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Genetic structure of Lake Magadi tilapia populations

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The control region of the mitochondrial DNA haplotype frequencies were significantly different among the two separate lagoon populations of *Oreochromis alcalicus grahami* in Lake Magadi and of *O. a. alcalicus* from lake Natron, and DNA fingerprint similarity indices were significantly higher for intra-population comparisons of the two Magadi lagoon populations and the Lake Natron population than the inter-population similarity indices among these populations. A modified F_{st} measure indicated population sub-division and the phylogeographic partitioning of the VNTR fragments observed were unique to specific populations further indicating substantial genetic differentiation. The lagoon populations within Lake Magadi demonstrated the same degree of genetic differentiation as either of these populations did to the outgroup (the Lake Natron population). There appears to be limited gene flow between Lake Magadi tilapia populations and this population structure has important implications for protecting locally adapted populations within this unique ecosystem.

Key words: Magadi; mitochondrial DNA; DNA fingerprinting; *Oreochromis alcalicus*.

INTRODUCTION

The cichlid species in the African Rift Valley lakes demonstrate extraordinary rates of morphological, behavioural and physiological evolution (Strürmbauer & Meyer, 1992; Meyer, 1993). This study focuses on *Oreochromis alcalicus* Boulenger, a tilapia endemic to two alkaline soda lakes of the Rift Valley. The sub-species *O. a. grahami* is found in Lake Magadi, Kenya and the sub-species *O. a. alcalicus* in Lake Natron, Tanzania. Once a continuous water body, Lake Magadi separated from Lake Natron approximately 10 000 years ago and has since developed a different geomorphology and water-chemistry (Goetz & Hillaire-Marcel, 1992). Lake Magadi water properties exhibit higher temperature, pH and alkalinity levels than Lake Natron. Magadi has a pH averaging 9·8–10·5 and total carbonate alkalinity >300 mequiv l^{-1}, and temperatures >40° C. Undoubtedly, Lake Magadi represents the most hostile environment that teleost fish have been found to inhabit. *O. a. grahami* has developed unusual morphological and physiological adaptations to the high pH and alkalinity. These include the excretion of urea rather than ammonia as their sole nitrogenous waste, through expression of the ornithine-urea cycle (Randall *et al.*, 1989; Wood *et al.*, 1994). This condition of 100% ureotely is unique among teleost fish and renders the sub-species of special evolutionary importance. In

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addition, the Magadi tilapia exhibit an extremely thin blood-water diffusion barrier (Maina, 1990; Laurent *et al.*, 1995; Maina *et al.*, 1996) and the use of the swim-bladder as a primitive air-breathing organ (Maina *et al.*, 1995). Essentially nothing is known about the physiology of the Natron tilapia.

In Lake Magadi, floating precipitate of sodium bicarbonate (trona), underlain by anoxic lake water, divides the *O. a. grahami* habitat into three main areas: Fish Springs Lagoon, South East Lagoon and South West Lagoon. Almost all research on the physiology of *O. a. grahami* has been performed on the Fish Springs fish which are unique relative to other teleosts in their extreme resistance to high environment pH, carbonate alkalinity, and temperature (Wood *et al.*, 1994; Maina *et al.*, 1996). One comparative morphological study identified differences in gill structure between the Fish Springs and South East Lagoon fish (Maina *et al.*, 1996). The Fish Springs Lagoon temperature is 32–37° C, *v.* South East Lagoon temperatures of between 37–45° C which may influence the availability of O_2 (Maina *et al.*, 1996).

The present study applied molecular genetic analysis to determine if two of the populations within Lake Magadi are genetically divergent from each other, and/or those of Lake Natron, and whether they should be considered as separate conservation units. Lake Magadi typically undergoes at least biannual flooding events that cover the trona with rain water thereby providing a potential route for the exchange of fish (Coe, 1966). The objective of this study was to determine the degree of population sub-division that has occurred between the two lagoon systems in Lake Magadi. The genetic differentiation that has accumulated in the 10 000 years of isolation between Lake Magadi and Lake Natron was also determined as a reference point.

Selecting the appropriate DNA marker to examine the genetic structure of populations is dependent on the genetic variation detected by a particular marker. African cichlid species have evolved phenotypically at such a high rate that the mutation rates of certain neutral markers, e.g. mitochondrial DNA, used in population studies does not demonstrate a corresponding increased rate of evolution (Strürmbauer & Meyer, 1992; Meyer, 1993). Although it is unlikely that new haplotype variants would have been generated at the mtDNA control region since Lakes Magadi and Natron separated, the maternal inheritance of this molecule causes it to be more susceptible to genetic drift (Moritz, 1994). Given the severity of the environments where these fish exist, it is highly probable that these populations have undergone significant reductions in their effective population size at various times. This would further increase the rate of differentiation causing the divergence of haplotype frequencies following the generation of boundaries among populations.

We also applied multi-locus DNA fingerprinting that detects hypervariable minisatellite DNA or variable number of tandem repeats (VNTR) loci. Multi-locus DNA fingerprinting has the potential for examining the genetic structure of natural populations which have been genetically differentiated over time-scales which other DNA markers may not detect. Population structuring is a factor that will decrease the measure of genetic similarity of the two populations through genetic drift (Cohen, 1990). If two populations are isolated from each other the prediction is that the similarity indices will be higher for pair-wise comparisons within a population than comparisons of individuals between

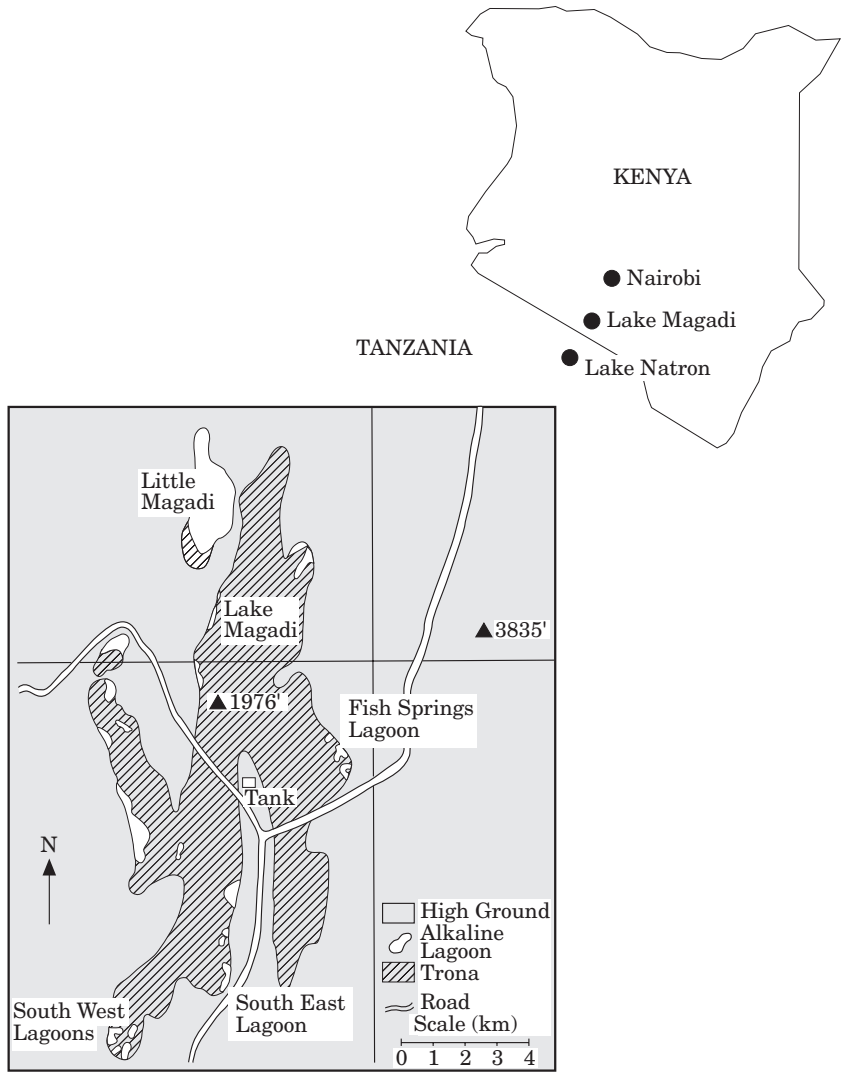

FIG. 1. Map of Lake Magadi, Kenya indicating the South East Lagoon, South West Lagoon and the Fish Springs Lagoon as well as the smaller Little Magadi to the north. Open water (alkaline lagoon) and trona are indicated on the map (adapted from Maina *et al.*, 1996).

populations. A number of previous studies have applied DNA fingerprinting to identify population structuring in natural populations (Gilbert *et al.*, 1990; Hoelzel & Dover, 1991; Degnan, 1993; Taylor & Bentzen, 1993; Rave, 1995; Refseth *et al.*, 1998).

MATERIALS AND METHODS

SAMPLE COLLECTION

The small size of the Lake Magadi lagoons (Fig. 1) limited sampling to the specific region within the lagoon that the fish inhabited. (Note that in Maina *et al.* (1996) the fish referred to as South West Lagoon were in fact collected at the South East lagoon.) At

both the Fish Springs Lagoon and South East Lagoon sites, fish were sampled from 15–22 January 1992, during a relatively dry period about 6 weeks after the little rains of December. A beach seine was pulled through each site to randomly collect fish. Heads were removed, and the bodies placed individually in lysis buffer (Guglich *et al.*, 1993). The Fish Springs Lagoon samples ($n=24$) were collected from a population numbering in the thousands. South East Lagoon samples ($n=16$) were collected from a small population numbering <1000 fish in total. Lake Natron ($n=20$) samples were included in this study as a control population with a known period of isolation from Lake Magadi. Lake Natron samples of *O. a. alcalicus* were kindly provided by Dr Jan Klein and were sampled according to Sültmann *et al.* (1995).

MITOCHONDRIAL DNA CONTROL REGION AMPLIFICATION AND ANALYSIS

The mtDNA control region haplotypes of Lake Magadi and Lake Natron tilapia were identified using single stranded conformational polymorphism (SSCP) analysis and DNA sequencing of the haplotypes. DNA was amplified under the following reaction conditions: 1 × PCR buffer (20 mM Tris-HCl pH 8·4, 50 mM KCl) with 2·0 mM $MgCl_2$ (GIBCOBRL), 0·5 U Taq polymerase (GIBCOBRL), 10 μM of primers 5′-TTCCACC TCTAACTCCAAAGCT-3′ and 5′-CCTGAAGTAGGAACCAGATG-3 (Lee *et al.*, 1995) and 25 ng of template DNA in a total volume of 20 μl. Amplification was performed under the following temperature regime: 95° C for 5 min, 55° C for 30 s and 72° C for 30 s. The amplified products were assessed by gel electrophoresis (Guglich *et al.*, 1993).

Single stranded conformational polymorphism (SSCP) analysis was performed according to above reaction conditions with the following exceptions: a total reaction volume of 10 μl was used; radioactively labelled (γ ^{33}P-dATP (ICN)) primers were added in addition to the unlabelled primers. Amplified products were electrophoresed through a nondenaturing acrylamide Model S2 gel (GIBCOBRL) (5% acrylamide [59 acrylamide : 1 bisacrylamide], 10% glycerol and 0·5 × TBE) for 16 h at room temperature. Any unique conformational polymorphisms were sequenced from one to five individual fish to confirm the SSCP patterns.

The software package AMOVA (Analysis of Molecular Variance, version 1.55; Excoffier, 1992) was used to test the hierarchical partitioning of genetic variation among the lagoon populations of Magadi and Lakes Magadi and Natron (Excoffier *et al.*, 1992). Statistical significance was tested in AMOVA using 9999 permutated matrices. The following statistics were analysed: φ_{CT} which analysed the variation among lake systems and examined the correlation between random haplotypes within a group of populations against random pairs of haplotypes from the whole species; φ_{SC} which analysed the variation of populations within lake systems and examined the correlation between random haplotypes against random pairs of haplotypes drawn from the lake; φ_{ST}, analogous to F_{ST}, analysed variation within populations and examined the correlation of random haplotypes within populations against random pairs of haplotypes from the species. Pairwise φ_{ST} tests were performed to assess population structuring.

DNA FINGERPRINT ANALYSIS

DNA fingerprints were generated using the minisatellite probes Jeffreys' 33.15 and *per* according to Guglich *et al.* (1993) using the restriction enzyme *Alu*I. Within and between population similarity indices (Lynch, 1991) were calculated from samples included on the same DNA fingerprint. Due to limitations in DNA quality and the need to score individuals from the same gel, a subset of samples was used from each population in generating the DNA fingerprints. Differences in the within and between population similarity indices were assessed statistically using a Mantel test (1967) on intra/inter-population similarity indices using the computer package NTSYS (Rohlf, 1993) and a modified F_{ST} measure (Wright, 1978) (F'_{ST}) (Lynch, 1991). The phylogenetic partitioning of VNTR alleles among the populations was determined by examining the number of common and unique VNTR alleles present in each population comparison. This

TABLE I. Frequencies of six control region haplotypes for *O. alcalicus* populations

Haplotype	Population		
	Fish Springs Lagoon, Lake Magadi	South East Lagoon, Lake Magadi	Lake Natron
A	9	12	0
B	7	4	11
C	5	0	0
D	0	0	2
E	3	0	6
F	0	0	1
Total	24	16	20

phylogeographic approach has been applied to determining the genetic divergence between sub-species of the cheetah (Mennotti-Raymond & O'Brien, 1993).

RESULTS AND DISCUSSION

Mitochondrial DNA haplotype diversity with *O. alcalicus* populations ranged from 2–4 (Table I) with a total of 6 (Fig. 2) partitioned among the Magadi and Natron populations examined. An analysis of the partitioning of the haplotype diversity (Table II) indicated 75% of the genetic variation was distributed within *O. alcalicus* populations. Only 8% of the variation was distributed between the Fish Springs Lagoon and South East Lagoon populations within Magadi and 17% of the observed variation was partitioned between the two lake systems. Genetic structuring was assessed by estimating pairwise φ_{ST} which indicated significant differences in all interpopulation comparisons (Table III).

Haplotypes B and E were present in both Lakes Magadi and Natron while each lake had two specific haplotypes (Table I). This pattern of haplotype distribution is consistent with two scenarios: (i) lineage sorting of haplotypes A and B is incomplete and ancestral haplotypes (A, C, D and F) are sorted into specific lakes and populations; or (ii) haplotypes B and E represent haplotypes pre-dating the separation of Lakes Magadi and Natron with the B form representing the ancestral type of newly derived haplotypes specific to each lake following the geologic separation of the watershed system. The prediction for the lineage sorting of haplotypes pre-dating the sub-speciation of *O. a. alcalicus* and *grahami* is that divergent haplotypes from the historic populations should be partitioned among populations (Duvernell & Turner, 1998). The similarity of lake-specific haplotypes (Fig. 3) supports lake-specific lineages that have diverged subsequent to isolation from the ancestral B haplotype. Although Lakes Magadi and Natron are estimated to have separated 10 000 years ago, it is possible that new haplotypes evolved subsequent to separation as the generation time of *O. alcalicus* has been estimated at *c.* 30 days.

To detemine the amount of genetic differentiation at VNTR loci among populations of *O. alcalicus*, the intra-population genetic similarity was compared with the inter-population genetic similarity. The prediction was that if animals

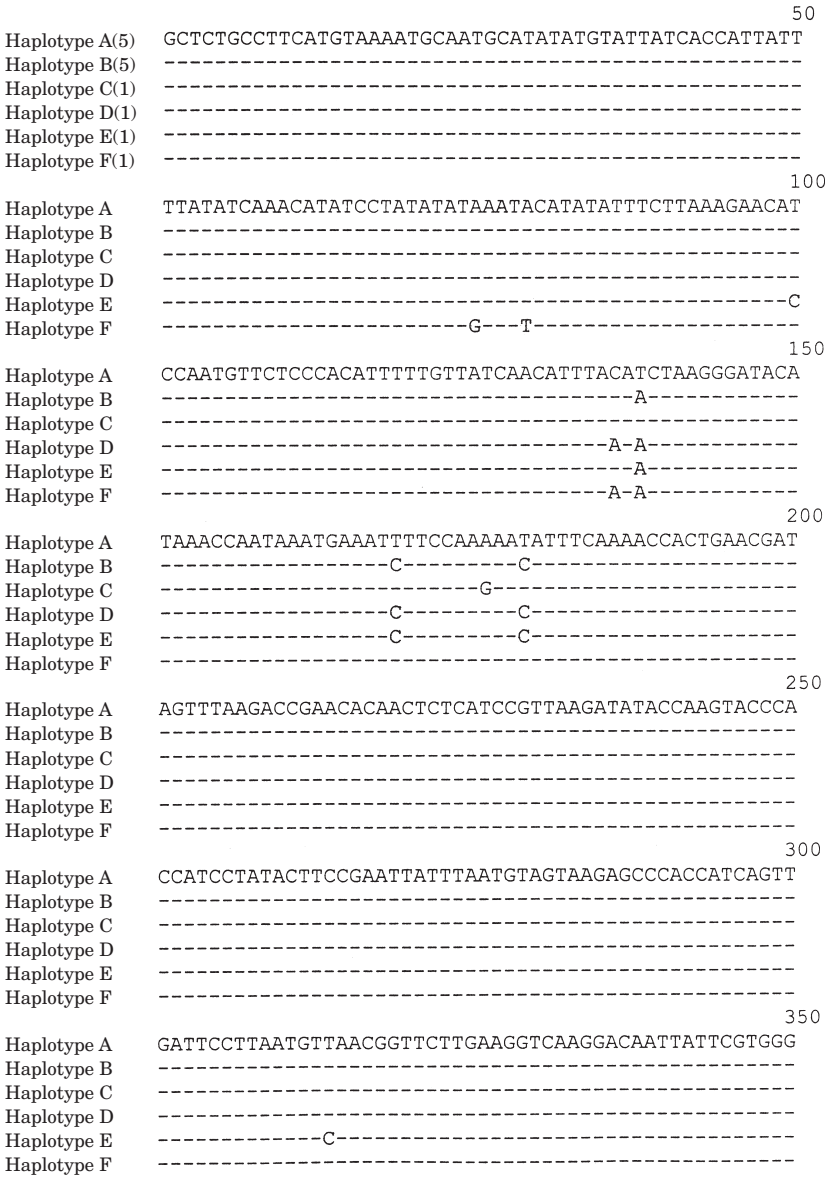

FIG. 2. DNA sequences of five control region haplotypes from *O. alcalicus*. Dashes indicate identical nucleotides of haplotype A and nucleotide substitutions have been identified based on differences from haplotype A. Numbers in parentheses indicate the number of fish sequences per haplotype.

from different geographic regions are structured into populations with limited or no gene flow, the S_{mean} value would be higher among individuals within the populations than among individuals between the populations. The S_{mean} values within the Magadi and Natron populations (Table IV) were considerably higher than S_{mean} values observed in interpopulation comparisons (Table V). The interpopulation comparisons between the Lake Magadi lagoons demonstrated equivalent levels of genetic variability to the levels observed between the

TABLE II. Analysis of variance of *O. a. alcalicus* and *O. a. grahami* estimated by AMOVA (Excoffier *et al.*, 1992) with 9999 permutations of the data

Variance component	Variance	% total	Measure	*P*-value
Among Lakes Magadi and Natron	0·140	16·97	φ_{CT}=0·170	<0·0001
Among populations within Lake Magadi	0·069	8·34	φ_{SC}=0·100	=0·0549
Within *O. alcalicus* populations	0·615	74·70	φ_{ST}=0·253	<0·0001

Partitioning of the genetic variation as measured by the variance, percentage (% total) of the genetic variation of *O. alcalicus* and φ_{CT}, φ_{SC} and φ_{ST} measures with corresponding *P*-values are provided for tilapia in Lakes Magadi and Natron.

TABLE III. Analysis of variance of *O. a. alcalicus* and *O. a. grahami* estimated by AMOVA (Excoffier *et al.*, 1992) with 9999 permutations of the data and pairwise population comparisons of φ_{ST} from AMOVA tests (below diagonal) and the probability (*P*-value) of generating a random value greater than the observed value (above diagonal) as an indicator of population structuring

Population	South East Lagoon	Fish Springs Lagoon	Lake Natron
South East Lagoon	—	0·0424	0·0000
Fish Springs Lagoon	0·1016	—	0·0071
Lake Natron	0·3979	0·1427	—

sub-species of *O. alcalicus* inhabiting separated lake systems. The Mantel test was used to compare the distribution of within population similarity indices with between population similarity indices. The three interpopulation comparisons, South East Lagoon and Fish Springs Lagoon and each lagoon population to the Lake Natron population, demonstrated significance levels of P=0·0001 for both minisatellite probes with approximate Mantel *t*-test statistic values in the range of $t = -0{\cdot}5215$ to $-6{\cdot}038$.

Genetic differentation was also detected among the tilapia populations using a modified version of Wright's index of population sub-division (F'_{ST}) (Lynch, 1991). An F'_{ST} value of 0·567 was calculated for all three populations when S_{mean} values for Jeffreys' 33.15 and *per* were combined. An F'_{ST} value of 0·585 was estimated for the two lagoon systems in Lake Magadi, again with the similarity values of the two probes pooled together. These values indicate that *c.* 57 and 59% of the genetic variation observed was the result of interpopulation variability. Substantial population differentiation is defined to exist with an F_{ST} value of 0·150, with a lower degree of population differentiation occurring below this value (Wright, 1978). The F'_{ST} values in this study were considerably higher than the level described by Wright (1978) as defining substantially differentiated populations, i.e. *c.* 15% interpopulation variance. Wright's definition was on allozyme data and the hypervariable nature of the VNTR loci in the modified calculation of population sub-division may not reflect an equivalent estimate. However, the equivalent F'_{ST} indices calculated in the outgroup comparison of

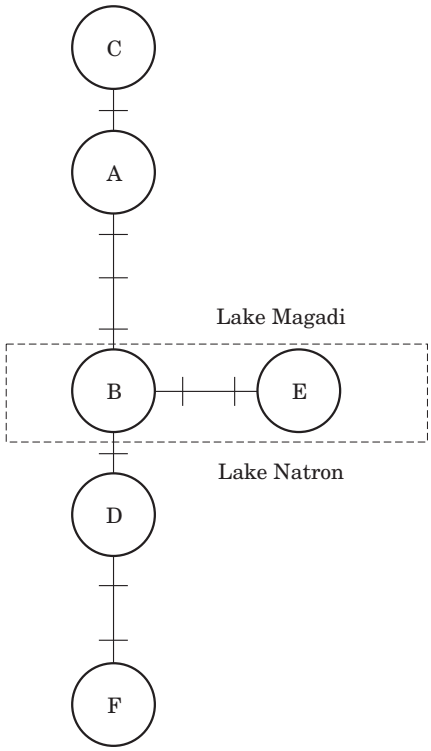

FIG. 3. Minimum-spanning tree of six control region haplotypes identified in *O. alcalicus*. Dashes indicate nucleotide substitution differences away from each haplotype. Haplotypes A and C were found only in Lake Magadi, haplotypes D and F were found only in the Lake Natron samples and haplotypes B and E were found in samples from both lakes.

known isolated populations, i.e. lake populations, and the Magadi lagoon population comparison, supported substantial isolation of the fish inhabiting the different lagoon systems.

A total of 129 and 104 polymorphic VNTR fragments were scored with Jeffreys' 33.15 and *per*, respectively, and were examined for their phylogenetic partitioning among the three populations. Of these fragments 11 (8·5%) were common to both Lake Natron and Lake Magadi tilapia with Jeffreys' 33.15 and 14 (13·5%) with *per*. The remaining 91·5 and 86·5% of the VNTR fragments were unique to each sub-species. A similar comparison of the two lagoon systems within Lake Magadi indicated 14·9 and 10·0% of the 94 VNTR fragments detected by Jeffrey's 33.15 and 140 detected by *per* were common to both populations with approximately 85 and 90%, respectively, of the VNTR fragments being specific to one of the two *O. a. grahami* populations. Therefore, a large portion of the allelic variation at the VNTR loci appears to have accumulated by mutation or differentiated by drift since the isolation of Lake Magadi. Similar levels of geographic partitioning and genetic divergence of the VNTR alleles were detected between the lagoon populations of Lake Magadi. This assessment differs from the F'_{ST} statistic in that the presence or absence of a VNTR allele is considered in the phylogenetic partitioning while Wright's measure of population sub-division considers the frequencies of the VNTR alleles.

TABLE IV. Levels of VNTR genetic variation within populations of *O. alcalicus* for the number of individual fish, the number of pair-wise comparisons used in calculating the mean similarity indices and an estimate of the number of VNTR loci (S_{mean}) (± S.E.) (Lynch, 1991)

Population comparison	Jeffreys' 33·15	*per*
South East Lagoon (*O. a. grahami*)		
Number of fish	8	8
Number of pair-wise comparisons	36	36
Number of VNTR fragments	19·1 ± 0·8	18·9 ± 1·4
Number of VNTR loci	11·2	10·4
S_{mean}	0·515 ± 0·106	0·334 ± 0·097
Fish Springs Lagoon (*O. a. grahami*)		
Number of fish	7	7
Number of pair-wise comparisons	28	28
Number of VNTR fragments	19·3 ± 0·9	21·3 ± 1·0
Number of VNTR loci	11·5	11·3
S_{mean}	0·318 ± 0·095	0·229 ± 0·079
Lake Natron (*O. a. alcalicus*)		
Number of fish	8	8
Number of pair-wise comparisons	36	36
Number of VNTR fragments	17·2 ± 2·1	16·7 ± 1·5
Number of VNTR loci	8·8	8·5
S_{mean}	0·180 ± 0·084	0·211 ± 0·091

The genetic differentiation at the VNTR loci within Lake Magadi occurring on the same scale as the outgroup comparison suggests an extended period of isolation between Fish Springs Lagoon and South East Lagoon tilapia. These results, coupled with the majority of mtDNA control region variation partitioned within populations suggest that the two Magadi populations do not form a single panmictic breeding group. Coe (1966) concluded that 'if it were not for the periodic gene flow allowed by flood connections, between otherwise isolated fish communities, no doubt sub-specific differences might well have arisen in the area.' The structuring of mtDNA haplotypes and VNTR alleles is not consistent with this hypothesis and the morphological gill adaptations observed between two lagoon populations (Maina *et al.*, 1996) strongly supports allopatric evolution in the absence of gene flow. Unfortunately, only one of the populations investigated in the present study has been subject to physiological investigation (Fish Springs Lagoon), while two have received morphological study (Fish Spring Lagoon and South East Lagoons).

Genetic diversity within *O. alcalicus* populations was generally low for the mtDNA control region. The number of control region haplotypes within the populations is on the low end of the number of haplotypes observed within other cichlid species (Meyer *et al.*, 1996; Agnese *et al.*, 1997). The haplotype diversity is also comparable to desert pupfish populations (genus: *Cyprinodon*) which form demographically isolated populations and demonstrate post-Pleistocene allopatric divergence with morphological, physiological and behavioural differences

TABLE V. Levels of genetic variation between populations of *O. alcalicus* for the number of individual fish, the number of pair-wise comparisons used in calculating the mean similarity indices and an estimate of the number of VNTR loci (S_{mean}) (± S.E.) (Lynch, 1991)

Population comparison	Jeffreys' 33·15	*per*
South East Lagoon and Fish Springs Lagoon, *O. a. grahami*		
Number of fish		
South East Lagoon	8	8
Fish Springs Lagoon	7	7
Number of pair-wise comparisons	56	56
Number of VNTR fragments	19·2 ± 0·7	20·1 ± 1·0
Number of VNTR loci	10·0	10·2
S_{mean}	0·132 ± 0·067	0·059 ± 0·048
South East Lagoon and Lake Natron, *O. a. grahami* and *O. a. alcalicus*		
Number of fish		
South East Lagoon	6	6
Lake Natron	6	6
Number of pair-wise comparisons	36	36
Number of VNTR fragments	15·0 ± 1·2	17·0 ± 0·8
Number of VNTR loci	7·8	8·8
S_{mean}	0·080 ± 0·054	0·056 ± 0·048
Fish Springs Lagoon and Lake Natron, *O. a. grahami* and *O. a. alcalicus*		
Number of fish		
Fish Springs Lagoon	6	6
Lake Natron	6	6
Number of pair-wise comparisons	36	36
Number of VNTR fragments	17·2 ± 2·1	16·7 ± 1·5
Number of VNTR loci	8·8	8·5
S_{mean}	0·084 ± 0·057	0·058 ± 0·049

among populations (Duvernell & Turner, 1998). The low haplotype diversity observed within desert pupfish populations suggested large ancestral populations followed by historical population bottlenecks. Furthermore, the VNTR variation within the Magadi lagoon population is comparable with levels observed in endangered species (Brock & White, 1992; Patenaude *et al.*, 1994; Schaeff *et al.*, 1997) which demonstrate low genetic variation as a result of low effective population sizes during bottleneck events.

The low levels of genetic variation at nuclear and mitochondrial genetic markers within Lake Magadi probably reflects the population histories within the isolated lagoon systems. These levels of genetic variation are consistent with low historical effective population sizes (N_e) and would be predicted during severe shifts in the alkalinity, temperature and pH subsequent to the separation of Lakes Magadi and Natron. Major geochemistry and geomorphological changes in Magadi would result in strong selection pressures on the fish populations resulting in potentially repeated population bottlenecks. This seems

consistent with the lower levels of genetic variation observed in the South East Lagoon, which has more severe environmental conditions, at least with respect to oxygen levels, than has the Fish Springs Lagoon. Additional lagoon systems within Lake Magadi will be examined for the presence of tilapia. Genetic characterization of these fish is planned to help assess further the degree of isolation and attempt to reconstruct the evolutionary history of *O. a. grahami* within Lake Magadi.

The physiology of Fish Springs Lagoon fish appears unique in a number of aspects relative to other teleosts. In addition to extreme resistance to high environment pH, carbonate alkalinity, and temperature (Reite *et al.*, 1974), these characteristics include 100% ureotely through complete expression of the ornithine-urea cycle enzymes in the liver (Randall *et al.*, 1989; Wood *et al.*, 1989, 1994). Associated with the latter is the greatest resistance to ammonia toxicity ever documented in a teleost fish (Walsh *et al.*, 1993), a feature which makes the gene pool of interest for intensive aquaculture, where ammonia toxicity is a common problem. The great tolerance to fluctuations in environmental O_2 levels (Narahara *et al.*, 1996), facilitated by an unusually high gill-surface area and short blood-to-water diffusion distance (Maina, 1990; Laurent *et al.*, 1995), is also relevant in this regard. Furthermore the Fish Springs Lagoon tilapia exhibit a remarkable ability for supplementary air-breathing through the swimbladder under hypoxic conditions (Maina *et al.*, 1995). Other remarkable features include the highest temperature-specific blood pHs (Johansen *et al.*, 1975; Wood *et al.*, 1994) and the highest routine metabolic rates (Franklin *et al.*, 1995; Narahara *et al.*, 1996) ever recorded for poikilothermic teleost fish, and an unusual insensitivity of the blood O_2 dissociation curve to pH fluctuations in the physiological range (Lykkeboe *et al.*, 1975; Narahara *et al.*, 1996).

Inasmuch as conditions are even more severe at the South East Lagoon, it seems likely that the same suite of unusual physiological adaptations will be expressed in these tilapia, perhaps to an even greater degree. In this regard it is noteworthy that the gills of these fish are anatomically different from those of Fish Springs Lagoon specimens with greater numbers of gill filaments and respiratory lamellae, resulting in a 2·5-fold greater weight-specific O_2-diffusing capacity (Maina *et al.*, 1996). Further, given the allopatric situation among the populations within Lake Magadi and the environmental differences between at least two microhabitats, i.e. the South East Lagoon and Fish Springs Lagoon, additional morphological and physiological differences may exist between these two populations and the yet uninvestigated South West Lagoon fish.

Ideally, protected status should be extended to each Magadi population. Apart from the present evidence of genetic differentiation, we know nothing about the Lake Natron population of *O. a. alcalicus*, which may share many, some, or none of the adaptations of *O. a. grahami*. Nevertheless, the uniqueness of the species as a whole should be sufficient to warrant the protection of this population as well. Presently, the protection of these populations is consistent with a classification of Management Units as described by Moritz (1994), as both mtDNA and nuclear markers demonstrate significant structuring among the populations. Management Units (MU) are the components of Evolutionary Significant Units (ESU): a biological population which is historically isolated and is distinguished by its presumed evolutionary significance (Ryder, 1986;

Waples, 1991; Dizon *et al.*, 1992). As further information is obtained on this species a classification of ESU may become appropriate or the taxonomic status *O. alcalicus* may warrant re-classification. A recent study of the Death Valley pupfish (Duvernell & Turner, 1998) has addressed the need to apply a broader definition of an ESU by integrating molecular and phenotypic data as to the adaptive significance of a population.

In a larger context, this study demonstrates the importance of considering microhabitats within the lakes of the African Rift Valley. As these lakes can exhibit extreme environmental conditions, fish that have adapted to life on the edge would maintain highly specialized adaptations, i.e. locally adapted genomes, ultimately resulting in allopatry based on an inability to survive or reproduce in neighbouring habitats. Assumption of gene flow among populations based on the absence of obvious physical barriers may be premature when the physicochemical and environmental conditions among habitats can act as effective barriers. Protection efforts should maintain the genetic variation that contributes to the biodiversity of these taxa and the evolutionary potential these fish represent.

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