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Genetic Variability in Natural Populations of Eurytopic Ostracod Candona neglecta Sars

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ABSTRACT—An electrophoretic survey of allozyme variation was conducted in four, highly polymorphic loci on nine populations of ostracod *Candona neglecta* Sars from three different environments: the profundal of post-glacial lakes, deep muddy bottom of the Baltic Sea and small astatic water bodies. The results suggest lack of genetic isolation between populations from lake profundal and the Baltic Sea. On the other hand a very distinct founder effect can be noted in the case of young, isolated populations from small astatic basins. It is suggested that a population inhabiting a large lake may be genetically subdivided due to differentiated eutrophication.

INTRODUCTION

Candona neglecta G.O. Sars, 1887, is a common eurytopic and amphigonic ostracod. It was described by Sars on the basis of individuals found in the Italian lake Garda and individuals raised in his aquaria out of dried mud from Algeria. It was later reported from several locations in Europe. Unfortunately, some data concern allied species, which only became obvious when the structural details of the penis are taken as a diagnostic feature (Petkovski, 1959; Sywula, 1974). *C. neglecta* is a peculiar species as it inhabits three extremely different types of environment: primarily, the profundal and, to a lesser extent, the littoral of post-glacial lakes; secondly, small permanent and temporary water bodies, limnocrene and helocrene swamps in particular; and finally the deep muddy and muddy-sandy bottom of the Baltic Sea (Sywula, 1974). Adult specimens, as well as eggs and larvae can not swim.

Each of the three types of environment imposes extremely different living conditions, which demand specific adaptations. One may thus put forward the hypothesis that populations inhabiting particular types of environment boast some genetic specificity or even make up groups of sibling species. The aim of this paper is to verify the above hypothesis.

MATERIALS AND METHODS

Samples were obtained from nine sites (Fig. 1, Table1), the site code also being the population code. The animals were collected using a fine mesh (100 Lμ) hand net (shallow waters) or a dredge (deep waters). Samples were taken to the laboratory, specimens being picked out immediately and frozen at −80°C.

Randomly selected adult specimens were sexed, homogenized and subjected to electrophoresis. Cellulose acetate electrophoresis was applied to study glucose phosphate isomerases (GPI, EC 5.3.1.19), leucine aminopeptidases (LAP, EC 3.4.11.1) and esterases (EST, EC 3.1.1). These procedures and staining methods were after Hebert and Beaton (1989). Each specimen was examined in respect of all three enzyme systems, alleles being designated according to the decreasing electrophoretic mobilities of the corresponding proteins. Esterases were evidently coded by several loci but only two of them could be genetically interpreted without special breeding data. None of the examined loci proved to be sex-linked.

Observed (direct count) and expected (based on Hardy-Weinberg equilibrium) heterozygosities were calculated and averaged across all loci for each population.

Representative samples of *C. neglecta* were tested for conformity with Hardy-Weinberg proportions using the χ^2 test for quality of fit. The fixation index F_{ST} was used to estimate the amount of inbreeding due to population subdivision: $F_{ST} = (H_T - H_S)/H_T$ (where H_S, the expected heterozygosity of an individual in an equivalent random mating subpopulation; H_T, the expected heterozygosity of an individual in an equivalent random mating total population).

F_{ST}-statistics were calculated according to Hartl and Clark (1989). The statistical significance of differences in the allele frequency between populations was tested by means of a likelihood-ratio test G^2 (Adam, 1987). Genetic identity (I) and genetic distance (D) between local populations were estimated after Nei (1972). The dendrogram was constructed by Unweighted Pair Group Method using Arithmetic Averages (UPGMA) (Sneath and Sokal, 1973).

Results

The obtained electrophoretic patterns were in accordance with the model predicted for dimeric proteins coded by one locus with four alleles in the case of Gpi, monomeric proteins coded by two loci with three alleles for two loci of Est and monomeric protein coded by one locus with two

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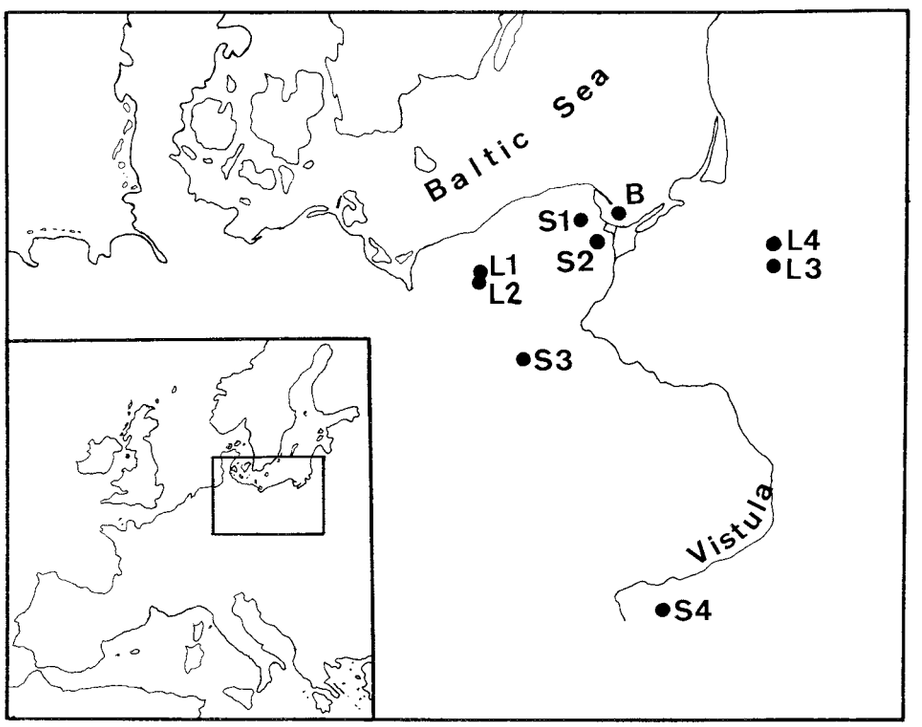

Fig. 1. Sampling localities of the analyzed populations of *Candona neglecta* (population codes as in Tab.1)

Table 1. Samples studied.

Type of environment	Location	Site code	Description	Sampling date
Baltic Sea	Gulf of Gdańsk off Sopot	B	depth 40 m, water temp.4°C–10°C, 8.1‰ S, muddy-sandy bottom	Dec. 1993
Post-glacial lakes	Lake Drawsko, Pomeranian Lake District	L1, L2	a post-glacial lake, 18.7 km^2, max.depth 79.7 m; L1 sample from profundal of the northern mesotrophic lake basin, depth 10 m, muddy bottom L2 sample from profundal of southern eutrophic lake basin, depth 33m, muddy bottom	May 1994
	Lake Rospuda Masurian Lake District	L3	a post-glacial lake, 3.4 km^2, max. depth 38.9 m; sample from profundal, depth 11–16 m, muddy bottom	Nov.1994
	Lake Szelment Wielki Suwałki Lake District	L4	a post-glacial lake, 3.6 km^2, max.depth 45.6 m; sample from profundal, depth 10–15 m, muddy bottom	Nov.1994
Small water bodies	Gdańsk-Oliwa	S1	a permanent puddle associated with a stream in the city park, frequently visited by wild ducks; depth 20–30 cm, muddy bottom with detritus	Nov.1994
	Lublewo (a village 15 km south of Gdańsk)	S2	a tiny water body in a peatbog meadow, seasonally drying out, clay- muddy bottom	May 1994
	Wiry (a village 15 km west of Poznań)	S3	seasonally drying up meadow helocrene, muddy bottom	May 1994
	Gorce mountains, Mount Turbacz slope	S4	permanent helocrene swamps of Olszowy stream at the edge of a forest at 1280 m a.s.l., muddy bottom	April 1994

Table 2. Allelic frequencies observed at the Gpi, Est, Lap loci in nine local populations (N,sample size; H,observed heterozygosity; $\bar{H}$, average heterozygosity over loci; χ^2,values calculated for quality of fit to Hardy-Weinberg expectations of genotype frequencies).

	Sampling localities								
alleles	B	L1	L2	L3	L4	S1	S2	S3	S4
Gpi[a]	0.0132	0.0217	0.0126	0.0095	–	0.0110	–	–	1.0000
Gpi[b]	0.3046	0.3206	0.5216	0.3619	0.0139	0.3514	1.0000	0.9259	–
Gpi[c]	0.6690	0.6577	0.4640	0.6286	0.9861	0.6376	–	0.0741	–
Gpi[d]	0.0132	–	0.0018	–	–	–	–	–	–
N	151	92	278	105	36	138	87	54	45
H	0.4636	0.5326	0.4712	0.5143	0.0278	0.4710	0.0000	0.1481	0.0000
χ^2	0.2718	1.9362	1.9726	0.7369	–	0.0008	–	0.0545	–
Est-1[a]	0.0649	0.0612	0.1379	0.1034	0.2222	0.2143	–	–	–
Est-1[b]	0.8442	0.6735	0.4828	0.6035	0.5000	0.4444	1.0000	1.0000	–
Est-1[c]	0.0909	0.2653	0.3793	0.2931	0.2778	0.3413	–	–	1.0000
N	77	49	29	29	9	63	55	26	27
H	0.2078	0.4898	0.6207	0.5517	0.3333	0.6190	0.0000	0.0000	0.0000
χ^2	1.9045	0.1401	0.4131	0.1189	–	0.8953	–	–	–
Est-2[a]	0.1544	0.1852	0.3939	0.1538	0.0357	0.2623	–	1.0000	1.0000
Est-2[b]	0.4780	0.5093	0.3258	0.5898	0.3214	0.4426	1.0000	–	–
Est-2[c]	0.3676	0.3055	0.2803	0.2564	0.6429	0.2951	–	–	–
N	68	54	66	39	14	61	47	23	9
H	0.6324	0.5370	0.7121	0.5641	0.4286	0.5902	0.0000	0.0000	0.0000
χ^2	0.1823	1.9895	1.6495	0.6101	–	1.8332	–	–	–
Lap[a]	0.5402	0.4896	0.4326	0.7456	0.5278	0.8116	0.7365	1.0000	0.8750
Lap[b]	0.4598	0.5104	0.5674	0.2544	0.4722	0.1884	0.2635	–	0.1250
N	112	96	141	57	18	69	74	18	16
H	0.4732	0.5417	0.4965	0.3333	0.6111	0.2609	0.3378	0.0000	0.1250
χ^2	0.2518	0.6744	0.0179	0.5129	–	0.6565	0.7883	–	–
$\bar{H}$	0.4443	0.5253	0.5751	0.4909	0.3502	0.4853	0.0845	0.0370	0.0313

alleles in the case of Lap. Of 12 alleles of four loci only one (Gpi[d]) was rare and found in only two populations. The others were common and found in at least six populations.

The allele frequencies and data on heterozygosity and χ^2-values connected with Hardy-Weinberg expectations are presented in Table 2. Not all of four electrophoretic phenotypes could be interpreted genetically in some individuals and therefore in Table 2 different numbers of individuals analysed are given with reference to particular loci. Two samples S3 and S4 were taken from small populations and although not very numerous they were statistically representative. On the other hand, sample L4 should not be treated as representative due to its small size as it could only be analysed partially. There are no grounds for rejecting the hypothesis assuming that all local populations were in Hardy-Weinberg equilibrium: for Gpi $p = 0.60 - 0.99$; for Est-1 $p = 0.50 - 0.95$; for Est-2 $p = 0.25 - 0.75$; for Lap $p = 0.25 - 0.99$. Six of nine examined local populations (five from large water-bodies and one from a small one) were polymorphic in reference to all four loci and highly heterozygotic; the remaining ones (from small water-bodies) being polymorphic in only one locus.

The values of the F_{ST} indicator are presented separately for all populations and for populations from large water-bodies in Table 3. These values were in general quite low for populations from large water-bodies (B, L1-L4) and high for those from small ones. Genetic identity and genetic distance of local populations are presented in Table 4; Fig.2 shows the populations clustering from the point of view of genetic identity. Our attention was drawn to the unexpected result that populations from large permanent water-bodies formed a single cluster, which also contained population S1 from a small water-body while other populations from small water-bodies were apart. Another unexpected result was that two samples from the same lake, one from mesotrophic basin L1 and the other from eutrophic basin L2, were relatively distant

Table 3. F_{ST}-values.

Alleles	Sampling localities	F_{ST}
Gpi[b]	B, L1-L3	0.0315
	B, L1-L3, S1-S4	0.3949
Gpi[c]	B, L1-L3,	0.0285
	B, L1-L3, S1-S4	0.3554
Est-1[b]	B, L1-L3	0.0635
	B, L1-L3, S1-S4	0.4132
Est-1[c]	B, L1-L3	0.0330
	B, L1-L3, S1-S4	0.4333
Est-2[a]	B, L1-L3	0.0579
	B, L1-L3, S1-S4	0.5580
Est-2[b]	B, L1-L3	0.0367
	B, L1-L3, S1-S4	0.3796
Lap[a]	B, L1-L3	0.0564
	B, L1-L3, S1-S4	0.1663

Table 4. Estimates of genetic identity (I) (above diagonal) and genetic distance (D) (below diagonal) between eight local populations based on data for four loci Gpi, Est-1, Est-2, Lap.

I / D	B	L1	L2	L3	S1	S2	S3	S4
B		0.9878	0.9086	0.9590	0.9210	0.7772	0.6216	0.3046
L1	0.0122		0.9444	0.9676	0.9369	0.7697	0.6063	0.3700
L2	0.0959	0.0572		0.9012	0.9111	0.7349	0.7186	0.4988
L3	0.0419	0.0330	0.1041		0.9787	0.8199	0.6586	0.4150
S1	0.0823	0.0652	0.0931	0.0215		0.7407	0.6770	0.5056
S2	0.2520	0.2618	0.3081	0.1985	0.3001		0.7346	0.2449
S3	0.4755	0.5003	0.3304	0.4176	0.3901	0.3084		0.4975
S4	1.1889	0.9943	0.6956	0.8795	0.6819	1.4068	0.6982	

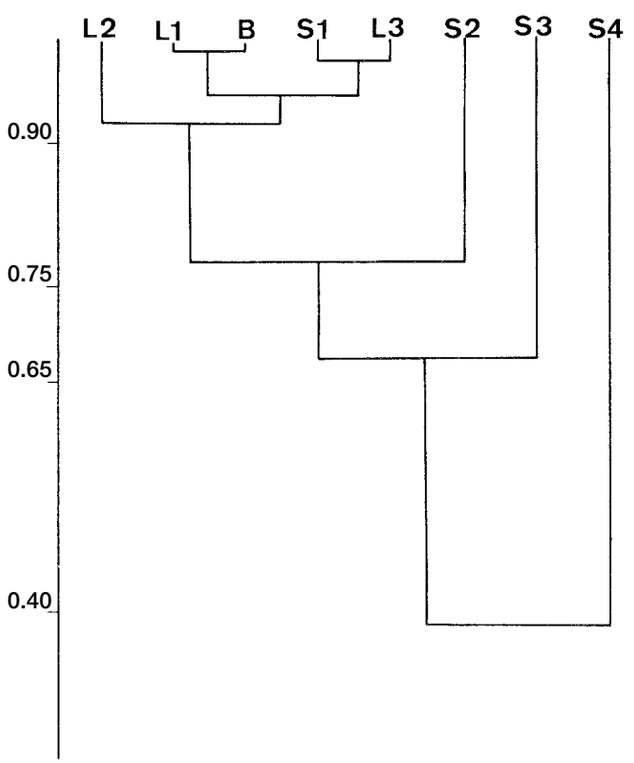

Fig. 2. Dendrogram of eight populations based on genetic identity (I) for Gpi, Est-1, Est-2, Lap loci (population codes as in Tab. 1)

from each other. The statistical significance of differences in the allele frequency between these two samples was checked by a G^2-test; 20.85 for locus Gpi, 2.23 for locus Est-1, 14.02 for locus Est-2 and 1.49 for Lap. The first and the third values were statistically important.

DISCUSSION

Four of the results obtained are noteworthy. At first there is the resemblance in fairly old populations from large water-bodies despite very different environmental conditions, on a deep muddy Baltic Sea bottom and in the profundal of freshwater post-glacial lakes (stations B, L1–L4) (see Tables 2–4, Fig.2). The genetic similarities within this set of populations (0.901–0.988) are not very high as compared with conspecific populations according to Ayala (1975). The low values of F_{ST} in this population set (Table 4) confirm the lack of genetic isolation between populations. In this context it is unlikely that the Central-European populations inhabiting the particular environment types are characterized by some genetic specificity or even make up a group of sibling species must be rejected. The examined ostracod can migrate only passively between lakes and Baltic Sea. The migration, probably mediated by birds and fish, might be an effective means of gene flow, which caused a fairly high level of genetic homogenization of quite old Central-European populations connected with large water bodies. A similar situation was found with a profundal ostracod *Cytherissa lacustris* Sars which is distantly related to *Candona neglecta* used in this study (Sywula, 1974; Sywula and Geiger,1990).

The second matter worth mentioning is the very distinct founder effect in the case of young, isolated populations from small astatic basins (stations S2 – S4). It can easily be traced by heterozygosity, genetic identity and F_{ST}.

Thirdly a different situation was noted at station S1. Although the basin is also young, small and astatic, the population had similar heterozygosity and allele frequencies to those of the old lake populations. (Tables 2,3, 4; Fig.2). The difference between this population and the remaining young populations seems to be caused by frequent visit of wild-duck to station S1; according to Michno B (unpublished data) the mean number of wild-ducks from October to April was as high as 180-323 in a single counting at station S1 (countings were conducted weekly in 1983-1993). This could possibly indicate the role of the birds in the passive migration of ostracods, which was discussed previously in respect of the species *Cytherissa lacustris* Sars (Sywula *et al.*,1994) and *Cyprideis torosa* (Jones) (Sywula *et al.*,1995).

The fourth item worth discussing is the significant difference in the allele frequencies within Lake Drawsko (stations L1, L2). This finding indicate that two basins of the lake are inhabited by two separate subpopulations of *C. neglecta*. As both basins are eutrophic to different extents, one may assume that it is a sign of a selection process connected with progressive eutrophication. A similar situation was noted in respect of ostracod *Cytherissa lacustris* Sars in an Alpine lake (Attersee) (Sywula and Geiger, 1990).

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