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Comparative molecular phylogeography of two *Xenopus* species, *X. gilli* and *X. laevis*, in the south-western Cape Province, South Africa

B. J. EVANS,*‡ J. C. MORALES,†‡ M. D. PICKER,§ D. B. KELLEY*‡ and D. J. MELNICK*†‡
*Departments of *Biological Sciences and †Anthropology and the ‡Center for Environmental Research and Conservation, Columbia University, New York, NY 10027, USA, §Department of Zoology, University of Cape Town, Rondebosch 7700, South Africa*

Abstract

Xenopus gilli **is a vulnerable anuran with a patchy distribution along the south-western coast of the Cape Province, South Africa. This species is sympatric with *Xenopus laevis laevis*, a widespread relative found over much of southern Africa. We examined the molecular phylogeography and population structure of the contact zone between these species to obtain information about historical biogeography and conservation management of this region. Analyses of the distribution, frequency, and cladistic and phenetic relationships among mitochondrial DNA haplotypes indicate that population subdivision is present in both taxa but that long-term isolation of sets of populations has occurred in *X. gilli* only. Haplotype and nucleotide diversity are also considerably higher within and among *X. gilli* ponds than *X. l. laevis* ponds in this region. We attribute the genetic segregation of *X. gilli* populations to ancient habitat fragmentation by ocean transgression into *X. gilli* habitat and to continued habitat alteration by human activity. The lower level of genetic diversity in *X. l. laevis* in this region is likely a result of a recent arrival of this taxon to the south-western Cape region relative to *X. gilli*. Population structure in *X. l. laevis* may be a result of isolation by distance. Clear evidence exists for at least two management units within *X. gilli* and strongly supports the establishment of protective measures east of False Bay in order to conserve a substantial portion of this species' extant genetic diversity.**

Keywords: *Xenopus gilli, Xenopus laevis*, comparative phylogeography, South Africa, conservation genetics, endangered species

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Introduction

Comparative phylogeography attempts to attribute similarities and differences in patterns of genetic polymorphism among taxa to features of their habitat and evolutionary history. A disjunct distribution of genetic polymorphism within the range of a taxon could arise either from the dispersal of an ancestor to an isolated locality or from the fragmentation of a continuous range into isolated populations (Wiley 1988). A major difference between these hypotheses is the age of the barrier that isolates populations relative to the age of the genetic disjunction. Dispersal of an ancestor to an isolated locality occurs in spite of a barrier which is older than the disjunction, whereas fragmentation of a continuous range occurs because a new barrier arises which is thus roughly contemporaneous with the genetic disjunction (Morrone & Crisci 1995). Congruent disjunct phylogeographic distribution of genetic polymorphism among multiple taxa supports the latter of these scenarios, a vicariance paradigm (Avise 1992; Joseph *et al*. 1995; Wiley 1988). However, although taxa may share a unique environment, differences among them such as gene flow, rates of molecular evolution, effective population size, or generation time could lead to differences in the distribution of genetic polymorphism (Zink 1996). Molecular phylogenies and analysis of allele frequencies and distributions can offer insight into the

Correspondence: Don Melnick, 452 Schermerhorn Extn., Department of Anthropology, Columbia University, New York, NY 10027, USA. Tel.: +1-212-854-3393; Fax: +1-212-854-3605. E-mail: djm7@columbia.edu

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biogeographical processes that influence evolution (Barton & Wilson 1995; Hudson 1990) and are thus useful tools for studies of comparative phylogeography.

A detailed assessment of the spatial distribution, evolutionary relationships, and evolutionary significance of populations also provides a basis on which conservation management decisions can be made. Molecular genetic studies of endangered taxa can be used to demarcate management units, which are sets of populations that are demographically independent, and evolutionarily significant units, which are historically isolated sets of populations (Moritz 1994, 1995). Multiple phylogeographic analyses of many species can identify regions of genetic endemism and/or diversity and provide a basis for broader, regionally based conservation strategies (Avise 1989, 1995; Erwin 1991).

Xenopus gilli, the Cape clawed frog, is a vulnerable anuran (IUCN 1994) that inhabits a small region along the south-western coast of the Cape Province, South Africa. It is sympatric with a small portion of the distribution of *Xenopus laevis laevis*, which occurs over much of southern Africa (Loumont 1984). Hybridization among these taxa has been investigated (Kobel *et al.* 1981; Picker 1985; Picker *et al.* 1996; Simmonds 1985) and is not associated with significant levels of introgression of mitochondrial or nuclear DNA (B. J. Evans *et al.* unpubl. data). The contact zone of these taxa is restricted to lowland fynbos habitat of the Cape Floral Kingdom of southern Africa, one of the six floral kingdoms of the world defined by their unique composition of plant life. In contrast to the vast habitat of *X. laevis*, the blackwater seepages characteristic of the fynbos biome inhabited by *X. gilli* are uncommon and easily disturbed by human activity. Habitat conversion is a likely cause of the decline of this uniquely adapted and narrowly distributed anuran (Kobel *et al.* 1981; Picker 1993; Picker *et al.* 1996).

One purpose of this study is to identify areas of evolutionary significance (Moritz 1994, 1995) within the range of *X. gilli* for use in conservation management. Another goal is to compare the molecular phylogeography of *X. gilli* and *X. l. laevis* to understand better the events that shaped the evolution of *Xenopus* species and other fauna in the south-western Cape region.

Materials and Methods

Sample collection

X. gilli was sampled from three main areas: the Cape Point Peninsula, a region east of Kleinmond, and an area further east about 40 km west of Cape Agulhas. *X. l. laevis* was sampled from areas within and surrounding the contemporary distribution of *X. gilli* and from an area north of Cape Town near Pinelands which is outside of the historical demarcation of the *X. gilli* distribution (Picker & de Villiers 1989) (Fig. 1). Some ponds contained both taxa, although most had only one. All animals were caught in seine nets and in collapsible funnel traps baited with bones in June, July and August of 1994. Approximately 200 µL of blood was obtained from the dorsal tarsus vein in the foot (Du Pasquier *et al.* 1985) without apparent harm to the animals. The DNA in the blood was preserved in an equal volume of a solution of 100 mM EDTA, 2% SDS, and 100 mM Tris and frozen until it could be extracted.

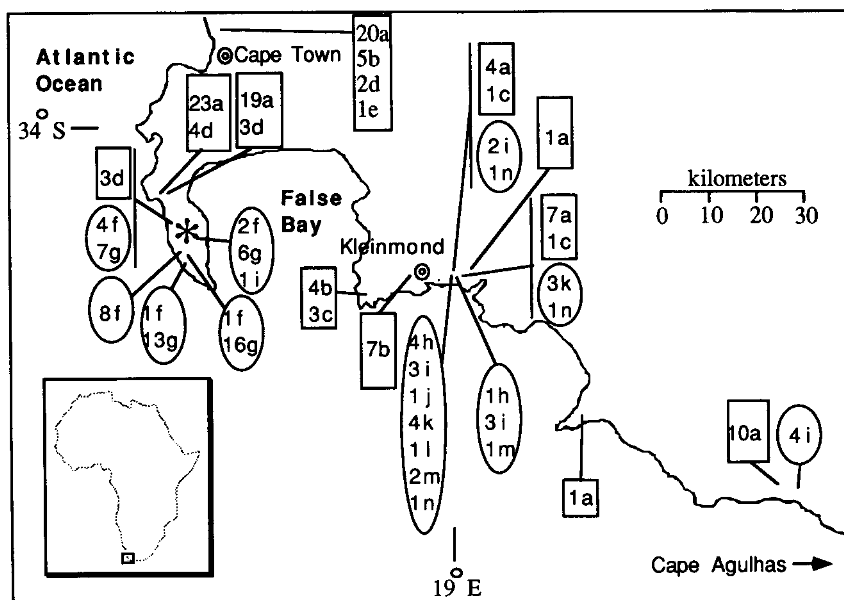

Fig. 1 Sampling localities and the distribution of mtDNA haplotypes of *Xenopus gilli* (ovals) and *Xenopus laevis laevis* (rectangles). Each letter represents a different mapped restriction enzyme profile as depicted in Table 1. Letters are preceded by the number of individuals with that haplotype. An asterisk indicates the location of the Cape Point Nature Reserve.

Additional tissue samples of *X. l. victorianus* from Rwanda, *X. l. sudanensis* from Nigeria, and *X. l. poweri* from Zambia were kindly provided by Dr H. R. Kobel from the University of Geneva, Switzerland. *Silurana tropicalis* tissue from Nigeria was obtained from a commercial source (Xenopus Express, Inc.). DNA was extracted from these tissue samples using a standard phenol and chloroform protocol (Sambrook *et al*. 1989).

DNA analysis

Mitochondrial DNA (mtDNA) is a nonrecombining, maternally inherited, rapidly evolving genome that is an informative tool for investigating molecular phylogeography (Avise *et al*. 1987). A 2694-bp section of the mtDNA including a small portion of the cytochrome B gene, the $tRNA^{Thr}$ and $tRNA^{Pro}$, the entire D-loop, and part of the $tRNA^{Phe}$ was amplified with the polymerase chain reaction using the primers BXenF2 (TCG CCT ACG CTA TCC TTC GAT C) which was labelled with biotin and XenB1 (GGA CCA AAC CTT TAT GCT TAC GG) which was unlabelled. These primers were designed from the complete sequence of the *X. laevis* mtDNA using the MacVector program (International Biosciences, Inc.) and span nucleotide number 17099–2196 in Roe *et al*. (1985). This region of the mtDNA has been used for phylogenetic and populations analysis of other anurans (Yang *et al*. 1994).

To evaluate the distribution of mtDNA haplotypes throughout the zone of contact of *X. l. laevis* and *X. gilli*, the amplified region of the mtDNA of 119 *X. l. laevis* and 90 *X. gilli* individuals was subjected to complete digestion with seven type II restriction endonucleases (*Aci*I, *Acc*I, *Ase*I, *Dde*I, *Dpn*II, *Hae*III and *Hpa*II) according to the manufacturer's protocols. The complete digestion products were then run on 1% agarose gels and stained with cybergreen (Molecular Probes, Inc.). The pattern of digested fragments for each enzyme was scored and compiled into a single haplotype for each individual. Restriction sites of each enzyme were mapped using a partial digestion technique for mapping unilaterally labelled amplified DNA (Morales *et al*. 1993). Partially digested unilaterally labelled DNA was run on 1.5% agarose gels next to a biotinylated size marker (Gibco, Inc.) and blotted to a nylon membrane. Labelled fragments were then visualized with a chemiluminescent reaction (Tropix, Inc.) followed by autoradiography. Approximate map positions were estimated based on the positions of labelled partially digested fragments relative to the size marker. Some *X. gilli* mtDNA haplotypes have an insertion in the D-loop near the XenB1 primer. This insertion did not complicate mapping and identification of homologous restriction sites because it is very near the end of the amplified region.

Data analysis

Character-based cladistic analysis was carried out on 63 mapped restriction sites in nine *X. gilli* haplotypes, five *X. l. laevis* haplotypes, three haplotypes of other *X. laevis* subspecies (*X. l. poweri*, *X. l. sudanensis* and *X. l. victorianus*), and one haplotype of *S. tropicalis* using the branch and bound search procedure of the program PAUP version 3.1.1 (Swofford 1993). Because the likelihood of a site loss is much greater than a site gain, Dollo parsimony was employed (DeBry & Slade 1985). A strict consensus tree was obtained from the eight most parsimonious trees using *S. tropicalis* as an outgroup (de Sa & Hillis 1990). To evaluate support for inferred evolutionary relationships, a 50% majority-rule bootstrap consensus tree was generated from a bootstrap analysis with 2000 replications (Hedges 1992), using the PAUP program. Decay indices for each monophyletic group of the bootstrap consensus tree, which represent the difference in tree lengths between the shortest trees that contain versus lack that group (Hillis *et al*. 1996), were calculated using the AUTODECAY program version 3.0, created by Eriksson & Wikstrom. Nucleotide distances among haplotypes were estimated from mapped restriction site data using the algorithms of Nei & Tajima (1983) and Nei & Miller (1990) with the weighting scheme of Nei & Tajima (1983), using the REAP program version 4.0 (McElroy *et al*. 1991). These formulas are appropriate for estimating genetic distances between nucleotide sequences that are less than 0.25 (McElroy *et al*. 1991).

Before comparing levels of diversity and divergence of a locus in different taxa, it is important to determine whether this locus has evolved at a similar rate in each taxon. The levels of divergence of the amplified section of the mtDNA were evaluated in each taxon using the relative rate test which compares the outgroup divergence distances of each of the ingroup taxa (Sarich & Wilson 1973; Li & Graur 1991). To compare the levels of divergence of the *X. gilli* species and the *X. l. laevis* subspecies in the Cape region, outgroup distances among nine *X. gilli* haplotypes were compared with outgroup distances among five *X. l. laevis* haplotypes with a Student's *t*-test, after establishing homoscedasticy, using version 3.1.5 of the JMP program (SAS Institute, Inc.). Before comparing levels of divergence between species, an average outgroup distance of five *X. l. laevis* haplotypes was calculated. This average value was grouped with those from three other subspecies of *X. laevis* (*X. l. poweri*, *X. l. sudanensis* and *X. l. victorianus*) to give equal weight to each subspecies, and then compared with the outgroup distance values of nine haplotypes of *X. gilli*. Student's *t*-tests were also used for comparing these distances, after establishing homoscedasticy, using the JMP program. To compare nucleotide divergence distances among haplotypes, a

neighbour-joining tree (Saitou & Nei 1987) was computed from the nucleotide distance matrix using the version 1.01 of the MEGA program (Kumar *et al*. 1993).

Geographic heterogeneity of mtDNA frequencies was evaluated with Monte Carlo simulations to generate the expected χ^2 distribution if the null hypothesis of mtDNA haplotype homogeneity were true (Roff & Bentzen 1989). This distribution was compared with the actual frequency of mtDNA haplotypes using the REAP program to estimate the probability that this frequency would occur by chance. This technique minimizes underestimation of the significance of population subdivision with small sample sizes or multiple rare alleles (Roff & Bentzen 1989). One thousand randomizations of haplotype frequency matrices grouped by pond and by location relative to False Bay (east or west) were carried out for each taxon. Analyses of pond haplotype frequencies throughout the study area were used to compare the molecular phylogeography of each taxon. Separate analyses of pond haplotype frequencies in ponds east of False Bay and in ponds west of False Bay were used to compare the populations in each of these regions. These regions were considered independently in both taxa based on results from cladistic analysis described below, which identified two clades in the *X. gilli* population that are almost completely geographically divided by False Bay. Separate analysis and comparison of these regions is also useful for conservation management of *X. gilli* as the Cape Point Nature Reserve, and any future reserves in the study area, are unlikely to span the Cape Flats region north of False Bay which is inhabited by a burgeoning human population.

Haplotype and nucleotide diversity within ponds and nucleotide diversity and divergence among ponds were estimated from distance and frequency data with the algorithms of Nei (1987) and Nei & Tajima (1981) using the REAP program. Haplotypes were analysed by pond for each taxon across the study area and by location relative to False Bay (east or west) for each taxon. Haplotype diversity within ponds is based on frequency distributions and the associated distance values among the haplotypes (McElroy *et al*. 1991). Nucleotide diversity is the average number of nucleotide substitutions per site within and among groups of DNA sequences (Nei & Miller 1990). Nucleotide divergence among ponds is the component of diversity not explained by within population polymorphism (Nei & Tajima 1981).

Results

Distribution of mtDNA variation

Nine *X. gilli* haplotypes were resolved from mapped sites of seven restriction enzymes in the amplified section of the mtDNA (Fig. 1, Table 1). Four different haplotypes were identified in the *X. l. laevis* individuals sampled within the range of *X. gilli* and a fifth haplotype present in one individual was identified in a locality north of Cape Town which is outside the range of *X. gilli* (Fig. 1). All *X. gilli* individuals, except one, collected on the west side of False Bay had one of two mtDNA haplotypes (f and g) and all individuals on the east side of False Bay had one of seven haplotypes (h, i, j, k, l, m or n) (Table 1). One out of 52 *X. gilli* individuals sampled in the Cape Point Peninsula had a mtDNA haplotype i that was the most common haplotype recorded in ponds east of False Bay. In contrast, most *X. l. laevis* individuals sampled throughout the study area had a single haplotype (a). Two haplotypes, (b and d), were also broadly distributed across this region although the two remaining rare haplotypes (c and e) were found only east and west of False Bay, respectively.

Table 1 Number of individuals, frequency, and location, east (E) or west (W) of False Bay of mtDNA haplotypes of *Xenopus gilli* and *Xenopus laevis laevis*. Each letter of the seven-letter haplotype code represents a different mapped restriction site profile for each of the seven enzymes used in alphabetical order. The letter in parentheses correspond to haplotype letters in Fig. 1

Species	Haplotype	No. individuals	Frequency	Location
X. l. laevis	(a) NAAAAAA	85	0.71	E and W
	(b) NAABAAA	16	0.13	E and W
	(c) NAAAABA	5	0.04	E
	(d) NAAABAA	12	0.10	W
	(e) NABABAA	1	0.01	W
X. gilli	(f) ABCCCCB	16	0.18	W
	(g) ABCGCCB	42	0.47	W
	(h) BADENDC	5	0.06	E
	(i) BADENDD	13	0.14	E and W
	(j) BADENDE	1	0.01	E
	(k) BADEEDD	7	0.08	E
	(l) BADEEDC	1	0.01	E
	(m) BAEENDD	3	0.03	E
	(n) BADENED	2	0.02	E

Cladistic analysis

The locations of mapped restriction sites used in the cladistic analysis are summarized in Appendix I. Bootstrap analysis supports the reciprocal monophyly of *X. laevis* and *X. gilli*, clusters *X. gilli* mtDNA haplotypes into two major clades, and clusters *X. l. laevis* haplotypes from the sample area into one unresolved clade (Fig. 2). Parsimony analysis generated eight most parsimonious trees of 116 steps. The topology of the strict consensus tree of these trees is the same as the bootstrap tree with the exception that *X. l. laevis* haplotypes a and c form a weakly supported monophyletic group as do *X. gilli* haplotypes i, m and n. The two major lineages of *X. gilli* mtDNA haplotypes are strongly supported by high bootstrap values and decay indices (Fig. 2), and are almost completely geographically partitioned with respect to False Bay. One *X. gilli* clade contains two closely related haplotypes (f and g) that occur only in the Cape Point Nature Reserve west of False Bay. All mtDNA sampled east of False Bay clusters in the other clade which contains seven closely related haplotypes (h, i, j, k, l, m, n). The only exception to the geographic partitioning of these clades is the single individual sampled west of False Bay with mtDNA haplotype (i) (Figs 1 and 2). The haplotypes of the predominantly eastern clade have an insertion of ≈ 90 base pairs in the mtDNA about 150 base pairs away from the XenB1 primer site, approximate nucleotide position 1742 in Roe *et al.* (1985). Although this feature was not used in the analysis of site data, it is a putative synapomorphy in the predominantly eastern *X. gilli* clade (Fig. 2). This insertion is in a different place from those described in the D-loop of other *Xenopus* species (Carr *et al.* 1987).

X. l. laevis haplotypes sampled in the study area all cluster in a single, unresolved clade (Fig. 2). The monophyly of the mtDNA of *X. l. sudanensis* and *X. l. poweri* with respect to *X. l. victorianus* and the monophyly of these subspecies with respect to *X. l. laevis* is supported by high bootstrap values and decay indices.

Levels of divergence

Genetic distances among mtDNA haplotypes calculated from mapped restriction sites are summarized in Table 2. Comparison of the outgroup distances of nine *X. gilli* mtDNA haplotypes ($x = 0.2211$, $\sigma = 0.0208$) and outgroup distances of four *X. laevis* subspecies mtDNA haplotypes ($x = 0.2434$, $\sigma = 0.0245$) reveals no significant difference ($P = 0.1484$). However, comparison of outgroup distances of *X. gilli* to the outgroup distance of only the *X. l. laevis* subspecies (i.e. leaving out all other *X. laevis* subspecies)

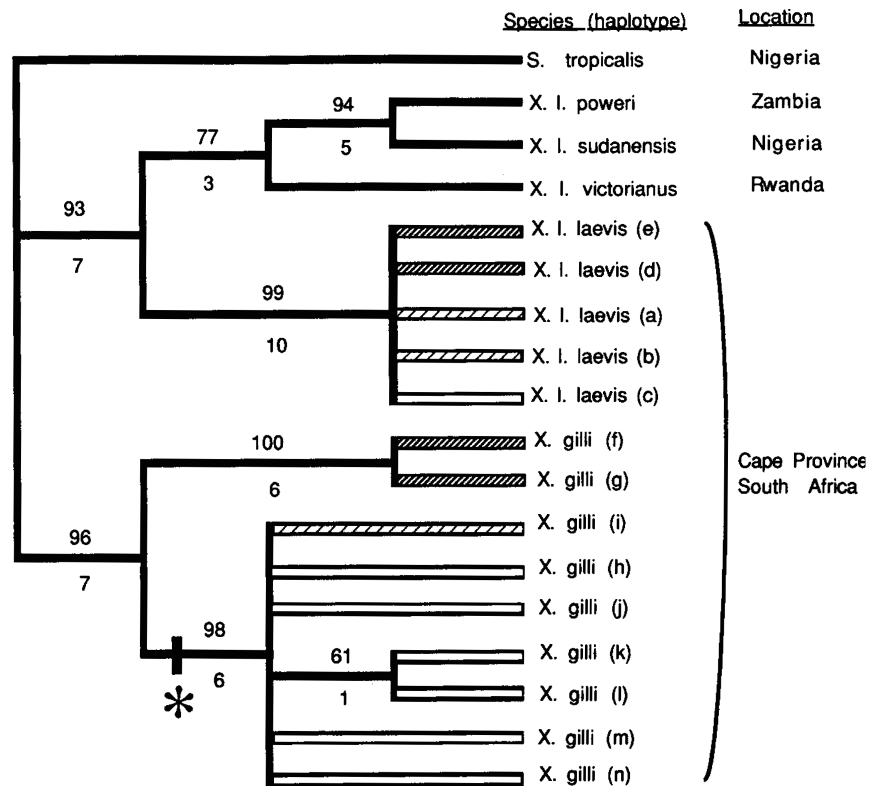

Fig. 2 Bootstrap 50% majority consensus tree with bootstrap values indicated above branches and decay indicies indicated below the branches. Haplotypes of *Xenopus laevis laevis* and *Xenopus gilli* present only west of False Bay have densely stripped terminal branches. Those on both sides of False Bay have stripped terminal branches. Those present only east of False Bay have outlined terminal branches. An asterisk indicates the branch along which a 90 base pair insertion in the D-loop of *X. gilli* is hypothesized to have occurred.

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Table 2 Nucleotide distance estimations among *Xenopus laevis*, *X gilli* and *Silurana tropicalis* mtDNA haplotypes

	S. tropicalis	*X. l. poweri*	*X. l. sudanensis*	*X. l. victorianus*	*X. l. laevis* a	*X. l. laevis* b	*X. l. laevis* c	*X. l. laevis* d	*X. l. laevis* e
S. tropicalis	0.0000								
X. l. poweri	0.2155	0.0000							
X. l. sudanensis	0.2533	0.0187	0.0000						
X. l. victorianus	0.2264	0.0978	0.0809	0.0000					
X. l. laevis a	0.2819	0.1280	0.1557	0.1405	0.0000				
X. l. laevis b	0.2552	0.1442	0.1741	0.1424	0.0111	0.0000			
X. l. laevis c	0.3368	0.1299	0.1600	0.1230	0.0174	0.0300	0.0000		
X. l. laevis d	0.2593	0.1328	0.1607	0.1462	0.0051	0.0162	0.0231	0.0000	
X. l. laevis e	0.2593	0.1328	0.1607	0.1462	0.0051	0.0057	0.0231	0.0102	0.0000
X. gilli f	0.1818	0.2191	0.1958	0.1883	0.2218	0.2236	0.2062	0.2033	0.2269
X. gilli g	0.2157	0.2893	0.2612	0.2536	0.2744	0.2787	0.2550	0.2446	0.2807
X. gilli h	0.2137	0.2422	0.2492	0.2031	0.1797	0.2181	0.1631	0.1852	0.1852
X. gilli i	0.2339	0.2727	0.2427	0.1965	0.1987	0.2387	0.1806	0.2046	0.2046
X. gilli j	0.2339	0.2727	0.2427	0.1965	0.2286	0.2700	0.2089	0.2350	0.2350
X. gilli k	0.2186	0.2852	0.2557	0.2096	0.2105	0.2494	0.1927	0.2163	0.2163
X. gilli l	0.2013	0.2534	0.2621	0.2160	0.1907	0.2280	0.1742	0.1960	0.1960
X. gilli m	0.2317	0.2717	0.2418	0.1933	0.1943	0.2324	0.1753	0.2005	0.2005
X. gilli n	0.2589	0.2664	0.2361	0.1899	0.1927	0.2333	0.1745	0.1987	0.1987

	X. gilli f	*X. gilli* g	*X. gilli* h	*X. gilli* i	*X. gilli* j	*X. gilli* k	*X. gilli* l	*X. gilli* m	*X. gilli* n
S. tropicalis									
X. l. poweri									
X. l. sudanensis									
X. l. victorianus									
X. l. laevis a									
X. l. laevis b									
X. l. laevis c									
X. l. laevis d									
X. l. laevis e									
X. gilli f	0.0000								
X. gilli g	0.0078	0.0000							
X. gilli h	0.0570	0.0716	0.0000						
X. gilli i	0.0527	0.0667	0.0048	0.0000					
X. gilli j	0.0638	0.0805	0.0152	0.0101	0.0000				
X. gilli k	0.0613	0.0764	0.0145	0.0096	0.0203	0.0000			
X. gilli l	0.0656	0.0813	0.0093	0.0145	0.0255	0.0045	0.0000		
X. gilli m	0.0593	0.0739	0.0099	0.0051	0.0154	0.0148	0.0197	0.0000	
X. gilli n	0.0593	0.0752	0.0101	0.0050	0.0159	0.0152	0.0203	0.0103	0.0000

indicates that *X. l. laevis* haplotypes are significantly more diverged from *S. tropicalis* ($x = 0.2785$, $\sigma = 0.0306$) than are the *X. gilli* haplotypes ($P = 0.0023$). This suggests that the average rate of evolution in the *X. l. laevis* subspecies has been slightly faster than that of *X. gilli* since the splitting of the *Xenopus* and *Silurana* clades. As the level of divergence of each species as a whole is similar since this split, the higher rate of divergence of the *X. l. laevis* subspecies may have occurred after this subspecies arose.

The levels of divergence among haplotypes within the *X. gilli* species and the *X. laevis* species can be seen with a neighbour-joining tree (Fig. 3). The topology of this tree is similar to that obtained from cladistic analysis (Fig. 2). Strong cladistic support exists for the bifurcation of *X. gilli* into a western clade and a predominately eastern clade. The two major evolutionary lineages in *X. gilli* which occur within the 300 km coastal range of *X. gilli* have a level of divergence similar to that seen among subspecies of *X. laevis* which occur over much of Africa south of the Sahara. It is difficult to relate these levels of divergence to time as estimates for the date of the split between *Xenopus* and its sister taxon *Silurana* vary from 30 to 40 million years ago (Bisbee *et al.* 1977) to 90 million years ago or more (Knochel *et al.* 1986; Graf 1996; Cannatella & de Sa 1993).

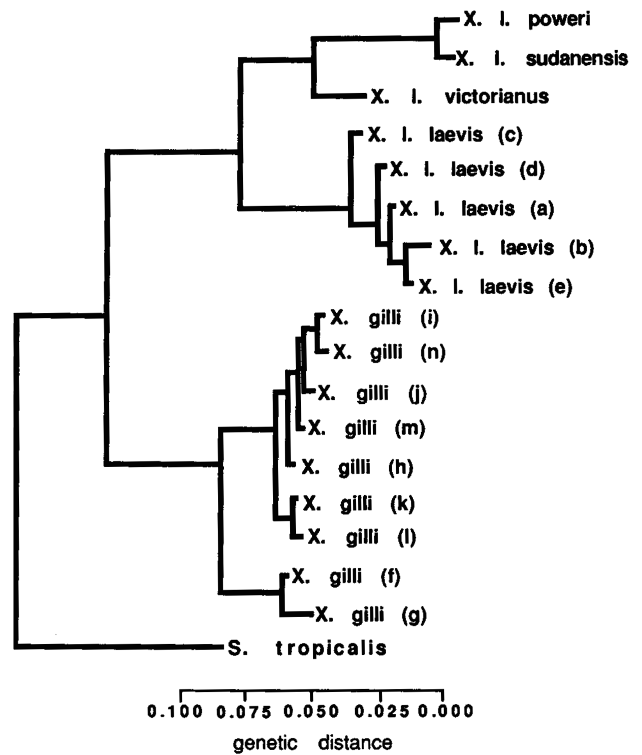

Fig. 3 Neighbour-joining tree of mtDNA haplotypes. A scale of nucleotide distance is included.

Population diversity and divergence

Monte Carlo simulations of *X. l. laevis* and *X. gilli* pond haplotype frequencies indicate that levels of geographic heterogeneity are significant among ponds in both species ($P < 0.05$) (Table 3). Separate Monte Carlo analyses of ponds on each side of False Bay indicate that significant geographic structure exists in *X. l. laevis* haplotype frequencies in eastern and in western ponds and in *X. gilli* haplotype frequencies in western ponds ($P < 0.05$), but not in *X. gilli* haplotype frequencies in ponds east of False Bay (Table 3).

Diversity and divergence analyses of each taxon indicate that the average haplotype and nucleotide diversity within ponds of *X. gilli* and nucleotide diversity and divergence among ponds of *X. gilli* are significantly larger than those of *X. l. laevis* ($P < 0.05$) (Table 3). Analysis of data grouped by location relative to False Bay indicates that haplotype diversity within ponds is significantly larger in *X. gilli* ponds east of False Bay than it is in *X. gilli* ponds west of False Bay and than *X. l. laevis* ponds east or west of False Bay ($P < 0.05$) (Table 3). This diversity reflects the large number of haplotypes identified in eastern *X. gilli* ponds (seven haplotypes) compared with western *X. gilli* ponds (three) and western and eastern *X. l. laevis* ponds (four and three, respectively) (Table 1). No difference was detected among haplotype diversity within *X. l. laevis* ponds in each region (Table 3).

Nucleotide diversity within ponds is higher east of False Bay in both *X. l. laevis* and *X. gilli*. Nucleotide diversity among ponds is also higher east of False Bay than those west of False Bay in both taxa. Nucleotide divergence among eastern *X. l. laevis* ponds is higher than among western *X. l. laevis* ponds, but in *X. gilli* it is higher among western ponds than eastern ponds. The latter geographical difference in nucleotide divergence is likely a result of the single predominantly eastern *X. gilli* haplotype that was present in a western *X. gilli* pond (Table 1). Were it not for this single individual, nucleotide diversity and divergence indices within and among ponds east of False Bay would be uniformly higher than within and among ponds west of False Bay in both taxa.

Discussion

Molecular phylogeography

Phenetic and cladistic analyses of the molecular variability among haplotypes suggest that the evolutionary history of *X. gilli* and *X. l. laevis* in the south-western Cape region is quite different. MtDNA of *X. gilli* comprises two ancient diverged evolutionary lineages on either side of False Bay that are geographically partitioned as a result of long-term barriers to gene flow. One individual was sampled in Cape Point Nature Reserve west of False Bay with a mtDNA haplotype from the predominantly eastern *X. gilli* clade. This individual could have been a product of a natural migration event from east to west, a descendant of a polymorphic ancestor population that colonized the western peninsula, or transported from an eastern locality by researchers studying this species.

Although the geographic distances between Kleinmond and the Cape Point populations and Kleinmond and the population near Cape Agulhas are approximately equal, the frequencies and relations among *X. gilli* haplotypes in these regions are very different. The mtDNA haplotype present in all individuals from the Cape Agulhas population is the one most commonly found in the Kleinmond ponds, but least commonly found in the Cape Point Nature Reserve. Ninety-eight per cent of the other *X. gilli* individuals in the Cape Point have one of two haplotypes that occur exclusively in these western ponds.

The sharp disjunction between *X. gilli* populations east and west of False Bay may have been caused by the

Table 3 Haplotype diversity and nucleotide diversity within ponds and nucleotide diversity and divergence among ponds *Xenopus laevis laevis* and *Xenopus gilli* analysed by taxon and by location relative to False Bay

	Within ponds		Among ponds	
Taxon	Haplotype diversity	Nucleotide diversity	Nucleotide diversity	Nucleotide divergence
X. gilli	0.3682 ± 0.0083*	0.0047 ± < 0.0001*	0.0387 ± < 0.0001*	0.0340 ± < 0.0001*
X. l. laevis	0.2305 ± 0.0044	0.0039 ± < 0.0001	0.0081 ± < 0.0001	0.0041 ± < 0.0001
Location				
X. gilli east	0.4846 ± 0.0192*	0.0049 ± < 0.0001*	0.0067 ± < 0.0001*	0.0018 ± < 0.0001
X. gilli west	0.2519 ± 0.0111	0.0045 ± < 0.0001	0.0066 ± < 0.0001	0.0020 ± < 0.0001*
X. l. laevis east	0.2233 ± 0.0105	0.0057 ± < 0.0001*	0.0105 ± < 0.0001*	0.0048 ± < 0.0001*
X. l. laevis west	0.2395 ± 0.0089	0.0017 ± < 0.0001	0.0039 ± < 0.0001	0.0022 ± < 0.0001

* indicates significantly large values in interspecific comparisons and intraspecific location comparisons.

fragmentation of a continuous lowland habitat by an oceanic transgression. Large expanses of coastal lowland were exposed by a major oceanic regression during the Oligocene and Early/Middle Miocene (Siesser & Dingle 1981). This area was then subsumed by a transgression during the Middle to Late Miocene, possibly exposed again in a brief regression during the Miocene–Early Pliocene, and inundated again during the Early Pliocene (Siesser & Dingle 1981; Hendey 1983; Rogers 1986). If oceanic transgression did fragment *X. gilli* habitat, then populations recorded north of False Bay near Citrusdale now thought to be extinct (Picker & de Villiers 1989) probably had eastern mtDNA haplotypes because this city is east of the late Tertiary sedimentary deposits north of False Bay (Hendey 1983). Disjunct population structure analogous to that observed in *X. gilli* has been noted in other herpetological fauna of this region and has been hypothesized to stem from the same geological events (Mouton 1986). A disjunct distribution of the Proteaceous floral subspecies *Leucospermum hypophyllocarpodendron hypophyllocarpodendron* similar to that of *X. gilli* has also been noted (Rourke 1972). This species may have dispersed over a Middle Pleistocene land bridge between the Cape Flats and Cape Agulhas during a period of low sea level (Rourke 1972). Although more recent land bridges may have facilitated *X. gilli* dispersal within eastern or western regions, the sharp geographical partitioning and high genetic distance between eastern and western haplotypes of *X. gilli* suggests that barriers to dispersal between these regions may have persisted through the Late Pliocene and Pleistocene.

In contrast to the disjunct distribution of these species, *X. l. laevis* is continuously distributed across the study area with a single haplotype most common on both sides of False Bay. Although largely homogeneous, significant population structure is present in divergent, less common haplotypes. Carr *et al*. (1987) hypothesize that a recent bottleneck of the effective number of females could account for the homogeneity of *X. l. laevis* mtDNA in the Cape region. Although possible, the associated 'mtDNA sweep' would have had to occur over a large portion of the south-western Cape Province which share closely related haplotypes (Grohovaz *et al*. 1996) and long enough ago for at least four other mtDNA haplotypes identified in this study to evolve. Another possibility is that *X. l. laevis* arrived to the south-western Cape Province recently relative to *X. gilli*. Subsequent to its arrival it may have been isolated from other populations of *X. l. laevis* by differences in climate arising in the early Pleistocene (Grohovaz *et al*. 1996). Examples of disparate phylogeographic histories analogous to that of *X. gilli* and *X. l. laevis* have been reported for other sympatric herpetofauna as well (Lamb *et al*. 1992).

The reciprocal monophyly of the mtDNA of *X. gilli* and *X. laevis* supports the hypothesis that introgression of mtDNA has not occurred at significant levels among these taxa (B. J. Evans *et al*. unpubl. data) even though these taxa mate to produce F1 hybrids (Kobel *et al*. 1981; Picker 1985; Simmonds 1985). This monophyly also supports species status of both taxa unless the fifth subspecies of *X. laevis*, *X. l. petersi*, for which there are no data in this study, clusters within the *X. gilli* mtDNA clade. The monophyly of *X. laevis* mtDNA examined in this study agrees with results from other studies of *X. laevis* mtDNA, as does the high level of divergence among the subspecies *X. l. laevis* and *X. l. victorianus* (Carr *et al*. 1987).

Population diversity and divergence

The results of this study indicate that population structure exists in both species and that, with the exception of haplotype diversity within *X. l. laevis* ponds and nucleotide divergence among *X. gilli* ponds, the region of the study area east of False Bay has higher diversity and divergence indices within and among ponds than the western region. However, the magnitude of this molecular diversity and divergence is much higher in *X. gilli* than in *X. l. laevis*, probably as a result of the antiquity of *X. gilli* mtDNA lineages relative to those of *X. l. laevis*. Because the population sampled in Cape Agulhas was homogeneous, the higher values of these indices in the east are not a consequence of the larger area of the eastern region. Higher diversity and divergence of mtDNA in this region could exist in the east if this area has previously been less genetically isolated from other populations than the western region.

Conservation

Human activity may be precipitating a world-wide decline of amphibian populations (Blaustein & Wake 1990; Pechmann *et al*. 1991). Although a significant discontinuity between the eastern and western extents of *X. gilli* habitat probably existed long before extensive human presence in this region, further fragmentation of *X. gilli* habitat as a result of human activity in the last 300 years is contributing to the decline of this endangered species (B. J. Evans *et al*. submitted; Kobel *et al*. 1981; Picker & de Villiers 1989). Reciprocal monophyly and significantly different haplotype frequencies of *X. gilli* populations on either side of False Bay indicate that each region is an evolutionarily significant unit and that at least two management units are present in this species (Moritz 1994, 1995). In conjunction with the existing Cape Point Nature Reserve, an additional reserve or protective measures within *X. gilli* habitat near Kleinmond would conserve much of the genetic diversity seen in this species and thus, a unique portion of the genetic

diversity of the genus. It would also help protect other endangered amphibians who share this unique habitat, such as the Micro frog (*Microbatrachella capensis*).

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Appendix 1 Location and presence of mapped restriction sites in the section of the mt DNA examined in this study

Enzyme	Approx. bp from primer BXenF2	Approx. bp in Roe *et al.* (1985)	*S. tropicalis*	*X. l. poweri*	*X. l. sudanensis*	*X. l. victorianus*	*X. l. laevis* a	*X. l. laevis* b	*X. l. laevis* c	*X. l. laevis* d	*X. l. laevis* e	*X. gilli* f	*X. gilli* g	*X. gilli* h	*X. gilli* i	*X. gilli* j	*X. gilli* k	*X. gilli* l	*X. gilli* m	*X. gilli n*
*Acc*I	1480	1026	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
	1430	976	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1350	896	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	400	17499	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
	280	17379	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
*Aci*I	1900	1446	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
	500	46	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	170	17296	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
*Ase*I	2300	1846	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2250	1796	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
	1750	1296	0	1	1	0	1	0	1	1	1	0	0	1	1	1	1	1	1	1
	1700	1246	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1660	1206	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
	1550	1096	1	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1

Appendix 1 *Continued*

Enzyme	Approx. bp from primer BXenF2	Approx. bp in Roe *et al.* (1985)	*S. tropicalis*	*X. l. poweri*	*X. l. sudanensis*	*X. l. victorianus*	*X. l. laevis* a	*X. l. laevis* b	*X. l. laevis* c	*X. l. laevis* d	*X. l. laevis* e	*X. gilli* f	*X. gilli* g	*X. gilli* h	*X. gilli* i	*X. gilli* j	*X. gilli* k	*X. gilli* l	*X. gilli* m	*X. gilli n*
	1500	1046	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
	1350	896	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	1
	1100	646	0	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1
	800	346	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	780	326	1	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
	730	276	1	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
	680	226	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1
*Dde*I	2400	1946	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2030	1576	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
	1930	1476	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
	1800	1346	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1750	1296	1	1	1	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0
	1700	1246	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1350	896	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1240	786	1	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0
	1100	646	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0
	870	416	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1
	700	246	0	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
	650	196	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	580	126	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
	500	46	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
	370	17469	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	300	17399	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	270	17369	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
	230	17329	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1
	200	17299	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
*Dpn*II	2070	1616	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
	1850	1396	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
	1600	1146	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	1550	1096	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1500	1046	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1370	916	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1270	816	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1000	516	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	700	246	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	650	196	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	400	17499	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
*Hae*III	1950	1496	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1900	1446	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1370	946	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	350	17449	1	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0
	200	17299	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0
*Hpa*II	2250	1796	1	1	0	0	1	1	1	1	1	0	0	1	0	0	0	1	0	0
	1900	1486	1	0	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1
	1800	1346	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	870	416	0	1	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
	840	386	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	350	17499	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
	300	17399	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1