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THE IMPACT OF GENETICS IN THE CONCEPT OF SUSTAINABLE AGRICULTURE

ABSTRACT

Recent development in molecular and quantitative genetics and genomics offer powerful tools for preservation and improvement of animal genetic resources. Many of these new tools were applied for assessment of the situation in rare and endangered populations. Therefore, objective and between populations comparable methods of evaluation of genetic variation as well as economic evaluation of biodiversity are urgently needed. Some examples in the past showed convincingly that application of novel technologies can contribute considerably to characterization and preservation of animal genetic resources. In addition to genetic and genomic tools, there is a number of techniques in reproduction biology which can efficiently support the efforts for preservation of animal genetic resources.

Keywords: genetic approach, genomics, reproductive technology, animal genetic resources

INTRODUCTION

In addition to its primary goal, production of food for constantly growing human population on earth, the modern agriculture has to address a number of questions which are of central interest for the society as a whole. Among them, the environmental issues and preservation of biodiversity represent the pivotal task for agriculture with the aim to enable efficient production and sustainable use of genetic resources. The strong preference for *high yield varieties* (HYV) in the industrialized agricultural production worldwide represents constant threat for biodiversity narrowing the space for further selection strategies. The Convention of Biological Diversity, signed by 192 countries at the United Nations Conference on Environment and Development in 1992 (Olesen et al, 2000), is a legal basis for international prevention of biodiversity based on three principal objectives:

- Conservation of world's biological diversity
- Sustainable use of its components and
- Fair and equitable sharing of the benefits to be derived from its use.

Although the sustainable use of animal genetic resources is widely recognized as a generally important task, its direct impact on modern animal breeding remains rather unclear.

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Recapitulating the development of agriculture and animal production systems we can define four important developmental stages defined as pioneering, production, increased productivity, and persistency. Based on the mechanistic word view, which has its roots in the work of classical scholars (Galileo, Copernicus, Newton, Descartes) and supplemented with great discoveries of Darwin, Pasteur and Mendel, the fundament of modern life sciences including agricultural sciences was created (Olesen et al, 2000).

RESULTS AND DISCUSSION

The concept of sustainable agriculture

The productivity in animal breeding has increased dramatically after 1950, as almost unlimited amounts of cheap grain were introduced into animal nutrition and principles of quantitative genetics were applied to animal selection. For this period the rapid increase of production per animal and a surplus of animal products on the market in developed countries are characteristic. At the end of the 20th century the switch to increased productivity per hectare agricultural land (and not per animal any more) as well as ecological concerns became important issues for the further development of animal production.

The definition of sustainable use of animal genetic resources (AnGenRes) includes the principle that AnGenRes should be judged as a part of a complex agricultural system, based on whole system analysis with the goal that animals should increase farm production per hectare agricultural land rather than production per animal. Breeding objectives should reflect producer's interests and improve the efficiency of production system. Introduction of new traits, like robustness is necessary and splitting of aggregate genotype into nonmarket- and market values (self supply, ecology) is necessary.

Food security is one of the major objectives of sustainable agricultural development. The depletion of plant and animal genetic resources decreases complexity of production systems (coffee plantages). The reduction in biodiversity increases vulnerability of agricultural systems and reduced biodiversity has negative effects on nutritional situation where high yield varieties (HYV) prevail, having the depletion of food quality as a consequence. From the philosophical prospective the definition of animal breeding goals for sustainable agriculture can provoke the conflict between the holistic approach and the reductionist one, where mechanistic technical reasoning hits the barrier of complete understanding of complex, non-exact systems. The other problem is related to the clash between objective and subjective judging on the complexity and reliability of causal interrelationships within agricultural systems.

Characterization of animal genetic resources and the role of biodiversity

Characterization of animal genetic resources is a global task which became one of important aims of the modern animal production. The legal background represent in addition to the Convention on Biological Diversity (1992), also the Interlaken Declaration on Animal Genetic Resources (2007), which has been put forward by FAO emphasizing that loss of genetic diversity deserves immediate action and therefore indicators for animal genetic diversity are urgently needed. In this context the Holmenkolen guidelines for sustainable aquaculture (1999) are an important document, because it promotes sustainability of animal breeding based on functional integrity.

In spite of the fact that phenotypic and genetic variation among different breeds of domestic animals is easily detected and recognized, there is still a critical question how to determine and objectively measure phenotypic and genetic variation within and between different breeds of domestic animals. In order to assess genetic variation, different types of data and approaches have been used:

- exterior data (height, weight, body mass and proportions)
- production data (milk yield, egg production, growth parameters, feed conversion, reproduction data)
- genotyping using conventional genetic markers (microsatellites, RFLPs, RAPD)
- genotyping using high density markers (SNP micro arrays).

Estimation of genetic distances based on phenotypic or genetic markers has been performed using different statistical tools and population statistics was used as a tool to understand the structure as well as the history of domestic animal populations (Ruane *et al*, 1999). Estimation of genetic distances between breeds became fashionable using different types of genetic markers. This has been particularly useful for the reconstruction of species/breed history (cattle, sheep, goat, chicken). Scientific community adopted recommendations in terms of the required number of animals (>25) and marker loci (25-30 MS) that should be used in such studies. However, the introduction of SNP chip technology resolved that problem quite efficiently and the use of standard SNP micro arrays (50k SNPs or more), which are in use for the purposes of genomic selection, offers sufficient amount of information to analyze fine structure of populations (enables detection of strains and color variants within the breed) and obviously makes separate genotyping of MS loci obsolete. Genetic profiling of populations in general can be used to demonstrate that populations that look and perform similarly are genetically different, but can not be used as an argument that populations that perform differently

are genetically the same. For the purpose of measuring biodiversity, the genetic indicator of biodiversity in farm animals has been proposed. The relationship is mainly dependant on the population size, proportion between sexes (1) and amount of genetic variability present in the population (2).

$$N_e = \frac{4N_m N_f}{(N_m + N_f)} \quad (1)$$

$$\left(\frac{1}{2N_e} \right) \sigma_g^2 = \text{Proportion of genetic variation lost in the next generation} \quad (2)$$

Considering only the number of individuals in animal populations, we would not be able to recognize threats for these populations which are caused by highly stratified population structure and unequal contribution of sexes to the next generation.

Tab. 1. Population census and effective population size of some high yield breeds

Cattle breed	Country	Period	Census population size*	Effective population size (N_e)	Reference
Holstein	Denmark	1983–1992	—	68	Sørensen <i>et al.</i> 2005
Holstein	Germany	1999	≈ 2 200 000	52	Koenig & Simianer 2006
Holstein	Denmark	1993–2003	≈ 3 700 000	49	Sørensen <i>et al.</i> 2005
Holstein	France	1988–1991 (?)	≈ 2 500 000	46	Boichard <i>et al.</i> 1996
Holstein	USA	1999	≈ 8 500 000	39	Weigel 2001
Jersey	Denmark	1977–1991	—	87	Sørensen <i>et al.</i> 2005
Jersey	Denmark	1993–2003	≈ 640 000	53	Sørensen <i>et al.</i> 2005
Jersey	USA	1999	≈ 550 000	30	Weigel 2001
Danish red	Denmark	1977–1998	—	157	Sørensen <i>et al.</i> 2005
Danish red	Denmark	2001–2003	≈ 560 000	47	Sørensen <i>et al.</i> 2005
Japanese black	Japan	1986–1990	—	30	Nomura <i>et al.</i> 2001
Japanese black	Japan	1993–1997	≈ 530 000	17	Nomura <i>et al.</i> 2001
Montbéliarde	France	1988–1991 (?)	≈ 700 000	125	Boichard <i>et al.</i> 1996
Abondance	France	1988–1991 (?)	≈ 65 000	106	Boichard <i>et al.</i> 1996
Normande	France	1988–1991 (?)	≈ 800 000	47	Boichard <i>et al.</i> 1996
Tarentaise	France	1988–1991 (?)	≈ 14 000	27	Boichard <i>et al.</i> 1996

From Taberlet *et al.*, 2008

Due to the fact that the numbers of animals in the most popular breeds of domestic animals are rather high, the general belief is that these breeds cannot be endangered. However, artificial insemination and rigorous selection enabled efficient spreading of favourable genes which originated from a relatively small number of high quality sires in large populations of highly productive animals. How this structuring of

populations effects the effective population size has been nicely shown by Taberlet *et al* (2008).

The national populations of Holstein cattle with the census over two million heads, have the effective population size of only several dozens, which is close, or even under the threshold set by FAO for endangered populations. This is a very specific situation which requires special measures to increase the effective populations size, mainly through the larger number of unrelated sires used for artificial insemination.

Economic evaluation of biodiversity

In spite of the fact that biodiversity is clearly recognized as an important component of our environment as well as agricultural systems, relatively few attempts were made to quantify the importance of biodiversity in economic terms. One of the most popular strategies is known as Weitzman's concept. It proposes to design biodiversity preservation strategy in four steps:

- Biodiversity assessment
- Economic evaluation of biodiversity
- Likelihood for improvement of the situation, due to our intervention
- Cost/benefit performance

Using modern technologies the first point does not represent a very difficult task, however, the second point is extremely difficult to perform and probably the most critical for the whole strategy and consequently crucial for the last point, cost/benefit analysis. In the recent time, as parameters for economic evaluation of biodiversity, the reduction of selection efficiency due to reduced genetic variability in highly selected herds, estimation of the depression, caused by higher level of inbreeding and reduction in robustness of high yield animals were mentioned as possible parameters for estimation of the economic value of biodiversity and consequently genetic variability.

Understanding genetics architecture of complex traits

Complex traits have complicated genetic structure which implies large numbers of genes which contribute in different proportions to the formation of the trait, whereby their mutual relationships reflect an extremely complicated functional network in which the simple additive mode of action certainly does not reflect the complexity of regulatory networks which are responsible for shaping these traits. Starting with the infinitesimal model of gene action as a basis for genetics of quantitative traits between 1950 to 1980, the introduction of the concept of quantitative

trait loci (QTL) reflected the development in molecular genetics and nourished the hope that we will be able to explain the vast majority of phenotypic variation with identification of 10-15 QTL per trait. Sobering results in the last few years, especially with the introduction of SNP chip technology and genomic selection, brought us closer to the infinitesimal model again but with the provision, that we will be able to find molecular explanation for subtle effects of large number of alleles affecting complex traits. Introduction of high throughput DNA technologies and development of bioinformatics opened an exciting period in which we will be able to better understand the genotype-phenotype relationship and to use this information for designing more efficient selection strategies and preservation strategies for still existing biodiversity.

Breeding and research strategies

One of the most important goals of breeding strategies in endangered populations is to increase the effective population size (N_e). This could be done by increasing the number of possibly unrelated individuals included in the reproduction of the population, by avoiding intense selection for special traits and by increasing the population size. In order to introduce important genetic material, which would improve the fitness of the population targeted introduction of desired genomic elements followed by back crossing seems to be an efficient method. To the limited extend, the exchange of genetic material between related populations can be applied. In this context, the local breeds represent a valuable source of haplotypes, adapted sometimes to very extreme environments.

Having this in mind, the question, where to search for most valuable genetic resources, arises. There play so called resource populations very important role. The resource populations are often extensively bred populations, without very focused breeding program and kept in isolation for some time. The special position among resource populations have populations, close to the domestication centers. In certain circumstances the populations in sporadic reproductive contact with wild relatives might be of particular interest. However, also synthetic populations of recent origin might be interesting resources of genetic variation, similar as individuals from hybridization zones in free living species.

The situation in which from the total of 3.831 breeds of domestic animals at the beginning of the 20th century, 16% are become extinct, 15% are rare and on average, one traditional breed dies out per week is quite obviously that targeted research of biodiversity and science based action is needed more than ever. Since the resources are insufficient, we

have to set priorities what to conserve. Therefore we need objective parameters for assessing global diversity of AnGenRes, which represent also a valuable research tool for identification of adaptive alleles.

Examples from breeding- and free living populations

In the following section two examples of successful application of genetic technology will be presented: Lipizzan horse and marble trout populations in Slovenia. In both cases, molecular data could be used first for breed- or population description and further for conservation plan proposals including monitoring of the efficacy of conservation actions (Dovc et al, 2004).

Lipizzan horse breed became the first pedigreed horse population where contribution of different lines was examined by DNA sequence analysis (Kavar et al, 1999). The pedigree data suggested very broad pool of mtDNA haplotypes as a consequence of numerous imports during the history of the breed. Indeed, 37 mitochondrial haplotypes, belonging to all four main equine mtDNA clusters, were identified in 56 Lipizzan maternal family lines (Kavar et al., 1999; Kavar et al, 2002). Comparison of molecular and pedigree data revealed, that some mares, founders of maternal lines, shared the same mtDNA haplotype. The distribution of mtDNA haplotypes among different Lipizzan studs reflects the intensity of exchange of breeding animals during the history of the breed. A good coverage of classical mare family lines was typical for traditional studs, whereas more remote studs, which were not included so frequently in these exchanges, showed very specific selection of only locally present mare family lines. However, more surprising was the fact that in some maternal lines more than one haplotype was found. This is an unambiguous proof that some pedigree errors occurred during the history of the breed. The minimal number of maternal pedigree errors in the whole Lipizzan pedigree was estimated to be 25, however it is possible that some pedigree errors remained hidden allowing even higher real number of errors. Based on this data, the proportion of animals having maternal parentage in their pedigree in disagreement with mtDNA haplotype in the present Lipizzan population is about 11%. Microsatellite data were used for estimation of microsatellite heterozygosity and genetic distances among Lipizzan studs and between Lipizzans and some other horse breeds. Analysis revealed clear clustering among studs and relative high degree of heterozygosity, comparable with other breeds, lagging only slightly behind much larger populations as for instance Holsteiner, Hannoveraner and Quarter Horses. From the point of the general usefulness of microsatellite markers in different horse breeds it is

important to stress that two MS loci, routinely used for parentage testing in horse (HMS3 and ASB2) have mutated priming sites in Lipizzans, with the consequence that null alleles and high discrepancy between expected and observed heterozygosity appeared at these loci in Lipizzans (Achman *et al*, 2001). However, the usefulness of microsatellite markers for controlling heterozygosity, inbreeding and paternity was confirmed. The availability of molecular data will enable consideration of rare mtDNA haplotypes and microsatellite alleles present in low frequencies as an important source of genetic diversity within the breed and will contribute to their preservation. This is especially important in a small, structured population for which the Lipizzan horse population is an outstanding example.

The marble trout has a restricted geographical range in the Po river basin in northern Italy and in the Adriatic river basin in former Yugoslavia and Albania, where it parapatrically coexist with indigenous brown trout. Natural hybridization between the two species was minimal (limited only to their contact zones) or none, maintaining undisturbed parallel evolution of both species. The situation has drastically changed when foreign strains of brown trout were introduced into the marble trout living space in the beginning of the last century. They built a reproductive bridge between the incipient species, which resulted in uncontrolled and unstoppable interspecies hybridization. In the late 1980s, hybrid swarms dominated the majority of Slovenian Adriatic river system and only few upper reaches were still inhabited with marble trout. An action plan for marble trout rehabilitation started in 1993. Its strategy was to establish a captive breeding programme with wild genitor stocks from pure marble trout populations, in order to release genetically pure individuals in the hybridization zone until almost all foreign alleles had been eliminated. In this sense, a diagnostic test for discrimination of marble trout from the hybrid swarms was required. Species determination based on morphology is due to susceptibility of morphological characters to environmental effects and invasive sampling inappropriate in such cases. Alternatively, molecular genetic approach can account for a variety of polymorphic characters that are not under selection pressure, and material collection is harmless for animals. Using the collection of marble trout informative markers, eight genetically pure populations of marble trout, separated from downstream hybrid populations by impenetrable barriers, were revealed in the Soča river basin (Snoj *et al*, 2000) providing material applicable for conservation strategies. The study based on 13 microsatellite loci, revealed that microsatellite allele number and heterozygosities of these eight populations were remarkably low compared

to genetic variation at microsatellite loci commonly observed in other fish species. Given the extremely low levels of genetic variability detected in those populations, identification of new genetic source of marble trout would be beneficial in order to augment the gene pool of progenitor stocks and to maximize genetic diversity of reintroduced populations. The use of specific genetic markers, developed for the marble trout population we were able to propose an efficient conservation programme, which took advantage from gene pool present in the hybrid zone and enabled use of resource populations which have been found in isolated tributaries based on genetic markers.

Animal genetic resources and modern genetics

The genomic era brought new approaches and many powerful technologies to animal science. From the genomic prospective we have for the first time the opportunity to use high throughput analyses which enable us to get for the first time an impression how do animals which differ in their phenotype differ at genomic level. However, the optimistic expectations underestimated heavily the complexity of the genome and the degree of genetic variation in domestic, and even to a greater degree, in free living populations. If we take as an example two unrelated animals, which belong to the same breed, but differ in a single trait, their genome sequence may differ in several million nucleotide positions which gives us no clue about the causal sequence for the observed phenotypic difference. This example illustrates well how important it is to select appropriate animal material and to define phenotypic traits as precisely as possible in genetic experiments. Based on new genomic technologies and analytical and computational tools which enable efficient processing of SNP and sequencing data, the idea of genomic selection was born. High throughput SNP typing using SNP micro arrays enables generation of complex genotypes containing information about 50.000 to 800.000 loci per animal for large number of animals (several tens of thousands to several hundreds of thousands) in a very short time. Statistical evaluation of these data allows establishment of statistical relationships between phenotypic traits and nucleotide sequence at defined positions through the entire genome. These relationships will after the initial learning phase replace collection of phenotypic data for selection purposes and allow selection purely based on DNA data. From the conceptual point of view this will bring us from the QTL principle which did not prove enough robustness and precision to be widely used across or at least within populations, back to the infinitesimal model, where we evaluate cumulative contribution of several hundred thousand of markers to the formation of a particular trait.

With the limitation to a certain causal locus, in farm animal species we already know about a dozen of such loci which significantly affect some traits, we can plan targeted introgression of favorable genetic variants into breeds which lack certain traits, the most illustrative examples are resistances to different diseases, using conventional breeding strategies. In the context of genetic diversity, this type of data allows us completely new insights into genetic richness of different breeds and genetic distances among them. This makes our decision about the most valuable animal genetic resources which should be preserved more objective, but not always substantially easier. New technologies allow us to identify new genotypes among phenotypically similar strains and to find valuable candidate genes for a number of adaptive traits.

Modern technology can help us to preserve and to improve the genetic base of our populations with potential for efficient production, but on the other side the advances in reproductive technologies give us new possibilities to design new solutions for *ex situ* preservation of genetic resources. This includes depositories of tissues (which generally have analytical value), depositories of germ cells (which open a chance for later reconstruction of the breed at extinction) and nuclear reprogramming and cloning (which even has potential for reconstruction of the breed from which only somatic cells, which may be frozen, are available).

CONCLUSION

In conservation actions the emphasis should be put on conservation of populations that are locally adapted and harbor different adaptive alleles. In a long term, the neutral diversity should be preserved. The special care deserve breeds, which are close to the domestication centers. Even unknown genetic variants may represent important genetic variation, necessary for further development of breeds and strains of farm animals.

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UTICAJ GENETIKE NA KONCEPT ODRŽIVE POLJOPRIVREDE

SAŽETAK

Skorašnji napredak molekularne i kvantitativne genetike i genomike pruža moćna sredstva za očuvanje i poboljšanje genetičkih resursa životinja. Mnogi od ovih novih sredstava primijenjeni su na procjenu situacije kada su u pitanju rijetke i ugrožene populacije. Stoga su hitno neophodne objektivne i međusobno uporedive metode evaluacije genetičkih varijacija između populacija, kao i ekonomsko vrjednovanje biodiverziteta. Neki primjeri iz prošlosti pokazali su ubjedljivo da primjena novih tehnologija može značajno da doprinese karakterizaciji i očuvanju genetičkih resursa životinja. Pored genetičkih i genomskih sredstava, postoji i određen broj tehnika u reproduktivnoj biologiji koji mogu efikasno podržati napore na očuvanju genetičkih resursa životinja.

Ključne riječi: genetički pristup, genomika, reproduktivna tehnologija, genetički resursi životinja