

A review on omics approaches, towards understanding environmental resilience of indigenous Nguni sheep: Implications for their conservation and breeding programs in South Africa

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ABSTRACT

Genetic heterogeneity, as the driver of evolutionary potential and counterbalance to inbreeding depression, is an important consideration for ensuring livestock adaptability, robustness and resilience to erratic and challenging environments. Accordingly, the low genetic diversity within exotic livestock breeds may suggest survival uncertainty and extinction risk, as agroecological environments are consistently changing due to global climate change. Indigenous livestock breeds, such as the southern African Nguni sheep, have been reported to be genetically and phenotypically diverse and adapted to their local environmental adversities, including high temperatures, drought, and diseases. Characterising and understanding the molecular determinants underpinning their robustness may assist in selecting and breeding for highly productive livestock progeny that are resilient to a broader spectrum of environmental challenges. Also, understanding indigenous livestock breeds genetic uniqueness will further emphasize their significance as a valuable genetic resource and strengthen the impetus for their conservation. This review first reiterates the concept of southern African indigenous sheep ecotype (Nguni sheep) as a valuable genetic resource for future breeding programmes against local environmental adversities, and further discuss the importance of understanding the genetic mechanisms underpinning their adaptative traits to southern Africa which have not been adequately explored. Some of the previously reported genes, associated with indigenous sheep breeds adaptation to southern African environments includes: *MYH9* (energy metabolism), *PITX1* (high altitude adaptation), *CREB3L2*, *CREB3*, *GNAQ*, *DCTN4* (thermoregulation), *EGLN1*, *EPAS1* (hypoxia associated genes). Finally, we highlighted contemporary omics techniques, commonly used in livestock genomic studies, that may assist in revealing the genomic basis of Nguni sheep unique genotypes of environmental resilience.

1. Introduction

The domestic sheep (*Ovis Aries*) is a ruminant mammal of the order Artiodactyla, which was domesticated about 11,000 years ago, as human civilisations transitioned from hunter-gatherer lifestyles to settled agrarian communities in the Fertile Crescent [1]. Small ruminants, particularly indigenous breeds, play a significant role to the livelihoods of human population in the tropics from socio-economic aspects [2–5]. Thus, emphasis on their genetic progress for increased productivity and reproductivity performance are crucial [6–9]. During

the past decades, the details of sheep domestication and phylogeography have been the subject of global discussion and investigation, in aim of gaining insights into the evolutionary history, migration patterns and adaptiveness to respective environments of these animals [10]. The consensus is that the Asian mouflon (*Ovis orientalis*) is the most likely wild ancestral species of present-day domestic sheep (Zeder, 2008 [11]). After domestication, sheep followed human migration and trade routes, and they were eventually distributed across the globe to different agroecological zones. Consequently, they evolved genetically as several mutations and allele frequencies fluctuated according to preferences of

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different forces of natural and artificial selection, founder events and bottlenecks, and introgressions they were subjected to Ref. [12]. This resulted in morphological and behavioural evolution, not only to align with specific farmer's needs, but also to respond to respective environmental adversities [13,14]. Accordingly, the contemporary global sheep population consist of a spectrum of sheep breeds of different socio-economic importance from subsistence farming in rural communities to commercial operations [15].

Subsistence farming is mostly dominated by indigenous breeds while commercial farmers prefer highly productive synthetic and developed breeds ("exotic breeds" will be used as an umbrella term for both synthetic and developed breeds in this paper) [16]. Synthetic breeds (also known as composite breeds) have been developed through cross-breeding programmes, where mating decisions are based on complementary desired characteristics across multiple generations [16,17]. Developed breeds refers to the exotic breeds that have mostly been artificially selected and bred, through conventional selective breeding, for a specific purpose. Indigenous breeds are a subset of locally adapted breeds where their level of adaptability is mostly due to natural selection and proportional to the time present in one or more of a specific region's traditional production systems or environments [17,18].

The increased demand of animal products during the past years has continued to pressure commercial sheep producers to implement farming systems that are characterised with high selection intensity for specific traits [19]. Commonly, these breeding systems are coupled with reduced effective population sizes, thus resulting in higher inbreeding levels and increased risk for the loss of advantageous alleles through drift [12]. This phenomenon has contributed significantly to achieving required phenotypic and genotypic homogeneity of highly productive exotic breed lineages for maximum and consistent productivity [19]. However, reduced genetic and phenotypic diversity in a population is associated with decreased animal fitness, robustness, and limited population evolutionary potential [20]. Prevailing assumption is, the current exotic sheep breeds populations may not be able to evolve with environmental transformations and its implications which is due to climate change (Aboul-Naga et al., [21,22]).

Adaptation traits (such as survival, disease resistance and reproduction) have been selected for through natural selection in the genomes of indigenous sheep breeds, whereas in exotic sheep breeds have been selected through artificial selection for traits such as growth and feed efficiency [19]. The Nguni ecotype sheep breeds (Zulu, Pedi and Swazi), amongst other local indigenous sheep breeds, were the first sheep breeds that arrived in southern African around 2000 years BP with the arrival of the Bantu tribes [23]. Since then, this sheep ecotype has thrived in local environments, primarily subjected to natural selection, representing their genetic architecture as an optimal blueprint which could elucidate the biological adaptations necessary for resilience to local environmental challenge (Gwala et al., [24,25]). This is substantiated by their elevated fitness under local natural conditions that has been reported high levels of phenotypic variability and genetic diversity ([26,27]; Gwala et al. [24,25]; Zanella et al., 2016 [28]). This is confirmed by population and genetic diversity studies where Pedi sheep showed the highest mean number of alleles (7.04) than both South African merino (5.96) and South African mutton merino (5.76) [29]. High phenotypic variation was also reported among different Zulu sheep populations on body measurements, such as body weight, tail length and circumference [30]. The utilisation of indigenous Nguni sheep as unique genetic resources may contribute significantly to ensuring sustainable breeding programmes towards producing high performance livestock breeds that are resilient to harsh local environments. However, to increase precision and efficiency of these breeding programmes through techniques such as marker-assisted breeding, indigenous breeds should be fully characterised and the genetic mechanism underpinning their adaptive traits better understood (Woolley et al., [31,32]).

The introduction of contemporary affordable high throughput whole genome sequencing technology offers researchers the opportunity to

genotype any organism and decipher their genotype-phenotype correlations ([15]; Yang et al., [33–36]). Such studies contribute significantly to understanding the genomic basis of specific advantageous phenotypic alterations brought by adaptation. Using Illumina HiSeq2000 paired-end reads, Ahbara et al. [37] reported for the 1st time several genes under selection in northeast African indigenous sheep genomes (Libyan, Sudanese and Ethiopian). Amongst those genes, there were thermoregulation (*CREB3L2*, *CREB3*, *GNAQ*, *DCTN4*) and hypoxia associated genes (*EGLN1*, *EPAS1*) which may be contributing to their environmental adaptation. In western African, 5 genes (*STX7*, *SCAMP1*, *RAB35*, *CD19*, *CTSS*) associated with trypanotolerance were reported under positive selection between Djallonke and Sahelian thin tailed indigenous sheep breeds of Ghana [35]. Nevertheless, the insight into genomic drivers underpinning sheep breeds' adaptation to specific environmental challenges is still inadequate and not fully investigated (Aboul-Naga et al., [21,22]). There are over 1000 sheep breeds reared across the globe under different environments and only a handful have been studied (Soma et al., [11,38–40]). Particularly, there is still a need to further genetically characterise indigenous breeds for insight into their genetic adaptation to respective environments [41]. The current review first reiterates the concept of southern African indigenous sheep ecotype (Nguni sheep) as a valuable genetic resource for future breeding programmes against local environmental adversities. We further discussed the importance of understanding the genetic mechanisms underpinning their adaptive traits to southern African environments, which have not been explored previously. Finally, contemporary genomic approaches commonly used in investigating genetic mechanisms that underpin livestock environmental resilience were also highlighted. We suggest that these approaches should be incorporated in future Zulu sheep genomic studies to understand their unique genotypes associated with their adaptation.

2. The overview of recent developments in ovine genomic resources

Since 2002, the International Sheep Genomics Consortium (ISGC) has been contributing significantly to global Ovine research community by developing public genomic resources such as several SNP chip arrays and ovine bacterial artificial chromosome (BAC) library to assist researchers decipher genotype/phenotype association [15]. However, with the introduction of whole genome sequencing, these genomic resources eventually became outmoded. In January 2009 during the ISGC workshop held in San Diego, California, the consortium agreed to initiate sequencing of the sheep reference genome [15]. In 2014 the first annotated sheep genome (Oar_v3.1) was published from sequencing two unrelated Texel sheep breed individuals (see Table 1) [42]. Accordingly, with the inclusion of Pacific Biosciences RSII (PacBio RSII) long-read data, in 2015 the contiguity of the assembly was improved (Oar_v4.0) [42].

Most recently, Ovine Functional Annotation Animal Genomes (FAANG) project sequenced one female of the Rambouillet sheep breed. Through a combination of Illumina short reads and PacBio RSII long reads, a new sheep reference genome assembly (Oar_rambouillet_v1.0) was released in 2017 (Davenport et al., [21,22]). The genome was revised and annotated in 2021 (ARS-UI_Ramb_v2.0) and updated in 2023 (ARS-UI_Ramb_v3.0) for most recent and accepted contiguous sheep reference genome assembly (Davenport et al., [21,22]). This genome size is comparable to other domestic (e.g. goats 2.9 Gb and cattle 2.8 Gb) and wild (e.g. Yarkand deer 2.6 Gb) species of the order Artiodactyla, with an estimated average of 21,713 protein coding genes across these species.

Surpassing the catalogued list in Table 1, there are over 50 Ovine genome assemblies available on the National Center for Biotechnology Information (NCBI) representing different breeds across the globe (<https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=9940>). Moreover, 19 of these assemblies are at chromosome level and represent different

Table 1
Available complete Ovine reference genomes from two different domestic sheep breeds.

Assemble ID	Oar_v3.1	Oar_v4.0	Oar_rambouillet_v1.0	ARS-UI_Ramb_v2.0
Breed	Texel	Texel	Rambouillet	Rambouillet
Total sequence length	2,860,496,367	2,615,499,683	2,869,897,780	2,628,146,905
Total un-gapped length	1,201,919,081	2,587,499,057	2,869,514,717	2,628,104,905
Gaps between scaffolds	139	0	0	0
Number of scaffolds	1331	5465	2640	142
Scaffold N50	34,092,587	100,009,711	107,697,089	101,274,418
Scaffold L50	26	8	8	8
Number of contigs	2,352,347	48,481	7485	226
Contigs N50	685	150,472	2,572,683	43,178,051
Contigs L50	545,914	5008	313	24
Reference	Jiang et al. [42]	[42]	Emily et al. (2017)	[22]
NCBI Accession number	PRJNA169880	PRJNA169880	PRJNA414087	PRJNA675594

breeds such as Polled Dorset, East Friesian, Romanov, Kermani, Chinese Merino, and Dorper from United Kingdom, Germany, Russia, Iran, China, and South Africa, respectively (Shernae et al., 2023). Mostly, these genomes are a mosaic of haplotypes with each haplotype segment derived from a single sheep individual of a particular breed. Although significant progress still to be made in sequencing and assembling *Ovis Aries* genomes for various breed from different agroecological zones across the globe but, *Ovis Aries* genetic diversity at species level is now represent. However, at breed level, the abundance of genome assemblies is still a limiting factor in understanding genetic variants associated with phenotypes of interest as some variants could be missed or misinterpreted if not present in the only assembled genome for that breed [43]. Perhaps, if the recent introduction of pan-genomics in sheep could be incorporated with copious genome assemblies at breed level then encompassing the entire repertoire of genes accessible not only at *Ovis Aries* species level but at breed level as well could be achieved [44]. The Pan-genome can be defined as a collection all DNA sequences of a species that contains sequences shared by all individuals (core genome) and is also able to display sequence information unique to each individual (variable or accessory genome) [45]. Contemporary, pan-genome assembly is becoming a new reference genome standard for mining the biological genetic variants [45]. For example, the first sheep pan-genome (2.75 Gb total size) constructed from 13 breeds reported by Li et al. [44], contained 137.7 Mb non-reference sequences and confirmed 865 population-stratified structural variants that can affect the expression of 304 genes related to different phenotypes and production traits. These reports further highlighted the suitability of pan-genome reference patterns in revealing the hidden structural variants and identifying causal variants that underly phenotypic variation, such as fat-tail in sheep.

3. Nguni sheep as a significant genetic resource and their current characterization status

Nguni sheep is a southern African indigenous sheep ecotype consisting of three breeds including Zulu, Pedi, and Swazi (Soma et al., [38–40]; Gwala et al., [24,25]). They have long, carrot shaped fat tails and are known for their resilience to high temperatures, ability to survive on low quality pasture, disease resistance, and tolerance to internal and external parasites [46]. Nguni sheep were brought to southern Africa with Bantu (Nguni) people who migrated from the north towards the south of the African continent [47]. In southern Africa, Bantu people with their incursion of sheep came through southern Rhodesia (today known as Zimbabwe) traveling towards the region, then known as the Transvaal (currently, consisting of the South African providences of Limpopo, North-West, Gauteng, and Mpumalanga), and spread to the southeast of modern-day South Africa [48]. To date, Nguni sheep breeds are mostly owned by local, rural farmers and are found and adapted to different regions of southeastern Africa and has been characterised and classified mainly based on their geographic location (see Fig. 1). The

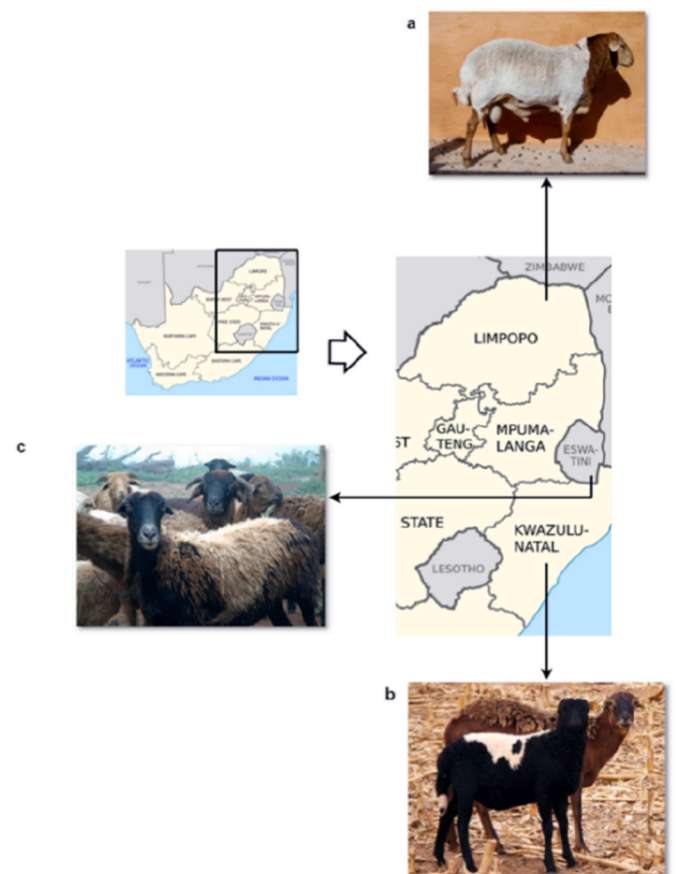


Fig. 1. Map showing geographic locations of southern African indigenous Nguni sheep breeds. A Pedi breed (Limpopo), b Zulu breed (KwaZulu-Natal) and, c Swazi breed (Swaziland).

Zulu breed is found in the eastern coastal province of South Africa, predominantly in the northern region of Kwa-Zulu Natal, while the Swazi breed is found in rural areas of Swaziland (Lebbie and Ramsay, 1999; Soma et al., [27,38–40,49]). The Pedi breed is found in the northmost province of South Africa (Limpopo) bordered by Mozambique, Zimbabwe, and Botswana (Campbell, 1995). Their isolation by distance and exposure to different climates over time could have contributed to their current genetic differentiation, therefore they are recognised as different breeds (Gwala et al., [24,25]).

There have been studies affirming the influence of environmental factors on the phenotypic heterogeneity observed in Nguni sheep populations, particularly in the Zulu breed. For instance, Kunene et al. [50, 51] conducted a study using linear body measurements to estimate live

weight of Nguni sheep, which confirmed differences in body measurements between Zulu rams from two distinct regions. The differences observed can be attributed to various environmental factors, including climate, forage availability, and local management practices, highlighting the adaptability of these sheep to their specific habitats. Moreover, genetic markers, including Random Amplified Polymorphic DNA (RAPD), microsatellites, and single nucleotide polymorphisms (SNPs), have been employed to underscore the genetic diversity within and among Nguni sheep populations. Research conducted by Gwala et al. [24,25] using RAPD provided evidence of significant genetic divergence in the Pedi breed compared to other Nguni breeds, indicating its distinct evolutionary path and genetic makeup. The author further highlighted notable proximity of Pedi sheep to exotic breeds which may suggest some introgression of exotic breeds with indigenous breeds. The need for targeted breeding strategies that can leverage this genetic diversity to enhance productivity while preserving indigenous traits.

The current literature on Nguni sheep genetic characterization establishes several key facts: (1) Nguni breeds exhibit greater genetic diversity and adaptability to the southern African environment compared to their exotic counterparts. This finding suggests that these breeds possess unique genetic traits that may be advantageous in coping with local conditions, such as disease resistance and climate adaptability. (2) The Pedi breed stands out as being phenotypically distinct when compared to other Nguni breeds as morphometric cluster analysis clustered Pedi sheep with Dorper than with other Nguni sheep Gwala et al. [24,25]. Also, Pedi had a significantly highest mean body weights (37.63) compared to Zulu (35.34) and Swazi (30.41). This divergence could be a result of specific selection pressures or environmental adaptations unique to the Pedi breed. (3) Additionally, the Pedi breed shows a higher genetic similarity to exotic breeds such as the Dorper, a locally developed synthetic breed. This relationship may provide opportunities for crossbreeding programs that aim to combine the hardiness of indigenous breeds with the growth traits of exotic breeds, ultimately improving livestock production in the region.

As one of the overall primary objectives of indigenous sheep breeds studies is to preserve their genetic uniqueness and heterogeneity, perhaps considering increasing their population gene pool could be crucial in achieving that. Within the Nguni sheep breeds context, between breeds ram exchange could contribute significantly to achieve elevated overall gene pool. However, there is still uncertainty in taking such progressive decisions because, for example, the proximity of Pedi breed to exotic breeds in this regard could have twofold influence. Firstly, Pedi sheep rams could lead to genetic erosion to “true” Nguni sheep genotypes if exchanged with for pure Nguni sheep breeding programs due to their introgressed gene pool. However, to the contrary there is a possibility that, Pedi sheep breed is the better version of Nguni sheep breeds which is more productive and adapted to local environments, and perhaps using their rams should be encouraged. Such decisions could only be taken once Nguni sheep genotypes and evolutionary history is fully explained and understood, precisely by using contemporary genetic tools to confirm their relatedness and breed specific possible contributions.

4. Socio-economic contributions of Nguni sheep within a local context

As mentioned earlier, sheep arrived in South Africa around 2000 years BP, with the arrival of the Buntu tribes [23]. Post-1652, the Dutch colony establishment at the Cape of the Good Hope introduced the first European sheep breed (Spanish Merino) in southern west Africa in 1789 to improve wool quality [23]. To expedite this initiative, in 1793 Spanish merino rams were imported for mixed breed development before the introduction of Australian merino in 1870. This was a strategic and essential initiative as these breeds contributed significantly to improving the local sheep production industry, especially as food production demands in South Africa also started to increase significant due

to human population growth after World War II in 1945. However, exotic breeds and their crosses were eventually prioritized commercially than indigenous breeds for their productive pre-eminence for specific breeding objectives, such as improving reproduction, meat and wool quality, dual meat and wool production, and milk production [52]. Contemporary, exotic sheep breeds found in southern African are highly productive due to their historic exposure to well-developed breeding programmes by exotic sheep producers compared to subsistence farmers owned local indigenous breeds [null]. Nevertheless, Nguni sheep breeds and other local indigenous breeds, although regarded less productive and mostly kept by rural and less knowledgeable subsistence farmers, they also contribute significantly as an income and affordable protein source for local communities (Kruger, 2011). Moreover, they are resilient to local diseases and can survive on low quality feed resources (no supplements required) hence resource lacking subsistence farmers find them cost effective to farm with [27].

Perhaps, Nguni sheep productivity could be improved through selective breeding practices, which has always been used in agricultural livestock production [53]. However, the question of concern is: “Are more robust animals less productive by putting their body condition before production?”. This could be true because, animals that are well adapted to thermal stress lose significant amount of blood plasma in mitigating the impact of hyperthermia through sweating and panting while compromised productivity as blood plasma contains electrolytes which are essential for production [54,55]. Furthermore, Macé et al. [56] reported that, the extent and temporal changes in profiles of body reserve mobilization and accretion which is part of the key mechanisms via which robustness is expressed is genetically linked. Selecting for better productivity in Nguni sheep therefore could mean sacrificing their robustness traits. Nevertheless, selection can still be applied in Nguni sheep populations to improve their productivity however, a pure-bred Nguni sheep conservation flock should still be maintained as a genetic reservoir and a reference in monitoring the selection flock performance. Although local farmers as primary custodians of these breeds lack technical skills and knowledge for such structured breeding programmes, trainings should be offered by relevant stakeholders to mitigate ongoing indiscriminate crossbreeding practises, leading to their genetic erosion and exacerbates their risk to extinction [null].

Nguni sheep’s resilience to many livestock production challenges within southern African agroecological zones cannot be over emphasised as it has also been highlighted to this point (Kunene et al., [50,51]; Soma et al., [27,38–40,57]). This implies that, their extinction will not only destabilize local communities monetary and affordable food resource, but the southern African breeding industry might also lose a valuable genetic reservoir that could contribute towards breeding in response to continuously exacerbating agricultural environmental conditions. Conservation initiatives must be addressed to counter tract this phenomenon. One of the previously reported feasible secondary conservation strategies for indigenous breeds is to improve their value by developing value chains for breed-specific products [12]. Furthermore, producing these breeds organically could improve their value as the preference for organic products is increasing recently [12]. These initiatives could improve Nguni sheep *in-situ* conservation as the farmers would be more interested in breeding them and conserving their genotypes.

5. Phenotypic and production traits in southern African sheep breeds

Linear body measurements such as wither height, heart girth, and live weight and scrotal circumference which are correlated with carcass weight and ram fertility, respectively, have been assessed in Zulu sheep breed [58,59]. Kunene et al. [58] findings confirmed low productivity in indigenous breeds, reporting 38 kg mature live weight for Zulu rams compared to 60–70 kg in yearling Merino sheep rams raised in pastoral systems [60]. Furthermore, a South African indigenous breed, Namaqua

Afrikaner, as one of the breeds that reached market readiness at a lower live weight compared to exotic breeds under feedlot conditions [61]. It is speculated that South African indigenous breeds' smaller body size is linked to their adaptation to local extreme heat stress, water scarcity, and limited quality pasture availability, as it reduces their overall energy requirements [62]. This is evident as Malan et al. [63] reported gene associated with exclusive energy metabolism (*MYH9*) in five South African indigenous breeds (Karakul, Damara, Pedi, Zulu and Namaqua Afrikaner) using Illumina Ovine SNP 50K BeadChip. Using the same genomic approach, Dzomba et al. [64] also reported directional selection (towards body confirmation and disease resistance) in Merino-derived sheep breeds of South Africa. Nevertheless, composite breeds such as the Meatmaster which is a cross between the Damara x Namaqua Afrikaner demonstrated cost effectiveness with shorter rearing periods in the feedlot [61]. They acquired the necessary subcutaneous fat cover for producing an A2 carcass more quickly than highly productive breeds like Merino and Dohne Merino [61]. Even though reproduction traits of indigenous breeds in southern Africa has not been extensively quantified, higher birth to weaning percentage, conception rate and udder health score was reported on Namaqua Afrikaner compared to exotic breeds such as South African Mutton Merino [65, 66].

6. Molecular studies on indigenous sheep breeds, insight into their genomic adaptation

To take advantage of the Nguni sheep traits of hardiness for breeding purposes, understanding the genomic basis of their unique capabilities is a prerequisite. However, the correlation between genotype and phenotype is a highly intricate phenomenon. For example, the epigenome comprising different mechanisms e.g. DNA methylation, remodelling, histone tail modifications, chromatin microRNAs and long non-coding RNAs, interact with environmental factors like nutrition, pathogens, and climate could influence the expression profile of genes and the emergence of specific phenotypes [67]. Moreover, multi-level interactions between the genome, epigenome and environmental factors might occur, resulting to altering phenotypes including health and production [68,68].

Historically, for insight into genes and mutations underpinning adaptation, researchers have always relied on forward genetic approaches [69]. However, with the recent molecular technology advancements, reverse genetic approach-based genome-scans has become feasible, enabling the identification of genetic alterations due to adaptation such as signatures of selection on any species or population. These sought-after approaches, which may also include transcriptomics and methylomics, disregards phenotypic data availability and could be contributory in studying genes associated with phenotypes of interest on less phenotypically explored organisms such as indigenous breeds [69]. Mwacharo et al. [70], using medium-density Chips, reported several genes involved in Djallonké sheep of Burkina Faso adaptation to humid areas of Western Africa. The authors reported various significantly enriched genes, distinct by their enrichment scores, Cluster 1 (*ALB*, *GC*, *AFP*, and *AFM*), Cluster 2 (*MC2R*, *CIB1*, *MC5R* and *CAVI*) and Cluster 3 (*LDLRAD4*, *LRP11*, *CFI* and *VLDLR*). Edea et al. [71,72] also reported candidate genes relevant to altitude adaptation on Ethiopian sheep populations. Different genomic regions were found harbouring candidate genes related to adaptation to high-altitude, such as *PITX1* at OAR5 (43.82–43.84 Mb), which Mudie et al. (2014) reported as crucial for cellular response to hypoxia. As agricultural environments are becoming more erratic and adverse due to climate change, these traits of adaptability could be of elevated significance in breeding programmes for robustness. However, inadequate effort is channelled in documenting genomic basis underpinning these traits. In southern Africa for example, there is limited literature focusing on such phenomenon within indigenous sheep breeds. According to Dzama [73], using appropriate genotypes, adapted and efficient indigenous animals, desired significant

genetic gains can be made in securing more climate-resilient in agricultural livestock. Below we discussed some of the contemporary genomic techniques that may provide insight into Nguni sheep mechanism of adaptation.

6.1. Genome assembly, towards local native breeds pan-genome

Since the introduction of first-generation sequencing [i.e. chain termination (Sanger) sequencing] in 1977, researchers and cohorts have continuously shown diligence towards achieve a cost-effective genome sequencing method [74]. This long sought-after goal was eventually achieved in 2006 with next generation sequencing (NGS) technology (the second-generation sequencing) [75]. The introduction of Pacific bioscience (Pac Bio), Single Molecule Real Time (SMRT), Illumina Tru-seq Synthetic Long-Read, and Oxford Nanopore Technologies sequencing platforms (third generation sequencing) further broke new grounds with affordable theatrically longer reads output at high throughput and acceptable accuracy level [76–79]. Today, researchers benefit from using both long and short reads in assembling and understanding characteristics of many complex eukaryote species' genomes for domestic and wild species [80,81]. In genomics, high-quality, annotated genome assemblies have become a valuable tool in understanding of molecular mechanisms, gene repertoires, genome architecture, and breed evolution (Shernae et al., 2023). Currently, there is no genome assembly representing southern African local indigenous sheep population, which could be the first step in understanding their genomic divergence due to local environments adaptation at whole genome level. Perhaps, the second step would be to build towards their pan-genome assembly which its exclusive significance as a genetic variant mining tool has been discussed earlier. Studies have shown that variable new genes of value and of research significance can be discovered through pan-genome analyses and using pan-genome references enhances read mapping ratio, transcriptome alignment efficiency, and the call rate of some rare and large variants [45]. Southern African local indigenous sheep breeds pan-genome analyses, therefore, could assist in capturing their genetic diversity, improve our understanding of captured explicit variants, and enhance insights into their evolutionary history which is important in breeding programmes development and application.

6.2. Genome-wide association studies

Genome-wide association study (GWAS) is designed to detect and elucidate association between genetic variants and a given trait [82]. Genomics have contributed significantly to understanding various human traits, including behaviour, infectious disease, and susceptibility [83,84]. Furthermore, GWAS continue to play a significant role in identification of quantitative trait locus (QTLs) associated with numerous complex diseases in human clinical genetics [85]. In livestock production, however, GWAS has mostly been used in mapping QTLs to traits of environmental adaptation, and economic importance like meat quality and quantity, calving ease, milk yield, fat and protein percentage, fertility traits, and egg production (Woolley et al., [31,32]). Using this technique, 36 different candidate genes harbouring 46 SNPs associated with heat tolerance were reported in Egyptian sheep breeds (Aboul-Naga et al., [21,22]). Moreover, in beef cattle, candidate genes involved in energy metabolism, angiogenesis, pantos phosphate pathway, heat shock protein response (*COX7C*, *RASA1*, *FBP1*) and thyroid hormone regulation (*FBP2*, *HSBP1*, *TRIP11*) were also identified using GWAS [86]. This technique could contribute to elucidate genes associated with Nguni sheep hardiness. Perhaps, the limiting factor would be that phenotypic data is a prerequisite for GWAS and Nguni sheep have not been well characterised phenotypically especially on breed specific characteristics there is very limited phenotypic data available ([27]; Aboul-Naga et al., [21,22]). The solution to this could be either, 1. Employ other techniques that do not require phenotypic data

for identification of candidate genes of adaptation such as Genome-wide detection of selection signature or, 2. Use relevant techniques to acquire adequate Nguni sheep breed specific phenotypic data for genome-wide analyses which could also be relevant when associating identified selection signatures with phenotypes of interest (Aboul-Naga et al., [21, 22]). Recent tremendous advancements in genetic tools have divulged alternative methods for mining genomic mechanisms underpinning phenotypes of interest which some also assist to bypass the need for phenotypic data and offer viable solutions to advance the identification of candidate genes of adaptation in livestock.

6.3. Genome-wide detection of selection signature

Selection strategies in livestock populations including natural selection, leaves unique genetic patterns or footprints (i.e. signature of selection) in the genomic regions subjected to selection [87]. These genomic region-specific patterns influence the population or breed phenotypic characteristics such as morphology, behaviour, adaptation to specific environment, and resilience to diseases [87]. With current desire to understand genotype-phenotype association, researchers have developed different approaches that may be used to identify signature of selection (SoS) in livestock based on methods such as linkage disequilibrium (LD), allele frequency spectrums (e.g. F_{st} outlier analysis), reduced local variability, and haplotype characteristics [88]. The manifestation of selection signatures is underpinned by the idea that, beneficial mutation in a population will be favoured by selection and eventually increase in frequency across the population through the phenomenon known as selective sweep or hitchhiking effect (Fig. 2) [89]. Consequently, the advantageous allele will increase in frequency towards fixation (soft selective sweep) or becomes completely fixed for the entire population (hard selective sweep) [87]. As it has been highlighted earlier, Nguni sheep breeds have evolved under natural selection as a major driving force thus could be harbouring selection signatures that may explain their resilience to their local environments. This technique has been used before in sheep species for identification of candidate genes of adaptation. For example, Edea et al. [71,72] reported candidate genes associated with cold adaptation in Ethiopian sheep populations. Within Nguni sheep context, unlike the GWAS discussed earlier, the is no limitations for this technique as it does not require phenotypic data for candidate genes identification.

6.4. Whole transcriptome sequencing

Different livestock individuals, breeds and species responds differently to distinct stimuli, biologically, and transcriptome studies (i.e. transcriptomics) has become the most widely used method to reveal links between gene expression and life phenomena [90]. Furthermore, the introduction of high throughput RNAseq technology post Maxam and Gilbert chemical degradation sequencing method has revolutionised transcriptomic analyses. High throughput RNAseq technology provides

large dynamic range and sensitivity, precision, unbiased quantification of transcripts and comprehensive coverage of all expressed sequences in a given tissue sample [91]. Evidently, RNAseq methods with appropriate bioinformatic analyses provides the opportunity to distinguish closely related transcripts from each other and efficiently identify and quantify splice variants, fused transcripts and mutants [90]. Bakhtiarizadeh et al. [92], through genome-wide gene expression profiling, compared transcriptomes between fat-tail and thin-tailed sheep breeds for insight into fat deposition in sheep tail. Several genes were found differently expressed between these breeds suggesting differences in their adipocyte regulations. Such results emphasize gene regulation (up and down) analyses as a tool that could be used to explain phenotypic expressions. Li et al. [93] also performed transcriptomic analyses of Hu Sheep liver to identify mRNAs, lncRNAs and pathways related to heat stress and reported significant changes in mRNAs, lncRNAs under different conditions of heat stress.

Transcriptomics, as a modern powerful technique should be incorporated to catalogue all adaptation related rare transcripts including post-transcriptional modifications such as splicing patterns and gene fusions [94]. As some phenotypic differences may be transcriptional related, this could expand the horizon in understanding indigenous breeds adaptation to adverse environments.

6.5. Epigenomics

The epigenome of an organism defines the mechanisms of molecular processes associated with the genome which influences the expression (up and down regulation) and activity of genes without modification of genomic sequence [95]. This is one of the mechanism organisms use to maintain homeostasis under different environmental circumstances [96]. Epigenomic status at any level such as within and/between populations, is determined by biological regulatory mechanisms, including DNA methylation (mostly DNA Cytosine Methylation in eukaryotes), histone modifications, chromatin structure changing, non-coding RNA expression [95,96]. Fan et al. [97] using deep whole-genome bisulfite sequencing reported, 11,522 differentially methylated genes in skeletal muscle cells between foetus and adult Hu sheep. The author further used comparable relationship between skeletal muscle development and meat production and quality for insight into Hu sheep meat productivity. Although Nguni sheep possess resilient characteristics such as the ability to survive on high roughage low quality feed, their productivity including carcass quality can be improved. Their epigenomic analyses may not only assist in understanding fundamental epigenomic molecular processes contributing to their adaptation but may also contribute to improving Nguni sheep productivity through selecting for better production associated epigenomic traits.

7. Conclusion

Domestication, breed formation and artificial selection, has left detectable signatures of selection in numerous regions of livestock genomes. Sheep is one of the most important livestock species as they are globally distributed and associated with respective socioeconomic significance. Nguni sheep plays a significant role for subsistence farmers of southern Africa as an affordable source of protein (food security). Thus, their insecurity has become a topic of interest due to their already highlighted significance, and this review has further discussed fundamental challenges underpinning their declining numbers and notable ongoing genetic erosion, attributed to subsistence farmers poor farming practices and indiscriminate crossbreeding respectively. However, this valuable animal genetic resource can still be secured from possible extinction if immediate actions could be taken including conservation initiatives strategies such as improving their value by developing value chains for their products including producing them organically as the preference for organic products is increasing recently. Furthermore, having Nguni sheep populations that are exposed to intense artificial

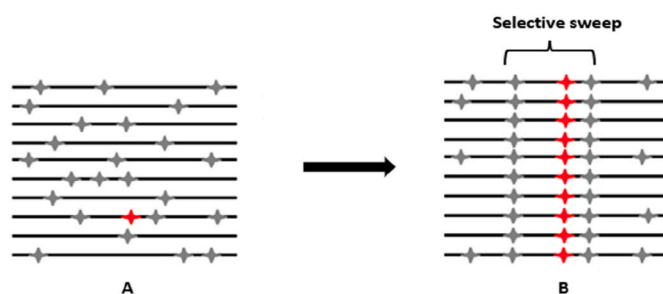


Fig. 2. A schematic illustration of beneficial mutation (red), before (A) and after (B) selection. As a new beneficial allele rises to high frequency, nearby linked alleles on the chromosome 'hitchhike' along with it to high frequency, creating a 'selective sweep'.

selection and strategic breeding programmes may assist improve their productivity for the future. Our literature analyses indicated that there are limited studies on revealing the genes/genomic regions that plays a role in the adaptation of southern African indigenous breeds to local environments. Further studies which may include pan-genome assembly for southern African indigenous breeds, encompassing the entire repertoire of genes accessible to these local breeds and improve the mining of their unique genetic variants of adaptation are suggested. So far with genomics, we have been able to identify some of the genes playing a role in adaptation in southern African indigenous sheep breeds, however this needs to be further verified using techniques such as GWAS studies as well. Thus, omics still have a role to play in this regard as a tool that could assist in divulging genomic regions which underpin Nguni sheep phenotypes of adaptability and could result in enhancing their productivity.

CRedit authorship contribution statement

Nkosinathi Nxumalo: Writing – original draft. **Clint Rhode:** Writing – review & editing, Supervision, Conceptualization. **Nokuthula Kunene:** Supervision. **Annelin Molotsi:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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