of biology and medicine. Genetic background greatly influences mouse lifespan and healthspan, so any particular intervention may affect these parameters to a greater or lesser extent depending on how penetrant it is on the chosen background. The considerable time and cost associated with mouse ageing studies limits the ability of researchers to investigate this in great detail, but genetic background should not be ignored when deciding on particular breeding strategies and the most appropriate controls. This is particularly relevant when using conditional mutants and the Cre-LoxP system or when reporter lines are maintained on different genetic backgrounds.

Most likely, large-scale and systematic studies will continue to be the key source of progress along these lines. For example, the International Knockout Mouse Consortium (http://www.mousephenotype.org/about-ikmc) has initiated a world-wide project to target every protein-coding gene in the mouse in embryonic stem cells and generate conditional and null mutants on the C57BL/6N background. One potential upshot of this approach might be that a longevity phenotype previously described for a particular mutant on the C57BL/6J background may not be faithfully recapitulated on the C57BL/6N background. This would

provide some idea of which genetic longevity interventions are more or less robust. Ultimately, however, to understand whether a mutation's effect is robust to any artefact of laboratory breeding, it is necessary to move beyond the C57BL/6 sub-strains and explore other mouse backgrounds.

Discussion

The burden of age-related disease has been rising steadily in recent decades, but there are some opportunities to translate biogerontological research into treating age-related disease in humans. The laboratory mouse has been a cornerstone of this research enterprise, with the implicit assumption that findings in mice will be sufficiently generalizable to inform our understanding of human biology. Here, we have explained that the validity of this assumption cannot be evaluated without reference to population-level factors that shape the genetic constitution of mouse populations maintained in laboratory captivity for decades (Table 1). The example of DR can be illustrative. The research literature is replete with statements indicating that DR has universally favourable effects on lifespan in rodents, with the impressive effect of "40% lifespan increase" often quoted. However, the effects of DR on median lifespan appear inconclusive in genetically diverse rhesus monkeys, with independent studies demonstrating either increased or decreased median lifespan. Our judgement is that these results are in fact in good agreement with an appropriate interpretation of existing mouse data, which has so far failed to demonstrate a significant positive effect on mean lifespan in genetically diverse mice that have not undergone inbreeding and/or adaptation to laboratory conditions (Harper et al. 2006). The example illustrates that mouse experiments can indeed be predictive of primate responses to anti-ageing interventions, but it is necessary to be aware of the role played by laboratory breeding and its effects on mouse genetics.

Although incorporating genetic heterogeneity into mouse studies adds another level of complexity to experimental design, important insights may be missed by focussing the majority of research effort on a narrow spectrum of mouse strains. There has been a recent successful drive to take advantage of the natural variation in lifespan within natural populations, including studies in long-lived animals such as naked mole rats (*Heterocephalus glaber*), ocean quahogs (*Arctica islandic)* and various bat and bird species. We are encouraged by this development, but emphasize that there remains ample opportunity to exploit intra-specific genetic variability using mice as a model system. Such work may

include studies utilizing less commonly studied mouse strains, as well as replication studies to determine if key findings can be observed in wild mice. At present, for example, the ITP policy treats lifespan extension in HET3 hybrid mice as an endpoint, with no second-step screening to determine if "successful" experiments can be replicated in other strains, particularly in wild mice that have not yet undergone adaptation to laboratory conditions. There is currently no evidence, for instance, that rapamycin, asprin, acarbose or 17α-estradiol are able to improve longevity in outbred wild mice. The above-mentioned lessons from DR responses in rhesus monkeys, however, may be fully applicable in this instance. Repeating longevity experiments in genetically diverse wild mice would not only provide additional validation and evidence to document lifespan-extending effects of such compounds, but would provide assurance that such positive effects are not influenced by any of the potential artefacts associated with laboratory breeding.

While we do not propose that wild mice or any mouse strain can provide a gold standard for ageing, there is a need for researchers and funders to be aware of strengths and limitations of different mouse models in relation to the question being asked. We have emphasized that the process of ageing is multifaceted and malleable by micro- and macro-evolutionary forces, which explains the considerable diversity in lifespan and age-related traits within and between species. In view of this diversity, there is a special need for ageing research to extend findings to more strains, and it cannot be expected that C57BL/6J or indeed any one mouse strain will be sufficient to judge the merits of a proposed anti-ageing intervention. Studies directed at elucidating basic mechanisms of ageing will also benefit from expanding the scope of mouse genetic diversity, since the exact roles of GH/IGF-1 signalling, mTOR signalling, sirtuins, inflammation, autophagy, oxidative stress, and other proposed ageing mechanisms are likely to vary among mouse strains, contributing to lifespan-limiting senescent processes in some strains but perhaps being less consequential in others.

We recognize that, as in much of biomedical research, there will be resistance to funding experiments that may generate negative findings, duplicate previous studies with a different mouse strain, lack hypotheses targeted towards molecular-level mechanisms, and/or utilize non-conventional models. This resistance will continue to buoy the use of male C57BL/6J in ageing research, partly because sacred tenets and foundational beliefs have been built upon a bedrock of experiments using this strain. We further expect that the initial steps associated with implementing new models, such as outbred wild mouse populations, will present

practical challenges for many labs. However, we envision tremendous opportunity for biogerontology to become a model discipline, leading the way for other fields of biomedicine, by doing the difficult work and troubleshooting needed to implement new research models on a large scale. If successful, biogerontology could truly become a translational science with bench-to-bedside research leading to the development of novel treatments that prevent rather than merely treat age-related disease in humans.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1. Genetic factors distinguishing laboratory mice from humans. The table lists factors influencing population structure in different types of mice (inbred, hybrid, wild) and modern humans. **Inbred mice** refers to genetically homogeneous strains maintained in the laboratory with successive generations of consanguineous mating (e.g., C57BL/6, ILSXISS). **Hybrid mice** refers to mice generated from the progeny of inbred line crosses (e.g., B6C3F1, HET3). **Wild mice** refers to outbred mice sampled directly from natural settings or the offspring of such mice reared in the laboratory for only a few generations without inbreeding. Y (yes) indicates relevance of a factor for ageing studies, whereas N (no) indicates a factor is not applicable or less important. Footnotes (1) – (9) provide further details.

	Inbred Mice	Hybrid Mice	Wild Mice	Human
Gene Flow[1]	N	Y	Y	Y
Genetic Drift[2]	Y	Y	Y	N
Founder Effects[3]	Y	Y	Y	N
Inbreeding Depression[4]	Y	N	N	N
Purging[5]	Y	Y	N	N
Heterosis (Hybrid vigor)[6]	N	Y	N	N
Outbreeding Depression[7]	N	Y	N	N
Laboratory Adaptation[8]	Y	Y	N	N
Mutation Accumulation[9]	Y	Y	Y	Y

[1]Gene flow is severely restricted to obtain high levels of inbreeding in mice, although in contrast hybrid mice are generated by extreme outcrosses of distantly related populations. Neither extreme is common in wild mice or humans.

[2]Genetic drift refers to random changes in gene frequencies due to small population size. It is most pronounced in small populations typical of laboratory-maintained rodents (inbred or hybrid). In wild mice, genetic drift may be less influential but likely important as a founder effect. Genetic drift is likely to be weak in humans due to large population sizes.

[3]Founder effects are a type of genetic drift occurring during population bottlenecks. It is common in laboratory rodents during the initiation of new colonies. Although the human genome appears to have been shaped by historical founder events, such founder events are less common in modern humans.

[4]Inbreeding depression (ID) for traits related to ageing may occur in some but not all inbred lineages. If present, ID is likely eliminated in hybrids. ID is possible in wild mouse

populations, but less likely due to larger effective population sizes. ID is also possible in humans, but much weaker due to the rarity of consanguineous mating.

[5]Inbreeding leads to unmasking of deleterious recessive alleles and purging via selection. Hybrid mice, although outbred, have a history of inbreeding and thus prior purging of deleterious recessives. In contrast, purging is less likely in wild mice or humans due to inbreeding avoidance and larger population sizes.

[6]Heterosis or hybrid vigor refers to improved fitness or function in hybrid progeny generated from the mating of distantly related inbred lineages. It may involve any trait and has been observed for growth rates, fecundity, age-related traits and lifespan.

[7]Outbreeding depression (OD) refers to loss of fitness or function in hybrid progeny generated from the mating of distantly related inbred lineages. OD is the opposite of heterosis and may occur due to disruption of co-adapted gene complexes that develop over time in isolated populations.

[8]Laboratory populations over time will likely experience some degree of adaptation to the artificial laboratory environment, potentially favoring early reproduction, early or excessive activation of the GH/IGF-1 axis, and decreased lifespan. In wild mice and humans, selection and adaptation also occur but involves different selection gradients compared to the laboratory environment.

[9]Lifespan of laboratory mice may be artificially curtailed shortly after the age of reproduction. This can exacerbate the "selection shadow" that normally prevents elimination of deleterious alleles with specific effects late in the lifespan. The can lead to the accumulation of spontaneous mutations and degrade healthspan and/or lifespan. The process would also be expected to contribute to senescence in wild derive mice and humans, but would be enhanced in laboratory-maintained mouse populations due to lifespan curtailment.