Evolutive observations on discontinuous populations of *Alcyonidium polyoum* (Hassall, 1841) (Bryozoa, Ctenostomida) along the coasts of Galicia and the Bay of Biscay

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Evolutive observations on discontinuous populations of Alcyonidium polyoum *(Hassall, 1841) (Bryozoa, Ctenostomida) along the coasts of Galicia and the Bay of Biscay.*– An electrophoretic study, using polyacrylamide gradient gels, was performed on various populations of *Alcyonidium polyoum* (Hassall, 1841) from Galicia and the Bay of Biscay. Independently of the fragmentation of the distribution area of the species, the populations are situated along a cline. Genetic isolation is beginning to appear in the populations from Arcachon and Ré Island, both geographically isolated localities. The population of Ré could represent an example of genetic drift.

Key words: *Alcyonidium*, Bryozoa, Galicia, Bay of Biscay, Geographic isolation.

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Introduction

Research performed since 1979 on the intraspecific variability of the ctenostomatous bryozoan *Alcyonidium polyoum* (Hassall, 1841) (= *A. gelatinosum* auct., cf. D'Hondt & Mawatari, 1986), using gradient polyacrylamide gels, has demonstrated the presence of intraspecific clines between the main natural geographical barriers (estuaries, large straits). Such barriers induce discontinuity in the clinal variations. The number of proteic bands (interpreted as allelic expressions) shared between each side of any barrier can be very high, and null for other enzymatic systems. This confirms that

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these populations belong to the same species (d'Hondt & Goyffon, 1986, 1987a, 1987b, 1989; d'Hondt et al., 1993; d'Hondt, 1995). Furthermore, each population presents a specific proteinogram, with a definite number of bands, in a characteristic pattern, constituting a diagnostic marker of the species, independently of the studied population and of its geographical origin. Four main groups (each divided into geographical subgroups) of populations have been identified: 1. The north Scandinavian pool (Sweden and Norway), isolated by the Kattegat; 2. The Danish pool, the southern geographical gap being situated on the Dutch coast; 3. The meridional pool (French and Spanish populations); 4. The British pool, with greater affinities with the two septentrional (1 and 2) populations than with the meridional population (3), including a peculiar Welsh population simultaneously presenting British, Irish and continental relationships (d'Hondt & Goyffon, 1991; Houzelot, 1993).

The present study fills a lacuna in our knowledge: the affinities of the populations from the Bay of Biscay and Galicia with those of Brittany. This coastal area of the European continent is of special interest, because many long portions of the littoral, uninhabitable for the *Alcyonidium*, are geographical barriers which isolate very small localities where the animals are present. This phenomenon induces a reproductive isolation between the discontinuous populations. The strait of Loire, spanning a large input of freshwater and alluvia, separates Brittany (previously studied by d'Hondt & Goyffon (1987)) from Charente-Maritime. Some islands situated off the coast are isolated by oceanic straits and strong currents. Charente-Maritime is isolated from Arcachon by the muddy beaches of Aiguillon Bay, the Gironde Estuary, and about a hundred kilometres of sandy beaches. Arcachon Bay is isolated from Galicia by another long sandy area, the Adour Estuary, and by the Biscay coast which has warmer waters. As the area studied in this paper therefore corresponds to a sequence of very different facies, only some of which are convenient for the life of the *Alcyonidium*, the hypothesis is that some indigenous populations of this species living in this area could be subject to independent evolution.

Specimens of *Alcyonidium polyoum* from Fouras (Charente-Maritime), Ré Island (Charente-Maritime), Arcachon Bay, Ribadeo (Galicia) and Vigo (Galicia) were studied.

Material and methods

Twenty to forty specimens of each population were examined. The method described by d'Hondt & Goyffon (1987a, 1989) was used. Each proteic extract was analysed on polyacrylamide gradient gels. After the migration, each gel was cut in strips and coloured by a specific reagent for a given enzymatic system. The results were analysed using Sörensen's coefficient of similarity (Houzelot, 1993) to allow the quantification of the primary results. Since the clines are not revealed by the calculation of similarity coefficients and need a qualitative examination, the phenograms were directly compared by eye to display the clines.

The prospected localities, all intertidal, were the following (fig. 1): A. Fouras ('La

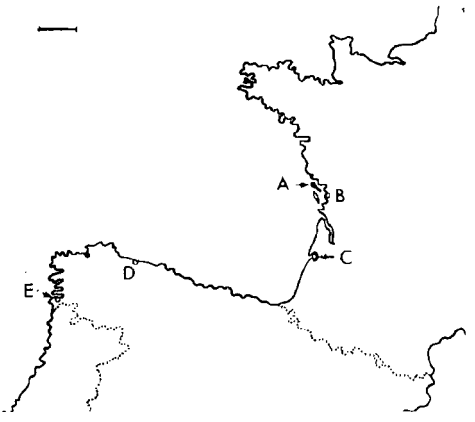


Fig. 1. Map of the localities: A. Fouras; B. Ré Island; C. Arcachon Bay; D. Ribadeo; E. Vigo. (Scale, 100 km).

Mapa de las localidades. (Para abreviaturas ver arriba). (Escala, 100 km).

Fumée', Charente-Maritime, France), with rather rough sea, on *Fucus serratus;* B. Ré Island ('Fier d'Ars', Charente-Maritime, France), somewhat calmer, on *Fucus serratus;* C. Arcachon Basin (east coast of 'Ile aux Oiseaux', Gironde, France), a calm area, on *Fucus vesiculosus* (*F. serratus* being disappearing in Arcachon); D. Ribadeo (northern coast of Galicia, Spain), a calm area, on *Fucus serratus;* E. Vigo (west coast of Galicia, Spain), also a calm area, on *Fucus serratus.*

The enzymatic systems considered herein are: 1. Acid phosphatase; 2. Non-specific esterases; 3. Leucine aminopeptidase; 4. Phosphoglucomutase; 5. Malate dehydrogenase; 6. Malic enzyme; 7. Glucose-6-phosphate isomerase; 8. Alkaline phosphatase. Proteinograms coloured with Coomassie blue, revealing the most concentrated proteins contained in a given extract, were also performed for each population. The formulae protocol follow D'HONDT & GOYFFON (1986, 1987a, 1991).

Results

Most profiles and proteinograms obtained for each enzymatic system consisted of shared proteic bands (and consequently alleles) (figs. 2,3), which attests the monospecificity:

1. Acid phosphatase (fig. 2.1): there is a N-S cline, the number of bands increasing from north to south. The phenotype of the Ré population is intermediate between those from Fouras and Arcachon, also characterized by a small number of bands.

2. Non-specific esterases (fig. 2.2): there is a cline with a greater number of the bands in the south, corresponding to proteins of high molecular weight, and a lower number of bands in the north corresponding to a low molecular weight. The population of Ré Island presents fewer proteic bands than the others, particularly those from Fouras and Arcachon. The reason might be, as for Acid-phosphatase, a genetic drift.

3. Leucine aminopeptidase (fig. 2.3): there is a cline, with substitution of groups of bands by others in relation to the distance. The number of bands with low molecular weight increases notably at Ribadeo. The population of Ré presents only two bands, neither of which are shared with Fouras, and only one with the population from Arcachon.

4. Phosphoglucomutase (fig. 2.4): the population from Ré Island lacks alleles in comparison with that from Fouras. Neither the two share bands with the Arcachon population. There is therefore a gap between Charente-Maritime and Arcachon. The latter constitutes the start of a cline to Ribadeo, then to Vigo, with a gradual increase of the number of proteic bands, particularly of low molecular weight. Spanish populations however present bands in common with those of Fouras.

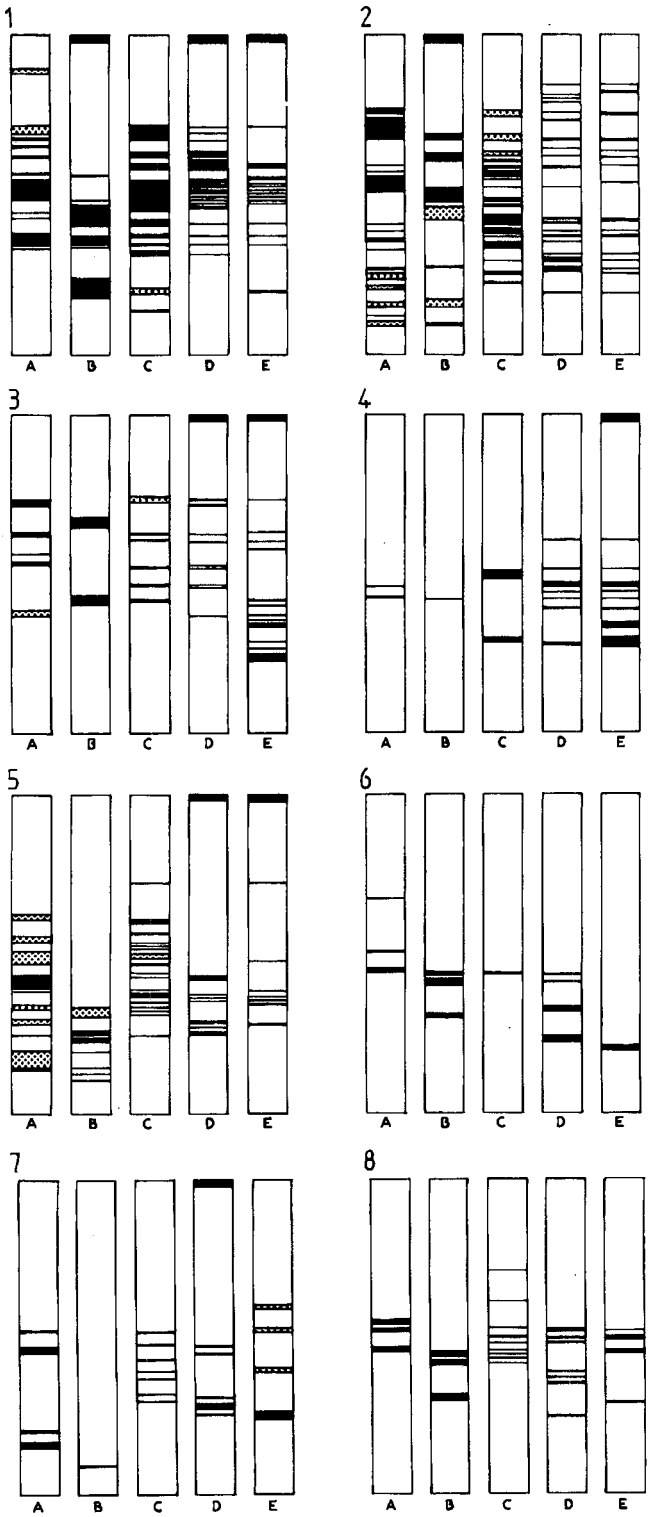
5. Malate dehydrogenase (fig. 2.5): a gradual substitution of associations of bands signifies an intraspecific clinal variation. The Ré population is almost devoid of the whole of the bands, with a high molecular weight present in the French populations of the continent (Fouras and Arcachon). Most of the proteic fractions with a high molecular weight are missing in the Spanish populations.

6. Malic enzyme (fig. 2.6): there is a clinal variation between Fouras and Ribadeo, but there are no shared bands between Ribadeo and Vigo (reproductive incompatibility), and a decrease to the south of the molecular weight of the corresponding proteins is observed. Only the Fouras population includes bands corresponding to proteins with a high molecular weight. The Arcachon population lacks most such bands.

7. Glucose-6-phosphate isomerase (fig. 2.7): there is a clinal variation for the continental populations, linking Fouras to Vigo, with substitution of associations of proteic bands. The populations from Ré Island and Fouras present reproductive isolation, and only one band with low molecular weight differing from the most southern populations.

8. Alkaline phosphatase (fig. 2.8): a clinal variation exists from Fouras to Ribadeo, with substitution of bands. Extra bands with high molecular weight are found in Arcachon.

9. Proteinograms (fig. 3): the numerous shared bands between the five populations confirm their monospecificity.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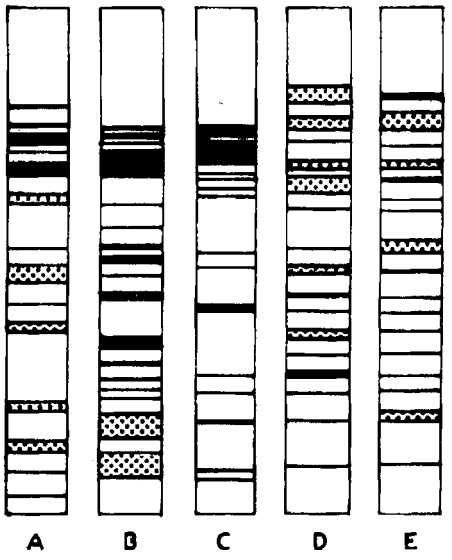

Fig. 3. Proteinograms. (For abbreviations see fig. 1).

Proteinograma. (Para abreviaturas, ver fig. 1).

Discussion

The gradient of porosity in the polyacrylamide gradient gels used in this work differs from those utilized in our previous research. It is therefore difficult to compare these results with those published earlier.

The interpretation of the phenograms is that the five populations belong to the same species. With rare exceptions, shared bands are observed in all populations for each of the enzymatic systems considered. A clinal variation between Fouras and Vigo is seen.

In six of the enzymatic systems, there are considerably fewer bands (acid phosphatase, non-specific esterases, leucine aminopeptidase, phosphoglucomutase, malate dehydrogenase, glucose-6-posphate isomerase) than in northern populations. In relation with acid phosphatase, non-specific esterases, phosphoglucomutase and malate dehydrogenase, the population in Ré is closest to that of Fouras; in the case of leucine aminopeptidase and alkaline phosphatase, the affinities of the Ré population are more related with the population from Arcachon. This indicates that the population from Ré is not situated within the cline Fouras-Vigo, but derives from the Fouras population. Moreover, each population has evolved independently from the others: in some enzymatic systems, one or two surnumerary bands can appear in one or two populations. Consequently, some alleles can disappear or not be expressed in a given portion of the coast, and reappear some distance away. This finding was demostrated in previous papers (D'HONDT & GOYFFON, 1989, 1991). The population from Ré Island has lost a significant proportion of its alleles in comparison with those of Arcachon and Fouras, perhaps as the consequence of a phenomenon of genetic drift, with geographic isolation producing reproductive isolation. The island of Ré is isolated from the continent by an arm of sea crossed by a violent current, preventing sporadic genetic exchanges. The *Alcyonidium polyoum* larvae are thus unable to metamorphose 1.5-2 h after breeding (D'HONDT, 1976). The period of com-

Fig. 2. Electrophoretic profiles obtained for each enzymatic system: 1. Acid phosphatase; 2. Non-specific esterases; 3. Leucine aminopeptidase; 4. Phosphoglucomutase; 5. Malate dehydrogenase; 6. Malic enzyme; 7. Glucose-6-phosphate isomerase; 8. Alkaline phosphatase. (For other abbreviations see fig. 1).

Perfiles electroforéticos obtenidos para cada sistema enzimático. (Para abreviaturas, ver arriba y fig. 1).

petence (during which the larva is able to metamorphose) is very short in this species (D'HONDT, 1981, 1982) and is acquired after a transitory free-living maturation period during which only very few larva swim. Metamorphosis is possible only in calm places. The population of *Alcyonidium* in Ré is situated at the west extremity of the island, far from the continent. The combination of these factors eliminates all genetic mixing between continental and insular populations.

The Arcachon population is isolated from that of Charente-Maritime by geographic and reproductive characters (no shared bands for phosphoglucomutase) and present bands (alleles) in common with the Spanish populations. The geographical barrier formed by the long sandy beaches of the districts of Charente-Maritime and Gironde, the flows of freshwater pouring in from the Gironde Estuary, and the violent current of the 'passes' of the Arcachon Bay, very likely constitute factors of isolation for the *Alcyonidium* of Arcachon. This barrier is probably more rigorous, and perhaps more recent, that the Estuary of Adour and the rocky coast of the Bay of Biscay. The Arcachon population therefore has greater affinities with Spanish populations than with those of Charente-Maritime. The population from Arcachon, characterized by the systems of phosphoglucomutase and malic enzyme, could have lost some alleles during evolution or be genetically 'polluted' by material from another origin. The presence at Arcachon (D'HONDT, 1974) of a bryozoan species from warm waters, *Scrupocellaria bertholleti*, may be related to the possible existence of some communication during the Messinian period between the Bay of Biscay and the Mediterranean Sea. The population of Arcachon differs more in fact from the Ré population than from the Galicia population. Both Arcachon and Ré populations constitute true vice-species *sensu* BERNARDI (1980) (cf. D'HONDT & GOYFFON, 1989), populations belonging to the same species, interfertile with some conspecific populations and intersterile with others, each constituting, with others, distinct boundaries or gradation zones according to the cases. The population from Ré Island presents a beginning of reproductive isolation, founded on the genetic incompatibility for only one enzymatic system (glucose-6-phosphate isomerase).

The clines are generally characterized by the disappearing, from south to the north, of many proteins of high molecular weight, and the presence of proteins with more reduced molecular weight. This observation confirms the preliminary results obtained by D'HONDT & GOYFFON (1987a), showing that all the northern populations studied (France, United Kingdom, Scandinavian countries) present proteic bands in various enzymatic systems widely distributed along the whole migration trail.

The differences between successive and discontinuous populations on the coasts of Bay of Biscay support the observations made in a previous study on the coastline of Wales (D'HONDT & GOYFFON, 1991) where geographic barriers (sometimes on short distances) induce an independent evolution of these populations and are factors of reproductive incompatibility between populations for many of the enzymatic systems studied. However, these differences are less accentuated than those determined by the main geographic barriers such as the English Channel and the Kattegat (D'HONDT & GOYFFON, 1989), and a little more evident than the differences between the subgroups of the cline in the Bailleron-Belgian frontier (D'HONDT & GOYFFON, 1989). This was predictable. However, the differences are not so ample as those found between the Angle and other Welsh populations (D'HONDT & GOYFFON, 1991).

Therefore, on the coast of the Bay of Biscay, where the area of distribution of *A. polyoum* is very fragmented and constitutes an interesting model for study, the geographic isolation or the insularity of some populations is not expressed by greater genetic differences than between French, Irish or Danish populations; in fact, the case of the Welsh population from Angle, in accentuated speciation, is very peculiar and exceptional. Another interesting finding in this study is the evidence of a reproductive isolation phenomenon correlated to the insularity. These conclusions should be compared in the future with those from an ongoing study, on discontinuous

and insular populations of *Alcyonidium polyoum* from the English Channel and Nordsee.

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Resumen

Observaciones evolutivas sobre poblaciones discontinuas de Alcyonidium polyoum *(Hassall, 1841) (Bryozoa, Ctenostomida) a lo largo de las costas de Galicia y de la bahía de Vizcaya*

El estudio electroforético, por medio de gel de polyacrylamida con gradación de porosidad, de poblaciones de *Alcyonidium polyoum* (Hassall, 1841) de la costa de Galicia y del mar Cantábrico, muestra que independiente de la fragmentación de la área de distribución de esta especie, los poblaciones están situadas sobre una clina.

Un principio de aislamiento genético tiene relación con las poblaciones de Arcachon y de la isla de Ré, localidades aisladas geográficamente. Posiblemente la población de Ré presenta un fenómeno de deriva genética.

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