CHARACTERISING HETEROZYGOSITY OF THE X CHROMOSOME IN THE AUSTRALIAN WAGYU POPULATION

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SUMMARY

A pedigree inbreeding coefficient is the probability that two alleles in an individual will be identical by descent which requires them to be homozygous for that locus. Homozygosity at the non-pseudo autosomal region (nPAR) of the sex chromosomes is complicated by their unique inheritance patterns. Heterozygosity at the nPAR X chromosome region is frequently used to predict the sex of genotyped animals for quality control purposes but the characteristics of the X chromosome in the Australian Wagyu population can make such sex predictions inconclusive.

INTRODUCTION

Managing the trade-offs between genetic diversity, inbreeding, and genetic gain is a known challenge in animal breeding programs. Pedigree based inbreeding coefficients provide an on-average indication of inbreeding across the entire genome but do not reflect the true genomic diversity, especially in specific genome locations that may be under more intense selection pressure. For example, non-identical twins, just like any siblings, will inherit different combinations of their parents' genomes. When their parents are related, it increases the chance of the twins inheriting more regions that are identical by descent (IBD). However, the specific IBD regions each twin inherits could differ due to the random assortment of genes, leading to unique genetic variations between the twins.

The X chromosome has a unique inheritance pattern in that mammalian males inherit only one copy of the X chromosome, from their dam. Loss of genetic diversity in the X chromosome can be exacerbated by widespread usage of a small number of sires, particularly in closed populations where a historical population bottleneck has reduced diversity. In other words, the X chromosome has a smaller effective population size than the autosomes which are not involved in determining the sex of an individual. A smaller effective population size means a faster accumulation of homozygosity for the same selection strategy.

Mammalian females inherit one X chromosome from each parent and typically inherit alleles that result in some of the loci being heterozygous, e.g., “AB”. Meanwhile males who inherit one X and one Y chromosome are said to be hemizygous and will appear to exhibit homozygosity at loci that are unique to the X chromosome, known as the non-pseudo autosomal region (nPAR).

When parents are related the offspring will exhibit increased rates of homozygosity and reduced genetic diversity relative to offspring of matings from unrelated parents. Consider the extreme case where a female’s father was also her maternal grand sire, i.e., her mother’s father. In that case, in the absence of recombination along the X chromosome, there would have been a 50% probability that two identical X chromosomes would have been inherited.

Reduction in genetic diversity and an increase in inbreeding within a population can have negative effects on the adaptability or fitness of the population. It can also impact genomic predictions which rely on X chromosome data. The X chromosome is routinely used to predict the likely gender of a genotype sample in order to assist with sample identification and quality control for curation and animal evaluation purposes (McClure *et al.* 2018). The heterozygosity of the nPAR

X chromosome SNPs is commonly used where a threshold value (or values) of heterozygosity indicates whether the "genotype sex" of a sample can be determined as male, female, or ambiguous. Reduced diversity in the X chromosome can lead to a higher rate of ambiguous or incorrect sex predictions for genotype samples of legitimate females.

This research characterises heterozygosity of X chromosomes in Australian Wagyu cattle.

MATERIALS AND METHODS

The data utilised in the study is the Australian Wagyu Association's genotype database containing > 323,000 SNP genotype samples representing more than 3,600 different chips or manifests.

The 280 nPAR X chromosome SNPs, the 101 PAR X SNPs, and 7 Y nPAR chromosome SNPs provided by McClure *et al*. 2018 were utilised for the study. The data set was first reduced to those samples with a raw locus call rate >= 0.95. Second, samples had to have been recorded as males or females in the pedigree and whose genotype samples were predicted as male or females respectively using the Irish Cattle Breeding Federation (ICBF) Y chromosome sex prediction described by McClure *et al*. 2018. This resulted in a final set of 73,814 female and 48,818 male samples. The test checks for called Y chromosome SNPs only on samples genotyped on chips where at least 6 Y chromosome SNPs were present, i.e., samples where no Y chromosome SNPs were available on the chip were discarded. The Y chromosome test was chosen due to its greater accuracy over the X chromosome sex test according to McClure *et al.* 2018 and Garrick 2019. Males required 6 or 7 called Y chromosome SNPs to be verified and females could not have more than 1 called Y chromosome SNP. The chrX sex test considers the heterozygosity of the nPAR X SNPs where if the heterozygosity is low (<= 5%) the sample is considered male, if high (>= 15%) considered female, with moderate heterozygosity (>5% and <15%) considered ambiguous.

The complete Australian Wagyu Association pedigree was utilised to compute pedigree inbreeding coefficients for all animals.

The heterozygosity of each sample was computed as the percentage of loci with heterozygote AB calls divided by the total number of loci with called values, e.g. #AB/(#AB+#AA+#BB).

The average pedigree inbreeding and chrX heterozygosity by birth year were computed as the simple mean of those coefficients across the sex verified genotyped animals born in that year.

The pedigree inbreeding coefficient calculation, genotype database extracts, sex tests, manipulations and analysis of genotype data were all undertaken using the "helical" command-line software package (Garrick *et al.* 2023).

RESULTS AND DISCUSSION

The animals with sex verified genotypes were examined by plotting the pedigree inbreeding coefficient against the nPAR and PAR X chromosome heterozygosities for females (Figure 1), and males (Figure 2). There is no evidence of a relationship between pedigree inbreeding and heterozygosity. The Pearson correlation for chrY verified females between pedigree inbreeding and heterozgosity was -0.28 for PAR X SNPs and -0.09 for nPAR X SNPs. For males the PAR and nPAR correlations with inbreeding are -0.009 and. -0.001 respectively. Interestingly, while the -0.28 correlation for PAR X SNPs for females is considered a low correlation, the value is notably higher compared to the nPAR X SNPs. The pedigree inbreeding coefficient is limited by recorded pedigree information, and while only 3,632 of the 73,814 genotyped females were missing either sire or dam information in the pedigree, without a complete pedigree inbreeding coefficients will be underestimated for some animals.

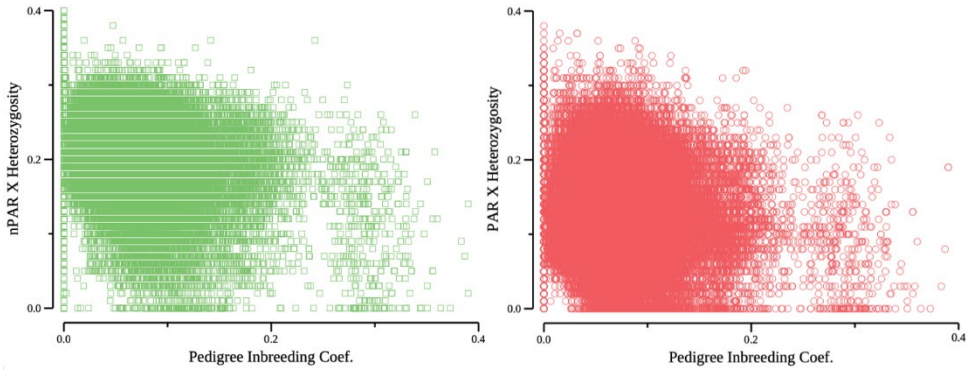

Figure 1. nPAR (left) and PAR (right) chrX heterozygosity versus pedigree inbreeding coefficients for chrY verified females

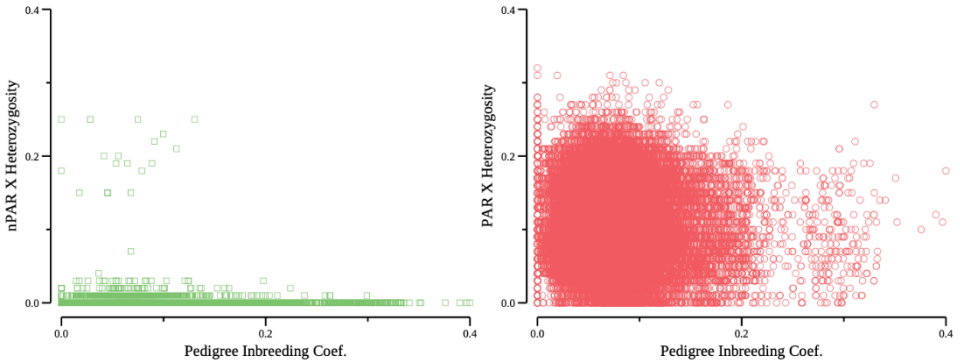

Figure 2. nPAR (left) and PAR (right) chrX heterozygosity versus pedigree inbreeding coefficients for chrY verified males

Comparing Figures 1 and 2 highlights the capability of the nPAR X chromosome SNPs to help confirm genotype samples originating from males and demonstrates no relationship between inbreeding and heterozygosity. However, using nPAR X chromosome heterozygosity alone, no clear demarcation point can be identified to accurately classify all females as some legitimate females exhibit low heterozygosity.

Consider a heifer who inherits two haplotypes on the nPAR X chromosome – one from the single copy in her sire, and one from one of the two copies in her dam. Cases of inbreeding where the X carried by her sire is unrelated to the X carried by the mother – for instance if her paternal and maternal grand sires were the same animal – then inbreeding will not be related to homozygosity. This is because her sire inherits his nPAR X chromosome from his mother. If however the heifers sire is also her maternal grand sire, then she may have inherited the same X regions from her sire and dam, and inbreeding will be related to homozygosity. A pedigree-based measure to characterise the unique inbreeding associated with the nPAR inheritance pattern may help management of nPAR X chromosome diversity.

Table 1 summarises nPAR X heterozygosity and inbreeding by year of birth for chrY verified females grouped by the chrX nPAR sex predictions of low, medium, and high heterozygosity. Approximately 1.2% of the females are not distinguishable from males according to the chrX sex prediction, while approximately 9.9% are ambiguous. The average inbreeding in the low heterozygosity group is over double that in the high heterozygosity group at 0.13 versus 0.06,

compared to 0.09 for the medium heterozygosity (ambiguous) group. The average pedigree inbreeding increased from roughly 0.03 to 0.07 over the last 20 years for the high heterozygosity group, while at the same time the number of females classified into this group dropped from ~98% to 90%.

Table 1. Grouped by the X chromosome heterozygosity as per chrX sex prediction class, we calculate: the average pedigree inbreeding (F), the mean heterozygosity of chrX (h_m), and the percentage of total individuals N within a birth year that are chrY verified females

nPAR X	**h <= 0.05 (predicted male sex)**			**0.15 > h > 0.05 (ambiguous)**			**h >= 0.15 (predicted female sex)**			
Birth yr.	h_m	F	% of N	h_m	F	% of N	h_m	F	% of N	N
2000	0	0	0.0	0.09	0.06	2.2	0.22	0.03	97.8	45
2002	0.05	0.03	0.9	0.11	0.1	2.6	0.2	0.03	96.6	117
2004	0	0	0.0	0.12	0.14	8.5	0.2	0.05	91.5	141
2006	0.03	0.09	0.8	0.12	0.08	5.1	0.2	0.05	94.1	389
2008	0.03	0.12	1.3	0.12	0.08	8.1	0.21	0.05	90.7	1190
2010	0.04	0.14	1.0	0.11	0.1	10.0	0.21	0.06	89.0	1171
2012	0.03	0.12	0.9	0.12	0.09	7.0	0.21	0.06	92.1	1716
2014	0.02	0.15	1.3	0.11	0.09	9.4	0.21	0.06	89.2	2965
2016	0.03	0.11	1.5	0.12	0.08	10.4	0.21	0.06	88.1	5153
2018	0.03	0.13	1.3	0.11	0.09	11.2	0.21	0.06	87.5	8523
2019	0.03	0.13	1.3	0.11	0.09	11.5	0.21	0.07	87.2	9211
2020	0.03	0.13	1.3	0.11	0.1	10.0	0.21	0.07	88.8	10914
2021	0.03	0.13	1.1	0.11	0.1	9.3	0.21	0.08	89.6	12412
2022	0.03	0.12	0.9	0.12	0.09	9.4	0.21	0.07	89.7	2584

CONCLUSIONS

The nPAR X chromosome has a unique inheritance pattern which means standard pedigree inbreeding coefficients cannot accurately characterise the probability of identical inheritance by descent of two alleles. A new pedigree-based inbreeding measure could account for the fact that homozygosity in the non-pseudoautosomal (nPAR) region of the X chromosome is expected only when both parents of an offspring share a common ancestor. This allows for the inheritance of identical nPAR X chromosome segments from both the sire and dam. Reduction in the heterozygosity of the nPAR X chromosome in the Australian Wagyu population over the last 20 years creates challenges in sex prediction associated with genotype quality control.

REFERENCES

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