Discordance between Genetic Structure and Morphological, Ecological, and Physiological Adaptation in Lake Magadi Tilapia

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ABSTRACT

The Magadi tilapia (*Alcolapia grahami*, formerly *Oreochromis alcalicus grahami*) is a remarkable example of teleost life in an extreme environment. Typical conditions include water pH = 10, titration alkalinity > 300 mM, osmolality = 525 mOsm, temperatures ranging from 23° to 42°C, and O_2 levels fluctuating diurnally between extreme hyperoxia and anoxia. A number of relatively small tilapia populations are present in various thermal spring lagoons around the margin of the lake separated by kilometers of solid trona crust (floating Na_2CO_3) underlain by anoxic water. Despite the apparent isolation of different populations, annual floods may provide opportunities for exchange of fish across the surface of the trona and subsequent gene flow. To assess the question of isolation among Lake Magadi populations, we analyzed the variable control region of the mitochondrial DNA (mtDNA) from six lagoons. A total of seven mtDNA haplotypes, including three common haplotypes, were observed in all six populations. Several of the Lake Magadi populations showed haplotype frequencies indicative of differentiation, while others showed very little. However, differentiation among lagoon populations was discordant with their geographical distribution along the shoreline. All populations exhibited the unusual trait of 100% ureotelism but specialized morphological and physiological characteristics were observed among several of the lagoon systems. In addition, distinct differences were observed in the osmolality among the lagoons with levels as high as 1,400–1,700 mOsm kg^{-1}, with corresponding differences in the natural levels of whole-body urea. These levels of osmotic pressure proved fatal to fish from less alkaline systems but remarkably were also fatal to the fish that inhabited lagoons with this water chemistry. Upon more detailed inspection, specific adaptations to differential conditions in the lagoon habitat were identified that allowed survival of these cichlids. Additional evidence against potential for gene flow among lagoons despite the sharing of common mtDNA haplotypes was that the osmolality of floodwaters following a heavy rain showed lethal levels exceeding 1,700 mOsm kg^{-1}. In isolation, different mtDNA haplotypes would be predicted to go to fixation in different populations due to rapid generation times and the small effective population sizes in a number of lagoons. We propose a model of balancing selection to maintain common mtDNA sequences through a common selection pressure among lagoons that is based on microhabitats utilized by the tilapia.

Introduction

African cichlids (family Cichlidae, suborder Labroidei, order Perciformes) comprise the most speciose assemblages of closely related vertebrates. A recent examination of "soda" tilapia in Lakes Natron and Magadi (Seegers and Tichy 1999; Seegers et al. 1999; Tichy and Seegers 1999) identified a small species flock within these lakes. The several species in Lake Natron demonstrate differences in mouthparts typical of other cichlid species flocks in the Rift Valley lakes. Although cichlid species have been characterized in a number of Rift Valley alkaline "soda lakes" (Trewavas 1983), the classification of species within these environments has focused on standard morphological differences, for example, jaw morphology and coloration.

The Lake Magadi tilapia in Kenya, *Alcolapia grahami* (Seegers and Tichy 1999), formerly *Oreochromis alcalicus grahami* (Fryers and Iles 1972; Trewavas 1983), has been well characterized with respect to the physiological adaptations required to inhabit

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one of the most severe of the soda lakes within the African Rift Valley. Typical conditions within Lake Magadi include water of pH 9.8–10.5, titration alkalinity >300 mM, osmolality 525 mOsm, temperatures as high as 42°C, O_2 levels fluctuating diurnally between extreme hyperoxia and virtual anoxia, and intense levels of avian predation and UV radiation (Narahara et al. 1996). Over the past 25 yr, the physiology of these cichlids has been studied in one lagoon population in detail, the Fish Springs Lagoon.

Of the many physiological adaptations to this severe environment, the most notable is the excretion of all N-waste as urea with no excretion of ammonia, presumably due to the difficulty of maintaining an outwardly directed P_{NH_3} gradient in this highly buffered alkaline water (Randall et al. 1989; Wood et al. 1989). This level of complete ureotelism is unique among teleost fish and is achieved by expression of the Krebs ornithine-urea cycle (OUC) throughout the white muscle mass (Lindley et al. 1999) as well as the liver (Randall et al. 1989; Walsh et al. 1993), and ureotely appears to be obligate in that even extended transfer to freshwater of low buffering capacity and circumneutral pH does not alter this 100% ureotelism (Wood et al. 2002*b*) . Exceptionally high urea excretion rates are made possible by a facilitated diffusion-type urea transporter that has been pharmacologically identified and cloned (Walsh et al. 2001). Tolerance of high environmental alkalinity is also facilitated by the regulation of exceptionally high blood and tissue pH (Johansen et al. 1975; Wood et al. 1994). These cichlids have further evolved a unique pyloric bypass system to allow drinking of alkaline lake water despite the presence of an acidic stomach (Narahara 2000; Bergman et al. 2003). The metabolic rate is exceptionally high, reflecting the high environmental temperatures and activity levels of these remarkable fish (Franklin et al. 1995; Narahara et al. 1996). Respiratory adaptations include a very high blood O_2 affinity and a high Q_{10} of metabolic rate adaptive to the severe diurnal temperature and O_2 cycle (Narahara et al. 1996), together with a thin diffusion distance (Maina 1990; Laurent et al. 1995) and an exceptionally high diffusing capacity of the gills for O_2 (Maina et al. 1996). Furthermore, the Lake Magadi tilapia is most unusual among cichlids in that it is capable of supplementary air-breathing during hypoxic conditions (Narahara et al. 1996), facilitated by a well-vascularized physostomus swim bladder that seems to serve as a primitive lung (Maina et al. 1995). Unique ionoregulatory strategies and accompanying gill structural specializations have also been described (Maloiy et al. 1978; Skadhauge et al. 1980; Eddy et al. 1981; Eddy and Maloiy 1984; Wright et al. 1990; Maina 1991; Laurent et al. 1995), and recently urea has been assessed to be a qualitatively important variable component of the tilapia's osmoregulatory complement (Wood et al. 2002*b*).

Perhaps most remarkable is that these unusual morphological and physiological adaptations in *A. grahami* may have evolved within the last 7,000–10,000 yr, when the water chemistry of the lake began to change since the drying of Paleolake Orolonga resulted in the separation of Lakes Natron and Magadi approximately 9,000 yr ago (Butzer et al. 1972; Roberts et al. 1993). Seegers et al. (1999) provide a full discussion of the geological history of the lakes.

Within Lake Magadi, a number of small populations exist in various thermal spring lagoons around the margin of the lake, in apparent isolation from one another, separated by kilometers of solid trona crust (floating Na_2CO_3) underlain by anoxic water (Coe 1966). Despite the apparent isolation of different populations, annual flooding of the trona surface by torrential rainfall may provide opportunities for the exchange of fish across the surface of the trona, resulting in subsequent gene flow. Early descriptions of these flooding events assumed homogenization of lagoon populations based on dead fish on the dried trona following the rains (Coe 1966). This presumed genetic exchange was claimed to have prevented speciation and even the accumulation of subspecific differences. However, subtle morphological differences in the size of the gills between two of the lagoon populations, the well-studied Fish Springs Lagoon and the less-studied South East Lagoon, suggested differentiation of the two populations (Maina et al. 1996). Differentiation between these two populations was confirmed using multilocus DNA fingerprinting and haplotype frequency differences at the mitochondrial DNA (mtDNA) control region (Wilson et al. 2000).

Therefore, the overall goal of this study was to identify additional Magadi tilapia populations, determine the environmental conditions of the specific lagoon systems they inhabit, identify specific adaptations in *Alcolapia* populations, and identify adaptations specific to lagoon systems. Classic cichlid taxonomic descriptions of coloration and jaw morphology, while appropriate in other Rift Valley Lakes such as Malawi, Tanganyka, and Victoria, may not accurately describe significant morphological, physiological, and behavioral adaptations to more severe environmental conditions such as those in Lake Magadi and other soda lakes. For example, Seegers et al. (1999) recently separated *A. grahami* of Lake Magadi from the Lake Natron species *A. alcalicus* based on different mitochondrial DNA (mtDNA) lineages and the allopatric (geographically separate) nature of the two lakes, as the morphology of these two fish was very similar.

An expedition to Lake Magadi in 1997 provided us with the opportunity to identify and characterize cichlid populations from six lagoons around Lake Magadi, as well as two additional populations from neighboring lakes, Little Magadi (only about 0.5 km to the northwest but elevated by 20 m above Lake Magadi) and Lake Natron, Tanzania (about 30 km to the south of Lake Magadi). The specific objectives of this study were to examine the hypothesis that gene flow is restricted and separate locally adapted populations are evolving by (1) quantitating the genetic relationship of *Alcolapia* populations with a focus on Magadi lagoon populations using the control region of the mitochondrial DNA; (2) describing the environmental condi-

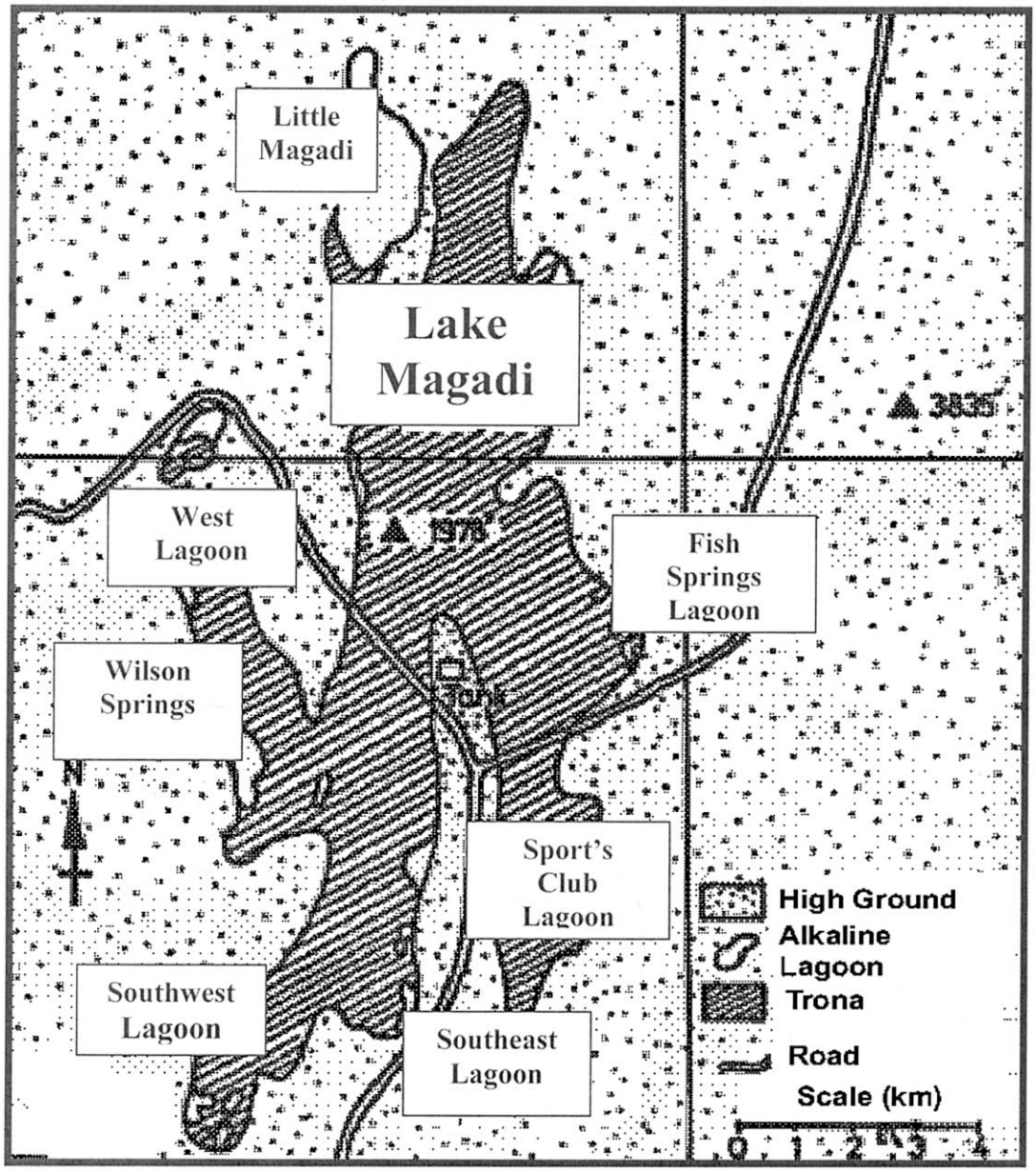

Figure 1. Map of Lake Magadi, Kenya, indicating the Fish Springs Lagoon, Sports Club Lagoon, South East Lagoon, South West Lagoon, Wilson Springs, West Lagoon, and Little Magadi. Open water (alkaline lagoon) and trona are indicated on the map; triangles indicate elevation in meters above sea level; "tank" refers to a water storage tank. *Horizontal line* = latitude 1°50′S; *vertical line* = longitude 36°20′E (adapted from Maina et al. 1996).

tions and chemistry of the water in which these populations live; (3) characterizing populations of the Lake Magadi basin, including Little Magadi, and Lake Natron using physiological, morphological, and behavioral characters (a particular focus here was on whether the fish were ureotelic and expressed the OUC); and (4) examining the concordance between the genetic differences and specific phenotypic differences.

Material and Methods

Samples of cichlids were collected in January and February 1997 using seine or hand nets from six lagoon populations in Lake Magadi, Kenya (Fig. 1): the Fish Springs Lagoon ($n = 22$); the Sports Club Lagoon ($n = 46$); the South East Lagoon ($n = 20$); the South West Lagoon ($n = 21$); the Wilson Springs La-

goon ($n = 11$); and the West Lagoon ($n = 17$). Fish from Little Magadi (taken near shore from the northern area of open water; $n = 21$) and Lake Natron, Tanzania (taken from a stream in the Shombole region running into the northern end the lake; $n = 27$), were also collected.

The mtDNA control region haplotypes of Lake Magadi, Little Magadi, and Lake Natron tilapia were identified using single-stranded conformational polymorphism (SSCP) analysis and DNA sequencing of the haplotypes according to the methods and nomenclature of Wilson et al. (2000). An analysis of molecular variance using the software package AMOVA, version 1.55 (Excoffier et al. 1992), was used to assess the partitioning of haplotypic genetic variation and population structure. The program Arlequin (Schneider et al. 1999) was used to generate a minimum-spanning tree (Excoffier et al. 1992).

To assess the demographic history of the *Alcolapia* populations, we examined parameters associated with mismatch distributions of pairwise sequence differences (Slatkin and Hudson 1991; Rogers and Harpending 1992; Rogers 1995). Population parameters were estimated using Arlequin (Schneider et al. 1999). We applied a nonlinear least-squared approach (Schneider and Excoffier 1999) to generate the test statistic sum of squares deviations (SSD) and estimate the population parameters based on the mismatch distributions. The parameter tau (τ) in mutational units was used to estimate the timing of past demographic events using the equation $\tau = 2\mu t$, where μ is the mutation rate for the sequence and t is the number of generations since the expansion (Excoffier and Schneider 1999). The parameter theta (θ_1) was used to assess the effective female population size using the equation $\theta_1 = 2\mu N_1$, where N_1 is the effective population size following the population expansion.

The water chemistry and conditions of each Magadi lagoon system and Little Magadi and Lake Natron sites were characterized for pH, temperature, total CO_2 concentrations, sodium, chloride, and osmolality (mOsm kg^{-1}). Additional measurements were taken along a transect near the Sports Club Lagoon site, following the flooding of Lake Magadi.

Water pH, temperature, total CO_2, chloride, and P_{O2} were measured on-site using equipment and methods identical to those described by Wood et al. (1994). Alkalinity, expressed as HCO_3^- equivalents (i.e., $[HCO_3^-] + 2[CO_3^=]$) was calculated from pH and total CO_2 measurements using values for αCO_2 and pK^I and pK^{II} at the appropriate temperature, chlorinity, and ionic strength from Skirrow (1975). Samples were frozen and transported back to McMaster University for measurement of sodium (by atomic absorption spectroscopy [AAS], Varian 1275-AA), osmolality (by vapor pressure osmometry, Wescor 5100A), and additional chloride determinations (by coulometric titration, Radiometer CMT10).

Fish were sampled from each of the identified field populations (with some exceptions) to determine the normal levels of whole-body ions (Na^+, Cl^-), whole-body urea-N, and hepatic enzyme activities. Individuals of each population (5–10) were killed by cephalic concussion, blotted dry, then immediately freeze-clamped in liquid nitrogen for later determination of whole-body urea-N, Na^+, and Cl^- concentrations. At the field laboratory, each frozen fish was weighed and then ground to a fine powder under liquid nitrogen, and a weighed aliquot of the frozen powder was extracted in nine volumes of 10% trichloracetic acid at 4°C for 30–60 min. The extract was centrifuged and assayed on-site for urea-N by the colorimetric diacetyl monoxime method (Rahmatullah and Boyde 1980). The extracts were returned to McMaster University where they were appropriately diluted and assayed for Na^+ by AAS (as above) and Cl^- by the colorimetric assay of Zall et al. (1956).

The liver from six additional fish from each population was immediately excised, freeze-clamped in liquid nitrogen, and weighed and prepared for transport in liquid nitrogen. Hepatosomatic index was calculated as the liver weight as a percentage of body weight. The activities of the following hepatic enzymes involved in the OUC and/or contributing to other aspects of nitrogen metabolism were determined at 30.0 ± 0.2°C, according to Mommsen and Walsh (1989): glutamine synthetase (GSase), ornithine-citrulline transcarbamoylase (OTC), arginase (ARG), glutamate dehydrogenase (GDH), alanine aminotransferase (AlaAT), and aspartate aminotransferase (AspAt). GLNase (glutaminase) was determined according to Curthoys and Lowry (1973), and argininosuccinate synthetase/argininosuccinate lyase (AS/AL; as a coupled reaction) and carbamoyl phosphate synthetase III (CPSase III) metabolism were determined according to Anderson and Walsh (1995).

Urea production appears to be a function of metabolic rate (Wood et al. 1994). Therefore, to determine the extent of ureotelism in the various populations, measurements of urea-N ($M_{Urea\text{-}N}$) and ammonia-N ($M_{Amm\text{-}N}$) excretion rates and O_2 consumption rates (Mo_2) were performed under conditions as close to those in the field as possible. Two approaches were used in parallel for some sites; for others, practical constraints dictated that only one or the other was used. Both employed the (500 mL) Tusker chamber system described by Wood et al. (1994). Immediately after capture, one batch of fish from each site ($N = 8$–10) were placed into individual Tusker chambers filled with water from Fish Springs Lagoon (see Table 1 for composition). This water had been preequilibrated with O_2 so as to allow the flux measurement to be started while the fish were being transported back to the field laboratory, a trip of 0.5–2.0 h (depending on the site) before the start of aeration. The other batch of fish from each site ($N = 8$–10) were transported back to the laboratory in a large bucket of their specific lagoon water, aerated with a battery-driven pump. Immediately upon return to the laboratory, the Tusker chambers were filled with this water, individual fish were added, aeration commenced, and the flux measurements were started. In both cases, total flux periods for urea-N and ammonia-N were 3–5 h; in the middle of the period, aeration was suspended, and the chamber sealed for 40 min to allow the measurement of O_2 consumption.

Table 1: Frequencies of mitochondrial DNA control region haplotypes from *Alcolapia* populations

mtDNA	Fish Springs Lagoon	Sports Club Lagoon	South East Lagoon	South West Lagoon	Wilson Springs	West Lagoon	Little Magadi	Lake Natron
A	10 (.455)	30 (.652)	11 (.550)	5 (.238)	6 (.545)	8 (.471)	0	0
B	10 (.455)	7 (.152)	6 (.300)	8 (.381)	3 (.273)	7 (.412)	20 (.952)	17 (.630)
C	2 (.090)	2 (.043)	2 (.105)	8 (.381)	1 (.090)	1 (.059)	0	0
D	0	0	0	0	0	0	0	7 (.259)
E	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	1 (.037)
G	0	0	0	0	0	1 (.059)	0	0
H	0	0	0	0	1 (.090)	0	0	0
I	0	6 (.130)	1 (.050)	0	0	0	0	0
J	0	1 (.022)	0	0	0	0	0	0
K	0	0	0	0	0	0	1 (.095)	0
L	0	0	0	0	0	0	0	1 (.037)
M	0	0	0	0	0	0	0	1 (.037)
Total	22	46	20	21	11	17	21	27

At the end of the experiment, exact time for the N-flux was noted, terminal water samples were taken for comparison to initial, and the fish were weighed. A chamber containing appropriate water but no fish served as a blank.

Urea-N and ammonia-N concentrations in water were measured colorimetrically on site by the diacetyl monoxime method of Rahmatullah and Boyde (1980) and the salicylate-hypochlorite method of Verdouw et al. (1978), respectively. In each case, it proved necessary to make up urea-N and ammonia-N standards in the appropriate lagoon water, and the assays were modified by the addition of an extra 1 mL of lagoon water to bring the volume to 3 mL so the samples could be read on a Spectronic 20 spectrophotometer (Bausch and Lomb). Nitrogen excretion rates ($M_{\text{Urea-N}}$, $M_{\text{Amm-N}}$) were calculated from the increases (blank-corrected) in water urea-N and ammonia-N concentrations over the entire flux period, factored by mass and time. Samples for water Po_2 were analyzed using a Radiometer pHM 71 gas analyzer and Radiometer O_2 electrode at the experimental temperature. Po_2 values were converted to O_2 concentrations using αO_2 values appropriate to the temperature and salinity from Boutilier et al. (1984). Mo_2 was calculated from the decrease (blank-corrected) in O_2 during the period of chamber closure, factored by mass and time.

In light of findings with respect to the chemistry at one site, Sports Club Lagoon, several tolerance tests were performed using water collected from the site. In addition, standardized tolerance tests were performed with each population (except Wilson Springs). The standardized test medium was designed to simulate a severe natural water chemistry and used Fish Spring Lagoon water (see Table 1 for composition) as a base. The test medium was made by adding sufficient NaCl to Fish Spring Lagoon water to raise measured Cl^- levels to 1,000 mM and sufficient processed "trona powder" (largely $NaHCO_3$ and $(Na)_2CO_3$ plus other minor natural salts, kindly supplied by Magadi Soda) to raise measured total CO_2 levels to 1,000 mM, respectively. The final Na^+ level was about 2,800 mmol L^{-1}, osmolality was approximately 3,300 mOsm, alkalinity was about 1,980 meq L^{-1}, and pH was approximately 10.35. In all tolerance tests, the water was oxygenated before use and equilibrated to 32°–35°C to ensure that only the osmotic pressure would be cause of toxicity. Freshly collected fish were returned to the field laboratory in their natural water as for the respirometry experiments. When possible, two tests were done: one test with fish collected the same day (allowed to settle for several hours) and a second test with fish acclimated to Fish Spings Lagoon water for at least 24 h. Fish were placed in a bucket containing 5 L of the test medium; timed observations of mortality were continued until all fish were dead, allowing calculation of median lethal time (LT50).

Data are expressed as means ± 1 SEM (N), where n = number of fish, and multiple comparisons performed by one-way ANOVA followed by the Bonferroni post hoc test for multiple comparisons to identify specific differences.

Results

Genetic Analysis

Mitochondrial control region haplotypes (350 bp) that were previously identified in Magadi/Natron tilapia (Wilson et al. 2000) were observed within the 1997 sample collection with the identification of a number of additional haplotypes. One haplotype from the previous study was not observed in the 1997 samples, specifically haplotype E (Wilson et al. 2000; Table 1; Fig. 2). Haplotype B was present in all the lagoon populations in Lake Magadi and both Little Magadi and Lake Natron. Haplotypes A and C were present in all the Lake Magadi lagoon

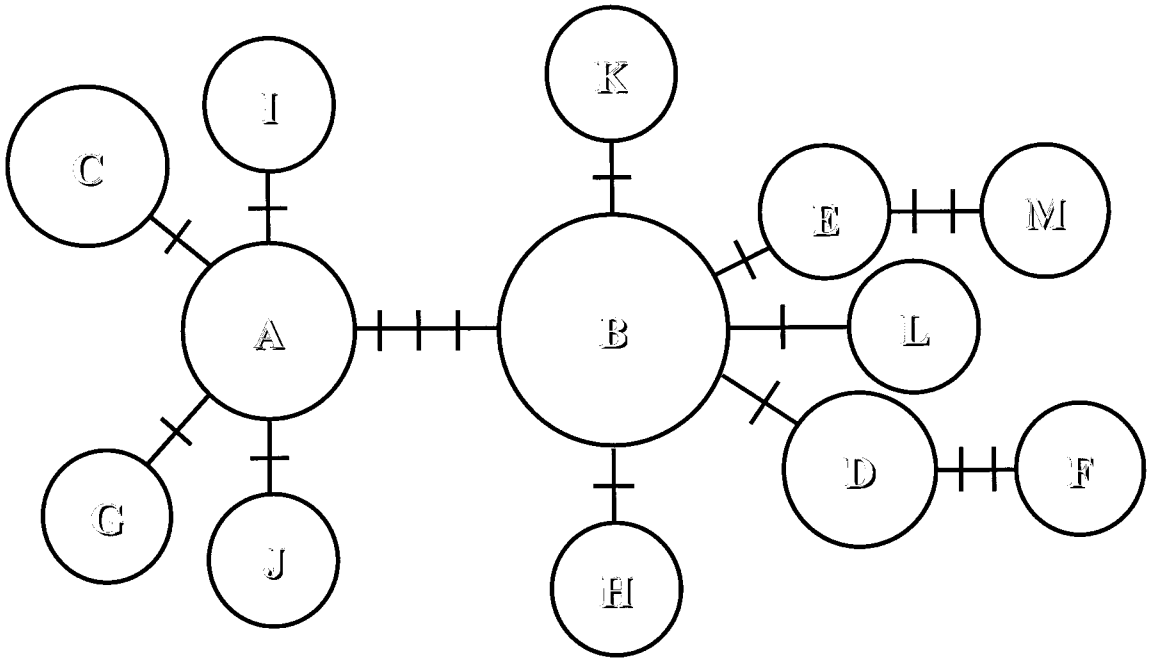

Figure 2. Minimum-spanning tree (Excoffier et al. 1992) of *Alcolapia* mitochondrial DNA control region sequences, where vertical lines indicate number of nucleotide sequence changes between haplotypes.

populations. Several lagoon- or lake-specific haplotypes were also identified.

Within each Magadi lagoon population, haplotypes A and B were the most prevalent sequences, with greater than 75% of the tilapia containing one of these two sequences in the majority of populations. Haplotype C was maintained at levels of 5%–10% and as high as 30% in the South West Lagoon population. Haplotype B was prevalent in the two other lake systems, Little Magadi and Lake Natron. A number of rarer haplotypes were observed in almost all the populations we examined.

We applied an analysis of molecular variance (AMOVA; Excoffier et al. 1992) to the haplotype data to assess the partitioning of genetic diversity into the various lakes and populations within the lake systems and the degree of population structuring among the different populations. The first comparison examined the partitioning of haplotypic diversity among *Alcolapia* species from Lakes Natron and the Magadi basin, including the lagoon systems of Lake Magadi and Little Magadi. The variance among lakes (11.13%) was slightly lower than the variance among the populations of the Magadi basin (16.79%), with the majority of the diversity (72.08%) being within populations. The ϕ_{ST} value of 0.279, analogous to Wright's F_{ST} statistic, indicates structuring consistent with the inclusion of three isolated lake systems. The second comparison examined partitioning within the Magadi lake basin by comparing Lake Magadi lagoon populations to Little Magadi. The variance between the two lakes (35.20%) was higher than the comparison that included Lake Natron. The variance existing among populations within the lakes was 4.34%, with 60.46% of the variance being partitioned within the tilapia populations. The ϕ_{ST} value of 0.395 was higher in the absence of Lake Natron when comparing the Magadi lakes. A ϕ_{ST} value of 0.055 was observed among the lagoon populations indicating minimal population structuring with 5.54% of the variance existing among lagoon populations and 94.46% existing within the lagoon populations.

Although the ϕ_{ST} value that included all the Magadi populations indicated low levels of structuring, we assessed the potential genetic exchange of fish among the specific Magadi lagoon populations by means of pairwise comparisons of populations in an AMOVA analysis (Excoffier et al. 1992). An assessment of structuring using a ϕ_{ST} statistic indicated differential amounts of structuring among the lagoon populations of Lake Magadi (Table 2). Our interpretation of the ϕ_{ST} estimates was that a value of approximately 0.050 suggested low levels of differentiation with levels higher than 0.100 supporting substantial levels of structuring (Wright 1978). All comparisons of the Magadi lagoon populations to the different lakes, Little Magadi and Natron, indicated substantial structuring. Within Lake Magadi, populations such as the South West Lagoon indicated some level of structuring among all the lagoon populations within Magadi. A number of pairwise comparisons indicated very low amounts of structuring between lagoon populations. The overall pattern of the potential movement of fish did not correspond well with geography, i.e., neighboring la-

Table 2: ANOVA of *Alcolapia* populations estimated by AMOVA (Excoffier et al. 1992) on pairwise comparisons of ϕ_{ST} as an indicator of population structure

	Fish Springs Lagoon	Sports Club Lagoon	South East Lagoon	South West Lagoon	Wilson Springs	West Lagoon	Little Magadi	Lake Natron
Fish Springs Lagoon	…							
Sports Club Lagoon	.0906	…						
South East Lagoon	−.0192	.0015	…					
South West Lagoon	.0559	.2111	.0844	…				
Wilson Springs	−.0295	−.0089	−.0654	.0701	…			
West Lagoon	−.0491	.0624	−.0347	.0643	−.0482	…		
Little Magadi	.3769	.5561	.5001	.3872	.5620	.4193	…	
Lake Natron	.1893	.3941	.2756	.1883	.2719	.1990	.1728	…

goon populations, which would be predicted to have more fish exchanged during heavy flooding.

An SSD statistic (Schneider and Excoffier 1999) was used to infer the demographic history of populations based on the mismatch distribution. The SSD statistic significantly rejected population expansion for Fish Springs and West Lagoon and approached significant rejection for South East, South West, and Wilson Springs (Table 3). Rejection of a population expansion in the above lagoons was further supported by overlap in the 90% and 95% confidence intervals of θ_0 and θ_1 in the SSD analysis using a coalescence model (Schneider et al. 1999). A past population expansion could not be rejected for Sports Club based on the mismatch distribution parameter and was supported for Little Magadi and Natron at all three parameters. Analyzing the mismatch distribution of all of the Magadi populations, assuming panmixia, indicated a rejection of population expansion consistent with the majority of lagoon populations analyzed independently.

Estimates of the time for specific demographic events required an estimate of the substitution rate. A rate of 5.56×10^{-8} site^{-1} yr^{-1} (Nagl et al. 2000) is the recent estimate substitution rate for cichlids, while 1.65×10^{-7} site^{-1} yr^{-1} (Ward et al. 1991; Excoffier and Schneider 1999) has been used as the substitution rate for the mtDNA control region of various taxa, including humans. We applied these rates to generate an estimate of the time of the historic demographic event (Table 3). The rate of 1.65×10^{-7} site^{-1} yr^{-1}, assuming a generation time of 60 d for *Alcolapia*, provided estimates of the age of historic demographic events to approximately 10,000 yr for all the Magadi lagoon populations and approximately 5,500 yr ago for Little Magadi and Lake Natron. As the majority of Magadi lagoon populations had expansion signals that were rejected, the values of the timing of the demographic event may be biased. A generation time of 60 d is a conservative estimate based on estimates of 42 d interpreted from Coe (1966).

Estimates of female effective population size (θ_1) from the mismatch distribution analysis were generated and compared to field observations (Table 4). Field estimates of female effective population size assumed gender ratio and an effective population/census population ratio of 50 : 50, although the ratio of effective-to-census population size may be as low as 10% in many natural populations. The population sizes in Little Magadi and Lake Natron were difficult to assess as they represented more lakelike systems. The three largest populations in Lake Magadi (Fish Springs, South West, and West Lagoons) were in reasonable proximity with both expected N_F (female effective

Table 3: Mismatch distribution analysis of expansion times (τ) and SSD statistics of population expansion and estimates of historic demographic events in *Alcolapia* populations

Population	τ[a]	SSD[b]	t (yr)[c]	t (yr)[d]	t (yr)[e]
Fish Springs	5.348	.020	173,636	28,752	9,759
Sports Club	5.526	.470	179,415	29,902	10,083
South East	5.445	.070	176,785	29,464	9,936
South West	5.855	.060	190,097	31,682	10,684
Wilson Springs	5.510	.070	178,896	29,816	10,054
West Lagoon	5.250	.010	170,454	28,409	9,580
Lake Magadi	5.344	.440	173,182	28,731	9,752
Litte Magadi	3.000	.180	97,402	16,233	5,474
Lake Natron	3.008	.300	97,662	16,172	5,489

[a] Expansion time (t) in years obtained from the expansion time in units of mutation rate ($\tau = 2\mu t$), assuming a generation time of 1 yr.

[b] Expansion time expressed in units of mutation rate ($\tau = 2\mu t$), where μ is the mutation rate and t is the number of generations since the expansion or demographic event. P values given for SSD statistic (Schneider and Excoffier 1999) estimated by simulated demographic parameters and compared to observed parameters. The P value is the proportion of expected distributions larger than the observed distribution.

[c] Expansion time (t) in years obtained from the expansion time in units of mutation rate ($\tau = 2\mu t$), assuming a mutation rate of 5.56×10^{-8} site^{-1} yr^{-1} and a generation time of 1 yr.

[d] Expansion time (t) in years obtained from the expansion time in units of mutation rate ($\tau = 2\mu t$), assuming a mutation rate of 5.56×10^{-8} site^{-1} yr^{-1} and a generation time of 60 d.

[e] Expansion time (t) in years obtained from the expansion time in units of mutation rate ($\tau = 2\mu t$), assuming a mutation rate of 1.65×10^{-7} site^{-1} yr^{-1} and a generation time of 60 d.

Table 4: Estimates of female effective population size (N_F) using the mismatch distribution parameter θ_1 and field estimates

Population	θ_1	Expected N_F[a]		Estimated Mean Time to Fixation (yr)	Field Estimate N_F	Estimated Mean Time to Fixation
		$\mu = 3.36 \times 10^{-7}$	$\mu = 9.90 \times 10^{-7}$			
Fish Springs	3.091	16,618	5,640	2,000	>5,000	1,500
Sports Club	5.526	21,709	10,083	3,500	250	100
South East	5.445	21,274	9,936	3,500	250	100
South West	3.821	20,543	6,972	2,400	>5,000	1,500
Wilson Springs	3.330	17,903	6,076	2,000	250	100
West Lagoon	3.457	18,586	6,308	2,000	>5,000	1,500
Lake Magadi	2.355	12,554	4,261	1,500	≈16,000	5,000
Little Magadi	.111	597	200	100	…[b]	…[b]
Lake Natron	1.573	8,457	2,870	1,000	…[b]	…[b]

Note. Estimated times to fixation of a single mitochondrial haplotype based on N_F were calculated.

[a] Estimate based on mismatch distributions where $\theta_1 = 2N_1\mu$, where θ_1 is the parameter for population size, N_1 is the present effective population size, and μ is the mutation rate per haplotype. The mutation rate per haplotype is estimated using the substitution rate per site by the number of base pairs being analyzed. Substitution rates of 5.56×10^{-8} and 1.67×10^{-7} were used based on cichlid (Nagl et al. 2000) and human control region estimates (Ward et al. 1991), respectively, and to account for the rapid generation time among Magadi tilapia, i.e., approximately 60 d (Coe et al. 1966).

[b] Lake conditions made field estimates difficult.

population size) and the field estimates numbering into thousands of fish. However, the three smaller populations (Sports Club, South East, and Wilson Springs Lagoons) had 10–20-fold lower field estimates than values from the mitochondrial mismatch distributions.

Based on both estimates of the female effective population size (N_F), the mean time to fixation was estimated for a given mtDNA haplotype (Kimura and Ohta 1969; Parker and Kornfield 1997). Consistent with estimates of τ and the formation of Lake Magadi 9,000 yr ago, the effective female population sizes were more consistent with field estimates in the large populations using a substitution rate approximately three times faster than recent published estimates for cichlids (Nagl et al. 2000; Table 4). Estimated fixation times for mitochondrial DNA at female effective population sizes (N_F) based on the accelerated substitution rate ranged from 100 yr (600 generations) in Little Magadi, to almost 3,500 yr in other populations. Fixation times based on field estimates ranged from 100 yr in small lagoon populations to 1,500 yr in larger populations. A discrepancy among expected and field estimates of N_F fixation was observed in the small lagoon populations of Sports Club Lagoon, South East Lagoon, and Wilson Springs. Assuming that lagoon fish were exchanged constantly every 2–3 generations within Lake Magadi, the estimates of expected and field N_F values would have resulted in fixation to one haplotype potentially twice within the history of the lake based on field estimates and several times based on the mismatch distribution population parameter N_1.

Morphological Characterization

Morphological differences were observed in this study among the *Alcolapia* populations. There were very distinct differences in relative gut length among four populations that were examined in detail. The mean gut length/body length ratio ranged from 2.956 ± 0.103 in Fish Springs Lagoon down to approximately 1.240 ± 0.147 in the South East Lagoon population ($P < 0.05$). South West Lagoon and Lake Natron fish exhibited intermediate ratios of approximately 1.8. Fish from Little Magadi were distinctly different in having a superior (i.e., upward slanted) mouth whereas all six Lake Magadi populations, as well as the Lake Natron fish, exhibited a simple terminal mouth.

Water Conditions at Different Sites and Physiological Characterization

The six Magadi lagoon systems, Little Magadi, and Lake Natron exhibited a relatively stable range of temperature (32°–42°C) and pH (9.1–10.1) but marked differences in osmolality, ionic concentrations, and alkalinity (Table 5). Using the Fish Spring Lagoon site as a reference, where the osmolality was about 50% that of full-strength seawater, osmolalities varied from less than half this value at Wilson Springs, to threefold this value at Little Magadi. Among the different sites, Na^+, Cl^-, total CO_2, and alkalinity also varied in approximately the same order as osmolality, although there was obviously not a constant ratio of ions among the various sites. Most notable were alkalinities in excess of 1,200 meq L^{-1} (in comparison to about 3 meq L^{-1}

Table 5: Values of water chemistry (for areas containing fish) measured from multiple replicates (typically 3–10)

Population	pH	ΣCO_2 (mmol L^{-1})	$[Cl^-]$ (mmol L^{-1})	$[Na^+]$ (mmol L^{-1})	Osmotic Pressure (mOsm kg^{-1})	Alkalinity (mEq L^{-1})
Fish Springs	9.86	216	113	355	581	378
Sports Club	10.05	835	693	978	1,465	1,625
South East	9.55	245	203	326	617	402
South West	9.65	226	190	454	884	380
Wilson Springs	9.60	120	46	183	278	184
West Lagoon	9.13	254	156	390	695	350
Little Magadi	9.32	834	192	372	1,689	1,251
Lake Natron	9.91	189	60	193	442	323

in full-strength seawater) in both Little Magadi Lake and Sport Club Lagoon (in Lake Magadi), associated with very different Na^+ and Cl^- concentrations but similar osmolalities at the two sites. The values reported in Table 5 for Sports Club Lagoon represent the maxima where fish occured; as outlined in detail subsequently, there was substantial chemical and physical heterogeneity within this environment.

Measurements of $M_{Urea\text{-}N}$ and $M_{Amm\text{-}N}$ excretion rates demonstrated that fish from all populations tested within Lake Magadi, and also both Lake Natron and Little Magadi, were 100% ureotelic (Fig. 3). $M_{Amm\text{-}N}$ was not significantly different from zero, but $M_{Urea\text{-}N}$ varied almost threefold among different populations (Fig. 3). There was no obvious relationship to the chemical severity of the environment, as indicated, for example, by osmolality (Fig. 3). $M_{Urea\text{-}N}$ data were normalized for differences in Mo_2 according to Wood et al. (1994), such that N/O_2 ratios in the range of 0.18–0.24 were typical (Fig. 4). Only the South East Lagoon fish exhibited significantly higher N/O_2 at 0.33, correlating with very high levels of ammonia-N in the water (271 μmol L^{-1}) due to the abundance of flamingo guano at this collection site. Water ammonia level was undetectable (<2 μmol L^{-1}) at all other sites.

For three populations (Little Magadi, South East Lagoon, and Lake Natron), measurements were started in the field in Fish Springs Lagoon water and also performed in their natural waters using separate batches of fish at the laboratory. While there were differences in absolute values of $M_{Urea\text{-}N}$ between the two measurements, these disappeared after normalization to Mo_2. Therefore, Figures 3 and 4 illustrate data for tests performed in the natural waters of each population except for Sports Club Lagoon (where the natural water proved toxic, as discussed below).

Assay of hepatic enzyme activities demonstrated a full complement of the enzymes of the Krebs OUC (CPSase III, OTC, AS/AL, ARG) as well as the N-feeder enzymes (GSase, ALAat, and ASPat) and GDH in all populations examined (Table 6). These included Little Magadi, Lake Natron, and four Lake Magadi populations—Fish Springs Lagoon, Sports Club Lagoon, South East Lagoon, and South West Lagoon. GLNase was low and variable. There were some significant variations in specific enzyme levels among populations, but nothing that correlated consistently with either $M_{Urea\text{-}N}$ (Figs. 3, 4) or the chemistry of the respective environments.

With the exception of Little Magadi, whole-body Na^+ and Cl concentrations were rather invariant among the different populations (Table 7), though the Wilson Springs fish, which inhabit the most dilute environment (Table 5), did have slightly lower whole-body ions. Apart from this, whole-body ion levels were not reflective of the concentrations in the water (Table 8), suggesting homeostatic regulation. Little Magadi fish were a clear exception (Table 9), with whole-body ions approximately threefold greater than those in the other populations, even though water Na^+ and Cl^- levels were comparable to levels at several other sites and by no means the highest overall (Table 7).

Variations in whole-body urea-N levels among populations were pronounced and much more consistent with water osmolarity variations: urea-N was lowest in Wilson Springs fish, where environmental osmolality was lowest, and highest in Little Magadi fish, where osmolality was highest (Table 7). Overall, there was a strong positive relationship (Fig. 5*A*) between whole-body urea-N and environmental osmolality, suggesting that urea plays a small but important role as a regulated osmolyte.

Surprisingly, we found that when fish from Sports Club Lagoon were collected and placed in what was ostensibly their own water (i.e., water collected from the site), they were all dead within 3 h, so it was impossible to run the metabolism experiments reported in Figures 3 and 4 in site water for this population. Furthermore, when fish from Fish Springs Lagoon were placed in Sports Club water, they were all dead within 5 min, suggesting a marked difference in tolerance between the two populations. These results stimulated us to evaluate the

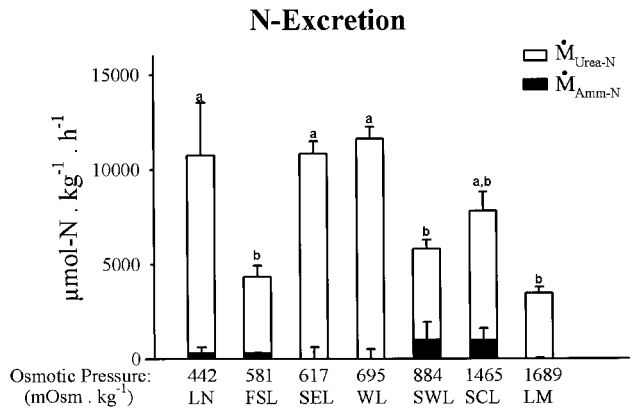

Figure 3. Mean rates of urea-N excretion ($\dot{M}_{Urea-N}$) and ammonia-N ($\dot{M}_{Amm-N}$) in different *Alcolapia* populations (*LN* = Lake Natron; *FSL* = Fish Springs Lagoon; *SEL* = South East Lagoon; *WL* = Western Lagoon; *SWL* = South West Lagoon; *SCL* = Sports Club Lagoon; *LM* = Little Magadi). The corresponding osmolalities of the environments are shown. Means + 1 SEM (N = 8–10). Means sharing the same letter are not significantly different.

tolerance of different populations to challenge with severe environmental chemistry.

The "toxic" water from Sports Club Lagoon had the following composition: Cl^- 1,025 mmol L^{-1}, Na^+ 2,400 mmol L^{-1}, total CO_2 1,066 mmol L^{-1}, alkalinity 2,130 meq L^{-1}, osmolality 3,400 mOsm kg^{-1}, and pH 10.03. At the time, we were concerned that cyanobacterial toxins in this natural water might be the cause of toxicity. Therefore, we made up an artificial standardized challenge medium as outlined in "Material and Methods," which replicated this chemistry: Cl^- 1,000 mmol L^{-1}, Na^+ 2,800 mmol L^{-1}, total CO_2 1,000 mmol L^{-1}, alkalinity 1,980 mequiv L^{-1}, osmolality 3,300 mOsm kg^{-1}, pH 10.35. Fish from all populations except West Lagoon were tested in separate, duplicate runs, and in all cases, 100% mortality occurred. Median lethal time (LT50) varied from a low of 2 min for Wilson Springs to a high of approximately 30 min for fish from Sports Club Lagoon and Little Magadi, with Fish Spring Lagoon fish intermediate at 11 min (Table 9). Among the different populations, LT50 was well correlated with the osmolality of their natural environment (cf. Table 5) and with whole-body urea concentration (Fig. 5*B*).

We then reexamined the water chemistry and fish distribution in the Sports Club Lagoon and found considerable heterogeneity in both. This whole lagoon in January–February 1997 was only about 165 m along the shore and 30 m wide, and fish appeared to be restricted to the inshore 10 m, where depth was only 2.5 to 5.0 cm. We identified two sources of spring-fed water: hot, very alkaline springs at the bottom of the lagoon (one sampled: 45.3°C, Cl^- 1,283 mmol L^{-1}, Na^+ 4,500 mmol L^{-1}, total CO_2 1,329 mmol L^{-1}, alkalinity 2,618 meq L^{-1}, osmolality >6,000 mOsm kg^{-1}, pH 9.91) and a single, much fresher water spring at the shoreline (37.0°C, Cl^- 20 mmol L^{-1}, Na^+ 102 mmol L^{-1}, total CO_2 48 mmol L^{-1}, alkalinity 69 meq L^{-1}, osmolality 153 mOsm kg^{-1}, pH 9.80) that provided cooler, less alkaline water along the lagoon surface. Evaporative cooling at the surface plus photosynthesis by free-living cyanobacteria in the surface waters was also occurring. Distinct temperature and O_2 gradients with depth were observed; for example, at one site 40.6°C, Po_2 290 Torr (approximately twice air saturation) at the surface and 44.5°C, Po_2 8 Torr (almost anoxic) at the 4.0 cm bottom. More importantly, there were great differences in Cl^- and total CO_2 concentrations with depth. For example, at one site the following values were found at the surface (Cl^- 71 mmol L^{-1}, total CO_2 123 mmol L^{-1}), whereas from 1.0 to 2.5 cm at the bottom, the values were more than 10-fold higher (Cl^- approximately 1,350 mmol L^{-1}, total CO_2 approximately 1,400 mmol L^{-1}.). At a second site, the differences were even more extreme. The "toxic" water we had collected had unavoidably consisted of mainly this deeper water.

To investigate the question of tolerance further, we ran a test with the Sports Club Lagoon fish to determine whether it was the NaCl or the $NaHCO_3$ + $(Na)_2CO_3$ (i.e., alkalinity) components, or both, which were toxic. Each component was tested separately on eight fish using Fish Spring Lagoon water dosed with NaCl, or dosed with trona powder to raise total CO_2. Both components separately caused 100% mortality within 45 min, though the LT50 was slightly shorter in the trona test.

Upon closer examination, it was clear that the Sports Club fish were exploiting a nontoxic surface layer no more than 1.0 cm deep, and congregating close to the shore edge at the source of the freshwater spring system for the majority of time we observed their behavior. Samples from this area provided the "typical" water chemistry reported for the Sports Club Lagoon in Table 5. When the observers made sudden movements, the fish would briefly enter into the deeper "toxic" water, although

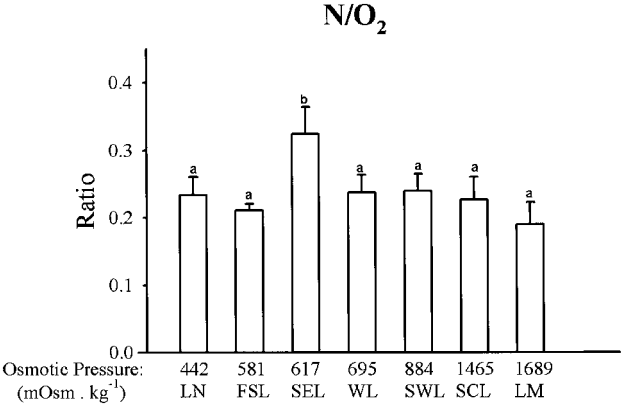

Figure 4. Mean N/O_2 ratios ($[M_{Urea-N}]/[Mo_2]$) in different *Alcolapia* populations. Abbreviations, sample sizes, and other information as in legend of Figure 3.

Table 6: Activities (μmols product/g wet weight of liver/min) of hepatic enzymes of the Krebs ornithine-urea cycle, associated enzymes of N-metabolism, and hepatosomatic index (HSI, % of body weight) in fish from different populations

Enzyme	Fish Springs (6)	Sports Club (3)	South East (6)	South West (6)	Little Magadi (6)	Lake Natron (6)
GSase	7.260 ± 1.220^{A}	5.040 ± .760^{AB}	6.320 ± .430^{A}	7.760 ± .620^{A}	4.260 ± .500^{B}	9.080 ± 1.340^{AC}
CPSase III	.044 ± .005^{A}	.029 ± .012^{A}	.024 ± .008^{A}	.050 ± .003^{A}	.039 ± .013^{A}	.054 ± .009^{A}
OTC	3.44 ± .17^{A}	4.93 ± .17^{B}	4.19 ± .60^{AB}	4.90 ± .31^{B}	3.32 ± .32^{A}	3.44 ± .30^{A}
AS/AL	.018 ± .005^{A}	.014 ± .001^{A}	.021 ± .007^{A}	.019 ± .008^{A}	.024 ± .010^{A}	.008 ± .004^{A}
ARG	36.51 ± 1.87^{A}	25.15 ± 2.72^{AB}	19.86 ± 1.29^{B}	25.57 ± 1.71^{AB}	34.16 ± 4.54^{AB}	26.46 ± 2.10^{AB}
GLNase	.430 ± .190^{A}	ND	.49 ± .16^{A}	.82 ± .16^{A}	.32 ± .17^{A}	.78 ± .25^{A}
GDH	19.49 ± 1.22^{A}	15.83 ± .76^{A}	20.30 ± 1.51^{A}	20.70 ± 1.09^{A}	19.89 ± 1.57^{A}	19.49 ± 1.51^{A}
ALAat	19.35 ± 1.50^{AB}	15.69 ± .54^{B}	18.6 ± 1.33^{AB}	25.04 ± 1.02^{A}	20.44 ± 1.81^{AB}	17.05 ± 1.60^{B}
ASPat	79.43 ± 3.15^{A}	55.89 ± 2.51^{B}	81.46 ± 4.13^{A}	96.48 ± 2.81^{C}	86.47 ± 4.61^{AC}	91.48 ± 4.97^{AC}
HSI	1.09 ± .07^{A}	3.11 ± .65^{AB}	1.91 ± .09^{B}	2.30 ± .16^{B}	1.64 ± .18^{AB}	1.83 ± .10^{B}

Note. Abbreviations include glutamine synthetase (GSase), ornithine-citrulline transcarbamoylase (OTC), arginase (ARG), glutamate dehydrogenase (GDH), alanine aminotransferase (AlaAT), aspartate aminotransferase (AspAt), glutaminase (GLNase), argininosuccinate synthetase/argininosuccinate lyase (AS/AL; as a coupled reaction), and carbamoyl phosphate synthetase III (CPSase III). Values given as means ± 1 SEM (*N*). Means sharing the same letter are not significantly different from one another.

this behavior diminished when a series of movements were made. Thus, the input of "fresher" water into this system appears to be crucial for the survival of this population.

A final laboratory challenge experiment examined the surface behavior in the Sports Club Lagoon fish and whether this behavior existed in another population from Lake Magadi, the Fish Springs Lagoon fish. In a bucket, we duplicated the stratified environment of Sports Club Lagoon by first adding a slightly concentrated version of the standardized toxic challenge medium, and then slowly layering onto it diluted Fish Spring Lagoon water. The stratification was confirmed with Cl^- readings from the surface layer (380 mmol L^{-1}) and depths of 2.5 cm (1,400 mmol L^{-1}) and 5.0 cm (1,400 mmol L^{-1}). The Sports Club cichlids ($n = 6$) swam slowly at the surface, one fish died at 30 min, another at 60 min, and no additional fish died by 4 h when the experiment was terminated. In a similar test with Fish Springs Lagoon fish, the cichlids did not swim at the surface and destroyed the stratification within minutes of starting the experiment. All died, with an LT50 of 18 min. In this test, the initial Cl^- concentrations were approximately 80 mmol L^{-1} (surface), 900 mmol L^{-1} (2.5 cm), and 900 mmol L^{-1} (5.0 cm), respectively.

A sudden rainstorm on the night of January 16–17, 1997, provided an opportunity to evaluate conditions on the flooded surface of the trona, a potential route for intermittent genetic interchange among populations. The transect of Table 8 was performed in a direction perpendicular to shore in the region of Sports Club Lagoon at about 1,600 hours, approximately 12 h after the end of the storm. At this time the depth of the floodwater on the trona was 2.0–6.0 cm, and the elevated levels of total CO_2, Na^+, Cl^-, alkalinity, osmolality, and pH indicated much dissolution of the trona into the rainwater. Based on the results of the standardized challenge tests, the measured water chemistry alone of the trona floodwater would likely have proved lethal (e.g., osmolality approaching 3,000 mOsm kg^{-1}, total CO_2 approaching 1,000 mol L^{-1}, alkalinity approaching 2,000 meq L^{-1}; Table 5), and this would have been exacerbated by temperatures significantly above the upper lethal limit of 42.5°C and a mean Po_2 below the lethal threshold of 16 Torr (cf. Narahara et al. 1996). Considering that the next nearest lagoon was more than 3 km away, the likelihood of fish surviving a journey through this extended zone of toxicity seems highly unlikely.

Discussion

Genetic Structure

Several population comparisons indicated some differentiation based on haplotype frequencies, while other comparisons showed very little differentiation indicative of gene flow among certain lagoons. The observed similarities and differentiation were discordant with the geographic distribution of lagoon systems along the shoreline (Fig. 1; Table 2). These findings contradict previous genetic structure analyses with mtDNA haplotypes and, more important, multilocus DNA fingerprinting analyzing nuclear minisatellite loci that indicated significant population structuring between two of the lagoon populations (Wilson et al. 2000). The discrepancy between the mtDNA results of the two studies may be the result of the lower sample size of one of the lagoon populations within the initial study. Despite this difference, the previous comparison indicated similar predominant A and B haplotype distribution within the two Magadi lagoon populations (Wilson et al. 2000).

The B sequence in all likelihood corresponds to the A1 mtDNA haplotype identified by Seegers et al. (1999) that was present in both Natron and Magadi lake systems. This haplotype was termed the "Orolonga haplotype," as it was interpreted to have existed within the Magadi/Natron paleolake and

Table 7: Whole-body urea-N, chloride, and sodium concentrations in fish from the different populations, sampled in their native waters

Population	Sample Size (N)	Whole-Body Urea Concentrations (μmol N kg^{-1})	[Cl$^-$] (mM kg^{-1})	[Na$^+$] (mM kg^{-1})
Fish Springs	9	22,397 ± 1,562^{AB}	73.5 ± 5.7^{A}	124.9 ± 13.2^{AB}
Sports Club	15	39,291 ± 3,102^{C}	85.3 ± 8.5^{A}	140.7 ± 35.2^{AB}
South East	10	25,633 ± 2,120^{B}	67.8 ± 3.8^{A}	91.6 ± 6.8^{B}
South West	8	35,339 ± 3,508^{C}	67.3 ± 1.9^{A}	121.7 ± 6.0^{A}
Wilson Springs	10	10,889 ± 628^{D}	57.4 ± 2.1^{B}	94.7 ± 6.1^{B}
Little Magadi	10	42,017 ± 2,918^{C}	167.7 ± 10.0^{C}	381.1 ± 32.2^{C}
Lake Natron	10	17,126 ± 2,119^{A}	82.1 ± 5.0^{A}	102.7 ± 12.4^{AB}

Note. Concentrations given as mean ± SEM. Means sharing the same letter are not significantly different from one another.

is the ancestral type for many of the existing haplotypes within the two lake systems at present. Also similar to findings in Seegers et al. (1999) is the presence of a number of Magadi-specific sequences grouping with the haplotype A, likely the corresponding sequence for A17 (Fig. 2). Unlike the findings of Seegers et al. (1999) several apparently Magadi-specific haplotypes appear to have been derived directly from B (A1; Seegers et al. 1999) and not exclusively the A haplotype.

The lack of significant differentiation among the lagoon populations in this study was further complicated by lagoon-specific characteristics (summarized in Table 10) that support locally adapted genomes existing in an allopatric situation within the different habitats. The common A and B haplotype distribution among all six Magadi lagoon populations was particularly surprising given that, in allopatry, we would predict that the lagoon populations should maintain different haplotypes through lineage sorting (Duvernell and Turner 1998). There are two alternative hypotheses to explain the common pattern of mtDNA haplotypes among all the lagoon systems. Firstly, gene flow is prevalent among the lagoon systems during the annual rains and flooding events. Second, a common selection pressure is acting on the mitochondrial DNA maintaining the haplotypes within isolated lagoon systems.

Extensive gene flow among the Magadi lagoon populations during annual flooding events could maintain the common haplotypes through the large metapopulation of tilapia that are subdivided into lagoon populations for the majority of the year and homogenized every three to six generations. This has been proposed by Coe (1966) for the Magadi tilapia; however, Coe's (1969) conclusion for Lake Natron during heavy rains was that deoxygenation of the water due to the flush of blue-green algae resulted in severe mortality and restricted fish to their springs and creeks. Tichy and Seegers (1999) have proposed that barriers to gene flow existed in Lake Natron as a result of deoxygenation and the lethal increase in concentrations of salt and brine, eventually resulting in the re-formation of the soda crust within 48 h. This explanation is consistent with our findings of extreme osmotic pressure following the flooding of Lake Magadi. For the Magadi situation it is likely that the high osmotic pressure of the floodwaters, lethal to all the tilapia we examined under experimental conditions (Table 8), acts as a barrier to dispersal and gene flow among lagoon populations. Although there is more extensive rainfall in the rainy season than we observed in late winter of the current study, the extremely thick trona (15 m in most cases) would appear to be an infinite reservoir of salts to produce this lethal water. The floodwater temperature is also likely an effective barrier to gene flow as a range of 39°–43°C surrounds the lethal limit for Magadi tilapia.

Assuming that gene flow occurs among the lagoons, Magadi tilapia would have to demonstrate phenotypic plasticity to adapt to the differential lagoon systems and express the morphological adaptations we observed, or sufficiently strong selection would be necessary to fix these characters despite gene flow. In general, there is not strong evidence for phenotypic plasticity among the cichlid species flocks for morphological characters. Adaptations in jaw morphology, the character most often studied in cichlids, have been shown to evolve independently (Ruber et al. 1999) and rapidly as a result of independent bones in the flexible jaw structure. However, although this flexibility is a mechanism for rapid divergence, ultimately reproductive isolation, whether allopatric, sympatic, or microallopatric (Meyer 1993), reinforces speciation within cichlid species flocks. The differing morphology we observed among several of the lagoon populations may support selection pressures acting on nuclear genes within each lagoon system, thereby differentiating into locally adapted genomes.

Water Conditions at Different Sites and Physiological Characterization

There was substantial variation in water conditions among the eight different sites studied (Table 5), presumably reflective of the characteristics of the influent hot springs. These measure-

Table 8: Values of water chemistry and temperature along a transect outward across the surface of the trona from Sports Club Lagoon, measured on January 17, 1997, approximately 12 h after a torrential rainfall

Transect Distance (m)	Temperature (°C)	pH	ΣCO_2 (mmol L^{-1})	$[Cl^-]$ (mmol L^{-1})	$[Na^+]$ (mmol L^{-1})	Osmotic Pressure (mOsm kg^{-1})	Alkalinity (mEq L^{-1})
0	37.0	9.97	360	507	1,145	1,627	688
30	39.5	9.91	569	667	1,310	1,858	1,099
50	41.5	9.88	731	865	1,531	2,119	1,421
100	44.6	10.01	1,007	632	1,724	2,316	1,956
150	43.8	10.05	597	622	1,897	2,634	1,167
200	42.0	9.94	638	655	2,414	3,058	1,239
300	40.1	9.93	469	520	2,166	2,967	900

Note. PO_2 averaged 11 Torr (range = 2.5 to 22.5) at the seven sites.

ments were all made at one time of the year (January–February) that intervenes between the "little rains" of December and the "big rains" of March–May. It would be of great interest to know how much variability occurs on a seasonal basis within individual sites, especially during the summer period of greatest aridity (cf. Coe 1966). Consistent features among all sites, however, were the presence of high pHs, alkalinities, and Na^+ and Cl^- levels. Osmolalities were also high but generally much lower than might be predicted by adding up all the measured electrolytes. This is probably explained by substantial ion pairing in solution at these high concentrations.

Fish Springs Lagoon is the only site where water chemistry has been studied previously, and the claim has often been made that the fish here live in one of the most hostile aquatic environments on earth (Johansen et al. 1975; Wood et al. 1994; Narahara et al. 1996). Clearly this claim must be reevaluated in light of the current findings that Sports Club Lagoon and Little Magadi have three- to fivefold higher alkalinities and osmolalities yet still support thriving cichlid populations. The exploitation of a less severe surface microhabitat may provide part of the explanation for the tolerance of the Sports Club Lagoon fish, but the same cannot apply in Little Magadi, which is an open lake. Little Magadi clearly deserves further chemical and biological study because the water ion ratios are very different from those at other sites, with much lower Na^+ and Cl^- relative to alkalinity and osmolality, suggesting the presence of substantial concentrations of other strong electrolytes.

An important physiological finding of this study is that the condition of 100% ureotelism, previously identified only in the Fish Springs population (Randall et al. 1989; Wood et al. 1989), was expressed in all seven of the *Alcolapia* populations (Figs. 3, 4) that were examined (Wilson Springs fish not tested). When expressed as the N/O_2 ratio, urea-N production was uniform across populations (Fig. 4), in accord with earlier work on the Fish Spring Lagoon population (Wood et al. 1994), indicating that urea-N production is normally a function of metabolic rate. The one exception, a high N/O_2 ratio in the South East Lagoon fish (Fig. 4) can be explained by the fact that these fish live in a high ammonia environment due to bacterial ammonia production from the guano-laden benthos. In our tests, these fish actually extracted sufficient ammonia-N from the water to explain the elevated urea-N production. Earlier, this potential

Table 9: Median lethal times (±SEM) for fish from the different populations in a standardized challenge test using two trials when possible

Population	Number of Fish Exposed Directly from Field Conditions	Number of Exposed Acclimated Fish	LT50 (min)
Fish Springs	10	10	11.0 ± 1.0
Sports Club	10	10	30.0 ± 6.0
South East	10	7	10.8 ± 1.3
South West	12	10	16.3 ± 7.8
Wilson Springs	8	…	2.0 ± .0
Little Magadi	11	10	29.5 ± 2.5
Lake Natron	11	…	8.0 ± .0

Note. The trials included fish tested within 1 d of their arrival to the laboratory and fish acclimated to Fish Springs water for at least 1 d (see text for details).

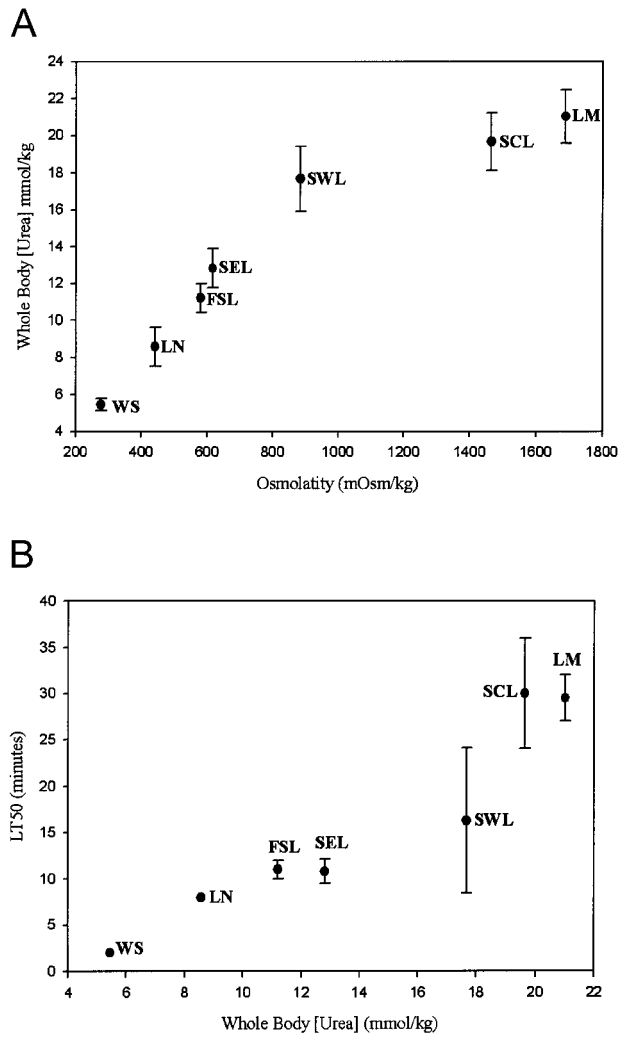

Figure 5. Relationships between (*A*) whole-body urea concentration and environmental osmolality and (*B*) median lethal time (*LT50*) and whole-body urea concentration in standardized challenge tests for the different populations. Means ± SEM. Abbreviations as in legend of Figure 3, and sample sizes as in Table 3 (*A*) and Table 4 (*B*).

for ammonia-N detoxification by elevated urea-N production was demonstrated in laboratory tests with Fish Spring Lagoon specimens where ammonia exposure stimulated urea-N excretion (Wood et al. 1989), but its significance was problematical because elevated environmental ammonia levels have never been found at this site. The South East Lagoon findings demonstrate that this phenomenon has real ecological significance and is an additional adaptive benefit of ureotelism.

These findings suggest that urea production may play a third adaptive role, that of contributing to osmoregulation. Whole-body urea levels correlated extremely well with environmental osmolality in the different populations, as well as with LT50 in the standardized challenge tests (Fig. 5). To date, osmoregulatory ability has been studied only in the Fish Springs Lagoon fish, which maintain an internal osmolality of about 375 mOsm kg^{-1} (Wright et al. 1990; Wood et al. 2002*a*, 2000*b*). The measured whole-body urea levels in these fish (~11 mmol kg^{-1}; Fig. 5), assuming a body water compartment of 65%–70%, accounts for about 5% of internal osmotic pressure. While this is relatively small, its contribution may be significant, as we have found that whole-body urea concentrations (in Fish Spring Lagoon specimens) can be rapidly adjusted in the face of sudden upward or downward osmotic challenge (Wood et al. 2002*a*). The same does not appear to be true of whole-body Na^+ and Cl^- concentrations that were fairly uniform in most populations, but threefold higher in Little Magadi fish (Table 7).

Maintenance of Mitochondrial DNA Haplotypes

The severe floodwater conditions and genetic, morphological, and physiological evidence supports isolation with differentiation as a result of selection pressures among isolated lagoons. These findings are consistent with the maintenance of mitochondrial DNA haplotypes as a result of a common selection pressure among the Magadi lagoon systems. The well-documented geological history of the Lake Magadi basin allows certain predictions of historic population events and the likelihood of maintaining mtDNA haplotypes.

Lake Orolonga was proposed to be present during a period of evolutionary stasis with one predominant tilapia form similar in appearance to *Alcolapia alcalicus* and *Alcolapia grahami* (Tichy and Seegers 1999). This period of stasis was followed by rapid morphological and "neutral" haplotype radiation (Seegers et al. 1999). Seegers et al. (1999) suggest that the *Alcolapia* species flocks may demonstrate elevated rates of mutation, potentially as a result of high environmental stress. We found that the estimates of past demographic events, based on the parameter τ, were consistent with the separation of Lake Orolonga 9,000–10,000 yr ago when using a substitution rate three times higher than presently published estimates for African cichlids (Nagl et al. 2000) and accounting for the rapid generation time of *Alcolapia grahami* (Coe 1966; Table 3). Given that the majority of haplotypes are specific to one or the other of the soda lakes, it seems reasonable that the generation of the haplotypes in both systems occurred following the separation of the paleolake, a point also made by Seegers et al. (1999). Calibrating the substitution rate with the geological evidence and accounting for the rapid generation in the tilapia provides the most biologically realistic estimate for this system.

Selection pressures would likely have intensified as the lake levels lowered, dividing the paleolake and shifting the water source more to the thermal hot springs and less on lake water from a more humid environment at the end of the Pleistocene. Selection would likely have further intensified in Lake Magadi 7,000 yr ago when the salinity increased and separation into

Table 10: Summary of genetic, adaptive, or distinct characteristics among *Alcolapia* populations from Lake Magadi lagoon populations, Lake Natron, and Little Magadi

Population	Character	Reference
Fish Springs Lagoon	DNA fingerprinting indicates significant differentiation with South East Lagoon; differentiation equivalent to comparison to Lake Natron fish	Wilson et al. 2000
Fish Springs Lagoon	Relatively large gut length	This study
South East Lagoon	DNA fingerprinting indicates significant differentiation with Fish Springs Lagoon; differentiation equivalent to comparison to Lake Natron fish	Wilson et al. 2000
South East Lagoon	Increased gill size and modified structure	Maina et al. 1996
South East Lagoon	Relatively small gut size	This study
Sports Club Lagoon	Increased tolerance to high osmotic pressure	This study
Sports Club Lagoon	Relatively high natural whole-body urea concentrations	This study
Sports Club Lagoon	Relatively high HSI values	This study
Sports Club Lagoon	Behavioral adaption to fresh surface water	This study
Little Magadi	Mouth morphology	This study
Little Magadi	Increased tolerance to high osmotic pressure	This study
Little Magadi	Relatively high natural whole-body urea concentrations and NaCl levels	This study
Lake Natron	Mouth morphology and coloration	Seegers et al. 1999; this study

lagoon systems began. Intense selection pressure would have potentially resulted in population bottlenecks both in the larger Lake Magadi basin, followed by the isolation of lagoon environments. Evidence for population bottlenecks was observed in two of the Magadi populations that were previously analyzed, the Fish Springs and South East Lagoons (Wilson et al. 2000). This was in contrast with Lake Natron tilapia that showed reasonably low levels of band-sharing and therefore higher levels of genetic variation not suggestive of past population bottlenecks. SSD statistics (Schneider and Excoffier 1999) did not support a historic population expansion in the majority of lagoon populations, consistent with potential bottlenecks. Independent population bottlenecks specific to lagoon systems and/or small effective population sizes are predicted to result in lineage sorting of mtDNA haplotypes (Duvernell and Turner 1998) resulting in differential haplotype distributions among isolated lagoons. The ubiquitous nature of the common A and B haplotypes and to a lesser extent the C haplotype suggests the maintenance of these haplotypes despite potential isolation, historic bottlenecks, and small effective population sizes.

Estimates of N_F based on nucleotide diversity and a mismatch distribution analyses appeared consistent with field estimates for the larger lagoon populations (Fish Springs, South West, and West Lagoon) in that the numbers were in the 5,000–10,000 range (Table 4). Discrepancies were apparent for estimates of the smaller lagoons, where highly conservative field estimates were no higher than 250 female breeding fish and estimates from mtDNA control region sequences were 20–30 times higher. Considering the rate that mtDNA haplotypes are predicted to go to fixation based on the rapid generation time, all the lagoons should have gone to fixation for one mtDNA a number of times within the history of Lake Magadi. Although this may argue for extensive gene flow and a large effective population size, it is important to note that N_F estimates based on τ and assuming a panmictic Magadi population revealed values three times lower than the combined field estimates. Furthermore, even assuming panmixia, fixation of a mtDNA haplotype should have occurred multiple times within Magadi since the formation of the lake, based on mismatch distribution estimates of N_F (Table 4).

The Sports Club Lagoon system is perhaps the best supporting evidence for the maintenance of common haplotypes in isolation. Population expansion was not excluded for the Sports Club Lagoon tilapia, a surprising result as this lagoon has the most severe environment and maintains one of the lowest effective population sizes. It is improbable that migration into this severe environment will occur from other sources given the results of the LT50 experiments and the observation of the behavioral adaptation to the less severe surface water from an alternative spring. As a result, this population may have maintained a small effective population size for a long period of time. With no influx of mtDNA haplotypes to maintain the observed distribution, this population should have gone to fixation to one of the observed haplotypes many times within its history.

Balancing Selection Acting on Mitochondrial DNA. In the absence of gene flow, the maintenance of two common haplotypes

in small populations may be explained by balancing selection where there is selective advantage to more than one mtDNA variant within heterogeneous microhabitats of the Magadi lagoon systems. Evidence for environmental heterogeneity of microhabitats was observed in the water chemistry and fish distribution in the Sports Club Lagoon. We identified both a source of hot alkaline water and a source of fresher water over the surface that provided a cooler, less alkaline environment. The ubiquitous maintenance of haplotypes A and B suggests that any environmental heterogeneity that maintains these haplotypes through balancing selection is common among all the Magadi lagoons.

We propose a model of balancing selection acting on a gene or genes on the mitochondrial DNA that is reflected in the control region haplotype distribution. The "Orolonga" haplotype (B, this study, and A1, according to Seegers et al. 1999) was predicted to represent the period of stasis before the separation of the paleolake and changes in the environmental conditions. This seems reasonable as it appears as the progenitor sequence for all the observed sequences in the two lakes. Assuming an accelerated substitution rate and generation time consistent with *Alcolapia*, the other common haplotype (A or A17, Seegers et al. 1999) evolved in the range consistent with the separation of Orolonga. Dry periods corresponding to low water levels would increase the exposure of Lake Magadi fish to water from thermal springs, predicted to be higher in temperature, salinity, alkalinity, and, therefore, osmotic pressure. We suggest that the mtDNA corresponding to haplotype A evolved in response to high osmotic pressure, although other possibilities exist including adaptations to anoxia, hyperoxia, and high temperature. The ubiquitous nature of haplotype B suggests that this sequence may be maintained for other properties common to Lakes Magadi, Natron, and Little Magadi. For the purpose of this model we consider additional haplotypes to be associated with either the A or the B mtDNA lineages, although additional selection pressure may be acting on functional sequence differences on those mtDNA molecules, for example, haplotype C.

Similar stable frequencies of two common haplotypes and rare endemic types were observed in natural populations of *Drosophila subobscura* (Garcia-Martinez 1998; Castro et al. 1999). Balancing selection was proposed as one of the explanations for the mtDNA haplotype distribution but negative Tajima's D scores did not support this, although this was not rejected based on the use of only a few segregating sites (Castro et al. 1999). Experiments on flies from natural populations of *D. subobscura* within caged conditions provided strong evidence for selection acting on the mtDNA as fixation to one haplotype occurred within 20 generations when flies were bred in captivity (Garcia-Martinez 1998).

Discordance between allozyme allele frequencies and both morphology and haplotype frequencies in spiders of the genus *Metepeira* supported balancing selection acting on allozyme loci (Piel and Nutt 2000). The original interpretation of the allozyme data supported extensive gene flow, while morphological and behavioral differences and mtDNA haplotype distributions suggested differentiation to the species level. Our findings show the opposite relationship with respect to nuclear versus mtDNA patterns; i.e., Magadi mtDNA demonstrate low differentiation while morphological, physiological, and behavioral characters and nuclear loci show significant differentiation. Evidence of selection acting on the mtDNA COI gene has been observed in deepwater vent clams (genus *Vesicomydae*; Peek et al. 2000), based on higher number of nonsynonymous to synonymous substitutions in more severe environmental conditions, conditions not dissimilar to the soda lakes. We are currently examining mtDNA genes including COI to determine if significant sequence differences may reflect differential selection pressures.

Conclusion

In summary, we identified discordant patterns between genotypic and phenotypic characters within the Lake Magadi tilapia and further identified evidence against gene flow despite the similar mtDNA haplotype patterns. One potential explanation for the discordant patterns we observed is balancing selection acting on a gene or genes on different mtDNA, represented by control region haplotypes, as an adaptation to heterogenous microenvironments within each Magadi lagoon system. These results demonstrate the need to consider the interpretation of genetic structure in the biological context of the system under investigation (Hedrick 1999). We are also presently examining other "neutral" DNA markers, specifically microsatellites, with the assumption they will provide a more complete assessment of genetic structure and potential isolation among the Magadi lagoons.

The findings in this study raise a number of important questions focusing on rates of adaptation, sequence substitution rates, and selection. It appears that cichlids from soda lakes may demonstrate rates of evolution in physiological adaptations comparable to morphological characters such as jaw morphology and coloration (Trewavas 1983; Seegers et al. 1999; Tichy and Seegers 1999). Also, generation time, while important in estimating various population parameters, may be extremely important in the evolution of *Alcolapia* tilapia to the high pH of the soda lakes. Larval phases of other teleosts express the OUC required for the excretion of urea (Wright et al. 1995). Adult Magadi and Natron tilapia may maintain a life cycle more consistent with a larval phase, maintaining this pathway, and potentially other important adaptations, within this environment. Furthermore, evidence of accelerated substitution rates and selection of mtDNA genes in stressful environments clearly warrants further study (Rand et al. 1994). Future expeditions should further characterize these fish and address some of the questions raised in this study. The Lake Magadi and Lake Na-

tron *Alcolapia* tilapia clearly represent important biological systems with which to study evolutionary processes.

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