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Genetic markers reveal a gradient of hybridization between cape hakes (*Merluccius capensis* and *Merluccius paradoxus*) in their sympatric geographic distribution

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ABSTRACT

The cape hakes *Merluccius capensis* and *Merluccius paradoxus* are important fishing resources for African countries such as Namibia and South Africa. In this study we have genetically analyzed adult samples from the overlapping distribution of these species. Eight microsatellite loci, the nuclear 5S rDNA locus and the Cytochrome Oxidase subunit I (COI) gene were employed as molecular markers. A North–South gradient of interspecific hybridization was found, with discordant mitochondrial and nuclear genotypes at the northernmost edge of *M. paradoxus* distribution. These results suggest intense introgression in North Benguela off the Namibian coast. Independent hake stock assessment is recommended in this region for sustainable management of this valuable resource.

1. Introduction

Hybrid zones may occur when two formerly separated species meet again (Avise and Wollenberg, 1997; Hewitt, 2001). They often arise at biogeographic borders and may occur for different taxa in what it is called a suture zone (Hewitt, 2000). In the marine realm hybrids between different animal species are relatively frequent (e.g. Gardner, 1997; Miralles et al., 2013; Palumbi, 1994; Srinivasa Rao and Lakshmi, 1999) because, among other reasons, many species have mass spawning and/or interspecific reproductive barriers may be weak. However, marine hybrid zones have been considered rare, perhaps because they have not been sufficiently studied (Arnold, 1997; Gardner, 1997). They have been reported for a few species, such as mussels of the genus *Mytilus* (e.g. Bierne et al., 2003, Riginos and Cunningham, 2005), redfish of the genus *Sebastes* (e.g. Roques et al., 2001), hakes of the genus *Merluccius* (Machado-Schiaffino et al., 2010) and some coral reef fishes (Hobbs et al., 2009). A variety of genetic consequences can result from hybridization (Seehausen, 2004, 2006). In cases of hybridization but no introgression, no genetic consequences are expected (this would be an evolutionary dead end). When there is introgression through unidirectional gene flow, one species will lose its genetic identity. Introgression through bi-directional gene flow will potentially result in reverse speciation (Seehausen, 2006). Finally, another possible outcome is hybrids becoming a new lineage (see Seehausen, 2004).

Hybridization is not expected to occur with the same frequency in all the areas where two species are sympatric. Hybrids are more frequent in marginal populations, where mate choice may be relaxed (e.g. Ritchie, 2007), and in the colonization front when one of the species is displacing or expanding its distribution (e.g. Carson and Templeton, 1984; Horreo et al., 2011). It also happens where the two sympatric species are unequally abundant (e.g. Arnold, 1997; Hobbs et al., 2009). In these cases asymmetric hybridization would be expected, the rarer species providing frequently the female in hybrid crosses (e.g. Wirtz, 1999).

Identification of hybrid zones is especially important for species subjected to exploitation because they may require a distinct management. Allendorf et al. (2001) have classified hybrid zones in six different types based on their origin (natural versus anthropogenic) and on the extent of introgression, with differential management and conservation priorities proposed for each of them. Cape hakes (*Merluccius capensis* and *Merluccius paradoxus*) are two of the most economically and ecologically important African fishing resources (Alheit and Pitcher, 1995; Boyer and Hampton, 2001), and have been subjected to sustainable management initiatives for the last decades (e.g. Butterworth and Rademeyer, 2005; Hutchings et al., 2009a). They overlap in the large part of their distributions, along the coastlines of Namibia and South Africa (Fig. 1), but they inhabit at different depths. *M. capensis* is known as the shallow cape hake while *M. paradoxus* is called the deep cape hake (Alheit and Pitcher, 1995).

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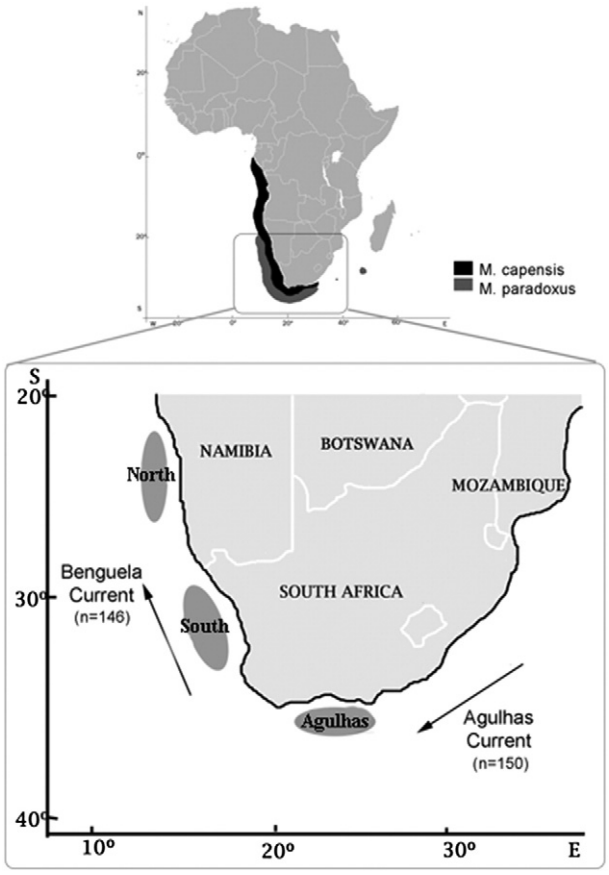

Fig. 1. *Merluccius capensis* and *Merluccius paradoxus* distribution range (above). Sampling areas in Agulhas, South Benguela and North Benguela are marked in dark in the enlarged section (below).

Cape hakes' population structure has been described by von der Heyden et al. (2007b): there are no barriers to dispersal between Namibian and South African waters for *M. capensis* while for *M. paradoxus* there are significant spatial population genetic differences. Spawning of the two cape hakes overlaps temporally. In South African waters, spawning occurs from August to March with two apparent peaks, the first at the end of the year for both species and the second in the austral autumn mainly for *M. paradoxus* (Assorov and Berembeim, 1983; Botha, 1986). In Namibian waters, *M. capensis* spawns throughout the year, more intensely between July and October, while by now there is no evidence of *M. paradoxus* spawning there (Alheit and Pitcher, 1995; Assorov and Berembeim, 1983; Kainge et al., 2007). Although little is known about the spawning behavior of these two species, reproductive barriers between them seem to exist, at least partially, due to depth. Von der Heyden et al. (2007a) and Stenevik et al. (2008) found eggs of *M. paradoxus* distributed in deeper waters than *M. capensis* eggs (with an average depth of 231 m and 348 m for *M. capensis* and *M. paradoxus* respectively). However, displacement of cape hakes has been reported in response to change in the oxygen content of bottom waters, *M. capensis* entering in contact with *M. paradoxus* (Hamukuaya et al., 1998). Since hybrid zones have been reported for other overlapping species of this genus (the North American hakes *Merluccius albidus* and *Merluccius bilinearis*; Machado-Schiaffino et al., 2010), it is theoretically possible that the same phenomenon occurs also for cape hakes.

The objective of this study was to examine the extent and direction of possible introgressive hybridization and to identify potential hybrid zones in cape hakes. For this purpose, adults of both species were sampled from different areas across the overlapping distribution and genotyped for eight microsatellite loci, the nuclear 5S rDNA locus and the Cytochrome Oxidase subunit I (COI) gene for genetic estimation of their hybrid status.

2. Materials and methods

2.1. Sampling

A total of 296 cape hakes, *M. capensis* and *M. paradoxus*, were collected during 2002–2003 from three different areas in the overlapping zone of both species in the south Atlantic Ocean (Fig. 1): two within the Benguela current (North and South, 11–14°E 22–26°S and 15–18°E 30–33°S, respectively) and one within the Agulhas current (20–24°E 34–36°S). They were taxonomically identified de visu by local experts. Tissue samples (muscle or fin biopsy of approx. 1 mm^3) were obtained from each individual and stored in absolute ethanol until analysis.

2.2. Genetic analysis

Eight microsatellite loci were assayed: Mmer-Hk3, Mmer-Hk9, Mmer-Hk20, Mmer-Hk29, Mmer-Hk34 (Morán et al., 1999), Mmer-UEAW01 (Rico et al., 1997), Maus7 and Maus32 (Machado-Schiaffino and Garcia-Vazquez, 2009). PCR conditions and protocols were slightly modified from Machado-Schiaffino et al. (2010) for optimizing amplification in *M. capensis* and *M. paradoxus* (Table 1). PCR products were separated using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems), with BigDye 3.1 Terminator system, in the Unit of Genetic Analysis of the University of Oviedo (Spain). Genotypes were determined employing the GeneMapper® Software Version 4.0.

The nuclear 5S rDNA coding gene was genotyped as described by Perez and Garcia-Vazquez (2004). *M. capensis* yields only one fragment of 371 nucleotides and *M. paradoxus* provides two fragments of 371 and 494 nucleotides. Fragment sizes were determined in 2% agarose gels by comparison with a DNA mass ladder.

The mitochondrial COI gene was amplified employing the primers COIFish-F1 and COIFish-R1 (Ward et al., 2005). PCR reactions were carried out accordingly with the protocols described by Ward et al. (2005). PCR products were visualized, purified and sequenced as described in Machado-Schiaffino et al. (2010). PCR products were visualized in 50 ml 2% agarose gels 3 μl of ethidium bromide (10 mg/ml). Stained bands were excised from the gel and DNA was purified with a Wizard SV Gel and PCR Clean up system (Promega) prior to sequencing. Automated fluorescence sequencing was performed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) in the Unit of Genetic Analysis of the University of Oviedo (Spain).

2.3. Data analysis

Microsatellite scoring errors, large allele dropout and null alleles were checked employing MICROCHECKER (van Oosterhout et al., 2004). GENEPOP (Raymond and Rousset, 1995) was employed to test for linkage disequilibrium and departure from Hardy–Weinberg equilibrium. Microsatellite variation parameters such as expected and observed heterozygosity were calculated with GENETIX Version 4.03 (Belkhir et al., 2004). FSTAT Version 2.9.3.2 (Goudet, 2001) was used to calculate microsatellite allelic richness. To identify individuals from each pure species, hybrids of first generation and backcrosses we employed NewHybrids (Anderson and Thompson, 2002), with settings of 300 000 Monte Carlo Markov Chain (MCMC) iterations after a burn-in period of 30 000 iterations. The Bayesian software STRUCTURE v.2.3.1 (Pritchard et al., 2000) was used to estimate the membership of each individual to each species with the "Admixture model" and $K = 2$ (two expected genetic units, one corresponding to each species), which assumes that individuals may have mixed ancestry. Settings were a burn-in period of 100 000 steps followed by 1 000 000 MCMC iterations. Since there is no clear consensus about the proportion of membership