

SPECIAL ARTICLE

Living inside the box: environmental effects on mouse models of human disease

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ABSTRACT

The impact of the laboratory environment on animal models of human disease, particularly the mouse, has recently come under intense scrutiny regarding both the reproducibility of such environments and their ability to accurately recapitulate elements of human environmental conditions. One common objection to the use of mice in highly controlled facilities is that humans live in much more diverse and stressful environments, which affects the expression and characteristics of disease phenotypes. In this Special Article, we review some of the known effects of the laboratory environment on mouse phenotypes and compare them with environmental effects on humans that modify phenotypes or, in some cases, have driven genetic adaptation. We conclude that the ‘boxes’ inhabited by mice and humans have much in common, but that, when attempting to tease out the effects of environment on phenotype, a controlled and, importantly, well-characterized environment is essential.

KEY WORDS: Husbandry, Diet, Environment, Stress, Mouse models, Human

“Little boxes, little boxes, little boxes all the same”
– Pete Seeger’s cover of Malvina Reynolds’ ‘Little Boxes’¹

Introduction

One question we often find ourselves asked is how can mice be realistic models for human diseases when they live in a box? The implication is that mice in laboratories have low psychological, metabolic and immunological stress levels compared with humans. This assumption is based on the concept that mice live in an environmentally controlled cage (a box) with free access to high-quality water (acidified or chlorinated tap water), without pathogens (specific-pathogen-free status), and *ad libitum* access to highly palatable and nutritious feed (also without pathogens as the food is usually pasteurized or sterilized). Laboratory mice live in a room that is environmentally controlled for temperature, humidity, light cycle and contaminants, as air is HEPA (high efficiency particulate air) filtered (Table 1). Aside from encounters with cage-mates and investigators, they are safe from predation. Lastly, mice usually have high-quality medical (veterinary) care. Fundamentally, laboratory mice live in ‘the comparatively plush environment of the shoebox

cage’ (Harper, 2008). Some have ‘doubted that commercial lab mice – selectively bred in sanitized environments – are good research analogues for people, who do not live in such clean conditions’ (Reardon, 2016; Beura et al., 2016). The phenotypes that researchers are interested in measuring and understanding are the consequence of a genotype expressed in a particular environment, and a repeatedly made argument is that mice in such a controlled environment cannot be expected to show the same phenotypes as humans even if the underlying genetics are the same. This assumes that there are huge differences between the manner in which mice are raised and how humans live.

However, when one is at the podium in a lecture hall it becomes obvious that the human population (the audience) is, in fact, living within a box (the lecture hall and, by extension, modern western homes and offices). These housing facilities are temperature controlled, air is ventilated and filtered, and lighting is controlled. Food is readily available (coffee, tea and cookies at the back of the lecture hall) and heavily regulated to ensure high nutritional quality and low to no pathogen exposure. Likewise, water from public sources is also highly controlled, frequently monitored for quality and treated to eliminate pathogens, and is readily available (*ad libitum* food and water). Medical care is, for the most part, readily available and of high quality. While these are generalities, they do represent how the majority of people live in developed countries.

Life in nature’s ‘box’


Mice and humans are both remarkably adaptable animals and can be found in a huge range of environments, showing adaptive responses to overcrowding, food and shelter availability, temperature and predation (Silver, 1995).

“The planet-wide spread of the house mouse in both man-made and natural habitats suggests an extreme reproductive adaptability, probably the most extreme among the mammals. Only humans are as adaptable (some would say less so).” (Bronson, 1984)

Much of the argument against using laboratory mice as models rests on the tacit assumption that there is a ‘normal’ environment for both humans and mice (Beura et al., 2016) – a ‘state of nature’ – and that, for mice, the laboratory environment is far removed from it, but that humans somehow live in a ‘natural environment’. This concept is self-evidently flawed, largely because, during human evolution, we have taken control of our own environment to a great extent, or evolved very specific behaviors or genetic traits to deal with it. For example, the evolution of skin pigmentation in hominins is accepted to have been dependent on the latitude at which populations live (Marciniak and Perry, 2017). Probably driven by the requirement for vitamin D synthesis, northern populations have lost their ancestral dark pigmentation. An exception to this seems to be the Inuit in the American Subarctic and far Northeast Asia, who have darker skin pigmentation than others living at the same latitude,

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¹Original words copyright 1962 Schroder Music Company (<https://people.wku.edu/charles.smith/MALVINA/mr094.htm>).

Table 1. Comparison between ‘lifestyles’ of humans in western affluent cultures and mice

Criterion	‘Modern’ humans	Laboratory mice
Live inside a box	Yes: house 1500+ ft ²	Yes: 300+ cm ²
Tap water (treated)	Yes	Yes
Nutritious food	Yes	Yes
<i>Ad libitum</i> access to food and water	Yes	Yes
Controlled environment (heated/air conditioned)	Yes	Yes
Pathogen exposure	Variable	Minimal
Medical care	Adequate	Exceptional
Vices	Optional	Offered (experimental design)
Light exposure (UV light/vitamin D)	Limited	Controlled
Sensory-rich environment needed	Yes	Yes
Population-density-induced stress	Yes	Yes
Space-induced stress	Maybe	No
Noise-induced stress	Yes	Yes
Social-status-associated stress	Yes	Yes
Intra-family/litter behavioral dependencies	Yes	Yes
Pheromone-dependent behavior/physiology	No	Yes

We take as comparison affluent western cultures, given the challenge that was the initial stimulus to this Special Article. However, it is important to note that many human populations do not have access to clean water, nutritious food and medical care, and are exposed to many transmissible diseases. This extreme range of human environmental conditions is important when considering the assessment of therapeutic efficacy and results of fundamental biomedical research. It raises an interesting question as to whether, historically, we have deliberately or unintentionally replicated aspects of western human society in the development of laboratory mouse husbandry techniques and experimental design.

such as Swedish populations. One hypothesis is that retention of darker pigmentation is a response to the requirement to protect from high UV exposure during the long and intense daylight periods of the arctic summer, but this is compensated for by a diet that is very rich in vitamin D (Schaebel et al., 2015). Concomitant selection for genetic variants associated with this diet and environment (Fumagalli et al., 2015) shows that this population has become extremely adapted both behaviorally and genetically. So the ‘box’, the environment in which the Inuit live, is very different from the ‘box’ that biological scientists in Maine or lawyers in Manhattan inhabit.

A parallel story for the mouse exists. Over the past 15,000 years, the genus’s close association with human beings has significantly affected its evolution. There is now clear evidence that commensal species of mice began living with humans at the time when humans became sedentary, and the anthropogenic environment created provided selective advantages for sympatric and commensal species of mice (Weissbrod et al., 2017). This close association has had demonstrable effects on phenotypes such as coat color, skull morphology and possibly behavioral traits associated with living in close proximity to humans and the other commensal animals under domestication at the time or earlier, such as dogs (Botigué et al., 2017) and cats (Otoni et al., 2017). As we discuss above, the mouse has colonized many habitats, with *Mus musculus domesticus* being the species most closely influenced by the human ‘box’. However, the reader must remember that the current strains of laboratory mice have also been bred from other species that have been associated in different ways and for different time periods with humans. The resulting range of laboratory strains available thus samples and recombines traits that evolved in quite different environments. Laboratory strains are best regarded as recombinants derived from three ancestral components: *M. musculus domesticus*, *Mus musculus musculus* and *Mus musculus castaneus* (Guénet and Bonhomme, 2003). The collaborative cross is now helping create further reassortment of the genetic variation available in inbred strains (Srivastava et al., 2017).

Life in the laboratory box

The idea that laboratory mice have been selected over a century for traits associated with both husbandry, such as fast maturation and

ease of handling, and with those of specific scientific interest, such as predisposition to the diseases for which they are used as models, is perhaps expected. However, strains differ considerably in their ‘wildness’ and aggression (Wahlsten et al., 2003). Some commonly used strains, such as SPRET/EiJ, are notoriously difficult to handle. The AKR strain, which shows a high incidence of leukemia, has a very high degree of intra-strain aggression (Simon, 1979). AKR was derived from a pet shop stock in the 1920s and SPRET/EiJ from wild captured *Mus spretus* stock from Spain in the late 1970s. It is unclear whether aggression was co-selected during the active selection for the desired strain characteristics in the research laboratory environment, or whether it was retained in these and other strains. However, it is quite evident that rearing in a laboratory environment does not automatically result in selection for docile mice.

An example of laboratory selection from wild-type variation is that of the progressive retinal degeneration phenotype associated with the *rd1* allele (*Pde6b^{rd1}*), first discovered in CBA mice and then as independent spontaneous mutations in C57BL/6 and C3H mice (Farber et al., 1994). These mice are used as a model for human retinal degenerative diseases such as autosomal recessive retinitis pigmentosa (OMIM:180072). Retinal degeneration mutations are found in many mouse strains, but it is unclear whether these have been selected by the laboratory environment itself or whether the environment is simply permissive for their spread through laboratory strains. It would seem likely that mice carrying these mutations would not have survived in the wild. However, they have been found in some recent wild-derived inbred strains, implying that the mutations may be present in some wild populations (Chang et al., 2002). Retinal degeneration mutations are similarly relatively common in the human population, with 1 in 2000–3000 individuals having some form of genetic retinal degenerative disease (Veleri et al., 2015).

Environmental carcinogens

Life in the box, whether mouse or human, has not always been as snug as people think. While we take this for granted, current laboratory animal husbandry practices were developed largely by trial and error. For example, mouse boxes evolved out of ‘shooks’, wooden boxes used to transport wild blueberries in Maine (E. Less,

personal communication; see Fig. 1). The lumber was readily available in pre-cut sizes, making it cheap and easy to create the boxes. To make them last, the wood was coated with creosote, but the mice then developed a high incidence of cancer (Boutwell and Bosch, 1958; Holme et al., 1999; Holstein, 1979; Schoket et al., 1988). This was not surprising, as a similar correlation was made nearly 200 years earlier for chimney sweep boys, who developed a high incidence of scrotal squamous cell carcinoma (Torrönen et al., 1989; Waldron, 1983). These wooden boxes were later replaced by stainless steel and, currently, by plastic boxes. A wide variety of bedding types continue to be used to house mice in these boxes. The different bedding types can affect breeding (Iturrian and Fink, 1968; Jackson et al., 2015; Tanaka et al., 2014), overall health (Becker et al., 2010; Ferrecchia et al., 2014; Horn et al., 2012; Potgieter and Wilke, 1996) or metabolism. This can lead to a wide variety of disorders, including cancer (Jacobs and Dieter, 1978; Li et al., 2009; Nielsen et al., 1986; Schoental, 1974; Torrönen et al., 1989; Vesell, 1967; Vlahakis, 1977). Living inside the box can lead to a variety of health problems in humans as well (Bond, 1966; Ritchie, 1946; Williams, 1948; Wilner et al., 1958).

Diet

Diet can have a profound effect on the phenotype of mice. One of the most studied dietary issues is the effect of high-fat diets on the development of atherosclerosis. Mice, normally fed a low-fat vegetarian-based diet, rarely if ever develop spontaneous atheromatous plaques in their arteries. Atherogenesis can be induced by placing the mice on high-fat diets, especially for lines that carry mutations involved in the pathogenesis of this disease (Chistiakov et al., 2017). Diet can modify the onset and severity of disease as well; for example, ectopic soft-tissue mineralization, a feature of pseudoxanthoma elasticum (Uitto et al., 2014) and several related diseases (Li and Uitto, 2013), can be exacerbated when mice are fed an experimental diet high in phosphate and low in magnesium content (Jiang and Uitto, 2012; Li et al., 2015). This so-called 'acceleration diet' shortens the time the investigated mice require to develop features of pseudoxanthoma elasticum. Consequently, adopting dietary modifications for humans with this condition are currently under investigation (<https://clinicaltrials.gov/ct2/show/NCT01525875>; Renie et al., 1984; Sherer et al. 2005).

Obesity and type 2 diabetes are a global problem in human populations. This disease has been recapitulated in mouse models, especially when using standardized high-fat diets (Heydemann, 2016).



Fig. 1. Historical development of the mouse 'shoe box'. Mouse facility at The Jackson Laboratory in the 1950s, showing racks containing the original double-sided wooden mouse boxes. The original wooden boxes were modifications of boxes used by the Maine blueberry industry to transport berries. In the early days, mice were shipped in wood containers with hardware cloth covering openings on the sides for aeration. Source: The Jackson Laboratory Archives.

Similar to humans, there are clear interactions between diet and exercise in mice (Nogueira et al., 2017), with different exercise regimes, such as swimming or treadmill running, ameliorating the physiological and pathological effects of a high-fat diet. Exercise-associated cardiac hypertrophy is also seen in both mice and humans (Evangelista et al., 2003). Interestingly, like humans, different strains of mice show different compliance levels with exercise regimes and, at the risk of sounding anthropomorphic, it is tempting to compare treadmill exercise avoidance behavior in C57BL/6 with our own human experience (Gibb et al., 2016).

One can argue that laboratory mice do not have access to human 'vices' or hedonic behaviors – rewarding stimuli gained through behavior – particularly those associated with dietary intake of types of food, alcohol and narcotics (Jimenez et al., 2017). In fact, mice are widely used for studies on voluntary self-administration of alcohol (Mayfield et al., 2016) and chocolate (Patrono et al., 2015), and have been shown to 'self-medicate' when repeatedly exposed to stressors (Mollenauer et al., 1993). So, while we limit access to such 'vices' by forced confinement, mice can have access and do take advantage of these opportunities, when available, just as humans do. It is of interest that feral mice will actively choose to wheel run in their natural environment when presented with a wheel and no other obvious reward (Meijer and Robbers, 2014), and seem to find the activity rewarding in itself.

Immunity and environmental pathogen exposure

There is evidence that the immune system of wild-living mice differs from that of laboratory strains, being highly activated and reflecting the constantly changing and heavy burden of pathogens to which the former are exposed. Their innate immune systems are however remarkably similar, with the exception that myeloid cells from wild-type mice do not seem to mount as aggressive a cytokine response *in vitro* as those from laboratory mice, suggesting selection for relative damping of the response in the wild (Abolins et al., 2017), and a novel population of myeloid cells seems to be absent from laboratory strains. The activity of the mouse adaptive immune system has been shown to change depending on early exposure to various pathogens (Tao and Reese, 2017). For example, co-housing with 'pet shop' mice, which are derived from free barn-living 'feral' populations, was shown to change the immune profile of laboratory mice, modifying it to one more closely resembling the adult human, with concomitant increased resistance to infection and altered T-cell kinetics (Beura et al., 2016). However, human populations do not all experience the same antigenic and disease environment, and this has wide-reaching consequences for the immune system (Grignolio et al., 2014). Transmissible disease accounts for about half of premature (<45 years) deaths in the developing world. In the developed world, sanitation and healthcare have radically reduced the number of pathogens to which humans are exposed (Guarner et al., 2006). Changes in lifestyle and environmental exposure, healthcare and antibiotic use have had profound effects on the human microbiome (Bloomfield et al., 2016), one negative consequence of which has been the increase in allergic and autoimmune disease, and possibly some types of neoplasia, such as acute lymphoblastic leukemia (ALL) (Bach, 2017; Greaves, 2009, 2018).

The microbiome, the large variety of microorganisms living in and on everyone and everything, can modulate host phenotypes no matter how carefully we attempt to control them. Spontaneous inflammatory bowel disease (IBD) was first identified in mice nearly 25 years ago (Sundberg et al., 1994). It was later shown to be modulated by *Helicobacter hepaticus*, an opportunist bacterial

species originally thought to be normal flora and thus unimportant (Cahill et al., 1997; Foltz et al., 1998; Ward et al., 1994). Modifications in the microbiome are now known to affect a wide variety of phenotypes in mice (Franklin and Ericsson, 2017; Su et al., 2016; Zachariassen et al., 2017) and humans (Bohn et al., 2017; Kuk et al., 2016; Lloyd-Price et al., 2016). So, just as in mice, we know that the antigenic environment in which we live affects our health, but this is different for different populations. Again, we find that humans live in ‘boxes’, but there are many different ones.

Effects of environment on ethologically appropriate behaviors

There has been much discussion about how to make a laboratory mouse ‘happy’ (Grimm, 2018) by enriching its environment. Attempts to assess mouse ‘happiness’ are subjectively anthropomorphic. Nevertheless, a reduction of stress and support for richer behaviors certainly produces a more ethologically appropriate set of behaviors and more normal physiology. There are many instances in which such environmental enrichment changes the outcome of experiments, and the association with a sensory-rich environment affects disease susceptibility and outcomes in human beings (Grimm, 2018). Manipulation of the cage environment can also be used to manipulate behaviors, and a wide variety of stressors can be added to a box cage for a mouse, such as turning a spotlight on and off (Aarts et al., 2015) or noise (Munzel et al., 2017), both of which are known physiological stressors in humans.

In addition to enrichment and cage size itself, pheromonal cues and housing density are all well-characterized factors that affect reproductive phenotypes. These factors are becoming a greater concern with the increasing use of ventilated cages, where pheromones cannot accumulate to any significant degree. It has long been known that putting an unknown (‘strange’) male or female in a box with another male or female will produce endocrine changes in both sexes, affecting behavior and physiology (Macrides et al., 1975; Nichols and Chevins, 1981). While only a few inbred strains are used in most of these studies, those that use more than one strain find that the responses vary between them (Heinla et al., 2018). Pheromones from urine or vaginal sites can modulate ultrasonic courtship in male mice (Nyby et al., 1977), and male urine can affect sexual maturation in females (Coppola and Vandenberg, 1985), presumably via pheromones released by the preputial and clitoral glands. To improve the success of timed matings when female and male mice are housed together for a limited time, breeding pairs can be maintained in the same box separated by a transparent partition for 3 days before setting up the timed mating. This permits male urine-borne pheromones to induce female estrus, thereby enabling the expression of male and female mating behaviors (Stiles et al., 2013).

These and related observations have raised concerns about sanitation practices in mouse husbandry that can alter pheromonal signaling and potentially cause confounding effects in the research. Even the organization of a mouse room, segregating male and female mice, could have a significant effect on experimental results (Bind et al., 2013; Lloyd et al., 2018). Evidence regarding the effects of human pheromones is highly controversial, but a recent paper suggests that this is one area where mice and humans might have rather distinct environmentally mediated communication mechanisms (Hare et al., 2017).

The cage environment also affects other aspects of reproduction. Parental interactions, both with pups and between mothers and fathers, are highly significant for both mice and humans (Glasper et al., 2018; Kentner et al., 2010; Liu et al., 2013), and there is an

increasing awareness of the importance of paternal interactions within the family or litter environment in both species.

The effect of housing and population density have complex but non-identical effects on mice and humans. Caging systems can affect the health and behavior of mice (Polissidis et al., 2017), although adverse physiological effects at stocking densities up to three times the recommended levels have been difficult to identify (Paigen et al., 2012; Morgan et al., 2014). The urban environment is reported to have a significant effect on human health and wellbeing. For example, noise and overcrowding (Park and Evans, 2016), and social interactions and status, all impact human disease susceptibility and wellbeing (Fassio et al., 2012; Lederbogen et al., 2011). On a species basis, however, it is difficult to assess and compare the relative severity of the physical environments to which humans and mice are exposed. Does 10 people living in a single room equate to 16 mice per shoebox cage with 31 cm² each?

Although it is difficult to disentangle the different elements of human urban life, the issues of housing space and population density are interesting. Group size and composition clearly affect the behavior of mice (Kappel et al., 2017; Lee et al., 2018), and crowded male mice of several strains show high levels of aggression. However, contrary to much received wisdom, the most important positive factor seems to be a rich physical and social environment, and not simply the space available, at least within the constraints of a laboratory setup (Bailoo et al., 2018). This is mirrored by a recent human study where, surprisingly, the size of houses *per se* has rather little effect on several indicators of overall subjective wellbeing (Foye, 2017); this is a consideration that is becoming more important in the UK particularly, which now has the smallest new housing stock in Western Europe (Morgan and Cruikshank, 2014). The question we need to ask is: how do we take into account this range of human environmental effects in clinical studies, just as we would with mice? In many ways, the impacts on ethologically appropriate behaviors of running a mouse experiment in a laboratory environment are much better recognized than those of the human environment in therapeutic clinical trials.

Heterogenization and population pharmacokinetics

One way of dealing with the unknown effects of environment – the ‘unknown unknowns’ – on experimental measurements or outcomes is to use data from a wide variety of randomly assigned or naturally occurring environments, or even genotypes. Embracing variability has the potential to make a study more reproducible and externally valid. This may either be done with systematized heterogenization, as proposed by Richter et al. (2010, 2009), or by combining studies conducted at different research sites (Kafkafi et al., 2005). Recently, Voelkl and colleagues (2018) reported simulated multi-laboratory studies where genetic and environmental variability, different strains, ages, housing conditions, etc., were used. This approach clearly has merits and it will be interesting to see how it might improve reproducibility in physiological, as well as behavioral, studies, where most effort has so far been focused (Karp, 2018). Interestingly, a similar strategy was developed for population pharmacokinetic studies in humans in the 1980s following concerns that the pharmacokinetics of new drugs were not being studied in relevant populations (Aarons, 1991). In this approach, data from all individuals in a population are evaluated simultaneously using nonlinear mixed-effects modeling (Mould and Upton, 2013). Such approaches actually depend on humans and mice living in a variety of boxes and the heterogeneity thus incorporated into the study can, in principle, make results much more robust.

Cauda

The challenge of investigating the impact of environment on phenotype is considerable, but to approach it by using ‘dirty’ mice in a stressful environment complicates the separation of the impact of any single aspect of the environment from another. The situation is similar when trying to establish the environmental impact on disease in humans. As we cannot put patients into a mouse house, we have to carry out demographic, ecological, toxicological and genetic studies to establish environmental impact. For both humans and laboratory mice, we argue that accurate recording, capture and characterization of environmental parameters is key to understanding how they affect phenotypic expression. Much effort was made in the European Mouse Disease Clinic (EUMODIC) and International Mouse Phenotyping Consortium (IPMC) to standardize assays and capture formal environmental metadata for phenotype assays carried out at multiple sites across the world. This has provided objective assessments of the impact of the experimental environment on reproducibility and insights into the interactions between phenotype and environment (de Angelis et al., 2015). This is inevitably a bootstrapping exercise that requires both mouse and human studies to move in lock-step towards a model replicating all or at least some of the human disease. As Robert Koch said, “remember gentlemen, a mouse is not a human being” (Greep, 1970). But, with renewed awareness of the importance of controlling and understanding the laboratory environment and its relationship to human environments, the mouse continues to be a powerful model.

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Competing interests

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References

- Aarons, L. (1991). Population pharmacokinetics: theory and practice. *Br. J. Clin. Pharmacol.* **32**, 669-670.
- Aarts, E., Maroteaux, G., Loos, M., Koopmans, B., Kovačević, J., Smit, A. B., Verhage, M., Sluis, S. and Neuro, B. M. P. C. (2015). The light spot test: measuring anxiety in mice in an automated home-cage environment. *Behav. Brain Res.* **294**, 123-130.
- Abolins, S., King, E. C., Lazarou, L., Weldon, L., Hughes, L., Drescher, P., Raynes, J. G., Hafalla, J. C. R., Viney, M. E. and Riley, E. M. (2017). The comparative immunology of wild and laboratory mice, *Mus musculus domesticus*. *Nat. Commun.* **8**, 14811.
- Bach, J.-F. (2017). The hygiene hypothesis in autoimmunity: the role of pathogens and commensals. *Nat. Rev. Immunol.* **18**, 105.
- Bailoo, J. D., Murphy, E., Varholick, J. A., Novak, J., Palme, R. and Würbel, H. (2018). Evaluation of the effects of space allowance on measures of animal welfare in laboratory mice. *Sci. Rep.* **8**, 713.
- Becker, C. E., Mathur, C. F. and Rehnberg, B. G. (2010). The effects of chronic exposure to common bedding materials on the metabolic rate and overall health of male CD-1 mice. *J. Appl. Anim. Welf. Sci.* **13**, 46-55.
- Beura, L. K., Hamilton, S. E., Bi, K., Schenkel, J. M., Odumade, O. A., Casey, K. A., Thompson, E. A., Fraser, K. A., Rosato, P. C., Filali-Mouhim, A. et al. (2016). Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature* **532**, 512-516.
- Bind, R. H., Minney, S. M., Rosenfeld, S. and Hallock, R. M. (2013). The role of pheromonal responses in rodent behavior: future directions for the development of laboratory protocols. *J. Am. Assoc. Lab. Anim. Sci.* **52**, 124-129.
- Bloomfield, S. F., Rook, G. A. W., Scott, E. A., Shanahan, F., Stanwell-Smith, R. and Turner, P. (2016). Time to abandon the hygiene hypothesis: new perspectives on allergic disease, the human microbiome, infectious disease prevention and the role of targeted hygiene. *Perspect Public Health* **136**, 213-224.
- Bohn, T., Desmarchelier, C., Dragsted, L. O., Nielsen, C. S., Stahl, W., Ruhl, R., Keijer, J. and Borel, P. (2017). Host-related factors explaining interindividual variability of carotenoid bioavailability and tissue concentrations in humans. *Mol. Nutr. Food Res.* **61**, 1600685.
- Bond, G. F. (1966). Effects of new and artificial environments on human physiology. *Arch. Environ. Health* **12**, 85-90.
- Botigué, L. R., Song, S., Scheu, A., Gopalan, S., Pendleton, A. L., Oetjens, M., Taravella, A. M., Seregély, T., Zeeb-Lanz, A., Arbogast, R.-M. et al. (2017). Ancient European dog genomes reveal continuity since the Early Neolithic. *Nat. Commun.* **8**, 16082.
- Boutwell, R. K. and Bosch, D. K. (1958). The carcinogenicity of creosote oil: its role in the induction of skin tumors in mice. *Cancer Res.* **18**, 1171-1175.
- Bronson, F. H. (1984). The adaptability of the house mouse. *Sci. Am.* **250**, 116-125.
- Cahill, R. J., Foltz, C. J., Fox, J. G., Dangler, C. A., Powrie, F. and Schauer, D. B. (1997). Inflammatory bowel disease: an immunity-mediated condition triggered by bacterial infection with *Helicobacter hepaticus*. *Infect. Immun.* **65**, 3126-3131.
- Chang, B., Hawes, N. L., Hurd, R. E., Davisson, M. T., Nusinowitz, S. and Heckenlively, J. R. (2002). Retinal degeneration mutants in the mouse. *Vision Res.* **42**, 517-525.
- Chistiakov, D. A., Myasoedova, V. A., Revin, V. V., Orekhov, A. N. and Bobryshev, Y. V. (2017). The phenomenon of atherosclerosis reversal and regression: lessons from animal models. *Exp. Mol. Pathol.* **102**, 138-145.
- Coppola, D. M. and Vandenberg, J. G. (1985). Effect of density, duration of grouping and age of urine stimulus on the puberty delay pheromone in female mice. *J. Reprod. Fertil.* **73**, 517-522.
- de Angelis, M. H., Nicholson, G., Selloum, M., White, J. K., Morgan, H., Ramirez-Solis, R., Sorg, T., Wells, S., Fuchs, H., Fray, M. et al. (2015). Analysis of mammalian gene function through broad-based phenotypic screens across a consortium of mouse clinics. *Nat. Genet.* **47**, 969-978.
- Evangelista, F. S., Brum, P. C. and Krieger, J. E. (2003). Duration-controlled swimming exercise training induces cardiac hypertrophy in mice. *Braz. J. Med. Biol. Res.* **36**, 1751-1759.
- Farber, D. B., Flannery, J. G. and Bowes-Rickman, C. (1994). The rd mouse story: seventy years of research on an animal model of inherited retinal degeneration. *Prog. Retin. Eye Res.* **13**, 31-64.
- Fassio, O., Rollero, C. and De Piccoli, N. (2012). Health, quality of life and population density: a preliminary study on “Contextualized” quality of life. *Soc. Indic. Res.* **110**, 479-488.
- Ferrecchia, C. E., Jensen, K. and Van Andel, R. (2014). Intracage ammonia levels in static and individually ventilated cages housing C57BL/6 mice on 4 bedding substrates. *J. Am. Assoc. Lab. Anim. Sci.* **53**, 146-151.
- Foltz, C. J., Fox, J. G., Cahill, R., Murphy, J. C., Yan, L., Shames, B. and Schauer, D. B. (1998). Spontaneous inflammatory bowel disease in multiple mutant mouse lines: association with colonization by *Helicobacter hepaticus*. *Helicobacter* **3**, 69-78.
- Foye, C. (2017). The relationship between size of living space and subjective well-being. *J. Happiness Stud.* **18**, 427-461.
- Franklin, C. L. and Ericsson, A. C. (2017). Microbiota and reproducibility of rodent models. *Lab. Anim. (NY)* **46**, 114-122.
- Fumagalli, M., Moltke, I., Grarup, N., Racimo, F., Bjerregaard, P., Jorgensen, M. E., Korneliusson, T. S., Gerbault, P., Skotte, L., Linneberg, A. et al. (2015). Greenlandic Inuit show genetic signatures of diet and climate adaptation. *Science* **349**, 1343-1347.
- Gibb, A. A., McNally, L. A., Riggs, D. W., Conklin, D. J., Bhatnagar, A. and Hill, B. G. (2016). FVB/NJ mice are a useful model for examining cardiac adaptations to treadmill exercise. *Front. Physiol.* **7**, 636.
- Glasper, E. R., Hyer, M. M. and Hunter, T. J. (2018). Enduring effects of paternal deprivation in California mice (*Peromyscus californicus*): behavioral dysfunction and sex-dependent alterations in hippocampal new cell survival. *Front. Behav. Neurosci.* **12**, 20.
- Greaves, M. (2009). The ‘delayed infection’ (aka ‘hygiene’) hypothesis for childhood leukaemia. In *The Hygiene Hypothesis and Darwinian Medicine* (ed. G. A. W. Rook), pp. 239-255. Basel: Birkhäuser Basel.
- Greaves, M. (2018). A causal mechanism for childhood acute lymphoblastic leukaemia. *Nat. Rev. Cancer.* **18**, 471-484.
- Greep, R. O. (1970). Animal models in biomedical research. *J. Anim. Sci.* **31**, 1235-1246.
- Grignolio, A., Mishto, M., Faria, A. M. C., Garagnani, P., Franceschi, C. and Trieri, P. (2014). Towards a liquid self: how time, geography, and life experiences reshape the biological identity. *Front. Immunol.* **5**, 153.
- Grimm, D. (2018). Are happy lab animals better for science? *Science* doi: 10.1126/science.aat2326.
- Guarner, F., Bourdet-Sicard, R., Brandtzaeg, P., Gill, H. S., McGuirk, P., van Eden, W., Versalovic, J., Weinstock, J. V. and Rook, G. A. (2006). Mechanisms of disease: the hygiene hypothesis revisited. *Nat. Clin. Pract. Gastroenterol. Hepatol.* **3**, 275-284.

- Guénet, J.-L. and Bonhomme, F. (2003). Wild mice: an ever-increasing contribution to a popular mammalian model. *Trends Genet.* **19**, 24-31.
- Hare, R. M., Schlatter, S., Rhodes, G. and Simmons, L. W. (2017). Putative sex-specific human pheromones do not affect gender perception, attractiveness ratings or unfaithfulness judgements of opposite sex faces. *R. Soc. Open Sci.* **4**, 160831.
- Harper, J. M. (2008). Wild-derived mouse stocks: an underappreciated tool for aging research. *Age* **30**, 135-145.
- Heinla, I., Ahlgren, J., Vasar, E. and Voikar, V. (2018). Behavioural characterization of C57BL/6N and BALB/c female mice in social home cage - Effect of mixed housing in complex environment. *Physiol. Behav.* **188**, 32-41.
- Heydemann, A. (2016). An overview of murine high fat diet as a model for type 2 diabetes mellitus. *J. Diabetes Res.* **2016**, 2902351.
- Holme, J. A., Refsnes, M. and Dybing, E. (1999). [Possible carcinogenic risk associated with production and use of creosote-treated wood]. *Tidsskr. Nor. Lægeforen.* **119**, 2664-2666.
- Holstein, J. (1979). *The First Fifty Years at the Jackson Laboratory*. Bar Harbor, ME, USA: The Jackson Laboratory.
- Horn, M. J., Hudson, S. V., Bostrom, L. A. and Cooper, D. M. (2012). Effects of cage density, sanitation frequency, and bedding type on animal wellbeing and health and cage environment in mice and rats. *J. Am. Assoc. Lab. Anim. Sci.* **51**, 781-788.
- Iturrian, W. B. and Fink, G. B. (1968). Comparison of bedding material: habitat preference of pregnant mice and reproductive performance. *Lab. Anim. Care* **18**, 160-164.
- Jackson, E., Demarest, K., Eckert, W. J., Cates-Gatto, C., Nadav, T., Cates, L. N., Howard, H. and Roberts, A. J. (2015). Aspen shaving versus chip bedding: effects on breeding and behavior. *Lab. Anim.* **49**, 46-56.
- Jacobs, B. B. and Dieter, D. K. (1978). Spontaneous hepatomas in mice inbred from Ha:ICR Swiss stock: effects of sex, cedar shavings in bedding, and immunization with fetal liver or hepatoma cells. *J. Natl. Cancer Inst.* **61**, 1531-1534.
- Jiang, Q. and Uitto, J. (2012). Restricting dietary magnesium accelerates ectopic connective tissue mineralization in a mouse model of pseudoxanthoma elasticum (Abcc6(-/-)). *Exp. Dermatol.* **21**, 694-699.
- Jimenez, S. M., Healy, A. F., Coelho, M. A., Brown, C. N., Kippin, T. E. and Szumlanski, K. K. (2017). Variability in prescription opioid intake and reinforcement amongst 129 substrains. *Genes Brain Behav.* **16**, 709-724.
- Kafkafi, N., Benjamini, Y., Sakov, A., Elmer, G. I. and Golani, I. (2005). Genotype-environment interactions in mouse behavior: a way out of the problem. *Proc. Natl. Acad. Sci. USA* **102**, 4619-4624.
- Kappel, S., Hawkins, P. and Mendl, M. T. (2017). To group or not to group? good practice for housing male laboratory mice. *Animals (Basel)* **7**, E88.
- Karp, N. A. (2018). Reproducible preclinical research—is embracing variability the answer? *PLoS Biol.* **16**, e2005413.
- Kentner, A. C., Abizaid, A. and Bielajew, C. (2010). Modeling dad: animal models of paternal behavior. *Neurosci. Biobehav. Rev.* **34**, 438-451.
- Kuk, S., Uyar, Y., Karaca, S. and Yazar, S. (2016). Microbiota: in health and in sickness, from birth to death. *Turkiye Parazitol. Derg.* **40**, 97-106.
- Lederbogen, F., Kirsch, P., Haddad, L., Streit, F., Tost, H., Schuch, P., Wust, S., Pruessner, J. C., Rietschel, M., Deuschle, M. et al. (2011). City living and urban upbringing affect neural social stress processing in humans. *Nature* **474**, 498-501.
- Lee, Y.-A., Obora, T., Bondonny, L., Toniolo, A., Miville, J., Yamaguchi, Y., Kato, A., Takita, M. and Goto, Y. (2018). The effects of housing density on social interactions and their correlations with serotonin in rodents and primates. *Sci. Rep.* **8**, 3497.
- Li, Q. and Uitto, J. (2013). Mineralization/anti-mineralization networks in the skin and vascular connective tissues. *Am. J. Pathol.* **183**, 10-18.
- Li, Z., Okano, S., Yoshinari, K., Miyamoto, T., Yamazoe, Y., Shinya, K., Ioku, K. and Kasai, N. (2009). Soft-hydrothermal processing of red cedar bedding reduces its induction of cytochrome P450 in mouse liver. *Lab. Anim.* **43**, 205-211.
- Li, Q., Kingman, J. and Uitto, J. (2015). Mineral content of the maternal diet influences ectopic mineralization in offspring of Abcc6(-/-) mice. *Cell Cycle* **14**, 3184-3189.
- Liu, H.-X., Lopatina, O., Higashida, C., Fujimoto, H., Akther, S., Inzhutova, A., Liang, M., Zhong, J., Tsuji, T., Yoshihara, T. et al. (2013). Displays of paternal mouse pup retrieval following communicative interaction with maternal mates. *Nat. Commun.* **4**, 1346.
- Lloyd, K. R., Yaghoubi, S. K., Makinson, R. A., McKee, S. E. and Reyes, T. M. (2018). Housing and testing in mixed-sex rooms increases motivation and accuracy during operant testing in both male and female mice. *Neurobiol. Learn. Mem.* **150**, 20-24.
- Lloyd-Price, J., Abu-Ali, G. and Huttenhower, C. (2016). The healthy human microbiome. *Genome. Med.* **8**, 51.
- Macrides, F., Bartke, A. and Dalterio, S. (1975). Strange females increase plasma testosterone levels in male mice. *Science* **189**, 1104-1106.
- Marciniak, S. and Perry, G. H. (2017). Harnessing ancient genomes to study the history of human adaptation. *Nat. Rev. Genet.* **18**, 659-674.
- Mayfield, J., Arends, M. A., Harris, R. A. and Blednov, Y. A. (2016). Genes and alcohol consumption: studies with mutant mice. *Int. Rev. Neurobiol.* **126**, 293-355.
- Meijer, J. H. and Robbers, Y. (2014). Wheel running in the wild. *Proc. R. Soc. B* **281**, 20140210.
- Mollnauer, S., Bryson, R., Robison, M., Sardo, J. and Coleman, C. (1993). EtOH self-administration in anticipation of noise stress in C57BL/6J mice. *Pharmacol. Biochem. Behav.* **46**, 35-38.
- Mo, C., Renoir, T. and Hannan, A. J. (2016). What's wrong with my mouse cage? Methodological considerations for modeling lifestyle factors and gene-environment interactions in mice. *J. Neurosci. Methods* **265**, 99-108.
- Morgan, J. L., Svenson, K. L., Lake, J. P., Zhang, W., Stearns, T. M., Marion, M. A., Peters, L. L., Paigen, B. and Donahue, L. R. (2014). Effects of housing density in five inbred strains of mice. *PLoS ONE* **9**, e90012.
- Morgan, M. and Cruickshank, H. (2014). Quantifying the extent of space shortages: English dwellings. *Building Research and Information* **42**, 710-724.
- Mould, D. R. and Upton, R. N. (2013). Basic concepts in population modeling, simulation, and model-based drug development-part 2: introduction to pharmacokinetic modeling methods. *CPT Pharmacometrics Syst. Pharmacol.* **2**, e38.
- Munzel, T., Daiber, A., Steven, S., Tran, L. P., Ullmann, E., Kossmann, S., Schmidt, F. P., Oelze, M., Xia, N., Li, H. et al. (2017). Effects of noise on vascular function, oxidative stress, and inflammation: mechanistic insight from studies in mice. *Eur. Heart J.* **38**, 2838-2849.
- Nichols, D. J. and Chevins, P. F. D. (1981). Adrenocortical responses and changes during the oestrous cycle in mice: effects of male presence, male urine and housing conditions. *J. Endocrinol.* **91**, 263-269.
- Nielsen, J. B., Andersen, O. and Svendsen, P. (1986). Hepatic O-deethylase activity in mice on different types of bedding. *Z Versuchstierkd* **28**, 69-75.
- Nogueira, P. A. S., Pereira, M. P., Soares, J. J. G., Filho, A. F. N., Tanimoto, I. M. F., Fonseca, I. A. T., Avelar, H. O., Botelho, F. V., Roever, L., Vieira, A. A. et al. (2017). Physiological adaptations induced by swimming in mice fed a high fat diet. *J. Exerc. Rehabil.* **13**, 284-291.
- Nyby, J., Wysocki, C. J., Whitney, G. and Dizinno, G. (1977). Pheromonal regulation of male mouse ultrasonic courtship (*Mus musculus*). *Anim. Behav.* **25**, 333-341.
- Otoni, C., Van Neer, W., De Cupere, B., Daligault, J., Guimaraes, S., Peters, J., Spassov, N., Prendergast, M. E., Boivin, N., Morales-Muñiz, A. et al. (2017). The palaeogenetics of cat dispersal in the ancient world. *Nat. Ecol. Evol.* **1**, 0139.
- Paigen, B., Svenson, K. L., Von Smith, R., Marion, M. A., Stearns, T., Peters, L. L. and Smith, A. L. (2012). Physiological effects of housing density on C57BL/6J mice over a 9-month period. *J. Anim. Sci.* **90**, 5182-5192.
- Park, G. and Evans, G. W. (2016). Environmental stressors, urban design and planning: implications for human behaviour and health. *J. Urban Design* **21**, 453-470.
- Patrono, E., Di Segni, M., Patella, L., Andolina, D., Valzania, A., Latagliata, E. C., Felsani, A., Pompili, A., Gasbarri, A., Puglisi-Allegra, S. et al. (2015). When chocolate seeking becomes compulsion: gene-environment interplay. *PLoS ONE* **10**, e0120191.
- Polissidis, A., Zelelak, S., Nikita, M., Alexakos, P., Stasinopoulou, M., Kakazanis, Z.-I. and Kostomitsopoulos, N. (2017). Assessing the exploratory and anxiety-related behaviors of mice. Do different caging systems affect the outcome of behavioral tests? *Physiol. Behav.* **177**, 68-73.
- Potgieter, F. J. and Wilke, P. I. (1996). The dust content, dust generation, ammonia production, and absorption properties of three different rodent bedding types. *Lab. Anim.* **30**, 79-87.
- Reardon, S. (2016). Dirty room-mates make lab mice more useful. *Nature* **532**, 294-295.
- Renie, W. A., Pyeritz, R. E., Combs, J. and Fine, S. L. (1984). Pseudoxanthoma elasticum: high calcium intake in early life correlates with severity. *Am. J. Med. Genet.* **19**, 235-244.
- Richter, S. H., Garner, J. P. and Würbel, H. (2009). Environmental standardization: cure or cause of poor reproducibility in animal experiments? *Nat. Methods* **6**, 257-261.
- Richter, S. H., Garner, J. P., Auer, C., Kunert, J. and Würbel, H. (2010). Systematic variation improves reproducibility of animal experiments. *Nat. Methods* **7**, 167.
- Ritchie, A. W. (1946). Housing; its effects on the nation's health. *Nurs. Times* **42**, 323.
- Schaebel, L. K., Bonefeld-Jørgensen, E. C., Laurberg, P., Vestergaard, H. and Andersen, S. (2015). Vitamin D-rich marine Inuit diet and markers of inflammation—a population-based survey in Greenland. *J. Nutr. Sci.* **4**, e40.
- Schoental, R. (1974). Letter: role of podophyllotoxin in the bedding and dietary zearalenone on incidence of spontaneous tumors in laboratory animals. *Cancer Res.* **34**, 2419-2420.
- Schoket, B., Hewer, A., Grover, P. L. and Phillips, D. H. (1988). Covalent binding of components of coal-tar, creosote and bitumen to the DNA of the skin and lungs of mice following topical application. *Carcinogenesis* **9**, 1253-1258.
- Sherer, D. W., Singer, G., Urbarri, J., Phelps, R. G., Sapadin, A. N., Freund, K. B., Yanuzzi, L., Fuchs, W. and Lebwohl, M. (2005). Oral phosphate binders in the treatment of pseudoxanthoma elasticum. *J. Am. Acad. Dermatol.* **53**, 610-615.
- Silver, L. M. (1995). *Mouse Genetics: Concepts and Applications*. Oxford: OUP.

- Simon, N. G.** (1979). The genetics of intermale aggressive behavior in mice: recent research and alternative strategies. *Neurosci. Biobehav. Rev.* **3**, 97-106.
- Srivastava, A., Morgan, A. P., Najarian, M. L., Sarsani, V. K., Sigmon, J. S., Shorter, J. R., Kashfeen, A., McMullan, R. C., Williams, L. H., Giusti-Rodríguez, P. et al.** (2017). Genomes of the mouse collaborative cross. *Genetics* **206**, 537-556.
- Stiles, R. J., Schrum, A. G. and Gil, D.** (2013). A co-housing strategy to improve fecundity of mice in timed matings. *Lab. Anim. (NY)* **42**, 62-65.
- Su, D., Nie, Y., Zhu, A., Chen, Z., Wu, P., Zhang, L., Luo, M., Sun, Q., Cai, L., Lai, Y. et al.** (2016). Vitamin D signaling through induction of paneth cell defensins maintains gut microbiota and improves metabolic disorders and hepatic steatosis in animal models. *Front. Physiol.* **7**, 498.
- Sundberg, J. P., Elson, C. O., Bedigian, H. and Birkenmeier, E. H.** (1994). Spontaneous, heritable colitis in a new substrain of C3H/HeJ mice. *Gastroenterology* **107**, 1726-1735.
- Tanaka, T., Ogata, A., Inomata, A. and Nakae, D.** (2014). Effects of different types of bedding materials on behavioral development in laboratory CD1 mice (*Mus musculus*). *Birth Defects Res. B Dev. Reprod. Toxicol.* **101**, 393-401.
- Tao, L. and Reese, T. A.** (2017). Making mouse models that reflect human immune responses. *Trends Immunol.* **38**, 181-193.
- Torronen, R., Pelkonen, K. and Karenlampi, S.** (1989). Enzyme-inducing and cytotoxic effects of wood-based materials used as bedding for laboratory animals. Comparison by a cell culture study. *Life Sci.* **45**, 559-565.
- Uitto, J., Jiang, Q., Váradi, A., Bercovitch, L. G. and Terry, S. F.** (2014). Pseudoxanthoma Elasticum: diagnostic features, classification, and treatment options. *Expert Opin Orphan Drugs* **2**, 567-577.
- Veleri, S., Lazar, C. H., Chang, B., Sieving, P. A., Banin, E. and Swaroop, A.** (2015). Biology and therapy of inherited retinal degenerative disease: insights from mouse models. *Dis. Model. Mech.* **8**, 109.
- Vesell, E. S.** (1967). Induction of drug-metabolizing enzymes in liver microsomes of mice and rats by softwood bedding. *Science* **157**, 1057-1058.
- Vlahakis, G.** (1977). Possible carcinogenic effects of cedar shavings in bedding of C3H-Avy fB mice. *J. Natl. Cancer Inst.* **58**, 149-150.
- Voelkl, B., Vogt, L., Sena, E. S. and Würbel, H.** (2018). Reproducibility of preclinical animal research improves with heterogeneity of study samples. *PLoS Biol.* **16**, e2003693.
- Wahlsten, D., Metten, P. and Crabbe, J. C.** (2003). A rating scale for wildness and ease of handling laboratory mice: results for 21 inbred strains tested in two laboratories. *Genes Brain Behav.* **2**, 71-79.
- Waldron, H. A.** (1983). A brief history of scrotal cancer. *Br. J. Ind. Med.* **40**, 390-401.
- Ward, J. M., Fox, J. G., Anver, M. R., Haines, D. C., George, C. V., Collins, M. J., Jr, Gorelick, P. L., Nagashima, K., Gonda, M. A., Gilden, R. V. et al.** (1994). Chronic active hepatitis and associated liver tumors in mice caused by a persistent bacterial infection with a novel *Helicobacter* species. *J. Natl. Cancer Inst.* **86**, 1222-1227.
- Weissbrod, L., Marshall, F. B., Valla, F. R., Khalaily, H., Bar-Oz, G., Auffray, J.-C., Vigne, J.-D. and Cucchi, T.** (2017). Origins of house mice in ecological niches created by settled hunter-gatherers in the Levant 15,000 y ago. *Proc. Natl Acad. Sci. USA* **114**, 4099.
- Williams, R. E.** (1948). Ventilation of dwellings and its effect on human health. *J. R. Sanit. Inst.* **68**, 583-592.
- Wilner, D. M., Walkley, R. P., Glasser, M. N. and Tayback, M.** (1958). The effects of housing quality on morbidity; preliminary findings of the Johns Hopkins longitudinal study. *Am. J. Public Health Nations Health* **48**, 1607-1615.
- Zachariassen, L. F., Krych, L., Engkilde, K., Nielsen, D. S., Kot, W., Hansen, C. H. F. and Hansen, A. K.** (2017). Sensitivity to oxazolone induced dermatitis is transferable with gut microbiota in mice. *Sci. Rep.* **7**, 44385.