Fine-scale population structure and inbreeding in Slovak Pinzgau cattle

V. Kukučková¹, R. Kasarda¹, N. Moravčíková¹, W. Nandolo² & G. Mészáros³

¹ Slovak University of Agriculture in Nitra, Department of Animal Genetics and Breeding Biology, Tr. A. Hlinku 2, 94976 Nitra, Slovakia
veron.kukuckova@gmail.com (Corresponding Author)
² Lilongwe University of Agriculture and Natural Resources, Department of Animal Science, P.O. Box 219, Lilongwe, Malawi
³ University of Natural Resources and Life Sciences, Department of Sustainable Agricultural Systems, Gregor-Mendel Strasse 33, 1180 Vienna, Austria

Summary

The aim of this study was to assess the fine-scale population structure and level of inbreeding in Slovak Pinzgau cattle as a preparatory step towards designing appropriate mating plans aimed at controlling the level of inbreeding and maintaining or increasing the current genetic diversity. The population structure was constructed from a genetic distance matrix using a network-visualization approach implemented in NetView, complemented with principal component analysis (PCA) and a genetic distance heatmap. Inbreeding was computed using runs of homozygosity (ROH). Relatively low genetic variability was observed in the population, especially in bulls. Most of the bulls clustered together except for one bull and his daughters. More than half of the mating combinations were between highly related individuals. Recent genomic inbreeding reached 0.85% in bulls and 0.94% in cows while historical inbreeding cover 5.69% of genome in bulls and 6.23% in cows. Despite the low variability, it is possible to design appropriate mating plans, and such plans are very necessary. The study has shown that such plans can be derived from observing the fine-scale structure of the population. The information from this study will be useful for farmers.

Keywords: avoidance of inbreeding, genetic diversity, kinship, mating

Introduction

Local breeds of livestock are of conservation significance as components of global biodiversity and as reservoirs of genetic variation relevant to the future sustainability of agriculture (Williams et al., 2016). Breeding of Slovak Pinzgau dual purpose breed has a long tradition in Slovakia. The population of purebred Slovak Pinzgau reached a maximum in 1978, and started to decline after 1990, mostly due to transformation processes in agriculture and widespread use of Holstein sires for crossbreeding (Kadlečík et al., 2013). The Slovak Pinzgau cattle are now classified as an endangered breed. One of the critical issues in the management of the breed is keeping inbreeding levels low. Typically, this can be achieved by having a well-designed mating plan (Kasarda et al., 2017), but such a plan requires in-depth understanding of the structure of the population.

Different statistical approaches have been developed to assess the relationships among populations and to allocate individuals to their respective populations. Membership of different individuals to population subgroups can be deduced using traditional principal component analysis (PCA). Neuditschko et al. (2012) proposed a high-definition network-
visualization approach which can correctly assign individuals to their respective breeds or sub-populations. The approach has an option for unsupervised clustering of all individuals based on genetic distances. The level of inbreeding can be computed from runs of homozygosity (ROH), which are consecutive stretches of homozygous SNP genotypes (Williams et al., 2016; Ferenčakovic et al., 2013).

The objective of this paper was to assess fine-scale population structure of Pinzgau cattle and the genomic inbreeding based on ROH of different minimum lengths as a preparatory step towards designing appropriate mating plans aimed at controlling the level of inbreeding and maintaining or increasing the current genetic diversity.

**Material and methods**

Pinzgau cattle from Austria (105 animals; Ferenčakovic et al., 2013) and Slovakia (115 cows, 37 bulls) were genotyped using Illumina BovineHD SNP Chip and Illumina BovineSNP50 v2 BeadChip. All common markers with unknown position as well as those assigned to sex chromosomes were removed. SNP data quality control was done using PLINK v1.09 (Purcell et al. 2007). Animals with more than 10% missing genotypes were excluded. A minor allele frequency threshold of 0.01 was used for network-visualization approach performed by NetView (Steining et al., 2016) and PCA. Further analyses were performed including information from 39,056 SNPs. For subsequent ROH calculation only markers with GenCall score > 0.7 or GenTrain score > 0.4 were used (45,936 SNPs). For 37 Slovak bulls and 108 cows, five inbreeding coefficients (F\textsubscript{ROH>1}, F\textsubscript{ROH>2}, F\textsubscript{ROH>4}, F\textsubscript{ROH>8} and F\textsubscript{ROH>16}) based on ROH of different minimum lengths were calculated.

**Results and discussion**

The Slovak and Austrian Pinzgau cattle created one admixed population expect for one bull with Canadian origin (Figure 1A). Figure 1B shows more details in the relationships within the Slovak population. The genetic distances of bull Carlo (red arrow) and his daughters from other individuals is evident. The population of Slovak bulls was characterized by relatively low genetic variability. Similarly, admixed sub-populations of bulls and cows were found based on PCA (Figure 1C). Only bull Carlo and most of his daughters created individual cluster and increased the genetic variability of the Slovak Pinzgau breed. The kinship of observed bulls with all examined cows is shown in Figure 1D. The heatmap shows kinship between the animals (increasing from white through yellow to red), where a red combination means an inappropriate mating combination. Less than half of all possible combinations would be suitable for mating.

Descriptive statistics of molecular inbreeding coefficients calculated from ROH are given in Table 1. ROH greater than 1Mb (historical inbreeding) cover on average 5.69% of genome in bulls and 6.23% in cows while inbreeding estimated by ROH >16Mb (recent inbreeding) reached 0.85% in bulls and 0.94% in cows. Generally, the value 1% means level of population endangerment. Ferenčaković et al. (2013) found genomic inbreeding in Austrian Pinzgau even higher, from 1.4% (F\textsubscript{ROH>16}) to 6.9% (F\textsubscript{ROH>1}). However, the Slovak Pinzgau have a much lower effective population size than the Austrian Pinzgau (Kukučková et al., 2017).
Figure 1A. Metapopulation of Slovak and Austrian Pinzgau visualized by network approach. B. Detailed view of sub-structures within the Slovak Pinzgau population. Austrian Pinzgau – pink, Slovak bulls – blue (Red arrow – bull Carlo - Canadian origin), Slovak cows – yellow, Slovak mothers of bulls – green. C. PCA plot of Slovak Pinzgau bulls (black dots), cows (blue dots), bull Carlo (green dot) and daughters of Carlo (red dots). D. Heatmap of genomic distances between 115 Slovak cows and 37 bulls. Red colour indicates stronger kinship.

There is need for systematic design of mating plans, and the study has shown that such plans can be derived from observing the fine-scale structure of the population. The results suggest that despite the low effective population size in the Pinzgau population, it is still possible to design matings that can lead to increased diversity (Figure 2).
Table 1. Descriptive statistics of the molecular inbreeding coefficients ($F_{ROH}$) calculated from runs of homozygosity with different size (>1, >2, >4, >8, and >16 Mb) in 37 Pinzgau bulls and 108 Pinzgau cows (in parentheses).

<table>
<thead>
<tr>
<th>$F_{ROH}$</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{ROH} &gt; 1$</td>
<td>0.0569</td>
<td>0.0252</td>
<td>0.0141</td>
<td>0.1815</td>
<td>0.0521</td>
<td>0.0617</td>
</tr>
<tr>
<td></td>
<td>(0.0623)</td>
<td>(0.0259)</td>
<td>(0.0202)</td>
<td>(0.1243)</td>
<td>(0.0536)</td>
<td>(0.0709)</td>
</tr>
<tr>
<td>$F_{ROH} &gt; 2$</td>
<td>0.0344</td>
<td>0.0241</td>
<td>0.0008</td>
<td>0.1535</td>
<td>0.0298</td>
<td>0.0389</td>
</tr>
<tr>
<td></td>
<td>(0.0368)</td>
<td>(0.0246)</td>
<td>(0.0009)</td>
<td>(0.0983)</td>
<td>(0.0286)</td>
<td>(0.0450)</td>
</tr>
<tr>
<td>$F_{ROH} &gt; 4$</td>
<td>0.0253</td>
<td>0.0220</td>
<td>0 (0)</td>
<td>0.1387</td>
<td>0.0212</td>
<td>0.0295</td>
</tr>
<tr>
<td></td>
<td>(0.0268)</td>
<td>(0.0220)</td>
<td>(0.0817)</td>
<td>(0.0195)</td>
<td>(0.0341)</td>
<td></td>
</tr>
<tr>
<td>$F_{ROH} &gt; 8$</td>
<td>0.0165</td>
<td>0.0192</td>
<td>0 (0)</td>
<td>0.1343</td>
<td>0.0128</td>
<td>0.0202</td>
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<tr>
<td></td>
<td>(0.0186)</td>
<td>(0.0176)</td>
<td>(0.0679)</td>
<td>(0.0128)</td>
<td>(0.0245)</td>
<td></td>
</tr>
<tr>
<td>$F_{ROH} &gt; 16$</td>
<td>0.0085</td>
<td>0.0151</td>
<td>0 (0)</td>
<td>0.1057</td>
<td>0.0057</td>
<td>0.0114</td>
</tr>
<tr>
<td></td>
<td>(0.0094)</td>
<td>(0.0109)</td>
<td>(0.0356)</td>
<td>(0.0058)</td>
<td>(0.0131)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. The potential mating pairs of 7 living sires and 11 mothers of bulls with molecular inbreeding coefficients ($F_{ROH} > d$) < 1% based on genomic distances. The most suitable combinations are coloured from white to yellow. Orange and red colour represent related or genetically similar individuals.

Conclusions

The population of Slovak bulls has low genetic variability. More than half of all possible mating combinations between the bulls and available cows are between highly related
individuals. These results suggest that despite the low effective population size in the Pinzgau population, it is still possible to design matings that can lead to increased diversity. This information will be very useful for farmers to make appropriate cattle mating decisions.

Acknowledgement

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