Major histocompatibility complex genetic diversity of Kenyan indigenous chicken populations based on microsatellite markers

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ABSTRACT: The study investigated the genetic make-up of indigenous chicken (IC) ecotypes based on the major histocompatibility complex (MHC) by genotyping microsatellite markers LEI0258 and MCW0371. Blood samples were collected from eight regions of Kenya; Kakamega, Siaya, West Pokot, Turkana, Bomet, Narok, Lamu and Taita-Taveta. In total 96 birds per population were sampled whereas 48 birds were genotyped. In total, 56 differently sized alleles were detected over all the populations. Cluster analysis based on LEI0258 and also in combination with MCW0371 indicated a clear IC subdivision into two genetically distinct groups. Two main population clusters were Lamu and others.

Keywords: Indigenous chicken ecotypes ; MHC ; Genetic diversity

Introduction

Indigenous chicken (*Gallus gallus domesticus*) are widely distributed throughout Africa and Asia under diversified geographical and agro-ecological conditions. Geographically isolated indigenous chicken (IC) populations are subjected to variable climatic conditions and each region is thought to host some unique types of chickens, hereafter called ecotypes. Such ecotypes are anticipated to possess unique combinations of alleles on genes that may constitute adaptation to local environment (Mwacharo et al., 2007). Kenyan IC ecotypes are known for good adaptability to harsh scavenging conditions and poor nutrition and tolerance to parasite and diseases in their habitat (Ngeno, 2011). The ecotypes may have evolved independently and become genetically diverged as a result of natural selection imposed by local adaptation to climate, parasites, diseases and nutrition in their habitat. In order to survive, these chicken have to harbour a large plasticity in their immune system to be able to withstand a large number of immune challenges. This plasticity in these IC might be explained by differences in the alleles of the genes in the major histocompatibility complex (MHC). The MHC is associated with immune response (Parmentier et al., 2004; Fulton et al., 2006; Nikbakht et al., 2013) and disease resistance (Lamont, 1989).The microsatellite marker LEI0258 is a well-studied marker of the MHC, and together with microsatellite marker MCW0371 explained already many immune haplotypes (Fulton et al., 2006). Marker LEI0258 has been used in several genetic diversity studies (Izadi et al., 2011; Chang et al., 2012). Relationship between the MHC and IC in different ecosystems has not been studied in depth. The objective of this study was to investigate MHC markers LEI0258 and MCW0371 in IC of different ecotypes in Kenya in order to quantify genetic differences within and between populations.

Materials and Methods

Sampling. Blood samples were collected from different regions (counties) of Kenya. The covered counties included; Kakamega (KK) and Siaya (BN) in the Western region, West Pokot (WP) and Turkana (TK) in the North Rift, Bomet (BM) and Narok (NR) in the South Rift, and Lamu (LM) and Taita-Taveta (TT) in the coastal region. Each county represents an ecotype. Two mature chickens per household located more than 0.5 km away were sampled resulting in a total of 768 birds (i.e. 96 samples per ecotype). One bird per household was genotyped to reduce the probability of sampling genetically related birds (i.e. 48 per ecotype). All samples were collected from free ranging IC populations. Blood samples (~2 mL in EDTA) were drawn from the wing vein of each bird.

DNA isolation, polymerase chain reaction (PCR) amplification and genotyping. Genomic DNA was obtained by standard phenol–chloroform extraction. Individuals were genotyped with LEI0258 and MCW0371 microsatellite markers located on chromosomes 16. The PCR as described by McConnell et al. (1999) and Fulton et al. (2006) was used. Primer sequences, fluorescent dyes and annealing temperatures (°C) given in supplementary material Table 1.

Table 1. Fluorescent dye, annealing temperatures (°C) and primer sequences

Marker	Fluorescent dye	Annealing temperature (°C)	Primer sequence
LEI0258	Fam	55	Forward: CACGCAGCAGAACTTGGTAAGG;Reverse:AGCTGTGCTCAGTCCTCAGTGC
MCW0371	Ned	55	Forward: TTTCATGGCATCCTAAGATGG;Reverse:CTGCTCCGAGCTGTAATCCTG

Statistical analyses. The MHC markers LEI0258 and MCW0371 were examined individually and were also examined together. Genetic diversity was assessed by calculating the number of alleles per marker and population (i.e. ecotype), observed (Ho) and expected (He) heterozygosity, inbreeding coefficients for the total population (Fit), coefficient of inbreeding between populations (Fst), and within-population inbreeding coefficient (Fis). GenAlex software version 6.5b5 (Peakall and Smouse, 2012) was used to estimate observed mean (Na) and effective (Ne) number of alleles, Ho and He per population. Population software version 1.2.32 (Langella, 1999) was used for allele frequency and private allele identification. The alleles were identified by their sizes. Population differentiation was estimated by fixation indices Fit, Fst and Fis for each marker across ecotypes according to the variance based method of Weir and Cockerham (1984) using FSTAT software Version 2.9.3.2 (Goudet, 2002). Structure software version 2.3.4 (Pritchard et al., 2000) with the Bayesian model-based clustering method for inferring population structure using multilocus genotypes was used. The program was run 100 times for each genetic cluster (K) value using the admixture and independent allele frequencies model with a burn-in period of 50,000 followed by 100,000 of Markov chain Monte Carlo iterations. Individuals were grouped into a predefined number of K clusters (K=1-8). Prior information on sampling locations was provided. Structure Harvester software version 0.6.93 (Earl and Vonholdt, 2011) was used to analyse structure output, to identify the optimal of clusters from K=1-8. The *ad hoc* statistic ΔK, based on the rate of change in the log probability between successive K values, was used to detect the true numbers of clusters (Evanno et al., 2005).

Results and Discussion

Genetic variability. Observed mean number of alleles (Na) for marker LEI0258 ranged between 20 (LM) to 27 (KK) whereas the effective mean number of alleles (Ne) ranged from 10.66 (TT) to 15.95 (BN) respectively (Table 2). In the 8 populations, 46 differently sized LEI0258 alleles (194–550 bp) were identified (Figure 1) with some ecotypes sharing common alleles. Allele sizes were consistent in the size range (182-552pb) reported by Charaza et al. (2013). The 46 LEI0258 alleles generated 229 genotypes (35 homozygous and the rest were heterozygous) in the eight populations. A total of 10 alleles (198-207 bp) were observed for MCW0371. Observed mean number (Na) of alleles per ecotype ranged from 8 for LM to 10 for NR. The TK ecotype had the lowest (4.3), and the BM the highest (6.5) Ne, respectively. The MHC markers were highly polymorphic. Observed (Na) and effective (Ne) mean number of alleles per population was higher for LEI0258 than MCW0371. High diversity of alleles for MHC markers LEI0258 might explain the plasticity of the IC chicken in coping with diseases.

Table 2. Observed mean (Na) and effective (Ne) number of alleles, observed (Ho) and expected (He) heterozygozity, and within-population inbreeding coefficient (Fis).

Marker	Ecotype	Na	Ne	Ho	He	Fis
LEI0258 (46 alleles)	BM	21.00	11.32	0.83	0.92	0.10
	KK	27.00	15.16	0.92	0.94	0.03
	LM	20.00	12.45	0.94	0.93	-0.01
	NR	25.00	14.54	0.88	0.94	0.07
	BN	26.00	15.95	0.98	0.95	-0.03
	TK	24.00	12.78	0.91	0.93	0.02
	TT	21.00	10.66	0.82	0.92	0.10
	WP	24.00	13.80	0.94	0.94	0.00
MCW0371 (10 alleles)	BM	9.00	6.52	0.88	0.85	-0.03
	KK	9.00	6.09	0.75	0.84	0.10
	LM	8.00	4.77	0.85	0.79	-0.08
	NR	10.00	5.16	0.81	0.81	0.00
	BN	9.00	5.45	0.83	0.82	-0.01
	TK	9.00	4.81	0.79	0.79	0.01
	TT	8.00	4.34	0.77	0.77	0.00
	WP	9.00	6.24	0.96	0.84	-0.14

BM = BM; KK = Kakamega; LM = Lamu; NR = Narok; BN = Siaya; TK = Turkana; TT = Taita-Taveta; WP = West Pokot

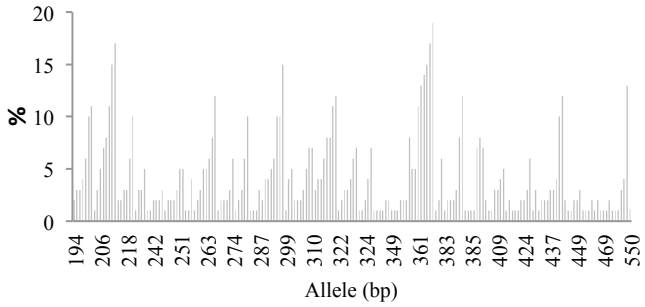

Figure 1. LEI0258 allele frequencies in the eight Kenyan indigenous chicken ecotypes

Allele sizes in marker LEI0258 can be used for distinguishing MHC haplotypes (Fulton et al., 2006). In comparison with the haplotypes reported by Fulton et al. (2006) and Charaza et al (2013), only three (BW3, B2 and B15) out of 15 were identified. The remaining unknown 13 haplotypes have not been published. LEI0258 alleles are correlated with traits and MHC haplotypes, nonetheless the same allele may be differently associated serologically with the defined MHC haplotype in the different populations (Izadi et al., 2011). Haplotype B2 identified is known to confer moderate resistance to Marek disease (Kaufman, 2000).

Several LEI0258 alleles were shared among ecotypes suggesting that they have been subjected to similar directional selection or due to recombination effect. The

majority of genotypes created by 46 LEI0258 alleles were heterozygotes. A higher frequency of heterozygous individuals at the LEI0258 marker suggests a higher antigen diversity being presented to T cells to mediate disease tolerance (Chazara et al., 2013). Marker MCW0371 revealed more alleles (198-207 bp) compared to 8 allele sizes (200-209 bp) reported by Fulton et al. (2006) but was consistent in 1-bp increments.

The observed heterozygosity ranged from 0.82 (in TT) to 0.98 (in BN) and the expected heterozygosity from 0.92 (in BM and TT) to 0.95 (in BN) in LEI0258 (Table 2). For MCW0371 the Ho ranged from 0.75 (in KK) to 0.96 (in WP) and the He from 0.77 (in TT) to 0.85 (in BM). LEI0258 Ho was higher than 0.86 for unselected chicken populations and lower than 1.00 for Red Jungle fowl found by Charaza et al. (2013). High and varied Ho and He in this study might be due to variation in population structure and origin. Differences in population structure could be arising from geographical isolation as well as variation in their ancestors. For instance, ancestors of LM ecotype have been anticipated to originate from Asia through Tanzania. Lamu ecotype local name is called 'Kuchi' which is similar to Kuchi ecotype in Tanzania. Kuchi chicken in Tanzania has similar prefecture as 'Kōchi' from Hamo halotype in Japan and its thought to be its origin (Lyimo et al., 2012). Wider genetic basis of IC could also be due to introduction of chicken from Europe.

The Fis for the population ranged from low positive to negative values (Table 2). Relatively low-positive Fis values indicated low level of inbreeding, maintained genetic variation within the IC ecotypes population. The Fis values per ecotype were below 5%, suggesting the IC populations were not endangered (Simon and Buchenauer, 1993; Ramadan et al., 2012).

Twelve new alleles were detected in marker LEI0258 in the IC population of Kenya (Figure 1). The new alleles were specific in only one of the ecotypes; LM (308, 406, 458 and 471), BN (453, 475 and 479), NR (445 and 482), TT (371), TK (396), and KK (432). Marker MCW0371 had no new alleles. The existence of specific new alleles such as observed with LEI0258 marker across the ecotypes indicated the presence of MHC genetic diversity between the ecotype populations. Variation in the numbers of new alleles between the ecotypes may be due to different origins of the populations. Diverse alleles in the studied IC could be a result of hybridized origin of Asian and European. Indigenous chicken are anticipated to have multiple origins from wild ancestor in South Asia and Island Southeast Asia (Lymo et al., 2012; Mwacharo et al., 2013) and introduced to Kenya through several entry points. Within Kenya, IC spread separately via multiple maritime and terrestrial routes and observed variation within ecotypes could be due to adaptation to environment provided by each specific route.

Population clustering. Population structure cluster analysis indicated a clear IC subdivision into genetically distinct populations (Table 3). Based on proportions of admixtures as shown by *ad hoc* statistic ΔK, IC can be divided into two clusters: LM (cluster 1) and all other ecotypes (cluster 2). Marker LEI0258 separately, and combination of LEI0258 with MCW0371, revealed two IC groups: LM and other populations. Marker MCW0371 alone did not produce clear clusters. The optimal K of 2 identified using ΔK approach was in agreement with findings by Desta et al. (2012) who reported the Kenyan population to belong to two genetic groups. Distinct IC groups could be associated with geographically and socio-economically isolation of populations as well as their ancestors. Presence of physical barriers such as mountains, rivers, and lakes separating the different communities and climatic conditions might have also contributed to the ecotype genetic variation.

Table 3. The Evanno table output for eight Kenyan indigenous chicken ecotypes for MHC markers LEI0258 and MCW0371 with K=1-8.

	K	Mean LnP (K)	Stdev LnP (K)	Ln'(K)	|Ln''(K)|	Delta K
MCW0371	1	-1496	0	—	—	—
	2	-1547	27	-51	4	0
	3	-1593	57	-47	44	1
	4	-1596	63	-2	14	0
	5	-1584	58	12	1	0
	6	-1572	43	12	29	1
	7	-1588	48	-17	41	1
	8	-1564	57	24	—	—
LEI0258	1	-13843	32033	—	—	—
	2	-2457	9	11386	11379	1302
	3	-2450	13	7	20	2
	4	-2462	18	-12	14	1
	5	-2461	14	1	5	0
	6	-2464	16	-4	3	0
	7	-2471	15	-6	1	0
	8	-2476	15	-5	—	—
LEI0258 and MCW0371	1	-3992	0	—	—	—
	2	-3817	2	175	84	40
	3	-3726	4	91	87	23
	4	-3723	6	4	0	0
	5	-3718	6	4	52	8
	6	-3766	11	-47	38	3
	7	-3851	13	-86	13	1
	8	-3950	16	-99	—	—

Conclusion

Kenyan indigenous chickens host many and highly diverse MHC alleles. The LEI0258 and MCW0371 markers revealed high allelic diversity within the MHC region. Cluster analysis based on combination of LEI0258 and MCW0371 and LEI0258 separately, indicated a clear IC subdivision into two genetically distinct groups. Lamu (one cluster) and others (cluster two).

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