Polymorphisms, Haplotype Variability and Neutrality Test of Bronze Locus in Turkey (*Meleagris gallopavo*)

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Summary

The bronze locus, identified as melanocortin-1 receptor (*MC1R*) gene, is involved in body coloration. We examined polymorphisms, haplotype variability and deviation from neutrality of bronze locus in Nigerian Indigenous Turkeys (NIT) using British United Turkeys (BUT) as control breed. Single-exon coding region of *MC1R* was sequenced. Polymorphisms were identified using MEGA v6 and CodonCode Aligner. *In silico* prediction of the functional effects of amino acid substitutions was done using SNAP2. Haplotypes were reconstructed using DnaSp v5. Eight polymorphisms were identified in *MC1R* gene of the birds with two novel polymorphisms: c.37C>A (neutral effect) and c.866T>G (gain-of-function) in NIT and BUT, respectively. Mutations c.450C>T and c.866T>G were unique to lavender NIT while c.186C>G was present in all birds. Two polymorphisms, c.866T>G and c.887C>T, were predicted to have functional effects. The highest genetic diversity was observed in lavender NIT while the least was observed in BUT. Fifteen (15) haplotypes were reconstructed, with *MC1R*BUT1 and *MC1R*BUT2 unique to BUT. Haplotype *MC1R**4 was absent in all the birds used in this study while *MC1R**5 was present in all NIT. Our study confirmed that *MC1R**2 carried black (B) allele. Two novel haplotypes (*MC1R*Nig1 and *MC1R*Nig2) identified in this study also carried *B* allele. *MC1R*Nig6, *MC1R*Nig7 and *MC1R*Nig8 were found to be unique to lavender. The novel polymorphisms and haplotypes identified in NIT and BUT could be used in differentiating them from other turkey breeds with the same plumage colours. Also, various polymorphisms and haplotypes identified in bronze locus of turkey are useful in breeding programmes aimed at developing or conserving different plumage colour types.

Key words

turkey, *B* allele, colour, haplotypes, lavender, polymorphisms

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Received: July 11, 2020 | Accepted: September 24, 2020

Introduction

Coat and plumage colours have intrigued animal breeders and geneticists for centuries (Mao et al., 2010). Body coloration has appeared most commonly in many sub disciplines of animal genetics such as sexual selection (Darwin, 1871; Andersson, 1994), geographical differentiation and speciation (Mayr, 1963), evolution of sexual dimorphism (Dunn et al., 2001), evolution of polymorphisms (Roulin, 2004), breed development, conservation and identification as well as adaptation (Mundy, 2005).

The knowledge of animal pigmentation genetics gained from studies of domesticated animals, particularly in model animals such as chicken and mice, provides a sound basis for examining the basis of colour variation in all animals (Mundy, 2005). Colours are used in avian species for physical protection (Burtt, 1986), protection from parasites (Goldstein et al., 2004), camouflaging and many signaling functions such as sexual signals, territoriality and dominance.

Many loci for body coloration have been reported and some candidate genes have been identified in birds and mammals (Mao et al., 2010). One of these loci is the bronze locus which was reported by Vidal et al. (2010b) to be melanocortin-1 receptor (*MC1R*) gene. The bronze locus has three alleles with the following order of dominance: *B* (black) > b^+ (wild bronze/lavender) > b^1 (black-winged bronze/lavender) (Asmundson, 1945). The *MC1R* is well known in the regulation of eumelanin/phaeomelanin switch (Andersson and Georges, 2004). The gene plays an important role in melanogenesis. It is a seven-transmembrane G-protein-coupled protein that is expressed exclusively in melanocytes (Mountjoy et al., 1992). The gene is found on chromosome 11 and encodes a 314 amino protein in only one exon in turkey birds. The variation of the gene has been studied in many animals including mouse (Robbins et al., 1993), cattle (Klungland et al., 1995), quail (Zhang et al., 2013), horse (Marklund et al., 1996), pig (Kijas et al., 1998, 2001; Mao et al., 2010), fox (Vage et al., 1997), turkey (Vidal et al., 2010b; Corso et al., 2017; Abdullah et al., 2019), chicken (Hoque et al., 2013) and guinea fowl (Vidal et al., 2010a).

Molecular characterization of *MC1R* gene is useful in breeding programmes aimed at developing or maintaining different colour varieties in species such as turkeys and other avian species in which the different and exquisite plumage colours have ornamental and selection values (Corso et al., 2017).

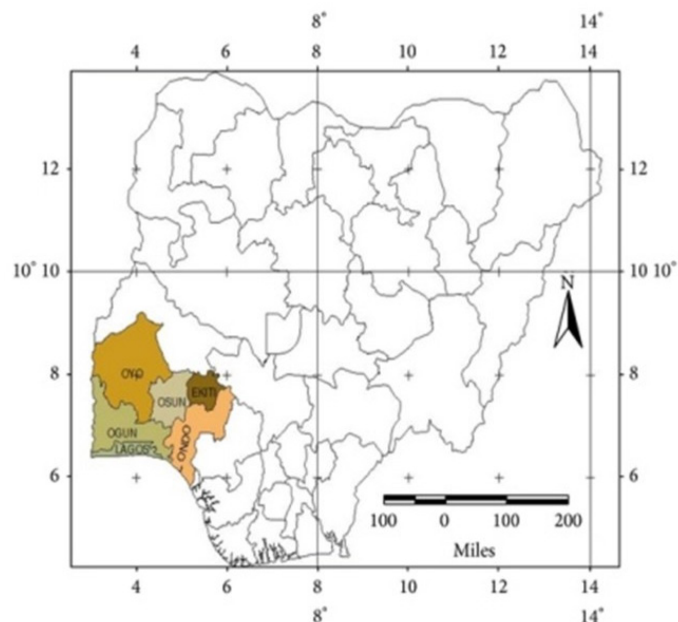
Domestic turkeys have been bred into many varieties with different plumage colour patterns and have been used in classical genetics studies to determine the genetic basis of colour phenotypes (Robertson et al., 1943). The Nigerian indigenous turkey is well adapted to wide range of climatic conditions and can be raised successfully almost everywhere if well fed and protected against diseases, predators and adverse weather conditions (Ogundipe and Dafwang, 1980; Ilori et al., 2009). These birds are excellent foragers and have three major feather colours which are black, lavender and white. Other turkey breeds found in Nigeria are locally adapted exotic turkeys including British United and Nicholas white turkeys, both of which are white in colour (Ilori et al., 2010).

In this study, we examined polymorphisms, haplotype variability and deviation from neutrality of bronze locus (*MC1R* gene) in Nigerian indigenous turkeys using British United turkeys as control breed for the purpose of breed conservation and identification. To the best of our knowledge, this study is the first attempt to examine polymorphisms, haplotype variability and deviation from neutrality of bronze locus in Nigerian indigenous turkeys.

Materials and Methods

Sampling Locations

Adult Nigerian indigenous turkeys were sampled from farms and different households in Southwest Nigeria (Fig. 1). Southwest geopolitical zone of Nigeria comprises six states which are Oyo, Ogun, Osun, Ondo, Ekiti and Lagos. The birds were sampled from farms or houses which are separated by around 9 km. One bird was sampled from each farm or house. The states are separated approximately by 200 km. The geographical description of the six states is presented in Table 1. The British united turkeys were sourced from the Turkey Breeding Unit of Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun State and other commercial farms in the six states.



Note: The coloured regions are the sampling locations

Figure 1. Map of Nigeria showing Southwest region (Source: Adediran et al. 2014)

Experimental Birds

One hundred and seventy-eight (178) adult birds consisting of 146 Nigerian indigenous (50 lavender, 46 white and 50 black) and 32 British united turkeys were used in this study (Table 2). British United turkeys were included as control breed having white plumage colour.

Blood Collection, Deoxyribonucleic Acid (DNA) Extraction, Polymerase Chain Reaction (PCR) Assays and Gene Sequencing

About 1.5 mL of blood was collected from brachial vein of each bird using needle and syringe. Individual blood sample was deposited in ethylenediaminetetraacetic acid bottle.

Table 1. Geographical description of sampling locations and number of birds sampled from each location

Geographical description	State					
	Lagos	Ogun	Ekiti	Oyo	Ondo	Osun
Landmass (km^2)	3,577	16,980	6,353	28,454	15,500	14,875
Altitude (m)	41	66	250	1200	344	320
Capital	Ikeja	Abeokuta	Ado-Ekiti	Ibadan	Akure	Osogbo
Coordinates	6°35'N 3°45'E	7°00'N 3°35'E	7°40'N 5°15'E	8°00'N 4°00'E	7°10'N 5°05'E	7°30'N 4°30'E
Season	Wet and dry	Wet and dry	Wet and dry	Wet and dry	Wet and dry	Wet and dry
Temperature (°C)	21.8-32.9	21-34	21-33	25-35	19-33	18-34
Humidity (%)	84	85	88	88	90	85
Major river	Lagos lagoon	Ogun	Ogbese, Ero, Osun, Ose	Oyan, Ogun, Oba, Otin, Osun	Owena, Ogbese, Ose, Oluwa	Osun, Oba
Number of birds sampled	8 lavender NIT 6 black NIT 8 white NIT 4 BUT	10 lavender NIT 8 black NIT 10 white NIT 12 BUT	8 lavender NIT 8 black NIT 8 white NIT 4 BUT	8 lavender NIT 8 black NIT 8 white NIT 4 BUT	8 lavender NIT 8 black NIT 8 white NIT 3 BUT	8 lavender NIT 8 black NIT 8 white NIT 5 BUT

Table 2. Experimental birds used in the study

Breed	Plumage colour	Code	Number
Nigerian indigenous turkey	Lavender	L	50
Nigerian indigenous turkey	White	W	46
Nigerian indigenous turkey	Black	B	50
British United turkey	White	E	32
Total			178

Genomic DNA (gDNA) was extracted from the blood at Biotechnology laboratory of the Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta from the birds using Zymo research Quick-gDNA™ MiniPrep kit following the manufacturer's instruction. The extracted gDNA was quantified for concentration and purity using Nanodrop spectrophotometer following the protocol described by Desjardins and Conklin (2010). The integrity of the gDNA was also verified using gel electrophoretic method by running 2 µL of gDNA samples on 1% agarose gel at 120 V for 20 minutes. Polymerase chain reaction (PCR) was carried out using tk_*MC1R*_Fwd 5'-ACATCCCTTCTGCCTCGTG-3' and tk_*MC1R*_Rev 5'-ATCTGTCCATCCACCCGTC-3' primers to amplify 1121 bp region covering 5'UTR, exon 1 (the single-exon coding sequence) and 3'UTR of turkey *MC1R*. For amplification, 5 µL of genomic DNA (~10-20 ng) was added to a reaction mixture containing 12.8 µL of nuclease free water, 2.5 µl of 1× PCR buffer, 1.5 µl of 1.5mM $MgCl_2$, 1 µl of 0.2mM dNTP, 1 µl of 0.4 UM forward primer, 1 µl of 0.4UM reverse primer and 0.2 µl of 2U/µL surf Hot Taq. The PCR conditions included initial denaturation at 96°C for 15 minutes, 35 cycles of final denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 70°C for 2 minute and final extension at 70°C for 5 minutes. The amplicon was purified with Magnetic Beads Carboxylate (MCLab, USA). The sequencing of PCR products was done with BigDye Terminator v. 3.1 using ABI 3730 XL DNA Analyzer (BioNeer, Daejeon, South Korea) following the supplier's protocol at STAB Vida Genetics Laboratory, Campus FCT UNL Edificio Departamental de Quimica, Laboratorio 009, 2829-516 Caparica, Portugal. One consensus sequence was generated from all the 178 sequences using CodonCode Aligner (http://www.codoncode.com/aligner) and submitted to GenBank with accession number MF360992.1.

Analyses of *MC1R* Gene

The 178 nucleotide sequences were trimmed and edited using Bioedit (Hall, 1999) and MEGA v6 (Tamura et al., 2013). The single nucleotide polymorphisms (SNPs) present in the single coding region (exon 1) of the gene were identified by aligning the exon with the reference exon 1 (ENSMGAG00000016832.1) downloaded from Ensembl database using Clustal W (Thompson et al., 1994) incorporated inside MEGA v6 software (Tamura et al., 2013). The SNPs were also confirmed using CodonCode Aligner (http://www.codoncode.com/aligner) and DnaSp v5 (Librado and Rozas, 2009). The resultant amino acid variation of each SNP was also predicted using CodonCode Aligner (http://www.codoncode.com/aligner). *In silico* prediction of the functional effects of amino acid variation was done using SNAP2 (Hecht et al., 2015). Other *in silico* prediction tools such as Polyphen predict functional effect of human non-synonymous SNPs while Panther was developed for predicting disease causing genetic variants with reference to a particular species with turkey not included as one of the species. SNAP2 predicts the functional effects of amino acid substitution using neural networks with no reference to a particular species.

Genetic diversity indices such as number of haplotypes, haplotype diversity, average number of nucleotide differences, nucleotide diversity, singleton variable site and parsimony informative site were estimated for the coding region of turkey *MC1R* gene using DnaSp v5 (Librado and Rozas, 2009).

Fu and Li's D*; Fu and Li's F* and Tajima's D tests were performed to test the coding region of the turkey *MC1R* gene deviation from neutrality using DnaSP v5 (Librado and Rozas, 2009).

Haplotypes present in coding region of *MC1R* gene in Nigerian indigenous and British United turkeys were reconstructed using DnaSP v5 (Librado and Rozas, 2009). DnaSp uses algorithms provided by PHASE, fastPHASE and HAPAR software in haplotype reconstruction. PHASE, fastPHASE, and HAPAR use coalescent-based Bayesian, patterns of linkage disequilibrium and pure parsimony approaches, respectively to infer haplotypes.

MEGA v6 software (Tamura et al., 2013) was used to determine the phylogenetic relationship among the obtained haplotypes. The phylogenetic tree was inferred using neighbor joining method. The reliability of the inferred tree was evaluated using bootstrap analysis of 1000 replications.

Results

Polymorphisms Identified in *MC1R* Gene

The polymorphisms identified in turkeys with different plumage colours were shown in Table 3 while the *in silico* prediction of their functional effects was presented in Table 4. A total of 8 polymorphisms were identified representing three synonymous and five nonsynonymous substitutions. Two novel mutations, c.37C>A (neutral mutation present in lavender and white Nigerian indigenous turkeys) and c.866T>G (gain-of-function mutation present only in lavender Nigerian indigenous turkeys), were identified in this study while the other six mutations have been previously identified by either Vidal et al. (2010b) or Corso et al. (2017). Mutations c.37C>A (neutral effect) and c.96G>A were identified only in lavender and white Nigerian indigenous turkeys. White British United turkeys had one unique polymorphism at position 411 while mutation c.186C>G was common to all the birds. Mutation c.887C>T was predicted to have effect on the resultant protein function with accuracy of 75% (Table 4).

Diversity of *MC1R* Gene in Nigerian Indigenous and British United Turkeys

Diversity indices of *MC1R* gene in Nigerian indigenous and British United turkeys with different colour variants are presented in Table 5. Lavender Nigerian indigenous turkeys had the highest number of haplotype and nucleotide diversity while the lowest nucleotide diversity was observed in white British United turkeys.

Test of Deviation of *MC1R* Gene from Neutrality

All the tests of deviation from neutrality indices obtained for all the colours were greater than 1, except Fu and Li's D of black Nigerian indigenous and white British United turkey as well as Tajima's D value of lavender Nigerian indigenous turkey (Table 6). The indices were also non significant ($p>0.05$).

Haplotypes Identified and Their Distribution

Fifteen haplotypes were constructed for *MC1R* gene of the birds (Tables 7 and 8). The haplotypes were designated *MC1R**1, *MC1R**2, *MC1R**3 and *MC1R**5 for those already identified by Vidal et al. (2010b); H6 for the haplotype identified by Corso et al. (2017); *MC1R*Nig1 – *MC1R*Nig8 for those identified in Nigerian indigenous turkeys and *MC1R*BUT1 - *MC1R*BUT2 for those identified in British United turkeys. The haplotypes were shared and unique to some plumage colours. *MC1R*Nig1, *MC1R*Nig2 and *MC1R**2 were specific to black Nigerian indigenous turkeys while *MC1R**5 was shared by all the Nigerian indigenous turkeys with different colour variants.

Table 3. Polymorphisms identified in *MC1R* gene of Nigerian indigenous and British United turkey with different plumage colours

SNP	Amino acid change	Type	L	W	B	E	Uniqueness	*Previous report
c.37C>A	p.Pro13Thr	Non-synonymous	Present	Present	Absent	Absent	L, W	FKR
c.96G>A	p.STP32Trp	Non-synonymous	Present	Present	Absent	Absent	L, W	Vidal et al. (2010b)
c.186C>G	p.Leu62Leu	Synonymous	Present	Present	Present	Present	L, W, B, E	Corso et al. (2017)
c.364A>T	p.Phe122Ile	Non-synonymous	Present	Present	Present	Absent	L, W, B	Vidal et al. (2010b), Corso et al. (2017)
c.411C>T	p.Ala137Ala	Synonymous	Absent	Absent	Absent	Present	E	Corso et al. (2017)
c.450C>T	p.Tyr150Tyr	Synonymous	Present	Absent	Absent	Absent	L	Vidal et al. (2010b), Corso et al. (2017)
c.866T>G	p.Val289Gly	Non-synonymous	Present	Absent	Absent	Absent	L	FKR
c.887C>T	p.Ala296Val	Non-synonymous	Present	Absent	Present	Absent	L, B	Corso et al. (2017)

Note: * - First known report

Table 4. Predicted functional effects of amino acid substitutions in *MC1R* gene of Nigerian indigenous and British United turkey

SNP	Amino acid change	Predicted effect	Score	Reliability (%)
c.37C>A	p.Pro13Thr	Neutral	-26	61
*c.96G>A	p.STP32Trp			
c.186C>G	p.Leu62Leu	Neutral	-99	97
c.364A>T	p.Phe122Ile	Neutral	-36	66
c.411C>T	p.Ala137Ala	Neutral	-99	97
c.450C>T	p.Tyr150Tyr	Neutral	-99	97
c.866T>G	p.Val289Gly	Effect	66	80
c.887C>T	p.Ala296Val	Effect	57	75

Note: * - Functional effects of c.96G>A(p.STP32Trp) was not predicted because STP which is stop codon is not an amino acid

Table 5. Diversity of *MC1R* gene in Nigerian indigenous and British United turkeys with different plumage colours

Diversity indices	Colour			
	Lavender Nigerian indigenous turkey	White Nigerian indigenous turkey	Black Nigerian indigenous turkey	White British United turkey
Number of sequences used	50	46	50	32
Number of sites analysed	945	945	945	945
Polymorphic site	7	4	3	2
Parsimony informative site	7	4	3	2
Singleton variable site	0	0	0	0
Number of haplotype	7	5	4	3
Haplotype diversity	0.722	0.758	0.745	0.677
Nucleotide diversity	2.2×10^{-3}	1.5×10^{-3}	1.1×10^{-3}	9.2×10^{-4}
Average number of nucleotide difference	2.078	1.412	1.047	0.871
Sequence conservation	0.993	0.996	0.997	0.998

Table 6. Test of deviation of *MC1R* gene in Nigerian indigenous and British United turkeys with different plumage colours from neutrality

Colour	Fu and Li's D	Fu and Li's F	Tajima's D
Lavender Nigerian indigenous turkey	1.24^{ns}	1.32^{ns}	0.87^{ns}
White Nigerian indigenous turkey	1.01^{ns}	1.27^{ns}	1.27^{ns}
Black Nigerian indigenous turkey	0.89^{ns}	1.13^{ns}	1.16^{ns}
White British United turkey	0.80^{ns}	1.16^{ns}	1.52^{ns}

Note: ^{ns} - not significant

Table 7. Haplotypes identified in *MC1R* gene of Nigerian indigenous and British United turkeys

		0	0	1	3	4	4	8	8
		3	9	8	6	1	5	6	8
		7	6	6	4	1	0	6	7
S/N	#Haplotype designation	C	G	G	T	C	T	T	C
1	*MC1R*Nig1	.	.	.	.	.	.	.	.
2	*MC1R*Nig2	.	.	C	.	.	.	.	T
3	*MC1R**2	.	.	C	.	.	.	.	.
4	*MC1R**5	.	.	C	A	.	.	.	.
5	H6	.	.	.	A	.	.	.	.
6	*MC1R**3	.	A	C	.	.	.	.	.
7	*MC1R*Nig3	A	.	C	.	.	.	.	.
8	*MC1R*Nig4	.	A	C	A	.	.	.	.
9	*MC1R*Nig5	.	A	.	A	.	.	.	.
10	*MC1R**1	.	.	C	A	.	C	.	.
11	*MC1R*Nig6	A	.	C	A	.	.	.	T
12	*MC1R*Nig7	A	.	.	A	.	C	.	.
13	*MC1R*Nig8	.	A	C	.	.	.	G	.
14	*MC1R*BUT1	.	.	.	A	.	C	.	.
15	*MC1R*BUT2	.	.	.	A	T	C	.	.

Note: # - *MC1R**4 is absent. *MC1R**1, *MC1R**2, *MC1R**3, *MC1R**4 and *MC1R**5 have been identified by Vidal et al. (2010a) while H6 was identified by Corso et al. (2017)

Table 8. Distribution of haplotype identified in Nigerian indigenous and British United turkeys with different plumage colours

Haplotype	Frequency	Lavender NIT (n=50)	White NIT (n=46)	Black NIT (n=50)	White BUT (n=32)
*MC1R*Nig1	9	-	-	9	-
*MC1R*Nig2	9	-	-	9	-
*MC1R**2	16	-	-	16	-
*MC1R**5	38	5	17	16	-
H6	9	-	9	-	-
*MC1R**3	37	25	12	-	-
*MC1R*Nig3	4	-	4	-	-
*MC1R*Nig4	4	-	4	-	-
*MC1R*Nig5	4	4	-	-	-
*MC1R**1	12	4	-	-	8
*MC1R*Nig6	4	4	-	-	-
*MC1R*Nig7	4	4	-	-	-
*MC1R*Nig8	4	4	-	-	-
*MC1R*BUT1	12	-	-	-	12
*MC1R*BUT2	12	-	-	-	12
Total	178	50	46	50	32

*MC1R*BUT1 and *MC1R*BUT2 were specific to white British United turkeys while *MC1R*Nig6, *MC1R*Nig7 and *MC1R*Nig8 were unique to lavender Nigerian indigenous turkeys. Haplotype *MC1R*Nig1 was observed in 9 out of 50 black Nigerian indigenous turkeys sampled while 12 out of 32 white British United turkeys have haplotype *MC1R*BUT1. Haplotypes *MC1R*Nig6, *MC1R*Nig7 and *MC1R*Nig8 were observed in 4 out of 50 lavender Nigerian indigenous turkeys used in this study.

Phylogenetic Relationship among *MC1R* Haplotypes

The phylogenetic relationship among *MC1R* haplotypes is shown in Fig. 2. Four clades were formed with haplotypes observed in *MC1R* gene of Nigerian birds clustering with other haplotypes except in Clade III which is occupied solely by *MC1R*Nig1. *MC1R*BUT1 and *MC1R*BUT2 formed sister taxa and clustered with *MC1R*Nig7 and *MC1R**1 in clade I.

Discussion

Polymorphisms in *MC1R* gene have been shown to evolve with different plumage colours. Mutation c.186C>G was identified in both British United and Nigerian indigenous turkeys suggesting its common roles in melanism of the two breeds. Non-synonymous polymorphisms c.37C>A and c.866T>G identified in our study are novel polymorphisms which have not been previously reported in *MC1R* gene of turkey birds. Novel melanic alleles have been proposed to have an immediate phenotypic effect and are usually available for selection. Novel melanic alleles have also been reported to be involved in adaptive potentials of organism to its environment (Mundy, 2005).

The presence of c.450C>T and c.866T>G in only lavender birds might indicate that these mutations are involved in *MC1R* activation in turkey birds with lavender colour. Activation of *MC1R* leads to increased synthesis of black or brown eumelanin, while low *MC1R* activity usually leads to increased synthesis of red or yellow phaeomelanin (Robbins et al., 1993).

The presence of only c.411C>T mutation in white British United turkeys was not surprising as this turkey breed has undergone many years of selection with white colour fixed for the breed identification. Many years of selection for the white colour in British United turkeys would have reduced the diversity of bronze locus in the breed.

Both c.364A>T and c.450C>T polymorphisms were present in lavender turkey. The implication of this is that c.450C>T or its combination with c.364A>T is involved in bronze/lavender colour formation while c.364A>T which was observed in all Nigerian indigenous turkeys is unique to some turkey breeds. *In silico* prediction of functional effect of p.Ile122Phe (c.364A>T) also revealed neutral effect of this polymorphism which may be the reason why the polymorphism is not specific to any plumage colour in Nigerian indigenous turkeys.

Mutation 96G>A is located in the extracellular domain 1 of *MC1R* gene (Prusis et al., 1997) and creates a premature stop codon. This particular mutation, in consequence, should involve a complete loss of function of the receptor. A neighbouring polymorphism, p.Arg28Cys reported by Nachman et al. (2003) in mouse is associated with light colour and thus a decrease in eumelanin production. Unsurprisingly, in Nigerian indigenous turkeys, expected lighter plumage (based on location of the

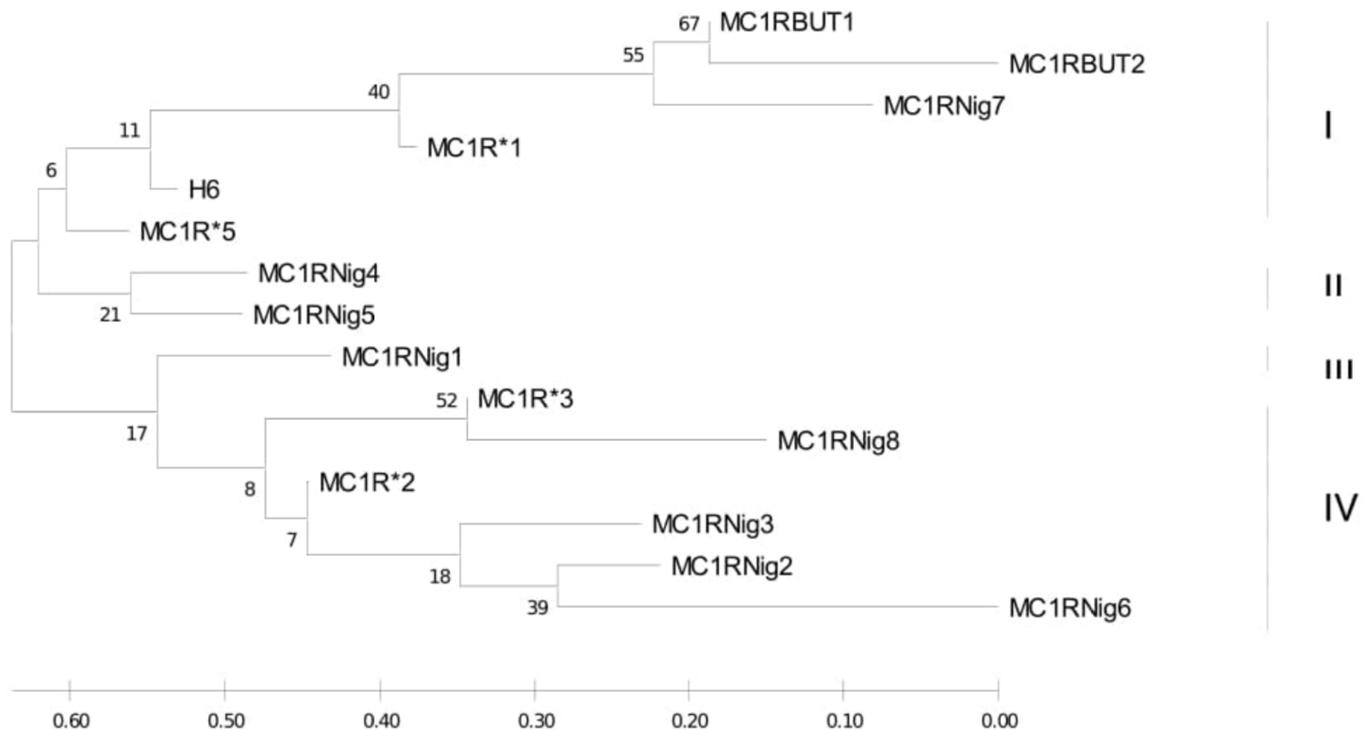


Figure 2. Phylogenetic relationship among MC1R haplotypes

mutation and its expected effect) was observed but surprisingly, this polymorphism is absent in British United turkeys. Our possible explanation to this phenomenon is that the genome of British United turkey has been greatly altered during the breed development or might have undergone selective sweeps as a result of several generations of selection for white plumage colour.

The diversity of *MC1R* gene was higher in lavender Nigerian indigenous turkeys compared with other colours. This kind of pattern was also observed by Guo et al. (2010), where Hebei chickens with yellow and black spots had greater genetic diversity. This rich genetic diversity observed in lavender turkeys might have resulted from the rich plumage colour diversity of lavender turkeys.

Plumage coloration is easy to observe and study, it is subjected to strong natural (Linnen et al., 2009; Van't Hof et al., 2011) or sexual selection (Uy et al., 2009) and shows a clear relationship to speciation (Hubbard et al., 2010). A test of deviation from neutrality can be used to detect non-neutral patterns of variations that would be expected if the plumage colour differences arose by selection. All the tests used in our study were non-significant, which suggested weak effects or false positives (Poelstra et al., 2013). The test of deviation values obtained for all colours was also positive, which is suggestive of balancing selection on the gene. Also, the positive test of deviation from neutrality indices obtained for both Nigerian indigenous and British United turkeys with different plumage colours was an indication of presence of low level of both low and high frequency mutations which can be linked to balancing selection and decrease in population size (Hahn et al., 2002). Positive Tajima's D values observed in all birds might have resulted from many haplotypes present in this region as positive Tajima's D values have been shown to be caused by presence of many haplotypes (Simonsen et al., 1995).

The haplotypes found in our study differed from one another by a small number of variations with some of the haplotypes shared among different colours while some are unique to different colours. Haplotypes *MC1R**1, *MC1R**2, *MC1R**3 and *MC1R**5 that were reported by Vidal et al. (2010b) were also observed in our study. Also, haplotype H6, reported by Corso et al. (2017) in Southern Brazilian turkeys, was also observed in our study. Bronze/lavender birds were observed to carry *MC1R**1 while black birds carried *MC1R**2. The presence of *MC1R**2 in only black Nigerian indigenous turkeys is not surprising as this haplotype has been found to carry black (*B*) allele. *MC1R*Nig1 and *MC1R*Nig2 were new alleles found in this study and they also carry *B* allele. The correspondence between bronze locus and *MC1R* gene has been confirmed by Vidal et al. (2010b) and all turkeys carrying *B* allele (which can be black, buff, chocolate and slate; either in homozygote *BB* or heterozygotes Bb^{+} and Bb^{l}) carried at least one copy of *MC1R**2 haplotype. The white turkeys (Nigerian indigenous and British United turkeys) carried many haplotypes which may be due to the fact that white colour birds carry many allelic combinations. White turkeys have the recessive allele c in homozygosity in an epistatic locus for colour and therefore may have any allele combination in the bronze locus (Robertson et al., 1943; Asmundson, 1945). The presence of *MC1R**5 haplotype in some black turkeys is not surprising as *B* allele is dominant over b^{+} which is an indication that the 16 black turkeys observed to carry haplotype *MC1R**5 in our study will have Bb^{+} genotype.

The phylogenetic relationship among the observed haplotypes revealed that four clades were formed with the novel Nigerian haplotypes present in all the clades. The *MCIR*Nig1 stayed solely on a separate clade. The possible explanation for uniqueness of this haplotype is that it is a recombinant haplotype that encodes the same amino acid sequence as *MC1R**2 and is considered as second form of B. Staying of *MC1R*Nig1 on its own separate clade suggests that this haplotype has some other roles in melanogenesis pathway (apart from formation of black feather) and some other physiological processes.

Conclusion

The novel polymorphisms and haplotypes identified in NIT and BUT could be used in differentiating them from other turkey breeds with the same plumage colours. Also, various polymorphisms and haplotypes identified in bronze locus of turkey are useful in breeding programmes aimed at developing or conserving different plumage colour types.

Acknowledgments

We are thankful to the Farm staff that assisted in collection of blood samples. We are also thankful to turkey farmers and home turkey breeders in the southwest region of Nigeria who allowed us to take blood samples from their birds.

Availability of Data

The *MC1R* gene sequence was submitted to GenBank with accession number: MF360992.1.

References

Abdullah M. M., Yousif A. N., Amen Q. A., Shaker Q. A. (2019). Detection of Melanocortin 1 Receptor (MC1R) Polymorphism and Its Relation to Plumage Colours in Local Turkey. *IOP Conference Series: Earth and Environmental Science* 388: 012033. doi: 10.1088/1755-1315/388/1/012033

Adediran O. A., Kolapo T. U., Uwalaka E. C. (2014). *Echinococcus Granulosus* Prevalence in Dogs in Southwest Nigeria. J Parasitol Res 2014: 124358. doi: 10.1155/2014/124358

Andersson L., Georges M. (2004). Domestic-Animal Genomics: Deciphering the Genetics of Complex Traits. Nat Rev Genet 5: 202–212. doi: 10.1038/nrg1294

Andersson M. (1994). *Sexual Selection*. Princeton: Princeton University Press.

Asmundson V. S. (1945). A Triple-Allele Series and Plumage Color in Turkeys. Genetics 30, 305–322

Burtt E. (1986). An Analysis of Physical, Physiological and Optical Aspects of Avian Coloration with Emphasis on Wood-Warblers. Ornithol Monogr 38: 1–126. doi: 10.2307/40166782

Corso J., Hepp D., Ledur M. C., Peixoto J. O., Fagundes N. J. R., Freitas, T. R. O. (2017). Genetic Variation of the Bronze Locus (*MC1R*) in Turkeys from Southern Brazil. Genet Mol Biol 40 (1): 104–108. doi: 10.1590/1678-4685-GMB-2016-0136

Darwin C. (1871). The Descent of Man and Selection in Relation to Sex. Princeton: Princeton University Press.

Desjardins P., Conklin D. (2010). Nanodrop Microvolume Quantification of Nucleic Acid. J Vis Exp 22 (45): 2565. doi: 10.3791/2565

Dunn P., Whittingham L., Pitcher T. (2001). Mating Systems, Sperm Competition and the Evolution of Sexual Dimorphism in Birds. Evolution 55: 161–175. doi: 10.1554/0014-3820(2001)055[0161:MSSCAT]2.0.CO;2

Goldstein G., Flory K., Browne B., Majid S., Ichida J. M., Burtt J. E. H. (2004). Bacterial Degradation of Black and White Feathers. The Auk 121: 656–659. doi: 10.1093/auk/121.3.656

Guo X. L., Li X. L., Li Y., Gu Z. L., Zheng C. S., Wei Z. H., Wang J. S., Zhou R. Y., Li L. H., Zheng H. Q. (2010). Genetic Variation of Chicken *MC1R* Gene in Different Plumage Colour Populations. Br Poult Sci 51: 734–739. doi: 10.1080/00071668.2010.518408

Hahn M. W., Rausher M. D., Cunningham, C. W. (2002). Distinguishing between Selection and Population Expansion in an Experimental Lineage of Bacteriophage T7. Genetics 161: 11–20

Hall T. A. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95–98

Hecht, M., Bromberg, Y., Rost, B. (2015). Better Prediction of Functional Effects for Sequence Variants. BMC Genomics 16 (Suppl 8): 51. doi: 10.1186/1471-2164-16-S8-S1

Hoque M. R., Jin S., Heo K. N., Kang B. S., Jo C., Lee, J. H. (2013). Investigation of *MC1R* SNPs and Their Relationships with Plumage Colors in Korean Native Chicken. Asian Austral J Anim Sci 26 (5): 625–629. doi: 10.5713/ajas.2012.12581

Hubbard J., Uy J., Hauber M., Hoekstra H. E., Safran, R. (2010) Vertebrate Pigmentation: from Underlying Genes to Adaptive Function. Trends Genet 26: 231–239. doi: 10.1016/j.tig.2010.02.002

Ilori B. M., Peters S. O., Aruleba D. D., Akano K., Wheto M. (2009) Heat Tolerance Traits among Pure and Crossbred Turkeys in Southwest Nigeria. Proceedings of the 14th Annual Conference of Animal Science Association of Nigeria, pp: 89-92

Ilori B. M., Peters S. O., Ikeobi C. O. N., Bamgbose A. M., Isidahomen C. E., Ozoje M. O. (2010). Comparative Assessment of Growth in Pure and Crossbred Turkeys in a Humid Tropical Environment. Int J Poult Sci 9(4): 368–375. doi: 10.3923/ijps.2010.368.375

Kijas J. M., Moller M., Plastow G., Andersson L. (2001). A Frame- Shift Mutation in *MC1R* and a High Frequency of Somatic Reversions Cause Black Spotting in Pigs. Genetics 158: 779–785

Kijas J. M., Wales R., Tornsten A., Chardon P, Moller M., Andersson L. (1998). Melanocortin Receptor 1 (*MC1R*) Mutations and Coat Color in Pigs. Genetics 150: 1177–1185

Klungland H., Vage D.I., Gomez-Raya L., Adalsteinsson S., Lien S. (1995). The Role of Melanocytes Stimulating Hormone (MSH) Receptor in Bovine Coat Color Determination. Mamm Genome 6: 636-639. doi: 10.1007/BF00352371

Librado P., Rozas, J. (2009). DnaSP v5: A Software for Comprehensive Analysis of DNA Polymorphism Data. Bioinformatics 25: 1451-1452. doi: 10.1093/bioinformatics/btp187

Linnen C. R., Kingsley E. P., Jensen J. D., Hoekstra H. E. (2009). On the Origin and Spread of an Adaptive Allele in Deer Mice. Science 325: 1095–1098. doi: 10.1126/science.1175826

Mao H., Ren J., Ding N., Xiao S., Huang L. (2010). Genetic Variation within Coat Color Genes of *MC1R* and ASIP in Chinese Brownish Red Tibetan Pigs. Anim Sci J 81(6): 630–634. doi: 10.1111/j.1740-0929.2010.00789.x

Marklund L., Moller M. J., Sandberg K., Andersson L. (1996). A Missense Mutation in the Gene for Melanocyte Stimulating Hormone Receptor (*MC1R*) is Associated with the Chestnut Coat Color in Horses. Mamm Genome 7: 895–899. doi: 10.1007/s003359900264

Mayr E. (1963). Animal Species and Their Evolution. Cambridge: Harvard University Press.

Mountjoy K. G., Robbins L. S., Mortrud M. T., Cone, R. D. (1992). The Cloning of a Family of Genes That Encode the Melanocortin Receptors. Science 257: 1248–1251. doi: 10.1126/science.1325670

Mundy N. I. (2005). A Window on the Genetics of Evolution: *MC1R* and Plumage Coloration in Birds. Pro R Soc B 272 (1573): 1633–1640. doi: 10.1098/rspb.2005.3107

Nachman M. W., Hoekstra H. E., D'Agostino S. L. (2003). The Genetic Basis of Adaptive Melanism in Pocket Mice. Proc Natl Acad Sci USA 100: 5268–5273. doi: 10.1073/pnas.0431157100

Ogundipe S. O., Dafwang, I. I. (1980). Turkey Production in Nigeria. NAERLS Bulletin 2: 2–22

Poelstra J., Ellegren H., Wolf J. (2013). An Extensive Candidate Gene Approach to Speciation: Diversity, Divergence and Linkage Disequilibrium in Candidate Pigmentation Genes across the European Crow Hybrid Zone. Heredity 111: 467–473. doi: 10.1038/hdy.2013.68

Prusis P., Schioth H. B., Muceniece R., Herzyk P., Afshar M., Hubbard R. E., Wikberg J. E. (1997). Modeling of the Three-Dimensional Structure of the Human Melanocortin 1 Receptor, Using an Automated Method and Docking of a Rigid Cyclic Melanocyte-Stimulating Hormone Core Peptide. J Mol Graph Model 15: 307–317. doi: 10.1016/s1093-3263(98)00004-7

Robbins L. S., Nadeau J. H., Johnson K. R., Kelly M. A., Roselli-Rehfuss L., Baack E., Mountjoy K. G., Cone R. D. (1993). Pigmentation Phenotypes of Variant Extension Locus Alleles Result from Point Mutations That Alter MSH Receptor Function. Cell 72: 827–834. doi: 10.1016/0092-8674(93)90572-8

Robertson W. R. B., Bohren B. B., Warren D. C. (1943). The Inheritance of Plumage Color in the Turkey. J Hered 34: 246–256. doi: 10.1093/oxfordjournals.jhered.a105298

Roulin A. (2004). The Evolution, Maintenance and Adaptive Function of Genetic Colour Polymorphism in Birds. Biol Rev Camb Philos Soc 79: 815–848. doi: 10.1017/s1464793104006487

Simonsen K. L., Churchill G. A., Aquadro C. F. (1995). Properties of Statistical Tests of Neutrality for DNA polymorphism Data. Genetics 141 (1): 413–429

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30: 2725–2729. doi: 10.1093/molbev/mst197

Thompson J. D., Higgins D. G., Gibson T. J. (1994). Clustal W: Improving the Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting, Position Specific Gap Penalties and Weight Matrix Choice. Nucleic Acids Res 22: 4673–4680. doi: 10.1093/nar/22.22.4673

Uy J. A. C., Moyle R. G., Filardi C. E. (2009). Plumage and Song Differences Mediate Species Recognition between Incipient Flycatcher Species of the Solomon Islands. Evolution 63: 153–164. doi: 10.1111/j.1558-5646.2008.00530.x

Vage D. I., Lu D., Klungland H., Lien S., Adalsteinsson S., Cone R. D. (1997). A Non-Epistatic Interaction of Agouti and Extension in the Fox, *Vulpes vulpes*. Nat Genet 15: 311–315. doi: 10.1038/ng0397-311

Van't Hof A., Edmonds N., Dalíková M., Marec F., Saccheri, I.J. (2011). Industrial Melanism in British Peppered Moths Has a Singular and Recent Mutational Origin. *Science* 332: 958–960. doi: 10.1126/science.1203043

Vidal O., Araguas R. M., Fernández R., Heras S., Sanz N., Pla C. (2010a). Melanism in Guinea Fowl (*Numida meleagris*) Is Associated with A Deletion of Phenylalanine-256 in the *MC1R* Gene. Anim Genet 41: 656–658. doi: 10.1111/j.1365-2052.2010.02056.x

Vidal O., Vinas J., Pla C. (2010b). Variability of the Melanocortin 1 Receptor (MC1R) Gene Explains the Segregation of the Bronze Locus in Turkey (*Meleagris gallopavo*). Poult Sci 89 (8): 1599–1602. doi: 10.3382/ps.2010-00726

Zhang X., Pang Y., Zhao S., Xu H., Li Y., Xu Y., Wang D. (2013). The Relationship of Plumage Colours with *MC1R* (Melanocortin 1 Receptor) and ASIP (Agouti Signaling Protein) in Japanese Quail (*Coturnix coturnix japonica*). Br Poult Sci 54(3): 306–311. doi: 10.1080/00071668.2013.780122

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