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Polymorphism of the Major Histocompatibility Complex and Genetic Structure of Southern African Village Chicken Populations

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Abstract: The chicken Major Histocompatibility Complex (MHC) is a cluster of 19 genes spanning 92 kb on chromosome 16 and plays an important role in immune response. It is highly polymorphic and has been used to study adaptive genetic diversity in chickens. LEI0258 micro satellite marker that is on the BF region of the MHC and was used to investigate adaptive genetic diversity and population structures of village chickens from South Africa (n = 77), Zimbabwe (n = 60) and Malawi (n = 30). These village chickens are raised under scavenging production systems that are characterized by frequent exposure to diseases pathogens as well as fluctuations in feed supplies. The number of alleles, allele frequency and heterozygosity levels were used to measure diversity within populations whilst the Wright's fixation indices were used to analyze the level of population sub-structuring. A total of 22 alleles ranging from 188-448 bp were observed. Number of alleles averaged 12 per population. Allele frequency ranged from 1.6-37.5% and H_O averaged 0.5. Wright's F statistics indicated high level of within population variability and absence of population sub-structuring amongst the South African, Malawian and Zimbabwean chickens. It was therefore concluded that LEI0258 is highly polymorphic and could be a useful marker for Southern African village chicken populations.

Key words: Genetic polymorphism, genetic adaptation, MHC, village chickens, population structure

INTRODUCTION

Village chickens are one of the most common and important livestock species in smallholder farming communities in Southern Africa and other developing countries (Ahlers *et al*., 2009). Such chickens are owned by most families in rural areas and mostly by the poorer households. This is because village chickens are far less costly to maintain as they usually fend for their own food or are supplemented with affordable household wastes and other less expensive feedstuffs such as crop residues (Kitalyi, 1996). They are kept in inexpensive, rudimentary types of housing structures and require little or no management (Ahlers *et al*., 2009). Village chickens normally play a significant role in household food security by providing meat and eggs for low-income families (Kitalyi, 1996). They help reduce problems of malnutrition in poor communities as they provide high quality protein and other nutrients. They are a source of income when their owners sell them to other community members (Moreki *et al*., 2011).

Chicken diseases are a major problem in village poultry production systems. Unlike commercial or intensively farmed chickens, village chickens run a high risk of contracting different kinds of diseases caused by parasites, viruses and bacteria. This is because the environment village chickens are raised in is not monitored and there are no strict biosecurity measures to control disease pathogens (Schou *et al*., 2010; Izadi *et al*., 2011). Village chickens scavenge for food from household wastes, maize by products and garden crops and they fend for water from unprotected sources. These feed and water sources are host to many pathogenic microorganisms and parasites that can cause diseases and chicken death when consumed (Kitalyi, 1996). In commercial chicken production systems most of these diseases are prevented by the use of tight biosecurity measures and other veterinary interventions (Janmaat and Morton, 2010). The treatment and control of these diseases normally entails the use of vaccines, antibiotics, antihelminths, culling and burning of infected chickens and normal preventative measures such as biosafety, disinfection, good hygiene as well as a balanced diet. Use of conventional medicines and biosecurity measures however are a challenge in village chicken production systems as (i) farmers do not have the finances to spend on veterinary drugs, (ii) there is

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limited access to veterinary extension services and (iii) village chickens are raised in mixed flocks that scavenge, making biosecurity control measures almost impossible to implement. As a result, treatment strategies are less successful in these informal production systems.

Genetic control strategies could be an alternative for disease control in the latter chicken production systems (Lamont, 1998). Areas where genetic intervention has been successful as a control measure include genetically engineered vaccines to control ticks in cattle as well as in the breeding for tick resistant cattle (Muhammad, 2008). By selecting chickens with genes conferring resistance to major diseases one can ensure that the chicken flocks stay uninfected even in the face of major outbreaks such as those that occur due to avian influenza and Newcastle diseases. One of the new approaches used as an alternative to the use of antibiotics or other veterinary interventions is genetic selection for animals with improved resistance/tolerance to diseases.

The Major histocompatibility complex (MHC) is a gene cluster that plays an important role in the immunology and disease resistance in chickens (Izadi *et al*., 2011). It is a gene cluster of 19 genes, 92 kb in size and is located on micro-chromosome 16 (Kaufman *et al*., 1999). The MHC has been associated with disease resistance and sensitivity to pathogens such as fowl cholera, coccidiosis and several others (Fulton *et al*., 2006). It is highly polymorphic and has been extensively used to study adaptive genetic diversity in a number of chicken populations (Fulton *et al*., 2006). Micro satellite markers such as the LEI0258, MCW0371, MHC-D and MHC-T can be used in studying the MHC (Sironi *et al*., 2010). It was concluded by (Nikbakht *et al*., 2013) that the LEI0258 marker is a useful method for studying village chickens as it has been proven to be highly diverse in the Iranian village chickens and that also this allelic data can be useful for conservation and other breeding programs to increase MHC gene diversity in chicken populations. This marker has proven to be polymorphic in African chickens and other lines where 50 alleles were observed in these populations (Chazara *et al*., 2013). The BF2 region of the MHC class I can also be sequenced to assist in the studying of the MHC genes and haplotypes (Livant and Ewald, 2005).

There is very limited information on the MHC haplotypes in Southern African village chicken populations. However, it is hypothesized that village chickens are likely to be endowed with high genetic variation in immune response genes as they are more exposed to harsh environments with pathogens and parasites in the absence of conventional health control programs (Izadi *et al*., 2011). Survival of village chickens in the face of many of these disease-causing pathogens would therefore depend on them having an inherent genetic response system to fight and resist infections. This study was aimed at (i) screening for polymorphism and MHC haplotypes in different village chicken populations of Malawi, South Africa and Zimbabwe and (ii) determining intra and inter-population diversity of Southern African village chicken populations using MHC micro satellite markers.

MATERIALS AND METHODS

Collection and extraction of genomic DNA: A random sample of chickens from the three participating countries were obtained from the South African farming regions of Limpopo (Limpopo District-Kwaaidraai village, n = 15), Northern Cape (Kgalagadi District-Bankara Sidibe village, n = 36) and Eastern Cape (Alfred Nzo District-Nkosana village, n = 26) the Zimbabwean agro-ecological zones (AEZ) (AEZ1 n = 30) and (AEZ5 n = 30) and Malawi (MAL n = 30). These chickens were sampled in previous studies by Muchadeyi *et al*. (2007), Mtileni *et al*. (2011). One chicken was sampled per household. Blood samples were collected from the wing vein onto FTA Micro Cards (Whatman Bio Science UK). DNA isolation was carried out following the modified Qiagen DNeasy blood and tissue kit extraction protocol (Sambrook and Russel, 2001). In summary, about 10-20 mg of bloodstained FTA micro cards were cut and placed in a 2 mL micro-centrifuge tube, 180 μL PBS and 20 μL of proteinase K were added to the blood samples and incubated overnight at 56°C in a heating block (Accublock™ Digital Dry bath Labnet. Labnet International Inc.). Subsequently, 200 μL buffered (containing guanidine hydrochloride) AL (lysis buffer) was added followed by 200 μL of absolute ethanol (100%). The mixture was transferred into a spin column tube and centrifuged (Spectrafuge 24D Labnet. Labnet International Inc.) and then washed with wash buffers (containing guanidine hydrochloride) AW1 and AW2. The DNA was eluted with 100 μL of buffer AE (elution buffer). The samples were run on 1% agarose gel with 4 μL of ethidium bromide at 120 V for 20 min. The gel was examined under UV light in a GelDox UVP Biospectrum® AC Imaging System.

Micro satellite marker genotyping: The PCR primers: forward 5-CACGCAGCAGAACTTGGTAAGG-3 and reverse 5-AGCTGTGCTCAGTCCTCAGTGC-3 were used for the amplification of LEI0258. The PCR reactions were performed in 7.5 μL reaction volumes containing 1 μL of 30 ng chicken genomic DNA, 10 pmol of each forward and reverse primers, PCR multiplex master mix and nuclease free water. The amplification was carried out in a GeneAmp 9700 thermocycler using the following conditions: an initial denaturation step at 94°C for 1 min, followed by 35 cycles at 92°C for 45 sec, annealing at 65°C for 30 sec, 72°C for 30 sec and a final extension of

10 min at 72°C. The cycling conditions were adopted from Sironi and co-workers (Sironi *et al*., 2010) and were optimized for this study.

Amplicon processing and allele-size scoring was performed and analyzed using an ABI 3130 XL genetic analyzer. The alleles were identified by their sizes (bp) and scored against the GeneScan LIZ 500 bp size standard using Genemapper, version 4 software (Applied Biosystems).

Statistical analysis

Marker polymorphism: Data was analyzed to give the total number of alleles as well as their distribution among the three populations using the Micro satellite Toolkit software (http://animalgenomics.ucd. ie/sdepark/ms-toolkit/). Allele frequency, defined as the proportion at which an allele occurs in a population, was also determined using the Micro satellite Toolkit Software.

Within population diversity: The micro satellite toolkit software was then used to generate the FSTAT input file. The FSTAT program was used to calculate the observed and expected heterozygosities of the three populations. For each population, expected heterozygosity was estimated using the formula:

$$H_e = 1 - \sum (p^2_i + q^2_i)$$

where, p is the allele frequency of allele 1 at one locus and q is the frequency of the alternate allele at the same locus using the FSTAT software program. Based on expected and observed heterozygosity, the inbreeding coefficient (F_{IS}) of each population was calculated using the formula in FSTAT program:

$$F_{IS} = \frac{H_e - H_o}{H_e}$$

where, H_e is the expected heterozygosity and H_o is the observed heterozygosity. The deviation of each population from the Hardy-Weinberg Equilibrium (HWE) was also tested using the FSTAT software program.

Between population diversity: The Weir and Cockerham statistic, which is a ratio of the variance in allele frequencies among populations to the overall variance in allele frequencies, was used to investigate between and within population variation using the FSTAT program. In addition, the pairwise F_{ST} values were used to differentiate between pairs of population variability.

RESULTS

Marker polymorphism: A total of 22 LEI0258 alleles were observed from the six populations under study, Allele sizes ranged from 188 to 448 bp. Alleles, 188, 204, 218, 232, 252, 267, 312 and 358 were shared by all the six populations and were therefore considered common alleles (Table 1). Other alleles were shared by some but, not all populations. For example, allele 276 was common to all the five populations except Eastern Cape whilst allele 290 was common to Limpopo, Northern Cape and the Zimbabwean AEZ1 ecotype.

Table 1: Allele frequencies (%) of LEI0258 in six chicken populations

	Population[1]						
Allele	EC	L	NC	MAL	AEZ1	AEZZ5	[2]Pop
188	7.7	7.1	3.1	12.5	20.7	12.9	6
204	11.5	3.6	15.6	26.8	12.1	33.3	6
218	3.9	7.1	7.8	14.3	3.5	5.6	6
232	1.9	3.6	1.6	37.5	0	22.2	5
236	0	0	4.7	0	0	0	1
252	13.7	10.7	3.1	0	13.8	9.3	5
267	13.5	10.7	3.1	1.8	6.9	9.3	6
276	0	14.3	4.7	3.6	12.1	5.6	5
290	0	3.6	1.6	0	6.9	0	3
297	3.9	0	0	0	0	0	1
298	0	3.6	0	0	1.7	1.9	3
312	17.3	28.6	21.9	3.6	18.9	0	5
322	3.9	0	1.6	0	3.5	0	3
334	1.9	0	0	0	0	0	1
350	3.9	0	1.6	0	0	0	2
358	9.6	10.7	18.8	0	0	0	3
374	3.9	0	0	0	0	0	1
378	0	0	1.6	0	0	0	1
386	0	0	1.6	0	0	0	1
396	0	3.6	3.1	0	0	0	2
438	1.9	0	0	0	0	0	1
448	1.9	0	1.6	0	0	0	2

[1]EC: Eastern Cape L: Limpopo NC: Northern Cape
MAL: Malawi AEZ1: Zimbabwe agro-ecological zone 1
AEZ5: Zimbabwe Agro-ecological Zone 5 [2]Pop: number of populations sharing an allele

Table 2: Number of alleles, heterozygosity and inbreeding per population[1]

	Population						
	EC	L	NC	MAL	AEZ1	AEZZ5	Total average
N	26	15	36	30	30	30	167
N_a	15	12	17	7	10	8	22
H_e	0.9	0.9	0.9	0.8	0.9	0.8	0.9
H_o	0.8	0.7	0.6	0.2	0.5	0.3	0.5
F_{IS}	0.2	0.2	0.4	0.7	0.5	0.6	0.4

[1]EC: Eastern Cape L: Limpopo NC: Northern Cape
MAL: Malawi AEZ1: Zimbabwe agro-ecological zone 1 AEZ5: Zimbabwe Agro-ecological Zone 5
N: Sample size N_a: Number of alleles H_e: expected heterozygosity
H_o: observed heterozygosity F_{IS}: inbreeding coefficient

Table 3: Pairwise F_{ST} values (below diagonal) between South African, Malawian and Zimbabwe village chicken populations[1] and F_{IS} (on the diagonal) per population

	EC	L	NC	MAL	AEZ1	AEZZ5
EC	0.2	-	-	-	-	-
L	0.0020	0.2	-	-	-	-
NC	0.0013	-0.0069	0.4	-	-	-
MAL	0.0997	0.1148	0.1012	0.7	-	-
AEZ1	0.0070	-0.0059	0.0290	0.1043	0.5	-
AEZZ5	0.0544	0.0896	0.0721	0.0021	0.0567	0.6

[1]EC: Eastern Cape L: Limpopo NC: Northern Cape
MAL: Malawi AEZ1: Zimbabwe agro-ecological zone 1 AEZ5: Zimbabwe agro-ecological Zone 5

Allele 396 was common to only two populations namely Limpopo and Northern Cape.

Table 1 also shows the allele frequencies of the 22 alleles in the six populations. Allele frequencies ranged from 1.6-37.5%. Allele 312 was the most frequent allele in South African Eastern Cape (17.3%), Limpopo (28.6%) and Northern Cape (18.9%) as well as Zimbabwean AEZ1 (3.6%) chicken populations. This allele was however less frequent in Malawian chickens (3.6%) and absent in the Zimbabwe AEZ5 ecotype. Allele 232 was less frequent in SA populations ranging from 1.6-3.6%, but was high in Malawian and Zimbabwean ecotype AEZ5 populations. This allele was absent in AEZ1. Three rare alleles (232, 396 and 448) were observed at a frequency of less than 5%.

A number of private alleles were also observed. For example, alleles, 297, 334, 374 and 438 were unique to the Eastern Cape chickens whilst alleles 236, 378 and 386 were only observed in the Northern Cape population. There were no private alleles observed in the Limpopo, Zimbabwean and Malawian populations.

Within population variation: Per population numbers of alleles are shown in Table 2. Northern Cape had the highest number of alleles (n = 17) whereas Malawi had the least (n = 7). Highest allele frequency was observed in the Malawian population (37.5 %) whilst lowest allele frequency was observed in the Northern Cape population (1.6 %). The expected heterozygosity per population was over 80% in all the populations (Table 3). Observed heterozygosity averaged 50.0% and Malawi had the least observed heterozygosity (20 %). Overall, all populations were inbred with an average inbreeding coefficient of 0.4. The Malawi chickens had the highest level of inbreeding (F_{IS} = 0.7) and Eastern Cape and Limpopo had the lowest inbreeding (F_{IS} = 0.2).

Between population variation: The Weir and Cockerham statistics showed that the total variation (F_{IT}) in the population was 0.5 of which 80% (F_{IS} = 0.4) was explained by within population variation. There was minimal between population differences as indicated by a 0.1 F_{ST} value. Table 3 shows that the pairwise F_{ST} between populations was very low ranging from -0.001 to 0.002. Together, the Weir and Cockerham statistics and pairwise F_{ST} values (Table 3) show an absence of population sub-structuring of the Southern African chickens.

DISCUSSION

The micro satellite marker LEI0258 was polymorphic in the village chicken populations. A total of 22 alleles were observed in 167 animals and most of the alleles were shared among the populations. The marker LEI0258 has also been reported to be highly polymorphic in other populations such as the commercial and traditional breeds analyzed by Izadi and co-workers (Izadi *et al*., 2011). A total of 26 LEI0258 alleles (182-552 bp) were also identified in the North American and European egg layer-type chickens (Fulton *et al*., 2006). Lwelamira and co-workers (Lwelamira *et al*., 2008) observed 22 and 23 LEI0258 alleles in 2 Tanzanian ecotypes and 19 alleles were identified in Vietnamese local chicken populations (Schou *et al*., 2010). Over 50 novel alleles were observed in Asian, European and West African chicken populations (Chazara *et al*., 2013) and according to (Izadi *et al*., 2011), MHC alleles tend to be shared among different populations.

The observed allele frequency in this study's populations (1.6-37.5%) showed that the LEI0258 alleles were well distributed within populations. A

considerable number of alleles were observed at moderate frequencies of above 20% and were equally represented among populations in the village chicken populations studied. Other alleles occurred at low frequencies below 5% and some of the alleles were considered rare because they were less frequent in populations (e.g., alleles 232, 396 and 448). Attention needs to be focused on these rare alleles in a population as they can easily be wiped out due to selection pressures or completely lost in a population due to genetic drift. Rare alleles can also be lost due to extinction of a population or death of the individuals that possess these alleles.

Allele 312 was the most frequent allele in the South African and Zimbabwean AEZ5 populations. This allele was observed at a highest frequency of 28.6% in the Limpopo population from South Africa. It was also shared by five village chicken populations and absent only in the Zimbabwean AEZ1 ecotype. High frequency of allele 312 implies that it confers a fitness or survival advantage to individuals carrying it resulting in it being selected for and occurring at elevated frequencies in most of the populations (Kuhnlein *et al*., 1997). It is therefore suggested that this allele be further investigated for association with disease and pathogen resistance in the village chicken populations.

It was observed that allele 232 was the least frequent allele at a frequency range of 1.6-3.6% in the six populations studied. There are several possibilities of such a low frequency. This allele could be a new mutation arising in a population and therefore found only in a few individuals. Such alleles pose a high chance of being lost out of the population due to genetic drift and death of the few individuals carrying it (Johnson *et al*., 2004). As such it will need to be conserved and propagated in the population. Alternatively, it could be that such an allele is associated with susceptibility to diseases and other selection pressures resulting in it not surviving the production challenges the chickens are challenged with (Leveque *et al*., 2003). Either way, this allele like the most frequent allele requires further investigation into its role and evolution in the populations.

In the populations studied there were also a number of private or unshared alleles that were observed in only one or few of the populations. Populations that have unique alleles should be considered for conservation to save such genetic variation. Limpopo and all Zimbabwe and Malawian populations did not have any private alleles and shared most of their alleles with the rest of the populations.

Results from this study indicated high level of within population diversity as indicated by number of alleles, allele frequencies and Weir and Cockerham statistics. The evaluation of genetic variation within and between the studied village chicken populations has been previously done using several autosomal neutral microsatellites (Muchadeyi *et al*., 2007; Mtileni *et al*., 2011). These previous studies showed that village chickens of Zimbabwe, Malawi, South Africa and Sudan had high within population genetic variation. This study extends the observed within population diversity to genes that could be under selection such as those on MHC. The major histocompatibility complex has been reported to be associated with disease resistance in a number of studies (Kaufman *et al*., 1999; Fulton *et al*., 2006; Muchadeyi *et al*., 2007). Polymorphism or variations of a population in the MHC regions are of great importance in conferring adaptive genetic resistance and flexibility in surviving the harsh and heterogeneous production environments. A population that is more polymorphic in this gene has greater chances of survival in the extreme village chicken production systems (Izadi *et al*., 2011; Lwelamira *et al*., 2008). Southern African chicken populations are equally genetically diverse as compared to other chicken populations from previous studies. Izadi and co-workers (Izadi *et al*., 2011) identified high within population diversity in Shiqi and Yellow Shiqi, whilst Fulton *et al*. (2006) reported on highly diverse Chinese chicken populations.

Expected and observed heterozygosity and number of alleles per population were also used to show within population genetic variation. The overall observed heterozygosity in South Africa, Zimbabwe and Malawi populations was ±0.5. A diverse population is expected to be highly heterozygous. A previous comparative study, demonstrated that village chickens were more heterozygous than commercial flocks (Izadi *et al*., 2011). A heterozygous population stands a better chance of resisting/tolerating multiple disease infections that challenge village chicken populations (Boonyanuwat *et al*., 2006).

Based on allele numbers and allele frequencies, lower levels of heterozygosity were observed than were actually expected thereby making these Southern African chicken populations generally inbred at the MHC locus. Inbreeding in the MHC region may be due to selection for specific alleles and allele combinations to fight the different diseases and pathogen challenges in the respective populations. As discussed before, village chickens are kept in challenging environments and are constantly exposed to parasites and diseases. Therefore in the face of diseases some alleles are naturally selected for whilst others are wiped out of the population. Such a scenario would therefore cause a deviation in genotype frequencies from those expected under the Hardy-Weinberg equilibrium.

There was also, based on the population differences in geographical locations, environmental conditions and the geographical separation, an expectation to observe

large between-population variations. However, results in this study showed that there were no genetic differences between the Eastern Cape, Limpopo and Northern Cape populations as well as chicken populations from Zimbabwe and Malawi. The Weir and Cockerham statistics showed that all the diversity found in these Southern African chickens was due to within population diversity and they were insignificantly low. This lack of population differentiation at the MHC locus implies that the South Africa, Zimbabwe and Malawi chickens populations all experience the same production challenges (disease, environmental stress, parasites etc.) resulting in the same MHC alleles and allele combinations being promoted or selected against. Lack of population sub structuring has also been observed using autosomal micro satellite markers (Muchadeyi *et al*., 2007; Mtileni *et al*., 2011).

Conclusion: Southern African village chicken populations were found to be polymorphic at the MHC region. Results from this study indicate that LEI0258 can be selected as a useful marker in these populations. The South African chickens had high within population and less between population diversity at the LEI0258 locus. Further studies are recommended to investigate alleles occurring at either high or low frequencies for their associations with resistance or susceptibility to diseases prevalent in these village chicken production systems.

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