Mammalian Biology (2021) 101:439–449
https://doi.org/10.1007/s42991-021-00126-1

ORIGINAL ARTICLE

Elucidation of coat colour genetics in blue wildebeest

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Received: 18 June 2020 / Accepted: 16 April 2021 / Published online: 14 May 2021

Abstract

The golden wildebeest, a colour variant of the blue wildebeest (*Connochaetes taurinus taurinus*), is one of the most common colour variant animals that South African game ranchers breed for. Based on pedigree records, the prevailing hypothesis is that the golden coat colour is an autosomal recessive trait. However, the genetic basis of the golden coat colour phenotype has not been investigated. A genome-wide association study (GWAS) was performed with 14 624 single nucleotide polymorphisms (SNPs) to identify putative candidate genes involved in blue wildebeest pigmentation. A total of 374 SNPs were significantly associated with coat colour (P value ≤ 0.001). Five of these SNPs mapped to four different *Bos taurus* orthologous genes that could be involved in pigmentation based on previous literature reports. An additional three SNPs with an association P value ≤ 0.05 mapped to well-known pigmentation genes and were also considered. Based on the reported biological function of the genes, the *myosin VC* (*MYO5C*), *myosin VIIA* (*MYO7A*), *solute carrier family 6 member 3* (*SLC6A3*), *solute carrier family 28 member 2* (*SLC28A2*), *dopamine receptor D2* (*DRD2*), *frizzled class receptor 4* (*FZD4*) and *tyrosinase* (*TYR*) genes are promising candidate genes that could contribute to coat colour determination in blue wildebeest. Based on the number of identified candidate genes, gene–gene interaction analysis, and their determined mode of inheritance, coat colour in blue wildebeest could rather be a quantitative threshold trait. This study provides a basis for further investigation on the genetic mechanisms of pigmentation in blue wildebeest.

Keywords Genome-wide association study · Single nucleotide polymorphism · Functional annotation · Inheritance model

Introduction

The South African wildlife industry is a multi-billion Rand (ZAR) enterprise centred on wildlife ranching activities that include the stocking, breeding, trading, and hunting of game animals (Cloete and Rosouw 2014). During the past decade, the industry has shifted from mainly focusing on hunting and ecotourism to the selective breeding of sought-after atypical colour variants, such as black impala (*Aepyceros melampus*) and golden wildebeest (*Connochaetes taurinus taurinus*; Miller et al. 2016). Although colour variant breeding has attracted the attention of both local and international investors, it has also received criticism from key stakeholders and role players in the industry. Some have questioned the conservation consequences of selecting for colour variations that are infrequently seen in the wild (Russo et al. 2019). Specific concerns regarding the intensive breeding of wildlife have been raised, which include the distortion of the natural process of evolution, the loss of genetic diversity due to isolation and inbreeding, the fixation of deleterious alleles, weakened resilience to environmental changes and reduced reproductive fitness of captive stock (Miller et al. 2016; Russo et al. 2019). Consequently, the colour variant market has declined considerably in recent years. Nevertheless, a large number of colour variant game animals still remain on wildlife ranches across South Africa (Coetzer and Grobler 2019).

A study by Taylor et al. (2016) identified the golden wildebeest, a colour variant of the blue wildebeest (*C. t. taurinus*), as one of the most common colour variant animals that game ranchers breed for in South Africa.

Unlike the silvery-blue coat colour of the wild-type blue wildebeest, the coat of the golden wildebeest is light

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Handling editor: J. Paul Grobler.

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golden-brown to yellow–brown in colour (Adetunji et al. 2018). In 2016, during the peak of the colour variant market, a blue wildebeest sold for an average auction price of ZAR 3 137 while a golden wildebeest sold for an average price of ZAR 418 090 (Cloete 2017). Nevertheless, despite the collapse of the colour variant market in recent years, golden wildebeest still had a higher average auction price (ZAR 8 394) than that of wild type blue wildebeest (ZAR 3 795) during the 2019 auction season (Vleissentraal 2019). Therefore, elucidation of the genetic mechanisms underlying this golden coat colour phenotype is of great interest. Based on pedigree analysis, the golden coat colour phenotype is believed to be the result of a simple autosomal recessive mutation (Taylor et al. 2016). However, the gene mutation and the molecular genetic basis of the golden coat colour phenotype, in general, has not been studied previously. Consequently, this mode of inheritance has not been confirmed. In addition, it is also not known if the causal variant of the golden coat colour is linked to other pleiotropic effects (Olivier 2015). Mutations influencing coat colour often have other phenotypic effects including susceptibility for skin diseases, disorders of the immune system, reproductive tract, and sensory organs. If the casual variant is linked to genes that are associated with pathological developments, it may pose significant economic drawbacks in terms of mortality, aesthetics and management costs (Adetunji et al. 2018).

Through the study of model organisms, great insights have been gained into the molecular mechanisms underlying melanin-based pigmentation. These studies have demonstrated that pigmentation biology and genetics are highly conserved across mammals (Hubbard et al. 2010). Melanocytes express a limited number of specific proteins that act in the melanogenesis cascade, with tyrosinase (TYR) as the rate-limiting enzyme, to produce the melanin biopolymer in mammals (D'Mello et al. 2016). Melanin pigments are synthesised and stored in melanosomes, which are lysosome-related organelles, before distribution to the surrounding keratinocytes (Cieslak et al. 2011; D'Mello et al. 2016). Mammalian melanocytes can produce two distinct types of melanin: the brownish-black eumelanin and the reddish-yellow pheomelanin. Coat colour variability is mainly determined by the ratio of eumelanin to pheomelanin, which is regulated by the melanocortin 1 receptor (MC1R) ligand system (D'Mello et al. 2016). Coat colour can, however, be modified by several other genes involved in the development and survival of pigment producing melanocytes, the regulation of pigment synthesis or in pigment transport and transfer (Cieslak et al. 2011). Currently, more than 150 genes are known to regulate pigmentation in mammals, either directly or indirectly, with updated lists of these genes being actively maintained by the European Society for Pigment Cell Research (ESPCR; http://www.espcr.org/).

Genetic research concerning the golden wildebeest will be able to facilitate breeding management and will also enable game ranchers to make informed decisions regarding the viability of golden wildebeest ranching (Russo et al. 2019). This study, therefore, aimed to (i) identify single nucleotide polymorphisms (SNPs) that are significantly associated with coat colour in blue wildebeest by performing a genome-wide association study (GWAS), (ii) identify whether any of the marker sequences that flank the significant SNPs mapped to genes with known functional roles in pigmentation, (iii) identify gene combinations that could play a role in determining coat colour in blue wildebeest, and (iv) elucidate the mode of inheritance of the golden coat colour variation.

Materials and methods

Study population: pedigree relations and genetic resources

Ninety-four blue wildebeest (*C. t. taurinus*) originating from a private game ranch (S24° 45.429′ E28° 27.162′) located in Limpopo Province, South Africa were selected for this association study after written informed consent was obtained from the rancher. The selected blue wildebeest included 24 wild-type blue wildebeest, 35 golden wildebeest and 35 so-called split wildebeest, which are phenotypically normal blue wildebeest that are presumed carriers of the golden causal variant based on parentage records. Individuals with the golden coat colour phenotype represented cases (affected individuals), whereas phenotypically normal blue wildebeest represented controls (unaffected individuals). The majority of the selected blue wildebeest could be grouped into one of 15 families based on shared sires and, therefore, some of the individuals in the population were half-sibs. Furthermore, five of the selected blue wildebeest were also the dams of offspring in other families in the study population.

DNA extraction

Samples consisted of either hair follicles (56 samples) or whole blood stored in EDTA (38 samples), which were originally submitted to Unistel Medical Laboratories (Pty) Ltd (Cape Town, South Africa) for routine microsatellite genotyping and parentage verification. Genomic DNA was isolated from each sample using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. The extracted DNA was quantified using a Nanodrop 1000 spectrophotometer and adjusted to 50 ng/µl in a final volume of 20 µl.

Genotyping and in silico mapping

All DNA samples were sent to Diversity Arrays Technology (Pty) Ltd in Canberra, Australia for genotyping using the DArTseq platform. The DArTseq approach, a variant of genotyping-by-sequencing (GBS), implements complexity reduction methods that effectively targets low-copy sequences of the genome (Melville et al. 2017). For detailed descriptions of the DArTseq protocol, refer to Kilian et al. (2012), Lal et al. (2017) and Melville et al. (2017). A set of 20 563 genome-wide SNPs, each located in a 69 bp marker/flanking sequence, were generated for this study population using the DArTseq platform. Genomic regions flanking these SNPs were aligned to the *Bos taurus* genome sequence assembly [UMD_3.1.1; Bovine Genome Database (http://bovinegenome.org/)] using blastn with default parameter settings (Van Deventer et al. 2020). Shorter alignments inherently have higher E-values and, therefore, a less stringent E-value threshold is usually required. Since the SNP marker sequences are only 69 bp in length, an E-value cut-off of $E \leq 1.0\text{E-}01$ was used for mapping. Of the marker sequences, 3 907 mapped to putative bovine orthologues genes.

Association analysis

The 20 563 SNPs were assessed for quality before the association analysis was conducted and SNPs were removed if they had a minor allele frequency (MAF) less than 0.05 and a call rate less than 0.80. For the remaining SNPs (14 624), Hardy–Weinberg equilibrium (HWE), allele frequencies and genotype frequencies were calculated for the total sample population, as well as for the case and control groups separately, using the software GenALEx version 6.503 (Peakall and Smouse 2006, 2012). Association between the SNPs and coat colour was assessed using the software UNPHASED version 3.1.7 (Dudbridge 2008). Since coat colour in blue wildebeest is a binary trait, a case–control design was implemented for the association study. To prevent confounding and to strengthen the power of the analysis, all known family relationships were taken into account. The default "Full" analysis model for genotypic tests was used for the association analysis. Statistical significance was evaluated by performing 1000 random permutations. Single nucleotide polymorphisms were considered to be significantly associated with coat colour in blue wildebeest for P value ≤ 0.001. In addition, SNPs that mapped to *B. taurus* genes with a known role in pigmentation (*i.e.,* occurs in the ESPCR pigment gene database) were considered statistically significant for P value ≤ 0.05. To estimate the genetic effect size of the statistically significant SNPs, the odds ratio (OR) for each genotype and upper and lower bound 95% confidence intervals (95% CI) were also calculated by the UNPHASED software.

The statistically significant SNPs were also analysed using the software SNPstats (Solé et al. 2006; https://www.snpstats.net/start.htm), to confirm the association. In addition, SNPstats assessed multiple inheritance models (dominant, over-dominant, co-dominant, recessive and additive) to evaluate the associations between the statistically significant SNPs and coat colour in blue wildebeest. Each inheritance model was assessed by performing an unconditional logistic regression analysis, and the best-fitting inheritance model for each polymorphism was identified based on the lowest Akaike Information Criterion (AIC) and/or Bayesian Information Criterion (BIC).

Assessment of linkage disequilibrium

The extent of linkage disequilibrium (LD) was assessed between the SNPs found to be significantly associated with coat colour. The standard descriptive LD parameters, D' and r^2, were estimated for all pairwise SNP combinations using the LD plot function in the software Haploview version 4.2 (Barrett et al. 2005). Using LD categories defined by Espigolan et al. (2013), pairwise SNP combinations were classified as exhibiting low LD ($r^2 \leq 0.16$), moderate LD ($0.16 < r^2 \leq 0.70$) or high LD ($r^2 > 0.70$). In addition, LD for all possible pairs of loci were also tested with probability tests in Genepop version 4.2 using default Markov chain parameters (Raymond and Rousset 1995; Rousset 2008). Linkage disequilibrium was considered to be significant for P values ≤ 0.05.

Functional annotation and identification of candidate loci

The marker sequences of the statistically significant SNPs were functionally annotated using the Blast2GO pipeline (Blast2GO version 5.5.1; Götz et al. 2008). The SNP flanking sequences were used to conduct a blastx search against the non-redundant (nr) National Center for Biotechnology Information (NCBI) sequence database prior to mapping for Gene Ontology (GO) terms. Default blastx parameters were used, however, a mammalian taxonomic filter was applied to make the blast search more time-efficient. Furthermore, similar as for the in silico mapping, blast matches were considered statistically significant for E values $\leq 1.0\text{E-}1$. Mapping and annotation of GO terms at level 2 were performed with default Blast2GO settings.

The marker sequences of the statistically significant SNPs with $P \leq 0.001$ were then matched, where possible, to the *B. taurus* orthologous genes identified in blue wildebeest. Each of these genes were further investigated to determine if they could be involved in pigmentation. Genes that could possibly play a role in pigmentation, based on previous literature reports, represented putative candidate genes for the determination of coat colour in blue wildebeest. In addition, *B. taurus* orthologous genes that occurred in the ESPCR database were also considered as putative candidate genes for coat colour determination if the corresponding SNP had a *P* value ≤ 0.05 for the association study. The orthologous genes that were identified as putative candidate genes for coat colour determination were then assigned GO terms using the PANTHER Classification System (Mi et al. 2019). The analyses were conducted using the Gene List Analysis tools available on the PANTHER website (http://www.pantherdb.org) and the GO-slim component terms for molecular function, biological processes and cellular components were retrieved for each of these putative candidate genes.

Gene–gene interaction analysis of candidate loci

All possible combinations of the candidate genes were assessed to determine if an interaction exists between any of these genes. The software UNPHASED version 3.1.7 (Dudbridge 2008) was used to test these combinations by performing gene–gene interaction analyses using the genotype tests option. A total of 1000 random permutations were performed to allow for multiple testing. Gene interactions were considered to be significant for *P* value ≤ 0.05.

Results

Association analysis

A total of 374 SNPs were significantly associated ($P \leq 0.001$) with coat colour in blue wildebeest. These SNPs are listed in Online Resource 1. In addition, three SNPs with an association *P* value ≤ 0.05 mapped to genes occurring in the ESPCR pigment gene database and were, therefore, also considered to be significantly associated with coat colour. The descriptive statistics for the 377 statistically significant SNP are summarised in Online Resource 2, while the descriptive statistics for the SNPs located in the individually identified putative candidate genes are presented in Table 1.

Assessment of linkage disequilibrium

Based on the LD categories defined by Espigolan et al. (2013), 69 219 (97.66%) pairwise SNP combinations were classified as exhibiting low LD, 1 642 (2.32%) were classified as exhibiting moderate LD and 15 (0.02%) were classified as exhibiting high LD. The average D' estimated for all pairwise SNP combinations was 0.39 [standard deviation (SD) = 0.27)], while the average r^2 was 0.04 (SD = 0.05). Based on the results of the probability tests, 34.16% of the 70 876 possible SNP combinations showed significant LD ($P \leq 0.05$).

Functional annotation and identification of candidate loci

Only 37 SNP sequences of the 377 statistically significant SNPs could be annotated and grouped into one or more of the three main Blast2GO Gene Ontology categories (Fig. 1). Among molecular functions, "binding" represented the most abundant category (22 sequences, 39%), followed by "catalytic activity" (16 sequences, 28%) and "transporter activity" (ten sequences, 18%). Among the biological processes, 19% (23 sequences) were annotated in both the "cellular process" and "metabolic process" categories and 15% (18 sequences) in the "regulation of biological process" and "biological regulation" categories. Single nucleotide polymorphism sequences associated with the "membrane" (22 sequences, 26%) and "membrane part" (17 sequences, 20%) represented the most dominant groups of the cellular component terms.

The marker sequences of the 374 significant SNPs ($P \leq 0.001$) were then matched, where possible, to the *B. taurus* orthologous genes previously identified in blue wildebeest. Of these significantly associated SNPs, 150 could be mapped to the bovine genome (Fig. 2). The bovine chromosomes with the highest number of mapped SNPs were chromosome 4, 5 and X. Of the 150 SNPs that mapped to the bovine genome, only 101 mapped to genes with known protein products. Each of these orthologous genes were further investigated to determine if they could be involved in pigmentation. A total of four genes [*myosin VC* (*MYO5C*), *myosin VIIA* (*MYO7A*), *solute carrier family 6 member 3* (*SLC6A3*) and *solute carrier family 28 member 2* (*SLC28A2*)] were identified as putative candidate loci for coat colour determination. The GO component terms, retrieved using the PANTHER classification system, for the putative candidate genes are presented in Online Resource 3. These candidate genes are mainly involved in myosin-dependent transport and the transmembrane transport of ions. Furthermore, an additional three orthologous genes [*dopamine receptor D2* (*DRD2*), *frizzled class receptor 4* (*FZD4*) and *tyrosinase* (*TYR*)] occurred in the ESPCR database of pigmentation genes and were also considered as putative candidate genes, because their corresponding SNPs had an association *P* value ≤ 0.05. Online Resource 4 presents the GO terms retrieved for these putative candidate genes. In total, seven genes were, thus, identified as putative candidate genes that could determine coat colour in blue wildebeest. The eight SNPs located in these genes are presented in Table 1. All SNPs located in the putative coat colour candidate loci fitted an additive model of inheritance best, except for two. These were SNP 100071798|F|0–48:A > G which has a dominant mode of inheritance according to the AIC and BIC values, and SNP 100025667|F|0–39:T > C and 100076096|F|0–31:A > G, which could either have a dominant or additive mode of inheritance.

Table 1 Summary statistics of the case–control association analysis for the SNPs associated with putative coat colour candidate genes

SNP ID	Alleles (Major/Minor Allele)	Blast *E* Value	Gene	MAF			Heterozygosity						HWE *P* value			Association *P* value	OR (95% CI)		Best-Fitting Model of Inheritance
				Case	Control	Total Cohort	Observed (Case)	Expected (Case)	Observed (Control)	Expected (Control)	Observed (Total Cohort)	Expected (Total Cohort)	Case	Control	Total Cohort				
100028101|F|0–43:C > A	C/A	2.63E-03	*MYO5C*	0.31	0.42	0.48	0.40	0.43	0.54	0.49	0.49	0.50	0.67	0.40	0.85	5.46E-04**	A/C: 2.48 (0.62–10.02)	C/C: 11.33 (2.62–49.05)	Additive
100076096|F|0–31:A > G	A/G	3.20E-02	*MYO7A*	0.41	0.00	0.16	0.24	0.48	0.00	0.00	0.09	0.27	2.71E-03*	1.00	7.68E-10 **	2.94E-08**	A/G: 2.63E + 09 (1.69E + 09 – 4.10E + 09)	G/G: 5.12E + 10 (3.35E + 10 – 7.83E + 10)	Dominant/ Additive
100025667|F|0–39:T > C	T/C	4.16E-07	*SLC6A3*	0.00	0.43	0.27	0.00	0.00	0.59	0.49	0.37	0.40	1.00	0.19	0.57	1.65E-12**	C/T: 2.62E + 07 (2.62E + 07 – 2.62E + 07)	T/T: 2.10E + 17 (2.10E + 17 – 2.10E + 17)	Dominant/ Additive
100071798|F|0–48:A > G	A/G	4.16E-07		0.50	0.28	0.36	0.59	0.50	0.24	0.40	0.37	0.46	0.30	2.57E-03*	0.06	1.85E-04**	A/G: 8.75 (2.82–27.17)	G/G: 5.10 (1.35–19.37)	Dominant
100048574|F|0–35:G > A	G/A	3.20E-02	*SLC28A2*	0.09	0.32	0.23	0.17	0.16	0.44	0.44	0.34	0.36	0.58	0.94	0.62	9.86E-04**	A/G: 1.71E + 08 (9.86E + 07 – 2.97E + 08)	G/G: 8.21E + 08 (4.73 + 08 – 1.42E + 09)	Additive
100075122|F|0–28:G > A	G/A	2.63E-03	*DRD2*	0.33	0.17	0.23	0.31	0.44	0.26	0.28	0.28	0.35	0.09	0.69	0.06	3.64E-02*	A/G: 0.24 (0.04–1.45)	G/G: 0.14 (0.02–0.75)	Additive
100028737|F|0–18:G > A	G/A	3.20E-02	*FZD4*	0.47	0.24	0.32	0.49	0.50	0.37	0.36	0.42	0.44	0.88	0.82	0.60	4.03E-03*	A/G: 0.28 (0.06–1.24)	G/G: 0.10 (0.02–0.47)	Additive
100070025|F|0–27:C > T	C/T	7.52E-04	*TYR*	0.35	0.13	0.21	0.21	0.45	0.14	0.22	0.17	0.33	2.00E-03 *	0.01*	4.11E-06**	9.64E-03*	C/T: 2.45 (0.76–7.92)	T/T: 7.47 (1.75–31.90)	Additive

Confidence interval (CI); dopamine receptor D2 (DRD2); frizzled class receptor 4 (FZD4); Hardy–Weinberg equilibrium (HWE); minor allele frequency (MAF); myosin VC (MYO5C); myosin VIIA (MYO7A); odds ratio (OR); single nucleotide polymorphism (SNP); solute carrier family 6 member 3 (SLC6A3); solute carrier family 28 member 2 (SLC28A2); tyrosinase (TYR)

*Statistical significance at *P* value cut-off of 0.05

**Statistical significance at *P* value cut-off of 0.001

Fig. 1 Gene ontology distribution of all single nucleotide polymorphisms (SNPs) significantly associated with coat colour in blue wildebeest. The results are summarised as follows: **a** molecular functions, **b** biological processes, **c** cellular components

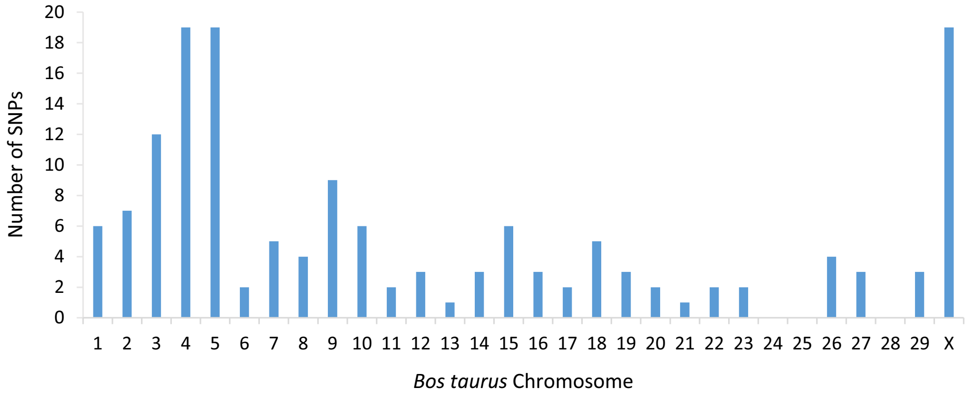

Fig. 2 Distribution of the single nucleotide polymorphisms (SNPs), significantly associated with coat colour in blue wildebeest (P value ≤ 0.001), across the *Bos taurus* chromosomes

Gene–gene interaction analysis of candidate loci

Coat colour is a polygenic trait and many of the coat colour-associated genes and their alleles often have epistatic interactions (Cieslak et al. 2011). However, none of the interactions between SNPs located in the identified coat colour candidate loci were found to be statistically significant (P values > 0.05).

Discussion

Despite golden wildebeest being one of the most common colour variant animals that game ranchers breed with in South Africa, the genetic basis of the golden coat colour phenotype has not been studied previously. Therefore, to elucidate the genetic underpinnings of coat colour in blue wildebeest, a GWAS was conducted with 14 624 SNPs. Of these SNPs, 377 were found to be significantly associated with coat colour.

Only 37 of the 377 significant SNP marker sequences could be functionally annotated. The SNP sequences that could not be functionally annotated could be located in genes that have not been fully annotated yet or could be located in non-coding regions. Studies have shown that SNPs located in non-coding regions could also have functional consequences by altering the binding site of transcriptional machinery (Spielmann and Mundlos 2016). Therefore, some of the SNPs that were not annotated could still influence coat colour in blue wildebeest by affecting the regulation of pigment gene expression. Moreover, tag SNPs in high LD with the causal variant could also show statistical association with coat colour if the actual functional variant has not been directly genotyped (Bush and Moore 2012). The natural decay of LD occurs at a much slower rate in inbreeding systems because there is a severe decrease in effective recombination and, consequently, genetic variants remain correlated over larger physical distances (Caldwell et al. 2006). The observed heterozygosity in the study population was lower than the expected heterozygosity, which indicates that there could indeed be inbreeding in the population which, in turn, could result in a higher level of LD (Online Resource 2). The level of LD was estimated between all significant SNP combinations to determine if a large number of the unannotated SNPs could indeed be tag SNPs of the causal variant. Generally, the pattern of LD between neighbouring markers are high and decreases with an increase in marker distance (Berihulay et al. 2019). However, because the positions of the significant SNPs relative to each other were not known in the current study, the LD between these markers could not be measured as a function of distance. As a result, the average D' and r^2 were estimated over all possible pairwise SNP combinations. Therefore, although the average D' and r^2 values seem low, it should be compared to the average LD observed across all chromosomes in other species. The LD observed in the current study was higher than the average LD generally observed in farmed domestic sheep (Al-Mamun et al. 2015; Alvarenga et al. 2018), but lower than that observed in cattle (Espigolan et al. 2013; Mustafa et al. 2018). Based on these comparisons, the number of tag SNPs in the current study is expected to be slightly higher than the number of tag SNPs in sheep association studies, but less than the number in cattle association studies. The percentage pairwise SNP found to be in significant LD (34.16%), based on the probability test P values, was also significantly more than the 14.58% reported in water buffalo (*Bubalus bubalis;* Nagarajan et al. 2009). In addition, although the significant SNPs were distributed throughout the *B. taurus* genome, more than a third (37.25%) of the mapped SNPs mapped to bovine chromosome 4, 5 and X, suggesting that these SNPs could possibly be physically linked.

Despite the large number of SNP sequences that could not be functionally annotated, the Blast2GO functional analysis is able to give an overview of the molecular functions, biological processes and cellular components involved in blue wildebeest pigmentation. Interestingly, the most prevalent cellular component was “membrane”, while binding and transporter activities also seem to play an important role in blue wildebeest pigmentation. Many previous studies have also identified genes coding for ion transport proteins as key regulators of melanin synthesis (*e.g.,* Bellono et al. 2016; Chao et al. 2017). These studies suggest that ion exchange might play a pivotal role in the regulation of pigmentation through the regulation of melanosomal pH. A study by Ancans et al. (2001) has shown that the activity of tyrosinase, the rate limiting enzyme in melanin synthesis, is optimal at a neutral pH. However, melanosomes, which are lysosome-related organelles, have an acidic pH. Therefore, melanogenesis is generally stimulated by an increase in melanosomal pH, while the activity of tyrosinase is gradually lost with decreasing pH (Cheli et al. 2009). Furthermore, studies by Ito et al. (2013) and Wakamatsu et al. (2017) have also found that an slightly acidic pH chemically shifts mixed melanogenesis to more pheomelanic states by suppressing the late stages of eumelanogenesis, that occur after the stages catalysed by tyrosinase.

A number of studies have specifically found mutations in *solute carrier* (*SLC*) genes to affect pigmentation by regulating pH (*e.g.,* Cook et al. 2008; Cieslak et al. 2011). Members of this gene family encode membrane-bound transporters that facilitate the movement of specific substrates, either against or with its concentration gradient (He et al. 2009). In the current study, three significantly associated SNPs were located in *SLC* genes, and were thus considered as putative candidate genes. The SNP in one of these *SLC* candidate genes could possibly result in a change in melanosomal pH, which in turn could result in a change in tyrosinase activity. Although, the gene–gene interaction analysis did not identify a significant interaction between the SNPs located in the *SLC* genes and the SNP that mapped to *TYR* (SNP 100070025|F|0–27:C > T), it does not undoubtedly prove that a *SLC* gene is not involved in the regulation of tyrosinase in blue wildebeest. Previous studies have shown that melanin synthesis does not always correlate with the level of *TYR* expression, and that TYR is also regulated post-transcriptionally. The subsequent processing of TYR to produce a mature, functional enzyme has been found to depend on the neutralisation of pH in the Golgi (Watabe et al. 2004). A change in pH caused by a SLC protein could, thus, possibly affect the maturation or functionality of TYR after translation, which in turn, could affect the rate of melanogenesis.

For SNP 100048574|F|0–35:G > A located in *SLC28A2*, each addition of the G-allele increased the odds of the resulting coat colour phenotype being golden (A/G genotype: OR 1.71E + 08; CI 9.86E + 07–2.97E + 08; G/G genotype: OR 8.21E + 08; CI 4.73E + 08–1.42E + 09). Furthermore, for SNP 100025667|F|0–39:T > C, located in *SLC6A3*, none of the golden wildebeest case individuals were carriers of the C-allele, and each addition of the T-allele also increased the odds of the coat colour being golden (C/T genotype: OR 2.62E + 07; 95% CI 2.62E + 07–2.62E + 07; T/T genotype: OR 2.10E + 17; CI 2.10E + 17–2.10E + 17). Both of these SNPs had large genetic size effects and are thus expected to greatly influence coat colour in blue wildebeest. It is, however, important to note that the size effects of these SNPs could possibly be overestimated due to the small study population. Furthermore, the other SNPs that mapped to *SLC6A3*, 100071798|F|0–48:A > G, also significantly deviated from HWE in the control group, but not for the case group. This deviation in the control group is likely the result of the lower frequency of the minor G-allele in the control group (0.28) compared to the total cohort (0.36) and the case group (0.50). The major A-allele of this SNP could thus possibly have a "protective" effect against the golden coat colour phenotype. If this SNP results in an increase in melanosomal pH, it could shift melanogenesis towards the synthesis of eumelanin, thereby reducing the odds of the coat colour phenotype being golden.

One of the significant SNPs also mapped to *TYR* and was thus also considered as a candidate gene for coat colour determination. Previous studies have reported that the required level of TYR activity is higher for the synthesis of eumelanin than for pheomelanin (Burchill et al. 1993). The golden coat colour phenotype is likely the result of increased red-yellow pheomelanin synthesis. If the SNP in the *TYR* gene results in reduced TYR activity, it might shift melanogenesis towards pheomelanin synthesis. The SNP that mapped to *TYR* deviated from HWE in the golden wildebeest case group, as well as in the control group. This deviation is likely due to the selection of case and control individuals based on phenotype, because the minor T-allele of the SNP was significantly increased in the case group (0.35) compared to the control group (0.13). Furthermore, the genotype distribution of the SNP fitted an additive model of inheritance best, with each addition of the minor *T*-allele increasing the probability of the resulting coat colour phenotype being golden (C/T genotype: OR 2.45; 95% CI 0.76–7.92; T/T genotype: OR 7.47; 95% CI 1.75–31.90). In addition, mutations in the *TYR* gene has been associated with a paler coat colour, due to reduced melanin production, and pleiotropic effects such as retinal functional abnormalities and behavioural changes (Reissmann and Ludwig 2013). Such pleiotropic effects have not been reported thus far in golden wildebeest. However, further investigation is needed to ensure that such negative effects do not occur.

Numerous proteins are involved in the sorting and trafficking of enzymes and structural proteins to melanosomes during melanin synthesis. Some actin-based myosin motors have been involved in this process. In the current study, two significantly associated SNPs (100028101|F|0–43:C > A; 100076096|F|0–31:G > A) mapped to genes coding for myosin motors, namely myosin VC and myosin VIIA, and were thus also considered as candidate genes for coat colour determination. The frequency of the C/C genotype for the *myosin VC* SNP (100028101|F|0–43:C > A) was significantly increased in the case group (0.53) compared to the control group (0.16). Carriers of the C-allele were also found to be significantly associated with a higher probability to result in the golden coat colour phenotype compared to the A/A homozygotes (OR 2.48; 95% CI 0.62–10.02). The Myosin Vs are well known for their role in the pigmentation of skin and hair. Myosin VC, specifically, has been found to function in the trafficking of integral proteins, such as tyrosinase-related protein (TRP) 1 and TRP2, to melanosomes (Bultema et al. 2014). Tyrosinase-related protein 1 and TRP2 play crucial roles in catalysing eumelanin-producing reactions. Therefore, if the C-allele of the SNP associated with *MYO5C* results in a transport protein with reduced functional activity compared to the wild-type protein, it may disrupt the synthesis of eumelanin in blue wildebeest. Furthermore, the SNP that mapped to myosin VII (100076096|F|0–31:A > G) significantly deviated from HWE in the case group, but not in the control group. The deviation observed in the case group is likely the result of the increased frequency of the minor G-allele (0.41) compared to the control group, which is homozygous for the major A-allele. The G-allele of this SNP could thus also possibly disrupt the synthesis or distribution of eumelanin. Studies have found myosin VII to be involved in the transport of retinal melanosomes and, therefore, also in the determination of eye pigmentation (Williams and Lopes 2011). Although the role of myosin VII has not been directly established in the determination of coat colour, the possibility of myosin VII playing a role in coat colour determination cannot be rejected. Additional studies are needed to determine the functional role of myosin VII in determining coat colour.

In addition, two of the SNPs located in candidate genes are not expected to have a large effect on coat colour determination because the genetic effect size of the genotypes were relatively small. These SNPs are SNP 100028737|F|0–18:G > A located in *FZD4* (A/G genotype: OR 0.28; 95% CI 0.06–1.24; G/G genotype: OR 0.10; 95% CI 0.02–0.47), and SNP 100075122|F|0–28:G > A located in *DRD2* (A/G genotype: OR 0.24; 95% CI 0.04–1.45; G/G genotype: OR 0.14; 95% CI 0.02–0.75). The *FZD4* candidate gene identified in the current study can possibly contribute to melanocyte development and/or the regulation of TYR activity by acting in Wnt signalling, while the D2 dopamine receptor could be indirectly involved in TYR regulation by regulating the synthesis of α-melanocyte stimulating hormone (αMSH), an agonist of MC1R (Yamaguchi et al. 1996; D'Mello et al. 2016).

As mentioned previously, the prevailing hypothesis is that the golden coat colour phenotype in blue wildebeest is a simple autosomal recessive Mendelian trait. However, based on the results of the current study, coat colour in blue wildebeest has a complex mode of inheritance with multiple genes, with different size effects, influencing the coat colour phenotype. The quantitative threshold model is commonly used to explain how multiple genes can influence the expression of binary traits (Moorad and Promislow 2011). Under the threshold model, the expressed phenotype for a dichotomous trait is wholly determined by an underlying, unobserved continuous trait referred to as "liability". Each of the loci involved in the phenotypic expression of the trait act additively on the scale of liability. Individuals with liability that exceeds a fixed threshold will express one phenotypic character state (affected), while individuals with liability below the threshold will express the alternate state (unaffected). Based on the results of the current study, coat colour in blue wildebeest could thus be a quantitative threshold trait mainly determined by *SLC6A3*, *SLC28A2* and *MYO7A*, because the SNPs located in these genes had the largest genetic size effects. In addition, a few minor genes with smaller size effects (*MYO5C*, *DRD2*, *FZD4*, *TYR*) are also expected to contribute to the trait. This particular inheritance model for coat colour in blue wildebeest is also supported by the gene–gene interaction analysis results which indicated that the trait is more likely to be additive across loci, with limited epistatic gene interactions. In addition, the majority of the SNPs located in the putative candidate loci fitted an additive mode of inheritance best, providing further support for the threshold inheritance model. This particular mode of inheritance for coat colour in a mammalian species has not been previously reported in published literature. It is possible that previous studies regarding coat colour inheritance in mammals may have overlooked this specific inheritance model due to study design or the use of a limited number of molecular markers. Nevertheless, due to the novelty of the findings in this preliminary study of coat colour in blue wildebeest, additional studies are required to confirm the mode of inheritance.

Conclusion

The current study represented the first genetic study to identify genes and SNPs associated with coat colour in blue wildebeest. Eight DArTseq SNP markers were identified as putative candidate loci for coat colour determination in blue wildebeest. These SNPs mapped to seven different candidate genes: *TYR*, *SLC6A3*, *SLC28A2 MYO5C*, *MYO7A*, *DRD2* and *FZD4*. Of these identified candidate genes, the *TYR* gene is the only gene, known from literature, to be associated with pleiotropic effects. However, none the negative effects associated with mutations in the *TYR* gene have thus far been reported in golden wildebeest. Furthermore, based on the results of the current study, coat colour in blue wildebeest could possibly be a quantitative threshold trait rather than a simple autosomal recessive trait as originally suspected.

Due to the short flanking sequences of the DArTseq SNPs, it was not possible to accurately determine whether any of the putative candidate SNPs for coat colour determination in blue wildebeest results in an amino acid change. Consequently, it will be important for future studies to assess the functional significance of the SNPs located in the putative candidate genes by determining if any of these SNPs result in amino acid changes or truncated proteins. Nevertheless, the findings of this preliminary study provide a valuable theoretical basis for further research regarding the genetic mechanisms of pigmentation in blue wildebeest.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42991-021-00126-1.

Acknowledgements The authors wish to thank Castle de Wildt (LBG Holdings) for providing consent for the use of their samples in this study. In addition, the authors would like to thank Dr. Barbara Picone for assisting with the mapping of the DArTseq marker sequences to the *B. taurus* genome. This study was funded by Unistel Medical Laboratories (Pty) Ltd and Stellenbosch University.

Funding This study was funded by Unistel Medical Laboratories (Pty) Ltd and Stellenbosch University.

Data availability The phenotypes and SNP dataset for this blue wildebeest population will be made available upon request for reproduction of results.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Ethical approval was not required, since the samples used for this study was not originally collected for this research project. Samples were initially submitted to Unistel Medical Laboratories for routine microsatellite genotyping and parentage testing purposes. Consent for use of the stored samples was obtained from the rancher after all analyses for record keeping purposes had been completed.

Consent to participate Written informed consent was obtained from the rancher to use make use of the samples for this study.

Consent for Publication Written informed consent was obtained from the rancher to publish the findings of the study.

References

Adetunji SA, Krecek RC, O'Dell N, Prozesky L, Steyl J, Arenas-Gamboa AM (2018) Melanoma in golden and king wildebeests. J Zoo Wildlife Med 49:134–142. https://doi.org/10.1638/2017-0059R1.1

Al-Mamun HA, Clark SA, Kwan P, Gondro C (2015) Genome-wide linkage disequilibrium and genetic diversity in five populations of Australian domestic sheep. Genet Sel Evol 47:90. https://doi.org/10.1186/s12711-015-0169-6

Alvarenga AB, Rovadoscki GA, Petrini J, Coutinho LL, Morota G, Spangler ML, Pinto LFB, Carvalho GGP, Mourão GB (2018) Linkage disequilibrium in Brazilian Santa Inês breed *Ovis aries*. Sci Rep 8:8851. https://doi.org/10.1038/s41598-018-27259-7

Ancans J, Tobin DJ, Hoogduijn MJ, Smit NP, Wakamatsu K, Thody AJ (2001) Melanosomal pH controls rate of melanogenesis, eumelanin/phaeomelanin ratio and melanosome maturation in melanocytes and melanoma cells. Exp Cell Res 268:26–35. https://doi.org/10.1006/excr.2001.5251

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265. https://doi.org/10.1093/bioinformatics/bth457

Bellono NW, Escobar IE, Oancea E (2016) A melanosomal two-pore sodium channel regulates pigmentation. Sci Rep 6:26570. https://doi.org/10.1038/srep26570

Berihulay H, Islam R, Jiang L, Ma Y (2019) Genome-wide linkage disequilibrium and the extent of effective population sizes in six Chinese goat populations using a 50K single nucleotide polymorphism panel. Animals 9:E350. https://doi.org/10.3390/ani9060350

Bultema JJ, Boyle JA, Malenke PB, Martin FE, Dell'Angelica EC, Cheney RE, Di Pietro SM (2014) Myosin Vc interacts with Rab32 and Rab38 proteins and works in the biogenesis and secretion of melanosomes. J Biol Chem 289:33513–33528. https://doi.org/10.1074/jbc.M114.578948

Burchill SA, Ito S, Thody AJ (1993) Effects of melanocyte-stimulating hormone on tyrosinase expression and melanin synthesis in hair follicular melanocytes of the mouse. J Endocrinol 137:189–195. https://doi.org/10.1677/joe.0.1370189

Bush WS, Moore JH (2012) Chapter 11: genome-wide association studies. PLoS Comput Biol 8:e1002822. https://doi.org/10.1371/journal.pcbi.1002822

Caldwell KS, Russell J, Langridge P, Powell W (2006) Extreme population-dependent linkage disequilibrium detected in an inbreeding plant species, *Hordeum vulgare*. Genetics 172:557–567. https://doi.org/10.1534/genetics.104.038489

Chao Y-K, Schludi V, Chen C-C, Butz E, Phuong Nguyen ON, Müller M, Krüger J, Kammerbauer C, Ben-Johny M, Vollmar AM, Berking C, Biel M, Wahl-Schott CA, Grimm C (2017) TPC2 polymorphisms associated with a hair pigmentation phenotype in humans result in gain of channel function by independent mechanisms. Proc Natl Acad Sci USA 114:E8595–E8602. https://doi.org/10.1073/pnas.1705739114

Cheli Y, Luciani F, Khaled M, Beuret L, Bille K, Gounon P, Ortonne J-P, Bertolotto C, Ballotti R (2009) αMSH and cyclic AMP elevating agents control melanosome pH through a protein kinase A-independent mechanism. J Biol Chem 284:18699–18706. https://doi.org/10.1074/jbc.M109.005819

Cieslak M, Reissmann M, Hofreiter M, Ludwig A (2011) Colours of domestication. Biol Rev 86:885–899

Cloete F (2017) Average prices: Wildlife auctions 2014–2016. Wildlife Stud Services, Wildlife Breeders Journal, pp 146–149

Cloete PC, Rosouw R (2014) The South African wildlife ranching sector: a social accounting matrix Leontief multiplier analysis. Acta Commer 14:1–10. https://doi.org/10.4102/ac.v14i2.225

Coetzer WG, Grobler JP (2019) Genetic variation among different springbok (*Antidorcas marsupialis*) colour variants. Mamm Biol 99:42–53. https://doi.org/10.1016/j.mambio.2019.10.006

Cook D, Brooks S, Bellone R, Bailey E (2008) Missense mutation in exon 2 of *SLC36A1* responsible for champagne dilution in horses. PLoS Genet 4:e1000195. https://doi.org/10.1371/journal.pgen.1000195

D'Mello SAN, Finlay GJ, Baguley BC, Askarian-Amiri ME (2016) Signaling pathways in melanogenesis. Int J Mol Sci 17:1144. https://doi.org/10.3390/ijms17071144

Dudbridge F (2008) Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. Hum Hered 66:87–98. https://doi.org/10.1159/000119108

Espigolan R, Baldi F, Boligon AA, Souza FRP, Gordo DGM, Tonussi RL, Cardoso DF, Oliveira HN, Tonhati H, Sargolzaei M, Schenkel FS, Calvalheiro R, Ferro JA, Albuquerque LG (2013) Study of whole genome linkage disequilibrium in Nellore cattle. BMC Genomics 14:305. https://doi.org/10.1186/1471-2164-14-305

Götz S, García-Gómez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, Robles M, Talón M, Dopazo J, Conesa A (2008) High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res 36:3420–3435. https://doi.org/10.1093/nar/gkn176

He L, Vasiliou K, Nebert DW (2009) Analysis and update of the human solute carrier (*SLC*) gene superfamily. Hum Genomics 3:195–206. https://doi.org/10.1186/1479-7364-3-2-195

Hubbard JK, Uy JAC, Hauber ME, Hoekstra HE, Safran RJ (2010) Vertebrate pigmentation: from underlying genes to adaptive function. Trends Genet 26:231–239. https://doi.org/10.1016/j.tig.2010.02.002

Ito S, Suzuki N, Takebayashi S, Commo S, Wakamatsu K (2013) Neutral pH and copper ions promote eumelanogenesis after the dopachrome stage. Pigment Cell Melanoma Res 26:817–825. https://doi.org/10.1111/pcmr.12137

Kilian A, Wenzl P, Huttner E, Carling J, Xia L, Blois H, Caig V, Heller-Uszynska K, Jaccoud D, Hopper C, Aschenbrenner-Kilian M, Evers M, Peng K, Cayla C, Hok P, Uszynski G (2012) Diversity arrays technology: a generic genome profiling technology on open platforms. In: Pompanon F, Bonin A (eds) Data production and analysis in population genomics: methods and protocols. Humana Press, New York, pp 67–89. https://doi.org/10.1007/978-1-61779-870-2_5

Lal MM, Southgate PC, Jerry DR, Bosserelle C, Zenger KR (2017) Swept away: ocean currents and seascape features influence genetic structure across the 18,000 Km Indo-Pacific distribution of a marine invertebrate, the black-lip pearl oyster *Pinctada margaritifera*. BMC Genomics 18:1–21. https://doi.org/10.1186/s12864-016-3410-y

Melville J, Haines ML, Boysen K, Hodkinson L, Kilian A, Smith Date KL, Potvin DA, Parris KM (2017) Identifying hybridization and admixture using SNPs: application of the DArTseq platform in phylogeographic research on vertebrates. R Soc Open Sci 4:161061. https://doi.org/10.1098/rsos.161061

Mi H, Muruganujan A, Huang X, Ebert D, Mills C, Guo X, Thomas PD (2019) Protocol update for large-scale genome and gene function analysis with the PANTHER classification system (v.14.0). Nat Protoc 14:703–721. https://doi.org/10.1038/s41596-019-0128-8

Miller SM, Clarke AB, Bloomer P, Guthrie AJ, Harper CK (2016) Evaluation of microsatellites for common ungulates in the South African wildlife industry. Conserv Genet Resour 8:329–341. https://doi.org/10.1007/s12686-016-0554-7

Moorad JA, Promislow DEL (2011) Evolutionary demography and quantitative genetics: age-specific survival as a threshold trait. Proc R Soc B 278:144–151. https://doi.org/10.1098/rspb.2010.0992

Mustafa H, Ahmad N, Heather HJ, Eui-Soo K, Khan WA, Ajmal A, Javed K, Pasha TN, Ali A, Kim JJ, Sonstegard TS (2018) Whole genome study of linkage disequilibrium in Sahiwal cattle. S Afr J Anim Sci 48:353–360. https://doi.org/10.4314/sajas.v48i2.16

Nagarajan M, Kumar N, Nishanth G, Haribaskar R, Paranthaman K, Gupta J, Mishra M, Vaidhegi R, Kumar S, Ranjan AK, Kumar S (2009) Microsatellite markers of water buffalo, *Bubalus bubalis*—Development, characterisation and linkage disequilibrium studies. BMC Genet 10:68. https://doi.org/10.1186/1471-2156-10-68

Olivier PA (2015) Colour variation of African Bovidae: Consequences for conservation and the wildlife ranching industry. Dissertation, University of the Free State.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Reissmann M, Ludwig A (2013) Pleiotropic effects of coat colour-associated mutations in humans, mice and other mammals. Semin Cell Dev Biol 24:576–586. https://doi.org/10.1016/j.semcdb.2013.03.014

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Resour 8:103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Russo I-RM, Hoban S, Bloomer P, Kotzé A, Segelbacher G, Rushworth I, Birss C, Bruford MW (2019) 'Intentional genetic manipulation' as a conservation threat. Conserv Genet Resour 11:237–247. https://doi.org/10.1007/s12686-018-0983-6

Solé X, Guinó E, Valls J, Iniesta R, Moreno V (2006) SNPStats: a web tool for the analysis of association studies. Bioinformatics 22:1928–1929. https://doi.org/10.1093/bioinformatics/btl268

Spielmann M, Mundlos S (2016) Looking beyond the genes: the role of non-coding variants in human disease. Hum Mol Genet 25:R157–R165. https://doi.org/10.1093/hmg/ddv259

Taylor WA, Lindsey PA, Davies-Mostert H (2016) An assessment of the economic, social and conservation value of the wildlife ranching industry and its potential to support the green economy in South Africa. The Endangered Wildlife Trust, South Africa

Van Deventer R, Rhode C, Marx M, Roodt-Wilding R (2020) The development of genome-wide single nucleotide polymorphisms in blue wildebeest using the DArTseq platform. Genomics 112:3455–3464. https://doi.org/10.1016/j.ygeno.2020.04.032

Vleissentraal (2019) 2019 Average game auction prices. URL https://vleissentraal.co.za/admin/statistics/2019%20-%20gemiddelde%20wildpryse%20-average%20game%20auction%20prices.pdf Accessed 20 Sep 2020.

Wakamatsu K, Nagao A, Watanabe M, Nakao K, Ito S (2017) Pheomelanogenesis is promoted at a weakly acidic pH. Pigment Cell Melanoma Res 30:372–377. https://doi.org/10.1111/pcmr.12587

Watabe H, Valencia JC, Yasumoto K-I, Kushimoto T, Ando H, Muller J, Vieira WD, Mizoguchi M, Appella E, Hearing VJ (2004) Regulation of tyrosinase processing and trafficking by organellar pH and by proteasome activity. J Biol Chem 279:7971–7981. https://doi.org/10.1074/jbc.M309714200

Williams DS, Lopes VS (2011) The many different cellular functions of MYO7A in the retina. Biochem Soc Trans 39:1207–1210. https://doi.org/10.1042/BST0391207

Yamaguchi H, Aiba A, Nakamura K, Nakao K, Sakagami H, Goto K, Kondo H, Katsuki M (1996) Dopamine D2 receptor plays a critical role in cell proliferation and proopiomelanocortin expression in the pituitary. Genes Cells 1:253–268. https://doi.org/10.1046/j.1365-2443.1996.d01-238.x