Population genetic structure of the common warthog (*Phacochoerus africanus*) in Uganda: evidence for a strong philopatry among warthogs and social structure breakdown in a disturbed population

Vincent B. Muwanika[1], Silvester Nyakaana[1], Hans R. Siegismund[2] and Peter Arctander[2]

[1]*Institute of Environment and Natural Resources, Makerere University, PO Box 7298, Kampala, Uganda and* [2]*Zoological Institute, Department of Evolutionary Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark*

Abstract

Fine-scale genetic structure of large mammals is rarely analysed. Yet it is potentially important in estimating gene flow between the now fragmented wildlife habitats and in predicting re-colonization following local extinction events. In this study, we examined the extent to which warthog populations from five localities in Uganda are genetically structured using both mitochondrial control region sequence and microsatellite allele length variation. Four of the localities (Queen Elizabeth, Murchison Falls, Lake Mburo and Kidepo Valley) are national parks with relatively good wildlife protection practices and the other (Luwero), not a protected area, is characterized by a great deal of hunting. In the total sample, significant genetic differentiation was observed at both the mtDNA locus ($F_{ST} = 0.68$; $P < 0.001$) and the microsatellite loci ($F_{ST} = 0.14$; $P < 0.001$). Despite the relatively short geographical distances between populations, significant genetic differentiation was observed in all pair-wise population comparisons at the two marker sets (mtDNA $F_{ST} =$ 0.21–0.79, $P < 0.001$; microsatellite $F_{ST} = 0.074$–0.191, $P < 0.001$). Significant heterozygote deficiency was observed at most loci within protected areas while no significant deviation from Hardy–Weinberg expectation was observed in the unprotected Luwero population. We explain these results in terms of: (i) a strong philopatry among warthogs, (ii) a Wahlund effect resulting from the sampling regime and (iii) break down of social structure in the disturbed Luwero population.

Key words: common warthog, philopatry, social structure

**Correspondence:* E-mail: vmuwanika@muienr.mak.ac.ug

Résumé

On analyse rarement la structure génétique fine des grands mammifères et pourtant il peut être important d'évaluer l'échange de gènes entre les habitats aujourd'hui fragmentés et de prévoir la recolonisation après des extinctions locales. Dans cette étude, nous avons examiné les structures génétiques de populations de phacochères venant de cinq sites ougandais en utilisant la séquence mitochondriale dans la région de contrôle et la variation microsatellite de la longueur d'un allèle. Quatre des sites (Queen Elizabeth, Murchison Falls, Lac Mburo et Kidepo Valley) sont des parcs nationaux avec une protection de la faune sauvage relativement bien appliquée et le dernier, Luwero, n'est pas une aire protégée et se caractérise par une chasse soutenue. Dans l'ensemble de l'échantillon, on a observé une différenciation génétique significative au locus du mtADN (FST = 0,68 ; $P < 0{,}001$) et aux locus du microsatellite (FST = 0,14; $P < 0{,}001$). Malgré les distances relativement courtes d'un point de vue géographique entre les populations, on a observé une différenciation génétique significative dans toutes les comparaisons de populations couplées pour les deux sets de marqueurs (mtADN FST = 0,21 à 0,79, $P < 0{,}001$; Microsatellite FST = 0,074 à 0,191, $P < 0{,}001$). Une déficience hétérozygote significative a été observée à la plupart des locus dans les aires protégées alors qu'on n'a observé aucune déviation significative par rapport au modèle de Hardy-Weinberg dans la population non protégée de Luwero. Nous expliquons ces résultats en termes de (i) forte philopatrie chez les phacochères, (ii) un effet Walhund résultant du régime d'échantillonnage, et (iii) une rupture de la structure sociale dans la population perturbée de Luwero.

Introduction

The common warthog (*Phacochoerus africanus*), is the most widespread pig species in Uganda. Its natural range extends across Uganda from the south-western, western to northern Uganda where grasslands and open woodland occur (Boitani *et al.*, 1999). Recently, there has been a significant reduction in the warthog range as a result of increased human population, poaching and farming activities. Currently, the range of surviving population is mainly confined to four protected areas: Kidepo Valley (KV), Queen Elizabeth (QE), Murchison Falls (MF) and Lake Mburo (MB) national parks. Remnants of a once widespread population are also found in the open woodlands of Luwero District (LR) in central Uganda. Luwero open woodland is a relatively small unprotected wildlife area surrounded by intensively farmed land. Within these woodlands, warthogs, like other large mammals are heavily hunted and have been eliminated from the surrounding farmed areas, for reasons that they are both a nuisance and a reservoir of livestock diseases.

Warthogs live in strong social units (clans) that consist of adult females, their offsprings and bachelor males. Both females and males are philopatric and relatively sedentary (Cumming, 1975; Somers, Penzhorn & Rasa, 1994). There is minimal contact between different animals coming from different clan areas and it is believed that clans seldom exchange members.

Populations of many mammalian species are organized into breeding social units maintained by some form of behavioral mechanism, which plays a key role in promoting or minimizing inbreeding (Greenwood, 1980). A social structure involving resident females and migrating males promotes inbreeding avoidance, while a clonal one may promote inbreeding. Social mechanisms therefore influence a substantial degree of genetic subdivision among populations. In some species such as elephants and buffaloes where females are philopatric, mitochondrial loci have been reported to show greater subdivision between populations than nuclear loci (Nyakaana & Arctander, 1999; Van Hooft, 2001). In the elephant where family units are matrilineal and males get expelled from the unit when they attain sexual maturity, matings tend to involve unrelated individuals resulting into higher levels of heterozygosity and genotypic proportions that accord to expected Hardy–Weinberg proportions. In other species such as the Alpine marmots that are highly social but with limited dispersal between groups, strong deficits in heterozygotes have been reported (Goossens *et al.*, 2001). In the latter case, the deficiency of heterozygotes was attributed to a Wahlund effect where well-differentiated subunits were sampled and treated as a single population. In the present study, we used nucleotide sequence variation within the mitochondrial control region and microsatellite allele length variation at seven nuclear microsatellite loci to study the population genetic structure of the common warthog from five different localities in Uganda.

Materials and methods

Sample collection and preservation

Ninety warthog samples were collected from five localities in Uganda (Fig. 1). The sample sizes, names and abbreviations of sampled localities are shown in the figure. Samples were obtained as small skin biopsies from free-ranging individuals, tissues from hunters or fresh dung. Of the eighteen samples obtained from KV, sixteen were dung, one was biopsy and another was a warthog tissue from a hunter. In MB, eleven were biopsies while the remaining three were dung samples. All samples from QE were skin biopsies while for LR, all were tissues from hunters. In MF, twelve samples were obtained as skin biopsies while seven were dung.

Re-sampling and sampling closely related individuals were minimized by obtaining only one sample from each group. In the field, samples were stored at ambient temperature in 25% dimethyl sulfoxide in saturated sodium chloride (Amos & Hoelzel, 1991) and were transferred to −80°C in the laboratory.

DNA extraction

Total genomic DNA was extracted from the samples by using standard procedures either involving treatment with sodium dodecyl sulphate and proteinase K, and subsequent phenol/chloroform extraction (Sambrook, Fritsch & Maniatis, 1989) or by use of Dneasy tissue kit (Qiagen; GmbH, Germany) following the manufacturers' protocol.

Mitochondrial DNA amplification and sequencing

Approximately 400 bp of the 5′ end segment of the control region was polymerase chain reaction (PCR)

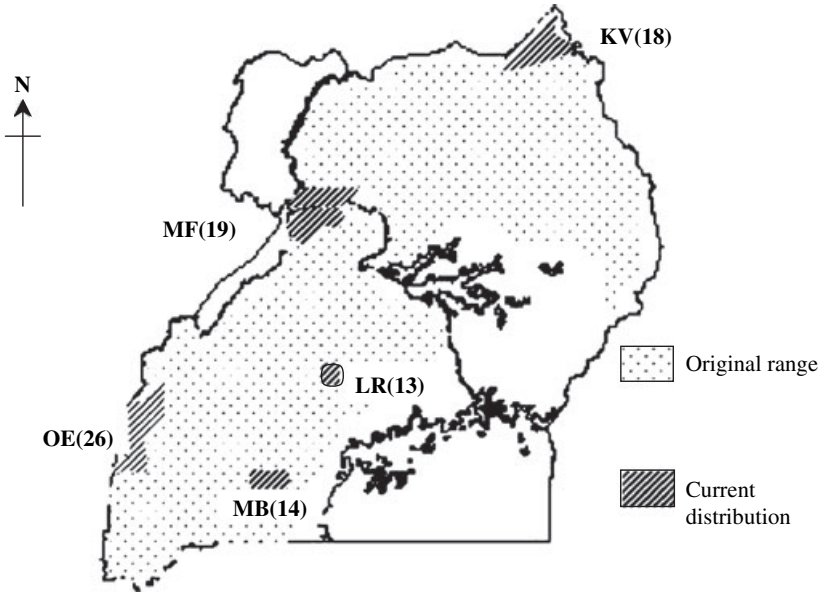

Fig 1 A schematic map of Uganda showing the original and current range of the common warthog, and areas from which samples were obtained. MF, Murchison Falls National Park; KV, Kidepo Valley National Park; QE, Queen Elizabeth National Park; MB, Lake Mburo National Park and LR, Luwero District. Figures in parentheses indicate the number of samples obtained from the area

amplified using primers MT4 (5′-CCTCCCTAAGACTCAAGGAAG-3′) (Arnason, Gullberg & Widegren, 1993) and PeaR (5′-AGTTCATAATTGAAACCCCCA-3′) (Muwanika *et al.*, 2003). Amplification was carried out in 50 μl reaction volumes containing 10 ng of total genomic DNA, 50 pmol of each of the primers, 5× PCR reaction buffer (Boehringer Mannheim GmbH, Germany), 50 pmol dNTPs and 1 U of *Taq* polymerase (Boehringer Mannheim GmbH). The PCR temperature profile was: one cycle of denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min and extension at 72°C for 1 min 30 s. One primer was 5′-end biotinylated, and the double-stranded PCR product was separated into single strands using streptavidin-coated paramagnetic beads (DYNAL A-S, Oslo, Norway). Single-stranded DNA was dissolved in distilled water and used as template for sequencing by the dideoxy chain-termination method (Sanger, Nicklen & Coulson, 1977) using sequenase kit version 2.0 (Amersham Pharmacia Biotech, Inc., Piscataway, USA), [α-^{35}] dATP (Amersham Pharmacia Biotech, Inc.) and a nonbiotinylated primer complementary to the template. Both strands were sequenced. Products of the sequencing reaction were electrophoresed in 6% polyacrylamide/7 M urea gel. The gel was fixed, dried exposed on a Kodak film (Kodak, New York, USA) for 24–48 h and read manually.

Sequence analysis

The extent of population subdivision was quantified using F_{ST} statistic computed by using the program ARLEQUIN version 2.0 (Schneider *et al.*, 2000). The statistical significance of F_{ST} was assessed with 1000 random permutations.

The evolutionary relationships between haplotypes were estimated using a minimum spanning network generated by using the program TCS version 1.13 (Clement, Posada & Crandall, 2000). This cladogram construction procedure is specifically designed to estimate the intraspecific gene trees where most of the haplotypes are present in the population.

Amplification of microsatellite loci

Seven polymorphic microsatellite loci, SW607, S0289, SW1682, SW1301, SW403, SW2419 and S0331 (Rohrer *et al.*, 1994, 1996), were used in this study. The seven loci are dinucleotide repeats and all but two (SW607 and SW2419), lie on different chromosomes. Amplifications were carried out in a 10 μl reaction volume containing 10 ng of total genomic DNA, 2 mM $MgCl_2$, 2 μM PCR GOLD buffer (Boehringer Mannheim GmbH), 0.2 pmol of each of the dNTPs, 0.2 pmol of each primer and 0.4 U of AmpliTaq GOLD DNA polymerase

(Boehringer Mannheim GmbH). PCR profiles comprised 94°C for 10 min of initial DNA denaturation and enzyme activation. This was followed by 28–34 cycles (28 for S0289; 30 for SW1682, SW607 and S0331; 32 for SW1301 and SW403; and 34 cycles for SW2419) of denaturation at 94°C for 30 s, annealing at 50–58°C (50°C for SW403; 52°C for SW607; 57°C for S0289 and 58°C for SW1882, SW1301 and SW2419) for 60 s and an extension at 72°C for 30 s. A final extension of 10 min followed all the reactions. All PCR reactions were run using dye-labelled primers (one primer in each primer set). The products were electrophoresed on 4% acrylamide gels using an ABI 377 (Perkin Elmer, Foster City, CA, USA) automated sequencer using ROX 500 as an internal standard

Statistical analysis of microsatellite data

GENEPOP version 3.1 (Raymond & Rousset, 1997) was used to test each locus in each population for departure from expected Hardy–Weinberg genotypic proportions by using the method of Guo & Thompson (1992) and also to test for linkage disequilibrium between loci. The Bonferroni correction for multiple comparisons was applied to the tests (Rice, 1989).

F_{IS} as a measure of heterozygote deficiency or excess (Wright, 1978) was estimated for each locus in each population. Weir & Cockerham's (1984) analogue of Wright's F_{ST} was computed by using ARLEQUIN version 2.0 (Schneider *et al.*, 2000) and used to quantify the extent of genetic differentiation between populations.

Results

Mitochondrial DNA variation

Sequence characteristics. The first 370 base pairs of the 5′ end of the mitochondrial control region were successfully amplified and sequenced in 84 individuals as follows: QE = 20, MF = 19, LR = 13, KV = 18 and MB = 14. In the 84 sequences, a total of 25 (6.8%) polymorphic sites defined thirteen haplotypes (Fig. 2). Sequences of these haplotypes were submitted to GenBank (accession nos. AY253812, AY253817, AY257399 and AY253820-AY253829). All individuals in QE had a single haplotype (QE1). This haplotype was observed in QE only. Haplotype MF15, the only shared haplotype, was found in MF, KV and LR. LR and KV populations had four haplotypes each. Three haplotypes out of the four in each of the two populations were area specific. In MF, three haplotypes were observed, two of which were area specific. Three haplotypes were observed in MB. All three haplotypes observed in MB were area specific. The evolutionary relationship between the different haplotypes is shown using a spanning network (Fig. 3). Apart from one haplotype (MF15) that is distributed in three of the localities, all the other haplotypes cluster according to their geographical origin.

Population structure. Analysis of population structure in the total sample revealed that approximately 68% of the total variation at the mitochondrial loci was partitioned among populations ($F_{ST} = 0.679$; $P < 0.001$). Significant

			Number of haplotypes in the populations				
		10 20					
		111 1112222222 22233					
		1145578448 9990134666 77825	QE	MF	LR	KV	MB
		5730152893 6890551169 45834					
1	QE01	CTATT-AACT GATATTTCCA ACATT	20	–	–	–	–
2	MF01	.......G.. .TATC.CT.. G..CC	–	4	–	–	–
3	MF02	.......G.. ....C.CT.. G..CC	–	13	–	–	–
4	MF15	....C.C... ...CC	–	2	9	7	–
5	LR01	....C.... ..G.C	–	–	1	–	–
6	LR02	....C.... C	–	–	2	–	–
7	LR04	....C.C... ..GCC	–	–	1	–	–
8	KV02	.C.C.C-.T. A.......TG	–	–	–	9	–
9	KV03	.C.C.C-.TA A.......TG	–	–	–	1	–
10	KV10	.... A.......T. .T...	–	–	–	1	–
11	MB01	..G.C..... ....G..	–	–	–	–	5
12	MB04	....C..... ....G..	–	–	–	–	8
13	MB07	T......... ....G..	–	–	–	–	1
Total number of individuals			20	19	13	18	14

Fig 2 Variable sites in the thirteen haplotypes found in 370 bp mitochondrial control region sequences of 84 warthog individuals from five localities in Uganda. The vertical numbers indicate the positions relative to the reference sequence QE01. Dots indicate an identical nucleotide at the position relative to the reference sequence, a hyphen (-) denotes a deletion/insertion. For abbreviations see Fig. 1

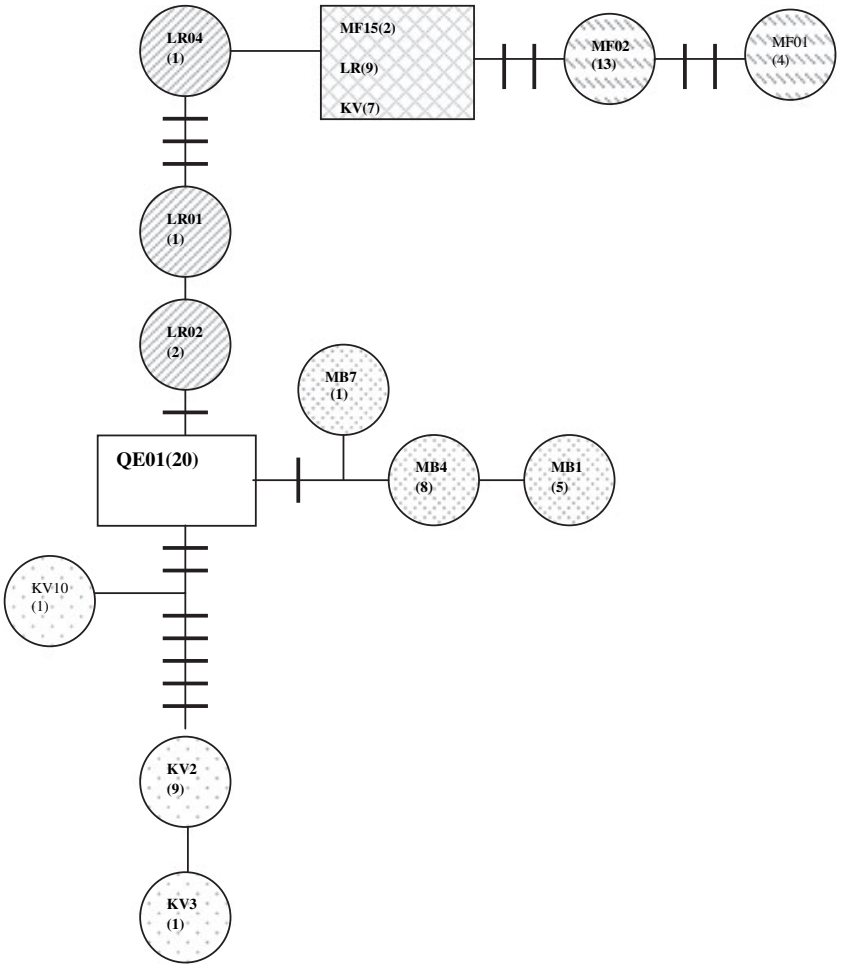

Fig 3 Haplotype network showing the phylogenetic relationships between the observed thirteen haplotypes. Hatch marks along branches indicate number of nucleotide differences in excess of one separating haplotypes. Rectangles denote inferred ancestral haplotype. The shared haplotype is also ancestral and is represented by a shaded rectangle. Haplotypes unique to populations are identified by differences in shading. Letters identify the area of origin and correspond to haplotypes in Fig. 2 while numbers in parentheses represent occurrence.

genetic differentiation was observed between all populations as indicated by the statistically significant F_{ST} values in all pair-wise population comparisons (Table 1). The extent of divergence between populations varied greatly, ranging from 21% (KV versus LR) to 79% (QE versus LR).

Microsatellite variation

Microsatellite allele length variation was scored at seven loci in four of the studied populations. Most of the KV samples consistently failed to amplify for most loci and were therefore not included in microsatellite analysis. A test for genotypic disequilibrium found genotypes at one locus independent of genotypes at another locus for each of the locus pairs in all the populations ($P \geq 0.087$). We therefore considered the seven loci as independent markers.

Three loci (SW607, S0331 and S02419; $P < 0.01$) in QE and one locus in MB (SW607, $P < 0.01$) showed significant departure from expected Hardy–Weinberg genotypic proportions after the Bonferroni corrections (Table 2). All the observed deviations from HW proportions were the result of heterozygote deficiency as indicated by the positive F_{IS} values (Table 2). Apart from only one locus in MF (SW1682, $F_{IS} = -0.022$), a positive F_{IS} value was observed for each locus in the protected populations (i.e. QE, MF and MB). In contrast, only three loci in LR showed positive F_{IS} values but the rest were negative (four of seven loci). The average F_{IS} observed in the protected populations (0.182–0.218) was more than ten times greater than that observed in the unprotected LR population (0.018).

Population structure. Analysis of population structure revealed that a significant amount of microsatellite variance ($F_{ST} = 0.144$, $P < 0.001$) resides among popula-

Table 1 Results of tests of population differentiation based on the control region sequence and microsatellite data

	F_{ST}	
	mtDNA sequence data	Microsatellite data (7 loci)
QE versus MF	0.75***	0.138***
QE versus MB	0.75***	0.191***
QE versus LR	0.79***	0.147***
MF versus MB	0.46***	0.139***
MF versus LR	0.45***	0.074***
MB versus LR	0.45***	0.143***
KV versus QE	0.70***	–
KV versus MF	0.41***	–
KV versus MB	0.40***	–
KV versus LR	0.21***	–

The level of significance shown as *** denotes significant at $P < 0.001$.

QE, Queen Elizabeth national park; MF, Murchison Falls national park; MB, Lake Mburo national park; LR, Luwero District; KV, Kidepo Valley national park.

Table 2 Summary of results of analysis of fixation indices per locus in each population

	Protected wildlife areas			Unprotected area
Locus	QE	MF	MB	LR
SW1301				
N	26	15	13	13
F_{IS}	0.058	0.378	0.269	0.253
$P_{(HW)}$	0.728	0.126	0.238	0.049
S0289				
N	26	15	13	13
F_{IS}	0.108	0.046	0.273	0.320
$P_{(HW)}$	0.169	0.267	0.071	0.085
SW2419				
N	26	15	13	13
F_{IS}	0.534	0.211	0.318	−0.173
$P_{(HW)}$	0.001	0.293	0.261	0.710
SW1682				
N	26	15	13	13
F_{IS}	0.253	−022	0.094	0.026
$P_{(HW)}$	0.056	0.749	0.471	0.609
SW403				
N	26	15	13	13
F_{IS}	0.079	–	0.207	−0.021
$P_{(HW)}$	1.000	–	0.523	0.667
S0331				
N	26	15	13	13
F_{IS}	0.272	0.222	0.260	−0.019
$P_{(HW)}$	0.005	0.072	0.483	0.168
SW607				
N	26	15	13	13
F_{IS}	0.224	0.254	0.1000	−0.055
$P_{(HW)}$	0.003	0.071	0.003	0.306
F_{IS} (AVE)	0.218	0.182	0.217	0.018

N, Number of individuals analysed; F_{IS}, Weir & Cockerham's (1984) analogue of Wright's fixation index; $P_{(HW)}$, single locus *P*-values. QE, Queen Elizabeth national park; MF, Murchison Falls national park; MB, Lake Mburo national park; LR, Luwero District.

tions. Wright's fixation index, F_{ST}, quantifying the proportion of genetic variation attributed to differences between populations, indicates significant genetic differentiation between all pair-wise population comparisons (Table 1), an observation consistent with results obtained from mtDNA sequence data. They range from 0.074 to 0.191 and indicate that a substantial amount of genetic variation is accounted for by differences between populations. These F_{ST} values are however more than three times less than those obtained from analysis of mtDNA sequence data. Allele frequency distributions in the four populations analysed indicate the occurrence of fifteen private alleles across the seven loci (Table 3). Six of the private alleles were scored in LR, four in MF, two in MB and three in QE. The most frequent private alleles were 179 bp scored at locus SW1682 in MB (0.538), and 171 bp at locus SW1301 (0.346) scored in LR. The rest of the private alleles occurred in low frequencies (≤0.107). Allele 101 bp of locus SW403 was the most frequent in all populations.

Discussion

High differentiation among populations

Analyses of population differentiation (Table 1) indicate that we sampled distinct subpopulations with differing allele (Table 3) and haplotype (Fig. 2) distributions. This is surprising for a large mammal considering the absence of a physical barrier and the small geographical distance between the populations. For instance, although the QE and LR, the QE and MB, and the MF and LR populations are only approximately 160 km apart from each other, there is a significant subdivision between them. Strong differentiation, without a physical barrier as observed in the warthog, can be interpreted in two ways: (i) habitat and population fragmentation, and (ii) inability of warthogs to disperse long distances.

Table 3 Observed allele frequency distribution in the warthog by locus and population

Locus/allele	QE	MF	MB	LR	Locus/allele	QE	MF	MB	LR	Locus/allele	QE	MF	MB	LR
SW1301					SW289					SW2419				
171				*0.346*	157		0.167	0.154	**0.423**	105		0.133		**0.423**
173		**0.467**	0.308	0.038	161	0.058	0.167	0.038	0.077	109	0.019	0.133		
175	0.019		0.155		165		0.033		0.115	111	**0.519**	**0.333**		0.308
177	**0.346**	0.133			167				*0.038*	113	0.269		0.038	
179	0.038			0.115	169	0.038	**0.400**	0.231	0.154	115	0.115		**0.500**	
181	*0.173*				171	**0.654**	0.200		0.038	117	0.058	0.133		0.115
183	0.115	0.033	0.038		179	0.019		**0.269**		119	0.019	0.033	0.462	0.038
185	0.154	0.033	**0.538**	0.192	181	0.115		0.038		121		*0.033*		
187	0.096	0.133		0.038	183	0.058		0.077		127		*0.133*		
189	0.038	0.167		0.077	187	*0.038*				129				*0.115*
191	*0.019*				189	0.019	0.033	0.192	0.154	135		*0.067*		
197		0.033		0.192										
SW1682					SW403					SW607				
147				*0.154*	97		0.033		0.154	157		0.033		0.154
149	0.058	0.067	0.115		99			0.193	0.038	167	0.019	0.233		0.115
151	0.192	0.100	0.038	**0.231**	101	**0.827**	**0.967**	**0.769**	**0.731**	169		*0.033*		
153				*0.038*	105	0.173		0.038	0.077	171	0.154	**0.333**	0.154	0.154
155		0.067	0.077							173	0.096	0.233	**0.385**	0.154
159	**0.635**	0.067	0.192	0.077	S0331					175	0.250	0.033	0.231	**0.231**
161	0.019	0.100	0.038	0.192	215		0.133	0.038	0.077	177	0.019			0.077
169		*0.033*			217		0.067		0.077	179	0.019		0.038	
171				*0.038*	219		0.067		0.115	181	**0.346**	0.067		0.115
173	0.052	0.233		0.192	221	**0.327**	**0.433**	0.346	0.077	183	0.096		0.192	
175	0.019	**0.300**		0.077	223	0.288	0.133	**0.385**	**0.308**					
177	*0.019*				225	0.288	0.167	0.038	0.269					
179			**0.538**		227	0.038			0.038					
					229			*0.192*						
					231	0.058			0.038					

Bold face in the allele frequency columns indicates most frequent allele in each population. Private alleles are indicated in italics.
QE, Queen Elizabeth national park; MF, Murchison Falls national park; MB, Lake Mburo national park; LR, Luwero District.

Random genetic drift in fragmented populations is expected to lead to an increase in the between-population component of genetic diversity (Nei, Maruyama & Chakraborty, 1975). Some recent studies in African large mammals have attributed differentiation among adjacent populations to rapid genetic drift following fragmentation (e.g. O'Ryan *et al.*, 1998; Whitehouse & Harley, 2001). This alone however, cannot adequately explain the level of differentiation (Table 1) and the strong structuring of haplotypes (Fig. 3) observed in the warthog because habitat and population fragmentation in Uganda are recent phenomena dating back probably <100 years. The observed differentiation and haplotype structuring is suggestive of a long-term limited dispersal between populations. Warthogs are known to be generally sedentary and living in small home ranges of 64–374 ha (Cumming, 1975; Kingdon, 1989). The expectation from animals exhibiting such habits is that of high levels of population differentiation. Our data clearly conform to this expectation and provide further evidence for a strong philopatry among warthogs. The results presented here therefore demonstrate a kind of genetic structure that discourages natural re-colonization through dispersal in case of a local extinction event.

The positive F_{IS}

In contrast to the striking pattern of heterozygosity excess observed in many large mammals such as buffaloes (Van Hooft, 2001), elephants (Nyakaana & Arctander, 1999),

most F_{IS} values observed in the warthog populations sampled from national parks were positive. A number of the positive F_{IS} values were significantly discordant with Hardy–Weinberg proportions (Table 2), indicating a general heterozygote deficiency among warthogs. Departure from Hardy–Weinberg proportions because of heterozygote deficiency could be attributed to null alleles (i.e. failure to amplify an allele), selection or assortative mating. Although these factors cannot be completely ruled out, they are the less likely explanations among warthogs because no such departure was observed in any single locus across all populations. The most likely explanation for heterozygote deficiency among warthogs is a Wahlund effect caused by sampling functionally independent social reproductive units. When units of sampling constitute amalgamations of functionally independent reproductive units, interpretation of F_{IS} is potentially confounded by a Wahlund effect whereby genetic variance between subgroups produces heterozygosity deficits relative to Hardy–Weinberg expectations for the group as a whole (Storz, Bhat & Kunz, 2001). Warthogs live in spatially recognizable units referred to as clans (Cumming, 1975; Kingdon, 1989). Different clans of warthogs tend to avoid each other. Under such a social organization, a sampling regime as the one used in this study where only one sample was obtained from each group or area could therefore easily result in positive F_{IS} values. Positive F_{IS} values that arise as an artefact of sampling design have also been reported in genetic studies of human populations, when units of sampling are hierarchical subdivisions of tribes that do not accurately reflect the true network of marital ties (Long, 1986).

There was, however, little or no evidence for a Wahlund effect in the LR population, as values of F_{IS} at most loci (four of seven loci) were negative with an overall value being slightly positive (Table 2). For a species with a well-structured social system where social units are strongly philopatric (Cumming, 1975), the apparent panmixia observed in the Luwero population is rather surprising. The most plausible explanation for this observation is that intensive hunting pressure in this unprotected population has led to the breakdown of the social structure of the family units whereby the remaining social units have amalgamated into one randomly mating population. Similar observations have been made in a recent study on social structure of elephants in Queen Elizabeth national park (Nyakaana *et al.*, 2001). In this case, breakdown of social structure because of poaching pressure has been suggested as the likely explanation of the observed apparent panmixia. It is therefore likely that the protected populations of QE, MF and MB have intact social structure among the family units while the social cohesion between members of family units in the Luwero population has been disrupted by the unregulated hunting pressure. These results should however be taken with caution because only one disturbed population is included in this study.

Acknowledgements

This work was funded by the Danish International Development Agency (DANIDA) under the Wildlife Genetics Project. We would like to thank the USDA-supported U.S. Pig Genome Coordination project for a contribution of four of the fluorescent primer sets used in this study. We are also greatly indebted to the Uganda Wildlife authority and the Wardens of Queen Elizabeth, Murchison Falls, Kidepo Valley and Lake Mburo National Parks for granting us permission to conduct research. Hunters kindly provided samples from Luwero.

References

Amos, W. & Hoelzel, A.R. (1991) Long term preservation of whale skin for DNA analysis. In: *Genetic Ecology of Whales and Dolphins* (Ed. A. R. Hoelzel). Report of the International Whaling Commission (Special Issue, 13), Cambridge.

Arnason, U., Gullberg, A. & Widegren, B. (1993) Cetacean mitochondrial control region sequences of all extant baleen whales and two sperm whale species. *Mol. Biol. Evol.* **10,** 960–970.

Boitani, L., Corsi, F., De Biase, A., Carranza, I.D., Ravagli, M., Reggiani, G., Sinibaldi, I. & Trapanese, P. (1999) *A Databank for the Conservation and Management of African Mammals*. Institute of Applied Ecology, Rome, Italy.

Clement, M., Posada, D. & Crandall, A.K. (2000) TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9,** 1657–1659.

Cumming, D.H. (1975) *A Field Study of the Ecology and Behavior of Warthog*. Museum memoir of Salisbury, Salisbury, U.K.

Goossens, B., Chikhi, L., Taberlet, P., Waits, P.L. & Allaine, D. (2001) Microsatellite analysis of genetic variation among and within Alpine marmot populations in the French Alps. *Mol. Ecol.* **10,** 41–52.

Greenwood, P.J. (1980) Mating systems, philopatry and dispersal in birds and mammals. *Anim. Behav.* **28,** 1140–1162.

Guo, S.W. & Thompson, E.A. (1992) Performing exact test for Hardy-Weinberg proportions for multiple alleles. *Biometrics* **48,** 361–372.

Kingdon, J. (1989) *East African Mammals. An Atlas of Evolution in Africa*, Vol. 11B. The University of Chicago, Chicago, IL.

Long, J.C. (1986) The allelic correlation structure of Gainj-Kalam-speaking people: I. The estimation and interpretation of Wright's F-statistics. *Genetics* **112,** 629–647.

Muwanika, B.V., Nyakaana, S., Siegismund, R.H. & Arctander, P. (2003) Phylogeography and population structure of the common warthog (*Phacochoerus africanus*) inferred from variation in mitochondrial DNA sequences and microsatellite loci. *Heredity* **91,** 361–372.

Nei, M., Maruyama, T. & Chakraborty, R. (1975) The bottleneck effect and genetic variability in populations. *Evolution* **29,** 1–10.

Nyakaana, S. & Arctander, P. (1999) Population genetic structure of the African elephant in Uganda based on variation at mitochondrial and nuclear loci: evidence for male-biased gene flow. *Mol. Ecol.* **8,** 1105–1115.

Nyakaana, S., Abe, L.E., Arctander, P. & Siegismund, R.H. (2001) DNA evidence for elephant social behavior breakdown in Queen Elizabeth National Park, Uganda. *Anim. Conserv.* **4,** 231–237.

O'Ryan, O.C., Harley, H.E., Bruford, W.M., Beaumont, M., Wayne, R. & Cherry, I.M. (1998) Microsatellite analysis of genetic diversity in fragmented South African buffalo populations. *Anim. Conserv.* **1,** 85–94.

Raymond, M. & Rousset, F. (1997) Genepop: Population genetics software for exact tests and ecumenicism. *J. Hered.* **86,** 248–249.

Rice, W.R. (1989) Analyzing tables of statistical tests. *Evolution* **43,** 223–225.

Rohrer, G.A., Alexander, J.L., Keele, W.J., Smith, P.T. & Beatie, W.C. (1994) A microsatellite linkage map of the porcine genome. *Genetics* **136,** 231–245.

Rohrer, G.A., Alexander, J.L., Hu, Z., Smith, T.P.L., Keele, W.J. & Beatie, W.C. (1996) Cloning and characterization of 414 polymorphic porcine microsatellites. *Anim. Genet.* **27,** 137–148.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*. Coldspring Harbor Laboratory Press, Coldspring Harbor, NY.

Sanger, F., Nicklen, S. & Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **74,** 5463–5467.

Schneider, S., Kueffer, J.-M., Roessli, D. & Excoffier, L. (2000) *Arlequin Ver.2.000: A Software for Population Genetic Data Analysis*. Genetics and Biometry Laboratory. Univ. of Geneva, Geneva, Switzerland.

Somers, J.M., Penzhorn, L.B. & Rasa, E.A. (1994) Home range size, range use and dispersal of warthogs in the eastern Cape, South Africa. *J. Afr. Zool.* **108,** 361–373.

Storz, F.J., Bhat, R.H. & Kunz, H.T. (2001) Genetic consequences of polygyny and social structure in an Indian fruit bat, *Cynopterus sphinx*. I. Inbreeding, outbreeding and population subdivision. *Evolution* **55,** 1215–1223.

Van Hooft, F.W. (2001) Population genetics of the African buffalo. From ecology to evolution. PhD Thesis, Department of Environmental Sciences, Wageningen University, Wageningen, The Netherlands.

Weir, B.S. & Cockerham, C.C. (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38,** 1358–1370.

Whitehouse, A. & Harley, H.E. (2001) Post-bottleneck diversity of elephant populations in South Africa, revealed using microsatellite analysis. *Mol. Ecol.* **10,** 2139–2149.

Wright, S. (1978) *Evolution and the Genetics of Populations. 4. Variability within and among Populations*. University of Chicago Press, Chicago, IL.

(Manuscript accepted 20 May 2006)

doi: 10.1111/j.1365-2028.2006.00671.x