SCI. MAR., 62(1-2): 141-149 SCIENTIA MARINA 1998

Biochemical genetics in the Argentinean squid, *Illex argentinus**

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SUMMARY: Previously reported results on natural populations of the Argentinean squid, *Illex argentinus*, urged further studies using electrophoretically detectable loci. The muscles of 202 adult squids caught in the Argentinean Sea were examined. Electrophoretic and staining methods for the 46 enzymes studied are given in detail. Forty-seven loci were detected (20 polymorphic), 45 of which are considered useable in population genetics screening. The expression of the loci coding for these enzymes is described and interpreted, and the presented results will serve as a basis for a more detailed examination of genetic variation in Argentinean squid populations.

Key words: Isozyme, squid, *Illex argentinus*.

RESUMEN: GENÉTICA BIOQUÍMICA DEL CALAMAR ARGENTINO, *ILLEX ARGENTINUS*– Estudios biológicos previos realizados en el calamar argentino, *Illex argentinus*, han sugerido la conveniencia de profundizar en el conocimiento de la estructura poblacional de la especie, utilizando el análisis genético de proteínas detectables electroforéticamente. Con esta finalidad se analizó el músculo esquelético de un total de 202 individuos capturados en el Mar Argentino. Se utilizó la técnica de electroforesis horizontal en gel de almidón al 11%. Se estudiaron 46 sistemas enzimáticos distintos. La expresión de los loci para estos enzimas son descritos e interpretados en detalle. Un total de 47 loci fueron detectados (20 polimórficos), de los cuales 45 se consideran utilizables para el análisis de la estructura poblacional de la especie.

Palabras clave: Isoenzimas, calamar, *Illex argentinus*.

INTRODUCTION

The Argentinean squid, *Illex argentinus* (Castellanos, 1960), is a cephalopod which belongs to the Ommastrephidae family and is one of the most important fishing resources in the South West Atlantic. Its current capture rate is more than 400,000 tons/year in areas off the continental shelf and the South Patagonian slope in the Argentinean Sea (Brunetti *et al*., 1996). It is a neritic-oceanic species which can be found between 23ºS and 53ºS, and the most frequented region is that between 35ºS and 52ºS at depths of between 80 and 400 metres (Castellanos, 1964; Brunetti and Perez Comas, 1989a, 1989b; Otero *et al*., 1981).

An exhaustive biological fishing study of this species has been carried out since 1981, a consequence of which has been the proposal of at least four spawning groups or stocks, based on the size structure, the length of the first maturity, spawning area and period (Brunetti, 1981, 1988; Nigmatullin, 1989). However, to date, the degree of genetic isolation between these groups is unknown. The current study consider the species' genetic variability and their population genetic structure.

*Received December 5, 1996. Accepted November 5, 1997.

The application of genetic analysis through the technique of enzymatic protein electrophoresis to the study of marine populations is standard practice nowadays and is extremely valuable in population identification (Allendorf *et al*., 1987; May and Krueger, 1990; Utter, 1991; Avise, 1994). However, work carried out on squid using this method is still scarce (Garthwaite *et al*., 1989; Carvalho and Pitcher, 1989; Carvalho *et al*., 1992), and this last study is the only published work on *I. argentinus* to date.

This current work presents the results of an extensive search for electrophoretically detectable enzymatic loci in the muscle of the Argentinean squid, which would serve as the basis for a more profound analysis that would provide greater knowledge of the population genetics structure of this species.

MATERIAL AND METHODS

A total of 202 individuals captured by the INIDEP research vessel "Capitán Oca Balda" during February of 1994 in the Argentinean Sea was analysed. Once each squid had been individualised, a piece of skeletal muscle was extracted from the mantle of each example and frozen on board at –20ºC. Later, the samples were transported to Spain in expanded polystyrene boxes with enough dry ice to ensure that they all arrived at their destination still completely frozen. Once they arrived, they were kept at –80ºC in the laboratory until their later electrophoretic analysis.

The sample analysis was performed using the technique of horizontal starch (11%) gel electrophoresis, following the description given by Aebersold *et al*. (1987). Nine different buffer systems were used:

1- *Continuous buffer AC*, modified from Clayton and Tretiak (1972).

2- *Discontinuous buffer TC/LB* (Ridgway *et al*., 1970).

3- *Continuous buffer TBE+NAD*, modified from Market and Faulhaber (1965).

4- *Continuous buffer TBE,* modified from Market and Faulhaber (1965).

5- *Continuous buffer TP*, modified from Guyomard and Krieg (1983).

6- *Discontinuous buffer Poulik* (Poulik, 1957).

7- *Continuous buffer TC* pH 6.5, modified from Shaw and Prasad (1970).

8- *Continuous buffer TC* pH 8.00, modified from Shaw and Prasad (1970).

9- *Discontinuous buffer TC/LB+TBE*, modified from Market and Faulhaber (1965) and Ridgway *et al*. (1970).

With respect to the stains, the ones used in this work corresponded to enzymatic systems which required the use of specific substrates for the reaction, in accordance with the protocols described by the following authors: Aebersold *et al*. (1987), Allendorf *et al*. (1977), Harris and Hopkinson (1976), Jorde *et al*. (1991), Morizot and Schmidt (1990), Pasteur *et al*. (1988).

Table 1 lists the analysed enzymes and indicates the ones which provided the best electrophoretic and staining conditions.

RESULTS

Genetic description and interpretation of zymograms

Forty-six different stains were tested, of which 13 did not show any activity and two gave a resolution that was insufficient for genetic interpretation. The remaining 31 however, produced consistent enzymatic phenotypes and enabled their genotype identification, producing a total of 45 loci that could be used in population studies.

The most conservative genetic explanation was adopted for estimating the number of loci and alleles that coded an enzyme, both when it was described for the first time and when there were differences with other authors, so that the necessary loci and alleles for their explanation would be minimum. The nomenclature used for the loci and allele designation follows the recent standards described by Shaklee *et al*. (1990). In those enzymes which presented more than one locus, the Arabic number suffix describes the different loci in increasing order of mobility with respect to the anode. The alleles for each locus were designated by their relative electrophoretic mobility with respect to the most common allele for this locus which was taken as **100*.

The zymograms obtained with the different enzymatic stains used together with their genetic interpretation are now described. In all cases, the interpretation which is given agrees with the current subunit structure of each enzyme. In spite of not having previous electrophoretic information for each enzyme, whenever possible, the results obtained in this work were compared to those described for the species earlier by Carvalho *et al*. (1992).

TABLE 1. Enzymes screened, electrophoretic buffers and stains tested. E.C.Nº: Enzyme Commission number. Key of the buffers (the order indicates the best resolution): 1: AC; 2: TC/LB; 3: TBE+NAD; 4: TBE; 5: TP; 6: Poulik; 7: TC pH 6.5; 8: TC pH 8; 9: TC/LB+TBE. Key of the stains: 1: Aebersold *et al*., 1987; 2: Allendorf *et al*., 1977; 3: Harris & Hopkinson, 1976; 4: Jorde *et al*., 1991; 5: Morizot & Schmidt, 1990; 6: Pasteur *et al*, 1988. # stain modified with agar 2%.

Enzyme	E.C.N°	Abbreviation	Buffer	Stain
Aspartate aminotransferase	2.6.1.1	AAT	2, 5	1
Acid phosphatase	3.1.3.2	ACP	5	3#
Adenosine deaminase	3.5.4.4.	ADA	8, 7, 1	1
Alcohol dehydrogenase	1.1.1.1	ADH	3	2#
Aconitate hydratase	4.2.1.3	AH	4	1
Adenylate kinase	2.7.4.3	AK	1	1
Alanine aminotransferase	2.6.1.2	ALAT	5	1
Catalase	1.11.1.6	CAT	5	1
Creatine kinase	2.7.3.2.	CK	8, 9	1
Diaphorase	1.6.4.3	DIA	9, 3	2#
Esterase	3.1.1.-	EST	6, 2	1
Esterase-D	3.1.-.-	ESTD	2	1
Fructuose bisphosphatase	3.1.3.11	FBP	1, 5	1
Fumarate hydratase	4.2.1.2	FH	3	1
beta-*N*-Acetilgalactosaminidase	3.2.1.53	bGALA	5	1
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	GAPDH	3, 5	3
alfa-Glucosidase	3.2.1.20	aGLU	5	1
N-Acetil-beta-glucosaminidase	3.2.1.30	bGLUA	5	4
Glutamate dehydrogenase	1.4.1.2	GLUDH	2	1
Glicerate dehydrogenase	1.1.1.29	GLYDH	1	5#
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	3, 1	1
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PDH	2, 1, 6	2
Glucose-6-phosphate isomerase	5.3.1.9	GPI	2	1
Hexokinase	2.7.1.1	HK	2	1
L-Iditol dehydrogenase	1.1.1.14	IDDH	2	2
Isocitrate dehydrogenase	1.1.1.42	IDHP	8, 7, 1	2#
L-Lactate dehydrogenase	1.1.1.27	LDH	3	2#
Lactoylglutathione lyase	4.4.1.5	LGL	6	1
alfa-Mannosidase	3.2.1.24	aMAN	5	4
Malate dehydrogenase	1.1.1.37	MDH	1, 3	2#
Malic enzyme (NAD+)	1.1.1.39	ME	3	2#
Malic enzyme (NADP+)	1.1.1.40	MEP	2	2#
Mannose-6-phosphate isomerase	5.3.1.-	MPI	4	3
Octanol dehydrogenase	1.1.1.73	ODH	9, 1, 2, 5	2#
Dipeptidase	3.4.-.-	PEPA	4	4
Tripeptide aminopeptidase	3.4.-.-	PEPB	4	4
Proline dipeptidase	3.4.-.-	PEPD	4	4
Peptidase-S	3.4.-.-	PEPS	4	4
Phosphogluconate dehydrogenase	1.1.1.44	PGDH	1	1
Phosphoglucomutase	5.4.2.2	PGM	8, 4	1
Pyruvate kinase	2.7.1.40	PK	2	6
Hidrógeno peróxido oxidoreductasa	1.11.1.7	POD	5	3
General (unidentified) protein		PROT	2	1
Superoxide dismutase	1.15.1.1	SOD	all	1
Tyrosine aminotransferase	2.6.1.5	TAT	5, 9	1
Xanthine dehydrogenase	1.1.1.204	XDH	3	2#

The enzymes analysed are presented already classified into three groups according to activity and degree of resolution.

Enzymes without activity

Of the 48 enzymatic stains used, the following 13 did not show any activity: Alcohol dehydrogenase (ADH), Aconitate hydratase (AH), Alanine aminotransferase (ALAT), Catalase (CAT), Esterase (EST), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), alfa-Glucosidase (aGLU), *N*-Acetyl-beta-glucosaminidase (bGLUA), Glycerate dehydrogenase (GLYDH), Hexokinase (HK), L-Lactate dehydrogenase (LDH), Lactoylglutathione lyase (LGL), Hydrogen peroxide oxidoreductase (POD).

Enzymes with little activity and insufficient resolution

Octanol dehydrogenase (ODH).

A cathodal zone of moderate activity, probably variable and with insufficient resolution was detected for this enzyme, but could not be genetically interpreted.

Xanthine dehydrogenase (XDH).

Presented an anodal zone of weak activity, with insufficient resolution that could not be genetically interpreted.

Enzymes with good activity and resolution

A list follows which describes the interpretation of the electrophoretic patterns that were obtained with the 31 remaining enzymatic stains.

Aspartate amino-transferase (AAT)

A total of two moderately active anodal zones were observed for this enzyme and this has been interpreted as the expression of two loci, *AAT-1** and *AAT-2**, both monomorphic.

Acid phosphatase (ACP)

An intensely active anodal zone, close to the origin, has been observed in this enzyme and it has been identified as the expression of the *ACP** locus where two alleles have been detected, **100* and **225*.

Adenosine deaminase (ADA)

A single variable anodal zone with moderate activity was observed for this enzyme and has been considered as a locus expression, *ADA**, with two alleles: **69* and **100*. The homozygotes showed a single band and the heterozygotes three bands, a characteristic pattern of dimeric proteins.

Adenylate kinase (AK)

This enzyme presented three anodal zones of differing intensity activity. The first very close to the origin, is a weak zone, quite unclear and with a very diffused aspect that could not be genetically interpreted. The second and third zones are moderately and intensely active respectively and have been identified as the expression of two loci *AK-1** and *AK-2**. None of these two loci presented allelic variation.

Creatine kinase (CK)

Three zones of activity with different intensity and resolution have been detected in this enzyme, each one is considered to be the expression of a locus: *CK-1**, *CK-2** and *CK-3**. Loci *CK-1** and *CK-2** have moderate activity and resolution and did not present any variation whatsoever in all the analysed individuals. Locus *CK-3** is variable with good activity and resolution and presented two alleles: **95* and **100*.

Diaphorase (DIA)

Two anodal zones of activity very close to the advancing electrophoretic front were observed for this enzyme. The first zone is weakly active and without any variation and has been considered as the expression of a single monomorphic locus *DIA-1**. The second zone is variable with intense activity and resolution, it has been identified as locus *DIA-2** in which three alleles have been detected: **95* ,**100* and **103*.

Esterase-D (ESTD)

A single anodal zone has been observed for this enzyme. It has good resolution without variation and has been interpreted as the expression of a monomorphic locus *ESTD**.

Fructose-biphosphatase (FBP)

A single anodal zone of moderate activity without variation was observed for this enzyme and it was interpreted as the expression of a monomorphic locus *FBP**.

Fumarate hydratase (FH)

This enzyme presents a single invariant anodal zone of moderate activity and intermediate resolution, that was defined as an *FH** locus.

beta-*N*-Acetylgalactosaminidase (bGALA)

This enzyme presents a single anodal zone of weak activity close to the origin with moderate resolution, which has been interpreted as a monomorphic locus *bGALA**.

Glutamate dehydrogenase (GLUDH)

A single anodal zone of weak activity was detected in this enzyme with moderate resolution. It was interpreted as the expression of a monomorphic locus *GLUDH**.

Grycerol-3-phosphate dehydrogenase (G3PDH)

A single anodal invariant zone of activity was detected in this enzyme that was assumed to reflect the expression of a single locus *G3PDH**.

Glucose-6-phosphate dehydrogenase (G6PDH)

A single variable zone of activity was detected in this enzyme. It has weak resolution and has been interpreted as the expression of a single locus *G6PDH**, with two alleles: **100* and **107*. The observed phenotypes correspond to a dimeric protein where the heterozygotes show three bands and the homozygotes one.

Glucose-6-phosphate isomerase (GPI)

A single anodal invariable zone with intense activity and good resolution was detected in this enzyme which was identified as the expression of a monomorphic locus *GPI**.

L-Iditol dehydrogenase (IDDH)

This enzyme presented a single anodal zone of activity which was variable with good resolution and was interpreted as the expression of a single locus *IDDH** with two alleles: **100* and **113*.

Isocitrate dehydrogenase (IDHP)

Two anodal zones were detected for this enzyme, both were variable with intense activity and good resolution. They were interpreted as the expression of two loci: *IDHP-1** with alleles **68* and **100;* and *IDHP-2** with alleles **94* and **100*. All the homozygotes present a phenotype with a single band, whereas the heterozygotes present three bands which is the pattern of the dimeric proteins.

alfa-Mannosidase (aMAN)

This enzyme presents a single anodal zone of activity. It has good resolution without variation and has been interpreted as a monomorphic locus *aMAN**.

Malate dehydrogenase (MDH)

This enzyme's zymograms present four anodal zones with different intensities that were interpreted as being the expression of three loci: *MDH-1**, *MDH-2** and *MDH-3**. The fourth zone corresponds to the interloci heterodimer between the first and second locus. The remaining heterodimers were not seen, possibly because they overlapped the expression of one of the other three loci. The intensely active locus *MDH-1** and moderately active locus *MDH-2**, did not present any variation in the total of analysed individuals. However, locus *MDH-3**, with intense activity and good resolution, presented two alleles **100* and **160*. The individual homozygotes presented a single band with three for the heterozygotes, which is typical of the dimeric proteins.

When using buffer number 3 (Table 1), the resulting zymograms for this enzyme are the equivalent, in activity and resolution zones, to those obtained for the malic enzyme (ME). In this case, the two loci detected in ME are identical to those designated *MDH-2** and *MDH-3**, both in allele quantity and individual variation.

Malic enzyme-NAD (ME)

This enzyme presents two intense anodal zones of activity with good resolution and each one is considered as the expression of a locus: *ME-1** and *ME-2**. Locus *ME-1** did not show any variation. However, two alleles were detected in locus *ME-2***100* and **160*, in which the individual homozygotes are observed with a single band and the heterozygotes with three, a characteristic of dimeric proteins. A zone of weak activity which is observed between both loci was considered to be the expression of the interloci heterodimers.

Malic enzyme-NADP (MEP)

A single variable anodal zone of intense activity and resolution has been detected for this enzyme, which is interpreted as the expression of the locus *MEP** with two alleles: **95* and **100*.

Mannose-6-phosphate isomerase (MPI)

Two anodal zones with good activity and resolution have been observed for this enzyme and each is considered to be the expression of loci *MPI-1** and *MPI-2**. Locus *MPI-1** did not show any variation

in the total of analysed individuals. Two alleles were detected in locus *MPI-2**: **85* and **100*. The slow allele showed the same electrophoretic mobility as locus *MPI-1**. All heterozygotes present two bands which are characteristic of monomeric proteins.

Dipeptidase (PEPA)

A single variable anodal zone of activity was observed with the glycyl-leucine dipeptide. It was interpreted as a single locus, *PEPA**, with three alleles: **95*, **100* and **105*.

Tripeptide aminopeptidase (PEPB)

A single anodal zone of moderate activity was observed for leucyl-glycyl-glycine tripeptide. There was no variation and it was interpreted as a single monomorphic locus *PEPB**.

Proline dipeptidase (PEPD)

The zymograms obtained with the phenylalanine-proline dipeptide presented a single variable anodal zone of activity that was considered to be the expression of a single locus *PEPD**, with three alleles: **80, *90* and **100*.

Peptidase-S (PEPS)

Three intense zones of activity were observed with the peptide leucyl-tyrosine and they were interpreted as the expression of three loci, *PEPS-1**, *PEPS-2** and *PEPS-3**. Locus *PEPS-1**, was very close to the origin and presented three alleles: **100*, **114* and **155*. Two alleles were detected in the remaining loci: **92* and **100* in locus *PEPS-2**, and **90* and **100* in locus *PEPS-3**.

6-Phosphogluconate dehydrogenase (PGDH)

This enzyme presents a single anodal zone of intense activity and good resolution which was interpreted as the expression of a locus *PGDH**. In the total of analysed examples, only one individual presented a phenotype with three bands which was interpreted as a heterozygote for the rapid variant **105*.

Phosphoglucomutase (PGM)

This enzyme presented a single anodal zone of intense activity with good resolution which has been interpreted as the expression of a locus *PGM** with three alleles: **64*, **100* and **114*. The homozygotes present a single band and the heterozygotes two, the typical expression of a monomeric protein.

Pyruvate kinase (PK)

Three anodal zones of activity with different intensities and resolutions were detected in the zymograms obtained for this enzyme. The one closest to the origin had moderate activity without any variation and was interpreted as the expression of a monomorphic locus *PK**. The two others presented weak activity and such very deficient resolution that they could not be genetically interpreted.

General protein (PROT)

Four invariant anodal zones of intense activity, with good resolution were detected for the general proteins which were interpreted as the expression of four loci: *GP-1**, *GP-2**, *GP-3** and *GP-4**.

Superoxide dismutase (SOD)

A single zone of intense activity and good resolution, without any variation, was observed for this enzyme which was interpreted as a monomorphic locus *SOD**.

Tyrosine aminotransferase (TAT)

Two anodal zones of activity were detected in this enzyme. The first had moderate activity and good resolution. It did not present any variation in the analysed individuals and is considered to be the expression of a monomorphic locus *TAT-1**. The second had intense activity and was interpreted as the expression of locus *TAT-2** with two alleles: **89* and **100*. The general pattern detected corresponds to a monomeric protein where the individual homozygotes present a single band and the heterozygotes two.

DISCUSSION

Selection of the loci for the study of genetic variability

The use of a considerable number of loci has been suggested for estimating the genetic variation

in natural populations (Nei, 1973; Nei and Roychoudhury, 1974). After several decades of applying electrophoretic techniques in fish, most authors consider that the use of a large number of loci is more important than the use of a great number of individuals (Gorman and Renzi, 1979). This is due to the variance which exists in the heterozygosis between the different loci in a species (Nei *et al*., 1976). With the aim of allowing the obtained loci to be used in later population analyses of the Argentinean squid, a large number of enzymatic stains were used, which in turn, provided a high number of well-resolved loci. At the same time, the sample used was also large (N=200) and this ensured the identification of as large a number as possible of allelic variants present in the species sampled area.

TABLE 2. Summary of the resolution for the studied enzymes (46) with observed loci (47) and detected alleles in *Illex argentinus*. [a] equivalent to *MDH-2**, [b] equivalent to *MDH-3**.

Enzyme	Locus	Resolution	Detected alleles
Aspartate aminotransferase	*AAT-1**	monomorphic	
	*AAT-2**	monomorphic	
Acid phosphatase	*ACP**	polymorphic	**100, **225*
Adenosine deaminase	*ADA**	polymorphic	**69, **100*
Alcohol dehydrogenase		no activity	
Aconitate hydratase		no activity	
Adenylate kinase	*AK-1**	monomorphic	
	*AK-2**	monomorphic	
Alanine aminotransferase		no activity	
Catalase		no activity	
Creatine kinase	*CK-1**	monomorphic	
	*CK-2**	monomorphic	
	*CK-3**	polymorphic	**95, **100*
Diaphorase	*DIA-1**	monomorphic	
	*DIA-2**	polymorphic	**95, **100, **103*
Esterase		no activity	
Esterase-D	*ESTD**	monomorphic	
Fructose bisphosfatase	*FBP**	monomorphic	
Fumarate hydratase	*FH**	monomorphic	
beta-*N*-Acetilgalactosaminidase	*bGALA**	monomorphic	
Glyceraldehyde-3-phosphate dehydrogenase		no activity	
alfa-Glucosidase		no activity	
N-Acetil-beta-glucosaminidase		no activity	
Glutamate dehydrogenase	*GLUDH**	monomorphic	
Glycerate dehydrogenase		no activity	
Glycerol-3-phosphate dehydrogenase	*G3PDH**	monomorphic	
Glucose-6-phosphate dehydrogenase	*G6PDH**	polymorphic	**100, **107*
Glucose-6-phosphate isomerase	*GPI**	monomorphic	
Hexokinase		no activity	
L-Iditol dehydrogenase	*IDDH**	polymorphic	**100, **113*
Isocitrate dehydrogenase	*IDHP-1**	polymorphic	**68, **100*
	*IDPH-2**	polymorphic	**94, **100*
L-Lactate dehydrogenase		no activity	
Lactoylglutathione lyase		no activity	
alfa-Mannosidase	*aMAN**	monomorphic	
Malate dehydrogenase	*MDH-1**	monomorphic	
	*MDH-2**	monomorphic	
	*MDH-3**	polymorphic	**100, **160*
Malic enzyme (NAD+)	*ME-1**	monomorphic[a]	
	*ME-2**	polymorphic[b]	**100, **160*
Malic enzyme (NADP+)	*MEP**	polymorphic	**95, **100*
Mannose-6-phosphate isomerase	*MPI-1**	monomorphic	
	*MPI-2**	polymorphic	**85, **100*
Octanol dehydrogenase		bad resolution	
Dipeptidase	*PEP-A**	polymorphic	**95, **100, **105*
Tripeptide aminopeptidase	*PEP-B**	monomorphic	
Proline dipeptidase	*PEP-D**	polymorphic	**80, **90, **100*
Peptidase-S	*PEP-S-1**	polymorphic	**100, **114, **155*
	*PEP-S-2**	polymorphic	**92, **100*
	*PEP-S-3**	polymorphic	**90, **100*
Phosphogluconate dehydrogenase	*PGDH**	polymorphic	**100, **105*
Phosphoglucomutase	*PGM**	polymorphic	**64, **100, **114*
Pyruvate kinase	*PK *	monomorphic	
Hydrogen peroxide oxidoreductase		no activity	
General protein	*PROT-1**	monomorphic	
	*PROT-2**	monomorphic	
	*PROT-3**	monomorphic	
	*PROT-4**	monomorphic	
Superoxide dismutase	*SOD**	monomorphic	
Tyrosine aminotransferase	*TAT-1**	monomorphic	
	*TAT-2**	polymorphic	**89, **100*
Xanthine dehydrogenase	*XDH**	bad resolution	

If the criterion of at least one locus being detected in those cases where there is activity in spite of insufficient resolution is used, then the total number of loci observed during this work was 52. However, not all these loci can be used in the study of genetic variability of the squid and their selection must be based on the following criteria: a) the enzyme resolution must be sufficiently clear to permit the observed phenotypes to be easily read and to allow their correct genotype interpretation. For these reasons, the stains with poor resolution or those where the observed variation could not be genetically interpreted (ODH, XDH) were rejected; b) one must take into account those loci which were detected in different stains and that were identical in mobility, quantity of alleles and observed individual variation (loci *1**, *2** of ME and *2**, *3** of MDH). Only the *ME* loci or the *MDH* loci should be used in population studies; c) all the loci which are going to be used must have been correctly resolved in the total of individuals. Even with these restrictions, a large number (45) of useful loci remain for the variability analysis in the Argentinean squid. Table 2 provides a summary of the resolution type for each of the studied enzymes, together with observed loci and detected alleles.

The principal use of this work compared to that of Carvalho *et al*. (1992), lies both in the greater number of resolved loci and the more detailed interpretation of the obtained zymograms. The latter is especially important for those research teams who would like to undertake more exhaustive future population studies of the Argentinean squid. There is a great need for understanding the patterns for genetic diversity in this species, both from an evolutionary point of view and for the management of the fishing grounds. It should be made clear that only two of the postulated four spawning groups were studies in this work. Any future studies which include all the known groups will probably reveal polymorphism in some of the loci that were shown to be only monomorphic by this work.

Measurement of genetic variability

Carvalho *et al*. (1992) reported identical results in the number of loci for the following enzymes: AAT, ADA, CK, DIA, ME, ESTD, FBP, FH, G3PDH, G6PDH, GPI, MPI, PEPB, PGDH and SOD. However, for the AK, IDHP, MDH and PGM enzymes, the number of detected loci in this work differ from those reported by Carvalho *et al*. (1992), probably due to the differing electrophoretic conditions employed in the two studies.

With respect to the detected genetic variability, 20 of the 47 resolved loci (P=0.42) demonstrated some form of polymorphism (*ACP**, *ADA**, *CK-3**, *DIA-2**, *G6PDH**, *IDDH**, *IDHP-1**, *IDHP-2**, *MDH-3**, *ME-2**, *MEP-1**, *MPI-2**, *PEPA**, *PEPD**, *PEPS-1**, *PEPS-2**, *PEPS-3**, *PGDH**, *PGM**, *TAT-2**), although only 6 (*ADA**, *DIA-2**, *IDHP-1**, *MEP**, *MPI-2**, *PGM**) are polymorphic according to the 95% criterion (P=0.13). These values are similar to those encountered by Carvalho *et al*. (1992), who obtained 12 polymorphic loci from 25 resolved loci of which 7 were polymorphic according to the 95% criterion.

ACKNOWLEDGEMENTS

We would like to express our thanks to Dr. N. E. Brunetti (INIDEP, Argentina) for collecting the samples and to F. Rivas for her technical assistance. B. Jerez received a fellowship from the Spanish Agency for International Co-operation (AECI) for his stay in Girona, Spain and assistance for the trip from UNESCO, ROSTLAC contract 883.501-4. This paper is a part of the PhD work of BJ at the Universidad de Mar del Plata, Argentina.

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Scient. ed.: J.M. Gili