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INBREEDING MEASURES FOR AUSTRALIAN WAGYU - METHODS AND IMPLICATIONS

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SUMMARY

This study analysed inbreeding patterns in the Australian Wagyu population using three complementary approaches: traditional pedigree-based coefficients, and genomic approaches based on runs of homozygosity (ROH), or runs of heterozygosity (ROHet). Data included a pedigree of >677,000 animals including 330,000 genotyped on the GGP100K platform. Results revealed that genomic inbreeding estimated from (ROH) consistently tracked about 10% higher than pedigree-based inbreeding, perhaps reflecting ancient inbreeding not captured in pedigree analysis. A steady increase in both metrics was observed from 2000-2024. These findings provide a foundation for implementing genomic-based mate selection strategies to optimise both genetic gain and maintenance of diversity in Wagyu breeding programs.

INTRODUCTION

The genetic architecture of modern cattle reflects their unique domestication history, with global populations descending from just two or three domestication events approximately 10,000 years ago (Pitt *et al*. 2018). These ancient population bottlenecks established a foundational level of inbreeding inherent in all contemporary cattle breeds. Against this historical backdrop, the intensification of modern breeding programs has heightened the importance of managing genetic diversity within specific populations, preventing inbreeding depression, and limiting the emergence of deleterious recessive alleles that could impact animal fitness, health, or welfare.

The Australian Wagyu population represents a compelling case study in genetic diversity management, having originated from a limited number of foundation sires exported from Japan in the 1990s. This relatively recent bottleneck, combined with the population's continued closure to outside genetics, creates unique challenges for maintaining genetic diversity while pursuing intensive selection for desired traits. Understanding the patterns and extent of inbreeding in this population is crucial for its long-term viability and continued genetic improvement.

Traditional approaches to monitoring inbreeding have relied on pedigree analysis, which provides expected relationships between animals based on their common ancestors. However, pedigree-based inbreeding coefficients are limited to the extent of pedigree recording that defines the known founders assumed to be unrelated and non-inbred, and cannot capture the actual segments of DNA inherited from common ancestors, nor can they identify specific genomic regions under selection pressure. The advent of high-density genomic data has enabled more precise characterisation of realised inbreeding through multiple complementary approaches.

This study characterises genetic diversity within the Australian Wagyu population using three distinct but complementary approaches: traditional pedigree-based inbreeding coefficients, runs of homozygosity (ROH), and runs of heterozygosity (ROHet). By comparing these metrics, we aim to provide a comprehensive assessment of inbreeding patterns and their implications for breeding program management. This research extends beyond previous work investigating X chromosome heterozygosity (Garrick *et al*. 2023) in the Australian Wagyu population.

MATERIALS AND METHODS

The data used in this study was a 2024 extract from the comprehensive Australian Wagyu Association (AWA) Helical database. The extract contains >591,500 SNP genotype samples originating from 951 different kinds of panels as defined by the genotype chip, version, manifest and service provider. The corresponding recorded pedigree of >677,000 animals included registered Japanese Black, Akaushi, Composite, and commercial animals.

Genotype samples were discarded from analyses if they had a raw call rate lower than 95% or if < 10% or > 90% of their SNPs were homozygous. SNPs were excluded if the minor allele frequency (MAF) was less than 15%. This higher than usual threshold was selected after exploratory analyses and serves to mitigate the effects of the smaller Japanese Black effective population size (Zhang and Banks 2021) and the founder bottleneck which exists outside of Japan. For this analysis, only animals genotyped on GGP100K chips were considered, amounting to 329,695 individuals with 64,180 informative SNPs after MAF filtering. A more strictly defined subset of that population was created by limiting animals to those with a recorded date of birth that were AWA registered Japanese Black (not Japanese Red, Akaushi, composite, or unregistered commercial animals), and that consisted of 94,279 individuals.

Traditional pedigree-based inbreeding coefficients were computed from the complete pedigree using an algorithm suitable for large populations (Meuwissen and Luo 1992).

Runs of homozygosity (ROH) were computed by assessing runs of homozygous alleles (SNP is AA or BB) with the following criteria:

1. Max gap between SNPs was 1,000,000 base pairs (1Mb)
2. Minimum ROH length was 500,000 base pairs (500kb)
3. Minimum number of SNPs in a run was 30 SNPs
4. Minimum SNP density was one SNP per 125,000 base pairs
5. 1 missing SNP was allowed within a run
6. 1 heterozygous SNP was allowed within a run

An ROH coefficient representing genomic inbreeding was computed as the length of the genome in ROH / 2.9 Gb which is the assumed length of the bovine genome. This inbreeding metric provides more detailed information than pedigree-based methods, which cannot account for ancient inbreeding events or missing pedigree information.

Runs of heterozygosity (ROHet) were computed by assessing runs of heterozygous alleles (SNP is AB) with the following criteria:

1. Minimum length of 250kb (250,000 base pairs)
2. Containing at least 15 SNPs
3. Maximum gap allowed between SNPs was 1Mb (1,000,000 base pairs)
4. 3 homozygous SNPs (AA or BB) were allowed within a run
5. 2 missing SNPs were allowed within a run

An ROHet coefficient was computed as the length of the genome in ROHet / 2.9 Gb. ROHet represent regions where an animal shows sustained heterozygosity which may in some cases be an artifact of the SNP chip design but in general should be indicative of genetic diversity or historical admixture in the context of analysing registered Japanese Black Wagyu individuals.

The Helical software system (Garrick *et al*. 2025) implements the analytical methods described above and was used to extract the data from the AWA Helical database, perform necessary quality controls, and to undertake the analyses.

RESULTS AND DISCUSSION

Founder animals in the pedigree were assumed non-inbred and unrelated so therefore comprise the base for pedigree inbreeding. Pedigree-based inbreeding coefficients averaged across each birth year are steadily increasing within the Japanese Black population. Observing Figure 1, genomic

inbreeding via ROH follows a nearly identical trend to pedigree-based inbreeding on the same individuals but with the mean inbreeding at each time point being roughly 10% higher than the pedigree inbreeding. This mean difference represents the ancient inbreeding existing in those founder pedigree animals which can be captured in descendants via genomic analysis. The variability from 2000-2006 may be attributed to the limited number of animals born in this period who had been genotyped on the GGP100K chip.

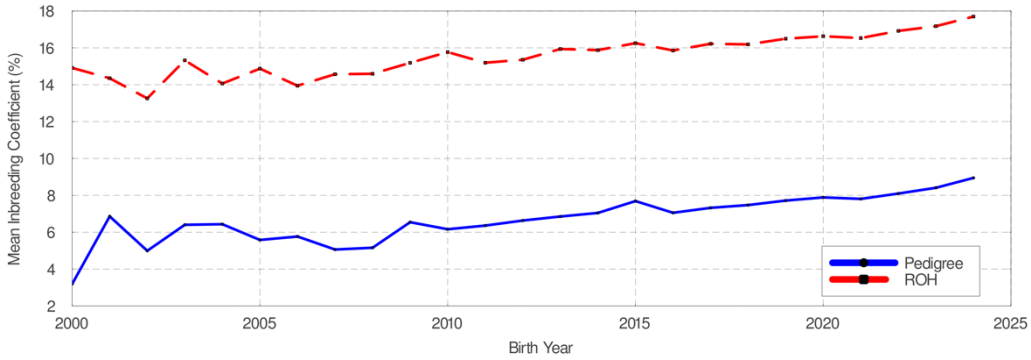

Figure 1. Mean inbreeding % by birth year for AWA registered Japanese Black Wagyu using a pedigree-based method (left axis) and genomic inbreeding (right axis)

Figure 2 shows that while there is a strong relationship between genomic and pedigree inbreeding, there also exists a wide distribution of genomic inbreeding (ROH) estimates for animals with the same pedigree inbreeding estimate. This distribution presents opportunities to utilise precision breeding to significantly improve the management of inbreeding over the long term, by assessing the specific genomic regions of homozygosity between potential mates.

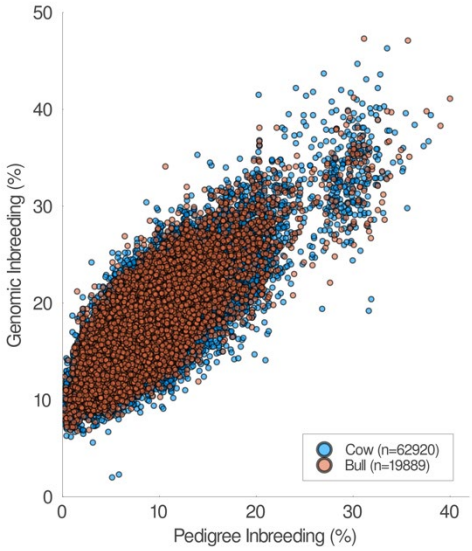

Figure 2. Comparison of genomic inbreeding % via ROH vs. pedigree inbreeding % for AWA 2020-2024 born genotyped registered Japanese Black Wagyu

Figure 3 visualises the distribution of ROHet versus ROH for the same set of animals. A negative correlation between ROH and ROHet is observed indicating that for increasing levels of ROH, the proportionate runs of heterozygosity decreases, however ROHet alone is not a good indicator for genomic inbreeding. For a given level of ROH, there exists a distribution of animals with widely varying ROHet. For example, animals with 20% ROH have between 5-9% of ROHet. These runs of heterozygosity present opportunities for mate selection which further account not just for animals with differing regions of ROH, but also to select mates with complementary regions of ROHet within the genome.

The increased uptake and interest in mate-allocation software reflects these opportunities in practice. Extensions of such tools to incorporate individual ROH and/or ROHet profiles could potentially target matings that maximise beneficial homozygosity in trait-specific regions while minimising overall ROH across the genome.

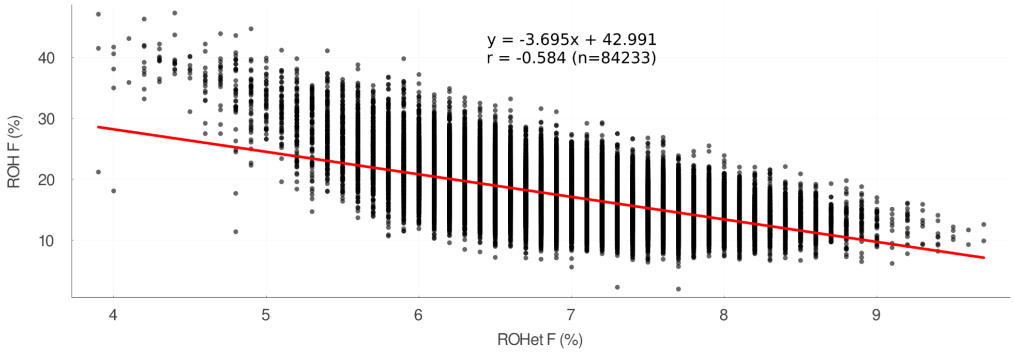

Figure 3. ROH versus ROHet for AWA 2020-2024 born genotyped registered Japanese Black Wagyu

CONCLUSION

The Japanese Black population registered with the Australian Wagyu Association shows increasing levels of inbreeding by birth year. Trends in genomic inbreeding estimates using an ROH analysis align well with trends in pedigree-based estimates but present new opportunities for mating decisions which optimise inbreeding and genetic gain within specific regions of the genome. While rising inbreeding warrants monitoring, the presence of heterozygous genome segments provides opportunities for maintaining genetic diversity. Future breeding decisions should focus on balancing genetic gain with maintenance of diversity, utilising specific ROH and ROHet segment information to guide mate selection with precision breeding. Implementation of these genomic tools in breeding programs could help optimise long-term genetic improvement while managing inbreeding accumulation.

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