Genetic Diversity among Nine Free Range Local Chicken Ecotypes in Tanzania Based on Microsatellite DNA Polymorphisms

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Abstract

The genetic diversity among nine free-range local chicken ecotypes identified in Tanzania was evaluated using 20 polymorphic microsatellites markers. The ecotypes were named Ching'wekwe, Mbeya, Morogoro-medium, Kuchi, Singamagazi, N'zenzegere, Pemba, Tanga, and Unguja. A manual microsatellite typing procedure was adopted. Of the 20 microsatellite loci, 16 were amplified by PCR and were highly polymorphic. Four to 15 alleles per locus and four to seven alleles per ecotype were detected. Gene diversity was very high (62 to 79%). The shortest genetic distance as measured by Nei's standard genetic distance (D_S) was between Kuchi and Unguja ecotypes (D_S distance = 0.0671) while the White Leghorn and Unguja ecotype (D_S distance= 0.9149) presented the longest genetic distance. A Neighbour-Joining dendogram based on D_S distance showed the local chicken ecotypes to assort into three clusters. The bootstrapping values within the dendogram ranged from 41 to 98%. These results indicate that the free-range local chicken ecotypes to exist in different genetic groups. The accurate genetic typing of this animal genetic resource is important in making rational decisions on the utilisation of the chickens for economical use without compromising the conservation of each unique ecotype adapted to a particular eco-climatic zone.

Key words: Genetic diversity, Free-range local chicken ecotypes, Microsatellites, Animal genetic resourcesIntroduction

In the developing world the term breed as it is formally defined has little meaning because of non-existence of pedigree information (Rege, 2003). However, there is a multitude of uniquely identified and named populations of animals (including chickens) that may be equated to breeds in the developed world (Sonaiya, 1990; Rege, 2003). Genetic characterisation of these named populations will be of great importance in preparing some guidelines for development, utilisation and conservation of these unique populations.

The diversity of the free-range local chickens (FRLC) has mostly been expressed in terms of phenotypic characteristics.

Variations in adult body weight, egg weight, reproduction performance

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and immune responses to various antigens have been reported (Gwakisa et al., 1994, Guèye, 1998, Msoffe et al., 2001, Msoffe et al., 2002). Very few studies have utilised genetic makers to evaluate the diversity of the FRLC (Okada et al., 1987; Horst, 1988; van Marle-Koster and Nel, 2000; Wimmers et al., 2000). In Bangladesh, Okada and others (1987), calculated the genetic distances of the local Bangladesh chickens using four blood groups and eight plasma proteins concluding that the genetic distances were narrow hence the local chickens in Bangladesh can be regarded as one breed.

DNA typing is a rapid and reliable method for differentiating individuals in genetically diverse populations (Bidwell, 1994; Parham and Ohta, 1996). Microsatellite DNA typing is one such method. Microsatellites are highly polymorphic and abundant in all vertebrate genomes and can easily be typed using polymerase chain reaction (PCR) and scored on an electrophoresis gel (Rincón et al., 2000). As a consequence, microsatellites have emerged as markers of choice in a number of genetic areas such as genome mapping, medical, ecological and evolutionary genetics (Primmer et al., 1997). Microsatellite DNA typing was used successful in genetic typing of the indigenous chickens in Africa, Asia and Latin America (Takahashi et al., 1998; van Marle-Koster and Nel, 2000; Wimmers et al., 2000).

Previous studies in Tanzania have indicated the presence of several local chicken ecotypes of diverse phenotypic and genotypic attributes (Msoffe et al., 2001; 2002; 2005). It is therefore justified to study the genotypic relatedness of these local chicken ecotypes as the first step in further characterization of the indigenous animal genetic resources in Tanzania.

This study was therefore aimed at assessing the genetic diversity among the local chicken ecotypes in Tanzania using microsatellite DNA polymorphisms. The established genetic groups will be a stepping-stone towards sound utilization, promotion and conservation of the uniquely adapted local chicken ecotypes.

Materials and Methods

Blood sampling

A total of 182 (about 20 samples per ecotype) blood samples were collected from nine free-range local chicken ecotypes of Tanzania mainland and Zanzibar. The chickens were obtained from villages of different ecological zones in Tanzania with no history of crossbreeding involving exotic chickens and were hence referred to as ecotypes. However, sampling was purposeful hence only households that possessed the desired ecotypes were sampled. More than one chicken were sampled in a household if they fulfilled the selection criteria. The chickens were either named after their areas of origin or else local names were used where available. The ecotypes origin and characteristics were described in previous studies by Msoffe et al. (2001; 2002). The ecotypes names were assigned a four letter code (in brackets) to allow easy handling during genetic data analysis. The ecotypes were: Ching'wekwe (CHING), Mbeya

(MBEY), Morogoro-medium (MORO), Kuchi (KUCH) and Singamagazi (SING). The Mbeya ecotype was later found (through farmer's and district livestock authorities narration) to be either purebred or crosses of Black Australorp breed imported from the neighbouring country of Malawi. Others ecotypes were: N'zenzegere (FRIZ), Pemba (PEMB), Tanga (TANG), and Unguja (UNGU). The areas selected covered five out of seven geographical zones in mainland Tanzania and both Zanzibar (Figure 1). It was only two geographical zones, Southern zone and the central zone that were not sampled due to some logistical challenges. A total of 13 White Leghorn (DAN) DNA samples (Courtesy of Dr. Helle R. Juul-Madsen, Research Centre Foulum, Denmark) were included as a reference population. Each DNA sample was analysed using all the 20 primer pairs.

DNA extraction

DNA extraction was done according to the procedure described by Sambrook et al. (1989) with some modifications to suit our laboratory conditions. A total of 50 µl packed red blood cells was drawn into a 1.5 ml eppendorf tube.

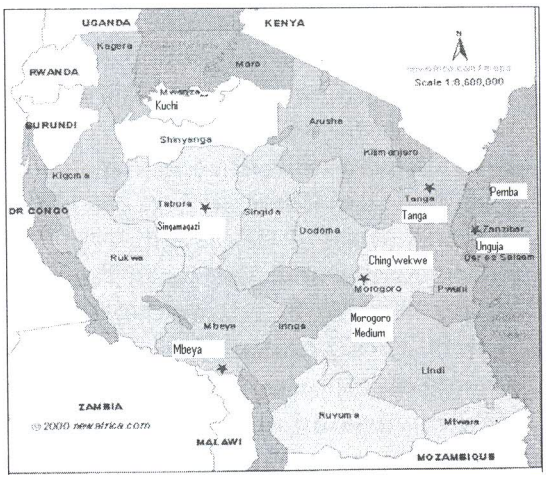

Fig. 1:

500 µl of TES buffer (0.2M Tris, 0.1M EDTA and 1% SDS) was added into the tube containing RBCs followed by 50µl of 20mg/ml Proteinase K (Sigma-Aldrich chemicals, St. Louis, USA). The mixture was incubated overnight in a 60^{0}C waterbath with occasional hand shaking. An aliquot of 0.4ml 5M Potassium Acetate per ml of the solution was added and this mixture was placed on ice for at least 30 minutes. The mixture was centrifuged at 12,000g for 10 minutes and the supernatant was transferred into a new tube. An equal volume of Phenol: Chloroform: Isoamyl alcohol (24: 23: 1; Sigma-Aldrich chemicals, St. Louis, USA) was added to the supernatant mixed gently until there was a formation of emulsion. Centrifugation at 12,000g for ten minutes was done and the mixture separated into lower organic phase and upper aqueous phase. The aqueous phase was carefully removed using a 1ml micropipette and transferred into a new tube. These two steps were repeated until no proteins (debris) were seen at the interphase. An equal

volume of Chloroform: Isoamylalcohol (23:1) was added to the clear aqueous phase, mixed and centrifuged at 12,000g for 10 minutes and the aqueous phase was obtained as above. An equal volume of Isopropanol was added very slowly at an angle, and then mixed by inversion.

The DNA was spooled using closed Pasteur pipette and was transferred into a new tube where the DNA was washed gently using 70% ethyl alcohol. In cases where DNA yield was not seen as a precipitate, the tube was centrifuged at 13,000g for 15 minutes, the supernatant was discarded (carefully without dislodging the DNA pellet) and the pellet was washed with ethyl alcohol as above. The DNA was allowed to dry at room temperature by leaving the tubes open for about one hour. Then the DNA was re-suspended in 50μl TE buffer (0.2M Tris, 0.1M EDTA) and was stored in the refrigerator at 4^0C.

Microsatellite loci and PCR conditions

A total of 20 microsatellite loci were used in this experiment. Ten microsatellite loci were chosen from the chicken genome database maintained at the Roslin Institute (http://www.ri.bbsrc.ac.uk/cgi-bin/microsatellite/microsearch.pl) accessed in June 2000. These were LEI0217, LEI0234, LEI0214, LEI0248, LEI0237, LEI.0193, LEI0040, LEI0093, LEI0043, and LEI0258. The microsatellite marker MCW005 was chosen from the MCW markers (Crooijmans et al., 1994). The other nine loci were from the population tester kit (http://poultry.mph.msu.edu) accessed in June 2000 a gift from the National Animal Genome Research Program (NAGRP courtesy of Dr. Hans Cheng). The nine included ADL0102, ADL0136, ADL0158, ADL0171, ADL0172, ADL0176, ADL0181, ADL0210, and ADL0267. A standard 30 to 35 cycles PCR was performed on a PTC-100 thermocycler (MJ research Inc, USA). An initial denaturation step was followed by 30 to 60 seconds annealing (with appropriate annealing temperature for each set of primers, Table 1), then extension at 72^oC for 1 minute. A final extension at 72^oC for 10 minutes was set for all primers. The PCR reagents concentrations are given in Table 2.

Table 1: Characteristics of the primers used in the study

Locus name	Primer sequence (5′-3′)	Annealing Temperature	Mg++ conc. (mM)§	Size (bp)¤
LEI0217	[1]F-GATGACTGAGAGAAATAACTTG R-AAATTACTGAGGCACAGGAG	51	1.5	198
LEI0234	F-ATGCATCAGATTGGTATTCAA R-CGTGGCTGTGAACAAATATG	55	1.5	289
LEI0214	F-TGCCT GT TT CT AG GA R-GATCAAGCACTGTATTTTATTC	50	2.5	159
LEI0248.	F-TTGCACTGAGACCAAATGTC R-CATAGATTTTCCTTAGTAGGTAACTTG	51	1.0	250
LEI0237	F-GTTAAGTGTTCTCTGATGTAGC R-CTTCAACTATAAAGCATAGCTG	53	1.0	224
LEI0193	F-AGCTGGAGGTAGAATCCTGAA R-ACAATCCCTTCCAACACACC	52	1.0	258
LEI0040	F-GCATTGCAGGTGGTGATAGGG R-TCAGCGCTCTTGAACTCCAGC	60	0.625	198
LEI0093	F-TCCTTGAAGTATTCCAAAGCTCA R-TCTCCTACTCCAGTGCCTTCA	60	0.625	143
LEI0043	F-CTTCCATGGCAGCTCAGCCT R-ATCACTCGCGGCCATTAGGA	60	0.625	139
ADL0102	F-TTCCACCTTTCTTTTTTATT R-GCTCCACTCCCTTCTAACCC	47	1.5	122
ADL0136	F-TGTCAAGCCCATCGTATCAC R-CCACCTCCTTCTCCTGTTCA	52	1.5	145
ADL0158	F-TGGCATGGTTGAGGAATACA R-TAGGTGCTGCACTGGAAATC	52	1.5	216
ADL0171	F-ACAGGATTCTTGAGATTTTT R-GGTCTTAGCAGTGTTTGTTT	46	1.5	104
ADL0172	F-CCCTACAACAAAGAGCAGTG R-CTATGGAATAAAATGGAAAT	49	1.5	154
ADL0176	F-TTGTGGATTCTGGTGGTAGC R-TTCTCCCGTAACACTCGTCA	52	1.5	192
ADL0181	F-CCAGTGAAATTCATCCTTTT R-CAATCTTTTGTGGGGTATGG	48	1.5	178
ADL0210	F-ACAGGAGGATAGTCACACAT R-GCCAAAAAGATGAATGAGTA	46	1.5	130
ADL0267	F-AAACCTCGATCAGGAAGCAT R-GTTATTCAAAGCCCCACCAC	50	1.5	117
MCW005	F-ACCTCCTGCTGGCAAATAAATTGC R-TCACTTTAGCTCCATCAGGATTCA	62	1.5	253
LEI0258	F- GGAATGGTTCAAGACGACGCAC R- AGCTGTGCTCAGTCCTCAGTGC	54	2.5	205

Note:

§Mg++ con. = The Magnesium ion concentration;

¤Size = the expected size (in base pairs) of the PCR product.

[1] F and R are the forward and reverse primers respectively.

Table 2: Composition of each PCR reaction mixture

Ingredient/concentration	Quantity, μl
dNTPs 200μM (dATP, dCTP, dGTP and dTTP)[1]	5
10X PCR buffer (100 mM Tris-HCl,50 mM KCl, 0.01% gelatin and 0.25% Tween 20)	2.5
$MgCl_2$ XmM	Y*
Primer 1 1 μM[2]	0.5
Primer 2 1 μM[2]	0.5
Double distilled water	Z§
Taq DNA polymerase (5units/μl) [1]	0.1
Template DNA 25ng/μl	2
Total	25

Note:
*Quantity of $MgCl_2$ depended on the concentration required by individual primer pairs
§Double distilled water was added to make the final volume of 25μl.
[1]Taq DNA polymerase, buffers and dNTPs (Armesham, Copenhagen, Denmark)
[2]Primer pairs (DNA Technology, Aarhus, Denmark)

Genotyping

PCR products were separated on 6% native polyacrylamide gels (Sigma-Aldrich, St. Louis, USA) using a Mini-Protean II® (Bio-Rad Italy,) electrophoretic equipment at 20V/cm for 40 minutes. A standard size marker, stepladder (Sigma-Aldrich, St. Louis USA) was included in each run. The amplicons were visualised by Silver staining (Rapley and McDonald, 1992). The size of the amplicons was determined using TotalLab® version 1.10 computer software (Nonlinear dynamics, Newcastle, UK). The data on PCR product size were then organised for statistical analysis using Microsatellite toolkit (Park, 2001).

Statistical analysis

The mean number of alleles per locus and the average observed and expected heterozygosities were used to assess the genetic diversity within populations. The differences between the expected and the observed heterozygosity were assessed using the X^2 test. The number of alleles per locus per population was obtained through direct counting. The mean observed and expected heterozygosities were calculated using the GENEPOP version 3.3 updated from v.1.2 (Raymond and Rousset, 1995). Deviations from the Hardy-Weinberg equilibrium (HWE) were tested either by an exact test for each locus per population using Guo and Thompson's (1992) Markov chain Monte Carlo algorithm that is conducted by the GENEPOP or for all loci and populations using Fisher's method also performed by GENEPOP.

The Nei's, (1972) standard genetic distance (D_S) was used to measure the genetic diversity among populations. The distance measure was calculated using the DISPAN programme (Ota, 1993). The phylogenetic tree of ecotype relationship was constructed using the Neighbour Joining (NJ) method with 100 bootstrap re-sampling (Saitou and Nei, 1987).

The coefficient of gene differentiation (G_{ST}) (Nei, 1973) was used to measure the genetic differentiation among the local chicken ecotypes and was estimated using the DISPAN programme.

Results

The genetic diversity of nine local chicken ecotypes from Tanzania and one reference breed (White Leghorn) were assessed by analysing 20 microsatellite loci. Primers for 16 loci provided specific products while the remaining four loci did not produce specific products hence were excluded in the analysis. Table 3 shows the number of alleles per locus and the allele size ranges for all the 16 loci analysed. All amplified loci were polymorphic and the total number of alleles detected was 152. Table 3 also shows a measure of genetic differentiation (G_{ST}). The G_{ST} value for all loci was 0.134 while the G_{ST} values for individual loci ranged from 0.071 (ADL181) to 0.313 (LEI258).

Table 3: Allele numbers and size ranges and measure of genetic differentiation (G_{ST}) for the 16 loci examined

Locus	Allele size range (bp)	Number of alleles	G_{ST}
ADL267	92 – 120	8	0.114
ADL102	96 – 138	11	0.194
ADL158	174 – 228	13	0111
ADL171	80 – 108	9	0.097
ADL172	130 – 188	14	0.089
ADL176	170 – 230	15	0.090
ADL181	164 – 196	9	0.071
ADL210	116 – 150	9	0.096
ADL136	124 – 200	12	0.086
LEI43	140 – 176	9	0.169
LEI40	180 – 264	9	0.223
MCW005	216 – 276	6	0.133
LEI217	184 – 280	11	0.140
LEI214	144 – 176	4	0.134
LEI234	288 – 380	5	0.108
LEI258	192 – 324	8	0.313
All loci	80 - 380	152	0.134

Table 4 shows the mean number of alleles per ecotype and the mean heterozygosity values that are measures of genetic variability within ecotypes. The mean number of alleles per ecotype ranged from 4.3 (White Leghorn) to 7.1 (Pemba ecotype). Except for the White Leghorn all local chicken ecotypes had mean allele number higher than five. The average gene diversity was lowest in the White Leghorn (0.621) and highest in the Ching'wekwe ecotype (0.794). The observed mean heterozygosities were lowest in White Leghorn (0.464) and highest in Singamagazi ecotype (0.688). There was high significant difference between the observed and expected heterozygosity ($X^2=\infty$).

The results for the test to determine the deviations from HWE for the different microsatellite loci are shown in Table 5. A total of 40 out 160 (25%) locus-ecotype combination did not deviate significantly from the HWE. The remaining 75% of the locus-ecotype combination significantly deviated from the HWE ($P \leq 0.0001$ to $P \leq 0.05$). The HWE test for all loci-all ecotypes combination revealed a high significant deviation. The inbreeding coefficients (f) were shown to be both positive and negative (Table 5).

The genetic distances between the local chicken ecotypes measured by the D_S distance method were used to construct the phylogenetic tree presented in figure 2. The shortest distance was between Kuchi and Unguja ecotypes (0.0671) while the longest distance was between White Leghorn and Unguja ecotype (0.9149). The genetic distance between the two isle ecotypes (Pemba and Unguja) was also very short (0.1163). The White Leghorn was only close to the Mbeya ecotype (0.2997), with the genetic distances exceeding 0.5 with all other ecotypes.

The phylogeny of all ecotypes indicated three main clusters. The first cluster comprised the two isle ecotypes (Pemba and Unguja), Kuchi and Ching'wekwe ecotype. The second cluster was made up of the Morogoro-medium, Singamagazi and N'zenzegere ecotypes. The third cluster included the Mbeya and Tanga ecotype together with the White Leghorn breed. The bootstrap values ranged from 41 to 98.

Table 4: Mean (±s.d.) allele number and heterozygosity values for local chicken ecotypes of Tanzania

Ecotype	MEAN NUMBER OF ALLELES	EXPECTED HETEROZYGOSITY	Observed heterozygosity
White Leghorn	4.31 ± 2.02	0.621 ± 0.0432	0.464 ± 0.0363
Ching'wekwe	5.94 ± 2.14	0.794 ± 0.0164	0.545 ± 0.0296
Kuchi	6.56 ± 1.93	0.789 ± 0.0251	0.594 ± 0.0304
Singamagazi	6.75 ± 2.72	0.775 ± 0.0244	0.688 ± 0.0254
Unguja	5.06 ± 1.65	0.736 ± 0.0509	0.579 ± 0.0379
Pemba	7.06 ± 2.46	0.788 ± 0.0236	0.602 ± 0.0276
Tanga	6.38 ± 3.20	0.771 ± 0.0304	0.064 ± 0.0316
N'zenzegere	6.69 ± 2.77	0.789 ± 0.0235	0.660 ± 0.0304
Mbeya	6.50 ± 1.71	0.757 ± 0.0294	0.532 ± 0.0298
Morogoro-medium	6.94 ± 2.95	0.778 ± 0.0248	0.586 ± 0.0288

**High significant difference between the observed and expected heterozygosity ($X^2=\infty$)*

Table 5: Test for HWE deviation and the inbreeding coefficients (*f*) in the local chicken ecotypes of Tanzania

Ecotype	Loci															
	ADL267	ADL102	ADL158	ADL171	ADL172	ADL176	ADL181	ADL210	ADL136	LEI40	LEI43	LEI214	LEI217	LEI234	LEI258	MCW05
DAN (9)	0.60 **	0.46 ***	0.73 ****	-0. 43 ****	- 0.26 ns	- 0.37 ns	0.29 ns	0.81 ****	- 0.00 ns	1 **	1 *	- 0.49 ns	0.49 ns	0.82 *	- 0.41 ns	1 ****
CHIN (13)	0.71 ****	0.70 ****	0.93 ****	- 0.07 ****	0.40 ****	0.11 ****	0.27 ***	0.45****	- 0.08 ****	0.09 **	0.88 ****	0.17 *	- 0.44 ns	0.35 ns	0.24 **	0.16 ns
KUCH (14)	- 0.03 ****	0.10 ****	- 0.21 **	- 0.17 **	0.10 ****	0.16 *	- 0.09 ns	-0.12 **	0.36 ****	1 ns	0.54 ***	0.71 *	0.36 *	0.58 ***	0.35 ****	0.25 ****
SING (10)	- 0.29 ****	- 0.19 ns	0.03 ns	- 0.43 ***	0.48 ****	- 0.10 ns	0.12 ****	-0.06 ns	0.19 ****	- 0.14 *	0.48 ****	0.52 *	0.37 ****	0.03 ns	0.44 ***	0.26 ns
UNGU (13)	- 0.06 *	0.02 **	0.12 ns	0.06 *	- 0.00 ***	0. 49 **	- 0.14 *	-0.20 ****	0.40 ****	0.47 *	0.78 ****	-	0.20 ns	0.72 **	- 0.13 **	0.72 **
PEMB (13)	- 0.13 **	0.30 ****	0.37 ****	- 0.07 **	0.01 ***	0.32 ***	- 0.48 ***	0.02 **	0.13 ****	0.65 ****	0.79 ****	1 ns	0.12 ns	0.49 **	0.14 ns	0.40 **
TANG (11)	- 0.17 **	0.27 ***	- 0.05 *	- 0.51 **	0.05 *	0.03 ns	0.13 ***	-0.35 *	0.03 **	1 ns	0.66 ****	0.11 ns	0.76 ****	0.26 ns	0.06 ns	0.6 **
FRIZ (10)	0.10 **	- 0.01 ns	- 0.17 ns	- 0.59 ***	0.06 ***	0.03 ns	- 0.05 ****	-0.01 **	- 0.11 ns	0.22 *	0.58 ****	0.57 ns	0.61 ****	0.65 ****	- 0.11 ns	1 ****
MBEY (12)	0.36 ****	0.23 ****	- 0.14 ns	- 0.45 *	0.15 ****	0.44 ****	0.58 ****	-0.09 ns	0.36 ****	0.93 ****	0.77 ****	- 0.15 ns	0.46 ****	0.36 ns	0.20 *	1 ****
MORO (13)	- 0.31 **	0.54 ****	- 0.06 ***	- 0.39 ns	0.40 ****	0.05 ****	0.95 ****	-0.24 ****	0.51 ****	0.14 ns	0.54 ****	0.75 *	0.17 ns	0.0 *	0.24 **	0.73 ***

DAN = White Leghorn, CHING=*Ching'wekwe*, KUCH=*Kuchi*, SING=*Singamagazi*, UNGU=*Unguja*, PEMB=*Pemba*, TANG=*Tanga*, FRIZ=*N'zenzegere*, MBEY=*Mbeya*, MORO=*Morogoro-medium*; Numbers in brackets indicate loci deviating from HWE; * P≤0.05, ** P≤0.01, *** P≤.001, **** P≤0.0001; ns not significant

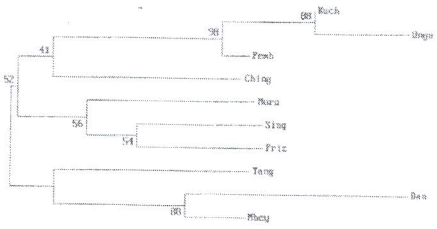

Figure 2: Unrooted neighbour-joining tree displaying the genetic relationship of nine local chicken ecotypes of Tanzania and a White Leghorn breed based on D_s genetic distance (Bootstrap values x100 re-sampling)

Discussion

The present study assessed into the genetic diversity both within and between the local chicken ecotypes of Tanzania using allele frequency information derived from microsatellite DNA analysis. It was observed that four out of the 20 selected microsatellite loci (20%) could not give specific products. Similar observations have been made by van Marle-Koster and Nel (2000), when characterizing the native Southern African chicken populations. In that study, four out of the 27 primer-pairs used (15%) did not produce specific products (Marle-Koster and Nel, 2000). Nevertheless, the results from these two studies are evidently different from a study by Pang and others (1999), who used chicken specific-primers to amplify Japanese quail microsatellite loci. In that study, 37 out 48 (77%) primer-pairs used could not produce specific products.

From the results, all the 16 loci were highly polymorphic with allele numbers ranging from 4 to 15 per locus (mostly >8). Similar studies by Wimmers et al. (2000), van Marle-Koster and Nel (2000), Vanhala et al. (1998) and Takahashi et al. (1998) revealed polymorphisms in the loci used but with fewer numbers of alleles per locus. For instance, in examining the genetic distinctiveness of African, Asian and South American local chickens using 22 microsatellite markers, Wimmers et al. (2000) detected two to 11 alleles per locus. Studies on genetic characterisation of the native Southern African chicken populations using 23 microsatellite markers (van Marle-Koster and Nel, 2000) showed the number of alleles per locus to range between two and 17. Vanhala et al. (1998) evaluating the genetic distance and variability between eight chicken lines using nine microsatellite markers found four to 13 alleles per locus. Using eight microsatellite markers to determine the genetic relatedness of the native Japanese breeds of chickens, Takahashi et al. (1998) detected two to 10 alleles per locus. All these studies are in general agreement with the current study although the methodology used was different. While the current study applied a manual genotyping method, all the other studies applied automated DNA sequencer.

The existence of >8 alleles per locus in the current study may be an indication of the high diversity among the local chickens in Tanzania. In a different study on genetic characterisation of indigenous goat populations from the sub-Saharan Africa a sufficiently high number of alleles per locus (8 to 23) were detected

(Chenyambuga, 2002). These results support the view of high polymorphisms in indigenous animal genetic resources.

The exact test for the HWE showed that 120 out of 160 locus-ecotype combinations deviated significantly from the equilibrium ($P \leq 0.0001$ to 0.05). Departure of this magnitude (75%) could not occur by chance alone. Similar observations were made by Chenyambuga (2002) where over 67% of locus-population combinations deviated significantly from the HWE. However, results from the current investigation differed from those by Vanhala et al. (1998) and that by Koskinen and Bredbacka, (2000) who reported deviations from HWE of 17 and 16% respectively.

Several factors may influence the deviation from the HWE including inbreeding or outbreeding, selection, migration or gene flow from external population, population sub-division and the presence of null alleles (Falconer and Mackay, 1996). It was noted in this study that the estimate for inbreeding coefficient (f) was mostly positive (113 out of 160). Positive (f) values are associated with heterozygosity deficiency and hence evidence of inbreeding. However the mostly likely cause of heterozygosity deficiency in this study was genotyping errors due to poor resolutions of alleles in some gels. Vanhala et al. (1998) had similar observations and associated wrong genotyping to the observed deviations from HWE observed in their study. The other factors such as outbreeding, migration and population sub-division may also have contributed to the observed deviations from the HWE. However it is unlikely that selection played any part because the samples were drawn from outbred populations and all ecotypes showed both positive and negative (f) values.

The mean number of alleles in different populations observed over a number of loci can be taken as a reliable indicator of genetic variability within the populations (MacHugh et al., 1997). There was high genetic variability within each local chicken ecotype as shown by mean number of alleles per ecotype that ranged from 4.31 (White Leghorn) to 7.06 (Pemba ecotype). The mean allele numbers per ecotype in this experiment were higher compared to those reported by van Marle-Koster and Nel, (2000) (2.3 to 4.3) or those reported by Vanhala et al. 1998 (2.4 to 5.7) indicating more diversity within the local chicken ecotypes of Tanzania. The main reason for the difference would be the inclusion of well-established chicken breeds (Vanhala et al., 1998), native chickens derived from exotic chicken crosses or local chickens whose crossbreeding status was not immediately known (van Marle-Koster and Nel, 2000).

According to Nei (1987), the expected heterozygosity (gene diversity) is a more appropriate measure of within population genetic variations. The expected heterozygosity values ranged from 62.1% in the White Leghorn to 79.4% in the Ching'wekwe ecotype. These are high values compared to those observed by Wimmers et al. 2000 (45 to 67%) and Vanhala et al. 1998 (37.8 to 67.1%).

The phylogenetic relationships between the local chicken ecotypes showed that the standard genetic distance measures used (D_S) was able to generally separate the local chicken ecotypes according to their geographical origin and historical background. This is consistent with work by Wimmers et al. (2000) who used a different distance measure (chord distance) to separate local chickens from different continents according to their geographical origins. Similarly a study by Hanslik and others (2000) showed that using Nei's genetic distance corrected for population size (Nei, 1978), Holstein Friesian cattle could be separated according to their geographical origins. The presence of the Kuchi ecotype in the same cluster with the Pemba and Unguja ecotypes was an unexpected finding. Their current geographical location does not explain their phylogenetic relatedness. However, it is historically known that during the slave trade era some inhabitants from the Mwanza and Tabora were taken to the isles en-route to the Middle East and America. It is possible therefore, that some of the freed slaves managed to take home some chickens from the isles when the slave trade was abolished. The argument that chickens were taken from the isles to mainland and not otherwise is based on the idea that slaves were most likely not allowed to carry any of their possessions. Secondly even today in Zanzibar a local chicken called Kuchi exists and it is mostly used for cockfighting, bringing the idea that probably these chickens were brought from Asia by the early traders. Takahashi et al. (1998) were able to use microsatellite markers to infer to the natural history of some Japanese native breeds of chicken. As seen in this study, the Mbeya ecotype and White Leghorn appeared on the same cluster. As indicated in the materials and method section, the Mbeya ecotype was composed of purebred or crosses of Black Australorp breed. Their genetic relatedness is most likely based on the two being selected egg laying breeds.

The authors are aware of some factors that might have negatively influenced the results that are presented in this study. The selection of microsatellite markers could be one of the factors especially the exclusion of FAO recommended markers due to reasons beyond the authors' control. Another factor is the low number of individuals typed per each ecotype. At the inception of the study the aim was to include at least 25 individuals from each ecotype. However, the practicability of this was hampered by financial and logistical constraints. Nonetheless, we believe that the results obtained here gives a clue on the understanding of the local chicken genetic resources in Tanzania and this information is worth sharing.

Accurate determination of the genetic variations within animal species is a fundamental step towards conservation of the animal genetic resources (Oldenbroek, 1999). Conservation of animal genetic resources would safeguard the mankind's future opportunities for such diverse issues as market demands, production environment, cultural, historical as well as research

(Gandini and Oldenbroek, 1999; Oldenbroek, 1999). The current study has established the genetic uniqueness between different local chicken ecotypes. This is a very basic step towards rational decision-making on the modalities of selective breeding (for economical gain) but without compromising the existence of each unique genetic resource. Future studies on local chickens in Tanzania will benefit in segregating the chickens according to these genetic groups in order to reduce the sampling errors inherent of treating local chickens as one genetic group.

Conclusion

It can be concluded from this study that local chickens in Tanzania are diverse and the diversity is associated with their geographical origin as well as their historical backgrounds. It is essential that before animal genetic resources conservation programmes are initiated, four steps (inventory, evaluation, choice and preservation) are considered (Patterson and Silverside, 2003). This study fulfils in part the inventory and evaluation steps. Efforts are therefore required in further documentation and evaluation of the different uniquely adapted chicken ecotypes before they are selected for preservation.

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