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Genetic Variation and Divergence in the Genus *Carcinus* (Crustacea, Decapoda)

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Abstract

The extent of genetic differentiation of the decapod crustaceans *Carcinus maenas* (L.) and *C. aestuarii* NARDO was studied in nine nearshore locations comprising various European marine and brackish-water habitats, (Norway, Germany, France, Spain, Italy and Yugoslavia), using starch gel electrophoresis. The heterozygosity for all 19 allozyme loci was 0.026–0.016 *(C. maenas)* and 0.011–0.003 *(C. aestuarii)*; one locus *(Pgm)* was the most polymorphic. The populations had rather low levels of genetic variability, comparable to those found in other decapods. In general, a light clinal variation in allele frequencies was discovered at the *Pgm* locus of *C. maenas* as latitude decreased. Because of similar morphologies and a closed genetic relationship ($I = 0.89$), the two forms should be considered subspecies. Therefore, the designation of the Atlantic subspecies *C. maenas maenas* is proposed, and the term of the Mediterranean subspecies *C. maenas aestuarii* is recommended.

1. Introduction

Electrophoretic investigations of proteins permit information to be obtained on the structure of populations at the genetic level. Gene-enzyme systems, which show a distinct polymorphism, can utilize knowledge of the genetic composition of populations to differentiate within or between distinct species. The electrophoretic variants, which are recorded with this technique in combination with enzyme-specific methods may be applied as genetic markers for population differentiation. The distribution supplies criteria on the dynamic events which determine the population structure, such as the diverse impacts of natural selection, gene flow, random genetic drift and other effects.

In order to assess the influence of these factors on the population structure, studies on two crustaceans (Decapoda, Portunidae) have been carried out. *Carcinus maenas* (LINNAEUS, 1758), the shore crab, is a typical coastal and sea-shore inhabitant of the Atlantic ocean, which lives in the tidal and in the sublittoral zone. *C. aestuarii* NARDO, 1847, the Mediterranean shore crab, occupies similar habitats in the Mediterranean Sea, Black Sea, Asow Sea as well as in the Canarian Sea (ZARIQUIEY ALVAREZ, 1968).

C. maenas has a distribution from northern Norway to Mauretania, and in the West, from Nova Scotia to Brasilia; recently, the species immigrated or was introduced into the Indopazific region (CHRISTIANSEN, 1969). Lately, it has been shown to occur in the Pazific Bight of San Francisco (California), where, since 1989, it has become abundant in shallow lagoons and spread throughout the central bays (COHEN *et al.*, 1995; GROZHOLZ and RUIZ, 1995). *C. maenas* is common in large parts of the continental Shelf and usually lives in shallow coastal or aestuarine waters. It occurs on soft, sandy or hard surfaces; it may be found under stones or seaweed along the shoreline. The diet consist of animal food of various origins;

only seldom is vegetable fare consumed. As an euryhaline crustacean species, *C. maenas* occasionally occurs in the Baltic Sea up to the border of the Darss peninsula (ca 8‰ S), while *C. aestuarii* also exists in the brackish-water of the Black Sea (ca 16–18‰ S) and the Asow Sea (ca 11‰ S).

In a short note, SHERBOURNE (1976) demonstrated evidence that a polymorphic locus encoding the enzyme PGM (phosphoglutomutase) is present in *C. maenas* in the Atlantic Ocean (Boothbay Harbor, Maine). According to this study, variations at the *Pgm* locus can be explained by the existence of three alleles. Other loci were not examined.

This investigation focusses on the population genetics of these decapods from the European area. Moreover, it is the aim of the study to determine if *C. maenas* and *C. aestuarii* can be distinguished electrophoretically and to estimate the scope of genetical differentiation at the species or subspecies level.

2. Material and Methods

Adult crustaceans (*C. maenas* and *C. aestuarii*) of either sex, were analysed electrophoretically. They were obtained from the following regions (Fig. 1): Bergen (Norway), Heligoland, Heikendorf (Federal Republic of Germany), Roscoff, Arcachon (France), Cadiz (Spain), Naples, Venice (Italy) and Rovinjo (Yugoslavia). At the last three localities, the collections consisted of *C. aestuarii* only. Salinities were between 30 and 37‰ S in nearly all marine habitats; reduced salinities (ca 15‰) were recorded only in the brackish-water sample Heikendorf on the Baltic Sea coast (Kiel Bight).

The majority of the captured animals were deep-frozen at –70 °C; the muscles of the claws and legs of the remaining animals were removed and deep-frozen at the same temperature.

Electrophoresis was performed on vertical 13% starch gels (Buchler Instruments). Electrophoretic buffer systems included the following methods: 1. Tris-citrate-buffer (TC) (AYALA *et al.*, 1972), 2. Tris-borate-buffer (TBE) (SCHOLL *et al.*, 1978) and 3. Histidine-buffer (HC) (WARD and BEARDMORE, 1977). The electrophoretic method was performed in a constant temperature room (4 °C) for 16–17 hours. The staining procedure of the slices took place directly following electrophoresis. Gels were stained according to slightly modified proceedings as described by HARRIS and HOPKINSON (1976) and SCHOLL *et al.* (1978). Mostly, the zymogrammes were photographed. No distinctive feature was observed that would distinguish between deep-frozen and fresh material.

The following enzyme systems, treated with agar-detector gels, were resolved: APK, GPT, MPI, PEP, PGI, and PGM. As a rule, the enzymes migrated in anodal direction. The most frequent allele was numbered "100". The designation for faster or slower bands in *C. maenas* and *C. aestuarii* were proportional to the migration of the reference allele.

Both populations of *Carcinus* were examined on each gel. Data were compared using the method of NEI (1972). Measures of genetic identity (I) and genetic distance ($D = -\ln I$) for all loci were used to quantify the degree of genetic divergence between the two taxa.

Data were analysed by the computer program BIOSYS-1 (Release 1.7) (SWOFFORD and SELANDER, 1981). The genetic structure among the populations can be calculated by means of WRIGHT's (cf. 1978) F statistic. The relation by the basic set of F statistics to variances within natural populations is considered. The formula of WRIGHT $F_{st} = 1/(1 + 4\,Nm)$ was used to estimate the gene flow from the values of genetic variances between populations (where N is the effective population size and m is the effective migration rate).

3. Results and Discussion

3.1. Genetic Differentiation

The most important issue in the investigations into differentiation of crustacean populations is to establish genetic polymorphism. Altogether, nine shore crab populations (*C. maenas* and *C. aestuarii*) from six European countries were studied. Using diverse buffer

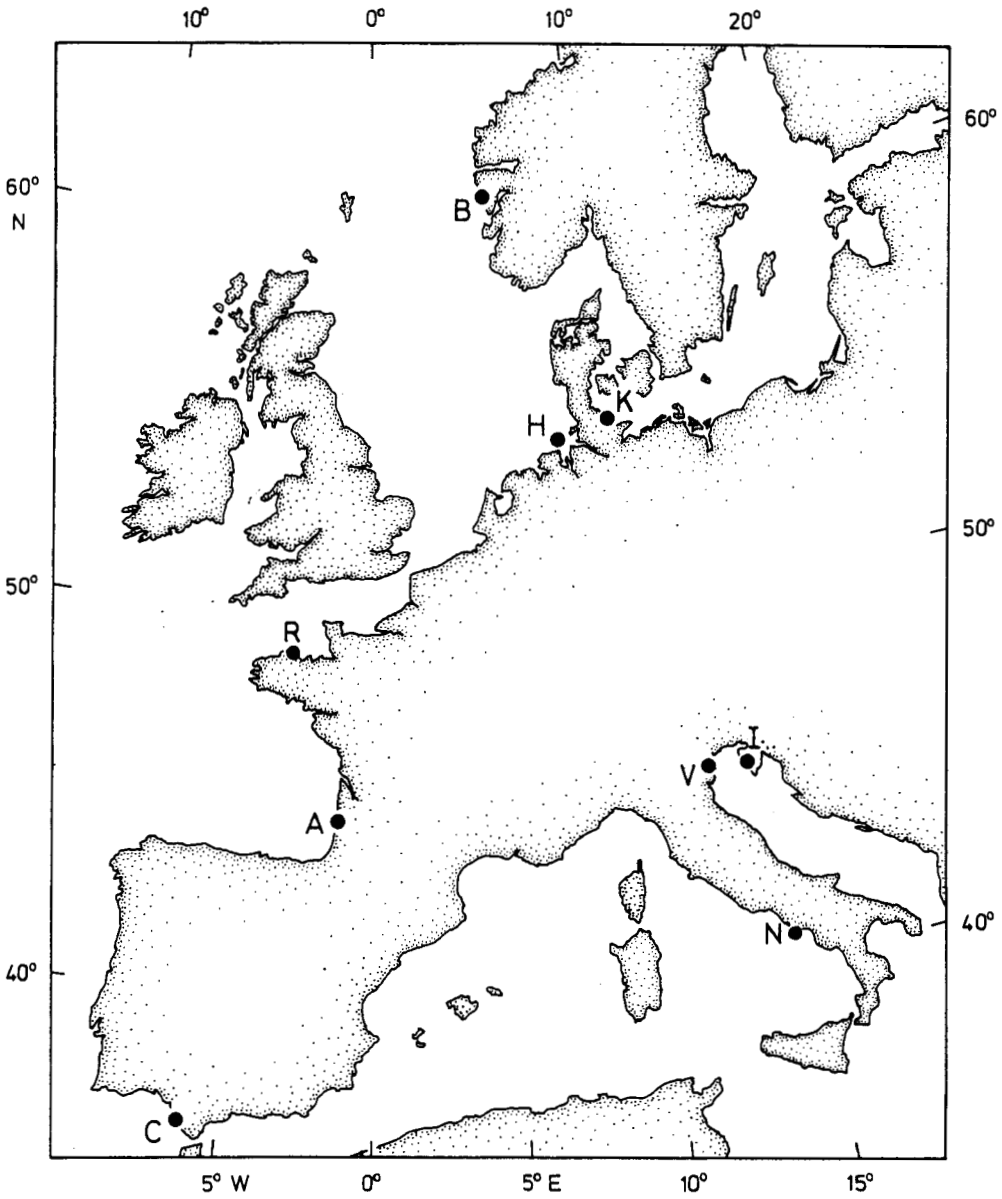

Figure 1. Location showing sampling localities of *Carcinus maenas*: K-Heikendorf, B-Bergen, H-Heligoland, R-Roscoff, A-Arcachon, C-Cadiz; *Carcinus aestuarii*: N-Naples, V-Venice, I-Rovinjo.

systems, sixteen gene enzymes from nineteen loci were investigated (Table 1). MDH-2 and PEP often stained less successfully and attempts to resolve these loci were abandoned; to some extent, these two loci were not considered in the calculations of genetic identity.

Loci that encoded ACON, FUM, GOT-1, α-GPD and PGI showed only negligible polymorphism (frequency of the most frequent band >0.95). Nearly all of the loci were monomorphic and the populations indicated very little genetic variability.

Table 1. System of gene-enzymes with abbreviations; European Commission (E.C.) numbers, electrophoretic methods and numbers of loci investigated.

Enzymes	Abbrev.	E.C. No.	Electrophoretic system	Loci
Aconitase	Acon	4.2.1.3	TC	1
Argininphosphokinase	Apk	2.7.3.3	TBE	1
Esterase	Est	3.1.1.1	TBE	1
Esterase-D	Est-D	3.1.1.2	TBE, TC	1
Fumarase	Fum	4.2.1.2	TC	1
Glutamate-oxaletate transaminase	Got-1, -2	2.6.1.1	TC	2
Glyceraldehydphosphat dehydrogenase	Gpt	2.6.1.2	TBE	1
Isocitrate dehydrogenase	Idh-1, -2	1.1.1.42	TC	2
Lactate dehydrogenase	Ldh	1.1.1.27	TC	1
Malate dehydrogenase	Mdh-1, -2	1.1.1.37	TC, HC	2
"Malic enzyme"	Me	1.1.1.40	TBE	1
Mannosephosphate isomerase	Mpi	5.3.1.8	TBE, HC	1
Peptidase	Pep	3.4.11/13	TC	1
Phosphoglucose isomerase	Pgi	5.3.1.9	HC, TBE	1
Phosphoglucomutase	Pgm	2.7.5.1	HC, TC, TBE	1
α-Glycerol-3-phosphat dehydrogenase	α-Gpd	1.1.1.8	TC	1

One locus encoding PGM showed a polymorphism at most localities. The population of the western Baltic Sea displayed the greatest variability while the populations of the Mediterranean Sea showed limited genetic variability (Table 2). The results indicated that the populations from the North Sea and Baltic Sea exhibited observed heterozygosity values of about 40%. On the other hand, the Atlantic populations had somewhat smaller observed heterozygoties, amounting to about 30% or less. *C. aestuarii* from the Mediterranean had heterozygosity values of 5–20%. Thus, the geographic differentiation of *C. maenas* consisted of a slight decrease in allele frequency at the *Pgm*-100 allele as latitude decreased. However, the dependence between two variables described by means of a regression analysis (allele frequency versus latitude) did not produce significant results. Nearly all the sam-

Table 2. PGM-allele frequencies of *Carcinus* in different European coastal regions. *Ho*-observed, *He*-expected HARDY-WEINBERG distribution. *N*-number of individuals *-significant departure from the HARDY-WEINBERG equilibrium ($P > 0.05$) and *F* values (with the exception of Venice).

Region	Alleles							*Ho*	*He*	*N*	F_{st}
	96	98	100	105	107	108	110				
Heikendorf			0.71		0.22		0.07	0.47	0.40	62	0.20
Bergen			0.73		0.13		0.14	0.45	0.30*	38	0.18
Heligoland	0.02		0.76		0.16		0.06	0.36	0.32	55	0.31
Roscoff			0.82		0.07		0.11	0.30	0.32	40	0.11
Arcachon			0.80		0.15		0.05	0.32	0.31	69	0.33
Cadiz			0.81		0.18		0.01	0.30	0.36	23	0.24
Naples		0.92	0.02	0.03		0.03		0.12	0.12	50	0.12
Venice		0.97		0.03				0.05	0.06	18	
Rovinjo		0.91		0.06		0.03		0.20	0.18	39	0.12

ples resulted in deviations, which were not significant from the HARDY-WEINBERG distribution. The values of heterozygosity for all 19 loci were 0.026 – 0.016 for *C. maenas* and 0.011 – 0.003 for *C. aestuarii*.

A full identity was discovered between *C. maenas* and *C. aestuarii* regarding the banding pattern of all but two loci. Only the polymorphic *Pgm* locus and the monomorphic *Gpt* locus revealed slight but specific differences. *Pgm* 100/100 allele (separation: TC- and HC-buffer) of the animals from the Atlantic, North and Baltic Sea wandered 2 mm further than that of the Mediterranean samples. In addition, the *Gpt* (separation: TBE-buffer) showed a different electrophoretic separation: in comparison with the Atlantic, North and Baltic Sea crustaceans, the Mediterranean crabs revealed an increase of 2 mm in the migration of gels. Thus, the extent of genetic variation by differences in mobilities are of particular value for characterisation of an organism at the species or subspecies level.

Gene flow estimates were calculated over the nearly entire sampling range. The values of statistics (Wright, 1978) for the *Pgm* locus are presented and show normal and moderately high levels of gene flow ($Nm = 0.27–2{,}02$). However, the data obtained to estimate gene flow should be interpreted with caution owing to the only banding pattern of the polymorphic *Pgm* locus in *Carcinus*.

Altogether, according to the present study, in European localities 6 (or perhaps 7) alleles at the *Pgm* locus were detected; SHERBOURNE (1976) was able to resolve only 3 alleles at the *Pgm* locus from his Boothbay Harbor (Atlantic East coast of USA) sample.

3.2. Divergence of the Populations

Up until the mid 20th century the term of *C. maenas* was applied to the two Portunidae populations. DEMEUSY and VEILLET (1953) were the first to point out differences between Atlantic and Mediterranean populations especially in the morphology of the cephalothorax, the extremities and the abdomen. They crossed Atlantic (origin: Manche) population with the Mediterranean (origin: Sète) population and the cross produced fertilized eggs. Whether the offspring would mature could not be checked because of the difficulties in rearing the larval stages. Finally, HOLTHUIS and GOTTLIEB (1958) introduced the name *C. mediterraneus* CZERNIASKY, 1884, for the Mediterranean form contrasting it to *C. maenas* as the Atlantic shore crab. ALMACA (1972) confirmed the biometric differences between the two species and he stressed the different dimensions of the carapace. Subsequently, MANNING and HOLTHUIS (1981) and LEWINSOHN and HOLTHUIS (1986) changed the name *mediterraneus* to *C. aestuarii* NARDO, 1847. However, COTTIGLIA (1983) considered the species name *aestuarii* as nomen nudum and recommended *mediterraneus* as ancient designation. We, on the other hand, suggest that the name *aestuarii* be retained for reasons given by us in the following section.

Although the species are very similar in appearance, they can be distinguished by a number of relatively minor characters (e.g. ZARIQUIEZ ALVAREZ, 1968; HOLTHUIS, 1987). The carapace of *C. aestuarii* is hexagonal; its upper side is smooth, slightly hairy, divided into discrete regions. The front border has three lobes and the middle one is slightly prominent. The antero-lateral margins are sliced into five teeth including an orbital angle, the last being the sharpest tooth. The chelipeds are strong, slightly asymmetrical, smooth, with only one strong, sharp tooth on the anterior region of the carpus. The walking legs are long and rather slender. The propodus is flattened more strongly in the last pair than in the preceding legs. The dactyle is flattened and lanceolate. The colour is variable; the upper side is generally deep green in adult specimens, while the underside is tinged with yellow or red.

The surface of the carapace of *C. maenas* is granular. The carapace is only slightly wider than long, with 5 less sharp antero-lateral teeth about the same in size; the granulate rostral arae is only slightly protruding and has a strongly reduced hair cover. The adults of the two

species have a strikingly different feature: in *C. maenas* the sexual pleopods (gonopods) are curved and divergent, while in *C. aestuarii* they are almost straight and parallel (see HOLTHUIS, 1987, p. 353, for an illustration of the ventrally visible abdomen).

In a large geographic area, populations can be so far apart that the gene flow between them is limited, thus causing the emergence of different characteristics. Measures of genetic similarity or identity (*I*) and genetic distance (*D*) may be used to quantify the degree of genetic divergence between taxa (NEI, 1972). These criteria allow an estimation of the number of gene substitutions per locus. For the taxonomic classification values of genetic identity (*I*) are generally 1 – 0.95 for populations, 0.98 – 0.84 for subspecies, and 0.90 – 0.13 for species (NEI, 1976). THORPE (1983) and THORPE and SOLE-CAVA (1994) reached a similar conclusion for the taxonomic classification of invertebrates. For all that, an estimate of genetic identity will provide more objective and potentially useful data for the identification of taxa.

To a large extent, on the basis of genetic identity, the populations of both species are structured fairly similarly. The genetic identity of *C. maenas* and *C. aestuarii* is about 0.89 (19 loci). From the findings cited above, this suggests that a taxonomic classification of subspecies is appropriate. The question arises whether the population are sufficiently large in order to designate these forms as species or subspecies. We reject the designation of species to these forms on the basis of genetic identity despite certain morphological differences. Therefore, we recommend the Atlantic form to be classified as *C. maenas maenas*, whereas the Mediterranean form should be classified as *C. maenas aestuarii*.

RICE and INGLE (1976, p. 103) also discussed the systematics of both closely related species within the scope of their morphological investigation on larval development. They confirmed that the two allopatric species do not differ essentially with regard to the diverse larval stages and came to the conclusion "that they should be accorded only by subspecific status, a course which would reflect both their close relationship and geographical separation."

In a vast geographic area a population can be so separated that the gene flow is reduced and thereby differences in characters appear. The contact range of the distribution of *Carcinus* probably lies in the Strait of Gibraltar, or in an adjoining sea region. Nothing is known about the potential transitions stages which might appear in this district. However, a geographic barrier in the Mediterranean exists, and this is the prerequisite for a divergent development.

3.3. Electrophoretic Consequences

Gel electrophoresis applies to separate proteins according to their relative mobilities in an electric field. Techniques of allozyme electrophoresis at a molecular level have been used to estimate genetic variability in a large number of organisms. Thus, the biochemical analysis of protein variation represents a modern tool to demonstrate the identification of individuals, to analyse population structure, to delineate species or subspecies and to reconstruct the phylogeny.

Electrophoretic variability has been studied in many populations of Decapoda Reptantia, comprising inhabitants of marine, brackish and freshwater environments (e.g. TRACEY *et al.*, 1975; HEDGECOCK *et al.*, 1975; GOOCH, 1977; TURNER and LYERLA, 1980; SHAKLEE and SALMOLLOW, 1984; FEVOLDEN and HESSEN, 1989; AYERBERG, 1990; LI *et al.*, 1993; STEVENS, 1994; MCMILLAN-JACKSTON *et al.*, 1994). Hypotheses such as random genetic drift and trophic stability have been suggested to account for the low genetic variability in the decapod crustaceans; the heterozygosity values of a diversity of species ranged from 0.000–0.128 (NELSON and HEDEGCOCK, 1980; HEDGECOCK *et al.*, 1982).

A comparison of the genetic variability among invertebrate groups demonstrated that the crustaceans have some of the lowest values of heterozygosity. The authors calculated a mean

heterozygosity of only 0.082 for 122 crustacean species investigated (NEVO *et al.*, 1984); altogether, these data correspond with the results on the low genetic variability of the genus *Carcinus*.

In the Atlantic Ocean and in the Mediterranean there are several decapods that occur in both seas. Compared to studies on the population structure of the Atlantic and Mediterranean *Carcinus* subspecies, investigations on other species from the two seas are still fragmentary. Biochemical genetic studies on *Crangon vulgaris*, a swimming decapod, have revealed that shrimp from Europaen habitats have the same alleles but different frequencies than shrimp from the Mediterranean (BULNHEIM and SCHWENZER, 1993).

Although the adult specimens of the two subspecies of *Carcinus* exhibit a characteristic mobility only on the sea bottom, the planktonic larvae display far more activity than the adult shore crabs. The development of the larval stages of *Carcinus* which lasts approximately 6 weeks at 15 °C (GRUNER, 1993) comprises four consecutive zoeas and one megalopa (Rice and INGLE, 1976; INGLE, 1992). Therefore, genetic differentiation is taking place despite the possibility of gene flow through the dispersal of the zoeal and megalopal stages.

The allele frequencies of *Pgm* of the genus *Carcinus* reveals a slight north-south gradient. The geographical pattern can not be attributed to chance or to artefact of sampling but a change of the frequency of the dominant allele. Pronounced clinal variation, i.e. displacement of allele frequencies, occurs in some other crustaceans: the hermit crab *Emerita talpoida* (CORBIN, 1977), the brackish-water amphipod *Gammarus zaddachi* (BULNHEIM and SCHOLL, 1981), the sandhopper *Talitrus saltator* (BULNHEIM and SCHOLL, 1986), the pea crab *Pinnotheres atrinicola* (STEVENS, 1991) and the blue crab *Callinectes sapidus* (MCMILLAN-JACKSTON *et al.*, 1994). These spatial differences in gene frequencies in allozyme distribution have been explained as gene flow and/or selective response to gradients of one or more environmental factors. At the *Pgm* locus, the mean temperature of sea-water which increases from north to south seems to be the determining factor. However, rather inadequate data are available on the selective forces acting along the cline and on the rate of gene flow among populations. In several decapods, for instance in *Callinectes sapidus*, homogeneity of allele frequency generally suggests high gene flow among populations (MCMILLON-JACKSON *et al.*, 1994), whereas significant heterogeneity indicates low gene flow.

The genetic basis of species formation is determined by evolutionary changes that occur in local populations. When a vast area is colonized, the diversity of populations is determined over a long period of time by genetic drift and selection. The allopatric speciation is sustained by continuation of racial differentiation and can be performed, in principle, by additional isolation. The geographical barrier splits a species with a wide range into two large or more subpopulations between which gene flow is restricted.

Studies of protein polymorphism have presented a variety of information about the structuring and differentiation of commercially exploited marine species. The inhabitants of the coasts, especially of southern regions, appreciate the shore crab as a cheap food. However, the intensive fishery of crabs is not worthwhile in comparison with the size of these portunid decapods (length of male carapace about 60 mm); only a small quantity of organic substance is edible. Altogether, the electrophoretic analysis of genetic diversity and the population structure at a molecular level is essential for decapod crustacean species of ecologic and economic importance.

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