OPEN ACCESS
Freely Available Online

JOURNAL OF ZOOLOGICAL RESEARCH
ISSN NO: 2694-2275

Editorial

DOI: 10.14302/issn.2694-2275.jzr-17-1878

Experimental Zoology. Advancements, Problems and Future Prospects.

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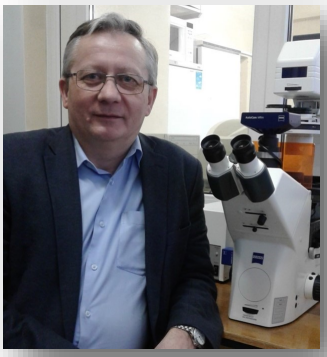

It is impossible to imagine the field of Zoology without the use of animal models. Achievements in this field are well known by the general public and is of genuine interest among scientists since the days of Karl Ernst von Baer (1792 – 1876) [1]. Through the careful collection of data through well-designed methodological approaches, the field of experimental Zoology went from a science that described basic characteristics of animals to a science that experimentally verify the accuracy of our knowledge about them. This mini-review presents a brief overview of the most important and relevant advances in this field over the last decades.

In the mid-twentieth century, the term "animal model" referred to a small number of species able to reproduce in the laboratory. This included: mice (Mus musculus), rats (Rattus norvegicus), guinea pigs (Cavia porcellus), rabbits (Oryctolagus cuniculus), dogs (Canis familiaris), fruit flies (Drosophila melanogaster), clawed frogs (Xenopus laevis , Xenopus tropicalis), salamanders (Ambystoma mexicanum), zebrafish (Danio rerio) among others. Development of standardized rules for the housing, feeding and breeding of different species have made an invaluable contribution to shape the modern guidelines on how to maintain animals models known as "Animal Rule"[2,3]. Methods used in modern studies to evaluate toxicity and efficacy of potential medicines is one great example of an important contribution that experimental Zoology of the 20th century produced[4]. The most daring experimental ideas were implemented in the development of transgenic salmon and mice models for medical research at the threshold of the 20th and 21st centuries [5,6].

AquAdvantage salmon was obtained using several methodological advances [7]. The stable line of genetically modified Atlantic salmon (Salmo salar) was first developed by direct microinjection of excess copies of a growth hormone gene into the zygote. This injected engineered gene contained the gene for a growth hormone made by Chinook salmon (Oncorhynchus tshawytscha) under control of an

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Keywords: animal models, gene, transgenic, mutation

Received : Nov 27, 2017 **Accepted :** Jan 02, 2018 **Published :** Jan 08, 2018

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anti-freeze protein promoter from Ocean pout (Zoarces americanus). For propagation of AquAdvantage, many famous modern approaches were used in aquaculture during the 20th century. These include activation of eggs by UV-irradiated sperm, obtaining diploids with both maternal haplotypes and reversion of sex and triploid production [7]. Further improvement of these methods is an important part of modern experimental ichthyology.

Experimental Zoology has spent over a hundred years developing mouse models of human diseases. A number of specific methodological approaches were tested through use of mice. These include: the generation of inbred strains, development of embryo transfer technology and the associated of micromanipulation including microinjections into zygotes, discoveries of molecular and genetic mechanisms of recombination and genome editing as well as the development of genetic engineering techniques.

Times have shown that obtaining inbred strains is an essential part of the experimental methodology associated with the study of many zoological species. Protocols for the generation of inbred mouse strain initiated by C. C. Little in 1909, are still considered the gold standard in this area. Today, the well-known strains are DBA, C57BL /6, BALB/C and some others. Long-term maintenance of these mice has revealed a number of problems associated with the mutations arising into individual allelic loci and genetic drift [8]. Therefore, current scientific reports indicate not only the name but also the origin of animals used to address this problem. Recently, presence of genetic differences between C57BL/6J and C57BL/6NCrlCrlj strains was revealed using single-nucleotide polymorphism genotyping [9]. The C57BL/6J strain bred at Jackson Laboratory. The C57BL/6NCrlCrlj strain bred at Charles River Laboratories Japan [9].

The advent of embryo transfer technology has radically changed the methodology behind animal model generation and development. This approach is used to eliminate pathogens from experimental mice populations as well as generating transgenic animals by gene transfer into the zygote [10, 11]. Today there are hundreds of strains generated in this way [12].

New types of models were developed after the discovery of site-specific Cre-Lox recombination [13]. This method relied on the ability of the enzyme Cre to initiate recombination between the two LoxP sites. The existing possibility to manipulate embryonic cells in vitro was the second important application using this approach [14]. Briefly, cells modified in vitro then injected into the inner cell mass (embryoblast) of blastocysts. The blastocysts were transplanted to pseudo-pregnant female mouse. The offspring screening was applied to detect genetically modified animal. This approach provided the ability to selectively inactivate expression of a target gene in the specific target tissue and in the whole organism according to the application demand.

Novel genome editing based on CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats -associated protein-9) nuclease complex is one of the actively discussed topics in the last few years [15]. The CRISPR/Cas9 nuclease complex can be introduced directly into the cell nucleus that is an important technical simplification for creating genetically modified animals. One documented disadvantage of the method is the mosaics obtained in the course of the experiment [16]. However, this method is still considered one of the most popular approaches for obtaining the animal models for all species.

It's impossible to imagine modern mouse models without mice carrying mutations that suppress the immune system. Currently, the widely known strains of mice are Nude, SCID, Rag1/Rag2-deficient, NOD scid gamma and some others [17].

Nude phenotype is associated with mutations mapped on chromosome 11 in mouse. The carriers of these mutations were found among mice from BALB/c strain in 1966 [18]. In addition, those carriers were characterized by the presence of rudiments of the thymus and poorly developed mammary gland. Dysfunction of the thymus leads to the lack of mature T lymphocytes, particularity in mice used to obtain the models. A special breeding scheme was applied to obtain homozygous mice, due to well documented side effect of developed mammary glands in females. To combat, this homozygous- males are mated with

heterozygous females at the final stage of obtaining homozygous progeny.

The strain of mice carrying a mutation of severe combined immunodeficiency (SCIDs) was originally obtained from the BALB/c strain [19]. Carriers or the mutations were identified between progenies of C-B-C17 mice sub-strain of BALB/c. Upon further investigation, inactivation of PRKDC gene leads to a shortage of mature T- and B-lymphocytes in these mice. Mice with the SCID phenotype are widely used in the creation of models.

The site-specific Cre-Lox recombination was used to create mice models with inactivated recombination-activating genes (Rag1/Rag2-deficient) [19, 20]. The main features of these mice are having small lymphoid organs with absence of mature B and T lymphocytes. They are often used as alternatives to Nude mice and SCID mice. The same approach was applied to develop strains with deletion in interleukin 2 receptor γ-chain gene [19, 21]. It leads to disruption of the differentiation of T lymphocytes and natural killer cells, as well as dysfunction of B-lymphocytes.

Full suppression of immune system function was achieved using a strain known as the non-obese diabetic (NOD) mouse. This strain develops spontaneous type 1 diabetes in a sex linked manner with high likelihood of incidence in females and less low incidence in males [22]. Since this model derived from NOD strain, it was named NOD scid gamma (NSGTM) and have genotype: NOD.Cg-Prkdc^{scid}il2rg^{tm1Wjl}/SzJ [18, 19]. Four genes regulating the immune system were inactivated in this strain. The resulting model was used to obtain humanized mice [19]. The humanized mice models have significantly improved the quality of preclinical studies of potential drugs.

Listed above are examples that have important conceptual, methodological and scientific value for the further developments in the field of experimental Zoology. Discussion of problems and achievements in this field is essential not only for biological researchers but also to the entire international community because almost every field uses model systems. The generation and maintenance of new models is an integral part of scientific progress.

Acknowledgment

I would like to thank N.A. Alimov for his critical comments and constructive suggestions.

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