Allozyme analysis of geographical and seasonal variation of *Illex coindetii* (Cephalopoda: Ommastrephidae) from central Mediterranean and Iberian Atlantic

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Previous morphological studies of *Illex coindetii* from the Mediterranean have shown both juvenile and mature individuals all year round suggesting an homogeneous population pattern, however, seasonal spawning variation seems to exist varying with geographical area. Present work analysed 240 individuals from the northern Tyrrhenian and Atlantic Iberian waters at the four year-round seasons using 33 presumptive enzyme-coding loci to compare geographical and temporal variances. Genetic variability was low ($H_o=0.02-0.04$). Moderate variation at some loci was found due to both temporal and geographical factors (12% of total genetic variance) and to temporal variance ($F_{ST}=0.004-0.015$). Unweighted pair-group method using arithmetic averages (UPGMA) and Multidimensional scaling–minimum spanning tree (MDS–MST) analyses after Cavalli-Sforza & Edwards (1967) chord genetic distances showed the summer Italian and the spring Atlantic samples as the most divergent among the whole.

INTRODUCTION

The species of Illicinae are likely to constitute a large portion (59%) of the annual world ommastrephid squid catches (1.3 million tn; FAO, 1998). *Illex coindetii* (Vérany, 1837) represents a valuable resource due to its abundance in specific areas of the Mediterranean and Atlantic Ocean, where multiple studies on its life cycle characteristics have been conducted (Sánchez et al., 1998). The species plays an important role in marine food webs providing prey items for demersal predators (Sánchez et al., 1998). However, information on the biology of *I. coindetii* throughout its distribution range, especially within the central Mediterranean waters, has been scarce (e.g. Belcari, 1996) and thorough research of its population structure is needed.

Previous analysis of length–frequency distributions for *I. coindetii* samples from western and central Mediterranean showed no evidence for the existence of distinct spawning populations (Belcari, 1996; González & Guerra, 1996; Sánchez et al., 1998). However, based on morphological analysis, some seasonal peaks of spawners individuals seem to exist and they may vary with geographical area (González & Guerra, 1996; Sánchez et al., 1998). Thus, individuals had a major peak in autumn (Sánchez et al., 1998), and in spring and summer at the Mediterranean and Iberian Atlantic, respectively (González & Guerra, 1996). Moreover, previous morphological studies had shown geographical variation in spite of no conclusive result in the population structure of *I. coindetii* throughout the Mediterranean and Atlantic waters (Hernández-García & Castro, 1998; Sánchez et al., 1998). Genetic information of the species may provide a valuable contribution to a better understanding on the degree to which

species are subdivided into effective populations or stocks, which is one of the most fundamental problems in any fishery (Sánchez et al., 1998).

There is a lack of genetic studies on Mediterranean versus Atlantic populations within ommastrephids. The analysis of geographical structure by electrophoretic characterization of allelic isozymes has been successful for other cephalopods (Maltagliati et al., 2002; Yeatman & Benzie, 1993), and particularly for other *Illex* species (Carvalho et al., 1992).

Temporal genetic population analysis has proved to be fruitful for objective stock identification on migrating teleosts (e.g. Waples & Teel, 1990) although this time-variable research has been absent for cephalopods. Many allozyme studies on marine populations have compared their genetic structure from different locations, under the assumption that the protein variation was temporally homogeneous and therefore geographically comparable (Moffett & Crozier, 1996). The present paper is aimed to evaluate potential geographic differentiation among populations of *I. coindetii* inhabiting the Tyrrhenian Sea and Iberian Atlantic waters, and to assess temporal stability of the allele frequencies out of 33 enzyme-coding loci from simultaneously captured samples through seasons within a year.

MATERIALS AND METHODS

Sampling

Sample codes, sampling areas, seasons and number of specimens per sample are shown in Table 1. Samples were freshly caught at local fishing auctions at Ribeira (north-

Table 1. *Sample codes, sampling area, sampling season and number of individuals (N) of analysed Illex coindetii.*

Code	Sampling Area	Season	N
IcW-W	NW Iberian Atlantic	Winter	22
IcP-W	Pisa, Tyrrhenian Sea	Winter	19
IcW-Sp	NW Iberian Atlantic	Spring	32
IcP-Sp	Pisa, Tyrrhenian Sea	Spring	33
IcW-Sum	NW Iberian Atlantic	Summer	33
IcP-Sum	Pisa, Tyrrhenian Sea	Summer	39
IcW-Aut	NW Iberian Atlantic	Autumn	26
IcP-Aut	Pisa, Tyrrhenian Sea	Autumn	36

western Iberian Atlantic coast) and Pisa (northern Tyrrhenian Sea, Italy) by bottom-trawlers. Over time sampling for the four seasons (1997–1998) analysis within each area was obtained simultaneously. Collected *Illex coindetii* individuals were both juvenile and mature and completed the one-year life cycle of the species, what excluded the possibility to have sampled different cohorts as it would be the case for teleost species. Specimens were kept at -20°C while shipping to the laboratory where they were deep-frozen at -80°C until processing. A total of 240 individuals were analysed by allozyme starch-gel electrophoresis.

Electrophoresis procedure

Horizontal starch-gel electrophoresis was carried out based on Murphy et al. (1996). A piece (11 × 11 mm) of mantle muscle was sliced out for homogenization with

0.01 M dithiothreitol (DTT) solution. The homogenate was centrifuged at 12,000g for 10 min at 4°C and the supernatant was embedded in Whatman strips no. 2 and 3. Hydrolysed-starch gels (12%, SIGMA starch) were run at constant voltage at 4°C . Enzyme systems routinely examined with adequate activity and resolution were shown in Table 2. The Tris-citrate, pH 8.0 buffer system (gel buffer dilution 1:11) was used for most of the enzymes (at 4.6 V cm^{-1}) except for ADA, G6PDH and GPI where Tris-borate-EDTA, 8.7 buffer was used (gel buffer dilution 1:9 for ADA with 3.6 V cm^{-1} and 1:5 for G6PDH and GPI with 10 V cm^{-1}) and for MEP and the locus *OPDH-3** where citrate morpholine, pH 7.4 buffer was employed. These enzymes were stained according to recipes in Murphy et al. (1996), with some variations for AAT, ACP, DDH, ESTD, IDHP, LAP, MPI, PEPA, PEPB, PEPD, PGDH, PGM, PK (e.g. Harris & Hopkinson, 1976). The 26 enzymes resolved 33 putative enzyme-coding loci.

Table 2. *Enzyme systems analysed with respective enzyme codes (E.C.).*

Enzyme system	E.C.
Aspartate Transaminase	AAT; 2.6.1.1.
Acid Phosphatase	ACP; 3.1.3.2.
Adenosine Deaminase	ADA; 3.5.4.4.
Adenylate Kinase	AK; 2.7.4.3.
Alanopine Dehydrogenase	ALPDH; 1.5.1.17.
Arginine Kinase	ARK; 2.7.3.3.
Hi-hydrolypoamide Transaminase	DDH; 1.8.1.4.
Carboxylic Ester Hydrolase (substrate: a-naphthyl acetate)	EST; 3.1.1.-.
Methylumbelliferyl-acetate Deacetylase	ESTD; 3.1.1.56.
Glycerol-3-phosphate Dehydrogenase (NAD^{+})	G3PDH; 1.1.1.8.
Glucose-6-phosphate Dehydrogenase	G6PDH; 1.1.1.49.
Glyceraldehyde-3-phosphate Dehydrogenase, phosphorylating	GAPDH; 1.2.1.12
Glucose-6-phosphate isomerase	GPI; 5.3.1.9.
L-Iditol 2-dehydrogenase	IDDH; 1.1.1.14.
Isocitrate Dehydrogenase (NADP^{+})	IDHP; 1.1.1.42.
L-Leucyl Aminopeptidase	LAP; 3.4.11.1.
Malate Dehydrogenase	MDH; 1.1.1.37.
Malate Dehydrogenase (oxaloacetate-decarboxylating) (NADP^{+})	MEP; 1.1.1.40.
Mannose-6-phosphate Isomerase	MPI; 5.3.1.8.
D-Octopine Dehydrogenase	OPDH; 1.5.1.11.
Cytosol non-specific Dipeptidase; substrate: gly-leu	PEPA; 3.4.13.18.
Tripeptide Aminopeptidase; substrate: leu-gly-gly	PEPB; 3.4.11.4.
X-pro Dipeptidase; substrate: phe-pro	PEPD; 3.4.13.9.
Phosphogluconate Dehydrogenase (decarboxylating)	PGDH; 1.1.1.44.
Phosphoglucomutase	PGM; 5.4.2.2.
Pyruvate Kinase	PK; 2.7.1.40.
Superoxide Dismutase	SOD; 1.15.1.1.

Table 3. Allele frequencies at 33 loci, sample sizes (N) and genetic variability indices: H_e (SE) unbiased estimate of mean expected heterozygosity; H_o (SE) mean observed heterozygosity; N_a (SE) mean number of alleles; and P_{95} , polymorphism to the 95% criteria) for *Illex coindettii* populations off north-western Iberian and Tyrrhenian Sea (IcW, IcP) waters in winter (W), spring (Sp), summer (Sum) and autumn (Aut) seasons. Sample codes are indicated in Table 1.

Locus	Population							
	IcW-W	IcP-W	IcW-Sp	IcP-Sp	IcW-Sum	IcP-Sum	IcW-Aut	IcP-Aut
<i>ACP*</i>								
(N)	20	19	32	32	31	36	26	35
*90	0.050	0.079	0.000	0.031	0.016	0.028	0.096	0.029
*100	0.950	0.921	0.984	0.969	0.952	0.931	0.865	0.971
*105	0.000	0.000	0.000	0.000	0.000	0.028	0.000	0.000
*110	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000
*115	0.000	0.000	0.016	0.000	0.000	0.014	0.038	0.000
<i>ADA*</i>								
(N)	20	19	17	15	10	38	26	24
*80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.979
<i>AK*</i>								
(N)	20	19	17	20	27	37	20	24
*80	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000
*100	1.000	1.000	1.000	1.000	1.000	0.986	1.000	1.000
<i>ALPDH*</i>								
(N)	20	19	32	32	31	36	16	34
*35	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015
*90	0.000	0.000	0.031	0.016	0.000	0.000	0.000	0.015
*95	0.000	0.000	0.000	0.016	0.016	0.000	0.000	0.000
*100	0.975	1.000	0.953	0.953	0.968	0.917	0.969	0.956
*105	0.025	0.000	0.000	0.016	0.016	0.042	0.031	0.015
*110	0.000	0.000	0.016	0.000	0.000	0.042	0.000	0.000
<i>DDH*</i>								
(N)	22	19	29	28	28	35	26	31
*60	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000
*100	1.000	1.000	1.000	1.000	1.000	0.986	1.000	1.000
<i>EST*</i>								
(N)	20	10	28	27	16	33	22	28
*95	0.000	0.000	0.036	0.019	0.000	0.000	0.000	0.000
*100	0.975	1.000	0.964	0.981	1.000	0.909	1.000	1.000
*105	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*110	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000
<i>ESTD*</i>								
(N)	20	19	25	32	32	38	26	34
*95	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000
*100	1.000	1.000	1.000	1.000	1.000	0.987	1.000	1.000
<i>G3PDH*</i>								
(N)	18	15	20	31	8	33	25	27
*90	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.074
*100	1.000	1.000	0.925	0.984	1.000	0.970	1.000	0.926
*110	0.000	0.000	0.000	0.016	0.000	0.030	0.000	0.000
*120	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000
<i>G6PDH*</i>								
(N)	22	17	26	24	28	31	26	28
*60	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*80	0.045	0.000	0.038	0.021	0.018	0.016	0.000	0.000
*90	0.000	0.000	0.000	0.000	0.000	0.081	0.000	0.018
*100	0.932	1.000	0.962	0.979	0.982	0.903	1.000	0.982
<i>IDDH*</i>								
(N)	22	18	30	24	32	37	26	34
*60	0.000	0.028	0.033	0.062	0.016	0.027	0.000	0.000
*100	0.977	0.944	0.950	0.938	0.953	0.973	0.981	0.985
*105	0.000	0.028	0.000	0.000	0.000	0.000	0.019	0.000
*110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015
*120	0.023	0.000	0.017	0.000	0.031	0.000	0.000	0.000
<i>IDHP-1*</i>								
(N)	20	19	31	31	31	38	26	33
*90	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*100	0.950	0.947	0.984	1.000	1.000	0.961	1.000	1.000
*105	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000

continued

Table 3. (Continued).

Locus	Population							
	IcW-W	IcP-W	IcW-Sp	IcP-Sp	IcW-Sum	IcP-Sum	IcW-Aut	IcP-Aut
<i>*110</i>	0.000	0.053	0.016	0.000	0.000	0.026	0.000	0.000
<i>IDHP-2*</i>								
(N)	15	13	29	25	31	30	24	29
<i>*90</i>	0.033	0.000	0.000	0.000	0.048	0.017	0.000	0.017
<i>*100</i>	0.967	1.000	0.931	1.000	0.952	0.983	0.979	0.966
<i>*105</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000
<i>*110</i>	0.000	0.000	0.069	0.000	0.000	0.000	0.000	0.017
<i>LAP*</i>								
(N)	20	18	23	30	32	31	12	26
<i>*100</i>	0.975	1.000	0.978	1.000	1.000	1.000	1.000	1.000
<i>*120</i>	0.025	0.000	0.022	0.000	0.000	0.000	0.000	0.000
<i>MDH-1*</i>								
(N)	22	19	32	31	32	37	26	35
<i>*90</i>	0.000	0.079	0.000	0.000	0.000	0.000	0.000	0.000
<i>*100</i>	1.000	0.921	1.000	1.000	1.000	1.000	1.000	0.986
<i>*120</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014
<i>MEP*</i>								
(N)	20	19	30	32	29	38	26	35
<i>*85</i>	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
<i>*100</i>	1.000	1.000	0.983	1.000	1.000	0.987	1.000	1.000
<i>*105</i>	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000
<i>MPI-2*</i>								
(N)	18	18	31	28	28	37	20	35
<i>*90</i>	0.000	0.028	0.000	0.000	0.018	0.041	0.000	0.014
<i>*100</i>	1.000	0.972	1.000	1.000	0.982	0.959	1.000	0.986
<i>OPDH-2*</i>								
(N)	22	19	30	31	30	38	20	33
<i>*90</i>	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000
<i>*100</i>	1.000	1.000	0.967	1.000	1.000	0.974	1.000	0.955
<i>*110</i>	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.045
<i>OPDH-3*</i>								
(N)	17	8	25	30	26	16	26	29
<i>*100</i>	0.912	1.000	1.000	0.983	0.981	1.000	1.000	0.983
<i>*105</i>	0.088	0.000	0.000	0.017	0.019	0.000	0.000	0.017
<i>PEPA*</i>								
(N)	14	13	28	30	32	26	20	35
<i>*80</i>	0.036	0.038	0.018	0.033	0.000	0.019	0.050	0.000
<i>*100</i>	0.964	0.962	0.982	0.967	1.000	0.981	0.950	1.000
<i>PEPB*</i>								
(N)	16	13	31	33	30	33	15	35
<i>*90</i>	0.000	0.000	0.016	0.015	0.000	0.030	0.000	0.000
<i>*100</i>	1.000	1.000	0.968	0.985	1.000	0.970	1.000	1.000
<i>*110</i>	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000
<i>PEPD*</i>								
(N)	22	17	31	25	30	26	20	34
<i>*90</i>	0.023	0.029	0.000	0.000	0.017	0.000	0.000	0.015
<i>*100</i>	0.977	0.971	1.000	0.980	0.983	1.000	1.000	0.985
<i>*110</i>	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000
<i>PGDH*</i>								
(N)	16	14	29	33	31	31	18	33
<i>*90</i>	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000
<i>*100</i>	0.969	0.964	0.931	1.000	1.000	0.952	1.000	1.000
<i>*110</i>	0.031	0.000	0.069	0.000	0.000	0.048	0.000	0.000
<i>PGM*</i>								
(N)	20	19	32	33	31	38	20	35
<i>*90</i>	0.100	0.026	0.156	0.061	0.129	0.092	0.050	0.129
<i>*100</i>	0.900	0.974	0.844	0.939	0.871	0.908	0.950	0.871
<i>H_c</i>	0.034	0.025	0.040	0.022	0.022	0.045	0.018	0.027
(SE)	0.009	0.008	0.011	0.006	0.008	0.010	0.008	0.009
<i>H_o</i>	0.035	0.022	0.031	0.020	0.021	0.044	0.019	0.025
(SE)	0.009	0.007	0.008	0.006	0.008	0.010	0.009	0.008
<i>N_a</i>	1.42	1.30	1.58	1.42	1.36	1.73	1.21	1.48
(SE)	0.10	0.09	0.12	0.12	0.11	0.13	0.08	0.12
<i>P₉₅</i>	15.15	12.12	15.15	6.06	3.03	15.15	9.09	6.06

Table 4. Nei's (1987) unbiased F -statistic estimates (F_{IS} and F_{ST}) for geographic (Tyrrhenian versus Atlantic) and temporal (Winter, Spring, Summer and Autumn) population differentiation of *Illex coindetii*. Differentiation at $G6PDH^*$, $MDH-1^*$, $PGHD^*$ and $G3PDH^*$ loci was significant ($P < 0.05$) after log-likelihood G -statistic testing within geographical, winter, spring and autumn comparisons, respectively.

Locus	Geographic		Winter		Spring		Summer		Autumn	
	F_{IS}	F_{ST}	F_{IS}	F_{ST}	F_{IS}	F_{ST}	F_{IS}	F_{ST}	F_{IS}	F_{ST}
ACP*	−0.039	−0.002	−0.046	−0.018	−0.001	0.000	−0.040	−0.002	−0.098	0.024
<i>ADA</i> *	0.001	−0.001							−0.016	0.007
<i>AK</i> *	0.000	0.000					−0.009	−0.001		
<i>ALPDH</i> *	0.026	−0.003	0.001	−0.001	0.153	−0.013	−0.050	0.003	−0.026	−0.001
<i>DDH</i> *	0.000	0.000					−0.009	−0.001		
<i>EST</i> *	−0.021	0.008	0.013	−0.014	−0.012	−0.013	−0.109	−0.046		
<i>ESTD</i> *	0.000	0.000					−0.007	0.005		
<i>G3PDH</i> *	0.249	−0.001			0.540	0.007	−0.026	0.012	−0.075	0.034
<i>G6PDH</i> *	−0.025	0.004	−0.029	0.028	−0.013	−0.015	−0.073	−0.002	−0.013	0.005
<i>IDDH</i> *	−0.023	−0.001	−0.012	−0.005	−0.036	−0.012	−0.032	0.004	−0.013	0.001
<i>IDHP-1</i> *	−0.011	0.000	−0.028	0.001	0.000	0.000	−0.031	0.012		
<i>IDHP-2</i> *	0.318	0.004	0.003	−0.003	1.000	0.033	−0.038	0.007	−0.020	0.002
<i>LAP</i> *	−0.006	0.006	0.001	−0.001	−0.003	0.003				
<i>MDH-1</i> *	0.496	0.007	0.653	0.040					−0.009	0.003
<i>MEP</i> *	0.000	−0.002			−0.001	0.001	−0.011	0.004		
<i>MPI-2</i> *	0.014	0.005	0.000	0.000			−0.029	0.006	−0.009	0.003
<i>OPDH-2</i> *	0.265	−0.003			−0.018	0.018	−0.022	0.008	0.654	0.019
<i>OPDH-3</i> *	−0.013	−0.003	−0.049	0.047	0.002	−0.002	−0.014	−0.009	−0.013	0.005
<i>PEPA</i> *	−0.016	−0.005	0.000	−0.038	−0.011	−0.012	−0.015	0.006	−0.048	0.022
<i>PEPB</i> *	−0.006	−0.003			−0.006	−0.010	−0.024	−0.015		
<i>PEPD</i> *	−0.006	−0.004	0.000	−0.026	−0.002	0.002	−0.013	0.005	−0.010	0.003
<i>PGDH</i> *	−0.017	−0.002	0.000	−0.017	−0.058	0.055	−0.039	−0.009		
<i>PGM</i> *	0.185	0.001	−0.066	0.021	0.197	0.028	0.321	0.001	0.065	0.015
<i>Mean</i>	0.082	0.000	0.027	0.004	0.182	0.008	0.013	0.008	0.017	0.015

two spring samples at *IDHP-2** and *ALPDH** loci, for the Iberian and Italian populations, respectively. However, such deviations were not significant after Bonferroni correction. The statistic F_{IS} showed no significant variation for HW within samples where alleles were permuted among individuals (Table 3).

Population differentiation

Average F_{ST} values were generally low for the overall, geographic and temporal comparisons (Table 4). Hierarchical analysis of gene differentiation showed that 12% of total variance was accounted for by the combination of both geographic and temporal (seasons) factors. Population differentiation due to the geographic variance (Atlantic Iberian versus Italian samples) was the lowest ($F_{ST}=0.000$, Table 4). Nei's (1987) unbiased F -statistic estimates (F_{ST}) showed variation at the loci *G6PDH** ($F_{ST}=0.004$, $P < 0.05$), *MDH-1** ($F_{ST}=0.040$, $P < 0.001$), *PGHD** ($F_{ST}=0.055$, $P < 0.001$) and *G3PDH** ($F_{ST}=0.034$, $P < 0.001$) after log-likelihood G -statistic testing within geographical, winter, spring and autumn comparisons, respectively (Table 4). For temporal testing adjustment of frequencies significance, a chi-squared test was applied (Workman & Niswander, 1970), where temporal variation in allele frequencies significantly occurred in spring and autumn samples (*PGDH**: $F_{ST}=0.036$, $\chi^2=14.76$,

$P < 0.005$ and, *G3PDH**: $F_{ST}=0.038$, $\chi^2=13.45$, $P < 0.005$; respectively).

Cavalli-Sforza & Edward's (CSE) (1967) chord (D_{cho}) genetic distances among samples were low ($D_{cho}=0.063$ – 0.101). The UPGMA analysis (after D_{cho} genetic distances) resulted in separating the summer and spring populations of Pisa and the Atlantic (IcPSum and IcWSp, respectively) from the bulk of the other six samples (Figure 1). Likely, MDS analysis together with the MST showed IcPSum, IcWSp and IcPW samples as the most genetically distant, with significant goodness of fit of D_{cho} values in the configuration space (0.05–0.10), which in turn showed dissimilarities among *Illex coindetii* samples in agreement with UPGMA results (Figure 2).

DISCUSSION

Genetic variability

Genetically low variable populations of *Illex coindetii* occurred at both Tyrrhenian and Atlantic locations and at the four seasons one-year round examined (see Table 3). Similar results were found in previous enzyme studies on *I. argentinus* possibly due to the type of genetic markers employed (Carvalho et al., 1992). General depressed variability seems to be present in cephalopods and mobile oceanic species, in contrast to other marine invertebrate organisms (Nevo, 1978). However, the presence of the moderately variable population of summer IcPSum at the

northern Tyrrhenian could not be associated with previous analysis due to the lack of genetic seasonal studies. Thus, variability parameters might be likely to correlate with time and location, although very few studies have been done for both components along genetically diverse populations at large geographical scales (Fujio et al., 1983).

Population differentiation

General genetic uniformity of the widely separated samples of *Illex coindetii* off the northern Tyrrhenian and Iberian Atlantic waters was indicated from low F_{ST} values and genetic distances. This was not surprising where a homogeneous pattern of population structure has been found morphologically in *I. coindetii* at the eastern Atlantic for the whole-year round (Sánchez et al., 1998), and at the western Atlantic versus Mediterranean (Zecchini et al., 1996). In spite of the existence of few polymorphic enzyme loci (present study), the low levels of sample differentiation in *I. coindetii* and in other marine invertebrates are consistent with the occurrence of a large homogeneous population over extensive dispersal areas throughout the year (Dawe & Brodziak, 1998). Nevertheless, some genetic variation was indicative in *I. coindetii* samples from the seasonal (up to $F_{ST}=0.015$) but not geographic variable ($F_{ST}=0.000$, present study). A temporal differentiation pattern in other marine populations has seldom been analysed (Maltagliati & Camilli, 2000), implying a conservative use of results interpretation for population structure conclusions (Waples, 1989). However, *I. coindetii* species have presented spawning peak activity that varied widely with areas and seasons along the Mediterranean and Atlantic (González & Guerra, 1996; Sánchez et al., 1998), under the possibility of spawning stocks structure. Hierarchical analysis of gene differentiation had showed that 12% of total variance existed by the combination of both geographic and temporal (seasons) factors, possibly at the most distant samples of summer in Italy (IcPSum) and of spring in the Atlantic (IcWSp). Similarly, some loci variation had occurred at certain times of the year (Table 4). A temporal morphological comparison had been previously made in individuals from the northern Tyrrhenian and from the southern Tuscan archipelago, being summer specimens distinct from spring samples (Belcari, 1996). Thus, the latter ultimately may support the existence of two or more groups of *I. coindetii* with different growth rates that depended on the hatching season (Belcari, 1996; Sánchez et al., 1998), or on the hydrological characteristics (Hernández-García & Castro, 1998). Thus, major maturation peaks in autumn and spring were revealed at the western Mediterranean (see Sánchez et al., 1998), which might correspond to the existence of different spawning groups. However, the slight genetic variation found for few loci in winter, spring and autumn, should not be indicative of population structuring (present study). From the UPGMA and MDS-MST results only the summer Italian *I. coindetii* IcPSum and spring Iberian IcWSp samples were genetically separated from the rest (64% bootstrap at the UGMA, see Figures 1 & 2). Previous genetic studies based on DNA techniques did not solve the population structure identification in its counterpart *I. argentinus* (Adcock et al., 1999). Furthermore, no consistent pattern of geographic or temporally

based stock structure was apparent using microsatellite markers on the latter species (Adcock et al., 1999), which did not confirm previous suggestions of cryptic species or defined stocks at a microgeographic level (Carvalho et al., 1992). Within the Mediterranean teuthofauna, there is no evidence of isolation because of the large water inflow to the local basins and the uniformity of salinity and temperature along the water column. Our results suggest, in conclusion, the presence of a homogeneous population structure of the migrating *I. coindetii* individuals at least from the central Mediterranean to the Iberian Atlantic, the summer Italian and the spring Atlantic samples being the most divergent among the whole. Incipient temporal variation may be affecting the population structure of *I. coindetii* at some degree, being of interest for further molecular investigation of the species.

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