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ENZYMATIC VARIABILITY OF MEDITERRANEAN SLIPPER LOBSTERS, *SCYLLARIDES LATUS*, FROM SICILIAN WATERS

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ABSTRACT

A genetic comparison of slipper lobsters, Scyllarides latus, *from different areas in Sicily was carried out, electrophoretically checking 22 enzymatic activity zones. The analysis has shown a low degree of variability among the specimens; therefore, non-local breeders might be used for restocking without harming the biodiversity.*

Key words: Crustacea, Decapoda, Scyllaridae, *Scyllarides*, genetics, electrophoresis

VARIABILITÀ ENZIMATICA NELLA MAGNOSA MEDITERRANEA, *SCYLLARIDES LATUS*, DI ACQUE SICILIANE

SINTESI

*L'articolo presenta il confronto genetico tra magnose (*Scyllarides latus*) provenienti da diverse aree siciliane, confronto effettuato con il controllo elettroforetico di 22 zone di attività enzimatica. L'analisi ha evidenziato un basso grado di variabilità tra gli individui, pertanto gli autori ipotizzano che esemplari allevati in altre località potrebbero venir usati nel ripopolamento, evitando effetti negativi sulla biodiversità.*

Parole chiave: Crustacea, Decapoda, Scyllaridae, *Scyllarides*, genetica, elettroforesi

INTRODUCTION

On the Sicilian coasts, restocking of the now rare Mediterranean slipper lobster, *Scyllarides latus*, could achieve stable results, since the decline of the resource seems due to a shortage in recruitment, not to environmental degradation (Bianchini *et al.*, 1998). Of course, a sound enhancement program should operate without disrupting the existing equilibrium.

To preserve the biological diversity, and to reduce the risks related to the introduction in the genetic pool of characteristics different from the local ones, it is necessary to make sure that brooders and seeding animals belong to the autochthonous population, or that their origin is genetically similar. With this in mind, morphological (Bianchini *et al.*, 1996) and karyological (Deiana *et al.*, 1997) studies may be used for screening, together with genetic analyses, based on PCR methods or on electrophoretic techniques.

This last approach is based on the notion that proteins under the effect of an electric field migrate along the medium in accordance to their net charge and their molecular weight; utilizing this phenomenon, the electrophoresis displays molecular differences due to aminoacidic substitutions or deletions. In fact, these differences arise from nucleotide mutations at DNA level of the structural gene, which produce isozymes (Hunter & Markert, 1957) migrating at different velocities. The isozymes, *i.e.* the multiple forms that an enzyme could assume, may depend on the presence of more than one locus codifying the enzyme (allozymes, following Prakash *et al.*, 1969), or on the effect of post-translational modifications on the formed polypeptic chains (Richardson *et al.*, 1986); a locus is considered polymorphic when the frequency of the most common allele is lower than 95%.

The last step in the analysis of enzymatic polymorphisms is the interpretation of the observed electrophoretic pattern, which requires special care in case of species not extensively studied (Richardson *et al.*, 1986).

MATERIALS AND METHODS

Thirty-three Mediterranean slipper lobsters, *Scyllarides latus*, coming from different Sicilian areas (Fig. 1) were examined; their morphometric data are given in Table 1.

The electrophoretic runs were commissioned to an external University laboratory, using funds provided by a national program on slipper lobster restocking (III triennial plan of the former Ministry of Merchant Marine).

Pereiopod muscles were used; samples were transported in liquid N_2 to the laboratory, where they were manually homogenized in water, centrifuged at 3000 rpm for 30 min, and stored at -70 °C.

The methodology used to study the *Scyllarides latus* population was the electrophoretic analysis of the enzymatic polymorphisms.

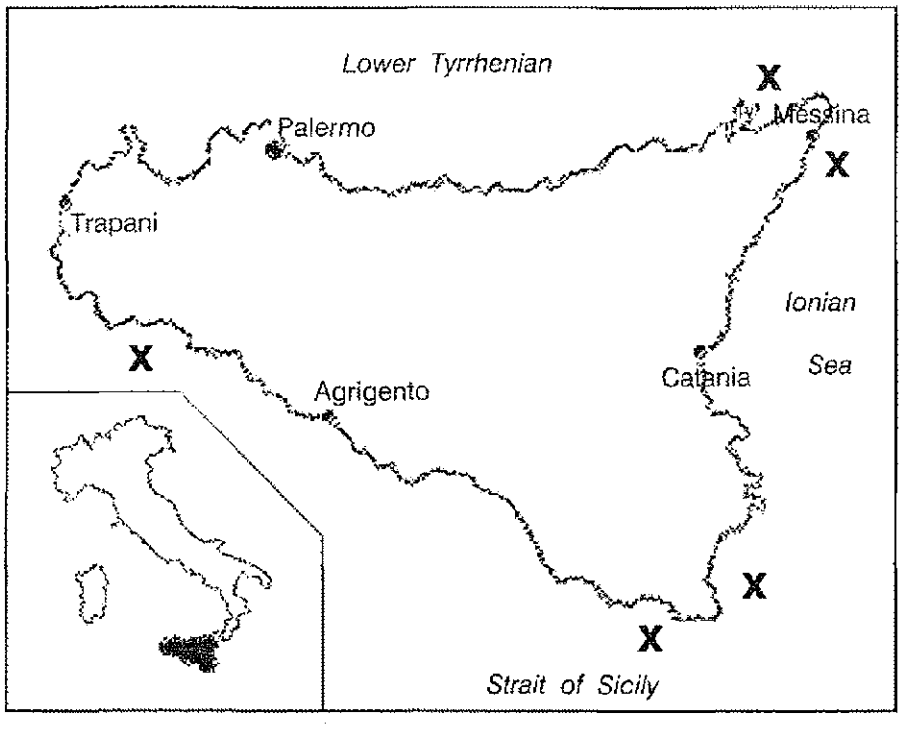

Fig. 1: Areas of collection (crosses) of the Sicilian "population" of slipper lobster *(Scyllarides latus).*
Fig. 1: Območja zbiranja (križci) sicilijanske "populacije" velikega nagajivca *(Scyllarides latus).*

Cellogel (cellulose acetate) was used as support; the electrophoretic runs were performed in Chemetron tanks filled with 250 ml of bridge buffer, holding 3 cellogel stripes (5.7x14 cm), pre-soaked for 15 min in a gel buffer equal to that of the run ("continuous buffer"). Each cellogel strip carried 5 samples; to allow comparison, the last sample of each strip was repeated on the next one.

At the end of the run, every strip was coloured with techniques specific for each enzyme, using buffered solutions with substrates, coenzymes and/or coupled enzymes and colours that bond with the final products. A thermostat was used for the enzymatic reaction; once the colour appeared, the reaction was stopped immersing the cellogel stripes in acetic acid (10%), and the stripes were sealed and kept at 4 °C.

Twenty enzymatic sets, accounting for 22 independently variable activity zones, *i.e.* accounted as the product of 22 loci, were examined. Table 2 reports the tested enzymes, their abbreviations, and the number of interpreted loci; the allele designation follows the numeric system based on the relative gel mobility of isozymes.

Table 3 reports, for each tested enzyme, the run conditions (buffers, times, applied tension in Volt, electrical input in mA, depending on the buffer ionic strength) and the colouring techniques, as well as the respective bibliographic references.

Tab. 1: Morphometric characteristics of the slipper lobsters** (Scyllarides latus) **analysed electrophoretically by sex (F = female, M = male).
Tab. 1: Morfometrične značilnosti velikih nagajivcev** (Scyllarides latus), **analizirane elektroforetično po spolu (F = moški, M = ženske).

	F	M
number	13	20
weight (g)		
mean	440.6	361.7
SD	191.0	76.7
min	258.0	237.0
max	940.0	523.0
carapace length (mm)		
mean	100.4	94.8
SD	13.2	7.0
min	88.6	81.7
max	132.2	106.7

RESULTS

The Sicilian slipper lobsters show a very modest electrophoretic variability in the 22 examined loci (Tab. 4): 20 loci are completely, or almost completely, fixed in the same allele, and the polymorphism is low in the other 2 loci (IDH and MDH-2) too.

Table 5 reports the estimates of the populational genetic variability, expressed as mean number of alleles per locus (N_a), percent of polymorphic loci (p), observed (H_o) and expected (H_e) heterozygosity (Nei, 1978). The studied population presents low values of polymorphism, with H_o=0.020, H_e=0.019, mean number of alleles per locus equal to 1.2 and percent of polymorphic loci equal to 9.1.

Moreover, the observed genotypic frequencies were compared with the expected frequencies, to display possible divergences from the Hardy-Weinberg equilibrium, per population and per locus (Tab. 6). Using the χ^2 analysis, the studied Sicilian population of *S. latus* results in substantial equilibrium in all the examined loci.

***Tab. 2: Enzymatic systems analysed electrophoretically in the slipper lobster** (Scyllarides latus).*
***Tab. 2: Elektroforetično analizirani encimatski sistemi velikega nagajivca** (Scyllarides latus).*

enzyme (Enzyme Commission number)	**abbreviation**	**No. of loci**
Alcohol dehydrogenase (1.1.1.1)	ADH	1
Adenilate chinase (2.7.4.3)	ADK	1
Aldolase (4.1.2.13)	ALDO	1
Creatine chinase (2.7.3.2)	CK	1
Esterase (3.1.1.1)	EST	1
Fructose-1,6-diphosphatase (3.1.3.11)	FDP	1
Fumarase (4.2.1.2)	FUM	2
Glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12)	GAPD	1
Glucose-phosphate isomerase (5.3.1.9)	GPI	1
Esochinase (2.7.1.1)	HK	1
Isocitrate dehydrogenase (1.1.1.42)	IDH	1
Lactate dehydrogenase (1.1.1.27)	LDH	1
Malate dehydrogenase (1.1.1.37)	MDH	2
Malic enzyme (1.1.1.40)	ME	1
Mannose-phosphate isomerase (5.3.1.8)	MPI	1
Amino-peptidase (3.4.11)	PEP	1
6-Phosphogluconate dehydrogenase (1.1.1.44)	6PGD	1
Phosphoglucomutase (2.7.5.1)	PGM	1
Trioso-phosphate isomerase (5.3.1.1)	TPI	1
Xantine dehydrogenase (1.2.1.37)	XDH	1

Total number of analyzed enzymes = 20
Total number of interpreted loci = 22

Tab. 3: Electrophoretic methods used for separation of the slipper lobster** (Scyllarides latus) **samples.
***Tab. 3: Elektroforetične metode, uporabljene za ločevanje primerkov velikega nagajivca** (Scyllarides latus).*

enzyme	buffer (conc.)	Volt/hour/mA	coloring technique (reference)
ADH	C (1x)	160/2:00/10	Richardson *et al.*, 1986
ADK	B (2 x)	160/2:30/18	Richardson *et al.*, 1986
ALDO	TEC 0.075 (1x)	160/2:00/14	Harris & Hopkinson, 1976
CK	C (2x)	160/2:00/18	Richardson *et al.*, 1986
EST	G (1x)	160/1:00/18	Richardson *et al.*, 1986
FDP	B (1x)	160/2:00/10	Richardson *et al.*, 1986
FUM	A (2x)	160/1:30/8	Richardson *et al.*, 1986
GAPD	C (1x)	160/2:00/10	Richardson *et al.*, 1986
GPI	C (1x)	160/2:00/10	Richardson *et al.*, 1986
HK	C (1x)	160/1:15/10	Richardson *et al.*, 1986
IDH	A (1x)	160/1:45/10	Ayala *et al.*, 1972
LDH	B (1x)	160/1:45/10	Selander *et al.*, 1971
MDH	C (1x)	160/1:30/10	Richardson *et al.*, 1986
ME	B (1x)	160/1:30/10	Richardson *et al.*, 1986
MPI	B (1x)	160/1:00/10	Richardson *et al.*, 1986
PEP	A (2x)	160/1:45/8	Richardson *et al.*, 1986
6PGD	B (1x)	160/1:30/10	Richardson *et al.*, 1986
PGM	C (1x)	160/1:45/8	Richardson *et al.*, 1986
TPI	B (1x)	160/2:00/10	Richardson *et al.*, 1986
XDH	F (1x)	160/1:45/18	Richardson *et al.*, 1986

Bibliographic references for the buffers:
A, B, C, F, G: Richardson *et al.*, 1986
TEC 0.075: Meera Khan, 1971

DISCUSSION

The electrophoretic analysis of the enzymatic polymorphisms has shown low levels of variability in the Sicilian population of *Scyllarides latus*. This result should not be interpreted as a bottleneck effect of the numerical scarcity of the Sicilian population, but may be structural to the taxon (Hardwick & Cline, 1984, 1985, 1986). In fact, preliminary unpublished results on two other populations, from Israel and from the Açores, seem to confirm a minimal heterozygosity. Moreover, this lack of genetic diversity is in substantial agreement with the pattern observed in other species of large-size decapod crustaceans (Hedgecock, 1987).

On the other hand, the apparent resemblance of the slipper lobster samples could be linked to peculiar variability patterns of the gene-enzyme systems hitherto tested; other kind of loci might display higher levels of variation.

The genetic analyses have shown a high similitude among the studied animals, coming from distant geographical locations: this fact suggests that non-local specimens could be used, as breeders or seeding stuff, in restocking and stock enhancement programs in impoverished areas, without exorbitant risks of genetic contamination and biodiversity reduction.

Nevertheless, the seemingly genetic homogeneity of the Mediterranean slipper lobsters needs further testing, perhaps with more sophisticated techniques (*e.g.* the analysis of microsatellite loci), before the actual introduction of allochthonous material.

CONCLUSIONS

On the Sicilian coasts, restocking of the now rare Mediterranean slipper lobster, *Scyllarides latus*, could achieve stable results, since the decline of the resource seems due to a shortage in recruitment, not to environmental degradation. To preserve the biodiversity, and to reduce the risks related to the introduction in the genetic pool of characteristics different from the local ones, it is necessary to make sure that brooders and seeding animals belong to the autochthonous population, or that their origin is genetically similar.

With this in mind, a genetic comparison of slipper lobsters, *Scyllarides latus*, from different areas in Sicily was carried out, electrophoretically checking 22 enzymatic activity zones (ADH, ADK, ALDO, CK, EST, FDP, FUM-1, FUM-2, GAPD, GPI, HK, IDH, LDH, MDH-1, MDH-2, ME, MPI, PEP-2, 6PGD, PGM, TPI, XDH).

Tab. 4: Frequencies of alleles for 22 loci in the Sicilian population of slipper lobster** (Scyllarides latus). **Legend: N = number of specimens; A = most common/only allele; B = second allele.
Tab. 4: Frekvence alel za 22 lokacij sicilijanske populacije velikega nagajivca** (Scyllarides latus). **Legenda: N = št. primerkov; A = najpogostejši/edini alel; B = drugi alel.

locus	
ADH	
N	29
A	1.000
ADK	
N	24
A	1.000
ALDO	
N	29
A	1.000
CK	
N	24
A	1.000
EST	
N	29
A	0.983
B	0.017
FDP	
N	27
A	1.000
FUM-1	
N	29
A	1.000
FUM-2	
N	29
A	0.983
B	0.017
GAPD	
N	29
A	1.000
GPI	
N	29
A	1.000
HK	
N	29
A	1.000

locus	
IDH	
N	29
A	0.948
B	0.052
LDH	
N	29
A	1.000
MDH-1	
N	29
A	0.983
B	0.017
MDH-2	
N	29
A	0.879
B	0.121
ME	
N	29
A	1.000
MPI	
N	29
A	1.000
PEP-2	
N	29
A	1.000
6PGD	
N	29
A	1.000
PGM	
N	29
A	1.000
TPI	
N	29
A	1.000
XDH	
N	29
A	1.000

***Tab. 5: Genetic variability estimates (± standard error) for 22 loci in the Sicilian population of slipper lobster** (Scyllarides latus).*
***Tab. 5: Ocene genetske variabilnosti (± standardna napaka) za 22 lokacij sicilijanske populacije velikega nagajivca** (Scyllarides latus).*

mean No. ind./populat. (± SD)	mean No. alleles/locus (N_a)	% polymorphic loci (p)	mean heterozygosis (± SD)	
			observed (H_o)	expected (H_e)
28.5 (0.3)	1.2 (0.1)	9.1	0.020 (0.012)	0.019 (0.011)

***Tab. 6: χ^2 test for the rejection of the Hardy-Weinberg equilibrium in the Sicilian population of slipper lobster** (Scyllarides latus).*
***Tab. 6: Test χ^2 za zavrnitev Hardy-Weinbergovega ravnovesja v sicilijanski populaciji velikega nagajivca** (Scyllarides latus).*

locus/class	observed	expected	χ^2	d.o.f.	P
EST			0.000	1	1.000
A-A	28	28.000			
A-B	1	1.000			
B-B	0	0.000			
FUM-2			0.000	1	1.000
A-A	28	28.000			
A-B	1	1.000			
B-B	0	0.000			
IDH			0.057	1	0.812
A-A	26	26.053			
A-B	3	2.895			
B-B	0	0.053			
MDH-1			0.000	1	1.000
A-A	28	28.000			
A-B	1	1.000			
B-B	0	0.000			
MDH-2			0.461	1	0.497
A-A	22	22.368			
A-B	7	6.263			
B-B	0	0.368			

The Sicilian slipper lobsters show a very modest electrophoretic variability in the examined loci. 20 loci are completely, or almost completely, fixed in the same allele, and the polymorphism is low in the other 2 loci (IDH and MDH-2) too. The observed heterozygosity (H_o) equals 0.020, and the expected heterozygosity (H_s) equals 0.019, the mean number of alleles per locus (N_a) is 1.2 and the percent of polymorphic loci is 9.1. Moreover, using the χ^2 analysis, the studied Sicilian population of *S. latus* results in substantial equilibrium in all the examined loci.

In fact, this lack of genetic diversity is in substantial agreement with the pattern observed in other species of large-size decapod crustaceans. The genetic analyses have shown a high similitude among the studied animals, coming from distant geographical locations: this result suggests that non-local specimens could be used, as breeders or seeding stuff, in restocking and stock enhancement programs in impoverished areas, without exorbitant risks of genetic contamination and biodiversity reduction.

ENCIMATSKA VARIABILNOST VELIKEGA NAGAJIVCA, *SCYLLARIDES LATUS*, V SICILIJANSKIH VODAH

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POVZETEK

V sicilijanskih obalnih vodah bi z vlaganjem zdaj redkega velikega nagajivca, Scyllarides latus, *dosegli brzkone dobre rezultate, saj vse kaže, da upadanja njegove populacije ne gre pripisati degradaciji tega morskega okolja. Toda da bi ohranili biodiverziteto v teh vodah in hkrati zmanjšali tveganja, povezana z vlaganjem osebkov v genetski "pool" z značilnostmi, ki se razlikujejo od lokalnih, bi bilo treba zagotoviti, da osebki za razplod teh rakov pripadajo avtohtoni populaciji, ali pa da je njihov izvor genetsko podoben lokalnemu.*

V ta namen smo opravili genetske primerjave med velikimi nagajivci, Scyllarides latus, *iz različnih območij sicilijanskih obalnih voda z elektroforetičnim pregledovanjem 22 encimatskih con aktivnosti (ADH, ADK, ALDO, CK, EST, FDP, FUM-1, FUM-2, GAPD, GPI, HK, IDH, LDH, MDH-1, MDH-2, ME, MPI, PEP-2, 6PGD, PGM, TPI, XDH).*

Na raziskanih lokalitetah je bila ugotovljena zelo skromna elektroforetična variabilnost velikih nagajivcev. 20 lokalitet je docela, ali skoraj docela, ustaljenih v istem alelu, polimorfizem pa je nizek tudi v dveh preostalih lokacijah (IDH in MDH-2). Opazovana heterozigotnost (H_o) je bila 0,020, pričakovana heterozigotnost (H_e) 0,019, srednje število alel na lokaliteto (N_a) 1,2 in odstotek polimorfične lokalitete 9,1. Poleg tega smo z analizo χ^2 ugotovili, da je preučevana sicilijanska populacija velikega nagajivca v precejšnjem ravnovesju na vseh pregledanih lokacijah.

Pravzaprav se to pomanjkanje genetske pestrosti v precejšnji meri ujema z vzorcem, opaženim pri drugih vrstah velikih dekapodnih rakov. Genetske analize so pokazale veliko podobnost med preučevanimi živalmi z oddaljenih geografskih lokacij, kar daje misliti, da bi za razplod in povečanje populacije v teh osiromašenih vodah lahko uporabili osebke iz drugih voda, in to brez večjih tveganj za genetsko "kontaminacijo" in zmanjšano biodiverziteto v sicilijanskih obalnih vodah.

Ključne besede: Crustacea, Decapoda, Scyllaridae, *Scyllarides*, genetika, elektroforeza

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