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Is neutral genetic diversity related to quantitative variation in semen traits in bulls?

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Abstract

Conservation decisions based on neutral genetic diversity have been observed to promote retention of useful quantitative variation in biological populations. An experiment was undertaken to determine the association between microsatellite marker polymorphisms and phenotypic variation in semen production and cryosurvival traits in bulls. Thirty-five ejaculates were collected from ten bulls of two breeds and evaluated before and after cryopreservation for several semen traits. The bulls were also genotyped using a set of sixteen bovine-specific microsatellite marker loci. Fixation indices (F_{ST}), heterozygosity and Nei's genetic distance measures were computed from allele frequency data for each of the bulls. Molecular and phenotypic data were used to compute tri-distance matrices for the ten bulls and correlated using Mantel's test in GenAlEx 6.5. The study revealed extensive heterogeneity in semen traits, heterozygosity and F_{ST} values among the bulls. Large pairwise phenotypic and genetic distances were also observed. Correlation between pairwise genetic distances and phenotypic distances was significant and highly positive for sperm viability ($r = .61$, $p < .001$) and moderately positive for sperm motility ($r = .40–42$, $p < .05$) variables. For sperm morphology, ejaculate volume and sperm concentration, correlation with genetic distances was positive, low and not significantly different from zero ($p > .05$). A tendency for a triangular-shaped relationship between genetic and phenotypic distances for post-thaw motility and viability traits was also observed. Accordingly, association with neutral genetic diversity was absent for semen production traits and moderate to highly positive for sperm cryosurvival traits. Given these findings, conservation decisions based on neutral genetic diversity may capture variation in some adaptive traits, but not others.

KEYWORDS

genetic distance, molecular markers, quantitative variation, Sanga cattle, semen traits

1 | INTRODUCTION

The evolutionary potential and adaptive ability of organisms to changing environmental and other production conditions depend on the presence of a wide variety of heritable genetic variants in populations. Genetic diversity implies the variety of alleles and genotypes and their frequencies in a population, species or group of species. Populations with wide genetic diversity for ecological traits are more resilient to climate-related shocks, survive better in challenging environmental biomes (Boettcher et al., 2010) and respond

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well to selection (Rege et al., 2011). The nature and strength of the association between measures of neutral diversity and variation in quantitative traits has been a subject of debate over the years. Since such studies generally show a low association between the two diversity measures, many researchers are of the opinion that they serve little purpose (Leinonen et al., 2008; Lewontin, 1984). Yet others have demonstrated that these studies are useful to evolutionary biology and population genetics. Comparative studies of adaptive and neutral diversity provide a means to understand the evolutionary forces underlying phenotypic patterns of variation in both natural and farmed species and assess whether sampling genetic resources for ex situ conservation based on neutral marker polymorphisms promotes retention of potentially useful variation (Zichello et al., 2018).

Genetic diversity has historically been inferred from the measurement of the phenotypic attributes of population members using morphological and productive markers (Ajmone-Marsan, 2010). Protein-based biochemical markers such as allozyme groups have also been used, but they suffer from low polymorphism levels. With the discovery of novel molecular biology tools, genetic diversity can now be directly quantified using uniparental genetic marker systems such as mitochondrial DNA sequences and *Y*-chromosomal haplotypes, and—or bi-parental markers such as microsatellites and single nucleotide polymorphisms (SNPs). Microsatellites are multiple copies of short tandem repeats, typically 2–6 nucleotide bases in length. Microsatellite polymorphism is a result of the differences in allele sizes due to variation in the number of repeats, which can range from 5 to 100 for any locus. They are powerful genetic markers, due to wide distribution in the genome, multi-allelic attributes, high level of polymorphism, robust analytical methodology and nuclear co-dominant inheritance pattern, which allow ease distinction between homozygous and heterozygous alleles. Typically, a sample of at least 15–30 animals is genotyped using panels of twelve or more marker loci to access population genetic diversity and relationships (Singh et al., 2014). The diversity of populations can be estimated using measures such as heterozygosity (H_O, H_E and H_T), fixation indices (F_{ST}, G_{ST} and θ) and genetic distance (D_A, D_S and D_R) parameters (FAO, 2011). The results are interpreted in light of genetic and historic events—migration, introgression, admixture, cross-breeding, bottlenecks, founder events and selection—that affect patterns of genetic diversity. One can also proceed to carry out an association study linking measures of genetic diversity and quantitative variation such as $Q_{ST,}$ coefficient of variation (CV), sum of eigenvalues (SEV) and heritability in traits of ecological or economic importance (Leinonen et al., 2008; Zichello et al., 2018).

Livestock are valuable to humankind through direct and indirect contribution to food production, income generation and provision of a variety of goods and services that support livelihoods and the national economy. Livestock diversity comprises a range of breeds, strains and ecotypes of poultry, cattle, goats, sheep, rabbits, fish, ostriches, crocodiles and other livestock species. This diversity is important for meeting the multiple needs of society, matching genotypes with varied production systems and coping with changes to climatic and production conditions (Wilson, 2009). Cattle genetic resources in southern Africa comprise some taurine (*Bos taurus*), zebuine (*Bos indicus*) and Sanga (*Bos taurus africanus*) genotypes (Frisch et al., 1997). Adapted Sanga type cattle breeds, which are characterized by cervico-thoracic neck humps, constitute the bulk of the cattle genetic resources in the region (Mapiye et al., 2019). These breeds harbour considerable genetic diversity and unique genetic variants (Gororo et al., 2018), and adaptive traits important for coping with undesirable changes to production and climatic conditions, and surviving emerging disease epidemics and parasites (Wilson, 2009). In the region, Sanga cattle are particularly valuable due to their diversified production ability, potential for multi-purpose use (Gororo et al., 2017; Rowlands et al., 2003), and possession of better fertility, fitness and health traits compared to exotic breeds (Moyo, 1997). However, native cattle genetic diversity is under threat due to inbreeding, unbalanced admixture with exotic breeds and neglect by stakeholders (Mapiye et al., 2019). Geographical concentration and numerical scarcity may also increase the vulnerability of native breed diversity to demographic bottlenecks such as disease epidemics, parasite outbreaks and droughts. As a result, ex situ conservation of such breeds is becoming increasingly important in the SADC region (AU-IBAR, 2019).

Conservation programmes seek to capture as much genetic diversity as possible in the gamete and embryo samples that are cryopreserved. A previous study observed breed and sire differences in sperm cryosurvival (Gororo et al., 2020), with negative implications for cryobanks and artificial reproduction programmes. In addition, some Sanga cattle breeds were observed to harbour considerable genetic diversity based on molecular marker genotyping (Gororo et al., 2018). It is not clear, however, to what extent molecular marker diversity is associated with quantitative variation in semen traits in these cattle breeds. Therefore, this study combined molecular marker with semen production and cryosurvival data to determine the relationship between neutral genetic and quantitative diversity in two Sanga cattle breeds. Secondly, the study sought to determine the impact of evolutionary forces on neutral and functional trait diversity in farmed livestock. It was hypothesized that molecular measures can be used as surrogates for quantitative variation in semen production and cryosurvival traits in bulls. If this hypothesis is true, then capturing neutral genetic diversity may help to retain diverse sperm phenotypes in sperm cryobanks for ex situ conservation of valuable cattle genetic resources.

2 | MATERIALS AND METHODS

2.1 | Study sites

The bulls used as blood and semen donors for this study were sampled from conservation populations at Grasslands and Makoholi Research Institutes, Zimbabwe. Zimbabwe is a subtropical country in southern Africa which experiences a tropical savanna type climate. The two study sites differ in their ecological conditions and

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vegetation type. Grasslands (18011`S, 31028`E) is located 67 km south-east of Harare in a highveld (1,600 m above sea level) area of the country. The institute is in Agro-Ecological Region II, experiences cool to warm (average 18°C) temperatures and receives high unimodal rainfall of 600–900 mm per annum (Vincent et al., 1960). Vegetation is described as *Hyperhenia tall grass-veld* dominated by perennial grasses that become course and less palatable in the dry season. Makoholi (190 50′ S, 300 46′E) is located in a lower altitude (1,204 m above sea level), lower rainfall (450–650 mm, Agro-Ecological Region IV) area, some 32 km north of Masvingo town. Its vegetation is described as sweet-veld dominated by annual grass species which remain palatable throughout the year.

2.2 | Animals

The bulls used in this study were a subsample of Sanga cattle genotyped in an earlier study (Gororo et al., 2018). Bulls were extensively managed on natural grazing with ad libitum access to water in the paddocks. During the dry season, bulls were fed on grass hay and a protein block. Breeding soundness evaluation was done at the onset of semen collection. Bulls with clinical inflammatory or congenital defects of the reproductive organs or scrotal circumference of less than 30 cm were excluded from semen collection. As a result, five Mashona and five Tuli bulls were retained for this study.

2.3 | Genotyping

Study animals were genotyped using a subset of bovine-specific microsatellite loci, as recommended by the Society for Animal Genetics (ISAG) and Food and Agriculture Organisation of the United Nations (FAO, 2011). The sixteen polymorphic markers used were BM1818, BM1824, BM2113, CSRM60, CSSM66, ETH10, ETH225, ETH3, ILST006, INRA23, RM067, SPS115, TGLA122, TGLA126, TGLA227 and TGLA53. Blood was collected by jugular venipuncture using 4 ml vacutainer tubes containing an anticoagulant (0.5 *M* EDTA), mixed homogeneously to avoid clotting, transported on ice and stored in a freezer at −20°C until required for analyses. Storage and DNA extraction were done at the Germplasm and Reproductive Technology Laboratory (GRTL), Chinhoyi University of Technology, Zimbabwe. DNA was extracted from peripheral blood lymphocytes using a commercial kit (Zymo Research, Germany). The presence of DNA was confirmed by 1.5% agarose gel electrophoresis using 1X TAE buffer on an OmniPAGE system (Biocom Direct, UK) and visualized on an Infinity ST5 Gel Documentation System (Vilber Lourmat, France). DNA samples were shipped to Veterinary Genetics Laboratory of the University of Pretoria in South Africa for genotyping. A 10 μl PCR reaction mixture was prepared with primer mix (2.8 μl, 0.08–1.00 *M*), molecular grade water (1.2 μl), Kapa master-mix (5 μl) and 20 ng template DNA sample (0.5–1 μl). DNA amplification was performed by multiplex PCR in a Perkin Elmer GeneAmp PCR System® 9700 (Applied Biosystems, CA, USA). The thermal cycler was programmed to run under the following conditions: 95°C for 3 min, 30 cycles of denaturation at 95°C for 15 s, specific marker annealing temperature for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 10 min and held at 4°C. PCR products were quantified using 3% agarose gel in TBE buffer stained with ethidium bromide and visualized under UV trans-illuminator. DNA fragments were separated by capillary electrophoresis in an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, USA). The GeneMapper version 4.1 was used to determine the fragment sizes in base pairs, and allele score data were converted to an MS Excel datasheet for statistical analyses.

2.4 | Phenotyping

Semen was collected during the cool dry season (May - August 2017) five times at two-week intervals from each bull using an electro-ejaculator standardized for bovine semen collection. Semen processing was carried out in a mobile laboratory in the field at the site of sample collection. Accepted ejaculates were initially diluted 1:1 (v/v) with freshly prepared Triladyl®, a commercial, one step egg-yolk Tris-citrate buffered semen extender (Minitub, Germany). Partially extended semen was then cooled to 4–5°C over 2 hr. During this period, gross sperm motility and concentration were evaluated to determine suitability for further processing and extender dilution rate to use, respectively. After 2 hr, the second extender fraction was added stepwise at three intervals of 10, 20 and 10 min, respectively, to bring sperm count down to a final storage concentration of ~100×10^6 sperm/ml. Fully extended semen was equilibrated for 2 hr at 4°C. Semen was then packaged into 0.25 ml PVC mini-straws by gentle suction, sealed and allowed to equilibrate for a further 20 min. The straws were frozen using the method of liquid nitrogen (LN_2) vapours and stored in a LN_2 flask for at least 48 hr before post-thaw semen analyses.

Semen was evaluated for semen production traits—ejaculate volume and concentration. Semen quality traits—gross motility, progressive motility, viability and sperm morphology—were evaluated in fresh and post-thaw samples. Ejaculate volume, sperm concentration and fresh motility were determined in the field, and cryosurvival traits were evaluated post-thaw at the laboratory. Sperm concentration was determined by a haemocytometer with an improved Neubauer chamber (Boerco, Germany). For sperm motility evaluation, a bright-field microscope with a pre-heated stage (37°C) was used to count the proportion of spermatozoa showing gross and progressive motility and estimate the rate of progressive linear motion for ten fields of view with groups of ~10 sperm per field. Motility rate was scored between 1 (very slow) and 5 (very fast). A sperm motility index (SMI) was then calculated for each ejaculate using the formula: SMI = [gross motility % + (20 × rate)]/2. Bright-field microscopy of eosin–nigrosin dry-mount stained smears was used for viability and morphology evaluation. The histological dual stain used was made up of 1.67 g eosin Y, 10 g nigrosin and 2.9 g sodium citrate dissolved in

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TABLE 1 Variability in semen production and cryosurvival traits in ten Sanga bulls kept under extensive conditions

Semen variable	Mean	StDev	CV %	*p*-Value
Semen production traits				
Ejaculate volume (ml)	10.2	2.98	29.2	.011*
Concentration ($\times 10^9$ sperm/ml)	1.090	0.776	71.1	.000***
Sperm cryosurvival traits				
Gross motility (%)	54.6	24.48	44.9	.000***
Progressive motility (%)	39.9	23.18	58.2	.000***
Sperm motility index (%)	57.7	22.36	38.7	.000***
Viable sperm (%)	75.3	8.35	11.1	.003**
Normal sperm (%)	91.2	2.70	3.0	.024*

Note: Significance—*$p < .05$; **$p < .01$; ***$p < .001$.

TABLE 2 Summary of genetic diversity parameters for ten bulls of two Sanga cattle breeds based on sixteen microsatellite markers

Parameter	Genetic notation	Breed		Global sample
		Mashona	Tuli	
Sample size	*n*	5	5	10
Number of loci genotyped	*n*	16	16	16
Observed number of alleles	No	58	69	82
Number of private alleles	PA	13	24	37
Mean number of alleles	*Na*	3.63	4.31	5.13
Shannon's Information Index	*I*	1.048	1.258	1.153
Marker polymorphism (PIC)	*PIC*	0.524	0.610	0.629
Observed heterozygosity	H_O	0.632	0.825	0.744
Expected heterozygosity	H_E	0.581	0.659	0.620
Unbiased heterozygosity	H_t	0.646	0.732	0.715
Fixation index	*F*	−0.162	−0.274	−0.218
Pairwise genetic distance (bulls)	D_A	20.00	19.70	23.04
Pairwise genetic distance (population)	D_A	0.000	0.000	0.343
Pairwise population differentiation	F_{ST}	0.000	0.000	0.082

100 ml distilled water. A 10 µl semen aliquot was mixed 1:1 (v/v) with the stain, smeared across a microscope slide, quickly air-dried and stored for later evaluation. At least 200 spermatozoa were counted per slide under bright-field microscopy (Amscope, USA) at ×1,000 and ×400 magnifications, respectively. Sperm displaying partial or complete purple staining were considered non-viable and those showing strict exclusion of stain were counted as viable. Each morphologically normal sperm was counted once, and each defect was counted separately even if multiple defects occurred on the same sperm cell.

2.5 | Statistical analyses

All semen data were subjected to descriptive statistics and analysis of variance (ANOVA) to determine the effects of bull and breed on each of the variables. *Post hoc* treatment of data was done using Fisher's LSD at the 5% level of significance. These analyses were carried out in the statistical program, Minitab version 17. For neutral genetic diversity, allele score and frequency data were used to estimate basic genetic parameters and fixation indices using FSTAT 2.9.4 (Goudet, 2005), as well as heterozygosity and Nei's standard genetic distances (D_A) in Arlequin 3.5 (Excoffier & Lischer, 2015). Marker and semen data were then used to compute tri-distance matrices for the ten bulls and correlated using Mantel's test in GenAlEx 6.5 (Peakall & Smouse, 2012). The pairwise tri-distance matrix for marker data was based on the covariance matrix of Nei's D_A, while that for semen data was based on absolute pairwise differences in mean semen trait values for the ten bulls. Pearson's correlation coefficient (R_{xy}) was then computed between the matrices by running the standardized Mantel statistic (r_M). The significance of the association was tested by running matrix permutation 10,000 times to obtain a randomization *p*-value of the null hypothesis of no association. The R_{xy} was declared statistically significant at $p < .05$.

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TABLE 3 Pairwise estimates of Nei's genetic distance (D_A) calculated using allele score data from sixteen microsatellite loci in ten bulls of two Sanga breeds

1	2	3	4	5	6	7	8	9	10	Bulls
0										**1**
19	0									**2**
23	20	0								**3**
23	24	24	0							**4**
15	20	20	24	0						**5**
18	19	19	23	15	0					**6**
27	28	28	24	28	27	0				**7**
19	16	24	20	20	19	20	0			**8**
20	23	27	27	23	22	27	23	0		**9**
19	24	24	28	20	19	28	24	15	0	**10**

Numbers in bold represent identity of the animals used in the study.

3 | RESULTS

3.1 | Variation in semen production and cryosurvival traits

Significant breed differences were observed for ejaculate characteristics (volume and concentration) as well as the proportion of post-thaw morphologically normal sperm. Semen volume averaged 10.2 ± 2.98 ml, ranging from 6.3 to 13.4 ml per ejaculate. Mean seminal concentration was 1.09 ± 0.776 × 10^9, with a range of 0.08 × 10^9 to 2.87 × 10^9 sperm/ml. Compared to Mashona, Tuli bulls produced greater (11.8 ± 0.31 ml versus 8.5 ± 0.38 ml, $p < .001$) and more concentrated (1.39 ± 0.09 versus 0.75 ± 0.10 × 10^9 sperm/ml, $p < .01$) ejaculates. The proportion of normal spermatozoa was higher ($p < .01$) in Mashona (92.3% ± 3.10%) compared to Tuli (90.3% ± 1.87%) bull semen, with an overall mean of 91.2% ± 2.70%. Breed differences in viability and motility traits did not reach statistical significance in post-thaw semen samples. The coefficient of variation (CV) was moderate to large and significant for most of the semen traits, except sperm morphology which showed a low but significant CV among the ten bulls evaluated (Table 1). There was a significant difference ($p < .05$) in the performance of the ten bulls in all traits evaluated. Some bulls produced semen of consistently poor cryosurvival compared to others.

3.2 | Neutral genetic diversity

Genetic parameters for the ten bulls that were genotyped using sixteen microsatellite markers are summarized in Table 2. A total of 82 alleles were observed from the sixteen loci studied in the global sample of ten animals, yielding a mean of 5.13 alleles per locus. Allelic diversity was lower in Mashona compared to Tuli bulls. Thirty-seven (45% of total) were unique to specific populations, with Tuli bulls having more breed private alleles compared to the Mashona bull population. No rare alleles (with a frequency <5%) were observed, possibly due to the small sample size.

TABLE 4 Pearson's correlation (R_{xy}) between pairwise genetic distances (D_S) and phenotypic distances in semen traits in Sanga bulls

Parameter	R_{xy}	*p*-Value	Significance
Ejaculate volume	.169	.143	ns
Sperm concentration	.070	.327	ns
Gross motility	.396	.010	*
Sperm motility index	.408	.005	**
Progressive motility	.420	.011	*
Viable sperm	.608	.000	***
Normal sperm	.105	.274	ns

Note: Significance—ns, non-significant $p > .05$; *$p < .05$; **$p < .01$; ***$p < .001$.

Genetic variability was assessed using heterozygosity and was estimated by the infinitesimal model (F_{ST}) and pairwise matrix of Nei's genetic distance (D_A) measures. The study showed significantly higher observed (0.742) compared to Hardy–Weinberg equilibrium (HWE) expected (0.620) heterozygosity proportions. Total heterozygosity (H_t) across the entire sample of 0.714 confirmed that the studied populations contain a high level of genetic diversity. Heterozygosity values were found to be higher in Tuli bulls, indicating wider genetic diversity among the sampled animals of this breed. Significant genetic variation was reflected by large differences in heterozygosity among the bulls. Excess of heterozygosity was also reflected in the fixation indices (*F*), which were moderately negative and significantly lower than zero for both Tuli and Mashona and the global sample. Shannon's information index (*I*) was moderate and significantly distant from zero. F_{ST} provides a measure of the proportion of the total genetic divergence that separates the two populations. In a situation of HWE, F_{ST} is expected to be equal to zero. Pairwise population F_{ST} (0.084) and genetic distance (D_A) (23.04) values showed moderate and significant ($p < .001$) genetic divergence of the two

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populations. Within the breeds, similar D_A values of 19.70 and 20.00 were observed ($p > .05$). Tri-distance matrices for marker (Table 3) and semen data were computed based on the global data sample. Pairwise matrices for semen data are given in the Supplementary Files. The pairwise matrix of D_A between the bulls revealed a low to moderate level of divergence between the bulls.

3.3 | Association between semen traits and neutral genetic distances

Results of the correlational study between genetic and semen quality distance matrices are given in Table 4. Pearson's correlation coefficient (R_{xy}) between pairwise genetic and phenotypic distances was positive for all traits studied. Correlation of the semen traits with genetic distances was high and significant for sperm viability ($r = .61$, $p < .001$), moderate and significant for motility ($r = .40–42$, $p < .05$) and low and non-significant for normal sperm morphology ($r = .11$, $p > .05$), ejaculate volume and sperm concentration. A trend towards a triangular relationship for sperm motility and viability variables was observed (Figure 1). Low marker distance values were associated with low phenotypic distances, whereas higher marker distances were corresponding to a wider range of phenotypic distances in these sperm quality traits.

4 | DISCUSSION

The present study explored the nature and strength of the relationship between neutral genetic differentiation and quantitative variation in semen production and cryosurvival phenotypes in bulls. Results showed that correlations between pairwise genetic and phenotypic distances were positive, moderate to high and significantly different from zero for sperm cryosurvival traits—sperm motility and viability. Conversely, correlations with neutral genetic distances were weak and non-significant for sperm morphology and semen production traits (ejaculate volume and sperm concentration). For both groups of traits, the direction of the correlation was positive. Although phenotypic distances in sperm cryosurvival traits showed a good congruency with neutral genetic diversity, there was a tendency towards a triangular relationship. There are not many neutral versus quantitative trait association studies in livestock with which to compare these results. The most comprehensive meta-analysis for association studies carried out in livestock was done by Hall et al. (2012). That review observed that most studies reported weak to moderate correlations between neutral and adaptive diversity measures in livestock. Correlations with genetic variation in neutral markers such as microsatellites and SNPs were significantly positive for puberty and fertility traits, growth traits, carcass traits and milk production, and negative for pre-weaning traits such as calving ease and survival to weaning. The small sample used in the present study may have had a negative impact on the reliability of the results. However, a sufficiently large number of marker loci were used, and the population genetic parameters from the ten animal subsample did not differ much from the original study (Gororo et al., 2018).

Low levels of association between quantitative trait and marker diversity are reported in many studies. A meta-analysis conducted by Reed and Frankham (2003) had observed that molecular measures explain only 15%–20% of the variation in quantitative traits, especially those associated with reproductive fitness. Earlier, the same researchers had observed a weak, but significantly greater than zero, relationship between single locus allozyme diversity and polygenic measures of quantitative variation (Reed & Frankham, 2001). Leinonen et al. (2008) carried out a review and meta-analysis of 64 empirical studies of quantitative trait and marker diversity among natural populations in 50 species. The

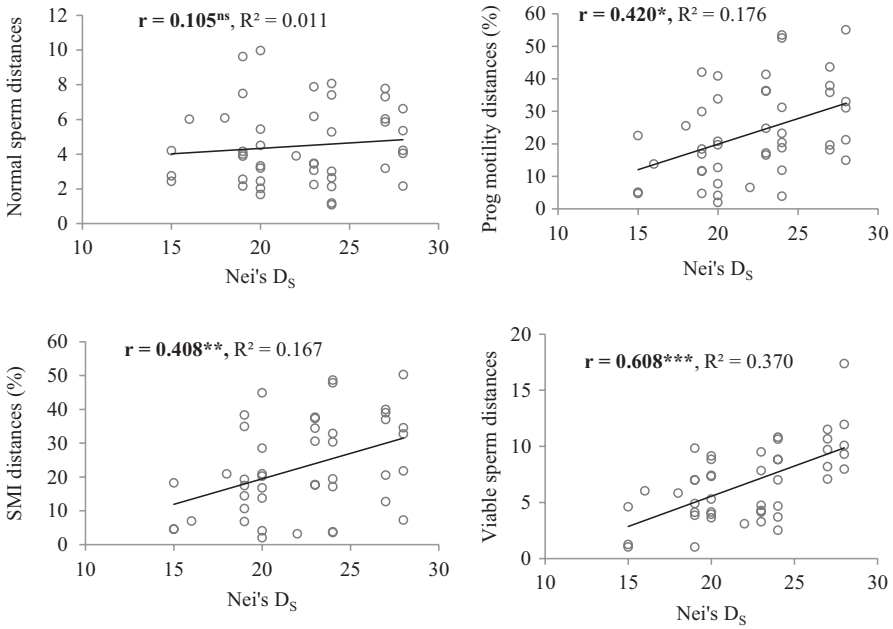

FIGURE 1 Scatterplot of relationships between phenotypic distances in semen traits and molecular microsatellite-based distances (Nei's D_S) in Sanga bulls. $^{ns}p > .05$, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

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study found a significant positive covariance between the indices of population differentiation for quantitative traits (Q_{ST}) and neutral genetic markers (F_{ST}) and that on average $Q_{ST} > F_{ST}$. Positive but weak relationships between molecular and quantitative measures of diversity have been reported for a number of traits in crop plants (Burstin & Charcosset, 1997; Gunjaca et al., 2008) and for reproductive and adaptive traits in livestock (Hall et al., 2012). However, a study by Zichello et al. (2018) used pairwise nucleotide diversity (π) and the sum of eigenvalues (SEV) to compare population genetic data from neutral autosomal loci with intraspecific cranial shape variations in humans. The study observed that global patterns of cranial variation are congruent with patterns of neutral genetic diversity. Most association studies between neutral and quantitative variation show a positive relationship, with the strength of that relationship varying between species and traits within species.

Findings from the present study may have important implications in evolutionary and conservation genetics for native breeds of livestock. The weak levels of association of semen production traits with neutral genetic distances indicate that evolutionary processes may not be affecting adaptive traits and neutral diversity in a similar fashion. Evolution in phenotypic traits related to fertility and fitness is largely driven by the interplay between selection and genetic drift, and the relationship may be weak or absent for polygenic traits under directional selection (Reed & Frankham, 2003). The Mashona and Tuli breeds used in the study have a unique history featuring adaptation to challenging subtropical biomes, several genetic bottlenecks, founder events and strict selection for type, adaptability and reproductive fitness (Harvey, 1987). Sample bulls were selected from in situ conservation populations under research station management conditions. These populations are small and fragmented, numbering a few hundred per site. It has been demonstrated that selection or random genetic drift results in considerable losses of genetic diversity for rare genetic variants in livestock (Eynard et al., 2016). Possible directional selection of functional loci related to male reproductive fitness such as semen production traits offers the most plausible explanation for observed lack of association with (nearly) neutral genetic data. Conversely, loci associated with sperm cryosurvival are not under direct selection, hence a good congruency with neutral genetic diversity. This indicates that neutral genetic diversity may be related to quantitative variation in some traits but not in others.

The nature of the relationship between quantitative distances and molecular marker distances may also be affected by the linkage disequilibrium (LD) between marker loci and the quantitative trait loci involved in the traits considered. It was demonstrated by Burstin and Charcosset (1997) that a poor association between molecular marker loci and quantitative trait loci leads to a low correlation between distances. In that case, polygenic inheritance and LD properties associated with kinship leads to a triangular relationship between marker distance and phenotypic distances. A strong relationship is expected in the case of LD, whereas the two distances vary independently in the absence of LD. In the latter case, high and low neutral marker distances can correspond to similar phenotypic distances. Inbreeding may also contribute to reduced phenotypic distances between animals, but was not seen to be present in the present sample based on measurements of heterozygosity and F_{ST} values. Variations in sperm cryosurvival traits showed a good congruency with neutral genetic diversity and a tendency towards a triangular relationship for sperm motility and viability traits. This triangular-shaped relationship was also reported by Rebourg et al. (2001) and Gunjaca et al. (2008) for molecular marker distances and quantitative trait distances in plant genetic research.

Generally, several factors are related to the low association of neutral and quantitative markers of diversity. Reproductive traits are polygenic and quantitative in nature and exhibit low to moderate heritability values. Their phenotypic expression is a composite result of additive genetic effects, dominance, epistasis and pleiotropic interactions, as well as large environmental effects (Burstin & Charcosset, 1997). Conversely, molecular markers, such as microsatellites only measure additive genetic variation, are functionally neutral and have lower amounts of mutational genetic variation and statistical power. Thus, molecular marker diversity may differ from variation in quantitative traits due to differential selection, higher mutation rates, large environmental variances and higher statistical power on the quantitative characters relative to molecular diversity measures (Reed & Frankham, 2003).

5 | CONCLUSIONS

Neutral genetic diversity was observed to have a weak association with quantitative variation in semen production traits and a good correspondence with variation in sperm cryosurvival traits in two breeds of Sanga cattle. These differences could be explained using population and evolutionary genetic models, including the effects of random genetic drift and directional selection. This indicates that neutral genetic diversity may be related to quantitative variation in some traits but not in others. Thus, capturing neutral genetic diversity may be effective in retaining diverse sperm phenotypes in sperm cryobanks for conservation of valuable animal genetic resources. However, given variability of the correlations across traits and the triangular nature of the relationship, the use of neutral marker loci as a proxy for phenotypic diversity in Sanga cattle semen should be approached with caution.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHORS CONTRIBUTIONS

EG and FPC conceived the study, collected data and carried out statistical analyses. EG prepared the draft manuscript. SMM and FC reviewed the draft manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL

This study was approved by the Academic Board of the Department of Animal Production and Technology, Chinhoyi University of Technology. Blood and semen samples were collected under supervisory guidance of a qualified veterinarian and according to the 'University Guidelines for Animal Handling and Sample Collection', which conforms to European Union Directive 2010/63 regarding the protection of animals used in scientific experiments.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author on request.

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