USE OF MOLECULAR MARKERS FOR EVALUATION OF GENETIC DIVERSITY AND IN ANIMAL PRODUCTION

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INTRODUCTION

Genetic markers provide information about allelic variation at a given locus. The increasing availability of molecular markers in farm animals allows the detailed analyses and evaluation of genetic diversity and furthermore the detection of genes influencing economically important traits. The majority of molecular markers used nowadays with high-throughput systems are microsatellite markers (simple tandem repeat, STR). Variable number of tandem repeats (VNTRs), random amplified polymorphic DNA (RAPD) markers, single strand conformation polymorphisms (SSCPs), restriction fragment length polymorphisms (RFLPs) and amplified fragment length polymorphisms (AFLPs) markers are not commonly applied in farm animals. Additionally within diversity and phylogeny studies specific mtDNA and Y chromosome markers are used for the identification of maternal and paternal lineages. Beyond diversity studies molecular markers are also used for mapping quantitative trait loci (QTL) and within marker assisted selection (MAS). Until recently microsatellites were the markers used for mapping quantitative trait loci for production and functional traits in farm animals (Hiendleder *et al*., 2003; Kühn *et al*., 2003) and tightly linked markers are used for marker assisted selection in practise. They are also the prerequisite for the identification of positional and functional candidate genes responsible for quantitative traits. The detailed use of molecular markers for the evaluation of genetic diversity and identification of economically important traits in animal production is presented on the basis of different examples.

EVALUATION OF GENETIC DIVERSITY

Livestock breeds have been formed by centuries of human and natural selection. Different breeds have been selected to fit several environmental conditions and human needs. The genetic diversity found in domestic breeds allows farmers to develop new characteristics in response to changes in environment, diseases or market conditions. Furthermore losing genetic diversity is a loss of civilization history. A considerable number of genetic distance studies for several livestock species were carried out during the last years by research teams from all over the world. Baumung *et al*. (2004) give a report about 87 different projects originated from 93 different countries. Most of these genetic distance studies were carried out for ruminants. They were mainly based on microsatellite loci, although a number of other polymorphic systems such as protein polymorphisms, blood groups, or other molecular marker systems were alternatively used (Baumung *et al*., 2004). The use of microsatellites has become a standard method to estimate neutral genetic diversity in livestock. To define species-specific standards, the International Society for Animal Genetics (ISAG) formed a FAO/ISAG advisory group on animal genetic diversity in 1995, which set up recommended species specific lists of microsatellite loci (about 30 per species) for cattle, chicken, sheep and swine to be used in diversity studies (http://dad.fao.org./). Peter *et al*. (2007) analysed the population structure and the genetic diversity of 57 European sheep breeds derived from 15 European countries. The analyses were based on microsatellite typing followed by principal component analysis and Bayesian model based clustering respectively. As a result of this study, distinct groups of sheep could be identified: Middle Eastern fat tailed sheep, south eastern European sheep and north western/western European sheep. Within the last group, two less distinct clusters comprised the Merinotype and Alpine breeds respectively.

As in sheep, most genetic diversity studies in cattle were first carried out on a national or regional scale and did not include genetic resources from other areas. For a more general understanding of diversity in the different species it is necessary to include reference samples and an overlapping set of markers/ standardized markerset to combine datasets from different analyses within metaanalyses. Markers such as microsatellites and AFLP were not only used to evaluate the genetic diversity but also to decide on conservation priorities of cattle breeds. Tapio *et al*.

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(2006) used the Weitzmann approach and the core set diversity measure of Eding *et al*. (2002) to quantify the contribution of each breed to the maximum amount of genetic diversity and to identify important breeds for the conservation of genetic diversity in Northern European cattle breeds. The European Cattle Diversity Consortium (2006) investigated the genetic distances between 69 European cattle breeds to indicate conservation priorities. In this study, two different methods of evaluation were used and combined: according to the Weitzman approach, contributions to genetic diversity are derived from genetic distances between populations. Alternatively diversity within and across populations is optimized by minimizing marker-estimated kinships. The authors discuss the prospects and limitations of marker assisted decisions on conservation priorities and conclude that current methods still need to be improved. The use of markers derived from genes within diversity studies can also lead to a deeper knowledge about the domestication and development of livestock species and breeds. For example, the analysis of the geographic distribution of casein haplotypes in cattle gave information about the consequences of the domestication process of modern cattle as well as the geographically differentiated natural or artificial selection respectively (Jann *et al*., 2004). Within this study Bos indicus specific casein haplotypes could be identified. The occurrence of these haplotypes in southern European breeds also suggests an introgression of indicine genes into taurine breeds during their development. Also the analysis of mitochondrial DNA or AFLP fingerprinting shed light on the process of domestication (e.g. Beja Peirera *et al*., 2006; Negrini *et al*., 2007) and allowed the characterisation of parallelisms in issues related to threats and conservation of domestic and wild animal species (Taberlet *et al*. 2007). As in cattle and sheep, a European project dealing with genetic diversity in pig breeds was established in the last years (Pig biodiversity Project II). The results of microsatellite typing followed by calculating the Reynolds distances present strong support for clustering of European pig breeds. Significant clustering of lines within main breeds was found (SanCristobal *et al*., 2006). Compared to other livestock species there are not many studies concerning the genetic diversity in camelids. Maté *et al*. (2005) investigated the genetic differentiation of four guanaco populations from Argentina and observed a high level of genetic diversity in this species.

USE OF MOLECULAR MARKERS IN ANIMAL PRODUCTION

With the help of molecular genetics, remarkable advances have been made over the last decades in the identification of loci and chromosomal regions containing loci that affect economically important traits in livestock production. The number of genetic markers is growing continuously and a main application of markers in animal production is the identification of QTL. Genome scans for QTL have been completed for all major livestock species (e.g. http://www.animalgemome.org/QTLdb/; http://bovineqtl.tamu.edu/index.html). If marker-QTL associations have been identified, MAS in breeding programmes can be applied with the aim of improving selection response (Meuwissen & Goddard, 1996). For a successful implementation of such QTL within selection programs, the identification of specific polymorphisms which are responsible for the observed effect i.e. the quantitative trait nucleotides (QTN) is needed (Ron &Weller, 2007). In German Holstein Cattle a whole genome scan was performed and QTLs for functional traits (Kühn *et al*., 2003), body conformation and behaviour identified (Hiendleder *et al*., 2003). Within the identical design Harder *et al*. (2006) detected QTL for lactation persistency traits. On the basis of a QTL identified on BTA 18 it could be shown using fine/comparative mapping strategies that it is possible to identify a candidate gene. DGAT1, the gene encoding diacylglycerol O-acyltransferase, was identified as candidate gene for milk production traits by Grisart *et al*. (2002) and Winter *et al*. (2002). In both studies a non conservative substitution of lysine by alanine (K232A) caused by an adenine/adenine to guanine/cytosine dinucleotide substitution is described. Since DGAT1 has main effects on fat content in milk the analyses of promoter variants of the bovine aS1-casein (CSN1S1) gene lead to a significant effect on the protein percentage in dairy cattle (Prinzenberg *et al*., 2003). The effect of DGAT could be further differentiated by including CYP11B1 variants (Kaupe *et al*., 2007). Not only quantitative traits were mapped via molecular markers but also genes for discrete traits. For example it was possible to map genes for genetic diseases like BLAD in cattle and to develop a DNA test to identify the unfavourable allele (Kriegesmann *et al*., 1997) and to identify the gene variant in pigs responsible for the maligne hyperthermia syndrome (MHS) which is closely related to meat quality (Fujii *et al*., 1991). Both tests are widely and successfully implemented within breeding programmes in the breeds affected and have reduced the frequency of the unfavourable allele. Furthermore internationally agreed molecular markers/marker sets are in the meantime used in parentage testing in nearly all farm animals worldwide. It was found that highly polymorphic microsatellites are more powerful for parentage control than the conventional use of biochemical markers like blood groups or biochemical marker systems (Glowatzki-Mullis *et al*., 1995). In addition, molecular markers are applied in the identification of source of animal/group on animal products and the determination of sex in embryos.

CONCLUSIONS

Molecular markers are used in genetic diversity studies which give us information about the kinships between

breeds and the domestication process. The favourite markers for these studies are mainly microsatellites but also AFLP, mtDNA or Y-chromosomal markers. On the other hand molecular markers already provide new opportunities to speed up selection of routinely measured traits or to select for new traits that are costly and/or difficult to record in farm animals and to improve animal production and productivity. The prospective availability of a large number of single nucleotide polymorphism (SNP) markers and high throughput technology for these markers will lead to a shift to SNP markers in near future. The potential to enhance rates of genetic improvement by using molecular information clearly exists. The full realisation of this potential will require investments in infrastructure and knowledge and will therefore be limited in the first step mainly to national/international important species/breeds within species. Moreover, it must be carefully targeted to provide optimal returns to breeding organisations and farmers.

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