Nontraditional models as research tools: The road not taken

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Abstract

Historical reasons resulted in almost exclusive use of a few species, most prominently *Mus musculus*, in becoming the mainstream models of biomedical research. This selection was not based on *Mus'* distinctive relevance to human disease but rather to the pre-existing availability of resources and tools for the species that were used as models, that has enabled their adoption for research in health sciences. Unless the utilization and range of nontraditional research models expand considerably, progress in biomedical research will remain restricted within the trajectory that has been set by the existing models, and of their ability to provide clinically relevant information.

Introduction

Biomedical research is dominated by studies involving limited species only, that are typically recognized as the traditional animal models (see Glossary). *Mus musculus* possesses the lion's share in this utility for reasons that are primarily historical and coincidental. For example, in 2018 according to the European commission, out of the 7,938,064 (48.9%) animals used totally in research and testing, 3,879,691 (48.9%) were mice, followed by 1,914,039 (23.1%) fish and 665,155 (8.3%) rats[1]. Early scientific advances have identified *Mus* as a suitable biological research model and soon, its use expanded to all fields of biomedical research because tools and resources were becoming increasingly available. Despite however its paramount contribution in the study of human disease, *Mus* possesses characteristics that cast some doubts regarding its ability to deliver clinically relevant information [1].

The aim of this article is to discuss some of these limitations and suggest that the inclusion of additional animal models, that today are considered as nontraditional, can be highly beneficial. With this suggestion, the contribution of *Mus* in biomedical sciences should not be understated since its utility as a model has shaped the biomedical field. Nor that the nontraditional models do not have limitations, such as their adaptation and usage in laboratory conditions, the lack of technologies and recourses that operate as a burden during experimentation, and unknown characteristics in their physiology and molecular profiles that can disqualify them as models. Nonetheless, today's progress in biomedical technologies render their adoption feasible or at least, worthy of exploring. This is also fully aligned with the recognition of personalized approaches as the direction of choice in biomedical practice.

Scientific Tradition and Mus as the model of choice in biomedicine

Modeling is essential in natural sciences including biomedicine, a fact that was epitomized by August Krough in his famous principle that stated that "For many problems there is an animal on which it can be most conveniently studied" [2]. There is a difference however between the biomedical sciences and the other natural sciences, on how their models were introduced and used: In other natural sciences the models are created by the experimentalists and reflect their current

[1] https://webgate.ec.europa.eu/envdataportal/content/alures/section1_number-of-animals.html

technological capabilities. In biomedicine however, the models have (biologically) evolved independently of the experimentalists and of their needs and have only been selected and recruited by them [3]. This creates limitations that reflect to biomedical research and its ability to produce clinically relevant information. Early advances in the fields of genetics, physiology and biochemistry, that already utilized mice, established a scientific tradition by generating tools and strategies that were readily applicable in health sciences research. From that point onwards, *Mus* is being used as the gold standard for experimental studies in biomedicine, not because of their unique relevance to human pathology, but rather because of historical and practical reasons. Tools and comparative data were becoming increasingly available, sustaining this choice of model and enabling the research enterprise in its entirety to proceed. Since about the 1980s, mice have represented the mainstream choice for securing funding and for publishing, in alignment with the overarching scientific standards and expectations. This path applies even to research in infectious pathologies at which the species-specificity of the host presents an objective obstacle. During the COVID-19 pandemic for example, an immediate response in developing an animal model was to sensitize *Mus* to SARS-CoV-2 infection by introducing hACE2 expression, to overcome the natural resistance of mice to the virus [4]. Analogous approaches are being employed for other infectious diseases, such as for HIV-associated pathologies at which mice are modified at the genetic and cellular level to be infectable by the human virus or the simian immunodeficiency virus (SIV) that can cause similar pathologies [5,6]. A dimension that needs to be considered and despite the limitations mandates the use of rodents, as compared to primates that get infected with SIV or cats that get infected with the feline immunodeficiency animals, is related to the ethical limitations in using them in research. In addition, practical limitations regarding animal size and life cycles also pose restrictions.

Notwithstanding the necessity at various instances, of following it, this trajectory, today, emerges as an oxymoron because the whole scientific, and indeed the cultural landscape of contemporary research recognizes the need for the advancement of personalized medicines and the focus on the individuality of patients [7]. Thus, at a time at which the aspired path of biomedical progress involves appreciation and exploitation of the specific differences of individual people and how these may impact their predisposition to disease and the efficiency of treatments, the experimental research findings on which such strategies are based, rely on limited models of genetic clones only. Unavoidably, from that perspective, opposing dynamics are sustained within

the ongoing research efforts, by which the preclinical results that are generated by using the traditional research models, produce information and knowledge that is seeking validation and application in genetically diverse human populations [8-10]. Furthermore, the quest for uniformity and the adherence to optimal - and thus stable – conditions of the conventional experimental setups, grows in expense of integration of the multitude of conditions that can impact human populations and their responses. For example, these might include social interactions, seasonality, exposure to a combination of diverse environmental stimuli, and everything pertinent to the widely perceived diversity in contexts [11-15].

Animal modeling and efficacy of the biomedical research enterprise

These dynamics are very likely to cause failures, the extent of which is hard to appreciate. Nonetheless, we can speculate on their extent, considering the effort and the resources that are allocated towards preclinical studies, and the small fraction of which ultimately result in clinically relevant applications or drugs. This is reflected in the high **attrition rate** during drug development that exceeds 90% [16-24]. The inadequacy of the existing models for preclinical research is recognized, in addition to the limited value of the oversimplified in vitro methodologies (25,26). Furthermore, indirect evidence at the population level for the suboptimal efficiency of this path can also be derived by the simple observation that life expectancy increased during the last decades, but the major contributors for this success were not the translation of preclinical research findings to human populations [27]. It was primarily due to changes in the **social determinants of health** and the progress in the management of infectious diseases [28-31]. Ironically, none of these were linked directly to, or had as a pre-requisite, systematic preclinical studies involving traditional animal models. Advances in social health determinants are related to the sociopolitical and economic progress that have improved hygiene and have rendered health care and preventive medicine accessible to an increasingly high fraction of the population. A series of animal studies exist that corroborate these advances but, in their majority, they are just confirmatory instead of drivers of the change. In infectious diseases, due to the specificity of most pathogens for their human hosts, progress does not depend on studies involving traditional animal models (32). Animal studies again exist that finetune the ongoing practices and illuminate the underlying mechanisms, but progress in this area is not dependent on animal studies per se.

Yet, progress in our understanding of the fundamental mechanisms of disease today is unequivocal, and so are our capabilities towards this direction, which further underscores the disparity in the translation of research findings into clinically relevant and directly applicable information. The acknowledgement of this limitation bears inherently on the notion that the mainstream efforts, that in principle rely on traditional animal models may be irrelevant or have minimal value for human health, especially when considering cost-efficacy.

A tradition in modeling and its intrinsic limitations

Two main reasons that are intrinsically linked to the models used to acquire scientific information likely account for this limitation in translational relevance. The first limitation relates to the species identity of the models that generates positive dynamics in their usage. Based mainly on reasons grounded in scientific tradition rather than a priori justification, studies typically use animals to address questions that may not be highly relevant, scientifically or clinically. By being traditional and to fulfil their function as the widely used gold standard, they mandate additional investment towards them, but they remain limited in their capacities and their ability to model human pathologies, and in addressing fundamental basic questions or needs (33). Various examples can illustrate this notion. Pigs receive increased attention in cariology research because they demonstrate anatomical similarity in structure and size with the human heart. As such, porcine models offer valuable information that mice cannot [34]. Eventually, pigs became used as a source for heart valve transplants a process that is continuously being improved. A caveat to this utility is that the lifespan of pigs is only 20 years, which is much shorter than the anticipated life expectancy of the patients undergoing the procedure [35-37]. For similar reasons relevant to scientific tradition, laboratory mice are being used for studies in aging and neurodegenerative diseases, despite the fact that they have a lifespan of only up to 3 years or less and have intrinsic resistance to age-dependent neuronal degeneration (38).

Mice are now being used for the study of nearly every pathology. This is a time when human population studies and the National Institutes of Health (NIH) increasingly appreciate the significance of emotional health and of social interactions in health outcomes (39). Because they are polygamous, laboratory mice exhibit only limited relevance on the impact of social connectedness in health outcomes. Mice do not develop pair bonds and do not exhibit paternal

care, and thus, the impact of loneliness is contextually very different from the chronic pathologies in humans that are exacerbated after the loss of a partner and they are intended to model [40-42]. Noteworthy, small rodents exhibiting monogamous behavior do exist, such as voles (genus *Microtus*) and deer mice (genus *Peromyscus*), that can be used to investigate the effects of social interactions in the study of various pathologies [43-45]. Yet, such non-traditional models are being used on a limited capacity (e.g. to study social interactions), and not being used to their full potential to study social interactions as modifiers of the outcomes of different pathologies. The limited utility of these models in the study of human disease is primarily related to the lack of specialized reagents and tools, and the skepticism that surrounds the clinical relevance of disease-related information that is produced by non-mainstream research models. Both reasons relate to the historical factors mentioned earlier.

We can only speculate what the potential impact of such historical inertia would have produced for biomedical sciences and specifically in the study of social interactions and their impacts on disease, if additional species like one or more of the monogamous rodents had received similar investment in tool development and attention as was dedicated to *Mus* (Clinician's corner). In this case, technology and baseline information would have accumulated such that researchers would have more diverse tools upon which they could rely, establishing a more direct ability to make biologically relevant comparisons to human disease, disorders, and health and wellbeing. In addition, the scientific community, investigators, and funding agencies, would have been more receptive to their utility under such conditions and settings. It is hard to imagine the lost progress for whole areas of research like preclinical drug testing and drug attrition.

Mice are also being used for the study of many cancers including breast cancer [46,47]. Yet, most human cancers of the breast are hormone-sensitive, and mice are required to receive exogenous estrogens, resulting in estrogen levels comparable to those occurring before menopause, albeit the disease is more common in postmenopausal women [48,49]. If animal models that could sustain hormone-sensitive breast cancer growth had been identified and used, it is likely that estrogen supplementation commonly used today would be seen as an irrational choice. The limitations of *Mus* in breast cancer research also becomes apparent by the low penetrance and the long latency of tumorigenesis in BRCA-mutant mice, despite these genes' central role in breast cancer in women [50]. Again in the cancer field, p53 germline mutations in humans - the most

common genetic defect of human cancers -, in humans are associated with the multicancer Li-Fraumeni syndrome while in mice they cause mainly lymphomas [51,52].

Cancer and behavioral sciences represent only two areas at which mice have limited ability to model human pathology and physiology. Analogous examples can be identified in the field of metabolism (53), immunology (54), neurobiology (55), and others.

Significant effort and resources are being continuously invested in adapting *Mus* towards pathologies of interest. For example mice have undergone humanization to mimic our immune system, or genetic modification, to generate loss- or gain-of-function mutants that develop human-like pathologies that are commonly found in humans and in humans and are modified by quantitative changes in gene expression [56-64]. Nonetheless, their similarity to human conditions and processes remains frequently elusive, and therefore, their ability to provide clinically meaningful results is tenuous. More importantly, by having an established tradition established that is restricted to the use of *Mus*, systematic efforts to identify naturally existing models for human pathologies are lagging, and the adherence to mainstream models remains the option of choice remains the option of choice out of convenience or lack of vision.

It should be acknowledged that at various instances the physiological differences between mice and humans, instead of a burden can be advantageous, and has fueled research that assisted in our better understanding of human disease. For example, the resistance of the cystic fibrosis gene-deficient mice to the disease as opposed to humans and pigs, prompted comparative biology studies and led to the identification of the adenosine triphosphatase gene as a therapeutic target (65). This example however, instead of being treated as a demonstration that mice may become eventually informative for all human conditions it should rather be seen as an example that illustrating the power of comparative biology and that the inclusion of diverse models is beneficial.

The second limitation is related to the genetic make-up of these models. In order to satisfy the demand for adherence to a uniform baseline for studies performed in different environments, traditional models require high genetic resemblance to each other, which can only be satisfied by using **inbred strains**. Inbred strains are the artificial products of selective breeding, which are considered identical living entities despite they continue to accumulate mutations, are not isogenic, and even their vendor may account for potential differences among them [66-68]. They are also described as wild type in the context of the experimental studies, albeit they do not exist in nature,

and their ability to survive in the wild is arguable. Yet, their phenotypes, again within the context of experimental studies, are thought to reflect the perceived normal, towards which the results of genetic, dietary, behavioral or other manipulations are compared. Their inbred nature, nonetheless, despite its advantages in mechanistic studies, contrasts with the natural human condition, both at the level of the individual patient that is highly heterozygous and at the level of the populations that are extremely genetically diverse. This happens while the concept of **hybrid vigor** or heterosis has been long known since Darwin [69]. And yet, the consequences of homogeneity in preclinical drug testing remain elusive [70,71]. Thus, while accurate information can be derived by preclinical studies, this information remains primarily applicable to the specific strain of mouse and the conditions under which it has been performed. Indeed, the relevance to humans and to their populations is unknown or speculative at best [72]. Partially this limitation was addressed by the introduction of genetically diversified mouse populations, yet these efforts are still restricted by limitations pertinent to *Mus'* physiology and the characteristics of the usually inbred specific mouse strains that have been used [73,74].

Seeking paradigm shifts by reappraisal of the research investment portfolio

Sound reasons for the historic availability of resources and technologies have established the specific trajectory for the process of scientific discovery. This has framed questions and research efforts and been limited by the study species and, therefore, has been limited by their specific characteristics. Thus, the information that can be obtained for biomedical science is only as good as the experimental system used to retrieve it. Today, however, this can change. Progress in genomics and high-throughput molecular technologies facilitate paradigm shifts in health sciences because they enable recruitment and adoption of a large array of organisms that can be used to model diseases and treatments (Box 1). Manipulating the genome of a mouse was once time-consuming, expensive, and highly specialized, and the pioneering methods were limited by stochastic problems in genome assembly and annotation. Furthermore, such work could only be done in mice for which the specific technology had been developed. Today this process is very rapid, cost efficient, precise, and can be applied readily to a wide range of organisms with high efficiency. Likewise, the progress in sequencing capabilities for high resolution genomes supported by advances in bioinformatic analyses, enables large scale, cost-effective and rapid

progress for several species of diverse and unknown genetic make-ups. The significance of using more than a single model in research is recognized by the fact that in drug development, safety studies frequently involve more than one model. Other approaches that alone or in combination with the non-traditional models possess great promise for breakthroughs involve innovative complex in vitro models (e.g., organoids, (multi)organ-on-chips, microphysiological systems) as well as in silico technologies (AI, machine learning, deep learning, digital twin technologies, in silico trials) that have been used successfully to support drug discovery, development and testing [75-77].

Despite this progress and the availability of such technologies, traditional models remain overwhelmingly preferred, notwithstanding their widely acknowledged limitations. From this perspective, instead of seeking new models, the improvement of the existing models is preferred. This can be attributed to the skepticism among investigators, reviewers and funding agencies against the non-mainstream outbred models. This skepticism is partly due to the perceived inability of such organisms to provide high resolution information of analytical and mechanistic value, which ironically is attributed to their diverse nature and the comparatively limited baseline information and tools that are currently available. Reviewers in scientific journals and panel members in study sections for funding agencies frequently anticipate information that is analogous in its detail to that of *Mus*, and question the informative value of studies that leverage novel systems that *Mus* can provide. And yet, the biological relevance of *Mus* is dogmatically unquestioned. This reflects the established scientific culture that remains highly analytical and **inductive**, despite the appreciated significance of synthetic approaches. To that end, investment in research efforts that are **deductive**, even if they are more "crude" in their nature, or involve "obscure" species, is rarely preferred. Such species, for example may develop obesity, cardiovascular disease, or cancer more similarly to humans, but with restricted ability dissecting the underlying process because tools such as specific antibodies do not exist yet. Thus, the investment towards the acquisition of more detailed information by using traditional models is prioritized and this choice remains more appealing, both career- and funding-wise..

Concluding remarks

The advocacy for nontraditional models should not be perceived as an attempt to diminish the paramount contribution of *Mus* in biosciences nor as an effort to ignore the fact that the whole biomedical field has been shaped by this species at an extent that it is practically impossible to appreciate the state of progress without referring to laboratory mice. Rather, it should be seen as an endeavor to expand the basis of the models used in biosciences to perform research that increases the chances to deliver breakthroughs, and sustain the associated risks and costs. The promotion and integration of nontraditional animal models in biomedicine remains a responsibility of the major funding agencies and investigators performing the science. Investment is needed to support a culture of scientific diversification. A plausible avenue forward to achieve this, is to promote large scale screening programs by which the relevance of different animal species to various clinical conditions will be explored. The initial goal of such programs should not be to deliver mechanistic information but rather to establish pools of models with relevance to disease. This way, both the critical mass of the researchers utilizing nontraditional models will increase, and the different conditions will be studied through the lens of different models, each of which may have its own advantages and limitations. Such expansion will not proceed on the basis that such models may eventually become traditional for certain conditions. This would have defeated their purpose and would again generate dynamics that have caused potential failures related to the use of a single species, such as *Mus musculus*, as a model. Rather, the adoption of any animal, *Mus* or other non-traditional animal models, should be promoted because it may better satisfy the demand of generating scientifically important and clinically useful information by a manner that is non-incremental, possessing high risk and the prospect of high return (see Outstanding Questions).

As Robert Frost (1874-1963) might have said, it is probably the time to consider taking the road not taken [78]. He lyrically described this in his homonymous poem:

[…I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood, and I—
I took the one less traveled by,
And that has made all the difference...]

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Declarations of interests

The University of South Carolina owns a patent (US 11,766,033 B2) on the utility of Peromyscus as cancer model. The author is inventor in this patent.

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Glossary

Deductive reasoning: it indicates the methodological approach in science by which general observations are used to draw specific conclusions. It is the process of going from the general to the specific.

Drug attrition rate: tt reflects the portion of the drugs that enter clinical trial testing but fail. It is estimated that is above 90% and for cancer drugs it is even higher.

Hybrid vigor (or heterosis): the enhanced performance and increased fitness that is recorded in hybrid strains.

Inbred strains: strains that are derived by successive brother-sister mating that results in homozygosity in all genetic characters. Variation in different traits is lower in inbred strains.

Inductive reasoning: it indicates the methodological approach in science by which specific observations are used to support generalized conclusions. It is the process of going from the specific to the general.

Social determinants of health: an umbrella term covering all environmental conditions at which people are born, live, learn, work, play, worship, and age (https://health.gov/healthypeople/priority-areas/social-determinants-health) (39)

Traditional models: animal species and strains that are commonly used in biomedical research. *Mus musculus* (laboratory mouse) is the most widely used mammal in health sciences research.

Box 1. A wide spectrum of species can be used as research models.

To get some appreciation of the breadth of species that can be used as models and how limiting the use of a single species can be, we should consider the following: *Mus musculus* is only one of more than 2,000 species of the order *Rodentia* (rodents) which includes 29 families and 468 genera [79,80]. Rodents vary in size and can range from a few grams (pygmy mice) to (capybaras). They have been adapted for living in diverse conditions and therefore their physiology and molecular profiles differ accordingly. Rats and hamsters also belong to this group of mammals. Other mammals that occasionally have been used in research studies as models are cats (Order: Carnivora, Family: Felidae), dogs (Order: Carnivora; Family: Canidae), pigs (Order: Artiodactyla, Family: Suidae), and others. Each of these species have their own characteristics that make them potentially suitable for the study of different conditions. Until recently, the use of these species in research was limited by the current state of the art of the existing knowledge. Today's advances can readily generate background information and methodologies to rapidly enable experimental studies.

Clinician's Corner. Oxytocin is a neuropeptide with an important role in the regulation of social interactions and connectedness. Studies in monogamous rodents (voles) were instrumental in our understanding of this activity of oxytocin in the context of pair bonding and the regulation of monogamous behavior. This information contributed to the initiation of clinical trials testing the beneficial effects of oxytocin in autism with promising results shown in some instances [81,82].