

## Short Communication

# Changes in the relationship between an endangered cattle breed and its mainstream sister breed over time

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## HIGHLIGHTS

- Resampling of genotypes allowed to estimate significance of fixation index.
- Birthdate based cohorts unraveled changes in relationship between two sister breeds.
- The proportion of opposing homozygotes was used to complete pedigree.
- More genotypes would be useful for this purpose.

## ARTICLE INFO

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## ABSTRACT

Because of lack of pedigree records, the history of the Red-Pied of the Ösling (RPO) breed, which is an endangered local red-pied cattle breed from Luxembourg, is not really known. However, it is assumed that there has been exchanges between the RPO and the Meuse-Rhine-Yssel (MRY), another red-pied cattle breed from The Netherlands. To validate this assumption, we estimated the changes in the relationships between the RPO and MRV breeds over time by the definition of cohorts with different birthdates in the MRV breed. The fixation indexes between the RPO breed and the different MRV cohorts, as well as a principal component analysis, showed that the RPO breed was more related to the oldest MRV animals, born before 1990, and to the youngest MRV animals, born after 2009. This confirmed the *a priori* known pattern of importation of MRV animals to the RPO population over time. Based on results obtained in a genomic relationship matrix and on the proportion of opposing homozygotes, we could infer sire-offspring relationships between an MRV sire and three RPO animals. To complete the pedigree of RPO animals even further and optimize exchanges between the MRV and RPO breeds, it would be interesting to have access to more MRV genotypes.

## 1. Introduction

Local breeds often tend to crossbreed with other similar-looking breeds in order to limit inbreeding rate and/or to maintain acceptable genetic progress in important traits. An extreme example is the replacement of local black and white populations by Holsteins through upgrading (Tatueva and Koltsov, 2023). Holstein animals with red-pied colour were also commonly used to improve local red-pied breeds, e.g., Simmental (Goncharenko et al., 2022), and red and white dual-purpose

populations (Schmidtman et al., 2021). Historically, before the influence of Holsteins, local red-pied dual-purpose cattle with very small population size were already collaborating with larger populations of red-pied breeds (Gengler and Wilmot, 2022). For example, from a previous study (Wilmot et al., 2023), we know that there has been admixture events between the Meuse-Rhine-Yssel (MRY) and the Red-Pied of the Ösling (RPO), a local breed originating from the North of Luxembourg.

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The RPO has an endangered breed status according to the European legislation. Only one breeder of this dual-purpose red-pied breed is officially registering its animals in the Herdbook of the breed. Moreover, little is known about the history of RPO and its pedigree is not complete because the RPO was not officially recognized for a long time: subsidies to sustain breeders who registers animals in the Herdbook were launched in 2014 through agri-environment climate measures. Therefore, based on the results previously obtained in Wilmot et al. (2023), showing admixture between RPO and MRY, a more detailed analysis of the genomic relationship between the RPO and MRY would help to unveil some parts of the history of the RPO breed. Moreover, inferring pedigree relationships between RPO and MRY, based on genomic relationships and counts of opposing homozygotes, could further clarify recent exchanges between both breeds.

The objective of this study was to estimate the changes in the relationship between the RPO and MRY breeds over time, based on estimated genomic relationships, and, by extension, to infer sire-offspring relationships between MRY and RPO animals.

## 2. Material and methods

Genotypes of 132 RPO and 292 MRY, coming from a previous study (Wilmot et al., 2023) were used. The same quality control (QC) as in Wilmot et al. (2023) was used and left 39,967 SNPs for further analyses. The QC was performed with Plink v.1.9 (Purcell and Chang, 2019).

### 2.1. Changes in the relationship between RPO and MRY over time

In order to investigate the changes in the genomic relationship between the RPO and MRY breeds over time, the MRY samples were divided into four cohorts, according to their birthdate. The MRY animals born before 1990 were referred as MRY1 ( $n = 64$ ), those born between 1990 and 1999 as MRY2 ( $n = 62$ ), those born between 2000 and 2009 as MRY3 ( $n = 117$ ) and those born after 2009 as MRY4 ( $n = 49$ ). As the birthdates of RPO animals were mostly spanning the last decade, they were kept as a single cohort. Moreover, the RPO animals were coming from a single breeder, the only one that is registering animals in the Herdbook of RPO. There might be other animals representative of the RPO breed in the Ösling region (North of Luxembourg) but they have not been discovered so far. The single population used in the current study was therefore well representing the RPO breed, as defined by the Herdbook.

We used Plink v.1.9 (Purcell and Chang, 2019) to estimate Weir & Cockerham's fixation index ( $F_{st}$ ) between the RPO population and the different MRY cohorts. To estimate if the  $F_{st}$  values between the RPO and the different MRY cohorts were significant, we made 1000 random samples of MRY, coming from all cohorts. The size of these random samples was the same as the smallest cohort of MRY, i.e.,  $n = 49$ . We computed  $F_{st}$  values between each of these random samples of MRY and the RPO population. We then computed a Bootstrap interval of 95 % for these  $F_{st}$  values. We considered that the  $F_{st}$  value between RPO and a specific MRY cohort was significant if outside the Bootstrap interval estimated on random samples of MRY. The rationale behind this strategy was to determine divergence/convergence that were more significant than what would have happened randomly. A principal component analysis (PCA) based on the matrix of correlation of genotypes of MRY and RPO animals was performed, using the FactoMineR package v.2.8 (Lê et al., 2008) of R software v.4.2.2 (R Core Team, 2022). Results were visualized with Rstudio 2022.02.1 + 461 (R Studio Team, 2023).

### 2.2. Pedigree discovery in RPO

Among the 132 RPO animals, 16 had no pedigree and another 16 had a missing sire. For these animals, we wanted to use the MRY population to discover the sire. For this purpose, the allelic frequencies (AF)

of MRY and RPO breeds were computed and then averaged across the breeds. Following the first method of VanRaden (2008), these averaged AF were then used for standardization of a genomic relationship matrix (GRM). This GRM was computed with the `calc_grm` program (Calus, 2016) available in the MiXBLUP software (ten Napel et al., 2021).

We extracted pairs of RPO-MRY animals with a genomic relationship higher than 0.10, to capture relationships as far as first-degree cousins which have an average relationship of 0.125. Based on values obtained with the GRM, it was possible to infer sire-offspring relationships for which the average relationship is 0.50. We therefore considered values higher than 0.45 as sire-offspring pairs. Pedigree, if available, and proportion of opposing homozygotes between two animals were used to confirm the sire-offspring relationship.

## 3. Results

Fig. 1 shows the first two principal components (PCs) of the PCA. The first PC explains the variation between the two breeds while the second PC explains the variation within the MRY breed. Table 1 shows the different  $F_{st}$  estimates between RPO and the different MRY cohorts. Both Fig. 1 and Table 1 show that the MRY1 cohort was more related to the MRY2 cohort than to the two other cohorts of MRY and more related to the MRY3 cohort than to the MRY4 cohort. As expected, MRY animals from one cohort were more related to animals from cohorts of MRY with closer birthdates than to cohorts of MRY animals with more distant birthdates. Therefore, based on the birthdate, there was a gradient of relationships within the MRY breed. Both Table 1 and Fig. 1 also demonstrated that the RPO population was more related to MRY1 and MRY4 than to MRY2 and MRY3. The Bootstrap interval of 95 % for  $F_{st}$  values between RPO and a random sample of MRY ranged between 0.028 and 0.033, meaning that the MRY2 and MRY3 cohorts significantly diverged from the RPO population.

Based on the GRM, the average relationship within RPO, excluding self-relatedness, was 0.030, while the average relationship of RPO with the different MRY populations was negative:  $-0.032$  with MRY1,  $-0.042$  with MRY2 and MRY3 and  $-0.034$  with MRY4, following the trend of  $F_{st}$  values. We detected 106 pairs of MRY and RPO animals with a genomic relationship higher than 0.10 and for which 47 different RPO and 26 different MRY were involved. It was also found that three pairs of MRY-RPO animals had a genomic relationship close to 0.47. The same MRY animal, from cohort MRY3, was involved in these three pairs, making highly probable that the three different RPO were half-siblings and this MRY animal, with Dutch and German origins, their sire. The proportion of opposing homozygotes between this MRY animal and the three RPO also confirmed the sire-offspring relationship (values of 0.015 %, 0.013 % and 0.023 %). The sire was confirmed by the available pedigree for one of the RPO.

Based on the GRM, we also found 12 pairs of MRY and RPO with genomic relationships between 0.20 and 0.25, suggesting either half-siblings or grandsire and second-generation offspring relationships. The available pedigree also confirmed five of these pairs as grandsire and grand offspring.

## 4. Discussion and conclusion

The study of the changes in the relationship between the RPO and MRY breeds over time showed that the RPO population was more related to the oldest and youngest cohorts of MRY (MRY1 and MRY4). This result confirmed the known history of the studied RPO population, raised by one breeder. Both RPO and MRY are known to originate from the same breed group (Wilmot et al., 2023). Importations of MRY animals to RPO were relatively common before 1990. However, they already started to slow down from the 1980's as most breeders of local red-pied breeds, of which MRY, turned to Holstein. Moreover, from 1982, the Luxembourgish Herdbook started to organise performance

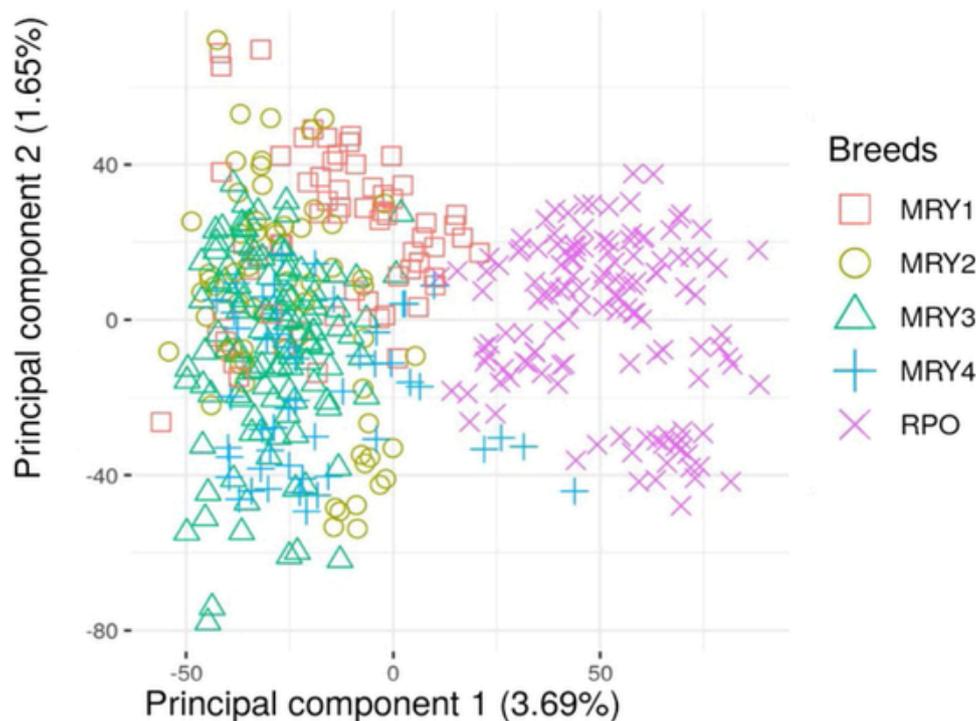


Fig. 1. First two components of a principal component analysis for the Red-Pied of the Ösling (RPO) and the different Meuse-Rhine-Yssel cohorts (MRY1 to MR4).

Table 1

Pairwise Weir & Cockerham's fixation index values between Meuse-Rhine-Yssel (MRY) cohorts and Red-Pied of the Ösling (RPO) breed.

Cohort/breed	MRY1	MRY2	MRY3	MRY4
MRY2	0.010			
MRY3	0.015	0.009		
MRY4	0.019	0.013	0.006	
RPO	0.032	0.036*	0.037*	0.031

MRY1 = cohort of Meuse-Rhine-Yssel animals born before 1990; MR2 = cohort of Meuse-Rhine-Yssel animals born between 1990 and 1999; MR3 = cohort of Meuse-Rhine-Yssel animals born between 2000 and 2009; MR4 = cohort of Meuse-Rhine-Yssel animals born after 2009; RPO = Red-Pied of the Ösling. \*: significant divergence between red-pied of the Ösling and a Meuse-Rhine-Yssel cohort.

recording with Germany and major breeding collaboration followed in 1990 with the beginning of genetic evaluations. Importation of Rotbunte DN (RDN) semen and animals, from Germany, to the RPO population were then favoured. This fact was also strengthened by the preference of the RPO's breeder for deep red colour and more beef-like animals compared to the MR type. Here it must be noticed that RDN was not collaborating with MR or importing MR animals at a large scale at that time. This explains why, between 1990 and 2009, the divergence between RPO and MR started to increase. However, during the last decade, the available RDN sires were more and more direct sons or grandsons of MR animals. Through this path, the RPO population started to import indirectly MR genetic material. The relationship between the RPO and MR populations therefore started to increase during the last decade. Direct importations also exist as illustrated by the MR sire discovered for three RPO animals.

However, until now, importation of animals or semen from the RDN or MR breeds to the RPO population is suboptimal because breeders are mainly making their choice of importations based on the phenotype of the animal (i.e., the Luxembourgish breeder prefers less white, more deep red animals with more meat), even if a genomic tool for breed as-

signment like in Wilmot et al. (2022) is also used to complement this first impression. It would therefore be necessary to optimize importations of animals from other breeds to the RPO population in terms of inbreeding rate, introgression of foreign alleles and genetic gain. For this purpose, a simulation of exchanges between the different breeds, like in Slagboom et al. (2022), can help to define the optimal number of MR and/or RDN sires to be imported in the RPO population.

As illustrated in this study, the common origin and the regular, direct, but also indirect, exchanges of animals and semen between the MR and RPO breeds can help to discover and/or correct the pedigree of RPO animals that can be relatively incomplete. With the help of a GRM and the proportion of opposing homozygotes, we could infer the sire of three RPO animals. The next steps to build the pedigree of this population could be the discovery of the maternal grand-sire, through the discovery of the sire, or by directly counting the number of opposing homozygotes between the maternal grand-sire and the offspring (VanRaden et al., 2013). An improved version of this parentage testing is using haplotypes instead of SNP conflicts counts. Our use of GRM to infer parentage and relationships is similar to the strategy of Munoz et al. (2014) or Moore et al. (2019) who used a realized relationship matrix to detect possible pedigree conflicts when the relationship of two animals was not within the expected range. Similar to us, Kaseja et al. (2022) combined the use of opposing homozygotes and GRM to check the pedigree. In their study, they defined a minimum threshold of relationships under which they can define pedigree errors. This minimum threshold is based on reference animals for which the relationship is known. However, in our case, as the pedigree of RPO animals was not very complete and that most of the genotyped RPO animals were born in the last decade, it was not possible to define a reference population with verified pedigree relationships. An interesting follow-up of the study would be to extend the screening of MR and RPO genotypes to infer pedigree relationships of RPO animals. This would be only possible if we have access to more MR genotypes.

There has been, there are and there will probably be importations of MR genetic material to the RPO population. Genomic relationships be-

tween both breeds can help to complete and correct the pedigree of RPO. Access to more MRY but also especially to RDN genotypes would help in this regard. Organizing exchanges of bulls and semen between these breeds in a manner that benefits the maintenance of the endangered RPO breed would be an important follow-up.

### Ethical approval

The SNP data for the animals included in this study were previously obtained on samples collected by concerned breeder associations based on relevant authorization by the different local authorities.

### Consent for publication

Not applicable

### Data availability

The data supporting the findings of this study cannot be made available as a whole. The corresponding author, upon reasonable request, will forward request to relevant data owners.

### Uncited references

R Studio Team, 2023.

### CRedit authorship contribution statement

**H. Wilmot:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **N. Gengler:** Writing – review & editing, Supervision, Methodology, Investigation. **J. Bormann:** Writing – review & editing, Investigation. **M.P.L. Calus:** Writing – review & editing, Supervision, Methodology, Investigation.

### Declaration of competing interest

The authors declare that they have no competing interests.

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