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# GENETIC DIFFERENTIATION AMONG GEOGRAPHIC POPULATIONS OF TWO SPECIES OF THE GENUS *GAMMARUS* : *G. INSENSIBILIS* AND *G. AEQUICAUDA* (CRUSTACEA, AMPHIPODA)

PATARNELLO T., B. BATTAGLIA and P.M. BISOL

Dipartimento di Biologia, via Trieste 75, 35121, Padova, Italy

*GAMMARUS*  
POLYMORPHISME GÉNÉTIQUE  
DISTANCE GÉNÉTIQUE

**RÉSUMÉ** – Des analyses électrophorétiques permettent de comparer la structure génétique de quelques populations géographiques (Adriatique septentrional et Méditerranée près de la côte française) de deux espèces de *Gammarus* : *G. insensibilis* Stock et *G. aequicauda* Martynov. Les résultats montrent que les populations de *G. insensibilis* sont très semblables entre elles, tandis que les populations de *G. aequicauda* semblent différentes. La distance génétique entre les deux espèces et entre les populations de chaque espèce a été également calculée. Une brève discussion porte sur les mécanismes qui peuvent être responsables des différences observées.

*GAMMARUS*  
GENETIC POLYMORPHISM  
GENETIC DISTANCE

**ABSTRACT** – The degree of genetic variation for the two species *Gammarus insensibilis* and *G. aequicauda* was analyzed by protein electrophoresis. The genetic variability was estimated in two populations for each species. In both cases an Italian and a French population were studied. The comparison of gene frequencies and the levels of heterozygosity indicates a greater degree of differentiation within *G. aequicauda* compared to *G. insensibilis*. The loci which are mainly responsible for the differences between the Italian and the French populations of *G. aequicauda* are *Mpi* and *Pgi*. The evaluation of the Hardy Weinberg equilibrium shows a deficiency of heterozygotes at the locus *Pgm* in both populations of *G. aequicauda*. The loci *Fh* and *Pgm* clearly discriminate between *G. aequicauda* and *G. insensibilis*. Genetic distances were calculated within and between species. The results are discussed in relation to the ecology of the two species.

## INTRODUCTION

The study of genetic strategies of adaptation to the environment is a central topic in evolutionary genetics. A common approach is to identify factors which may affect genetic variation. It consists of examining correlations between the genetic structure of populations and environmental parameters.

This method of research has successfully produced a few studies which described biochemical genetic mechanisms of adaptation (DiMichele and Powers, 1982; Watt, 1983; Koehn and Hilbish, 1987). More typically, it has provided useful informations concerning the genetic structure of several species, permitting estimation of phylogenetic relationships as well as discrimination of geographic population structure within species (Oxford and Rollinson, 1983).

The analysis of population structure by electrophoretic techniques has been successfully employed in the study of several marine species (for a review see Battaglia and Beardmore, 1978). On some occasions, it has proven useful in revealing differentiation among populations not detectable from the morphological analyses (Battaglia, 1982).

The influence of the environment on evolutionary processes may be more easily detected in stressful environments such as marginal marine habitats (Koehn and Bayne, 1989). Brackish lagoons, typical marginal marine environments, experience remarkable daily and seasonal variation in temperature, salinity and oxygen.

In this paper we report on a study of genetic variability in Italian and French populations of two species belonging to the genus *Gammarus* : *G. insensibilis* Stock, 1966 and *G. aequicauda*

(Martynov, 1931). The first description of these amphipods as distinct species was provided by Stock (1967) who based his classification on a reduced number of key morphological characters.

Although both species live in brackish water, they exhibit some ecological differences. Studies conducted on both natural (Janssen *et al.*, 1979) and laboratory (Brun, 1971) populations revealed a higher tolerance of *G. aequicauda* to wide range of temperature and salinities than *G. insensibilis*. Therefore *G. aequicauda* may be considered to inhabit environments more variable than those experienced by *G. insensibilis*.

## MATERIALS AND METHODS

Samples of *G. insensibilis* were collected in the lagoon of Venice (Italy), near San Felice island, and in the lagoon of Salses-Leucate (southern France). *G. aequicauda* was sampled in the lagoon of Venice (Piovini) and in the lagoon of Canet-Saint Nazaire, which belongs to the same lagoon network of Salses-Leucate in southern France.

The two species were sampled in Italy during November 1987, and in France during August 1988 and stored at  $-40^{\circ}\text{C}$ .

Electrophoretic analyses were carried out on starch gels according to Selander *et al.* (1971).

The following enzymes were analyzed: amylase (AMY, E.C. 3.2.1.1), aminopeptidase (AP, E.C. 3.4.-.-), arginine kinase (APK, E.C. 2.7.3.3), esterase (EST, E.C. 3.4.1.1), fumarase (FH, E.C. 4.2.1.2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, E.C. 1.2.1.12), glutamate-oxalacetate transaminase (GOT, E.C. 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49), hexokinase (HK, E.C. 2.7.1.1), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), leucine aminopeptidase (LAP, E.C. 3.4.11), malate dehydrogenase (MDH, E.C. 1.1.1.37), malic enzyme (ME, E.C. 1.1.1.40), mannose-6-phosphate isomerase (MPI, E.C. 5.3.1.8), glucose-6-phosphate isomerase (PGI, E.C. 5.3.1.9), phosphoglucomutase (PGM, E.C. 2.7.5.1), pyruvate kinase (PK, E.C. 2.7.1.40), triose phosphate isomerase (TPI, E.C. 5.3.1.1), and xanthine dehydrogenase (XDH, E.C. 1.1.1.204).

Fourteen loci were classified in all four populations, as well as additional loci as indicated in Table I.

## RESULTS

Table II lists the loci monomorphic for all populations. Gene frequencies, levels of heterozygosity, and the evaluation of the fit to Hardy-Weinberg equilibrium are reported in Table III for

Table I – List of the loci analyzed in two populations of the species *G. insensibilis* and *G. aequicauda*.

<i>G. insensibilis</i>		<i>G. aequicauda</i>	
Venice	Salses Leucate	Venice	Canet S. Nazaire
<b>Common Loci</b>			
AP	AP	AP	AP
APK	APK	APK	APK
EST-1	EST-1	EST-1	EST-1
EST-2	EST-2	EST-2	EST-2
FH	FH	FH	FH
GAPDH	GAPDH	GAPDH	GAPDH
GOT-1	GOT-1	GOT-1	GOT-1
HK	HK	HK	HK
LAP-1	LAP-1	LAP-1	LAP-1
MDH-1	MDH-1	MDH-1	MDH-1
MPI	MPI	MPI	MPI
PGI	PGI	PGI	PGI
PGM	PGM	PGM	PGM
XDH	XDH	XDH	XDH
<b>Other scored loci</b>			
AMY-1	-	-	-
AMY-2	-	-	-
G6PDH	-	-	-
-	LAP-2	LAP-2	LAP-2
ME	ME	-	-
PK	-	-	-
TPI	-	TPI	-
-	MDH-2	MDH-2	MDH-2
-	-	IDH	IDH
-	-	GOT-2	-

Table II. – List of monomorphic loci in two populations of the species *G. insensibilis* and *G. aequicauda*. G.i.(Ve) = *G. insensibilis* from the lagoon of Venice; G.i.(L) = *G. insensibilis* from Salses Leucate; G.a.(Ve) = *G. aequicauda* from the lagoon of Venice; G.a.(C) = *G. aequicauda* from Canet Saint Nazaire;

G.i.(Ve)	G.i.(L)	G.a.(Ve)	G.a.(C)
AP	AP	AP	AP
APK	APK	APK	APK
EST-2	EST-2	EST-2	EST-2
GOT-1	GOT-1	GOT-1	GOT-1
LAP-1	LAP-1	LAP-1	LAP-1
XDH-1	XDA-1	XDH-1	XDH-1
AMY-1	-	-	-
AMY-2	-	-	-
ME	ME	-	-
G6PDH	-	-	-
PK	-	-	-
TPI	-	TPI	-
MDH-2	MDH-2	MDH-2	-
IDH	IDH	-	-

loci which are polymorphic in at least one of the four populations.

The loci most polymorphic in the *G. insensibilis* population from the lagoon of Venice are *Mpi* and *Pgi*, with five and six alleles classified, respectively. The level of observed heterozygosity is 9 % for *Mpi* and 45 % for *Pgi*. The other polymorphic loci are *Fh*, *Mdh-1* and *Gapdh*. The mean heterozygosity observed over 20 loci is 3 % compared to the expected values of 3.5 % (Table IV A).

A similar situation characterizes the genetic structure of the French population of *G. insensibilis*. The most polymorphic locus is again *Pgi* with an observed heterozygosity of 45 %, while *Mpi* in this population shows only 1.9 % observed heterozygosity (Table III). The mean values of polymorphism are calculated over 17 loci and are not significantly different from those found in the Italian population of this species (Table IV A).

The gene frequencies and the heterozygosity of both populations of *G. aequicauda* are reported in Table III. In the Italian sample, *Mpi*, *Pgi*, and *Pgm* are highly polymorphic, and the observed heterozygosities are 53.5 %, 60.0 %, and 31.4 %, respectively. The population from Canet Saint Nazaire exhibits some differences for these loci: *Mpi* is monomorphic, and the observed heterozygosity for *Pgi* is only 9.4 %. In contrast, *Pgm* in this population is more heterozygous than in the Italian sample ( $H_{obs} = 57.6\%$ ). These differences are responsible for the lower mean heterozygosity found in the French population of *G. aequicauda* ( $H_{obs} = 3.8\%$ ) compared to the Italian one ( $H_{obs} = 7.8\%$ ).

With regard to Hardy-Weinberg equilibrium expectations, PGM shows heterozygote deficiencies in both populations of *G. aequicauda*. The deficiency is significant in the Italian sample (Table III).

Nei's genetic distance (D) (1972) calculated for 14 common loci indicates greater differentiation between the two populations of *G. aequicauda* ( $D = 0.0399$ ) than between the Italian and the French populations of *G. insensibilis* ( $D = 0.0014$ ) (Table IV B, Figure 1). The genetic distance between *G. insensibilis* and *G. aequicauda* is 0.14 (Fig. 1).

## DISCUSSION

The analyses of enzymatic polymorphism in the two species points out similarities in their genetic structure. The level of heterozygosity is very low in both species. Nevertheless, some differences are obvious. The loci *Fh* and *Pgm* discrimi-

Table III. - Gene frequencies, observed and expected heterozygosity, and evaluation of the Hardy-Weinberg equilibrium for loci polymorphic in two populations of the species *G. insensibilis* and *G. aequicauda*. G.i. = *Gammarus insensibilis*; G.a. = *Gammarus aequicauda*; Ve = lagoon of Venice; L = Salses-Leucate; C = Canet-Saint Nazaire. Hobs = observed heterozygosity; Hexp = expected heterozygosity; 2N = number of scored genes; P(H-W) = Hardy-Weinberg equilibrium; 1,2,3,4,5,6 = gene frequencies of the alleles 1,2,3,4,5, classified according to their electrophoretic mobility.

	1	2	3	4	5	6	2N	Hobs	Hexp	P(H-W)
<b>EST-1</b>										
G.i.(Ve)	0	1					200			
G.i.(L)	0	1					106			
G.a.(Ve)	0	1					100			
G.a.(C)	0.011	0.989					94	0.021	0.021	>0.95
<b>FH</b>										
G.i.(Ve)	0.010	0.990					200	0	0.020	>0.10
G.i.(L)	0.009	0.991					106	0.019	0.018	>0.95
G.a.(Ve)	1	0					100	0	0	
G.a.(C)	0.989	0.011					94	0.021	0.022	>0.90
<b>GAPDH</b>										
G.i.(Ve)	0.005	0.995	0				200	0.010	0.010	>0.95
G.i.(L)	0	0.972	0.028				106	0.057	0.054	>0.95
G.a.(Ve)	0	0.986	0.014				144	0.015	0.015	>0.90
G.a.(C)	0	0.989	0.011				94	0.021	0.022	>0.90
<b>HK</b>										
G.i.(Ve)	1	0					200			
G.i.(L)	1	0					106			
G.a.(Ve)	0.980	0.020					100	0.040	0.039	>0.90
G.a.(C)	1	0					94			
<b>LAP-2</b>										
G.i.(Ve)	-	-					-			
G.i.(L)	0.991	0.009					106	0.019	0.018	>0.95
G.a.(Ve)	1	0					100			
G.a.(C)	1	0					94			
<b>MDH-1</b>										
G.i.(Ve)	0.025	0.975					200	0.050	0.049	>0.80
G.i.(L)	0.010	0.990					96	0.021	0.021	>0.95
G.a.(Ve)	0	1					88			
G.a.(C)	0	1					88			
<b>MPI</b>										
G.i.(Ve)	0.005	0.015	0.955	0.010	0.015		200	0.090	0.087	>0.95
G.i.(L)	0	0	0.991	0.009	0		106	0.019	0.016	>0.95
G.a.(Ve)	0	0.563	0.430	0.007	0		142	0.535	0.498	>0.50
G.a.(C)	0	0	1	0	0		86	0	0	
<b>PGI</b>										
G.i.(Ve)	0.005	0.030	0.580	0.375	0.005	0.005	200	0.450	0.524	>0.10
G.i.(L)	0	0.146	0.622	0.230	0	0	102	0.459	0.538	>0.20
G.a.(Ve)	0	0.429	0.500	0.043	0.028	0	140	0.600	0.563	>0.50
G.a.(C)	0	0.019	0.934	0.047	0		106	0.094	0.125	>0.50
<b>PGM</b>										
G.i.(Ve)	1	0					200	0	0	
G.i.(L)	1	0					106	0	0	
G.a.(Ve)	0.010	0.353	0.431	0.186	0.020		102	0.314	0.655	<0.01
G.a.(C)	0.076	0.390	0.347	0.187	0		118	0.576	0.687	>0.05

Table IV. – A, Mean values and standard deviations of the genetic polymorphism in two populations of the species *G. insensibilis* and *G. aequicauda*. A locus is considered polymorphic when the frequency of the rare allele is > 0.01. B, Genetic identity and genetic distance between two populations of the species *G. insensibilis* and *G. aequicauda*. Gi(Ve) = *G. insensibilis* from the lagoon of Venice; Gi(L) = *G. insensibilis* from Salses Leucate; Ga(Ve) = *G. aequicauda* from the lagoon of Venice; Ga(C) = *G. aequicauda* from Canet Saint Nazaire.

A	<i>G. insensibilis</i>		<i>G. aequicauda</i>	
	Venice	Salses Leucat	Venice	Canet S. Nazaire
n° of individuals	100	55	50	47
n° of enzymes	18	14	15	14
n° of loci	20	17	19	17
Mean n° of genes sampled per locus	187.5 ± 31.93	94.12 ± 8.01	107.16 ± 20.51	94.12 ± 8.02
Mean n° of alleles per locus	1.60 ± 1.39	1.47 ± 0.87	1.58 ± 1.17	1.47 ± 0.87
% of polymorphic loci	20.0	29.4	26.3	29.4
Mean observed heterozygosity	0.030 ± 0.101	0.038 ± 0.120	0.078 ± 0.187	0.038 ± 0.120
Mean expected heterozygosity	0.035 ± 0.117	0.043 ± 0.131	0.094 ± 0.215	0.043 ± 0.121

B	Gi(Ve)	Gi(L)	Ga(Ve)	Ga(C)
Gi(Ve)	***	0.999	0.839	0.875
Gi(L)	0.001	***	0.842	0.878
Ga(Ve)	0.176	0.172	***	0.961
Ga(C)	0.134	0.130	0.040	***

Below diagonal: Nei's genetic distance (Nei, 1972);  
Above diagonal: Nei's genetic identity (Nei, 1972).

nate clearly between *G. insensibilis* and *G. aequicauda*. Moreover, the locus *Pgm* is polymorphic only in the latter species and shows a deficit of heterozygotes in both populations analyzed. The exact evolutionary cause of this deficit does not emerge from the present data and could be due to selective forces or stochastic factors. The fact that a deficiency is found in both Italian and French populations of *G. aequicauda* suggests the possibility of genotypic selection.

The genetic distance estimates show a higher degree of differentiation within *G. aequicauda* than within the two populations of *G. insensibilis*. In *G. aequicauda*, previous studies conducted on a sample from Sigean (a brackish water basin belonging to the same lagoon network that includes Canet Saint Nazaire) confirm the low genetic polymorphism characterizing the French populations ( $H_{obs} = 0.038$ ) (Bisol *et al.*, 1987).

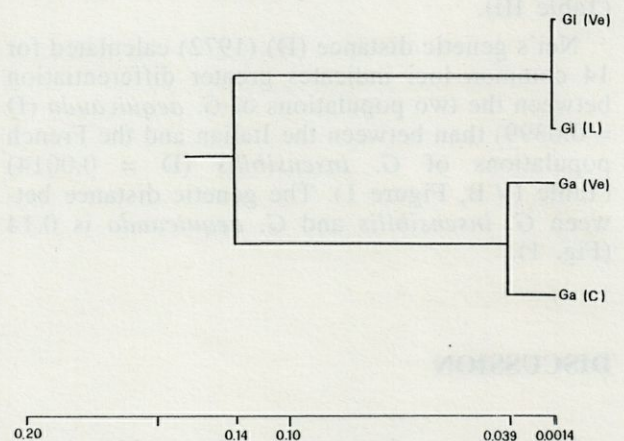


Fig. 1. – Phylogenetic relationship between two populations of the species *G. insensibilis* and *G. aequicauda* based on Nei's (1972) genetic distance.

The comparison of the two samples of *G. aequicauda* in the present study reveals that a large degree of the differentiation is due to the loci *Mpi* and *Pgi* loci. The first locus is monomorphic in the sample from Canet-Saint Nazaire whereas the heterozygosity observed in the Venetian population exceeds 50%. A similar situation characterizes the locus *Pgi* where the polymorphism of the Italian population is obviously higher compared with the French one.

The tendency for greater differentiation within the *G. aequicauda* populations could be a consequence of its ecology. This species lives in a very marginal environment as shown in natural and laboratory studies (Brun, 1971; Janssen, 1979). The salinity tolerated by *G. aequicauda* ranges from 1‰ to 39‰, whereas *G. insensibilis* can live between salinities of 15‰ and 37‰.

Experiments conducted at controlled temperatures on samples of both species from the same location in Italy as in the present paper, demonstrate mortalities of approximately 50% in *G. insensibilis* after 36 hrs at 27°C, whereas *G. aequicauda* exhibited mortalities of only 10% under these conditions (Patarnello, unpublished). This confirms that *G. aequicauda* is able to survive at more stressful conditions where selective mechanisms of genetic adaptation may play a stronger role (Koehn and Bayne, 1990).

The environmental characteristics of the habitats where the two populations of *G. aequicauda* have been collected show different degrees of marginality. This may be relevant in explaining the degree of genetic differentiation observed. Canet-Saint Nazaire is a small closed basin of shallow brackish water with wide annual fluctuation of both temperature and salinity. The latter ranges from low values of 7‰ during the winter to high values of 30‰ during the summer. Water temperature is strongly influenced by air temperature due to the shallow depths of the water combined with restricted circulation caused by the sea grass *Ruppia maritima*. During the summer the temperature can exceed 32°C while in the winter it may drop below 5°C (Herve', 1978). In contrast, the Italian population of *G. aequicauda* was collected from Piovini, an area far from the open sea but directly connected with the main basin of the Lagoon of Venice. The annual range of variation of both temperature and salinity are, on average, narrower than Canet-Saint Nazaire (Brunetti *et al.*, 1983). These differences may account for the more stressful environment in the basin of Canet Saint-Nazaire.

The lower level of polymorphism observed here in *G. aequicauda* may be the result of stronger selective pressures favoring the fixation, or increased frequency, of alleles which allow greater physiological flexibility. It is not possible from the present data to exclude that the greater degree of

differentiation observed within the two populations of *G. aequicauda* is the consequence of stochastic factors, namely genetic drift or founder events. However, evidence for selection acting on *PGI* and *MPI* in other species of amphipods (Patarnello *et al.*, 1989, McDonald, 1989) suggests the possibility that in marginal environments, such as the brackish water lagoons discussed here, selection drives allele frequencies.

Further analyses of other natural populations of *G. insensibilis* and *G. aequicauda* from different geographic areas are needed in order to confirm the greater degree of differentiation within *G. aequicauda* compared with *G. insensibilis*. Moreover, biochemical characterization of *MPI* and *PGI* genotypes in relation to their metabolic roles is necessary in order to evaluate if, under stressful environmental condition, these genotypes possess differential fitness.

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