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SHORT NOTE

Evidence of Genetic Divergence Between Two Brackish-Water Gammaridean Sibling Species

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ABSTRACT: The genetic distinctiveness of the morphologically very similar amphipods *Gammarus zaddachi* Sexton and *G. salinus* Spooner was assessed by means of enzyme electrophoresis. Samples from sympatric populations collected in the Baltic Sea area were used for interspecific comparisons between allele frequencies at three polymorphic loci (phosphoglucose isomerase, glutamate oxalacetate transaminase, arginine phosphokinase). The results obtained demonstrate that populations of both gammarids can be distinguished at the enzyme level, indicating their reproductive isolation.

In recent years several biochemical methods have been introduced as suitable tools for the study of taxonomic and phylogenetic relationships between species, thereby complementing traditional morphological diagnosis. Among the techniques used, the separation of enzymes by means of gel electrophoresis has been shown to be of great relevance for distinguishing species and assessing their relatedness (cf. Avise, 1974). The measures applied for evaluating interspecific similarities or dissimilarities are based on relative electrophoretic mobilities of homologous enzymes and on levels of allozyme variation.

In this paper we report on results of an electrophoretic investigation designed to estimate the genetic relationships between two euryhaline amphipods, *Gammarus zaddachi* Sexton and *G. salinus* Spooner. The taxonomic status of these closely related species has long been disputed. *G. zaddachi*, first described by Sexton (1912), was later demonstrated to comprise a complex of three forms. On the basis of structural and ecological differences detected, Segerstråle (1947) and Spooner (1947) distinguished three subspecies – *Gammarus z. zaddachi, G. z. salinus*, and *G. z. oceanicus*. As a consequence of unsuccessful interbreeding experiments (Spooner, 1947) and as a result of further comparative analysis, Kinne (1954) upgraded them to species level.

In contrast to *Gammarus oceanicus, G. zaddachi* and *G. salinus* are very similar in morphological characters and geographical range, both exhibiting an east-atlantic, boreal distribution. Some differences exist with regard to habitat selection (e. g. Kinne, 1954; den Hartog, 1964; Fenchel and Kolding, 1979). Inhabiting coastal and estuarine areas, *G. zaddachi* occurs predominantly in oligohaline waters, whereas *G. salinus* prefers mesohaline waters. Zones of species overlap exist, particularly in the Baltic Sea at low salinities.

In a study on the *Gammarus* fauna of the Isefjord (Denmark), Rasmussen (1973) expressed doubts on the validity of the established species system. He noticed considerable variability of external morphological characters hitherto used for distinguishing between the above-named species and the related *Gammarus locusta* and *G. duebeni*. Since Rasmussen did not substantiate this view in more detail, a study on enzyme electrophoresis was initiated in order to explore the genetic distinctiveness of the gammarids in question. This report, concentrating on the biochemical species discrimination between *G. zaddachi* and *G. salinus*, is part of an investigation on the genetic variation between geographic populations of both gammarids (Bulnheim and Scholl, in preparation).

Based on the techniques of vertical starch-gel electrophoresis, allozyme frequency data were sampled from selected gene loci. The following enzymes, encoded by three loci, were assayed from supernatants of tissue homogenates: phosphoglucose isomerase (PGI), glutamate oxalacetate transaminase (GOT), and arginine phosphokinase (APK). The electrophoretic separation was accomplished by use of Tris-citrate buffer (Ayala et al., 1972, for PGI and GOT) and Tris-borate EDTA buffer (Scholl et al., 1978, for APK). Gels (Connaught starch-hydrolyzed, 14 %) were stained for PGI, GOT (following Scholl et al., 1978, herein further details on procedures) and APK (staining method as described by Scholl et al. for creatine phosphokinase except for the substitution of creatine phosphate by 10 mg arginine phosphate).

Both amphipods share identical electrophoretic mobilities for the three enzymes tested as evidenced

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Table 1. *Gammarus zaddachi* and *G. salinus*. Distribution of allele frequencies at three polymorphic loci (glutamate oxalacetate transaminase = GOT, phosphoglucose isomerase = PGI, arginine phosphokinase = APK) in samples from four sympatric populations. Alleles assigned according to relative anodal mobility. Phenotype frequencies compared between both species by use of χ^2 test (level of significance $P = 0.05$). n. s. = not significant, H = observed frequency of heterozygotes, N = number of individuals examined

Collecting site	Date	Species	PGI							GOT				APK				
			91	94	100	103	N	H	P	96	100	N	H	P	95	100	N	H	P
Landwehr	IV. 1979	*G. zaddachi*		0.14	0.86		18	0.17			1.00	18	0.00		0.44	0.56	18	0.44	
		G. salinus		0.22	0.66	0.12	47	0.43	n.s.	0.13	0.87	47	0.09	n.s.		1.00	40	0.00	<0.001
Pelzerhaken	IV. 1978	*G. zaddachi*		0.02	0.92	0.06	82	0.12			1.00	97	0.00		0.02	0.98	97	0.03	
		G. salinus		0.17	0.77	0.06	90	0.34	<0.001	0.09	0.91	90	0.16	<0.001		1.00	90	0.00	n.s.
Stralsund	IX. 1979	*G. zaddachi*		0.07	0.90	0.03	36	0.14			1.00	36	0.00		0.03	0.97	36	0.06	
		G.salinus	0.02	0.10	0.85	0.04	26	0.23	n.s.		1.00	26	0.00	n.s.		1.00	25	0.00	n.s.
Tvärminne	IX. 1979	*G. zaddachi*			0.97	0.03	56	0.05		0.02	0.98	56	0.04		0.02	0.98	42	0.05	
		G. salinus		0.14	0.67	0.19	56	0.27	<0.01	0.03	0.97	56	0.05	n.s.		1.00	55	0.00	n.s.

by comparisons of the zymogram patterns obtained. Interspecific differences, however, exist with regard to enzyme polymorphism in allopatric as well as in sympatric populations. These genetic differences are documented in Table 1 for four sympatric populations from various localities of the Baltic Sea area: Landwehr (Kiel Canal, FRG), Pelzerhaken (Lübeck Bay, FRG), Stralsund (GDR), Tvärminne (Finland). The two species co-occurred at the four localities examined except for the latter station, where a distance of less than 500 m was recorded between the collecting sites of both amphipods.

Table 1 lists the allele frequencies, including the observed proportion of heterozygotes. The phenotype distributions observed fit Hardy-Weinberg expectations in all cases. PGI is polymorphic in both forms; however, the level of heterozygosity is high in *Gammarus salinus* but low in *G. zaddachi*. GOT is commonly monomorphic in *G. zaddachi* but moderately polymorphic in *G. salinus*. APK is consistently monomorphic in the latter species but polymorphic in *G. zaddachi*. In the Landwehr population the APK-allele 95 has a frequency of 0.44 in *G. zaddachi*, whereas none of the *G. salinus* individuals examined carries this allele.

Statistical comparisons between phenotype distributions in sympatric *Gammarus zaddachi* and *G. salinus* populations yield significant differences at either one or two of the enzyme loci studied, except for the sample obtained from Stralsund. However, in this population considerable interspecific differences could be detected by scoring another enzyme, mannose-6-phosphate isomerase (M6PI), which is polymorphic in both species.

The results obtained demonstrate that populations of *Gammarus zaddachi* and *G. salinus* can be consistently distinguished at the enzyme level. As shown by the data on allozyme variation in sympatric populations they are reproductively isolated. Hence, they represent genetically distinct species. This confirms previous observations on interfertility (Spooner, 1947), although interspecific precopulations may occur (Kinne, 1954; Schulze and Arndt, 1971). In addition, the information available indicates that the pronounced morphological similarity between *G. zaddachi* and *G. salinus* corresponds to a functional similarity in terms of growth rates, moulting frequencies, egg production and embryonic development (Kinne, 1961) as well as to various physiological responses and resistance capacities (Bulnheim, 1979). Kinne (1961) assumed that *G. zaddachi* and *G. salinus* represent two relatively young species which recently have become genetically independent from each other. The above-reported data and further unpublished evidence, also obtained from electrophoretic analysis, confirm this

suggestion. They indicate little genetic divergence between the two congeners under consideration, thus emphasizing their sibling character.

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