A GENOME-ASSISTED MOET DESIGN FOR INBREEDING PREVENTION IN THE ENDANGERED MURNAU-WERDENFELSER CATTLE

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ABSTRACT

Murnau-Werdenfelser cattle are an indigenous breed from Upper Bavaria in Germany. The population is endangered and is supported by the Bavarian government. The aim of the study was to select optimal unrelated mating partners for the Multiple Ovulation and Embryo Transfer (MOET) procedure and to present a new genomic technology to breeders. Samples from 143 animals of the Murnau-Werdenfelser breed were genotyped with the Illumina BovineSNP50 BeadChip using standard procedures. Genome-wide realized relationships in the form of an identity by descent (IBD) matrix among 143 individuals were estimated based on 50,864 SNP markers. In order to facilitate the discussion with breeders and to provide a basis for mating decisions, the individual DPSdistance matrix was presented in two-dimensional (2D) space. Based on the positions of both parents in the 2D plot, the potential positions of all offspring were derived. For each included donor dam, a separate 2D plot was prepared. Breeders accepted this graphical method well and used it smoothly for selection of the optimal MOET mates.

Key words: cattle, endangered breeds, Murnau-Werdenfelser cattle, genetics, genetic relationship, genetic distances, IBD, MOET

1 INTRODUCTION

Murnau-Werdenfelser dual purpose cattle are an indigenous breed from Germany that is widespread in the mountain region of Upper Bavaria. They probably originated from a crossing of the native local population with Oberinntaler cattle. Later, some introgression of Franconian yellow and Braunvieh cattle took place. The medium body framed cattle are yellow to reddish brown in coat colour. On average, cows produce 4200 kg of milk with 3.8 % fat per lactation. The milk is very suitable for cheese production (Sambraus, 2010).

In the second half of the 19th century, the breed was famous for the draft ability of its oxen. A herdbook has existed since 1927. In 1936, the population counted more than 30,000 animals. The Independent Breeding Organisation for Murnau-Werdenfelser cattle was founded in 1952. Gradually, the Murnau-Werdenfelser breed was replaced by Simmental in the east and Braunvieh cattle in the west of Bavaria and the population size decreased dramatically (Sambraus, 2010).

In the middle of the 1970s, nine sires from three different lines were used for artificial insemination. Unfortunately, till 1984, these sires were not used to the same extent and at the same time. In natural service, there were only sires from one line. Breeders started to complain of inbreeding depression. Consequently, the breeding organisation approved of an introgression of the southeastern French Tarentaise breed in 1986. They imported semen from four Tarentaise sires that was tentatively used on only six herdbook farms. The population size of Murnau-Werdenfelser cattle at that time was estimated to be 526 animals with 239 cows on 74 farms (Schedel, 1986, 1987). Later, in 2005, the breeding organisation decided again to use Tarentaise for artificial insemination at

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a larger scale. All crossed offspring were recorded in the herdbook (Kinzelmann and Schedel, 2006).

In 2007, there were just 130 farms with animals recorded in the herdbook. The keeping of the breed is supported by the Bavarian government (Sambraus, 2010). Since 2008, 7663 semen samples from 17 bulls that belong to three bloodlines have been included in the State genetic reserves. Likewise, the breeding organisation decided to conserve purebred Murnau-Werdenfelser animals without introgression of foreign breeds. For this purpose, they chose an advanced reproduction technique named Multiple Ovulation and Embryo Transfer (MOET). Embryo Transfer (ET) uses Follicle Stimulating Hormone (FSH) to cause the ovaries to release more than one egg in a cycle what increasing the reproduction rate of females. Multiple eggs can be fertilised inside the cow and collected non-surgically after mating or artificial insemination. Embryos can be transferred into surrogate mothers (recipients) who are at the same stage of their cycle but have not been mated. Many of these embryos are accepted by the recipients and a normal pregnancy occurs. Alternatively, embryos can be frozen for sale or transferred later (Pistory, 2009). As a result of the first use of MOET in the years from 2009 to 2011, 20 offspring (17 sires, three cows) were born. Some sires were included in semen production and they have been used in the population until today.

In the recent years, genotyping with a large number of markers brought the opportunity to estimate the genetic relationship among animals more accurately compared to the pedigree data, which is especially important in highly related, inbred and endangered populations.

In 2016, it was decided to repeat the MOET offspring production to provide new sires for semen production. The fact that all sires and breeding dams in the Murnau-Werdenfelser cattle breed were genotyped with the Illumina BovineSNP50 BeadChip opened up the opportunity to use genomic data to search the most unrelated mating partners. Additionally, the breeding organization had stored semen from old ET sires in the gene bank and they selected the best dams from the population as donors.

The aim of the study was to select optimal unrelated mating partners and to present a new genomic technology to breeders in a user-friendly way.

2 MATERIALS AND METHODS

2.1 SAMPLES AND GENOTYPING

In the course of the previous and the current project, semen and tissue samples from 143 animals of the Murnau-Werdenfelser cattle breed were collected (39 sires and 104 dams). The animals were born between 1971 and 2015. All 143 animals were genotyped with the Illumina BovineSNP50 BeadChip array using standard procedures (http://www.ilumina.com). Quality control procedures excluded SNPs with genotyping errors (based on the available genotypes of relatives), unknown chromosomal position according to the *Bos taurus* genome assembly UMD 3.1 (http://www.cbcb.umd.edu/research/bos_taurus_assembly.shtml#1; autosomal SNPs only), call rate < 95 %, minor allele frequency < 0.025, and departure from Hardy-Weinberg equilibrium within the breed ($P < 0.01$). Finally, 50,864 autosomal SNPs with an average marker density of 49.15 kb were considered for analyses.

2.2 GENOME-WIDE RELATIONSHIP MATRIX

Genome-wide realized relationships in the form of an identity by descent (IBD) matrix among 143 individuals were estimated using the method of Powell *et al.* (2010), which was applied to 50,864 SNP genotypes. The result was a Unified additive relationship (UAR; 143x143) matrix. Despite known UAR values for all genotyped animals, only UAR values among sires that were ready for artificial insemination and living dams that were selected as donors were extracted.

2.3 CLUSTER ANALYSIS

The estimation of genetic distances between animals ($D_{PS} = \ln(PS)$) was based on the proportion of shared alleles (PS; Bowcock *et al.*, 1994). A heuristic approach, as described in Veit-Kensch *et al.* (2007), was used to present the individual D_{PS} distance matrix in two-dimensional (2D) space. The graphical presentations of 2D plots of the D_{PS} distance matrix were generated using the R programming language (R Development Core Team, 2008).

2.4 POTENTIAL POSITION OF THE MOET OFF-SPRING IN THE 2D PLOT

In the 2D plot, living dams and sires with IBD values of less than 0.05 that provided as potential parents of the MOET offspring were preselected. Due to the large genetic distances, the preselected mates were, in the majority of the cases, distantly positioned in the 2D plot. Based on the theory and the empirical observations from the first MOET (2009 to 2011), the potential positions of the

future offspring in the 2D plot can be precisely deduced. The placement of all potential offspring in the 2D space provided the basis for mating decisions and facilitated the dialogue between breeders and geneticists. In the course of this discussion, the most important "regions" in the 2D plot that were interpreted as regions of the diversity space (e.g. Medugorac *et al.*, 2009) could be explored and the optimal positions of future breeding sires could be determined in a more comprehensible manner.

3   RESULTS AND DISCUSSION

3.1   GENETIC RELATIONSHIP MATRIX

An essential difference between realized and expected genetic relationships between individuals is always to be expected. The expected relationship between full-sibs is always 0.5. In reality, however, some full-sibs are more related (e.g. 0.6) than others (e.g. 0.4). This depends on chance alone, but the average expected value is 0.5. Realized genetic relationships do not consider the pedigree data but count chromosomal fragments that are identical by descent (IBD) between two individuals instead. With

the large number of whole-genome genotypes (SNPs) that are available, realized genetic relationships become more accurate compared to the expected relationships that are estimated from the known pedigree information of the animals recorded in the herdbook.

Consequently, a special matrix in three different colours was constructed to facilitate interpretation for the breeders, the owners of the donor dams (Fig. 1). Within the matrix, IBD (UAR) values higher than 0.100 were marked in red, IBD values between 0.050 and 0.099 in yellow, and IBD values less than 0.050 in green. Only sire-dam pairs with an IBD value less than 0.050 were considered as potential parents of the MOET offspring.

3.2   CLUSTER ANALYSIS

The individual D_{PS} distance matrix among all included Murnau-Werdenfelser animals is shown in 2D space in Figure 2. Sires for AI (light blue) and donor dams (orange) are marked with different colours to gain a clear overview which proportion of the 2D space is covered by the newly selected donor dams. As demonstrated by Simčič *et al.* (2015), the projection of the multidimen-

Legend: Lab-ID – laboratory number, Inb – Inbreeding, MWF – Murnau-Werdenfelser, IBD – identical by descent, red box - IBD higher than 0.100, yellow box – IBD from 0.050 to 0.099, green box - IBD less than 0.050

Figure 1: The IBD matrix among potential mating pairs for the MOET offspring
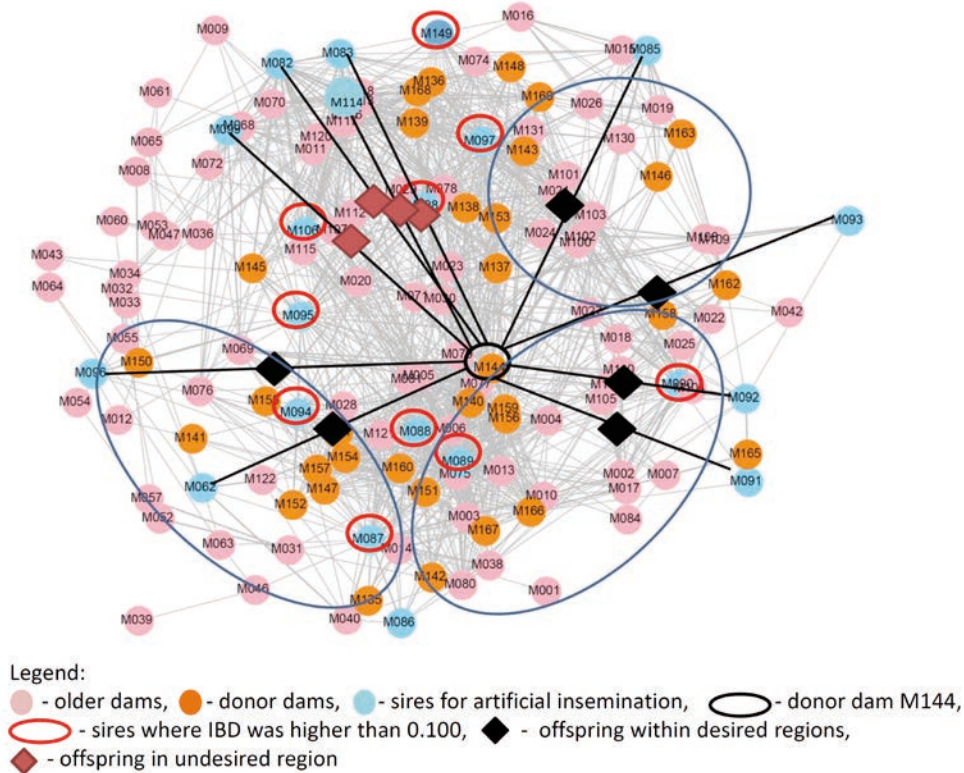
Figure 2: Individual DPS distance matrix with the locations of potential offspring from donor dams in two-dimensional space. The three most important regions where future AI bulls should be placed were marked by ellipses.

sional D_{PS} distance matrix in 2D space is capable to reflect the most important relations among animals of one or many subpopulations.

3.3 POTENTIAL POSITION OF THE MOET OFF-SPRING IN THE 2D PLOT

After intensive discussion of the entire diversity space of Murnau-Werdenfelser cattle, the breeders agreed on the three most important "regions" in which future AI sires should be placed (Fig. 2). For each of the considered donor dams, a single 2D plot was prepared. Figure 2 exemplifies such a plot in which the donor dam M144 is connected to all unrelated potential sires. Based on the position of the dam and all potential sires in the 2D plot, the positions of all potential offspring were derived. As illustrated in Figure 2, only a small proportion of matings will result in an offspring that is placed in one of the three most important "regions" of the diversity space for selection of the future AI sires. Complementary use of the future sires that are placed in these three "regions" should reduce inbreeding in the next generation

of Murnau-Werdenfelser cattle. In those regions of the diversity space that were considered less important at this stage, sires that had recently been used over AI centres were overrepresented.

4 CONCLUSIONS

In this current, practically relevant project, we present the results in a visual way that was as simple and user-friendly as possible. For each included donor dam, a separate 2D plot was prepared. The breeders accepted this graphical method well and used it smoothly for selection of the MOET mating pairs; the practical implementation of this method thus is already in progress. Genotyping with a large number of markers brought the opportunity to estimate the realised genetic relationships among animals more accurately and independent of the presence and quality of the pedigree data. Therefore, this genome-assisted MOET design could also be used in other highly related, inbred and endangered populations, especially when pedigree data are not available.

5   NOTE AND ACKNOWLEDGEMENTS

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