Research on the genetics of South African sardine *Sardinops sagax*

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Previous research

Research into the genetics of South African sardine was conducted during the 1990s as part of larger studies to examine the phylogeography of the monotypic genus *Sardinops* through analysis of mitochondrial DNA from specimens collected off southern Africa (Indian and Atlantic waters), Australia and New Zealand, Japan, and the Pacific coast of North and South America (Okazaki *et al*. 1996, Bowen and Grant 1997, Grant *et al*. 1998). These three studies provided similar results of strong geographic structuring of mtDNA lineages and three monophyletic clades corresponding to (a) South Africa and Australia, (b) Chile and California, and (c) Japan, leading Grant *et al* (1998) to recommend three subspecies designations: *Sardinops sagax ocellatus* in southern Africa, Australia and New Zealand; *Sardinops sagax sagax* in Chile, Peru, Ecuador, Mexico, the United States and Canada; and *Sardinops sagax melanostictus* in China, Korea, Japan and Russia (Kasapidis 2014).

More recently, Shannon (2014) used a novel mitochondrial DNA marker (NADH dehydrogenase subunit 2; ND2) and seven microsatellite DNA loci to test the hypothesis of multiple sardine stocks off South Africa, with samples collected off the South African West and South Coast in 2007 and 2008, and from Namibia, and the South African West, South and East Coast in 2009. Mitochondrial DNA from 33 individuals collected in 2007 and 53 collected in 2009 showed high levels of haplotype diversity (27 and 41 for 2007 and 2009, respectively) and a star-like haplotype network with few shared alleles, which has previously been reported for sardine (Bowen and Grant 1997, Grant and Bowen 1998). No geographic structuring was found but sample sizes were small given the high genetic heterogeneity observed. Microsatellite loci also showed high heterogeneity and individual variability in sardine collected in all three years, with an Analysis of Molecular Variance showing that the majority of the genetic variance occurred at the level of the individual (Shannon 2014). These mitochondrial and microsatellite data do not indicate the occurrence of genotypically-differentiated sardine stocks off South Africa (Shannon 2014) but suggest possible microgeographic structuring that is temporally unstable. This was deemed to support the sweepstakes hypothesis, under which a small number of adult individuals can be disproportionately responsible for successful recruitment in subsequent years, which results in a pattern of small patches of genetically differentiated groups, which are not consistent across years, in an otherwise genetically well-mixed population (Hampton 2014).

Present research

A project that aims to examine South African sardine stock structure using genomics received funding from the NRF for a 3-year period and was initiated in 2014 (Teske 2013). This project will use recent advances in DNA sequencing technology (Next-Generation Sequencing or NGS) to generate

data from hundreds of thousands of loci, as opposed to the few loci analysed previously. It is possible that the apparent lack of genetic structure in South African sardine observed by Shannon (2014) is a consequence of genotyping a small number of selectively neutral loci, which may be too uninformative for the study of a species that might be anticipated to show only minimal genetic divergence between stocks given a large population size, moderate to high levels of gene flow, and boom and bust abundance cycles (Teske 2013). Hence the genomic approach offers a substantial advantage over previous molecular studies as it is more likely to capture the few loci that show genetic structure should such structure have only developed recently. Additionally, the genomic approach enables the identification of gene regions that are involved in adaptation to regional environmental conditions and which are more likely to show divergence, than are selectively neutral loci. NGS is particularly powerful for examining the genetic structure of neritic species with large population sizes, moderate to high levels of gene flow and recent demographic histories, and has been successfully used to detect subtle genetic differentiation linked to environmental parameters in Atlantic herring *Clupea harengus*, a high dispersal species that appears to be panmictic on the basis of selectively neutral markers (Limborg *et al*. 2012). Those authors analysed 281 single nucleotide polymorphisms (SNPs) and combined genome scan and landscape genetic analyses to identify four genetically distinct groups of herring, and reported a complex pattern of varying spatial variation among outlier loci likely reflecting adaptation to local environments.

The identification of gene regions involved in adaptation to regional environmental conditions is particularly relevant to South African sardine, because this population occurs in two different, temperature-defined marine bioregions off South Africa throughout the year (the cool temperate bioregion off the west coast and the warm temperature bioregion off the south coast), and also in a third bioregion (the subtropical bioregion off the east coast) during the winter KZN sardine run. Marine biogeography is believed to be strongly shaped by water temperature and considered likely to be the direct result of a species' thermal tolerance range (Portner *et al.* 2007), implying that populations of ectothermic organisms are primarily sub-divided into evolutionary units adapted to different temperature-defined water masses (Teske 2013). Applying a genomic approach to South African sardine will also provide sufficient information for "close-kin identifications" as estimating relatedness is one of the planned analyses (P. Teske, pers. comm.).

Samples of sardine liver and muscle tissue, and fin clips, were collected for genomic analysis from a total of 80 fish caught from 16 midwater trawls during the May 2014 pelagic recruit survey. Fish ranged in size from 12.4 to 21.7 cm CL but were mostly >16.0 cm CL, and were collected from an area extending from just north of Doring Bay to just east of Mossel Bay, providing good coverage of the putative western sardine stock and some coverage of the putative southern stock. Tissue from 10 adult fish sampled from a purse-seine catch taken off Port Elizabeth (southern and/or eastern stock) in October 2014 has also been collected, and further samples are presently being collected during the November 2014 pelagic spawner survey. Samples from the May 2014 survey have been sent to the University of Johannesburg for DNA and RNA assessment, with good quality DNA being obtained. Samples of sardine muscle tissue only collected during the July 2005 sardine run survey (eastern stock) have also been sent to UJ but their quality and potential for use has yet to be assessed. DNA extraction and library collection will be carried out at Flinders University, Australia, and sequencing at the Genome Quebec Centre in Vancouver, Canada.

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