Genetic diversity of grasscutter (*Thryonomys swinderianus, Rodentia, Hystricomorpha*) in Ghana based on microsatellite markers

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Abstract

Grasscutter (*Thryonomys swinderianus*) is a fairly large rodent that inhabits sub-Saharan Africa. There are very limited ecological studies on the grasscutter despite its importance as a protein resource. The objective of this study was to apply novel microsatellite markers to determine the genetic structure and diversity of grasscutter populations in Ghana. A total of 66 hair samples were collected from grasscutters in three main agro-ecological zones of Ghana, namely Guinea Savanna ($n = 19$), Forest ($n = 16$) and Coastal Savanna ($n = 16$) as well as Volta Region ($n = 15$). Samples were genotyped at 12 polymorphic loci and the results showed relatively high diversity ($MN_A = 7.3$, $H_E = 0.745$) within populations. Phylogenetic analysis revealed that Forest population is closer to the Coastal Savanna population than the other populations whilst Volta Region population is closer to the Guinea Savanna population than the other populations. Pairwise F_{ST} values however indicated that all populations were significantly differentiated ($p < 0.01$). STRUCTURE clustering analysis showed that Volta population split from the Guinea Savanna population. Grasscutter populations in Ghana are genetically differentiated according to agro-ecological zones and the Volta Lake could be serving as a barrier to gene flow.

Introduction

Grasscutter (*Thryonomys swinderianus*) which is also known as the greater cane rat is a fairly large rodent that inhabits sub-Saharan Africa, mainly Western, Central, Southern and some parts of East Africa (Annor *et al*., 2009). The species is hunted aggressively in West Africa because it is a delicacy (Annor *et al*., 2009). There have been efforts over the years to domesticate this species to make it more easily available for the people in West Africa and subsequently in other parts of the continent (Asibey, 1981; Jori *et al*., 1995; Addo *et al*., 2002; Mensah & Okeyo, 2006; Annor *et al*., 2008). Many techniques including the use of fire are employed to hunt the species (Adu *et al*., 1999). The use of fire most often gets out of hand leading to bushfires that destroy the habitats of many

wildlife species, thereby raising serious biodiversity and environmental issues.

Even though there are concerns about the techniques used for hunting the grasscutter in the wild, grasscutter game meat trade continues to flourish, making significant contributions to the economies of African countries where this meat is consumed (Ntiamoa-Baidu, 1997). It is therefore imperative that the grasscutter be given the attention it deserves so that it can contribute even more to those economies without jeopardizing wildlife diversity and the environment in general. To date, very little is known about the ecology, demographics and population dynamics including dispersal and breeding structure of grasscutters in the wild. Determining the genetic structure and variability of grasscutter populations may therefore shed light on some of these mechanisms.

Since their discovery, microsatellite markers have been employed by ecologists to investigate the ecology of many taxa in order to answer questions relating to effective population size, kinship, migration rates, demographic bottleneck consequences, effect of landscape and threat status (Selkoe & Toonen, 2006). Microsatellites are the most widely used markers for molecular ecological studies to date. They are selectively neutral and follow Mendelian inheritance pattern. Furthermore, they can be used to analyse ancient degraded DNA or DNA from non-invasive samples such as hair and faeces thereby making them invaluable tools for ecologists (Taberlet *et al*., 1999).

Owing to the development of novel microsatellite markers (Adenyo *et al*., 2012), we aim to characterise the study populations and understand their dynamics in order to provide useful information for their management and for the purpose of future conservation if the need arises. There are many rivers in Ghana, including the Volta River which has formed a lake (Volta Lake) due to a hydroelectric power dam constructed in the 1960s at Akosombo. We envisaged that this lake might have a profound effect on the fauna populations (including the grasscutter) in terms of their dispersal.

Since mitochondrial and nuclear markers have different modes of inheritance and rates of evolution, they sometimes present conflicting results depending on the characteristics of the populations being studied (Flanders *et al*., 2009). Mechanisms such as sex-biased dispersal, incomplete lineage sorting, homoplasy and effective population size may have different influences on inferences made on populations, depending on whether mitochondrial or nuclear markers are used (Clutton-Brock, 1989; Colbert *et al*., 2001; Hewitt, 2004). In a recent study using mitochondrial DNA marker (D-loop), we found that in spite of geographic distance, Guinea Savanna and Coastal Savanna populations of grasscutters are closer than the Forest population (Adenyo *et al*., 2013). The objective of the current study was to determine the genetic diversity of grasscutters in Ghana using microsatellite markers. Specific questions addressed by this study were whether or not grasscutter populations inhabiting different agro-ecological zones in Ghana were significantly differentiated and whether or not the Volta Lake had an effect on the population structure of grasscutters.

Materials and methods

Location

In Ghana, there are three main agro-ecological zones that differ in climate and vegetation. These are the Guinea Savanna zone which has grasses, shrubs and sparse trees with unimodal rainfall, the Forest zone which has bimodal rainfall and the Coastal Savanna which has similar vegetation as the Guinea Savanna but has a bimodal rainfall (*http://www.fao.org/docrep/008/a0013e/a0013e05.htm*). In this study, we used novel microsatellite markers of the grasscutter to assess the genetic diversity and structure of their populations in the different agro-ecological zones.

Sampling and DNA extraction

A total of 66 hair samples were collected from grasscutters hunted from the wild by hunters and farmers in three agro-ecological zones of Ghana. In the Guinea Savanna zone, samples were taken from around the Mole National Park ($n = 10$) and Tamale ($n = 9$). Sixteen samples were collected from a bushmeat market in Kumasi which is located in the Forest zone. In the Coastal Savanna zone, samples were taken from Mankessim ($n = 5$), Jukwa ($n = 7$) and Accra ($n = 4$). Additionally, eight samples were taken from Nkwanta and seven from Afajato area, both located in the Volta Region which is on the eastern side of the Volta Lake. This was done in order to test the effect of the Volta Lake on the grasscutter populations in the Guinea Savanna zone. The sampling locations are shown in Fig. 1. DNA was extracted from 1–2 mm hair root clippings of 15–20 hair pieces per animal using Instagene Matrix (Bio-Rad Laboratories, USA). The quantity and quality of DNA was measured using NanoDrop spectrophotometer (Thermo Scientific, USA). The DNA was then stored at -30 ^{o}C until ready for use.

Microsatellite genotyping

Twelve highly polymorphic microsatellite markers including three tetra-repeats and nine di-repeats (*Tsw02, Tsw03, Tsw06, Tsw07, Tsw08, Tsw09, Tsw11, Tsw13, Tsw16, Tsw19*, *Tsw21* and *Tsw23*) were selected from a previously published panel (Adenyo *et al.*, 2012) for genotyping. Multiplex PCR was conducted with 3–5 primers per reaction using the QIAGEN Multiplex PCR Kit (QIAGEN, Germany) under the following conditions: initial denaturation at 95 ^{o}C for 15 minutes, 35 cycles at 94 ^{o}C for 30 seconds, 55 ^{o}C or 60 ^{o}C for 90 seconds and a final extension at 60 ^{o}C for 30 minutes. PCR mixture contained 20 ng of DNA template, 0.2 µM of each primer of which the forward primers were fluorescently labelled (6FAM, HEX and NED), 0.1 µg of T4 Gene 32 Protein (Nippon Gene, Japan) and 2x QIAGEN Multiplex PCR Master Mix in a total volume of 10 µL. PCR was performed on GeneAmp PCR System 9700 (Applied Biosystems, Singapore). Each reaction was repeated two to three times to confirm the genotype of each individual. The PCR products were thereafter diluted (1:100) and electrophoresed on an ABI 3130 xl DNA

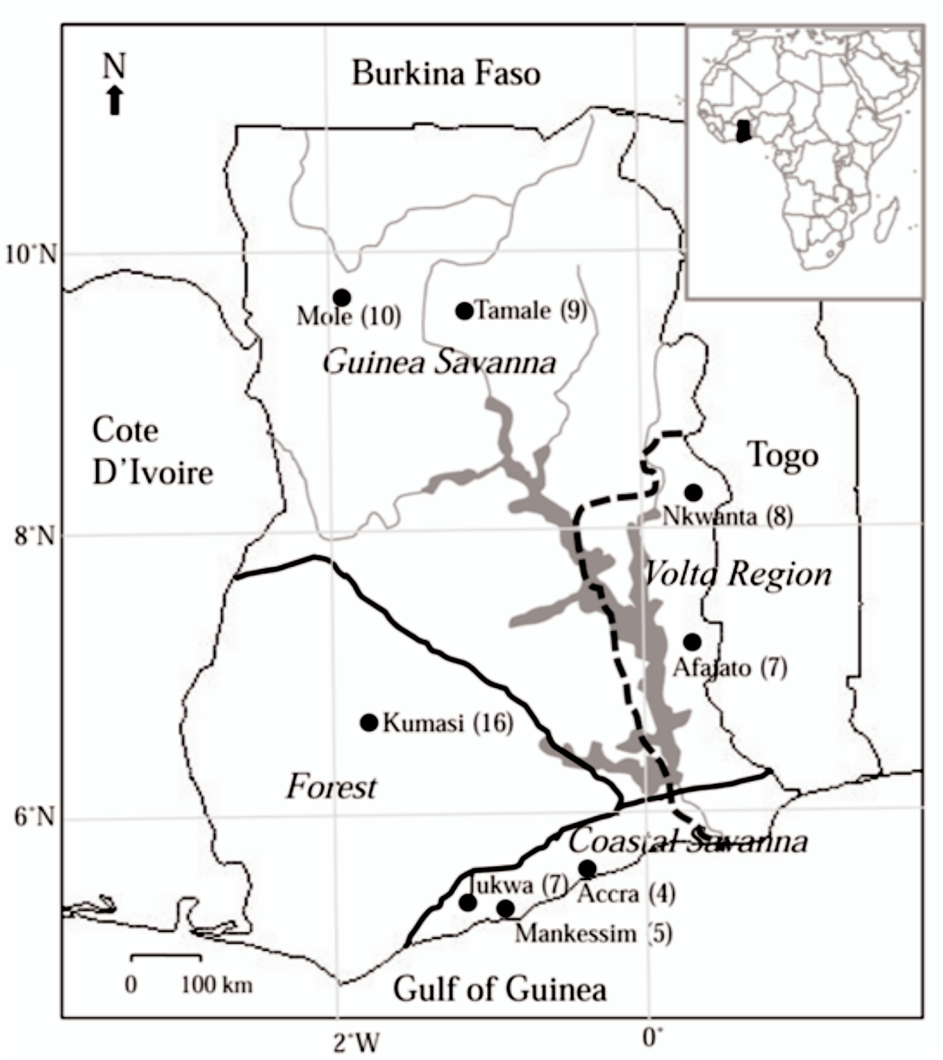

Fig. 1. The map of Ghana showing different agro-ecological zones and sampling locations. The numbers in parenthesis indicate the number of samples collected at each location. Nkwanta and Afajato are located in the Volta Region which is shown with the dash line.

Sequencer (Applied Biosystems, USA) and the fragment sizes were scored with 400 HD Rox size standard using Peak Scanner Software ver 1.0 (Applied Biosystems, USA).

Data analysis

The data were checked for the presence of null alleles and stuttering at all loci using the software program MICROCHECKER (Van Oosterhout *et al*., 2004). Number of alleles (N_A), allele frequencies, observed

(H_O) and expected heterozygosities (H_E) and fixation indices F_{IS}, F_{IT} and F_{ST} as well as deviations from Hardy-Weinberg equilibrium (HWE) were determined using GenAlEx ver 6.41 (Peakall & Smouse, 2006). These population parameters were calculated for each population for all loci and also across populations for each locus. Polymorphism information content (PIC; Botstein *et al*., 1980) was determined for all loci using Microsatellite Toolkit ver 3.1.1 (Park, 2001). Allelic richness, which is an estimation of the mean number of alleles per locus corrected by sample size, was estimated with FSTAT ver 2.9.3 software (Goudet, 2002).

Phylogenetic analysis was conducted with POPULATIONS ver 1.2.30 (Langella, 1999) using Nei's minimum genetic distance, D_m, with 1000 bootstraps over loci after which the results were viewed with TreeView (Page, 1996). To infer population structure, the STRUCTURE software ver 2.3 (Pritchard *et al*., 2000; Falush *et al*., 2003) was employed using the admixed ancestry and correlated allele frequency model. The program was run after burn-in period of 10,000 and Markov Chain Monte Carlo (MCMC) replication of 100,000. The number of putative populations, K, was set to range from 2 to 8 and number of iterations for each value of K was 50. No *a priori* population information was used in running the programme. Each individual was assigned to a cluster based on the highest likelihood of membership. Results from STRUCTURE were zipped and used as input file for the web-based programme STRUCTURE HARVESTER (Earl & vonHoldt, 2012) to calculate posterior probability values, ln Pr(X/K). The best value of K or the most likely number of clusters was inferred from "K which was calculated by the method of Evanno *et al*. (2005). Results were then combined using the program CLUMPP ver 1.1.2 (Jakobsson & Rosenberg, 2007) which determines symmetric similarity coefficient between pairs of runs and averages individual membership proportions. Summary barplots were then generated using DISTRUCT ver 1.1 (Rosenberg, 2004).

To estimate the extent of exchange of individuals between populations, current migration was assessed using GENECLASS ver 2.0 (Piry *et al*., 2004). This program is able to detect migrants through a Bayesian method using multilocus genotype data (Rannala & Mountain, 1997). As a test statistic, it estimates likelihood L = L_home/L_max, where L_home is the likelihood that the individual's genotype belongs to the population from which the individual was sampled and L_max is the highest likelihood of the genotype in any population (Paetkau *et al*., 2004). The program was run with Monte-Carlo resampling algorithm and number of simulated individuals was pegged at 10,000 ($a = 0.01$).

The rate and direction of migration among the populations were estimated using a Bayesian approach implemented in the program BAYESASS ver 3.0.3 (Wilson & Rannala, 2003). It was run with MCMC algorithm of 10,000,000 iterations sampled every 100 steps after 1,000,000 iterations were discarded as burn-in to

ensure that the chain simulation reaches stationary distribution before sampling. The program was run three times with different seeds for each run to ensure concordance. The default seed of 10 was used initially followed by 100 and lastly 1000.

We tested for isolation by distance by determining the correlation between pairwise differentiation and geographic distance. Geographic distances between the locations (in km) were obtained by converting decimal degrees coordinates into distance matrix using Geographic Distance Matrix Generator ver 1.2.3 (*http://biodiversityinformatics.amnh.org/open-source/gdmg*). Pairwise geographic and genetic distances were log transformed and a Mantel test was conducted using IBDWS ver 3.14 (Jensen *et al*., 2005) to assess the correlation between geographic distance and pairwise genetic differentiation.

Results

Microsatellite characteristics and genetic diversity

There were a total of 133 alleles ranging from 8–16 with a mean of 7.3 per locus across populations. Eleven private alleles were found in Guinea Savanna, two in Forest, 10 in Coastal Savanna and nine in the Volta Region. H_O and H_E ranged from 0.460 for *Tsw09* to 0.847 for *Tsw16* and 0.452 for *Tws09* to 0.866 for *Tsw08*, respectively (Table 1). These results indicate that all the markers used were polymorphic. Apparently, *Tsw09* was the least polymorphic among the panel of markers used in this study. Allelic richness ranged from 5.499 for *Tsw02* to 11.435 for *Tsw08*. The range of PIC followed the same pattern as allelic richness (Table 1). Three loci deviated from HWE in the Volta Region population (*Tsw06*, *Tsw13* and *Tsw19*), two loci deviated from HWE in each of Guinea Savanna (*Tsw09 and Tsw11*) and Forest (*Tsw11 and Tsw13*) populations whereas in the Coastal Savanna, only locus *Tsw08* deviated from HWE. H_O and H_E were 0.780 and 0.789 for Guinea Savanna, 0.735 and 0.760 for Forest, 0.729 and 0.740 for Coastal Savanna, 0.679 and 0.690 for Volta Region respectively. Wright's fixation indices, F_{IS}, F_{IT} and F_{ST} for each locus over populations are listed in Table 1. F_{IS} which indicates heterozygote deficiency and represents an indicator of the level of inbreeding, was found to be low (0.009–0.031) for all populations, indicating that the populations are not inbred (Table 2).

Population structure and isolation by distance

Phylogenetic analysis revealed that the Forest population is closer to the Coastal Savanna population than the other populations whilst Volta Region population is closer to the Guinea Savanna population than the other populations (Fig. 2). These results are supported by fairly high bootstrap value (90%). Pairwise F_{ST} values however indicated that all populations were significantly differentiated ($p < 0.01$) after Bonferroni correction for multiple testing (Rice, 1989) (Table 3).

STRUCTURE clustering analysis showed a more detailed structure of the

TABLE 1
Profile of 12 microsatellite loci

Locus	N_A	H_O	H_E	F_{IS}	F_{IT}	F_{ST}	*Allelic richness*	*PIC*
Tsw02	9	0.571	0.557	-0.025	0.004	0.028	5.499	0.549
Tsw03	12	0.786	0.801	0.019	0.078	0.060	7.883	0.835
Tsw06	10	0.718	0.776	0.075	0.117	0.046	7.106	0.791
Tsw07	8	0.710	0.729	0.026	0.124	0.100	6.167	0.786
Tsw08	16	0.802	0.866	0.074	0.117	0.047	11.435	0.902
Tsw09	8	0.460	0.452	-0.017	0.320	0.332	5.517	0.633
Tsw11	14	0.691	0.826	0.163	0.229	0.079	9.968	0.889
Tsw13	8	0.747	0.709	-0.055	0.020	0.071	6.290	0.733
Tsw16	12	0.847	0.819	-0.034	0.005	0.038	8.213	0.834
Tsw19	11	0.805	0.782	-0.029	0.028	0.056	7.729	0.811
Tsw21	13	0.828	0.827	-0.001	0.042	0.043	8.574	0.852
Tsw23	12	0.803	0.794	-0.012	0.039	0.050	8.046	0.817
Mean	7.3	0.731	0.745	0.015	0.094	0.079		
SE	0.3	0.023	0.020	0.018	0.028	0.024		

N_A: number of alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, F_{IS}: fixation coefficient of an individual within a subpopulation, F_{IT}: fixation coefficient of an individual within the total population, F_{ST}: fixation coefficient of a subpopulation within the total population, *PIC*: polymorphism information content.

TABLE 2
Characteristics of grasscutter populations

Population	*n*	$MN_A \pm SE$	$N_E \pm SE$	*Private*	$H_O \pm SE$	$H_E \pm SE$	$F_{IS} \pm SE$
Guinea Savanna	19	8.333 ± 0.620	5.250 ± 0.444	11	0.780 ± 0.042	0.789 ± 0.024	0.009 ± 0.047
Forest	16	7.167 ± 0.613	4.735 ± 0.438	2	0.735 ± 0.033	0.760 ± 0.030	0.031 ± 0.031
Coastal Savanna	16	7.417 ± 0.596	4.796 ± 0.542	10	0.729 ± 0.054	0.740 ± 0.047	0.009 ± 0.043
Volta Region	15	6.500 ± 0.571	3.914 ± 0.451	9	0.679 ± 0.053	0.690 ± 0.050	0.007 ± 0.043

MN_A: mean number of alleles, N_E: effective number of alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, F_{IS}: fixation coefficient of an individual within a subpopulation, SE: standard error.

populations (Fig. 3). At K = 2, the pattern of clustering was similar to the clustering observed in the Neighbour-joining tree. However, at K = 3, the Volta Region population split from the Guinea Savanna population whilst the Forest and the Coastal Savanna populations remained together. Interestingly, no clear structure was observed at K = 4. Moreover, using the method developed by Evanno *et al.* (2005), the highest "K was found at K = 3 suggesting that there were three distinct clusters.

No significant correlation was found between genetic differentiation and geographic distance (Mantel test, $r = 0.29$, $p > 0.05$) even though some tendency can be seen (Fig. 4).

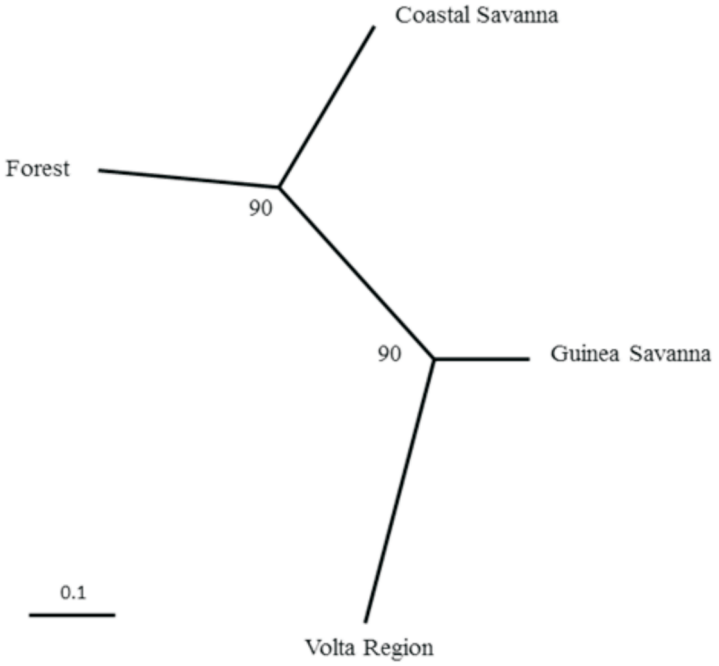

Fig 2 Neighbour-joining tree of grasscutter populations in Ghana based on Nei's minimum genetic distance, *Dm*. Bootstrap values are shown (1000 bootstraps were conducted over loci).

TABLE 3
Matrix of pairwise F_{ST} (below diagonal) and Nei's genetic distance (above diagonal).

	Guinea Savanna	Forest	Coastal Savanna	Volta Region
Guinea Savanna		0.253	0.234	0.203
Forest	0.043		0.118	0.411
Coastal Savanna	0.044	0.032		0.462
Volta Region	0.047	0.075	0.087	

All pairwise F_{ST} values are significant ($p < 0.01$)

Migration rate

Four migrants were detected in the dataset by GENECLASS including two females, one male and one individual of unknown sex. One individual in the Volta population was detected as a migrant from the Guinea Savanna, two were detected in the Coastal Savanna (one from Volta and the other from Forest) and one individual detected in the Forest originating from Coastal Savanna. The rates of migration between populations are presented in Table 4. Except for migration rate between Coastal Savanna and Forest, none of the

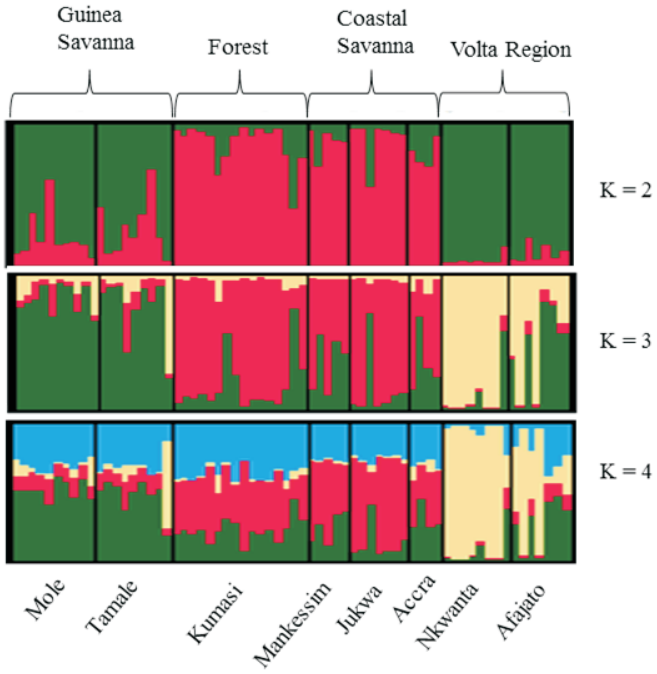

Fig 3 Structure clustering of grasscutter populations in Ghana. Sampling localities are shown below (separated by black vertical lines) while populations are indicated above the diagram. Each colour represents a cluster and individuals in each cluster are represented by bars.

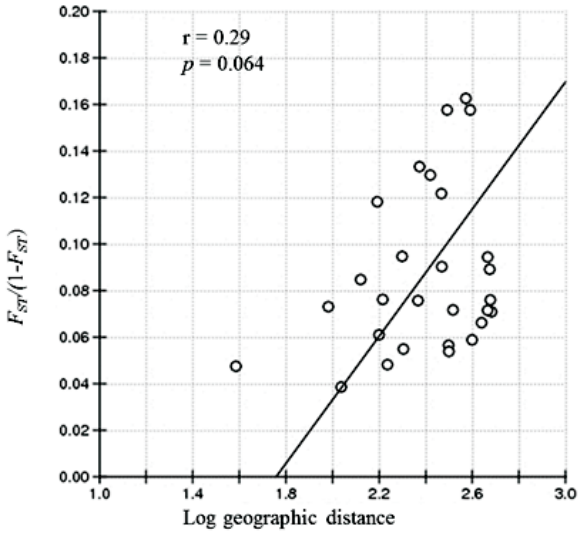

Fig. 4. Correlation between genetic differentiation and geographic distance of grasscutter populations in Ghana.

TABLE 4
Mean (95% confidence interval) migration rates among populations of grasscutter

Migration into:	*Migration from:* *Guinea Savanna*	*Forest*	*Coastal Savanna*	*Volta Region*
Guinea Savanna	0.7678 (-0.015–0.051)	0.0178	0.0377 (-0.029–0.105)	0.1767 (0.000–0.346)
Forest	0.0145 (-0.013–0.042)	0.7010	**0.2650 (0.206–0.324)**	0.0196 (-0.015–0.055)
Coastal Savanna	0.0297 (-0.02–0.081)	0.0409 (0.023–0.105)	0.9057	0.0237 (-0.018–0.066)
Volta Region	0.0302 (-0.023–0.083)	0.0253 (-0.018–0.068)	0.0220 (-0.019–0.063)	0.9226

Columns correspond to populations from which individuals migrated whilst rows correspond to populations from which individuals were sampled. Values in parentheses indicate 95% confidence interval (CI). The value in bold shows 95% CI that is significantly different from zero. Values along the diagonal indicate the proportion of individuals derived from the source population.

population pairs recorded migration rate that was significantly different from zero.

Discussion

In this study we found significant differentiation among all pairs of populations which is not entirely in agreement with mitochondrial D-loop results that we reported previously even though the same populations were used (Adenyo *et al*., 2013). In our previous results, we found that the Guinea Savanna and the Coastal Savanna populations are essentially one Savanna population because we did not find any significant differentiation between them, whereas in the current study we found significant differentiation between them ($p < 0.01$). However, since mitochondrial DNA is maternally inherited, our previous results represented only meternal gene flow (uni-parental). The current results show that Coastal Savanna is rather closer to Forest than to Guinea Savanna. This probably reflects effective dispersal of females even though grasscutters like most mammals are expected to exhibit male-biased dispersal. This can be seen from the perspective of the breeding structure of the grasscutter which usually consists of one male with multiple females and their offspring (Ewer, 1969; Opara, 2010). Even though the mechanisms of dispersal are not well understood and therefore need thorough investigation, we can deduce from our results that long distance female-mediated gene flow is possible in the grasscutter. This could also be aided by their high prolificacy. Even though moderate average litter sizes of 3-5 have been reported, litter size of up to 15 is possible in the grasscutter (Ewer, 1969; Asibey, 1981; Adu *et al*., 1999), which makes it less likely that all females would stay in their natal groups. A north-south dispersal may be more likely as conditions

in the north are harsh and food resources are less compared to the south. We found that genetic diversity within Guinea Savanna is slightly higher than in other populations, which is in agreement with our previous mitochondrial results (Adenyo *et al*., 2013).

The disparity or discordance between the mitochondrial and microsatellite patterns, which is defined as significant difference in the patterns of differentiation between the two markers, can be attributed to sex-biased dispersal or some demographic events (e.g. genetic drift) that can cause biased change of either of the markers (Toews & Brelsford, 2012), which are known to have different mutation rates. The mutation rate of microsatellites is higher than that of mitochondrial DNA, which could explain why the microsatellites could detect a differentiation between Guinea Savanna and Coastal Savanna that the D-loop sequence data could not detect in our previous results. This situation is not unique to the grasscutter but has been reported in some other rodents such as field voles, common voles, Dalton's mouse, Douglas red squirrels and American red squirrels (Braaker & Heckel, 2009; Bryja *et al*., 2010; Beysard *et al*., 2011; Chavez *et al*., 2011).

The fact that we detected four migrants indicates that the populations are not entirely isolated but exchange individuals, albeit at a minimal rate. Both male and female migrants were detected, which confirms that not only males but also females disperse from their natal groups. Even though the direction of migration cannot be ascertained because the migration rates were not significant (except Coastal Savanna-Forest), a cursory look at the migrant individuals detected shows that Forest and Guinea Savanna exchange individuals in either direction whilst one direction (Guinea Savanna-Volta Region) was observed between Guinea Savanna and Volta. Interestingly, no migrant was detected in the Guinea Savanna population. This result suggests a preferential north-south migration and indicates gene flow among the different populations which agrees with previous mitochondrial results where we detected two common haplotypes among the populations (Adenyo *et al*., 2013). Also, only two private alleles were found in the Forest population in the current result indicating that the Forest population is relatively new, supporting the assertion that the grasscutter is mainly a Savanna species (Jori *et al*., 1995).

Water bodies such as rivers are known to form barriers that limit gene flow. For instance, it has been shown that a river served as an effective barrier to gene flow in European ground squirrels in Serbia (Ćosić *et al*., 2013) as well as the entire species range (Řičanová *et al*., 2013). Also, Bryja *et al*. (2010) found that rivers in West Africa form effective barriers to gene flow in Dalton's mouse. Similar observations were previously made by Nicolas *et al*. (2008) for *Praomys sp* and Dobigny *et al*. (2005) for *Taterillus sp* in the West African sub-region. The effect of the Volta River was clearly evident in the study of Brouat *et al*. (2009) on *Mastomys erythroleucus*. All these studies mentioned above indicate that rivers can be effective barriers to dispersal.

The splitting of the Volta Region population from the Guinea Savanna population is interesting and noteworthy, as it suggests the effect of human activity on a natural population. The grasscutter Guinea Savanna and Volta populations could have represented one population in the recent past. However, in 1962, a hydro-electric power plant was constructed at Akosombo on river Volta to serve the populace and budding industries in Ghana. As a result, a large man-made lake (the largest in the world), spanning about 400 km in length and 21 km wide, was created. This probably had some impact on animal populations by hindering gene flow across the lake. Even though grasscutters are good swimmers (Opara, 2010), we do not expect that they can swim across such a wide lake. We speculate that this situation may not affect only grasscutters but also many other mammals that inhabit Ghana. This may not be significant for grasscutter populations because they are very prolific and agile. However, there could be profound effects of such separation on the population viability of large mammals, especially primates which have low reproduction rate. The effects of construction of such dams on fauna populations should therefore be considered when embarking on such efforts in the future in order to safeguard populations from extinction.

No tendency of isolation-by-distance was found among the grasscutter populations (Fig. 4) which is in agreement with mitochondrial results (Adenyo *et al*., 2013). This is probably due to limited number of sampling localities. More extensive sampling may show otherwise, because presence of isolation-by-distance has been shown in other rodents on a regional scale (Nicolas *et al*., 2008; Bryja *et al*., 2010). It will be interesting to scale up this study by including samples from different places in the species geographic range to get a better view of the grasscutter's phylogeography. An advanced technique such as the use of ddRAD markers which was recently developed could aid in large scale phylogeographic study of this species of immense agricultural and ecological importance (Adenyo *et al*., 2017).

In this study, we have shown one way of applying the novel microsatellite markers developed for the grasscutter (Adenyo *et al*., 2012). We have shown that the markers can be used to address ecological questions such as genetic diversity, population structure and dispersal of the grasscutter in the wild. It can be concluded that grasscutter populations in Ghana are genetically differentiated according to agro-ecological zones and the Volta Lake serves as a barrier to dispersal. As far as genetics of the grasscutter is concerned, this is the first time microsatellite markers have been used to study the populations in Ghana and of course the first of its kind in the species range. These results will form a baseline from which inferences can be made for comparison in future phylogeographic studies on the grasscutter. As we advance the course of domestication, there will be the need to determine the effect of domestication on the genetic structure of the grasscutter by comparing the domestic populations to their wild counterparts.

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