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Homozygosity indicators in canine MHC region of the Standard Poodle and Leonberger populations

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Dog breeds are the leading examples of artificial selection, with sometimes extreme changes between the wolf-like phenotypes and current breeds. This increased selection pressure manifest in increased homozygosity throughout the genome, including the major histocompatibility complex (MHC) with large influence on the immune system. The MHC region in 98 Leonberger and 37 Standard Poodle dogs was examined using single nucleotide polymorphism (SNP) data. The overall homozygosity levels and via the runs of homozygosity (ROH) were calculated as indicators to assess the MHC regions, compared to other random parts of the dog genome. High proportion of homozygosity was observed in all examined chromosomes, ranging from 58 to 78%. The ROH was preferred to the overall level of homozygosity, as it showed the variability within the MHC regions. The homozygosity was even lower at the locations of the genes with a known effect on the immune response, confirming previous findings.

Keywords: Run of homozygosity, SNP, Dog, Dog leukocyte antigen

1 Introduction

Domestication of animal species has led to animals going through artificial selection through many consecutive generations in order to attain desired phenotypic and behavioural attributes, which led to significant variance between and within domestic animal species (Rodenburg and Turner, 2012). Dog breeds in particular are leading examples of artificial selection, with sometimes extreme changes between the wolf-like phenotypes and current breeds (Brown et al., 2019). Due to economic and aesthetic reasons, breeders have created a diversity of dogs of different sizes, which led to increased inbreeding and sometimes to change natural characteristics.

The dog chosen for the paper were the Leonberger and the Standard Poodle, also known as the Großpudel in German speaking countries. Both the Leonberger and the Standard Poodle originated from Germany, even if the Standard Poodle is often thought to be French. The Leonbergers have a large muscular body, and apart from being companions, they can be used as guard or rescue dogs. The breed was established by crossing a black and white Newfoundland (or Landseer) bitch with a long-haired male from the Hospice of the Saint Bernard (St. Bernard). After a few generations, a white Phyrenäen dog was added. It is assumed that the Leonberger breed has 7 founders. In the 1880s the Leonberger population went almost extinct because of hunger and war, causing a bottleneck. There was a need to repopulate those breeds, and in order to do so, breeders had to intensify selection and inbreeding (Khatib, 2015). The Leonberger population in Austria is around 500, with nine males and 11 females used in the breeding.

The Poodles were initially used as water retrievers. After the increase of their popularity and standardization of the breed in France the use of the dogs focused on exploitation of their intelligence as performance assistance dogs, apart from their iconic role as companion animals. The Poodles were very much appreciated in the USA and Britain in 1950s, which led to intensive breeding, which in turn

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led to undesirable consequences such as eyesight problems (Biniok, 2008). Biniok (2008) further explains that after some years of being intensively bred, the Poodles lost their popularity among owners. This caused a reduction in inbreeding thus the population's genetic diversity increased. Historical background has an effect on the dog's health, and this can be observed in certain region of the genome. Currently there are almost 600 Standard Poodles in Austria, with 22 females and 27 males actively used in breeding.

From the health perspective the artificial selection can be both beneficial and harmful to the animal welfare, and can affect the animal health (van Rooijen, 2014). The major histocompatibility complex (MHC) is a region of the genome with a tightly knitted cluster of genes vital for the immune system, regulating adaptive and distinctive immune systems (Kennedy et al., 2011, Klein, 1986, Wagner, 2003). Kennedy et al. (2011) explained that MHC is vital to our understanding when it comes to vulnerability to diseases, reaction to vaccines and the development of the autoimmunity.

The region is made up of three genetic regions which are classified as Class I, Class II and Class III, where Class I and II contain polymorphic genes and Class III contains genes such as TNF-alpha (Kennedy, 2009). In the *Canidae* family, mostly in canine, the MHC is where the most of the Dog Leukocyte Antigen (DLA) can be found, specifically in the MHC Class I and Class II (Kennedy et al., 2001, Safra et al., 2011, Wagner, 1999).

Alleles and haplotypes of DLA have been linked to a variety of autoimmune syndromes in dogs, comprising also the MHC Class II genes (Safra et al., 2011). In general, the major DLA section has been linked to chromosome (Chr) 12 (Barth et al., 2016, Safra et al., 2011, Xiao et al., 2016, Yuhki et al., 2007a). The DLA-12, DLA-64, DLA-79 and DLA-88 positions have been analysed the literature (Table 1).

Table 1 The location of the DLA in the MHC regions for *Canis lupus familiaris* (Dog)

Class	Name	Chromosome	Location/Mb	Reference
MHC Class I	DLA-88	12	0.892 – 0.896	(Graumann et al., 1998; Wagner et al., 2000; Xiao et al., 2016)
MHC Class I	DLA-12	12	0.933 – 0.937	(Burnett et al., 1997; Wagner, 2003)
MHC Class I	DLA-64	12	0.984 – 0.987	(Burnett et al., 1997; Wagner, 2003)
MHC Class II	DLA-DRA1	12	2.13 – 2.14	(Debenham et al., 2005; Wagner et al., 1995)
MHC Class I b	DLA-79	18	41.14 – 41.17	(Burnett and Geraghty, 1995)

Analysing run of homozygosity (ROH), for the past ten years, has become the avant-garde methodology to assess inbreeding in both animal and human populations (Meyermans et al., 2020). ROH are adjoining region of the genome where there are extended genomic stretches in homozygotes in an individual (Ceballos et al., 2018, Rebelato et al., 2018) and can be observed in an individual when it inherits two copies of ancestral haplotype, subsequently making this haplotype homozygous by descent (Ceballos et al., 2018). Those regions can be detected by molecular markers and through analysis can provide beneficial information such as accurate inbreeding estimates, evaluation of a population, evolution of a species and to identify signatures of selection (Rebelato et al., 2018).

Taking all this in consideration, it will be interesting to improve our understanding of the MHC region and the DLA in relation to ROH and homozygosity indicators. The aim of the study is to assess the level of inbreeding in the Leonberger and Standard Poodle breeds, and to find whether there is a relationship between ROH and homozygosity indicators and Canine MHC. As the dog health depends on the MHC region, there is a possibility to help breeders to make better decision regarding the improvement of their population by observing the genomic regions with DLA 88, DLA-12 and DLA-64 and the DLA-DRA1 on Chr 12 and DLA-79 on Chr 18.

2 Material and methods

2.1 Breeds SNP data and data preparation

The single nucleotide polymorphism (SNP) of 98 Leonberger (42 male and 56 female) and 37 Standard Poodle (13 male and 24 female) dogs genotyped with the Illumina CanineHD BeadChip was provided by FERAGEN GmbH company. PLINK 1.9 (Chang et al., 2015) was used for data preparation and calculation of overall homozygosity indicators, being one of the most popular tools for analyzing SNP genotypes and ROH both in human and animal populations (Meyermans et al., 2020).

The SNPs data for both dog breeds went through quality control (QC) following parameters to remove any data that could affect further analysis, i.e. include only SNPs and individuals with at least 90% genotyping rate and individuals which were complying to the p-value threshold of 0.00001. For this QC minor allele frequency was not considered, in order to account for SNPs fixed in the whole population, which account as valid parts of the ROH segments. After QC, 207,661 SNPs in the Standard Poodle and 207,045 SNPs in the Leonberger were left. The QC output data for Leonberger and Stadard Poodle were used for the next steps.

Chr 12 and Chr 18 were a deliberate choice as the canine MHC region and DLA-79 are positioned on the respective chromosome. Furthermore, the same genomic region was extracted from three random chromosomes, namely Chr 3, Chr 20 and Chr 32 as a control.

2.2 Analysis of homozygosity and ROH of the different regions

For each chromosome, a 6.0 Mb region was extracted from 0 Mb to 6.0 Mb position on Chr 3, 12, 20 and 32, and from 38.0 Mb to 44.0 Mb on Chr 18. The nucleotide map position started at the beginning of the chromosome and therefore it did not include the centromere, which is also not considered on the NCBI database used to locate the position of the genes. The MHC Class I and Class II were localised according to the results from Yuhki et al. (2007) and the NCBI database.

The proportion of homozygosity was calculated as the average of the number of observed homozygosity for each individual to the number of non-missing SNPs per individuals in each dog group; the latter were obtained using the function --het in PLINK 1.9. ROH was analysed using the cgaTOH software (Zhang et al., 2013) with ROH segments that contained more than 20 SNPs having a minimum length of 0.5 Mb. Missing SNPs and heterozygosity were not allowed in the runs. Results from the cgaTOH software were used to calculate the average length of runs in Mb in each breed for the selected chromosome.

The visualizations were done with the R software (R Core Team, 2013) and Microsoft Excel 2010.

3 Results and discussion

3.1 The MHC region position

Yuhki et al. (2007b) and Safra et al. (2011) have localised the MHC regions between 3.0 to 6.0 Mb measuring the length of the region after the centromere. The centromere was not considered in the NCBI database in reference to this region, which may lead to confusions. Therefore, for our research we defined the different regions by starting at the end of the centromere in measuring the length of our 6Mb long regions. This led to positioning the MHC Class I region in the window of 0.26 Mb to 1.04 Mb and the MHC Class II between 2.09 and 2.86 Mb.

The highest number of SNPs were observed in Chr 12 with an average of 901 SNPs followed by Chr 18 with an average of 670 SNPs, and the lowest on Chr 3, 20 and 32 with an average of 513 SNPs (Figure 1). The higher number of SNPs in Chr 12 and 18 might be explained with the presence of the highly polymorphic region MHC region (Barth et al., 2016, Wagner et al., 2000, Wagner et al.,1995).

3. 2 Homozygosity indicators

The average length of runs in both breeds ranged from 0 to 6 Mb (Figure 2). Chr 12 in the Standard Poodle seemed to have higher average ROH than the Leonberger, whereas the Leonberger had a higher average run on Chr 18 than the Standard Poodle. Furthermore, Figure 2 illustrates that some of the dogs had the whole studied chromosome segment in a homozygote state. We can also observe that the average length of run for both breeds on Chr 3, 20 and 32 was also different.

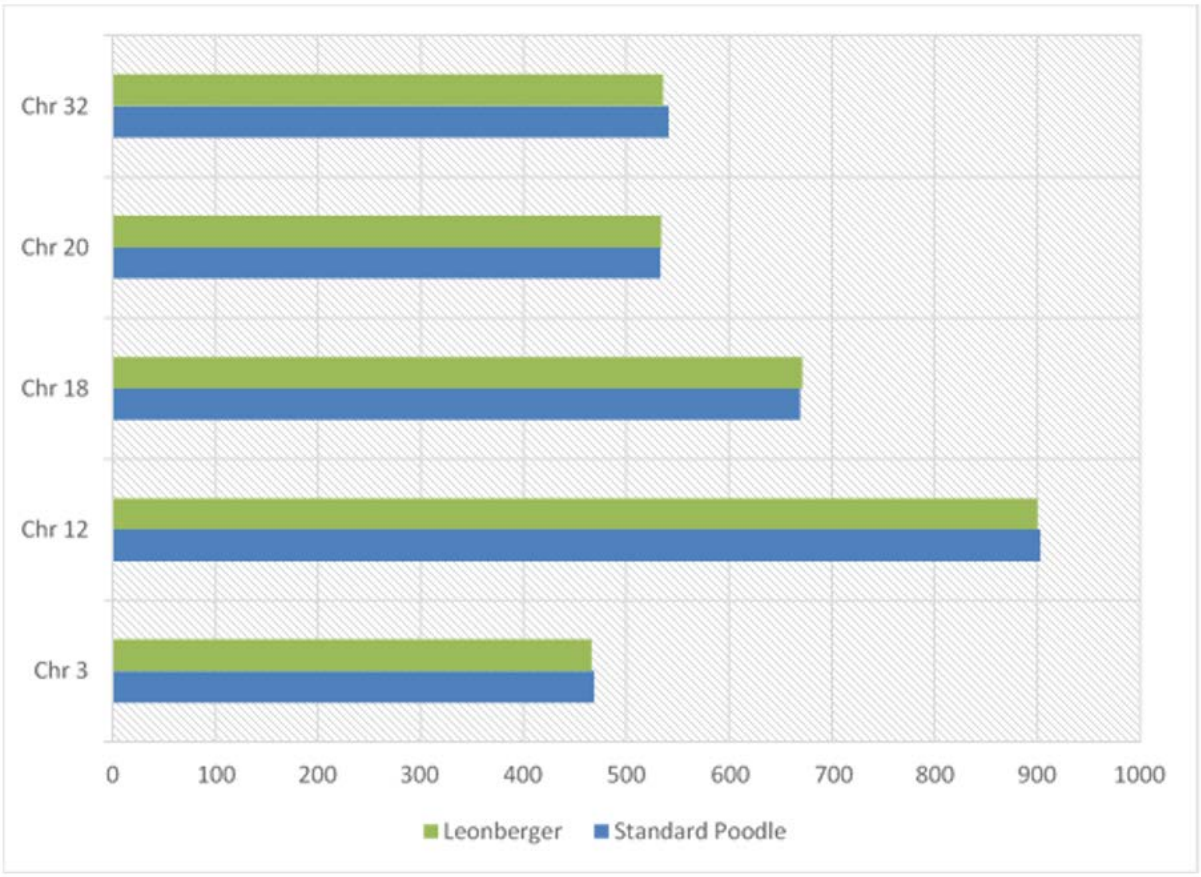

Figure 1 Number of SNPs per chromosome in the 6 Mb region of the selected chromosomes

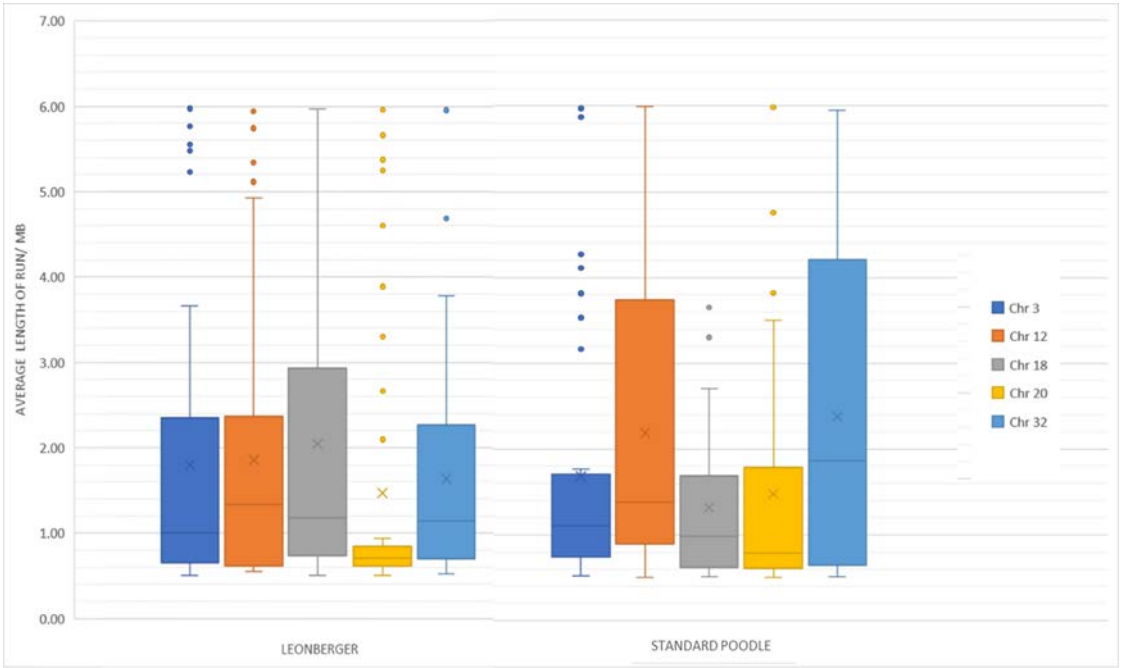

Figure 1 Average length of run in the 6Mb region on selected chromosomes in both dog breeds

Surprisingly, high proportion of homozygosity was observed in both the chromosomes with MHC regions and the chromosomes used for comparison ranging from 58 to 78% (Figure 3). The high level of homozygotes can be easily explained by the history of these dog breeds as mentioned in the introduction. Furthermore, the population size of these two dog breeds was relatively small and this increased allele fixation via drift, which in turn resulted in the increase of homozygotes. Considering all these points, we can explain the high proportion of homozygosity as the consequence of inbreeding and genetic drift in small populations (Millstein, 2017, Wright, 1932).

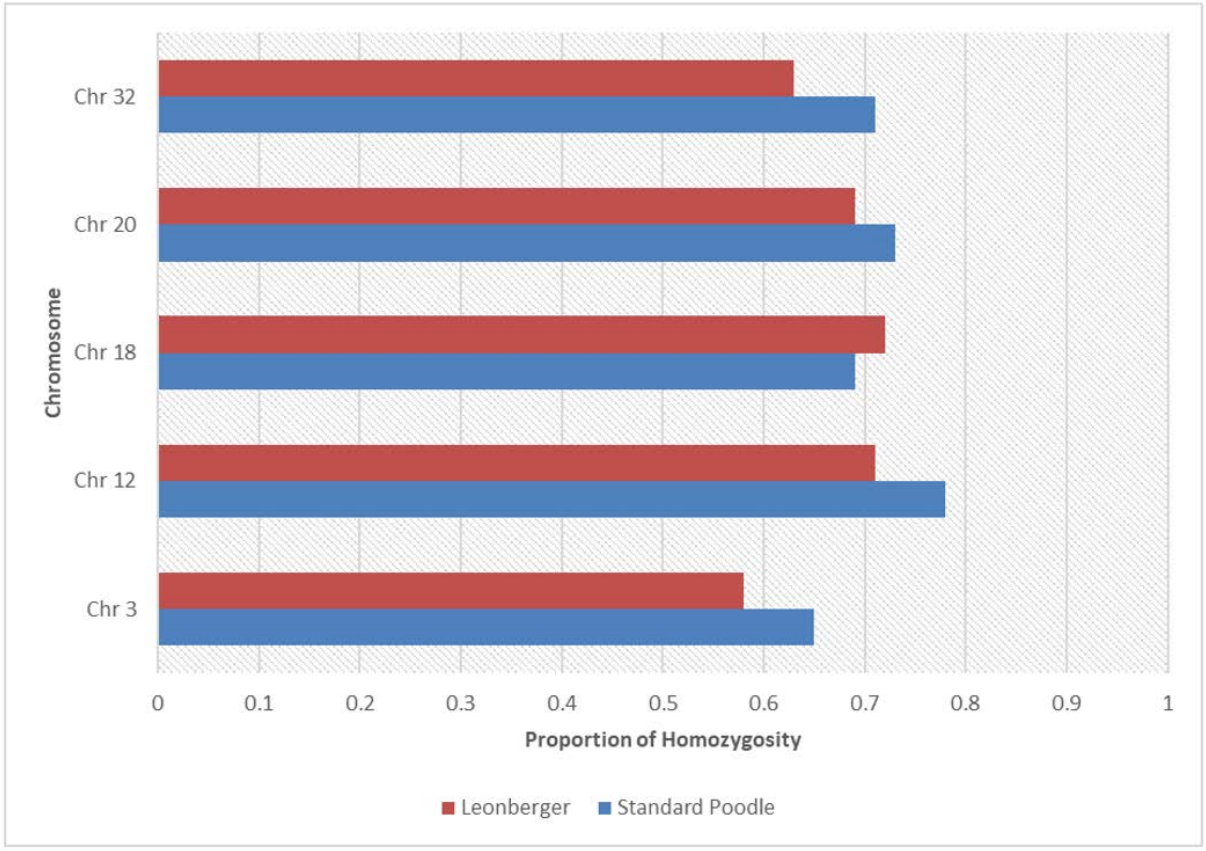

Figure 3 Proportion of homozygosity on selected chromosomes in the 6 Mb region

3.3 Proportion of SNPs in a run

The proportion of SNPs in ROH are shown in Figure 4. In order to ease the comparison of the MHC regions to other chromosomes, a window between the coordinates 0.26 Mb to 1.04 Mb and a second region between 2.09 Mb and 2.86 Mb, representing the position of the MHC Class I and Class II, respectively, on Chr 12, were used on Chr 3, 20 and 32.

Within the Leonberger breed the proportion of SNPs were lower in the MHC region with an overall proportion of less than 0.38 compared to Chr 3 and 20 which showed a proportion of 0.26 to 0.38 and 0.31 to 0.97, respectively. The lowest overall proportion was observed on Chr 32 where the whole 6 Mb segment was a region of low incidence of ROH with less than 0.26. Interestingly, this low proportion of ROH on Chr 32 appeared in both dog breeds. In the MHC region, where the clusters DLA-88, DLA-12 and DLA-66 are located, the proportion of SNPs in ROH was around 0.38 which was comparable to Chr 3 with also 0.38, but much lower than the almost fixed region on Chr 20 with the proportion SNPs in ROH of 0.98.

Within the Standard Poodle population, the proportion of a SNP in a run varied between 0.27 and 0.68. In the MHC Class I region the proportion of ROH ranged from 0.27 to 0.32, whereas in the same position on Chr 3, 20 and 32 it ranged from 0.16 to 0.52, 0.32 to 0.54 and 0.11 to 0.14, respectively. The MHC Class I region showed a higher proportion range compared to the other regions except on Chr 32 where it was the lowest. In the segment of the DLA-88, DLA-12 and DLA-64 on Chr 12, the proportion of SNPs in ROH was 0.30 whereas the same position on the Chr 3, 20 and 32 showed a

proportion of 0.43, 0.43 and 0.14, respectively. The segment containing the important genes also showed a lower homozygosity proportion compared to the other chromosomal regions except on Chr 32.

On the other hand, the MHC Class II region had a proportion of ROH which ranged from 0.24 to 0.27 in the Leonberger and 0.37 to 0.46 in the Standard Poodle. On the same position, in Chr 3, 20 and 32, the proportion varied from 0.30 to 0.38, 0.34 to 0.49 and 0.20 to 0.27, respectively, in Leonberger and from 0.30 to 0.37, 0.38 to 0.49 and 0.24 to 0.27, respectively, in the Standard Poodle. The proportion was very different between the breeds for the same exact region.

The DLA-DRA1 which was positioned in the MHC Class II has a proportion of 0.24 and 0.46 in the Leonberger and the Standard Poodle, respectively. On Chr 3, 20 and 32, with the same position, the proportion was 0.32, 0.34 and 0.26 in the Leonberger, and 0.30, 0.41 and 0.27 in the Standard Poodle, respectively. A low proportion of SNPs in ROH was observed in the Leonberger at the DLA-DRA1 region compared to the other chromosome, whereas in the Standard Poodle the DLA-DRA1 region was surprisingly more homozygous compared to the other chromosomes. In the MHC Class I the proportion of ROH was similar in both breeds. Sudden drop in homozygosity was observed around the MHC Class I and Class II which might be a result of selection in action. Most dogs seem to have a recombination event nearly the MHC regions which causes the large difference in homozygosity proportions.

On Chr 18, the proportion of homozygosity in the DLA-79 regions was 0.39 and 0.31 in the Leonberger and the Standard Poodle. The proportion was relatively high compared to the overall 6.0 Mb region, but as around the MHC Class I and Class II, sudden drops in ROH were observed.

An overall comparison of the MHC Class I and Class II on Chr 12 and DLA-79 on Chr 18 of the Leonberger and the Standard Poodle, shows that these regions had rather low homozygosity compared to the other chromosomes. Results also illustrate that the overall homozygosity on all chromosomes in both dog breeds were comparable, and that the homozygosity in MHC Class I and Class II depended on the dog breeds.

4 Conclusions

This paper examined the homozygosity within the MHC region in two dog populations. While the overall level of homozygosity in the MHC region of Chr 12 was 0.71 and 0.78, the exact segments of the MHC Class I and Class II showed homozygosity proportion of around 0.2 to 0.4 using the ROH approach. Recombination brake points could be detected with ROH. The incidence of ROH was lower in the MHC regions compared to other examined parts of the genome. In conclusion, the ROH measures were favoured to assess the MHC regions, as it gave more insight into the variability of homozygosity in the region, in particular at the location of MHC genes.

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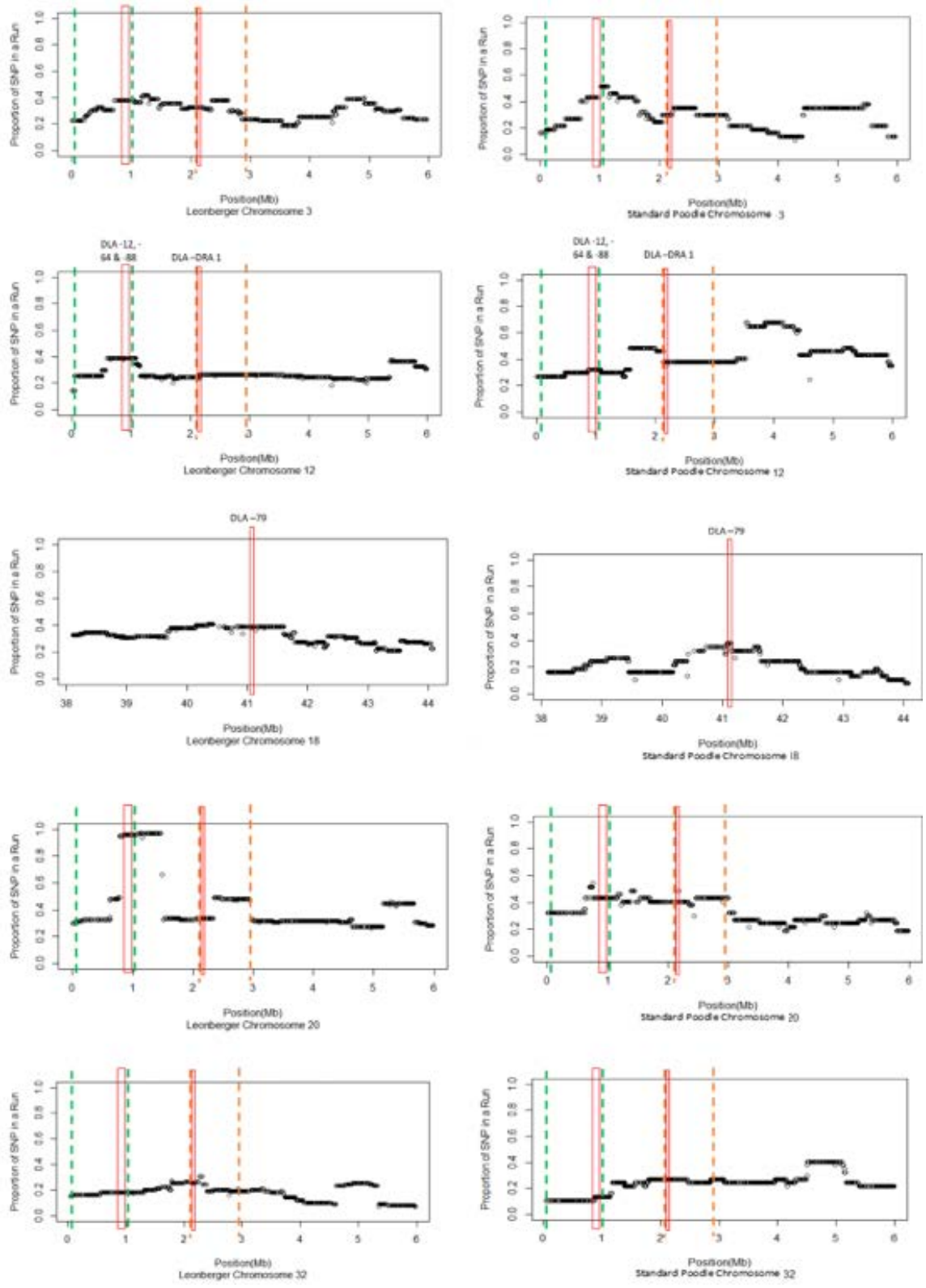

Figure 4 Proportion of SNPs in ROH on of the selected chromosomes in the Leonberger and Standard Poodle. The dashed lines on Chr 12 denote the MHC regions, and the full vertical blocks on Chr 12 and 18 denote the location of the DLA genes. The same locations are highlighted on other chromosomes for better comparison

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