A lack of genetic variation in commercially bred Nile crocodiles (*Crocodylus niloticus*) in the North-West Province of South Africa

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Abstract

Although much is known about the genetic variation of crocodilians, very little is known about the levels of variation in Nile crocodiles. The purpose of this study was to compare the low levels of genetic variation reported for wild crocodile species with levels in 50 sub-adult crocodiles bred for commercial utilisation. Gene products of 52 protein-coding loci in *Crocodylus niloticus* were examined by horizontal starch gel electrophoresis. No detectable genetic variation was observed at any of these loci, which possibly indicates captive inbreeding or represents the natural state of the wild population where the breeding stock was captured. Possible explanations for this unexpected result are discussed.

Introduction

The Nile crocodile is endemic to Africa and has been classified as vulnerable in the *South African Red Data Book* for reptiles and amphibians (Branch, 1988). Populations of crocodiles have been severely depleted in recent years due primarily to the reduction of riverine habitat induced by the construction of dams, weirs and irrigation schemes (Jacobsen, 1988). This, along with the flooding of nesting banks, pollution of water sources and competition with man due to incompatibility with livestock farming has led to the fragmentation of breeding populations and a subsequent decrease in hatchling numbers (Jacobsen, 1988). Although poaching is no longer considered a threat (Blake and Jacobsen, 1992), some crocodiles are still poached from reserves either for their skins or for traditional medicine (Leslie, 1997). In 1992, there were only an estimated 8 000 Nile crocodiles remaining in the wild in South Africa.

The Nile crocodile is of considerable economic importance as its hide is in great demand in the leather trade (Patterson, 1987). More recently, the flesh of the crocodile has become a gourmet dish, highly valued both in South Africa and abroad. In 1992, the Convention on International Trade in Endangered Species of the World Fauna and Flora (CITES) downlisted South Africa to Appendix II (Mulder, 1992). This made trade in captive-bred crocodile products legal. The increasing demand for crocodile products and the corresponding decrease in numbers led to the establishment of crocodile farms. They protect wild populations from hunting by supplying an easily accessible source of crocodiles for the market, create job opportunities, are usually tourist attractions and play an educational role. The abundance of crocodiles on farms has also been identified as a reservoir for the re-establishment of crocodiles in suitable habitats (Jacobsen, 1988; Mulder, 1992) if the environmental causal factors of decline could be removed or reduced. Although conservation authorities would support such measures should they become necessary, the genetic implications of such a strategy must first be assessed. For example, there is concern that the breeding practices employed at crocodile farms may change the genetic variation in captive crocodiles. Due to the possible negative effects of inbreeding or other changes in a population's genetic structure, an estimate of the genetic variability within reserve populations and populations intended for supplementation of protected populations may help in formulating management strategies and prevent deleterious consequences. The aim of this study was to determine if crocodiles bred for commercial purposes had different levels of genetic variation when compared to values previously reported for wild crocodiles.

Materials and methods

Nile crocodiles were sampled from a captive-bred population at the Kwena Gardens crocodile farm near Sun City in the North West Province, South Africa (25º30'S; 27º04'E). The original breeding stock was collected in the Okavango Delta, Botswana. Tissue samples from the heart, kidney and liver were collected from 50 offspring during a routine slaughter of one-and-a-half to two year old crocodiles (size: 1.0 to 1.75 m) of the F_1 generation. Muscle samples were obtained from the flesh remaining on the hide when it was removed. As the muscle did not always adhere to the skin, muscle samples were only obtained from 30 of the 50 individuals. Blood samples were collected from 10 individuals when the spinal column was severed for comparison with other tissue samples as blood is a non-invasive sampling technique. Following electrophoretic analysis it was determined that blood did not provide sufficient loci for the purposes of this investigation. All the samples were frozen and transported to the laboratory for analysis by horizontal starch gel electrophoresis.

The following buffer systems were used to separate the enzymes investigated: **MF**- a continuous Tris (0.18 M), boric acid (0.1 M) and EDTA (0.004 M) buffer system (pH 8.6) (Markert and Faulhaber, 1965); **TC**- a continuous Tris (0.3 M) and citric acid (0.1 M) buffer system (pH 6.9) (Whitt, 1970) and **A**- a continuous Tris (900 mM), boric acid (500 mM), EDTA (20 mM) and magnesium chloride (40 mM) buffer system (pH 8.6) (Gonchurenko et al., 1992). Two tissue extracts for each individual were prepared from

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a mixture of all the tissues and diluted 1:1 either with distilled water or a 0.25 M sucrose solution (as recommended by Lawson et al., 1989) for comparison. The tissue extracts were analysed by starch gel electrophoresis using 10% **MF** buffer or 12 % **TC** or **A** buffered gels. Extracts of either muscle or heart were used when the results proved unsatisfactory due to the dilution factor. The enzymes were separated using a 50 mA current supply. The gels were sliced into four equal slices and each of these slices was stained for a different enzyme using the staining techniques of Harris and Hopkinson (1976). The staining patterns were then interpreted and the loci were numbered from the cathode to the anode (according to Van der Bank et al., 1992), i.e. the zones of activity closest to the origin were designated as the first locus, with the more anodal loci indicated by progressively higher numbers. The resultant alleles in each zone or locus are also labelled from the origin, according to the relative mobilities of the gene products they encode.

Results

The proteins, locus abbreviations, enzyme commission numbers, tissues and buffers giving the best results for *C. niloticus* are listed in Table 1. Twenty-one of the 26 proteins stained for provided interpretable results for 52 protein-coding loci. The enzymes that did not show sufficient activity or resolution for interpretation were alcohol dehydrogenase (E.C. 1.1.1.1), hexokinase (E.C. 2.7.1.1), mannose-6-phosphate isomerase (E.C. 5.3.1.8), nucleoside phosphorylase (E.C. 2.4.2.1) and L-iditol dehydrogenase (E.C. 1.1.1.14). The tissues homogenised with distilled water provided the best resolution.

None of the loci resolved displayed allelic polymorphism (see Fig. 1). The average heterozygosity (H) value was zero, the average number of alleles per locus (A) was one and the proportion of polymorphic loci (P) was zero.

Discussion

Although it is almost impossible to examine the variability of the genome as a whole, allozyme data can, if used comparatively, serve as an estimate of variability amongst the structural loci coding for soluble proteins (Lawson et al., 1989). For instance, vertebrate heterozygosity has an average value of 0.060, whereas reptile heterozygosity has been estimated at 0.047 (Nevo, 1978). Low levels of genetic variation in crocodilians have been reported in previous research (Table 2), and it was established that species of *Crocodylus* are closely related (Densmore, 1983; Menzies and Kushlan, 1991). Species appear to be distinguishable from each other on the quantitative basis of gene frequencies, rather than fixed allelic differences. This also appears to be the only distinguishing factor in previous studies where various populations of American alligators (Adams et al., 1980), American crocodiles (Menzies and Kushlan, 1991) and Nile crocodiles (Jurgens et al., 1994) were compared.

Genetic variation in American alligators (*Alligator mississipiensis*) varies from 0.009 to 0.034, from 0.058 to 0.158 in American crocodiles (*C. acutus*) and from 0.011 to 0.042 in Nile crocodiles (Table 2). Therefore a comparison of genetic variation in the Nile crocodile with that of alligators shows them to be remarkably similar and the genetic variation in American crocodiles is thought to be higher due to their more variable habitat (i.e. estuarine vs. freshwater habitat) (Menzies and Kushlan, 1991).

The two previous studies of Nile crocodiles by Lawson et al. (1989) and Jurgens et al. (1994) revealed variation at two and three loci respectively. Lawson and his colleagues investigated 27 protein coding loci and detected polymorphism at the glucose phosphate isomerase (GPI) and erythrocytic acid phosphatase (EAP) loci. Jurgens and colleagues investigated 52 loci and only detected polymorphism at the mannose-6-phosphate isomerase (MPI-1), 6-phosphogluconate dehydrogenase (PGD-1) and general protein (PROT-1) loci. This study investigated 52 protein coding loci (including the above-mentioned GPI, MPI, PGD and PROT loci) and found no polymorphism. Although low levels of genetic variation are expected for crocodilians, our result is unusual since it indicates no variation in the population studied. We expected to either find more variation due to the optimal, artificial environmental conditions provided at the farm that could minimise the selection pressure and lead to a reduction in gene frequencies or a reduction in natural levels of variation due to changes in gender ratios and male dominance.

Possible reasons for low levels of variation in wild populations of crocodilians

Low genetic variability can be attributed to one or a combination of factors (Simpson, 1953; Cavalli-Sforza and Bodmer, 1971). One of these factors is genetic drift, where the population size is small or periodically reduced (bottleneck effect), or the population is derived from one or a small group of individuals (founder effect). Initially it was thought that the low level of variation in wild alligators was due to genetic bottlenecking, but this hypothesis has since been rejected (Gartside et al., 1977; Menzies et al., 1979). In the absence of evidence for the bottleneck effect in the Nile crocodile it was also rejected as a probable cause for the similarly low levels of genetic variation within this species (Lawson et al., 1989). It is now thought that the low levels of variation in crocodilians are determined by their environment (Gartside et al., 1977). These low levels of variation are considered to be an indication of directional selection in response to environmental factors, otherwise known as the ecological theory (Baker and Stebbins, 1965; Levins, 1968; Gartside et al., 1977; Adams et al., 1980). In other words, the low level of genetic variability represents adaptation to a narrow niche within a relatively stable environment (Lawson et al., 1989). If the fixation of many genes is a response to long periods of environmental stability, the high levels of homozygosity may not be a disadvantage since it is an approach to optimal adaptation (Gartside et al., 1977). This tends to coincide with Nevo's (1978) hypothesis that environmental variables are the determinants of genetic variation. Although it is not possible to eliminate a population collapse or a genetic mechanism as determinants of low heterogenity, natural selection is considered the most probable. Whatever the relationship between heterogenity in a population and its environment, the rapid recovery of some alligator populations suggests that these low levels are compatible with the maintenance and expansion of a healthy population over the short term (Chabreck, 1967).

Variation may also be reduced by genetic systems that limit variability, i.e. reduced crossing over. It is not possible to discount reduced frequency of crossing over or some other genetic mechanism or breeding system as a possible factor contributing to the low heterozygosity in alligators (Gartside et al., 1977) and other crocodilians.

The third possible cause of low variation is directional selection that favours a certain genotype. Directional selection induced by man is an unlikely mechanism in wild populations. Although crocodile hunting for the skin trade will tend to remove the older and larger individuals selectively, it is unlikely that this will discriminate against a certain protein phenotype (Gartside et al.,

TABLE 1
PROTEINS STAINED FOR, ENZYME COMMISSION NUMBERS (E.C. NO.), LOCUS ABBREVIATIONS, BUFFERS AND TISSUES GIVING BEST RESULTS (SEE MATERIALS AND METHODS FOR BUFFER ABBREVIATIONS)

Protein	E.C. No.	Locus	Buffer	Tissue
1 Adenylate kinase	2.7.4.3	AK-1 to 3	TC-12%	X, H, M
2 Aspartate aminotransferase	2.6.1.1	AAT-1,-2	MF-10%	H, M
3 Creatine kinase	2.7.3.2	CK-1 to 3	MF-10%	X, H, M
4 Esterase	3.1.1.-	EST-1 to 3 EST-4	A-12%, MF-10% A-12%	X, H, M, Bl
5 General protein		PT-1, -2 to 9	MF-10%	M, H, Bl
6 Glucose dehydrogenase	1.1.1.47	GLD-1,-2	A-12%	H, M, Bl
7 Glucose-6-phosphate isomerase	3.5.1.9	GPI-1,-2	MF-10%	X, H, M, Bl
8 Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	GAP-1, -2	A-12%	H, M
9 Glycerol-3-phosphate dehydrogenase	1.1.1.8	GPD	MF-10%	X, H, M
10 Isocitrate dehydrogenase	1.1.1.42	IDH-1 IDH-2	A-12% MF-10%	X, H, M X, H, M
11 L-Lactate dehydrogenase	1.1.1.27	LDH-1,-2	MF-10%	X, H, M
12 Malate dehydrogenase	1.1.1.37	MDH-1, -2	MF-10%	X, H, M, Bl
13 Malic enzyme	1.1.1.38	ME	MF-10%	X, H, M
14 Peptidase Substrate: Glycyl-L-leucine Leucyl-glycyl-glycine Leucyl-tyrosine Phenylalanyl-proline	3.4.-.-	 PEP-A PEP-B1,-2 PEP-S1 to -3 PEP-D1,-2	 TC-12% A-12% RW-12%, MF-10% MF-10%	 H, Bl X, Bl M, H, Bl M, H
15 Peroxidase	1.11.1.7	PER-1 PER-2, -3 PER-4 PER-5 PER-6	A-12% MF-10% MF-10% MF-10% A-12%	Bl Bl X, H, M, Bl X, H, M, Bl X, H, M, Bl
16 6-Phosphogluconate dehydrogenase	1.1.1.44	PGD	MF-10%	H, M, Bl
17 Phosphoglucomutase	5.4.2.2	PGM	TC-12%	X, H, M
18 Superoxide dismutase	1.15.1.1	SOD-1,-2	MF-10%	X, H, M

H= heart, M= muscle, Bl= blood and X= mixed tissue sample.

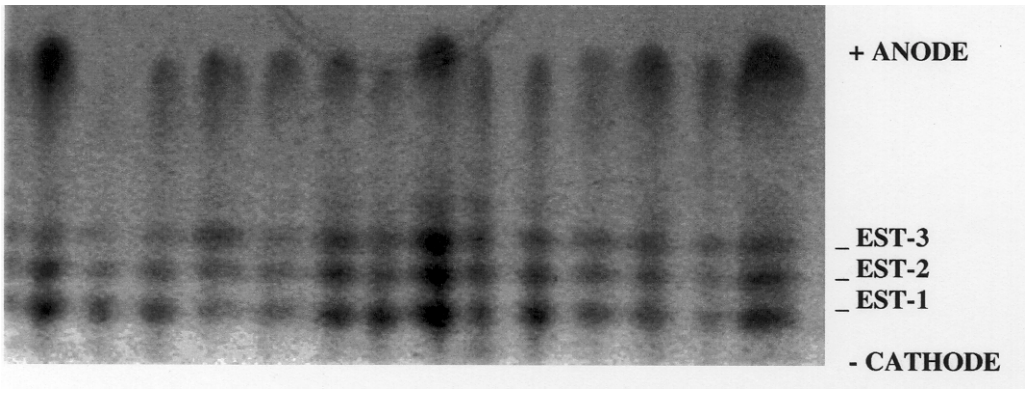

Figure 1
A MF buffered gel (10%) stained for esterase (E.C no. 3.1.1.-)

TABLE 2
COMPARATIVE POPULATION STATISTIC VALUES FOR THE TOTAL NUMBER OF LOCI STUDIED, THE NUMBER OF POLYMORPHIC LOCI, A (AVERAGE NUMBER OF ALLELES PER LOCUS), P (PROPORTION OF POLYMORPHIC LOCI) AND H (HETEROZYGOSITY AT ALL LOCI) FOR THREE SPECIES OF CROCODILIANS (ADAPTED FROM LAWSON ET AL., 1989)

Source	Species	Locality	No. of loci (No. polymorphic)	A	P	H
Gartside et al. (1977)	*A.m* (wild)	Louisiana, USA	49 (3)	1.08	0.06	0.021±0.012
Menzies et al. (1979)	*A.m* (wild)	Florida, USA	44 (2)	1.08	0.045	0.009
Adams et al. (1980)	*A.m* (wild)	Louisiana, USA	27 (2)	1.07	0.074	0.012
Adams et al. (1980)	*A.m* (wild)	Florida, USA	21 (4)	1.19	0.191	0.022
Adams et al. (1980)	*A.m* (wild)	South Carolina, USA	27 (5)	1.15	0.186	0.034
Menzies and Kushlan (1991)	*C.a* (wild)	Florida, USA	32 (19)	_	0.594	0.117± 0.017
Menzies and Kushlan (1991)	*C.a* (wild)	Jamaica	32 (13)	_	0.406	0.055± 0.009
Menzies and Kushlan (1991)	*C.a* (wild)	Dominican Republic	32 (19)	_	0.594	0.158 ±0.039
Lawson et al. (1989)	*C.n* (wild)	Gonarezhou National Park, Zimbabwe	27 (2)	1.07	0.074	0.011
Jurgens et al. (1994)	*C.n* (farm)	Rustenburg, RSA	51 (3)	1.12	0.078	0.035
Jurgens et al. (1994)	*C.n* (wild)	St. Lucia, RSA	28 (2)	1.22	0.174	0.042
This study	*C.n* (farm)	N.W. Province, RSA	52 (0)	1.00	0.000	0.000

A.m. = Alligator mississipiensis; C.a. = Crocodylus acutus; *C.n. = Crocodylus niloticus*

1977) and the crocodile hunting carried out in South Africa in the 1960s was not selective at all. Therefore, the overall impact of hunting on the genetic variation of the total population is likely to be limited as hunting will randomly remove genotypes from the population.

Another possible cause of decreased variation is breeding systems that reduce variability, such as inbreeding, and behaviour such as male dominance. These factors have a negligible effect in natural populations were the number of individuals is large and there is a wide variety of mates. Since there is usually an average of six to eight females to every male in wild populations (Deeming and Ferguson, 1989; Marais, 1990), even non-dominant males may mate with a proportion of the females.

Commercial crocodile farms and their potential impact on genetic variation

In South Africa it was, until recently, illegal to collect or capture crocodile eggs, hatchlings and adult crocodiles in the wild and move them to farms for commercial purposes (Marais, 1991). Farms established in this period were encouraged to purchase their breeding stock from existing farms or from neighbouring countries and this had to be done within a framework of national and international laws concerning crocodiles. At farms where the breeding stock is obtained from a single source (i.e. in the present study), the greatest potential danger is inbreeding and its possible associated decrease in production. Since most farms obtain their breeding stocks from many different sources, intensive breeding for commercial purposes could lead to an increase in the variation present in the offspring (outbreeding). In addition, the optimal, artificial environmental conditions at the farm could minimise selection to maintain diverse gene frequencies. These effects may have important ecological consequences if crocodiles are released in order to supplement wild populations or if some crocodiles manage to escape from the farms during flooding or due to negligence.

Since observed heterozygosities in commercially bred Nile crocodiles vary from zero (this study) to 0.035 (Jurgens et al., 1994), whereas heterozygosity varies between 0.011 (Lawson et al., 1989) and 0.042 (Jurgens et al., 1994) for wild crocodiles, it would appear that captive-bred and wild populations differ in their quantitative genetics. Some of this variation may be of adaptational value in the wild populations, but the variation in captive-bred populations may reflect either the natural state of their source population (which is often unknown by South African crocodile farmers) or indicate interference by man. These results have important implications for the conservation of crocodilian populations. An example of this would be the increased number of amelanistic crocodiles, which exhibit lighter skins, observed at farms. These crocodiles are very rare in the wild, but are relatively common on crocodile farms where they are highly prized for their unusual hides.

In the presence of directional selection, the loss of genetic variability within natural populations of a normally outbreeding species can often be attributed to the founder effect or genetic drift (Lawson et al., 1989). For practical reasons a small founder population is established as breeding stock from individuals that were selected at the time of capture for some trait such as size, gender or physical condition. This will limit the amount of variation in the founder population (the founder effect) and may lead to some form of genetic drift. In this study, the wild breeding stock was captured in the Okavango Delta in Botswana. No additional breeding stock has been added since the establishment of the farm, which would amplify the founder effect in the small breeding population and encourage a decrease in variation (genetic drift). The population size is small and the ratio of males to females is different to that encountered in the wild (Schmidt, 1998). Often the crocodiles are kept in ponds where there are three or four males with

20 to 40 females, whereas in the wild there are an average of six to eight females per male (Deeming and Ferguson, 1989; Marais, 1990). Under these conditions even normal crocodile behaviour such as male dominance has an increased impact in the smaller populations maintained on farms. The dominant male tends to defend his territory and females aggressively (Schmidt, 1998). The dominant male will mate more often and for a longer season and tend to pass on his genes more often than the smaller or less aggressive males (Deeming and Ferguson, 1989). Male dominance is more pronounced on crocodile farms where breeding groups are confined, as it is easier for the dominant male to force two or three other males into submission than in the wild where there are many more sexually mature males. Over time, this would result in a change in the total genetic variation in the captive-bred population in comparison to wild populations, and could lead to genetic inbreeding as documented in this study.

The apparent lack of any form of variation (not even the low levels expected for crocodilians) in the slaughtered stock studied could be due to inbreeding, genetic drift as a result of the small founder population, or could indicate the natural state of the population in the area where the breeding stock was captured. Inbreeding can lead to a deterioration of certain attributes, such as fertility, vigour and resistance to disease (inbreeding depression) (Ayala, 1982) and this could lead to decreased hatchling success and retarded growth and development of the hatchlings that survive. This, in turn, could lead to decreased production and will have a negative impact on the farm as a whole. These crocodiles may then be unsuitable for release into the wild. The farm management of Kwena Gardens considers inbreeding to be unlikely since only 1% of their hatchlings are deformed and this has been attributed to temperature fluctuations (Schmidt, 1998). Approximately 9 to 15% of the eggs laid are infertile and do not hatch, which is within the range of 7 to 30% considered normal (Smith and Marais, 1993). It seems most likely that the remarkable lack of variation can be attributed to genetic drift associated with the small founder population size captured in the Okavango Delta and/or the apparent dominance of one male over the females in his enclosure. The lack of genetic variation in the F_1 generation is probably due to the history of intensive exploitation of Nile crocodiles in Botswana. Botswana's crocodile population has undergone three periods of heavy commercial exploitation: 1957-1969 (50 000 crocodiles shot for their hides), 1974-1975 (940 adults shot) and from 1983-1988 (1 053 adults captured and 14 000 eggs collected) (Graham et al., 1992). The exploitation so dramatically reduced crocodile numbers that it became necessary for the Botswana Department of Wildlife and National Parks to stop commercial hunting in 1975 since is was not economically viable (Graham et al., 1992). The lack of variation as a result of intensive exploitation has been maintained by the use of only the original founder population for breeding purposes and unnatural breeding conditions.

To conclude, from the standpoint of conserving natural gene pools of Nile crocodiles, the supplementation of wild populations with captive-bred crocodiles should be given the utmost consideration and the genetic composition of the source of supplementary crocodiles adequately assessed. The conservation of wild crocodile populations should ideally be undertaken on a populational basis (Menzies and Kushlan, 1991), although restocking measures may be unavoidable. Sections of selected rivers and dams may be protected by either purchase or agreement with landowners on both banks, allowing breeding populations of crocodiles to establish themselves once the causal factors of their decline have been removed or reduced (Jacobsen, 1988).

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