Phylogeographic structure of *Octopus vulgaris* in South Africa revisited: identification of a second lineage near Durban harbour

P. R. Teske, A. Oosthuizen, I. Papadopoulos, N. P. Barker

Abstract

In a previous study that investigated genetic structure of *Octopus vulgaris* along the South African coast by sequencing the mitochondrial cytochrome oxidase III gene (COIII), all sequences generated were identical. Such a finding is unusual, because mitochondrial DNA mutates quickly, and several marine invertebrates present in southern Africa show considerable genetic variation and structure. We reanalysed the samples using two different mitochondrial markers, namely cytochrome oxidase I (COI) and the large ribosomal subunit (16S rRNA). Sequences of both these markers showed variation. The conclusion of the previous study, that South Africa's *O. vulgaris* population is characterised by a lack of genetic structure along the coast, is rejected. Some specimens from Durban (southeast Africa) were genetically more different from those found in the remainder of the country than were specimens from other regions (Tristan da Cunha and Senegal). We suggest that the lineage in Durban may have been recently introduced.

Introduction

The amount of genetic structuring in marine organisms is generally considered to be negatively correlated with dispersal ability (Palumbi *1994*). Recent genetic studies on passively dispersing southern African marine invertebrates have identified considerable phylogeographic subdivision along the coast, with several widely distributed species comprising two or more regional lineages (Ridgway *1998*; Teske et al. *2006*; Zardi et al. *2007*). In contrast, most species that are able to disperse actively are characterised by little or no genetic structure (Tolley et al. *2005*; Klopper *2005*; Norton *2006*; but see Gopal et al. *2006*), suggesting that a species' degree of genetic structuring along the South African coast depends to a large extent on its mode of dispersal. In a previous study on the South African population of *Octopus vulgaris*, Oosthuizen et al. (*2004*) identified only a single mitochondrial COIII haplotype among 35 samples collected between Hout Bay (west coast) and Port Alfred (south coast) and concluded that a single genetic population exists in South Africa. Such a conclusion is reasonable given that newly hatched *O. vulgaris* are planktonic for approximately 50–60 days (Villanueva *1995*) and adults are known to migrate (Mangold and Boletzky *1973*; Smale and Buchan *1981*). However, the fact that all sequences were identical is problematic, as mitochondrial DNA mutates quickly and a long-established population should show some variation. There are several possible explanations for the lack of genetic diversity: (a) the southern African *O. vulgaris* population has undergone a recent genetic bottleneck or founder event; (b) mitochondrial DNA in *O. vulgaris* evolves more slowly than in other marine organisms; (c) the low amount of genetic diversity is a sampling artifact (this is unlikely, because the number of samples collected was similar to those in other studies); (d) there has been a contamination problem during the amplification process (this is also unlikely, because the sequence generated was identical to one independently generated by another researcher [Warnke *1999*]) and (e) the COIII sequences generated previously are not actually of mitochondrial origin, but are amplicons of a numt (i.e. a nuclear mitochondrial pseudogene that has arisen from mitochondrial DNA being integrated into the nucleus), which exhibits a considerably lower mutation rate than the homologous mitochondrial marker. The COIII sequence previously generated (Accession No. AJ250487) does not contain any stop codons or shifts of the reading frame, which would indicate that it could be from a non-functional numt. However, if the insertion into the nucleus has taken place recently, then insufficient time has passed for such mutations to take place.

In this study, we reanalysed some of the samples used by Oosthuizen et al. (*2004*) to determine which of the possible reasons for the lack of genetic variation is the most likely, by amplifying partial sequences of COI and 16S rRNA and determining whether these alternative markers showed higher levels of genetic variation. We also sequenced some additional samples from South Africa and elsewhere.

Materials and methods

Sampling and laboratory work

We sequenced a portion of the COI gene and of the 16S rRNA of three randomly selected samples each from three localities in the region previously sampled by Oosthuizen et al. (*2004*): Hout Bay, Struisbaai and Port Elizabeth (southwest and south coast; Fig. 1). In addition, we sequenced eight samples from South Africa's east coast (seven from Durban and one from Umhlanga), as well as a number of specimens from populations that are closely related to the South African lineage of *Octopus vulgaris* (Oosthuizen et al. *2004*). These originated from the South Atlantic island of Tristan da Cunha (three specimens), Senegal (West Africa; three specimens) and Spain (Atlantic coast and Mediterranean coast; three and one specimens, respectively). Extraction, amplification and sequencing of molecular markers followed protocols described previously (Teske et al. *2004*, *2006*). The COI gene was amplified using primers LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′; Folmer et al. *1994*) and 16S rRNA was amplified using primers 16SarL (5′-CGC CTG TTT ATC AAA AAC AT-3′) and 16SbrH (5′-CGG GTC TGA ACT CAG ATC ACG T-3′; Palumbi *1996*).

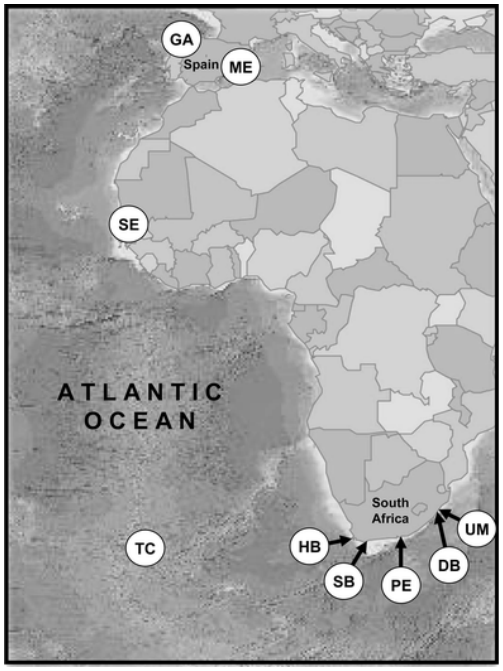

Fig. 1 A map of the eastern Atlantic Ocean showing sampling sites. Acronyms represent the following localities: *GA*, Galicia (Atlantic Ocean coast of Spain); *ME*, Mediterranean coast of Spain; *SE*, Senegal; *TC*, Tristan da Cunha; *HB*, Hout Bay; *SB*, Struisbaai; *PE*, Port Elizabeth; *DB*, Durban; *UM*, Umhlanga

Data analyses

Sequences were aligned by eye in MEGA version 3.1 (Kumar et al. *2004*). The 16SrRNA sequences included a short microsatellite, which was excluded from the analyses because it was not phylogenetically informative (when the microsatellite was excluded, sequences were grouped according to geographical region; when it was included, all geographical structure was lost). A minimum spanning haplotype network of combined COI and 16S rRNA sequences was constructed using Arlequin 3.1 (Excoffier et al. *2005*).

To determine whether mitochondrial DNA in *Octopus vulgaris* evolves more slowly than in other marine invertebrates, we carried out pairwise relative rate tests between COI and COIII sequences of *Octopus* with those of other cephalopods using the programme RRTree (Robinson-Rechavi and Huchon *2000*). Tests were performed

separately for synonymous and non-synonymous substitutions by computing the parameters B4 (number of synonymous transversions per fourfold degenerate site) and Ka (number of non-synonymous substitutions per non-synonymous site). Cephalopod species for comparison were selected on the basis that they originated from regional lineages that showed genetic variation. The evolutionary rate of the COI sequences of all *Octopus vulgaris* specimens generated in this study were simultaneously compared with that of two squid species, *Loligo pealei* and *L. plei* (Herke and Foltz *2002*). In the case of the COIII sequences, we compared *Octopus vulgaris* sequences available on GenBank with those of *Sepia apama* (Kassahn et al. *2003*). In both cases, *Nautilus macromphalus* was specified as outgroup. Accession numbers of all sequences used for the relative rate tests are listed in "Appendix 1".

Results

A total of 23 COI sequences and 24 16S rRNA were generated. These were submitted to GenBank (accession numbers DQ683205–DQ683251). Eighteen unique 16S rRNA haplotypes (or 7 when gaps were excluded) and six unique COI haplotypes were recovered. The length of aligned 16S rRNA sequences of *O. vulgaris* was 508 base pairs, and the COI sequences were 526 base pairs in length. The South African population was characterised by a dominant haplotype that was found at all five sampling localities (Fig. 2). Two other South African haplotypes differed from it by a single nucleotide substitution, as did the specimens from Tristan da Cunha and the specimens from Senegal. Two of the individuals from Durban had combined COI/16S rRNA sequences that were as different from the main South African clade as were the specimens from Spain. The maximum number of pairwise differences between sequences of South African samples was seven (1.3%) for COI and two (0.4%) for 16S rRNA, exluding gaps. The 16S rRNA sequence of the seventh *Octopus* specimen collected in Durban (Accession No. DQ683251) revealed that this individual had been misidentified and was actually a member of the tropical species *O. cyanea* (its COI gene was not sequenced). The 16S rRNA sequence of this sample differed from that of a previously published sequence of a specimen from the Mariana Islands (Accession No. AJ252779) by a single nucleotide substitution.

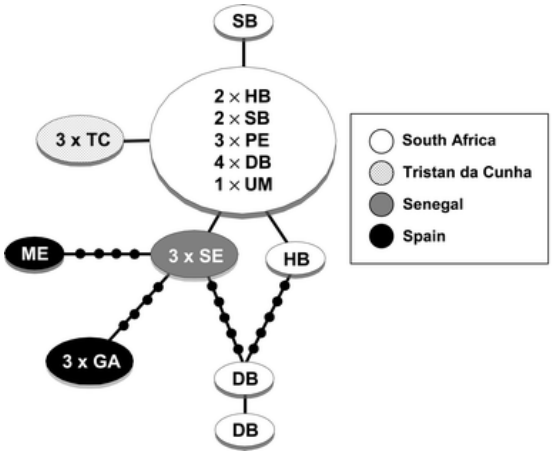

Fig. 2 A minimum-spanning haplotype network constructed from combined COI and 16S rRNA sequences of *Octopus vulgaris* from South Africa, Tristan da Cunha, Senegal and Spain. *Small black circles* are interior node haplotypes not present in the samples. Acronyms represent the following populations: *HB*, Hout Bay; *SB*, Struisbaai; *PE*, Port Elizabeth; *DB*, Durban; *SE*, Senegal; *TC*, Tristan da Cunha; *GA*, Galicia (Atlantic coast of Spain); *ME*, Mediterranean coast of Spain

Relative rate tests comparing COI sequences of *Octopus vulgaris* generated in this study with the squid species *Loligo pealei* and *L. plei*, as well as those comparing COIII sequences of *Octopus vulgaris* and *Sepia apama*, were non-significant (COI, B4: $P = 1.0$; Ka: $P = 0.5$; COIII, B4: $P = 1.0$; Ka: $P = 0.7$).

Discussion

The low amount of genetic differentiation among combined COI and 16S rRNA sequences of the majority of South African samples indicates that the lack of variation found in the previous study is unlikely to have been due to the amplification of a numt. Morever, there is no support for the hypothesis that mitochondrial DNA evolves more slowly in *Octopus vulgaris* than in regional lineages of other cephalopods that showed higher levels of genetic variation. This suggests that South Africa's *Octopus vulgaris* population has either experienced a recent genetic bottleneck or has recently been founded.

It is as yet difficult to interpret the presence of two genetically different lineages of *O. vulgaris* in southern Africa. The fact that one lineage was very abundant along most of the coast, whereas the other has so far only been found in Durban, suggests that the latter may have been recently introduced. Its presence in the vicinity of one of Africa's busiest harbours could be an indication that it arrived there in a ship's ballast water. On the other hand, the low genetic variation in the more common lineage, and its close association with populations from fairly distant localities, could be an indication of a recent founder event. More sampling is necessary to determine whether the less common lineage found in Durban has recently been introduced (in which case identical mitochondrial haplotypes should be found in other regions), or whether it could be a long-established lineage that has been replaced in most of its southern African habitat by a more recent arrival (in which case it should be genetically distinct from other populations). BLAST searches of GenBank using BLASTN 2.2.14 (Altschul et al. *1997*) revealed that the COI and 16S rRNA sequences of the two specimens found near Durban harbour are distinct from all of the *O. vulgaris* sequences that have so far been submitted (generated from samples collected in Europe, Senegal, South Africa, Tristan da Cunha, the Americas and Japan). This suggests that further sampling needs to be conducted in regions from which no genetic data on *O. vulgaris* are as yet available, such as East Africa, India and Southeast Asia.

Acknowledgments We are grateful to Francisco Santaclara (Vigo, Spain) for providing tissue samples from Spain and Senegal, to James Glass for providing samples from Tristan da Cunha, and to Pierre Pradervand for providing samples from Durban and Umhlanga. We also thank three anonymous referees for some useful comments that considerably improved the quality of this manuscript. This study was supported by a Claude Harris Leon Foundation postdoctoral research fellowship awarded to PRT, Rhodes University and an NRF grant to NPB (GUN 2069119). The experiments conducted in this study comply with current South African laws.

Appendix 1

List of sequences used for pairwise relative rate tests; COI: *Nautilus macromphalus* DQ472026, *Loligo pealei* AF207909–AF207926 and AY039621–AY039623, *L. plei* AF207927–AF207947 and AY039619–AY039620; COIII: *N. macromphalus* DQ472026, *Octopus vulgaris* AJ012121–AJ012127, AJ250476–AJ250479, AJ250481, AJ250487, AJ616311, AJ616312, *Sepia apama* AY294336–AY294353.

References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3:294–299

Gopal K, Tolley KA, Groeneveld JC, Matthee CA (2006) Mitochondrial DNA variation in spiny lobster Palinurus delagoae suggests genetically structured populations in the southwestern Indian Ocean. Mar Ecol Prog Ser 319:191–198

Herke SW, Foltz DW (2002) Phylogeography of two squid (Loligo pealei and L. plei) in the Gulf of Mexico and northwestern Atlantic Ocean. Mar Biol 140:103–115

Kassahn K, Donnellan S, Fowler A, Hall K, Adams M, Shaw P (2003) Molecular and morphological analyses of the cuttlefish Sepia apama indicate a complex population structure. Mar Biol 143:947–962

Klopper AW (2005) Intraspecific genetic variation in the percoid teleosts Argyrosomus japonicus (Temminck and Schlegel, 1843) and Pomadasys commersonnii (Lacepède, 1801) as inferred from the mitochondrial control region. M. Sc. thesis, University of Pretoria, South Africa

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163

Mangold K, Boletzky S (1973) New data on the reproductive biology and growth of Octopus vulgaris. Mar Biol 19:7–12

Norton O (2006) The population structure of two estuarine fish species along the southern African coastline. M. Sc. Thesis, Rhodes University, South Africa

Oosthuizen A, Jiwaji M, Shaw P (2004) Genetic analysis of the Octopus vulgaris population on the coast of South Africa. S Afr J Sci 100:603–607

Palumbi SR (1994) Genetic divergence, reproductive isolation and marine speciation. Annual Rev Ecol Syst 25:547–572

Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK (eds) Molecular systematics. Sinauer Associates, Sunderland, pp 205–247

Ridgway TM, Steward BA, Branch GM, Hodgson AN (1998) Morphological and genetic differentiation of Patella granularis (Gastropoda: Patellidae): recognition of two sibling species along the coast of southern Africa. J Zool Lond 245:317–333

Robinson-Rechavi M, Huchon D (2000) RRTree: relative-rate tests between groups of sequences on a phylogenetic tree. Bioinformatics 16:296–297

Smale M, Buchan P (1981) Biology of Octopus vulgaris off the east coast of South Africa. Mar Biol 65:1–12

Teske PR, Cherry MI, Matthee CA (2004) The evolutionary history of seahorses (Syngnathidae: Hippocampus): molecular data suggest a West Pacific origin and two invasions of the Atlantic Ocean. Mol Phylogenet Evol 30:273–286

Teske PR, McQuaid CD, Froneman PW, Barker NP (2006) Impacts of marine biogeographic boundaries on phylogeographic patterns of three South African estuarine crustaceans. Mar Ecol Prog Ser 314:283–293

Tolley KA, Groeneveld JC, Gopal K, Matthee CA (2005) Mitochondrial DNA panmixia in spiny lobster Palinurus gilchristi suggests a population expansion. Mar Ecol Prog Ser 297:225–231

Villanueva R (1995) Experimental rearing and growth of planktonic Octopus vulgaris from hatching to settlement. Can J Fish Aquat Sci 52:2639–2650

Warnke K (1999) Diversität des Artenkomplexes Octopus cf. vulgaris Cuvier, 1797 in Beziehung zu seiner Verbreitung an der Ost- und Westküste Lateinamerikas. PhD thesis, Universität Bremen, Shaker Verlag, Aachen

Zardi GI, McQuaid CD, Teske PR, Barker NP (2007) Unexpected genetic structure in indigenous (Perna perna) and invasive (Mytilus galloprovincialis) mussel populations in South
Africa. Mar Ecol Prog Ser (in press)