Population divergence in East African coelacanths

Kathrin P. Lampert[1], Hans Fricke[2,3], Karen Hissmann[3], Jürgen Schauer[3], Katrin Blassmann[4,5], Benjamin P. Ngatunga[6], and Manfred Schartl[4,*]

The coelacanth, *Latimeria chalumnae*, occurs at the Eastern coast of Africa from South Africa up to Kenya. It is often referred to as a living fossil mainly because of its nearly unchanged morphology since the Middle Devonian. As it is a close relative to the last common ancestor of fish and tetrapods, molecular studies mostly focussed on their phylogenetic relationships. We now present a population genetic study based on 71 adults from the whole known range of the species. Despite an overall low genetic diversity, there is evidence for divergence of local populations. We assume that originally the coelacanths at the East African Coast derived from the Comoros population, but have since then diversified into additional independent populations: one in South Africa and another in Tanzania. Unexpectedly, we find a split of the Comoran coelacanths into two sympatric subpopulations. Despite its undeniably slow evolutionary rate, the coelacanth still diversifies and is therefore able to adapt to new environmental conditions.

Latimeria chalumnae is of prime interest for evolutionary biology, but is a rare and endangered species and efforts for its conservation are underway [1]. Data on population dynamics and gene flow will help to determine the survival capacity of coelacanth populations in Africa and add to focusing conservation efforts. We studied intraspecific genetic variation by using standard microsatellite-based analyses. For 70 coelacanth genotyping was possible for at least 9 out of 14 analysed microsatellite loci, which showed significant levels of differentiation (Supplemental information). Also, assignment analyses uncovered a subdivision of the samples (Figure 1A,B) into different populations that correlated with three geographic regions: Comoros, Tanzania, and South Africa. Unexpectedly, the Comoros harbour two different genetic groups.

Notwithstanding this clear population differentiation, overall microsatellite allelic diversity was low (median 5 alleles/locus). The probability of identity for all markers combined, however, was $2.88*10^{-8}$, rendering a random occurrence of identical genotypes very unlikely. Private alleles were found in 10 of the 14 loci: 12 alleles were specific for the Comoros, three for South Africa and 13 for Tanzania. Heterozygosity rate differed greatly between individuals but overall was rather low (0.44 ± 0.18).

Mitochondrial D-loop sequences from 62 individuals [2,3], including 13 new specimens from Tanzania, were analyzed. They confirmed the very low genetic diversity in *Latimeria chalumnae*. Only 9 very similar haplotypes were found (Figure 1C) and within the 726 bp alignment only 8 positions were variable (Supplemental information). Haplotypes differed maximally at five positions (average 2.7 = 0.37%; Figure 1C). Consequently, the mean genetic distance (Kimura two parameter distance) between haplotypes was similarly low: 0.0038 ± 0.002. Seven of the eight exchanges were transitions and six were A to G mutations (Supplemental information). This low level of haplotype and mutation diversity appears counterintuitive considering the species' long existence. Either the evolutionary rate in *Latimeria chalumnae* is extremely slow [4] or haplotypes may have diverged rather recently after the species went through a bottleneck.

Haplotype frequencies varied substantially. H3 was most common (almost 50% of all individuals) and might therefore be the ancestral haplotype. It has also the broadest geographic distribution being found in Tanzania, the Comoros, Madagascar and South Africa. H1 and H5 were found in two regions, while all other haplotypes were restricted to a single site (Figure 1C,D; Supplemental information).

Field sites differed significantly in haplotype variability (permutation test $Z^* = 6.823$; $p = 0.001$). Most individuals came from the Comoros and three different haplotypes were identified here. In Tanzania within 15 individuals 6 haplotypes were found. In Kenya a haplotype (H5) was found that was also present only in northern Tanzania. H4 was only found in Mozambique and H6 was restricted to South Africa (Figure 1D, Table S2). High levels of differentiation were found between South Africa and the Comoros and slightly lower levels between the Comoros and Tanzania and South Africa and Tanzania (Supplemental information). This is in agreement with our microsatellite results and confirms a recent study [5] on a different set of specimens from Tanzania, where, based on mitochondrial sequences a population divergence of Tanzanian Latimeria from the Comoros was proposed. However, due to the variable (sometimes very low) numbers of individuals available and the fact that mitochondrial DNA is inherited as a single matrilineal locus, these data should be taken with some caution. For instance, our microsatellite analyses did not confirm a separation of northern vs. southern Tanzanian coelacanths [5].

Our results shed new light on the ecology of *Latimeria*. Initially it was assumed that there was only one single viable population at the Comoros harbouring 300 to 400 individuals. Sporadic captures in Mozambique and Madagascar were attributed to individuals that were accidentally transported by strong currents [3,6]. The finding of a number of individuals in Tanzania and South Africa, however, and a more thorough investigation of the ecology of these sites revealed small but viable populations in these areas [1,4,7]. Our genetic data support the hypothesis that these populations might initially have come from the Comoros. However, since they were separated they genetically diverged from their ancestral population. In fact, constant gene flow between the African coelacanth populations seems unlikely as the geographic distance between e.g. South Africa and the Comoros is large and at least in one direction movement is restricted by strong currents [3,6].

While allopatric diversification is rather common, the separation of the Comoros population into two genetically distinct groups is much harder to understand. This split is even more pronounced in the microsatellite data but the nuclear genotype groups do not correlate with certain mtDNA haplotypes. The two Comoran subpopulations cannot be separated based on collection sites and the geography of the region. Ecological factors might act as strong disruptive selection pressures. However, such factors still need to be investigated.

Coelacanths are generally viewed as evolutionary relics. Levels of

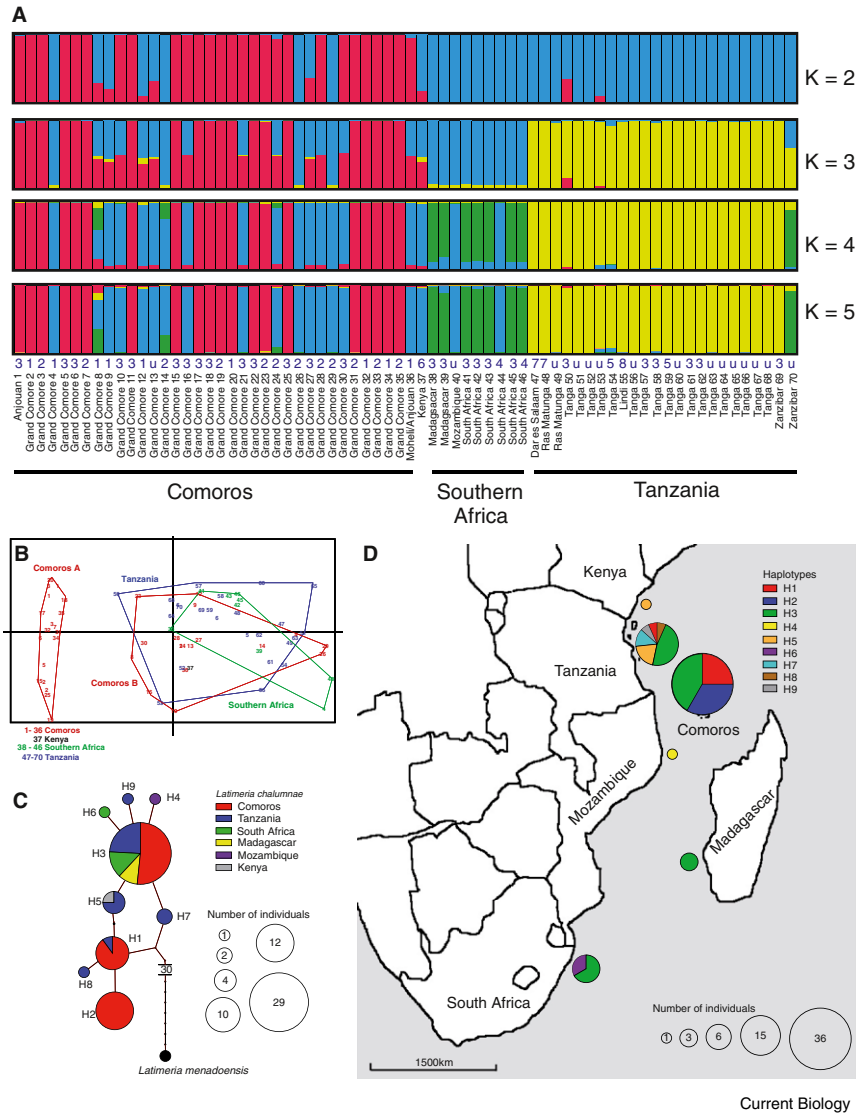

Figure 1. Coelacanth population genetics.
(A) Genetic clustering analysis for K = 2 to K = 5. Each column represents an individual with the fraction of each cluster (color coded) given on the y-axis. STRUCTURE as well as TESS proposed 3 to 5 clusters as the most likely number of genetically separated groups in the sample. K = 4 seems to be the most informative clustering. The population from the Comoros is split in two different groups from the beginning (K = 2), suggesting a strong sub-division of this rather large population. The Tanzania samples split next (K = 3) and there seems to be one more genetically distinct population in South Africa (K = 4). A raise to K = 5 did not reveal any more splits. (B) Principal component analysis of the studied individuals. The x-axis (first component) is significant ($p = 0.001$), the y-axis (second component) is not significant. Different geographic regions are color coded. This graph confirms the results from the STRUCTURE analyses. (C) Haplotype network for all 9 mtDNA control region haplotypes identified in *Latimeria chalumnae* (H1 to H9). For comparison, the connection to the mtDNA control region haplotype of *the* Indonesian coelacanth*, Latimeria menadoensis,* is given. Different colors depict the geographic area where the respective haplotype was found. The size of the circle correlates with the number of individuals found to have this particular haplotype. (D) Geographic distribution of *L. chalumnae* haplotypes. Different haplotypes are depicted in different colors. The circle diameter correlates with the number of individuals investigated at this location.

population divergence and allelic diversity are low and confirm the assumed slow rate of molecular evolution in coelacanths. Obviously, even such slow evolutionary rates allow for local adaptation. As shown earlier for coelacanths [2,8] and recently for cycad plants [9], near extinction need not be an evolutionary dead end.

Supplemental Information

Supplemental Information (experimental procedures and two tables) can be found with this article online at doi:10.1016/j.cub.2012.04.053.

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[1]University of Bochum, Evolutionary Ecology and Biodiversity of Animals, Universitaetsstr. 150, D-44780 Bochum, Germany. [2]Max-Planck-Institut für Marine Mikrobiologie, Bremen, Germany. [3]Leibniz-Institut für Meereswissenschaften, Kiel, Germany. [4]University of Wuerzburg, Physiological Chemistry I, Biocenter, Am Hubland, D-97074 Wuerzburg, Germany. [5]Present address: Department of Environmental Sciences, University of Basel, St Johanns-Vorstadt 10, 4056 Basel, Switzerland. [6]Tanzania Fisheries Research Institute, P.O. Box 9750, Dar es Salaam, Tanzania.
*E-mail: phch1@biozentrum.uni-wuerzburg.de