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Genetic Diversity of Helmeted Guineafowl (*Numida meleagris*) Based on Microsatellite Analysis

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Characterization of the genetic diversity of indigenous animal populations is a prerequisite for providing needed information for the conservation of useful genotypes against future uncertainties in the face of daunting global challenges such as climate change, emerging diseases, population growth, and rising consumer demands. In this study, a total of 232 helmeted guineafowls (*Numida meleagris*) sampled from three populations in Ghana, one population in Benin and two populations in Japan were genotyped across six autosomal microsatellite loci. Three vulturine guineafowls (*Acryllium vulturinum*) were included as outgroup. A total of 66 alleles were observed with an average of 11.0 alleles per locus. The indigenous West African populations (Ghana and Benin) were more genetically diverse (N_a=9.8; H_o=0.457) but less differentiated (F_{ST}=0.162) compared to the non-indigenous populations in Japan (N_a=4.2; H_o=0.236; F_{ST}=0.389). The information from this study would be useful for selection and improvement programs necessary for the sustainable exploitation of this agriculturally and commercially important species as a suitable alternative to chicken.

Key words: genetic diversity, Ghana, helmeted guineafowl, microsatellite

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Introduction

Chicken (*Gallus gallus*), Japanese quail (*Coturnix japonica*), turkey (*Meleagris gallopavo*), helmeted guineafowl (*Numida meleagris*) and ring-necked pheasant (*Phasianus colchicus*) belonging to the order Galliformes are agriculturally and commercially important poultry species throughout the world. Among these species, the helmeted guineafowl is valued for its fine-flavored meat that resembles the meat of animals hunted for food. Its domestication is believed to have occurred in the southern part of the Sahara, particularly in West Africa (Crawford, 1990), where it is widely distributed in the savannah areas. In spite of its African origin, it is able to thrive in all climates and is reared commercially in Europe, America and Asia. In Ghana, the helmeted guineafowls thrive mostly in the northern savannah zone where wild and feral populations still exist. Their production, mainly by small-scale rural farmers, account for about 25% of the entire poultry population in that zone, and they have a high cultural value in addition to the central role they play in ensuring food security.

Despite the importance of this species, especially in its area of origin, little is known about its genetic diversity. Currently, research work on the genetic variation of poultry species is becoming increasingly important to characterize the genetic structure of local populations. This serves as an important first step to reveal the uniqueness of these populations and to identify valuable genetic resources for conservation against future needs. In the face of daunting global challenges such as climate change, emerging diseases, population growth, and rising consumer demands, it is likely that new genotypes would be required in the future. Characterization and conservation of animal genetic resources are thus necessary to ensure future food security. Such studies are facilitated by the use of molecular tools, particularly microsatellite markers.

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Among Galliformes poultry species, however, many genetic diversity studies have been reported in chicken (Hillel *et al.,* 2003; Granevitze *et al.,* 2007; Muchadeyi *et al.,* 2007; Mwacharo *et al.,* 2007; Tadano *et al.,* 2007; Osei-Amponsah *et al.,* in press), for which many microsatellite markers have been developed. Unfortunately, the helmeted guineafowl genome is the least studied among Galliformes poultry species and to date, no original microsatellite markers have been developed for this species. To date, attempts to apply markers from other Galliformes poultry species such as chicken and the Japanese quail to the helmeted guineafowl have yielded limited success (Kayang *et al.,* 2002; Nahashon *et al.,* 2008). Thus, apart from a study using randomly amplified polymorphic DNA (RAPD) markers, which showed low genetic variation among varieties of guineafowl in India (Sharma *et al.,* 1998), no reports are available on molecular characterization of this species.

This study forms one of the methods to analyse genetic variation in guineafowl. Helmeted guineafowls sampled from three populations in Ghana, one population in Benin, and two populations in Japan were genotyped across six microsatellite loci in order to investigate the genetic diversity and relationships of populations living in and outside their native habitat. Such information would provide a foundation for developing sustainable genetic improvement and conservation programs aimed at enhancing the growth and reproductive traits, meat quality, as well as disease resistance of this agriculturally and commercially valuable species.

Materials and Methods

Sample Collection

A total of 232 helmeted guineafowl samples were obtained from six populations in Ghana, Benin, and Japan. In Ghana, sampling was done in three populations. Two of these, Upper-West Region (Ghana/UWR; $n=43$) and Northern Region (Ghana/NR; $n=94$), were located in the northern Interior Savannah zone but were separated by a distance of about 300 km. They were kept by farmers on a free-range basis. The third population was maintained at the Agricultural Research Center, University of Ghana, Legon (Ghana/ARC Legon; $n=26$), located in the southern Coastal Savannah zone of the country. The samples from Benin ($n=27$) were obtained from a population maintained at Département des productions animales, l'Ecole Polytechnique d'Abomey-Calavi (Benin). The indigenous populations in Ghana and Benin were not purebred varieties but mixed, with the *Pearl* (wild-type) variety being predominant. In Japan, non-indigenous samples were collected from a commercial stock of the *Pearl* variety maintained at JAFRA TRADING CO., LTD., Ibaraki Prefecture (Japan/JAFRA; $n=35$) and a *White* variety population kept at Kobe Kachoen, Kobe City (Japan/Kobe Kachoen; $n=7$). In addition, three vulturine guineafowl (*Acryllium vulturinum*) samples obtained from Kyoto City Zoo, Kyoto, Japan, were included to serve as an outgroup population. The helmeted guineafowl samples thus obtained could be considered as two broad groups: the indigenous West African populations comprising the Ghanaian and Beninois samples ($n=190$), and the non-indigenous Japanese populations ($n=42$).

DNA Extraction and Choice of Microsatellite Markers

Genomic DNA was extracted from blood or feather samples using the QIAGEN DNeasy Tissue kit (QIAGEN, Valencia, CA, USA). In the absence of guineafowl-specific microsatellite markers, we chose five autosomal microsatellites that were originally developed from Japanese quail and found to cross-amplify the orthologous regions in helmeted guineafowl (Kayang *et al.,* 2000; Kayang *et al.,* 2002). In addition, we developed markers based on glutamine repeat polymorphism in the exons of chicken genes. We accomplished this by searching for (CAG)n repeats using the Ensembl Genome Browser (http: //www.ensembl.org/index.html) and then we designed primer-pairs to flank the repeats. In this way, two chicken-originated markers, *KUC0001* (GenBank Accession No. AB526216) and *KUC0002* (GenBank Accession No. AB 526217) were successfully developed that could amplify the orthologous loci in guineafowl (*KUC* denotes *K*yoto *U*niversity *C*hicken). *KUC0001* was developed from chicken Ensemble protein-coding gene *ENSGALG00000014545* located on *GGA1* and orthologous to human *ZFR* (Zinc finger RNA-binding protein), while *KUC0002* was developed from chicken Ensemble protein-coding gene *ENSGALG 00000017281* (*IPI00588817.2*) located on *GGA1* and orthologous to human *KCNA6* (potassium voltage-gated channel subfamily A member 6). The forward and reverse primers were 5′-GTGGATCTGGGGTGGTTTGGGA-GGTTG-3′ and 5′-AGCAGCAGCAACAGAAACAG-GCAGTGG-3′ for *KUC0001* and 5′-CCCACTGCTGG-TGCTGGCCCTT-3′ and 5′-GCTGCTGGCCACCATT-GTTGCTGCT-3′ for *KUC0002,* respectively. However, no polymorphism was detected at the *KUC0002* locus and so this marker was excluded from further analysis. A total of six autosomal microsatellite markers (five from Japanese quail and one from chicken) were, therefore, used in the genetic diversity studies of the guineafowls.

Microsatellite Genotyping

The six markers were used in multiplex PCR reactions with sets of three primer-pairs employing the QIAGEN Multiplex PCR kit (QIAGEN). PCR was carried out in 10 μl reactions containing 20 ng of DNA template, 0.2 μM of each primer and 1x QIAGEN Multiplex PCR Master Mix. After an initial incubation of 95℃ for 15 min, PCR amplification was performed for 30 cycles consisting of 94℃ for 30 sec, 55℃ or 60℃ for 90 sec, 72℃ for 60 sec, followed by a final extension of 60℃ for 30 min. Subsequently, the PCR products were electrophoresed on an ABI 3100 DNA Sequencer (Applied Biosystems Division, Foster City, CA, USA) and the sizes of the fragments were estimated based on fluorescently labelled forward primers (FAM, NED, and HEX) using the GENESCAN and GENOTYPER software (Applied Biosystems).

Table 1. **Observed (N_a) and effective (N_e) number of alleles, observed (H_o) and expected (H_e) heterozygosities, and within-population (F_{IS}), between-population (F_{ST}), and overall population (F_{IT}) inbreeding coefficients across the six guineafowl populations excluding the vulturine guineafowl outgroup (which belongs to a different genus/species)**

Locus	Chromosome	N_a	N_e	H_o	H_e	F_{IS}	F_{ST}	F_{IT}
GUJ0001	*CJA27*	8	1.5	0.200	0.317	0.068	0.397	0.438
GUJ0017	*CJA01*	8	3.0	0.485	0.663	0.114	0.330	0.406
GUJ0059	*CJA05*	12	7.7	0.685	0.873	0.136	0.285	0.382
GUJ0066	*CJA02*	28	7.0	0.625	0.860	0.186	0.175	0.329
GUJ0084	*CJA02*	8	1.6	0.336	0.375	−0.341	0.492	0.319
*KUC0001**	*GGA1*	2	1.2	0.177	0.162	−0.235	0.133	−0.071
Mean**		11.0±8.9	3.7±3.0	0.418±0.215	0.542±0.300	−0.012±0.090	0.301±0.055	0.302±0.077

* *KUC* denotes *K*yoto *U*niversity *C*hicken.
** The means are given±SD for N_a, N_e, H_o, and H_e, and±SE for F_{IS}, F_{ST}, and F_{IT}.

Table 2. **Mean observed (N_a) and effective (N_e) number of alleles, observed (H_o) and expected (H_e) heterozygosities per population and within-population (F_{IS}), between-population (F_{ST}), and overall population (F_{IT}) inbreeding coefficients**

	n	N_a±SD	N_e±SD	H_o±SD	H_e±SD	F_{IS}±SE	F_{ST}±SE	F_{IT}±SE
West African populations	190	9.8±8.6	3.8±3.2	0.457±0.219	0.549±0.287	0.094±0.076	0.162±0.068	0.075±0.012
Ghana/UWR	43	6.5±3.4	3.2±2.3	0.541±0.260	0.569±0.256			
Ghana/NR	94	7.7±7.9	3.7±3.4	0.428±0.243	0.517±0.308			
Ghana/ARC Legon	26	4.3±3.1	2.7±2.1	0.397±0.295	0.441±0.340			
Benin	27	4.7±3.2	2.7±1.6	0.482±0.166	0.532±0.245			
Japanese populations	42	4.2±3.9	2.4±1.9	0.236±0.241	0.400±0.352	0.247±0.086	0.389±0.150	0.188±0.142
Japan/JAFRA	35	3.5±2.7	2.2±1.7	0.232±0.242	0.351±0.365			
Japan/Kobe Kachoen	7	2.3±1.8	2.0±1.4	0.262±0.292	0.335±0.389			
Vulturine guineafowl	3	1.8±0.8	1.6±0.5	0.467±0.447	0.347±0.318	−0.615±0.200	−0.615±0.200	0.000

Data Analysis

The total number of observed alleles, allele frequencies, average number of observed and effective alleles per locus, observed and expected heterozygosities, and inbreeding coefficients (F_{IS}) per population were determined using POPGENE version 1.32 software (Yeh and Boyle, 1997). Wright's fixation indices (F_{IS}, F_{ST}, and F_{IT}) (Weir and Cockerham, 1984) were calculated to quantify the partitioning of variance between and within populations.

To determine the proportion of genetic variability due to population substructuring, pairwise F_{ST} values were computed for all pairs of the six helmeted guineafowl populations using GENEPOP version 3.4 software (Raymond and Rousset, 1995). Pairwise Nei's genetic distances were also calculated to estimate genetic relatedness using POPGENE. To determine phylogenetic relationships, Reynolds' genetic distances (Reynolds *et al.,* 1983) among pairs of the seven populations were computed and a consensus phylogenetic tree of populations with 1,000 bootstraps over loci was generated using POPULATIONS version 1.2.30 software (http: //bioinformatics.org/~tryphon/populations/).

Results and Discussion

A total of 232 helmeted guineafowls and three vulturine guineafowls were genotyped across six autosomal microsatellite loci. Owing to the dearth of markers for the guineafowls and the difficulty of developing original microsatellite markers in the helmeted guineafowl based on the first author's experience, we were limited to using cross-species markers from the Japanese quail and chicken. The quail-specific markers had been previously tested and found to amplify the orthologous regions in the helmeted guineafowl (Kayang *et al.,* 2002).

In all, 66 alleles were observed with an average of 11.0 alleles per locus (Table 1). The mean effective number of alleles per locus was however 3.7. The observed heterozygosity of the markers ranged from 0.177 (*KUC0001*) to 0.685 (*GUJ0059*) with a mean of 0.418, indicating that the markers are sufficiently polymorphic to determine genetic diversity in the helmeted and vulturine guineafowl populations studied. The lower polymorphism of the first chicken-originated marker *KUC0001* could be due to the fact that it was developed from the exon, which is a relatively conserved region. Indeed, the second marker *KUC0002* that was similarly developed was monomorphic when tested in the studied populations and was, therefore, excluded from the analysis. These results affirm the usefulness of this cross-species quail microsatellite panel for genetic diversity studies in the helmeted guineafowl.

Across loci, the mean observed and effective number of alleles were higher in the indigenous West African popula-

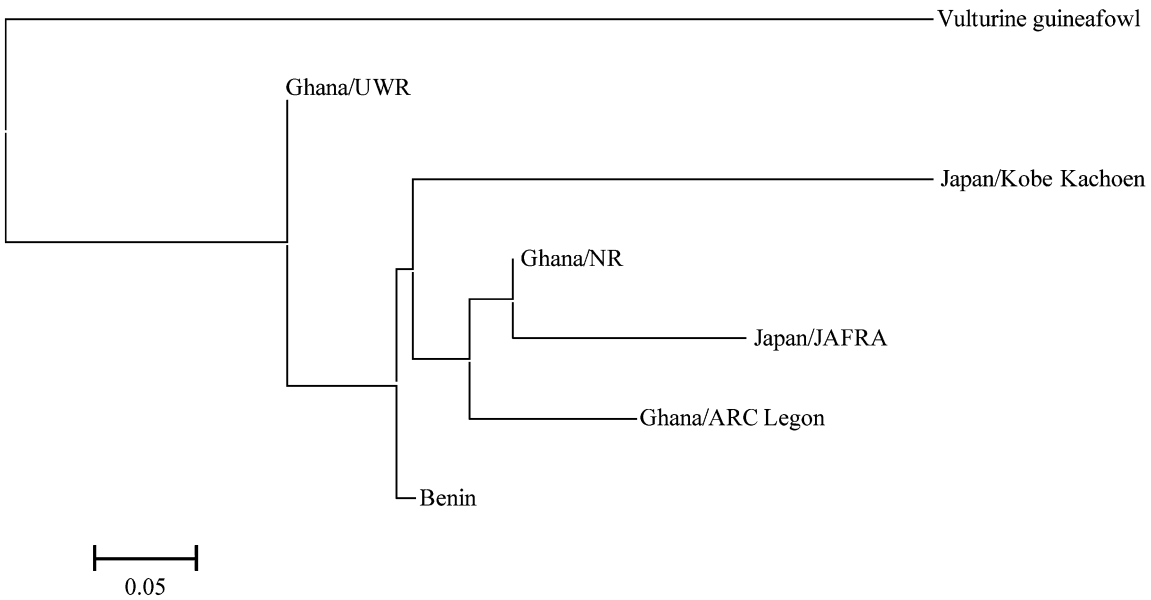

Fig. 1. **Neighbor-joining tree showing phylogenetic relationships of guineafowl populations sampled from Ghana, Benin and Japan.** Ghana/UWR=Upper-West Region, Ghana/NR=Northern Region, Ghana/ARC Legon=Agricultural Research Center, University of Ghana, Legon, Japan/JAFRA=JAFRA TRADING CO., LTD., Ibaraki Prefecture, Japan. The consensus tree was generated with 1,000 bootstraps over loci but the bootstrap values were all below 50 and, therefore, not shown in the diagram. The tree is drawn to scale, with branch lengths indicating the amount of divergence between nodes in the tree. The scale bar represents an amount of evolutionary change corresponding to 0.05 nucleotide substitutions per site.

tions (9.8 and 3.8) than in the non-indigenous Japanese populations (4.2 and 2.4), respectively (Table 2). Similarly, the mean observed and expected heterozygosities were greater in the West African populations (0.457 and 0.549) than in the Japanese populations (0.236 and 0.400), respectively. Low levels of genetic diversity have been reported for guineafowl varieties outside their area of origin. Based on RAPD markers, Sharma *et al.* (1998) observed a low level of genetic variation within and among *Lavender, Pearl* (wild type), and *White* helmeted guineafowl varieties in India and attributed this to a small founder population and many years of multiplication without selective breeding. The helmeted guineafowl stocks in Japan were also constituted from small founder populations and therefore, due to population size, genetic drift, inbreeding and selection, a reduction in genetic diversity occurred.

These observations were supported by Wright's fixation indices, which showed that within-population inbreeding (F_{IS}) was greater in the Japanese populations (0.247) compared to the West African populations (0.094) (Table 2). However, the two Japanese populations were clearly differentiated ($F_{ST}=0.389$) whereas there was little differentiation among the four West African populations ($F_{ST}=0.162$). These findings probably reflect the free-range management system without artificial selection in the West African populations as opposed to directed selection in the Japanese populations. Indeed, based on similar studies in chicken, negligible or absence of population differentiation has been reported for indigenous free-ranging populations in Africa (Muchadeyi *et al.,* 2007; Osei-Amponsah *et al.,* in press).

As shown in Table 3, the pairwise Nei's genetic distances between the six helmeted guineafowl populations ranged from 0.076 (Ghana/NR–Japan/JAFRA) to 0.427 (Ghana/UWR–Japan/Kobe Kachoen). The closest pair was thus the Ghana/NR population in Ghana and the commercial population in Japan. Similarly, the genic differentiation (F_{ST}) values were least in the Ghana/UWR–Benin pair (0.059) but highest in the Japan/JAFRA–Japan/Kobe Kachoen pair (0.335). These results are supported by the clustering in the neighbor-joining tree where Ghana/NR and Japan/JAFRA is the closest pair on one branch (Fig. 1). The foundation stock of Japan/JAFRA was originally introduced from a commercial stock in France and it is most probable that the French stock was derived from a West African population.

Compared to other Galliformes poultry species, the helmeted guineafowl genome has received little attention and no original markers have been developed for population genetic studies in this species. Therefore, this is the first report using cross-species microsatellite markers from the Japanese quail and chicken to estimate the genetic diversity across diverse populations of the helmeted guineafowl. The results clearly show that the indigenous West

Table 3. **Nei's genetic distance (above diagonal) and pairwise F_{ST} (below diagonal) estimates for all loci between seven populations (six *Numida meleagris* and one *Acryllium vulturinum*)**

Population	Ghana/ UWR	Ghana/NR	Ghana/ARC Legon	Benin	Japan/ JAFRA	Japan/Kobe Kachoen	Vulturine guineafowl
Ghana/UWR		0.089	0.169	0.091	0.138	0.427	1.185
Ghana/NR	0.065		0.079	0.094	0.076	0.329	1.169
Ghana/ARC Legon	0.128	0.069		0.129	0.182	0.379	1.169
Benin	0.059	0.069	0.107		0.144	0.332	1.255
Japan/JAFRA	0.138	0.085	0.205	0.151		0.336	1.269
Japan/Kobe Kachoen	0.256	0.231	0.307	0.235	0.335		0.911
Vulturine guineafowl	0.430	0.467	0.542	0.466	0.577	0.593	

African populations are more genetically diverse but less differentiated compared to the non-indigenous populations in Japan. The information from this study would be useful for selection and improvement programs necessary for the sustainable exploitation of this agriculturally and commercially important species (of African origin) as a suitable alternative to chicken (of Asian origin). However, more efforts need to be directed at developing original guineafowl-specific microsatellite markers to expand the scope of the study in the helmeted guineafowl.

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