Y chromosome genetic diversity and breed relationships in native Polish cattle assessed by microsatellite markers

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Abstract: Cattle provide a wide range of products and services to humans; thus the significance of conservation of livestock genetic resources is evident. We analyzed the genetic variability of five Y-chromosomal microsatellites in 395 bulls representing six cattle breeds (Polish Red, Polish Whitebacks, Polish Red-and-White, Polish Black-and-White, Polish Holstein Friesian, and Simmental). We identified three haplotypes in the paternal gene pool of analyzed populations. Haplotype diversity was low and frequencies ranged from 9.87% to 100%. In Polish autochthonous breeds (Polish Red, Polish Whitebacks), we detected two and three haplotypes. The network analysis revealed that tested bulls of both breeds were distributed in two clearly separated haplogroups. The dominant frequency in the Polish breeds had the haplotype also prevalent in the majority of European cattle. Only three Whitebacks carried a haplotype not identified until now in any international cattle breed. We showed that partitioning of genetic variation among breeds can be mainly explained by their assignment to past improvement crossings and reflected the low male effective population size. Our findings point also to a great importance of preservation of genetic diversity in indigenous cattle breeds.

Key words: Bos taurus, genetic diversity, Y-specific microsatellites, conservation

1. Introduction

Recognition of genetic diversity of livestock species is important when considering uniqueness of breeds and defining conservation priorities. Although Y-chromosome diversity is lower than autosomal, and is additionally reduced by a common use of a small number of selected males in many livestock species, it has been shown that the studies of male lineages added much to what can be inferred only from mtDNA and autosomal variation (Edwards et al., 2000; Lindgren et al., 2004; Anderung et al., 2005; Götherström et al., 2005; Edwards et al., 2007; Li et al., 2007; Ginja et al., 2009; Pérez-Pardal et al., 2010b). Moreover, while mtDNA variants stay mostly within the herd, Y-chromosomal variants may reflect the origin of sires as influenced by introgression and upgrading of cattle. Cattle history inferred from mitochondrial DNA markers suggests that cattle domestication involved at least two genetically distinct auroch species (Bos primigenius) in the Near East and the Indus Valley (Loftus et al., 1994; Troy et al., 2001). A similar division of modern domesticated cattle into the two main phylogenetic branches (taurine and zebu) was reported based on analysis of Y-chromosome haplotypes (Kantanen et al., 2009). Pérez-Pardal et al. (2010a) distinguished three main groups of sires separated by evolutionary time that clearly predated domestication. These three groups were consistent with the haplogroups previously identified by Götherström et al. (2005): Y1 (more frequent in northwestern Europe) and Y2 (dominant in southern Europe and Anatolia) in taurine cattle (Bos taurus), and Y3 being exclusive to zebu cattle (Bos indicus). Registered specific geographic diversity of the Y-microsatellite haplotypes reflected, as expected, a distinct origin and history of individual haplotypes/breeds.

Since cattle domestication in the Neolithic age, about 800 breeds of cattle have been raised, of which only 38% are currently not at risk of extinction (FAO, 2007). Moreover, highly productive breeds have replaced local ones in many regions of the world. Accordingly, two issues regarding the value of local cattle breeds are worth emphasizing. First, recent studies indicate that not the number of genes, but their complicated regulation is the essence of somatic complexity and determines all biological properties (Mattick et al., 2010). Secondly, in the face of growing genetic erosion, low-production breeds may be fundamental for the effective conservation of farm animal genetic resources. The indigenous breeds

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are usually characterized by a combined type of utility and good adaptation to the local environmental conditions. Moreover, they have historical and cultural value, and expand opportunities for rural development. Two cattle breeds (Polish Whitebacks and Polish Red) analyzed in the present study are indigenous Polish breeds derived from primitive cattle once residing in the northeast of Europe (Polish Whitebacks; Prawocheński and Kączkowski, 1926) or from the eastern part of Central Europe and Scandinavia (Polish Red; Adametz, 1901). While the herdbook for the Polish Red breed was established in 1895, the contemporary herdbook for Polish Whitebacks was founded only in 2003. Polish Red was also the first breed of cattle covered by the National Rare Livestock Breeds Preservation Programme (NRLBPP; since 1999). More cattle breeds were included later, such as Whiteback (2003), Polish Red-and-White, and Polish Black-and-White (2007–2008).

The present territorial division has become a barrier that separated phylogenetically related populations and restricted gene flow in many regions of the world, e.g., Whitebacks, which also survived in Lithuania. This is also evident in the case of red cattle from different countries (including Ukraine, Latvia, Lithuania, and Estonia). The appropriate selection of animals for the restoration program requires great effort and responsibility, since past crosses with sires of international breeds significantly hamper breeding management. An example of specific difficulties in assessing the contribution of foreign breeds in the gene pool of the protected population could be the Polish Whitebacks, considered almost extinct in the 1970s. Its variety, defined as lowland cattle (Zulawki cattle), became completely extinct in the first half of the 20th century. In the first actions taken regarding restoration in the mid-1990s, only about 50 individuals that corresponded to the description of the breed were inventoried. Similar difficulties, although to a much smaller extent, occurred at the beginning of the Polish Red cattle preservation program and problems with pure breed material still exist.

The aim of this work was to investigate Y-chromosomal haplotype type and diversity in Polish indigenous cattle breeds covered by NRLBPP using five Y-chromosome microsatellite loci. Specifically, we aimed to determine the paternal gene pools of the analyzed breeds and the influences of foreign breeds during improvement crossings. In addition, we investigated the phylogenetic relationships of the Polish cattle with other Eurasian breeds using the haplotypes available in the literature.

2. Materials and methods
Blood samples were collected as follows: Polish Red (PR, n = 81), Polish Whitebacks (PW, n = 21), Polish Red-and-White (PRW, n = 80), Polish Black-and-White (PBW, n = 80), Polish Holstein Friesian of the black-white variety (PHF, n = 106), and Simmental bulls (SIM, n = 27) used in the past for the improvement crossings with Polish local breeds. Two male samples of Rathi cattle (RAT, India) were analyzed as zebu cattle representatives. Four breeds of cattle (PR, PW, PRW, and PBW) were covered by NRLBPP. Twenty-five cows (PR, n = 15; PBW, n = 10) were also screened as a control group to test for the male specificity of Y-chromosomal microsatellites. Pedigree information was used for sample selection to minimize the degree of relatedness.

We extracted DNA from blood samples using the Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer’s recommendations. We amplified five Y-chromosomal microsatellites: INRA124, INRA189, BM861 (Edwards et al., 2000), BYM-1 (Ward et al., 2001), and DYZ1 (Kantanen et al., 2009) using 50–100 ng of DNA, the given amount of each primer (6 pmol for BYM-1 and DYZ1; 15 pmol for INRA189; 1.5 pmol for INRA124, and 3 pmol for BM861), and 1X AmpliTaq Gold 360 PCR Master Mix (Life Technologies, USA). The markers were PCR-amplified with annealing temperatures of 66 °C (BYM-1) and 60 °C (DYZ-1). INRA124, INRA189, and BM861 were typed in a multiplex PCR as described in Edwards et al. (2000). Fragment size of amplified DNA was determined on ABI 3130 Genetic Analyzer (Life Technologies, USA) using the GeneMapper software (version 4.0) and an internal size standard (GeneScan 500 LIZ Size Standard, Life Technologies, USA). The consistency of the sizes of different alleles was standardized by using breeds with fixed alleles analyzed by other authors (Kantanen et al., 2009). No amplification product was obtained when using control female DNA.

Allele frequencies for Y-chromosomal loci were determined by standard gene counting. The number of Y-haplotypes and haplotype frequency were calculated for allelic combinations (INRA124-INRA189-BM861-DYZ1-BYM1) recorded as haplotypes using GENALEX v.6.5 (Peakall and Smouse, 2006). In addition to quantifying genetic differentiation, GENALEX v.6.5 was used to calculate haploid genetic diversity (h). The F ST calculations were made with ARLEQUIN v.3.5 (Excoffier et al., 2005). Genetic diversity between populations was assessed by the analysis of molecular variance (AMOVA), using the same software. We considered two hierarchical levels: 1) populations clustered into groups and 2) individuals clustered into populations. The analyzed breeds were subdivided into two groups. The first consisted of two old autochthonous breeds (PR and PW), and the second consisted of three breeds having their roots in Holstein Friesian cattle (PRW, PBW, and PHF). The significance of the variance components and fixation indices was then tested using 10,000 permutations with Bonferroni correction. Single locus gene diversity values were calculated over all populations using ARLEQUIN v.3.5.
A median-joining (MJ) network was constructed using NETWORK 4.6.1.2 (www.fluxus-engineering.com) and the algorithm of Bandelt et al. (1999). The differences in genetic variance at five analyzed loci were compensated by giving the lowest weights to the loci with the highest variances: INRA124, INRA189, BM861, DYZ-1, and BYM-1 were given weights of 9, 7, 10, 7, and 10, respectively. Otherwise, program default settings were applied in the network construction. The haplotype nomenclature was modeled on Kantanen et al. (2009). Results for the two zebu bulls were chosen to represent the Bos indicus alleles for Y-chromosome microsatellites and used for haplotype network construction. The results of Rathi cattle were excluded from the analyses of genetic diversity because we were more interested in analyzing the population structure of Polish autochthonous breeds.

3. Results

We identified seven alleles in six cattle populations from Poland and three alleles in zebu cattle. All Y-chromosomes of the cattle from Poland were classified to be taurine (detection of taurine-specific allele INRA124 (132 bp), Edwards et al., 2000; Hanotte et al., 2000) while Y-chromosomes of two RAT bulls were classified to be zebu (detection of zebu-specific allele INRA124 (130 bp) (Edwards et al., 2000; Hanotte et al., 2000) and INRA189 (88 bp), Edwards et al., 2000). At the Y-chromosomal markers in taurine cattle we detected two alleles at INRA189 and BYM-1, and single alleles each at BM861, DYZ-1, and INRA124. The allele INRA189 (102 bp) was found in 27 SIM and in 12 bulls of Polish autochthonous breeds (8 PR and 4 PW).

We observed a total of four different haplotypes in the analyzed populations: three haplotypes in taurine cattle and a single haplotype in zebu cattle (Table 1). Overall, four breeds reared in Poland (PBW, PR, PHF, and SIM) exhibited only a single haplotype, while in the autochthonous breeds we detected two (PR) and three (PW) haplotypes. The most common H11 haplotype (INRA124 (132 bp)-INRA189 (98 bp)-BM861 (158 bp)-DYZ-1 (366 bp)-BYM-1 (258 bp)) had a total frequency of 89.37% and was observed in 353 bulls from five breeds. The second most common haplotype, termed H16 (INRA124 (132 bp)-INRA189 (102 bp)-BM861 (158 bp)-DYZ-1(360 bp)-BYM-1 (258 bp)), had a frequency of 9.87% and was detected in three breeds (39 bulls in total). The remaining H8 haplotype (INRA124 (132 bp)-INRA189 (98 bp)-BM861 (158 bp)-DYZ-1 (360 bp)-BYM-1 (258 bp)) was detected with low frequency (0.76%) only in three bulls of the Polish Whitebacks.

When inspecting genetic variation within breeds, PW was the most diverse breed in terms of haplotype number and haploid diversity. The haploid diversity calculated for the entire Y-data set was low (0.037 ± 0.019). The haploid diversity calculated per breed varied from 0.000 to 0.151 where more than one haplotype was found (Table 1). The locus-wise F ST estimates calculated from taurine data ranged from 0.192 (INRA189) to 0.277 (DYZ-1), with an average distance value of 0.239 (INRA124, BM861, and BYM-1 were not considered in the calculation). Average distance values for each breed compared to the rest of the breeds indicated high differentiation of the breeds (Table 1). Average pairwise values of FST showed that all populations (except for the PHF, PBW, and PRW) were significantly different (Table 2). In our studies the genetic

<table>
<thead>
<tr>
<th>Breed</th>
<th>N</th>
<th>Haplotype frequency (%)</th>
<th></th>
<th></th>
<th></th>
<th>h</th>
<th></th>
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<tbody>
<tr>
<td>PW</td>
<td>21</td>
<td>14.28</td>
<td>66.68</td>
<td>19.04</td>
<td>0.151</td>
<td>0.467</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>81</td>
<td>90.13</td>
<td>9.87</td>
<td>0.071</td>
<td>0.247</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBW</td>
<td>80</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.312</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRW</td>
<td>80</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.312</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHF</td>
<td>106</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.327</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIM</td>
<td>27</td>
<td>100</td>
<td>0</td>
<td>0.922</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAT</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0.941</td>
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differences among cattle populations within two tested groups using Y-chromosome DNA markers obtained by the AMOVA analysis explained 5.166% of the total genetic variation, while 73.499% of the variation was explained among analyzed six breeds on population level (Table 3).

Relationships among Y-chromosome haplotypes found in the analyzed six breeds and haplotypes published earlier by Kantanen et al. (2009) are shown in the MJ network in the Figure. The network of Y-haplotypes formed a structure of three phylogenetic haplogroups. In our data

Table 3. AMOVA for Y chromosome haplotypes identified in six breeds of cattle reared in Poland.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Variance components (%)</th>
<th>F statistics</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Among groups</td>
<td>Among breeds within groups</td>
</tr>
<tr>
<td>Historical origin:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polish local breeds: PR, PW</td>
<td>2</td>
<td>18.794</td>
<td>5.166</td>
</tr>
<tr>
<td>PBW, PRW, PHF</td>
<td>3</td>
<td>Overall</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>5</td>
<td>18.794</td>
<td>5.166</td>
</tr>
<tr>
<td>Breeds reared in Poland: PW, PR, PBW, PRW, PHF, SIM</td>
<td>6</td>
<td>-</td>
<td>73.499</td>
</tr>
</tbody>
</table>

Figure. Network representation of the haplotypes identified in six cattle breeds reared in Poland and in Rathi cattle. The haplotypes of Kantanen et al. (2009) were used as reference. The circle size is proportional to the haplotype frequency. Breed groups are represented as follows: PR (vertical), PW (cross), PRW (backward diagonal), PBW (forward diagonal), PHF (diagonal cross), SIM (horizontal), Rathi (solid white), and reference haplotypes (solid gray).
the majority of sires (from all but the SIM breed) carried the haplotype H11. All SIM sires, eight of PR, and four of PW shared the haplotype H16 and fell into the second haplogroup together with PW sires carrying the H8 haplotype (within two mutational steps from the H16). The most striking feature of the network was the location of sires of the two indigenous Polish breeds (PR and PW) in two different haplogroups.

4. Discussion

For the presented study we used the set of five cattle Y-specific microsatellites to assess diversity on the male path in six cattle breeds reared in Poland. Y-chromosomal microsatellites used in our study coincided with those previously used in Portuguese cattle (Ginja et al., 2009), Ethiopian cattle (Li et al., 2007), the Lidia breed (Cortes et al., 2011), 60 breeds of Eurasian cattle (Kantanen et al., 2009), 138 breeds of different geographical origin (Edwards et al., 2011), and 45 cattle populations from Europe and Africa (Pérez-Pardal et al., 2010b). We observed low diversity at three markers (single alleles at INRA124, BM861, and BYM-1). Similar results have been also reported by others (Ginja et al., 2009; Cortes et al., 2011). This points to the overall low diversity of the Y-chromosome markers, which may be due to either a low mutation rate or selection affecting the allelic diversity. It has been also shown that particular microsatellite alleles were either indicus-specific allele (INRA189 (88 bp) (Edwards et al., 2000), INRA124 (130 bp) (Edwards et al., 2000; Hanotte et al., 2000), and BM861 (156 bp) (Edwards et al., 2000; Özşensoy et al., 2014)) or were found only in a few individuals from indigenous breeds of different geographical origin (INRA189 (90 bp), INRA189 (82 bp) (Kantanen et al., 2009); INRA124 (134 bp) (Özşensoy et al., 2014)). Regarding studies of Y-chromosomal microsatellites, up until now the highest diversity was observed in the INRA189 marker in a wide range of cattle breeds (Ginja et al., 2009; Kantanen et al., 2009; Cortes et al., 2011; Edwards et al., 2011).

Haploid diversity found in our data set was lower than previously reported: 0.064 (Kantanen et al., 2009), 0.042 (Ginja et al., 2009), 0.099 (Li et al., 2007), and much lower than 0.42 (Cortes et al., 2011). Our results may be biased downwards due to the incomplete overlap of the markers used for the analysis. Moreover, three breeds analyzed here (PHF, PBW, and PRW) have their roots in an international cattle breed. In turn, restitution programs of PR, and especially of the PW breed, were made with a small number of males. Specifically, the current Polish Whitebacks population consists of only just over 300 cows entered in the book and 17 bulls allowed to breed (Kasprzak et al., 2014). Moreover, little is known about the paternal genetic influences at the beginning of the PW restitution program. Furthermore, geographical structuring in taurine cattle populations is also compatible with an overall poor genetic differentiation of the Y chromosome (Kantanen et al., 2009).

Compared with mtDNA, the small number of males used for breeding and male-mediated crossbreeding has accelerated the loss of Y-chromosomal variation in domestic cattle. As a consequence, it led to the genetic dilution of many local breeds worldwide. For instance, several Scandinavian, Russian, and Ukrainian cattle have been influenced by gene flow from commercial cattle breeds (Kantanen et al., 2009). A similar pattern of haplotype frequency distribution with a few haplotypes represented in higher frequency has been also described in other domestic animal species, e.g., in horses (Lindgren et al., 2004) and sheep (Meadows et al., 2006). In our study, one (H11) of the three identified haplotypes represented 89.37% of the total frequency. Samples obtained from PR and PW revealed that the H11 haplotype was present in the majority of specimens, which suggested weak genetic distinctiveness of patrilines and may confirm the share of bulls of foreign breeds in the current gene pools of these two old Polish local breeds. In addition, the high Y-specific marker F ST values were in agreement with the higher proportion of the total variance explained by the genetic differences among the breeds on a population level. This may reflect the low male effective population size and the loss of the significant portion of paternal genetic diversity before the systematic conservation program was implemented. All Simmental bulls typed in our analysis and in Kantanen et al. (2009) had the haplotype H16; however, in the study by Götherström et al. (2005), Simmental bulls were distributed in both Y1 and Y2 haplogroups. The presence of haplotype H16 in PW, being the only haplotype found in SIM until now (Kantanen et al., 2009; present study), should be considered a genetic signature of the crossbreeding with this breed rather than an indication of their common ancestral origin. Although the H16 haplotype was previously identified in other local breeds from Eastern Europe and Anatolia (Kantanen et al., 2009), Simmentals have been the most widely distributed cattle breed worldwide and were used for cross-breeding with many local breeds. Interestingly, three sires of PW bore haplotype H8 that was earlier found exclusively in Ukrainian Whiteheaded cattle (Kantanen et al., 2009). This suggests that the H8 haplotype could be indigenous in the origin of the local breeds from this region.

The network analyses revealed that PR and PW bulls fell into two clearly separated haplogroups. This also confirmed the share of foreign male material in the present gene pool of these breeds. Similarly, the common use of elite bulls of Holstein Friesian cattle in breeding schemes resulted in elevated frequency of the H11 in
European cattle (Kantanen et al., 2009). All PHF, PBW, and PRW bulls carried this haplotype, too. In previous studies that used autosomal markers, Holstein Friesian cattle showed a comparatively wide gene exchange with all nine cattle populations analyzed, including PR, PBW, and PRW (Grzybowski and Prusak, 2004a). The greatest gene migration was observed between the PBW and PRW cattle. Since the upgrading of cattle is usually done using a small number of bulls, we obtained reduced levels of haplotype diversity (and prevalence of H11 haplotype) in male material. On the other hand, the analysis of autosomal microsatellites showed the uniqueness of the gene pool of PR cattle included in NRLBPP, with five private alleles identified (Grzybowski and Prusak, 2004b). Similar results were reported by Sawicka-Zugaj and Litwińczuk (2012), with ten private alleles identified each in PR and PW. These studies indicated that both old Polish autochthonous breeds, PR and PW, still hold a significant proportion of the old population specificity despite the crossing with other breeds applied in the past. One explanation for these Y-chromosomal/autosomal discrepancies might be the greater maternal (as opposed to paternal) genetic contribution to the gene pool of the present PR and PW populations.

In the present study, we evaluated the paternal gene pool of Polish cattle breeds covered by the NRLBPP and demonstrated that Y-chromosomal microsatellites can be successfully used to recognize paternal components in local cattle breeds when breeding history is difficult to track back (scarce or no pedigree data at the start of the restoration program). Our findings also pointed to the limited paternal origin of Polish local cattle populations and evident male-mediated gene flow from foreign breeds. Similar problems associated with genetic dilution affected many local livestock breeds, while, on the other hand, demand for the local specific products is still increasing worldwide. Integration of data derived from different types of genetic markers (nuclear, Y-chromosome, and mitochondrial) could provide wider insight into the historical and present populations of autochthonous cattle breeds. It may also facilitate the determination of the conservation priorities and proper breeding schemes.

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