SCI. MAR., 65 (1): 81-84 SCIENTIA MARINA 2001

NOTE

Species identification of two sympatric hakes by allozymic markers*

M. I. ROLDÁN and C. PLA

Laboratori d'Ictiologia Genètica, Universitat de Girona, Campus Montilivi, E-17071 Girona, Spain. FAX: 34-972-418277.
E-mail: dbmrb@fc.udg.es

SUMMARY: Samples were collected from hake species *Merluccius australis* and *M. hubbsi* in the south west Atlantic Ocean. Enzyme electrophoretic analysis of the eye, liver and muscle revealed 5 out of 33 genetic loci with species-specific allelic frequencies. These five loci provide a set of genetic markers for individual classification.

Key words: identification, allozymes, hake, *Merluccius australis, M. hubbsi,* southwest Atlantic Ocean.

INTRODUCTION

The morphological similarities among species and the uncertainty of phylogenetic relationships suggest the application of molecular techniques. These methods have provided critical insights towards resolving similar problems in teleost taxa. Allozyme electrophoresis has been a widely applied molecular method for comparing levels of genetic divergence between populations and between taxa (e.g. Grant *et al*., 1999). The findings have direct implications on species management and taxa biogeography. Allozyme differences are also useful for inexpensive and uncomplicated species identification using isolated tissues (e.g. fish fillets), or intact individuals of morphologically indistinct taxa (Utter *et al*., 1974; Shaklee *et al*., 1982). These applications are the topic of this communication. Two southwestern Atlantic hakes, *M. australis* (Southern hake) and *M. hubbsi* (Argentinean hake), have overlapping distributions in the austral zone of the Argentinean Sea, with intense commercial harvesting throughout the ranges of both species (Fig. 1). Immature fish of these species are difficult to distinguish by simple observation, and a portion of catches reported as Argentinean hake are believed to be Southern hake (FAO, 1997). Data presented in this work provide a tool for resolving the identification of catches reported from the south-west Atlantic fishery.

*Received April 18, 2000. Accepted July 27, 2000.

MATERIALS AND METHODS

We examined morphologically distinct adults of both species (86 Argentinean hake and 24 Southern hake) captured together in the Argentine Sea (Fig. 1) by Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata, Argentina. They were immediately frozen with dry ice and stored at -80ºC prior to electrophoretic analysis. Tissue extraction, electrophoresis and procedures for visualising pro-

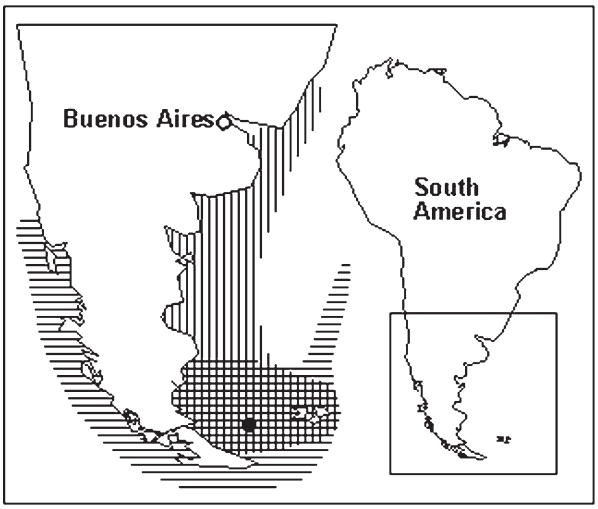

FIG. 1. – Distribution of *Merluccius australis* (horizontal lines) and *M. hubbsi* (vertical lines) from Cohen *et al*., 1990. Solid circle: sampling site.

teins followed the methods outlined in Roldán *et al*. (1998). Extracts from eye, liver and skeletal muscle were electrophoretically screened for resolution and activity with buffer systems described in García-Marín *et al*. (1991) (Table 1). Genetic nomenclature follows Shaklee *et al*. (1990). Alleles were designated by their mobilities relative to the most common allele in *M. hubbsi*, which was designated *100 for each locus. All data analysis was computed by BIOSYS (Swofford and Selander, 1981).

RESULTS AND DISCUSSION

We interpreted the banding patterns of 20 enzyme systems to be a reflection of 33 genetic loci (Table 1). Fifteen out of 33 were monomorphic, based on the identical phenotypes for all individuals tested in both species. Variation was detected for the remaining 18 loci between species (Table 2). The genetic distance (Nei, 1972) 0.157 indicated a close between-species relationship, compared to a distance of 0.583 (31 loci; Grant *et al*., 1988) between two sympatric South African hakes (*M. capensis* and *M. paradoxus*). The expected heterozygosity (Nei, 1978) for both species shows the highest values among the species' genera (*M. australis*=0.099; *M. hubbsi*=0.089) (Roldán *et al*., 1999).

Many of the loci are useful markers for identifying specific catches throughout allelic frequencies

TABLE 1. – Enzyme systems, loci abbreviations and tissues with strongest expression. M, skeletal muscle; L, liver; E, eye.

Enzyme	E C no.	Loci	Tissue	Polymorphic
Adenylate kinase	2.7.4.3	*AK**	M	No
Creatine kinase	2.7.3.2	*CK**	M	Yes
Esterase	3.1.1.-	*EST-1**	M, E	No
		*EST-2**	M, E	Yes
		*EST-3**	M, E	No
		*EST-4**	M, E	Yes
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-1**	M	No
		*GAPDH-2**	E	No
		*GAPDH-3**	E	No
Glycerate dehydrogenase	1.1.1.29	*GLYDH-1**	L	Yes
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH**	M	Yes
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-1**	M	Yes
		*GPI-2**	E	Yes
Glutathione reductase	1.6.4.2	*GR-2**	L	Yes
Isocitrate dehydrogenase	1.1.1.42	*IDHP-1**	M	Yes
		*IDHP-2**	L, E	Yes
L-Lactate dehydrogenase	1.1.1.27	*LDH-A**	M	No
		*LDH-B**	M	No
		*LDH-C**	L	No
Lactoylglutathione lyase	4.4.1.5	*LGL**	M	No
Malate dehydrogenase	1.1.1.37	*MDH-2**	M	No
		*MDH-3**	M, L	No
Malic enzyme (NADP+)	1.1.1.40	*MEP-1**	M	Yes
		*MEP-2**	M	No
		*MEP-3**	L, E	Yes
Peptidase-A (Glycyl-Leucine)	3.4.-.-	*PEP-A**	M, E	Yes
Peptidase-B (Leucyl-Glycyl-Glycine)	3.4.-.-	*PEP-B-1**	M, E	Yes
		*PEP-B-2**	M, E	No
Peptidase-S (Leucyl-Tyrosine)	3.4.-.-	*PEP-S-1**	M, E	Yes
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	M	Yes
Phosphoglucomutase	5.4.2.2	*PGM**	M	Yes
Pyruvate kinase	2.7.1.40	*PK-3**	L	No
Superoxide dismutase	1.15.1.1	*SOD**	L, E, M	Yes

TABLE 2. – Allelic frequencies of 18 polymorphic loci of *M. australis* and *M. hubbsi* from the Argentinian Sea. (N).

Locus	allele	*M. australis* (24)	*M. hubbsi* (86)
*CK**	*100	0.729	1.000
	*115	0.271	
*EST-2**	*90	0.979	0.250
	*100	0.021	0.430
	*110		0.320
*EST-4**	*90		0.198
	*95	0.375	
	*100	0.625	0.750
	*110		0.052
*GLYDH-1**	*100	1.000	0.994
	*115		0.006
*G3PDH**	*100		0.959
	*110	1.000	0.041
*GPI-1**	*-200	0.021	0.012
	*100	0.958	0.977
	*300	0.021	0.012
*GPI-2**	*85	0.063	0.006
	*100	0.917	0.977
	*125	0.021	0.017
*GR-2**	*83		0.006
	*100	1.000	0.994
*IDHP-1**	*65	0.021	
	*75	0.083	0.093
	*100	0.896	0.907
*IDHP-2**	*100	0.021	0.884
	*115	0.875	0.116
	*135	0.104	
*MEP-1**	*95	0.083	
	*100	0.917	1.000
*MEP-3**	*80		0.006
	*100	1.000	0.994
*PEP-A**	*85		0.017
	*100	0.854	0.983
	*105	0.146	
*PEP-B-1**	*80	0.979	0.006
	*100	0.021	0.994
*PEP-S-1**	*95		0.140
	*100	0.896	0.849
	*110	0.104	0.012
*PGDH**	*20	0.813	
	*28	0.167	
	*32	0.021	0.006
	*55		0.076
	*75		0.314
	*90		0.029
	*100		0.267
	*110		0.017
	*120		0.076
	*130		0.134
	*145		0.035
	*155		0.047
*PGM**	*70		0.006
	*90	0.063	0.029
	*100	0.833	0.936
	*110	0.104	0.029
*SOD**	*100		0.959
	*160	0.271	0.041
	*230	0.708	
	*315	0.021	

(see Table 2). Distinct allelic frequencies at five loci (*G3PDH**, *IDHP-2**, *PEP-B-1**, *PGDH** and *SOD**) provide a set of genetic markers for individual classification. However, joint analysis can be made from a single gel without additional effort. We suggest combined genotyping at *G3PDH** and *PGDH** muscular loci on AC (pH=7) buffer as a fast identification method. This two locus screening makes it possible to distinguish occasional intraspecies polymorphism (heterozygosity at one or the other locus) from interspecies hybridisation (heterozygosity at both loci). This method will distinguish frozen and fresh processed fish, such as uncooked minced and fillets or ambiguous whole fishes of *M. australis* and *M. hubbsi* with almost complete confidence.

This current work also presents the results of an extensive search for electrophoretically detectable enzymatic loci in muscle, liver and eye of *M. australis* and *M. hubbsi*, which would serve as the basis for a deeper analysis that would provide greater knowledge of the population structure of both species. At this moment, despite their economic importance, few allozymic data useful for population differentiation are available on these two species. Roldán (1991) working with muscle on *M. hubbsi* reported four polymorphic loci and Smith *et al*. (1979) reported two polymorphic loci on *M. australis* from New Zealand.

ACKNOWLEDGEMENTS

We thank B. Jerez and A. Malaspina (INIDEP, Argentina) for helping to collect samples and J.L. García-Marín and F. Utter for helpful comments on the manuscript.

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Scient. ed.: J. Lleonart