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Allozyme characterization of the Norway lobster, *Nephorps norvegicus*, of two Adriatic trawling grounds

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Samples of Norway lobsters (Nephrops norvegicus) *collected in two Adriatic trawling grounds - where they give origin to populations with marked biometrical and biological differences - have been electrophoretically analyzed. No genetic differentiation between the two populations was detected, suggesting that the observed biological differences have not a genetical basis but are merely due to environmental factors.*

INTRODUCTION

The Norway lobster *Nephrops norvegicus* has been extensively studied for sexual dimorphism and geographic variation in many North Sea and Eastern Atlantic areas (see FARMER, 1975). Morphometric data on Mediterranean stocks are only available for Catalonian Sea (SARDA *et al.*, 1981) the Thyrrenian Sea (MATTA, 1959; RELINI-ORSI and RELINI, 1985). In the Adriatic Sea the species has received particular attention for ecological and biological parameters. The available data show that in this area *N. norvegicus* colonizes bottoms with a wide depth range, where it builds up population with marked differences in density, mean length, growth, size at first sexual marurity (KARLOVAC, 1953; GAMULIN-BRIDA *at al.*, 1972; FROGLIA and GRAMITTO, 1981, 1988).

Two Adriatic trawling grounds (Fig. 1) appear to be particularly interesting as they show very great differences among the above mentioned biological parameters: namely an area representative of the central Adriatic "deep water Nephrops grounds", located in the western part of the Pomo pit (depth 200-250 m), supporting a denser population of smaller specimens in cooler waters, and a second one 13 miles North-West off Ancona, 50-60 m deep, representative of the Adriatic "Shallow water Nephrops grounds", characterized by large, more scattered animals. The hypothesis that noticed differences are mainly due to environmental factors has been suggested (FROGLIA and GRAMITTO, 1987).

In order to decide whether the observed biological differences have a major environmental basis or, rather, a genetic one, a detailed gene-enzyme system characterization has been undertaken. Actually, allozyme analysis has been widely exploited in Decapods (see HEDGECOCK *et al.*, 1982) and proven very useful in assessing genetic differences at generic and specific ranks. In particular, the Astacidea, have been quite extensively investigated, but, to our knowledge, no data are available for *Nephrops* or closely related genera; therefore, this represents the first contribution to the topic.

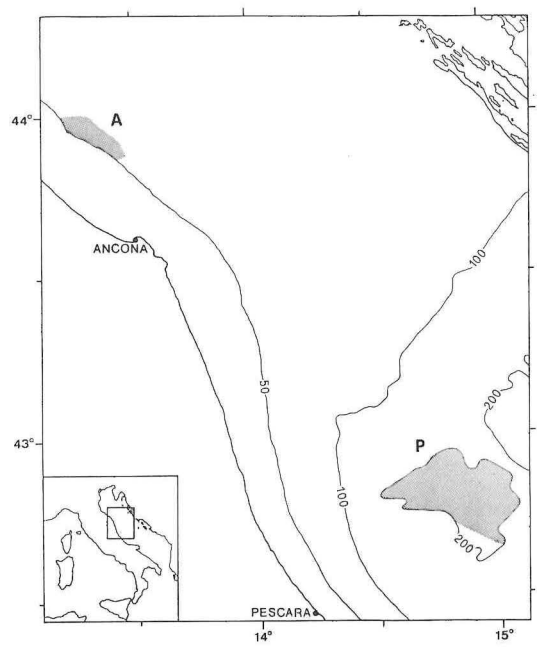

Fig. 1. Trawling grounds sampled for *Nephrops norvegicus* (A: off Ancona; P: Pomo pit).

MATERIALS AND METHODS

Samples were collected with the ship R/v "S. Lo Bianco", using an Italian bottom trawl with 32 mm streched meshes in the cod-end, in April and June 1988 (Pomo pit: 20 females and 15 males; off Ancona: 18 females and 15 males).

For allozyme analysis a sample of chela muscle from each lobster was homogenized with a glass rod in an equal volume of 0.1 M Tris - HCL buffer solution, pH 7.5, containing 0.1 x 10^{-2} M Na-EDTA, 0.6 x 10^{-3} M NAD, 0.5 x 10^{-3} M NADP, 1 x 10^{-3} M Mercaptoethanol. After centrifugation at 12.00 rpm for 5 minutes, the clear supernatant was used for the electrophoretic run, carried out on cellulose acetate membrane (Cellogel, Labometrics) in Shandon chambers (Mod. 600) for 3-3.30 hours at 3oC at constant voltage (220 V). Best results were obtained with TEC "0.075" run buffer (MEERA KHAN, 1971). Staining procedures employed in this study were, with minor modifications, as follows: LDH - SHAW and PRASAD, 1970; 6PGDH, G6PDH - MEERA KHAN, 1971; GOT - SELANDER *et al.*, 1971; a GPDH, ME, G3PDH - AYALA *et al.*, 1972; ADK, MPI, PGI - VAN SOMERET *et al.*, 1974; MDH, IDH, PGM - MEERA KHAN *et al.*, 1982. For MDH, ME and GOT two and for MPI three indipendent loci were scored, so that a total number of 18 loci were analyzed, although not all of them for each specimen. It is to be noted that the enzymes have been chosen considering the available data on their polymorphism and heterozygosity (HEDGECOCK *et al.*, 1982).

Phenotypic, genetic and allele nomenclature, as well as proportion of polymorphic loci (P) and mean expected heterozigosity per locus ($\overline{H}_{exp}$) calculations, were performed according to HEDGECOOCK *et al.* (1982), while the effective number of alleles per locus (A) was estimated as reported by FERGUSON (1980). Finally, genetic distance (D) and identity (I) were calculated according to Nei's method (1972).

RESULTS

No differences in allelic frequencies were evidenced between sexes in each sample. The minimum sample size is 12 animals for the *Gpdh locus* (Table 1), while for the remaining loci it is larger, with an average number of analyzed per locus of 26 Pomo pit and 23 for off Ancona populations.

The two samples of *N. norvegicus* are monomorphic for the same allele at 11 out of 18 loci. They show (Table 1) slightly different allelic frequencies at *Got*-2 and *Pgi* loci. No diagnostic loci exist between the two samples and private alleles occur at a very low rate being found in the Pomo pit popultion at the α*Gpdh* and *Pgm* loci (102 and 96, respectively) and in the Ancona sample at *Me*-2 and *G6pdh* loci (103 and 104, respectively).

As predictable from allelic data, genetic identity is very high (I = 0.998) and distance very low (D - 0.002), clearly indicating a difference of interpopulational level.

The comparison between observed and expected heterozigosity at a single locus, reported in Table 1, shows a highly significant deficiency of heterozygotes at the *G6pdh* locus for both populations (X^2_1 = 23.29 with P < 0.001 for Pomo sample; X^2_3 = 25.01 with P < 0.001 for Ancona sample); the same applies for the Pomo

Table 1. Allele frequencies at the 7 polymorphic loci found in the two populations of *Nephrops norvegicus* analyzed (SPOM = Pomo pit; SANC = off Ancona); *Ldh*, *Mdh*-1, *Mdh*-2, *Me*-1, *Idh*, *G3pdh*, *Got*-1, *Adk*, *Mpi*-1, *Mpi*-2, *Mpi*-3 are monomorphic for the same allele. Observed (H_{obs}) and expected (H_{exp}) heterozigosity and number of assa- yed genomes (i. e. twice the number of analyzed animals = n) per locus are

LOCUS	ALLELES	SPOM	SANC
αGpdh	n	24	40
	100	0.96	1.00
	102	0.04	– –
	H_{obs}	0.083	0.000
	H_{exp}	0.080	0.000
Me-2	n	66	40
	100	1.00	0.975
	103	– –	0.025
	H_{obs}	0.000	0.050
	H_{exp}	0.000	0.049
6Pgdh	n	66	62
	100	0.97	0.97
	104	0.03	0.03
	H_{obs}	0.061	0.064
	H_{exp}	0.059	0.062
G6pdh	n	66	50
	100	0.895	0.94
	104	– –	0.02
	108	0.105	0.04
	H_{obs}	0.030	0.040
	H_{exp}	0.190	0.114
Got-2	n	56	56
	100	0.93	0.965
	106	0.07	0.035
	H_{obs}	0.143	0.071
	H_{exp}	0.133	0.069
Pgm	n	60	58
	96	0.015	– –
	100	0.985	1.00
	H_{obs}	0.033	0.000
	H_{exp}	0.033	0.000
Pgi	n	48	50
	97	0.02	0.02
	100	0.94	0.78
	103	0.04	0.20
	H_{obs}	0.083	0.280
	H_{exp}	0.119	0.351

sample at the *Pgi* locus (X23 = 23.47 with P 0.001). However, mean obverved heterozygosity of both samples is in agreement with the expected one, the differences being not significant X21 = 2.198, with P 0.10 for Pomo pit sample; X21 = 0.768, with P 0.30, for Ancona population (Table 2).

The Pomo sample shows a higher number of polymorphic loci when compared to the coastal one (Table 2).

Table 2. Genetic parameters of the two analyzed samples (SPOM = Pomo pit; SANC = off Ancona; H_{obs} : mean observed heterozygosity, per locus; H_{exp} : mean expected heterozygosity per locus; A: mean effective number of alleles per locus; P: proportion of polymorphic loci).

	SPOM	SANC
$\overline{H}_{obs}$	0.023	0.027
$\overline{H}_{exp}$	0.033	0.036
A	1.04	1.05
P	0.33	0.28

DISCUSSION

The identity and distance values obtained do not support differentiation between the Pomo and Ancona samples higher than population level and therefore electrophoretical data well agree with the suggestion that their biometrical and biological features have an environmental determination.

Actually, fully comparable distance and identity figures have been obtained from conspecific population analysis of *Homarus americanus* (TRACEY *et al.* 1975).

On a wider comparative evalution scale, *N. norvegicus* allozyme pattern is in good agreement with the corresponding picture obtained for Astacura (see HEDGECOCK *et al.*, 1982). In particular, Adriatic *N. norvegicus* shows the typically low heterozygosity value of the Decapods so far analyzed; possibly, the ecological and population genetic factors envisaged for Penaeid Prawns by MULLEY and LATTER (1980) could be of importance for the Norway lobster too, in determining the observed population structure.

However, two genetic parameters appear to be at variance in the Adriatic Norway lobster. First, the mean number of alleles per locus in our samples (A = 1.04, 1.05; Table 2) is much lower than that reported by HEDGECOCK *et al.* (1982) for Astacidea and Palinura (A = 1.24 with a range 1.11-1.49). We must remember though, that the A value we give for *N. norvegicus* is the mean *effective* one, and we do not know whether the same evaluation method has been followed for the above reported groups. Second, the proportion of polymorphic loci in our samples (P = 0.33 and 0.28; Table 2) in clearly higher than the mean value reported for Astacidea and Palinura (P = 0.185), and actually beyond their range (0.100-0.276) (HEDGECOCK *at al.*, 1982).

Finally, we have no factual explanation for the heterozygote deficiency at the *G6pdh* locus in both populations and at the *Pgi* locus for the Pomo pit sample; we can just suggest that these geno-phenotypes have a direct or indirect differential survival.

On the whole, the hypothesis of environmental factors playing a major role in realizing the biological differences noticed between the Pomo pit and off Ancona areas (FROGLIA and GRAMITTO, 1987) appears to receive a definitive evidence from gene-enzyme system analysis.

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Alozimska karakterizacija škampa, *Nephrops norvegicus*, sa dva ribolovna područja Jadranskog mora

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KRATKI SADRŽAJ

Jadranske populacije škampa *(Nephrops norvegicus)* pokazuju vrlo izrazite razlike u nekolicini biometrijskih karaktera. Još uvijek je pod znakom pitanja da li su one prouzročene okolišem ili genetskog porijekla. Elektroforetski smo analizirali 18 genetsko-enzimskih sustava dva uzorka škampa iz Jadranskog mora čiji se slijedeći parametri izrazito razlikuju: raspršena plitkovodna populacija kočarskog područja 13 Nm sjeverozapadno od Ankone s velikim primjercima i gusta populacija s malim primjercima iz dubokih voda Jabučke kotline.

Genetska je analiza pokazala da ne postoje razlike koje nadilaze razlike na nivou populacije (D = 0.002) između ova dva uzorka. Njihove razlike nastale su samo zbog različitosti okoliša u kome se kreću. Jedna šira usporedba je pokazala da jadranski *N. norvegicus* imaju iste genetske karakteristike kao i Astacura, iako je proporcija polimorfizma *N. norvegicus* znatno veća od srednjih vrijednosti objavljenih za Astacidea-e i Palinura-e.