African Journal of Biotechnology Vol. 4 (8), pp. 768-771, August 2005
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2005 Academic Journals

Full Length Research Paper

Genetic structure among the local chicken ecotypes of Tanzania based on microsatellite DNA typing

P. L. M. Msoffe[1]*, M. M. A. Mtambo[1], U. M. Minga[2,3], H. R. Juul-Madsen[4] and P. S. Gwakisa[2]

[1]Department of Veterinary Medicine and Public Health, P.O. Box 3021, Morogoro Tanzania.
[2]Department of Veterinary Microbiology and Parasitology, P. O. Box 3019, Morogoro, Tanzania.
[3]Open University of Tanzania, P. O. Box 23409, Dar es salaam, Tanzania.
[4]Danish Institute of Agricultural Sciences, Research Centre Foulum, Tjele, Denmark.

Accepted 21 June, 2005

A study was conducted to evaluate the genetic structure of local chicken ecotypes of Tanzania using 20 polymorphic microsatellite DNA markers. A standard PCR was followed by manual genotyping (6% native polyacrylamide gel visualized by silver staining). Phylogenetic analysis of 13 individuals from each of the nine ecotypes named *Ching'wekwe, Kuchi, Mbeya, Morogoro-medium, N'zenzegere, Pemba, Singamagazi, Unguja* and a White Leghorn breed was performed. The ten populations assorted into nine clusters with chickens from the same ecotype often clustering together. It is concluded that there is high genetic relatedness within indigenous chicken ecotype than between ecotypes. This offers a basic step towards rational decision-making on the modalities of selective breeding without compromising the existence of each unique genetic resource.

Key words: Local-chicken, ecotypes, microsatellites, DNA, PCR.

INTRODUCTION

The diversity of the local chickens reported is mostly on phenotypes including adult body weight, egg weight, reproduction performance and immune responses to various diseases (Gueye, 1998; Msoffe et al., 2001, 2004). Limited reports have addressed the genetic diversity of the local chickens (Horst, 1988; van Marle-Koster and Nel, 2000; Wimmers et al., 2000). DNA based typing methods provide a rapid and reliable method for differentiating individuals in a genetically diverse population (Bidwell, 1994; Parham and Ohta, 1996). Among the DNA methods, microsatellite DNA typing has provided better and reliable results (van Marle-Koster and Nel, 2000; Wimmers et al., 2000). Microsatellites are highly polymorphic and abundant molecular markers that are easily typed using PCR and scored on an electrophoresis gel (Rincon et al., 2000).

Accurate determination of the genetic variations within animal species is a fundamental step towards conservation of the animal genetic resources (Oldenbroek, 1999). Therefore, this study aimed at establishing the genetic structure of the local chickens from the nine ecotypes based on manual microsatellite DNA typing.

MATERIALS AND METHODS

A total of 130 (13 from each population) genomic DNA samples were extracted by salt method (Sambrook et al., 1989) from nine free-range local chicken ecotypes of Tanzania and one reference population White Leghorn (DAN). The description of the chickens was done earlier (Msoffe et al., 2001, 2004). The chicken ecotypes were locally named *Ching'wekwe* (*CHIN*), *Mbeya (MBEY)*, *Morogoro-medium (MORO)*, *Kuchi (KUCH)*, *Singamagazi (SING)*, *N'zenzegere (FRIZ), Pemba (PEMB), Tanga (TANG)*, and *Unguja (UNGU).* A four-letter or three-letter for White Leghorn (shown in brackets) code was assigned for each population for easy data handling.

Twenty microsatellites markers were chosen from the public genome database of the Roslin Institute (10) (http://www.ri.bbsrc.ac.uk/cgi-bin/microsatellite/microsearch.pl) (LEI0217, LEI0234, LEI0214, LEI0248, LEI0237, LEI.0193, LEI0040, LEI0093, LEI0043, and LEI0258), nine from the population tester kit (http://poultry.mph.msu.edu) (ADL0102,

*Corresponding Author. E-Mail: msoffepl@suanet.ac.tz ,makengamsoffe@yahoo.co.uk.

Table 1. Placement of individual local chickens into clusters resulting from individual chicken dendogram for the local chicken ecotypes of Tanzania.

Ecotypes	Clusters									Total
	A	B	C	D	E	F	G	H	I	
Ching'wekwe	11	0	0	0	0	0	0	0	2	13
Kuchi	0	0	10	0	0	0	0	0	3	13
Singamagazi	0	0	0	0	0	0	6	7	0	13
Unguja	0	0	7	5	0	0	0	1	0	13
Pemba	1	0	0	9	0	0	0	1	2	13
Tanga	0	13	0	0	0	0	0	0	0	13
N'zenzegere	0	7	0	0	0	0	6	0	0	13
Mbeya	0	0	0	0	0	13	0	0	0	13
Morogoro-M*	2	0	0	0	0	2	9	0	0	13
White Leghorn	0	0	0	0	13	0	0	0	0	13
Total	14	20	17	14	13	15	21	9	7	130

*Morogoro-medium ecotype.

ADL0136, ADL0158, ADL0171, ADL0172, ADL0176, ADL0181, ADL0210, and ADL0267) and one microsatellite marker (MCW005) from the MCW markers (Crooijmans et al., 1994). A standard PCR was performed on a PTC-100 thermocycler (MJ research Inc, USA) following the published protocol for each marker. Each 25 µl reaction mixture contained 50 ng template DNA, 200 µM of each dNTP, 10X PCR buffer (100 mM Tris-HCL, 50 mM KCL, 0.01% gelatin and 0.25% Tween 20), 1 µM of each primer pair, 5 units/µl Taq polymerase, and $MgCl_2$ (0.625 – 2.5 mM). An initial denaturation step at 94°C - 96°C was followed by 30 to 35 cycles at 94°C/96°C for 1 min, appropriate annealing temperature (46 - 62°C) for 30 s to 2 min, extension at 72°C for 1 to 3 min and finally a 10 min extension at 72°C. Each DNA sample was run through all the 20 primer pairs.

Amplicons were electrophoresed on 6% native polyacrylamide gels (Sigma-Aldrich, St. Louis, USA) using a Mini-Protean II® (Bio-Rad Italy,) equipment for 40 min at 20 V/cm and visualized by silver staining (Rapley and McDonald, 1992). A standard size marker, step ladder (Sigma-Aldrich, St. Louis USA) was included in each run. The size (bp) of the amplicons was determined using TotalLab® version 1.10 (Nonlinear dynamics, Newcastle, UK). The data on amplicon size were organised using microsatellite toolkit embedded in Microsoft Excel® (Park, 2001). The data were analysed using DISPAN, MICROSAT, PHYLIP and TREEVIEW programmes. A distance matrix for all 130 individuals was generated by MICROSAT program, processed by PHYLIP program and a dendogram was built using individual birds as operational taxonomic units.

RESULTS AND DISCUSSION

The genetic structure of the local chicken ecotypes is shown in Figure 1. Nine clusters (A to I) were formed with most chickens from same ecotypes clustering together. Hundred percent (13/13) (Table 1) of chickens from the *Mbeya*, *Tanga* ecotypes and the White Leghorn clustered in the F, B and E clusters respectively. Eleven (84.6%) of the 13 chickens from *Ching'wekwe* ecotype clustered in the A cluster, while 77% (10/13) of chickens from the *Kuchi* ecotype clustered together (cluster C) with the remaining 23% clustering in cluster I. Chickens from the *Morogoro-medium* and *Pemba* ecotypes had each 69% (9/13) of individuals clustering together (cluster G and D, respectively). Individuals from the *N'zenzegere* ecotype were located on clusters B (54%) and G (46%) while those from the *Unguja* ecotype were found in clusters C (54%) and D (38.5%). Cluster I was assigned to seven birds of which two belonged to the *Ching'wekwe* and *Pemba* ecotypes each and three belonged to the *Kuchi* ecotypes.

These findings indicate the existence of genotypes within each ecotype. Nonetheless, being outbred populations, the sharing of genetic materials between ecotypes was equally evident (Figure 1). With the majority of chickens clustering within ecotypes, there is further indication for limited interbreeding between the ecotypes due to their geographical separation or preferential mate selection. Other studies (Chenyambuga et al., 2002), attributed the distinctness between one local breed of goat (Newala) and the rest to the lack of communication between Newala and other parts of Tanzania. This study contributes to the body of existing knowledge on local chickens genetics (van Marle-Koster and Nel, 2000; Wimmers et al., 2000).

The current study has established the possibility for genetic uniqueness between the local chicken ecotypes. However, these preliminary results should be interpreted with caution because among other things, the number of birds, the number of loci as the well as the manual scoring of microsatellites were potential sources of errors. Further studies using more birds per population and more microsallite markers especially those recommended by FAO are necessary to validate the current findings. The current study offers the basic step towards rational decision-making on the modalities of selective breeding

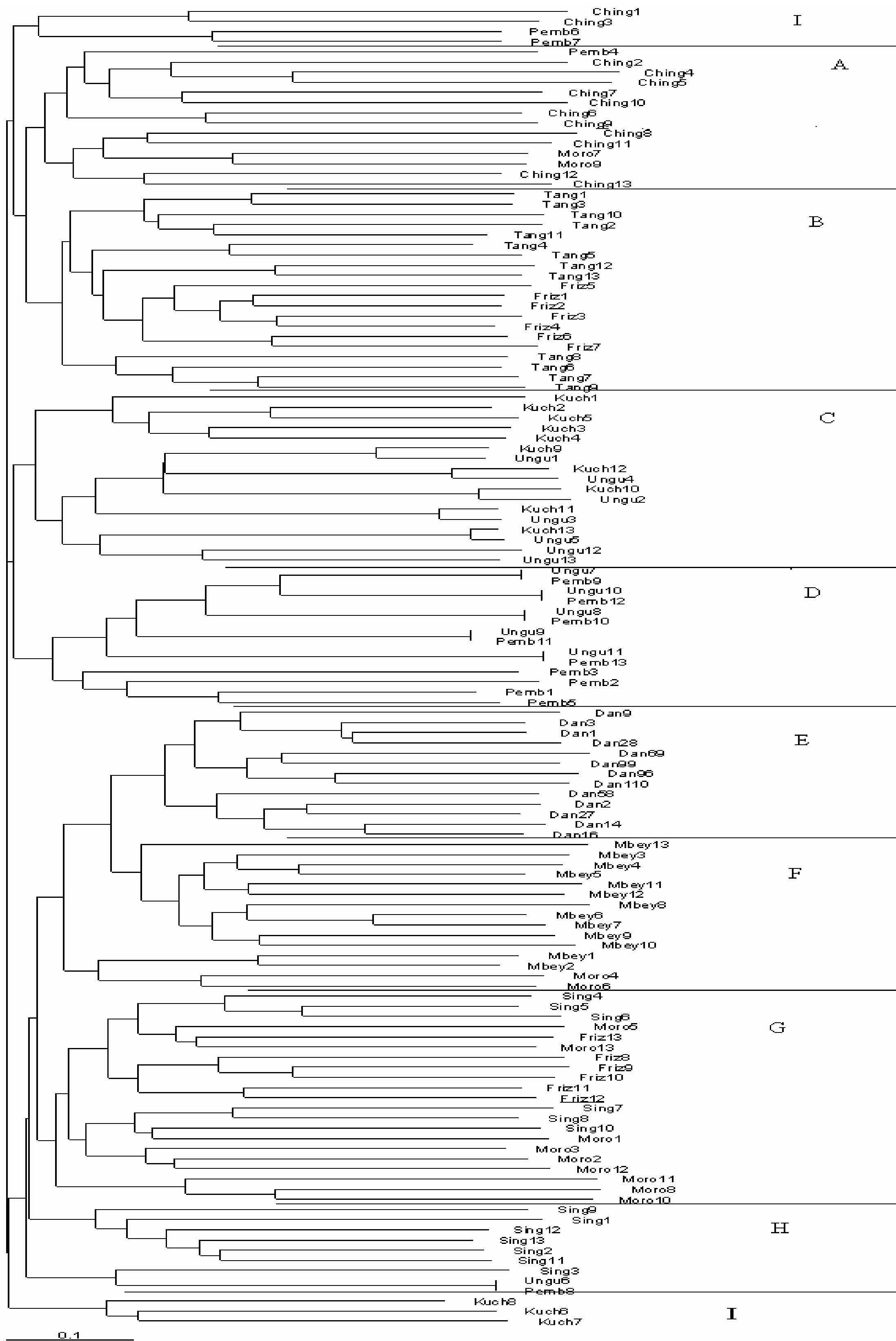

Figure 1. A Neighbour-Joining tree based on allele sharing distances among 130 individual local chicken ecotypes of Tanzania and White Leghorn breed.
Note: A – I represent different clusters.

without compromising the existence of each unique genetic resource.

ACKNOWLEDGEMENTS

This study was supported by DANIDA under the ENRECA project "Improvement of Health and Productivity of Rural Chickens in Africa". The authors are grateful for the support. Nonliner Dynamics are thanked for the TotalLab® analytical software. National Animal Genome Research Program (Dr. Hans Cheng) for the population tester kit.

REFERENCE

Bidwell J (1994). Advances in DNA-based HLA-typing methods. *Immunology Today* 15, 303-307.

Chenyambuga SWPC, Watts J, Hirbo SJ, Kemp O, Hanotte G C, Kifaro PS, Gwaskisa JEO, Rege , P H Petersen (2002). Analysis of genetic diversity and relationships of Tanzanian local goat populations using microsatellite DNA markers. Tanzania J. Agric. Sciences*,* 5 (1): 29 – 38.

Guèye EF (1998). Village egg and fowl meat production in Africa. World's Poultry Science Journa 54, 73-86.

Msoffe PLM, Mtambo MMA, Minga UM, Olsen JE, Juul-Madsen HR, Gwakisa PS, Mutayoba SK , Katule AM (2004) Productivity and reproductive performance of the free-range local domestic fowl ecotypes in Tanzania. Livestock Research for Rural Development. Vol.16, Art.#67.http://www.cipav.org.co/lrrd/lrrd16/9/msof16067.htm.

Msoffe PLM, Minga UM, Olsen JE., Yongolo MGS, Juul-Madsen HR, Gwakisa PS , Mtambo MMA (2001). Phenotypes including immunocompetence in scavenging local chicken ecotypes of Tanzania. Tropical Animal Health and Production 33, 341-354

Oldenbroek JK (1999). Introduction. In: Genebanks and Conservation of Farm Animal Genetic Resource, DLO, Institute for Animal Science, Lelystad, the Netherlands. pp. 1-9.

Parham P, Ohta T (1996). Population biology of antigen presentation by MHC class I molecules. Science 272, 67-74.

Park SDE (2001) Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection [Ph.D. thesis], Uni. of Dublin

Rapley E, McDonald B (1992). Microsallites: The genomic road map. Today's Life Science, 62-65.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning: A loboratory manual 1st edition, Cold Spring Harbour Laboratory Press, vol. 2, pp. 9.16-9.23.

Sneath PHA, Sokal RR (1973). Numerical taxonomy. W. H. Freeman and Co. San Francisco, California, USA. p. 573.

Van Marle-Koster E, Nel LH (2000). Genetic characterisation of native Southern African chickens populations: Evaluation and selection of polymorphic microsatellites markers. South African J. Animal Science, 30, 1-8.

Wimmers K, Ponsuksili S, Hardge T, Valle-Zarate A, Mathur PK, Horst P (2000). Genetic distinctness of African, Asian and South American local chickens. Anim. Genet. 31: 159-165.