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Genetic diversity of domesticated and wild Sudanese guinea fowl (*Numida meleagris*) based on microsatellite markers

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Abstract. Genetic diversity was investigated among four Sudanese domesticated guinea fowl populations collected in different regions of Sudan: the states of Blue Nile (BL), Gezira and Khartoum (G), Kassala and Gedaref (KG), and West and North Kordofan (N). In addition, one wild population from Dinder National Park (D) was included. From 25 microsatellites chosen, 10 were informative and used for the current study. A total of 107 alleles were found with observed heterozygosity between 0.364 and 0.494. The populations kept on farms showed high genetic identity with values between 0.9269 and 0.9601. Neighbor-joining tree analysis and STRUCTURE modeling showed that the wild population clearly differs from the populations kept on farms.

1 Introduction

In the sub-Saharan regions of the African continent there are several species of poultry mainly represented by chickens, guinea fowl, ducks and turkeys. In these regions, poultry production plays an important socioeconomic role in the the resource-poor households as a cheap source of protein and cash income. The helmeted guinea fowl (*Numida meleagris*) belongs to the family *Phasianidae* and the subfamily *Numidinae* and is one of six guinea fowl species found only in Africa and Arabia. Within the helmeted guinea fowl, nine different subspecies are known. The subspecies found in Sudan is named “bristle-nosed guinea fowl” (*Numidia meleagris meleagris*) (Moreki, 2009).

In most parts of Africa, guinea fowl are reared mainly under extensive (free-range or traditional) systems at subsistence level with low levels of input resulting in low productivity. Keeping the domesticated birds in free-range systems provides the opportunity of mixing with wild ecotypes (Moreki and Radikara, 2013). Compared to chicken the meat of guinea fowl fetches higher prices, so it could be a potential tool to reduce rural poverty (Kusina et al., 2012). Furthermore, guinea fowl are resistant to most poultry diseases at adult age and require less labor and management than chickens (Sayila, 2009). To improve the economic situation and the income, especially in the rural areas, guinea fowl breeding should be supported to open new poultry markets in Africa (Moreki and Radikara, 2013).

In Sudan wild as well as domesticated guinea fowl are found in the area of poor and rich savanna. The wild type occurs in several conserved national parks, among which Dinder National Park is the most important, conserved since 1935 in an area of 10 000 km^2 (www.unesco.org). Birds living in national parks have no genetic exchange with other populations. Domesticated guinea fowl are mainly kept in backyard free-range systems by small farmers. Farmers do not control the mating of the birds: during the reproduction season between May and September, the birds gather in large flocks in the nearest forest or bushes and then one male and

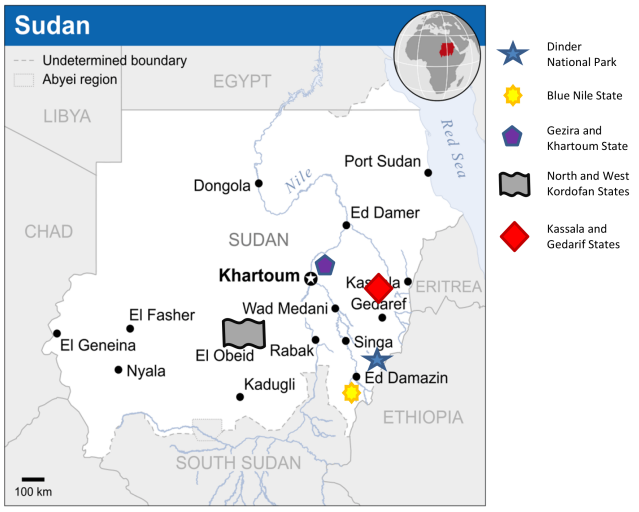

Figure 1. Map of Sudan (based on an OCHA map) edited to show the locations of the different populations of guinea fowl included in the current study.

one female typically pair and remain in close association through the breeding season (Elbin et al., 1986).

Although the identification of genetic resources and the prevention of further loss of genetic variation is an important task, there have been only a few studies worldwide dealing with genetic diversity in guinea fowl. Kayang et al. (2010) investigated the genetic structure of guinea fowl populations from Ghana, Benin and Japan using six microsatellites. The authors stated that the indigenous West African populations were genetically more diverse compared to the non-indigenous populations in Japan. The analyses of Indian guinea fowl populations by Prakash et al. (2013) using RAPD (randomly amplified polymorphic DNA) as well as the molecular characterization of the major histocompatibility complex (MHC) class I region in guinea fowl (Singh et al., 2010) showed low genetic diversity compared to other poultry species.

As early as 1992, a FAO workshop on the development of the guinea fowl as a semi-domestic producer of meat and eggs in the dry regions of West Africa considered that it could be important in the future for the production of meat that guinea fowl are able survive and produce in areas unsuitable for conventional domestic livestock breeding. In this context and for developing a breeding concept for guinea fowl, the first step is to describe the genetic differences between the populations in the country.

Therefore, the aim of the current study was to analyze the genetic structure of four different Sudanese guinea fowl populations and the genetic differentiation among these populations using microsatellite markers. In addition the difference between the wild population and the domesticated populations should be investigated. The results of this study could contribute to distinguishing between different local types of guinea fowl in Sudan.

2 Material and methods

2.1 Study area and sample collection

The animals from which blood samples were collected originated from different regions of Sudan representing different agroecological zones. Five populations of guinea fowl were collected and named according to the region of origin: BL ($n = 40$) from the state of Blue Nile, D ($n = 37$) from Dinder National Park, G ($n = 31$) from the states of Gezira and Khartoum, KG ($n = 39$) from the state of Kassala, and N ($n = 37$) from the states of North and West Kordofan (Fig. 1). Blood samples were collected at slaughter from $n = 184$ guinea fowl regardless of sex using Whatman™ FTA™ blood filter cards (WB120238-GE Healthcare UK Limited) and stored at room temperature until DNA extraction. According to other diversity studies (e.g., Peter et al., 2007; Al-Qamashoui et al., 2014) not more than two animals per farm were taken in order to minimize the percentage of related individuals.

2.2 Microsatellite analysis

After the extraction of genomic DNA using phenol–chloroform according to Sambrook et al. (1989), the DNA quality and quantity were checked by means of a ND-1000 NanoDrop spectrophotometer (NanoDrop Technologies Inc., USA) and the DNA was stored until use at −20 °C. The following microsatellites were chosen: 10 microsatellites (ADL278, MCW222, ADL112, MCW295, MCW14, MCW183,GUJ123, MCW330, MCW69 and MCW248) recommended by the FAO for diversity studies in chickens; 12 microsatellites (GUJ01, GUJ13, GUJ17, GUJ21, GUJ59, GUJ66, GUJ84, GUJ86, GUJ29, GUJ61, GUJ91 and GUJ94) developed by Kayang et al. (2002) for helmeted guinea fowl, Japanese quails and chickens; and 3 microsatellites (NMG10, NMG13 and NMG17) developed by Botchway at al. (2013) for guinea fowl. The reverse primer of each microsatellite was labeled with a fluorescent dye at the 5′ end. PCR was performed in a final volume of 15 µL containing 50 ng of template DNA, 10 pmol of each primer, 1× PCR buffer (Promega, Mannheim, Germany), 1–2.5 mM $MgCl_2$ (Promega; for the specific concentration see Table S1 in the Supplement), 0.2 mM dNTPs (Life Technologies, GmbH, Darmstadt, Germany) and 0.5 U Taq Polymerase (Promega). PCR amplification was carried out in the following steps: initial denaturation (95 °C for 240 s) followed by 35 cycles with 95 °C for 15 s, x °C for 30 s (where x is the annealing temperature for each primer used; see Table S1 in the Supplement) and 72 °C for 45 s, and a final extension at 72 °C for 300 s. Microsatellite analysis was performed on an ABI 3130 automated sequencer (Applied Biosystems, Darmstadt, Ger-

Table 1. Mean number of alleles (MNA), observed (H_O) and expected (H_E) heterozygosity, and F_{IS} values based on 11 microsatellites in four domesticated (BL, G, KG, N) and the wild population (D).

Population	No. of animals	MNA	H_O	H_E	F_{IS}
BL	40	5.6	0.488	0.557	0.124
D	37	8.0	0.494	0.606	0.137
G	31	4.5	0.364	0.500	0.277
KG	39	4.6	0.386	0.538	0.286
N	37	5.4	0.388	0.501	0.230

many) using GeneMapper version 4.0 (Applied Biosystems) for genotyping.

2.3 Statistical analysis

Allele frequencies of all loci; observed, expected and average heterozygosity; genetic identity; genetic distances; and the Hardy–Weinberg equilibrium were calculated using Popgene version 1.31 (Yeh et al., 1997). The program ML-NullFreq (Kalinowsky and Taper, 2006) was used to test for the occurrence of null alleles. This program includes, in contrast to other software packages, not only the heterozygote deficiency but also missing values in the estimation procedure. For description of the genetic differentiation, Nei's genetic distance (Nei, 1972) was estimated to define the genetic difference between the populations. All F statistics were computed using FSTAT (Goudet, 1995). Furthermore, a neighbor-joining consensus tree was constructed using SplitsTree version 4.13.1 (Huson et al., 2006). The STRUCTURE software package (version 2.3; Pritchard et al., 2000) was used to determine the most likely number of partitions in the data set. The most probable number of K is characterized by the maximum value of the natural logarithm of the probability (Pr) of the observed genotypic array (G), given a preassigned number of clusters (K) in the data set ($\ln \Pr(G|K)$). Ten independent runs for $K = 2, 3, 4$ and 5 were carried out with a burn-in length of 20 000 followed by 100 000 iterations.

3 Results and discussion

In total 25 microsatellites from three different sources were selected for the analysis, but only 11 of them were polymorphic in our study. In detail, the following results were observed: we chose 10 microsatellites recommended by the FAO for diversity studies in chickens in order to have the chance to compare the results with chicken diversity studies, but only the microsatellites MCW69 and MCW222 from this panel were polymorphic in the guinea fowl samples used. From the 12 microsatellites chosen from the panel of Kayang et al. (2002), 7 microsatellites (GUJ1, GUJ13, GUJ17, GUJ59, GUJ66, GUJ84, GUJ86) were polymorphic in the populations used. From the microsatellites (NMG10, NMG13 and NMG17) developed especially for guinea fowl by Botchway et al. (2013), the markers NMG13 and NMG17 were polymorphic in the samples of the current study. In total, 14 microsatellites were not informative for the diversity analysis because they were monomorphic. This confirms a previous study of Nahashon et al. (2008), where 50 % of chicken microsatellites and 47 % of quail microsatellites were polymorphic in guinea fowl.

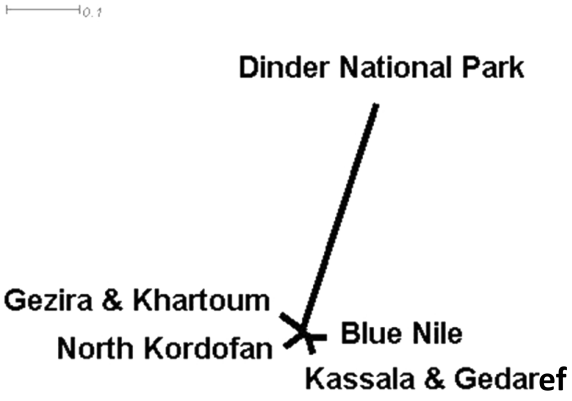

Figure 2. Unrooted neighbor-joining consensus tree depicting the relationship of four domesticated Sudanese guinea fowl populations and a wild population at Dinder National Park based on 10 microsatellite markers using Nei's (1972) genetic distances.

Table 2. Genetic distance (below the diagonal divide) and genetic identity (above the diagonal divide) according to Nei (1972) between the four domesticated populations (BL, G, KG, N) and the wild population (D).

Population	BL	D	G	KG	N
BL	–	0.7543	0.9269	0.9601	0.9400
D	0.2820	–	0.6643	0.7327	0.7610
G	0.0759	0.4091	–	0.9548	0.9311
KG	0.0408	0.3111	0.0462	–	0.9534
N	0.0619	0.2732	0.0714	0.0477	–

At 11 microsatellite loci, 107 alleles were found in total; the number of alleles per locus varied from 3 alleles at locus NMG17 to 36 alleles at locus GUJ66 (Table S1). Across loci the highest mean observed (0.494) and expected (0.606) heterozygosity was detected in population D, the wild population from Dinder National Park (Table 1).

Within the microsatellite marker NMG 17, deviation from Hardy–Weinberg equilibrium was observed across all populations and the analysis with ML-NullFreq indicates the occurrence of null alleles in this marker. This microsatellite was developed especially for guinea fowl together, with 30 others, by Botchway et al. (2013). The authors mentioned the occurrence of null alleles in 15 of these markers but not for

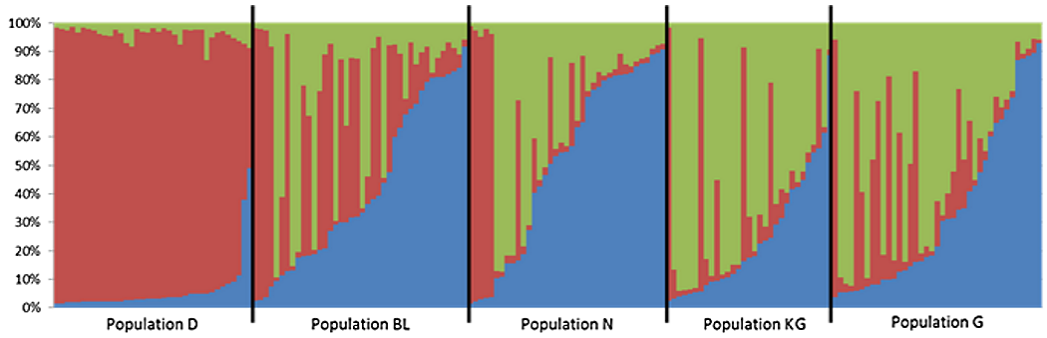

Figure 3. Clustering diagram based on STRUCTURE analysis of the five guinea fowl populations for $K = 3$. Each individual is represented by a vertical line, which is partitioned into $K = 3$ colored segments that represent the individual's estimated membership fractions in K clusters using the **Q** matrix of the run with the best similarity. Black lines separate different populations (D: Dinder National Park; BL: Blue Nile; N: North and West Kordofan; KG: Kassala and Gedaref; G: Gezira and Khartoum).

NMG17. In contrast to Botchway et al., we found strong evidence for null alleles within the marker NMG17 and therefore this microsatellite was excluded from further analysis. All populations showed positive F_{IS} values, whereas the highest value was observed in the population KG with 0.286 and the lowest (0.124) in population BL (Table 1). Generally, positive F_{IS} values indicate a heterozygote deficiency which suggests inbreeding within the population (Wright, 1951). In most African countries, guinea fowl are kept mainly in extensive systems: the farmers house their guinea fowl during the night and allow them to scavenge the whole day (i.e., Kusina et al., 2012). Animals are raised on farms and kept in villages, which may lead to reduction of chances of natural mating between unrelated flocks from other regions and which will also increase the opportunity for inbreeding. This may be an explanation for the relatively high F_{IS} values in the current study.

The lowest genetic distance was observed between populations BL and KG (0.0408) and the highest between populations D and G (0.4091). The genetic distances and the genetic identity according to Nei (1972) are summarized in Table 2. In total, the four domesticated populations showed high genetic similarity (genetic distances between 0.0408 and 0.0759), which confirms the results of Kayang et al. (2010,) who found genetic distances between West African guinea fowl populations of between 0.079 and 0.169. Also, studies using RAPD to describe the genetic diversity in guinea fowl have found high genetic similarity between three guinea fowl populations in India (Sharma et al., 1998) as well as between white and grey guinea fowl in Poland (Bawej et al., 2012). In summary, these results show that there is clearly only little genetic variation between guinea fowl populations.

Based on the genetic distances, an unrooted neighbor-joining tree was constructed (Fig. 2). Regarding the tree, it is clear that the population from Dinder National Park differs from the four populations kept on farms in the different regions. The domesticated populations are genetically similar even though they come from geographical different locations. This similarity is also shown by the high genetic identity of the four populations BL, G, KG and N with values between 0.9601 and 0.9269 (Table 2).

STRUCTURE was used to demonstrate the presence of distinct genetic populations.

Over the five replicates for each K the highest mean values for $\ln \Pr(G|K)$ were obtained for $K = 3$. By assuming $K = 3$, three groups of populations were defined (Fig. 3). Group 1 was mainly found in the Dinder National Park population and is representative of the wild type of guinea fowl. Aside from the first cluster, which is associated with the wild population, the other populations showed a mixture of cluster 2 and 3, whereas the second cluster had a greater part within populations BL and N and the third cluster in populations KG and G.

Similar to the study of Tadano et al. (2014) comparing microsatellite variation between red jungle fowl and commercial chicken lines, the wild population in our study differs from the domesticated populations. Knowing that, in the analysis of Berthouly et al. (2009), wild chickens and phenotypically similar domestic chickens were in the same cluster of the STRUCTURE analyses, it is remarkable that, in our analyses, the wild population differs clearly from the domesticated populations although they are phenotypically similar.

Muchadeyi et al. (2007) and Mtileni et al. (2011) proposed that large effective population sizes as well as continuous gene flow may be forces responsible for the lack of population differentiations among the local chicken genotypes in their studies. This may also be a reason in the current study why a clear differentiation between the domesticated populations was not possible. Apart from the wild population, we could not find a substructure associated with the geographic location of the fowl. Our finding is similar to the results of Muchadeyi et al. (2007), who could not observe such a substructure in Zimbabwean chicken populations, just like the results of Al-Qamashoui et al. (2014), who demon-

strated an absence of substructures in Omani chickens. Also, in Sudan, like in other sub-Saharan regions, the connectivity of rural and nomadic communities during the seasons could contribute to gene flow between the populations.

The current study shows the possibility to distinguish between farm-kept and wild guinea fowl populations via microsatellite analysis, but it is not possible to find great differences between local breeds or ecotypes. Because of the genetic and phenotypic similarity of the domesticated guinea fowl populations, it will not be necessary to consider different ecotypes in future breeding programs.

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