

## Biochemical-genetic approach towards the systematics of lechwe *Kobus leche*

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Red lechwe *Kobus leche leche* Gray, 1850 ( $n = 3$ ), black lechwe *K. l. smithermanni* Lydekker, 1900 ( $n = 10$ ) and Kafue lechwe *K. l. kafuensis* Haltenorth, 1963 ( $n = 19$ ) from Zambia were examined for genetic variability and differentiation at 30 presumptive structural loci using horizontal starch gel electrophoresis. Values of polymorphism ( $P = 10.0$ – $16.7\%$ ) and average heterozygosity ( $H = 6.3$ – $7.9\%$ ) were within the range commonly found in ungulates. Genetic variability was lowest in the red lechwe, which may be due to a genetic bottleneck the Zambia population experienced some 50 years ago. Relative ( $F_{ST} = 21\%$ ) and absolute (Nei's 1978,  $D = 0.020$ – $0.023$ ) genetic differentiation were in accordance with the subspecies status proposed for red lechwe, black lechwe, and Kafue lechwe on the basis of morphological characters.

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### Introduction

Despite a large number of extant taxa, little is known of electrophoretic variability and differentiation in antelopes. A study of the biochemical systematics of representatives of several subfamilies and tribes of antelopes was reported by Georgiadis *et al.* (1990), and genetic variability and differentiation within the *Hippotraginae* was evaluated by Grobler and Van der Bank (1993). Electrophoretic variability has been assessed in populations of Speke's gazelle *Gazella spekei* (Templeton *et al.* 1987), Arabian oryx *Oryx leucoryx* (Vassart *et al.* 1991), the sand gazelle *Gazella leptoceros* (Granjon *et al.* 1991), the blesbok *Damaliscus dorcax*, and the springbok *Antidorcas marsupialis* (Bigalke *et al.* 1993). In many taxa subspecies have been described on the basis of superficial morphological differences. Almost no data are available to assess their validity from the perspective of population genetics.

Lechwe *Kobus leche* Gray, 1850 are one of the most aquatic antelope species of Africa. They are entirely restricted to seasonally inundated flood plains in the Central Basin of Africa south of the equator, spending much time wading in the water that represents their refugial habitat (Child and von Richter 1969, Williamson 1990). This species has been subdivided into three subspecies, red lechwe *K. l. leche* Gray, 1850, black lechwe *K. l. smithermanni* Lydekker, 1900, and Kafue lechwe *K. l. kafuensis* Haltenorth, 1963. A fourth subspecies, Roberts' lechwe *K. l. robertsi* Rothschild, 1907, is now extinct (Ansell 1960, 1968, Dorst and Dandelot 1970). Black and Kafue lechwe live exclusively in Zambia. Whereas the population of the former increased from 21 500 animals in 1975 (Grimsdell and Bell 1975) to 41 400 animals in 1983 (Jeffery 1984), that of Kafue lechwe decreased from 94 000 animals in 1973 (Bell *et al.* 1973) to 40 000 in 1986 (Spinage 1986). The range of red lechwe is more extensive and includes parts of Angola, Botswana, Namibia, and Zaire. Populations have fluctuated widely, for example in Zambia a decline to no more than 70 individuals in 1948 was followed by an increase to some 3400 animals in 1985 (VVAA 1989).

The three subspecies have been separated mainly on the basis of coat colouration. Red lechwe has a bright chestnut fulvus colouration, only the legs being marked with black. The Kafue lechwe has dark shoulder patches in adult males, and the black lechwe is blackish brown on back and sides. Horns of the Kafue lechwe seem to be slightly thinner than in the other two subspecies (Dorst and Dandelot 1970).

The present study examines taxonomic relationships of lechwe at the biochemical genetic level, and provides a preliminary characterization of genetic variability within the taxa.

## Materials and methods

We obtained samples of skeletal muscle of red lechwe ( $n = 3$ , collection areas Lukanga swamps, Kabwe District in the Central Province), black lechwe ( $n = 10$ , collection areas Bangweulu swamps, Mpika District in the Northern Province), and Kafue lechwe ( $n = 19$ , collection areas Lochinvar, Monze District, Blue Lagoon, Mumbwa District in Central and Southern Provinces) from Zambia. All animals were shot with rifle from long distance, ie they were not chased, by licensed hunters. Samples were frozen within 4–8 hours after the death of animals at  $-18^{\circ}\text{C}$  and shipped frozen to the laboratory. Preparation of tissue extracts, electrophoretic and staining procedures were performed according to routine methods (Hartl and Höger 1986, Grillitsch *et al.* 1992). The enzyme systems screened, the loci scored, and buffer systems used are given in Table 1. Interpretation of electrophoretic patterns followed Harris and Hopkinson (1976), and Harris (1980). The calculation of indices of genetic variability and differentiation was supported by the BIOSYS programme package (release 1.7) of Swofford and Selander (1989).



Table 1. Enzyme systems screened and presumptive loci scored in three subspecies of lechwe. Buffer systems: P – continuous phosphate buffer, pH = 7.4; TC – continuous tris-citrate buffer, pH = 8.0 (for details see Grillitsch *et al.* 1992).

Enzyme system (abbreviation, E.C. number, buffer system used)	Locus
Lactate dehydrogenase (LDH, 1.1.1.27, P)	<i>Ldh-1, -2</i>
Malate dehydrogenase (MDH, 1.1.1.37, TC)	<i>Mdh-1, -2</i>
Malic enzyme (ME, 1.1.1.40, TC)	<i>Me-1, -2</i>
Isocitrate dehydrogenase (IDH, 1.1.1.42, TC)	<i>Idh-1, -2</i>
6-Phosphogluconate dehydrogenase (PGD, 1.1.1.44, P)	<i>Pgd</i>
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1.2.1.12, TC)	<i>Gapdh</i>
NADH-diaphorase (DIA, 1.6.2.2, TC)	<i>Dia</i>
Catalase (CAT, 1.11.1.6, TC)	<i>Cat</i>
Superoxide dismutase (SOD, 1.15.1.1, P)	<i>Sod-1, -2</i>
Aspartate aminotransferase (AAT, 2.6.1.1, TC)	<i>Aat-1, -2</i>
Glutamate-pyruvate-transaminase (GPT, 2.6.1.2, TC)	<i>Gpt</i>
Pyruvate kinase (PK, 2.7.1.40, TC)	<i>Pk</i>
Adenylate kinase (AK, 2.7.4.3, TC)	<i>Ak-1, -2</i>
Phosphoglucomutase (PGM, 2.7.5.1, P)	<i>Pgm-1</i>
Esterases (ES, 3.1.1.1, P)	<i>Es-d, Es-1</i>
Peptidases (PEP, 3.4.11, P)	<i>Pep</i>
Aminoacylase-1 (ACY-1, 3.5.1.14, TC)	<i>Acy-1</i>
Adenosine deaminase (ADA, 3.5.4.4, TC)	<i>Ada</i>
Aldolase (ALDO, 4.1.2.13, TC)	<i>Aldo</i>
Fumarate hydratase (FH, 4.2.1.2, TC)	<i>Fh</i>
Mannosephosphate isomerase (MPI, 5.3.1.8, P)	<i>Mpi</i>
Glucosephosphate isomerase (GPI, 5.3.1.9, P)	<i>Gpi</i>

## Results

Five out of 30 presumptive structural loci were polymorphic and differentially diagnostic, namely (tetrameric structure of the corresponding allozymes as inferred from heterozygous phenotypes in parentheses): *Me-1* (4), *Acy-1* (2), *Ada* (1), *Mpi* (1), and *Gpi* (2). Allelic frequencies, the proportion of polymorphic loci ( $P$ , 99% criterion), the mean number of alleles per locus ( $A$ ), expected (Nei's 1978, unbiased  $H$ ) and observed ( $H_o$ ) average heterozygosity, and the inbreeding coefficient ( $F_{IS}$ ) are given in Table 2. In black lechwe, genotype frequencies at *Gpi* showed a significant deficiency of heterozygotes ( $p = 0.003$ , exact probability). Of the total gene diversity, 21% ( $F_{ST} = 0.212$ ) was due to diversity among populations (subspecies). Nei's (1978) unbiased genetic distances and modified Rogers distances (Wright 1978) are given in Table 3.

Table 2. Allelic frequencies and indices of genetic variation in three subspecies of lechwe.  $p$  – allelic frequency,  $h$  ( $h_0$ ) – expected (observed) single locus heterozygosity (Nei's 1978, unbiased  $h$ ),  $P$  – proportion of polymorphic loci,  $A$  – mean number of alleles per locus,  $H$  ( $H_0$ ) – expected (observed) average heterozygosity (Nei's 1978, unbiased  $H$ ),  $F_{IS}$  – inbreeding coefficient. Overall values are calculated over 30 loci.

Locus	Allele	Black lechwe ( $n = 10$ )		Red lechwe ( $n = 3$ )		Kafue lechwe ( $n = 19$ )	
		$p$		$p$		$p$	
<i>Me-1</i>	<i>a</i>	0.700	$h = 0.468$	1.0	$h = 0.0$	0.632	$h = 0.526$
	<i>b</i>	0.250	$h_0 = 0.500$	0.0	$h_0 = 0.0$	0.211	$h_0 = 0.546$
	<i>c</i>	0.050		0.0		0.157	
<i>Acy-1</i>	<i>a</i>	0.400	$h = 0.721$	0.500	$h = 0.600$	0.184	$h = 0.605$
	<i>b</i>	0.100	$h_0 = 0.400$	0.0	$h_0 = 0.333$	0.0	$h_0 = 0.737$
	<i>c</i>	0.150		0.0		0.289	
<i>Ada</i>	<i>d</i>	0.350		0.500		0.527	
	<i>a</i>	0.850	$h = 0.268$	0.500	$h = 0.600$	0.842	$h = 0.273$
<i>Mpi</i>	<i>b</i>	0.150	$h_0 = 0.300$	0.500	$h_0 = 1.0$	0.158	$h_0 = 0.316$
	<i>a</i>	1.0	$h = 0.0$	0.167	$h = 0.733$	0.342	$h = 0.462$
<i>Gpi</i>	<i>b</i>	0.0	$h_0 = 0.0$	0.500	$h_0 = 0.333$	0.658	$h_0 = 0.474$
	<i>c</i>	0.0		0.333		0.0	
	<i>a</i>	0.300	$h = 0.442$	0.0	$h = 0.0$	0.658	$h = 0.462$
	<i>b</i>	0.700	$h_0 = 0.0$	1.0	$h_0 = 0.0$	0.342	$h_0 = 0.474$
<i>P</i>		0.133		0.100		0.167	
<i>A</i>		1.23		1.13		1.23	
<i>H</i>		0.063		0.064		0.079	
$H_0$		0.040		0.056		0.084	
$F_{IS}$		0.365		0.125		-0.063	

Table 3. Genetic distances among three subspecies of lechwe. Above diagonal: modified Rogers distances (Wright 1978), below diagonal: Nei's (1978) unbiased genetic distances.

	Black lechwe	Red lechwe	Kafue lechwe
Black lechwe	–	0.168	0.144
Red lechwe	0.023	–	0.166
Kafue lechwe	0.020	0.023	–

## Discussion

Both average heterozygosity and the proportion of loci polymorphic in the three subspecies of lechwe are within the range commonly found in ungulates (see Hartl and Pucek 1994, for review). The lower  $P$ - and  $A$ -values in the red lechwe may be



due to a bottleneck the population in Zambia experienced some decades ago (see Introduction), which is supported by high frequencies of variant alleles at the polymorphic loci. It is typical for genetic bottlenecks that most of the rare alleles are lost whereas the frequencies of some of them may be dramatically raised (cf Nei *et al.* 1975). Since values of average heterozygosity are similar when constituted either by a few highly polymorphic or many slightly polymorphic loci (Table 2),  $H$  alone is not a very powerful indicator of genetic depletion (see Hartl and Pucek 1994, for further discussion). However, due to small sample size in the red lechwe, the lower  $P$ - and  $A$ -values need to be proven by further investigation. In the black lechwe, the lack of heterozygotes at the *Gpi* locus and the highly positive  $F_{IS}$ -value suggest some subdivision within the Zambian population of this subspecies. Alternatively, these results may be explained by introgression of the Kafue lechwe alleles into populations of the black lechwe. Unfortunately, biological information on lechwe populations in Zambia is inadequate to support or refute these hypotheses.

Red, black and Kafue lechwe proved to be closely related, showing a genetic distance and an  $F_{ST}$ -value of a magnitude comparable to that found among subspecies in other ungulate taxa. For example, Gyllensten *et al.* (1983) reported Nei distances up to 0.025 and a  $G_{ST}$  ( $F_{ST}$ ) of about 22% among subspecies of European red deer *Cervus elaphus*. The results of the present study are in accordance with the fact that morphological differences among the three lechwe subspecies are slight and involve little but variation in patterns of coat colour.

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