ELECTROPHORETIC HETEROGENEITY IN HEDISTE DIVERSICOLOR (ANNELIDA: POLYCHAETA) WITHIN AND BETWEEN ESTUARIES IN NORTHERN FRANCE

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SOLUBLE PROTEINS
TWO-DIMENSIONAL ELECTROPHORESIS
ESTERASES
NEREIDIDAE
ESTUARIES
HEDISTE DIVERSICOLOR

ABSTRACT. – We compared the general protein and non-specific esterases band patterns of different populations of the polychaete annelid *Hediste diversicolor* (O.F. Müller) (Nereididae) located at different spatial scales in the estuaries of Aa, Canche, Authie and Rance (France). Our results indicate a restricted gene flow between populations located in different estuaries but also within the same estuaries. These results could be explained by the particular life cycle of this species ("bentho-pelagic" life cycle with a brief semi-pelagic larval phase) which does not favour a great dispersion of larvae and by local marine currents and eroding channels which canalize an oriented dissemination of larvae. In a preliminary step, intra-population banding pattern variability was assessed in two populations by high resolution two-dimensional electrophoresis analysis of proteins. Our results show a great inter-individual variability in protein profiles. These results are in good concordance with the hypothesis of the existence of a restricted gene flow between populations.

PROTÉINES SOLUBLES
ELECTROPHORÈSE BIDIMENSIONNELLE
ESTÉRASES
NEREIDIDAE
ESTUAIRES
HEDISTE DIVERSICOLOR

RÉSUMÉ. – Nous avons comparé les profils électrophorétiques des protéines générales et des estérases non-spécifiques de différentes populations de l'Annélide Polychète *Hediste diversicolor* (O.F. Müller) (Nereididae) localisées à différentes échelles d'espace dans les estuaires de l'Aa, de la Canche, de l'Authie et de la Rance (France). Nos résultats indiquent un flux génétique restreint entre les populations localisées dans les différents estuaires ainsi qu'à l'intérieur même des estuaires. Ces résultats pourraient être expliqués par la nature particulière du cycle de vie de cette espèce (cycle « bentho-pélagique » avec une phase larvaire semi-pélagique brève) qui ne favorise pas la dispersion des larves ainsi que par les courants marins locaux et les chenaux d'irrigation qui canalisent une dissémination orientée des larves. Lors d'une étape préliminaire, la variabilité intra-population des profils électrophorétiques de deux populations a été appréciée après analyse par électrophorèse bidimensionnelle hautement résolutive des protéines. Nos résultats montrent une grande variabilité inter-individuelle des profils électrophorétiques et sont en parfait accord avec l'hypothèse selon laquelle il existerait un flux génétique restreint entre les populations.

INTRODUCTION

Among nereidid polychaetes, *Hediste diversicolor* (O.F. Müller, 1776) (= *Nereis diversicolor*) is a wide-spread species in the intertidal zone of marine and brackish waters throughout Europe. Its range extends from the Baltic sea to Morocco and the Mediterranean, Black and (by introduction) Caspian Seas (Fauvel 1923, Clay 1967, Smith 1977). Moreover, *H. diversicolor* is a constant species of the *Macoma baltica* community and occurs in all European estuaries. This species is able to tolerate great variations of temperature (Ivleva 1970, Wolff 1973) and salinity (Wolff 1973, Neuhoff 1979) and to survive to drastic conditions of hypoxia (Wells & Dales 1951, Kristensen 1983). As a consequence, *H. diversicolor* is able to settle in naturally-fluctuant environments such as the upper waters of estuaries. *H. diversicolor* is an infaunal species which inhabits sandy muds but also gravels, clays and even turf (Rullier 1959, Amanieux 1967, Clay 1967, Cazaux 1970, Elkaim 1974, Desprez 1981, Bachelet 1987) where it builds U or

Y-shape burrows (Lambert & Retière 1987). According to Goerke (1971) and Fauchald & Jumars (1979), this species is omnivorous. Its individual feeding behaviour consists of two different strategies, one in which mucous is secreted to collect food particles as a pellet form at the opening of the burrow and the other without mucous secretion but direct food ingestion (Esnault *et al.* 1990).

H. diversicolor populations are conspicuous elements of benthos in intertidal mudflats and estuaries; they form an important part of the food supply for various birds and bottom-dwelling fishes. This species is a potentially valuable organism for long-term monitoring programs and as a bioindicator (Scaps 1997, Scaps & Borot 2000) and is also used commercially as a bait (Scaps 1992, Olive 1994, Gambi *et al.* 1994), So, it is of prime importance to characterize populations within and between estuaries.

Some authors have studied genetic differentiation in shallow brackish-water polychaetes of the family Nereididae. Abbiati & Maltagliati (1992) have showed that two populations of *Neanthes succinea* from the Tyrrhenian Sea and the Adriatic Sea are reproductively isolated. Fong & Garthwaite (1994) have compared biochemically using ten allozyme loci three morphologically similar species of the polychaete genus *Hediste* [*H. limnicola*, *H. diversicolor* and *H. japonica*] respectively from the west coast of North America, Europe and Japan. These authors have found that these three taxa are genetically distinct and constitute valid species. *H. limnicola* recognized previously as a self-fertilizing hermaphrodite is quite polymorphic in the four populations examined and these authors suggest that cross-fertilization must occur in the field. More recently, Sato & Masuda (1997) have demonstrated genetic differentiation in two sibling species of the polychaete *Hediste japonica*. Concerning *H. diversicolor*, previous studies have showed allozyme evidence of genetic differentiation between populations from the North Sea and the Baltic Sea (Röhner *et al.* 1997) and from the Western Mediterranean (Abbiati & Maltagliati 1996). Moreover, some authors have found a substantial degree of genetic differentiation between estuaries (Hateley *et al.* 1992, Fong & Garthwaite 1994).

In order to study the relationships between genetic structure and mode of reproduction and dispersal, we compared the general protein band and non-specific esterase band patterns of different populations of *H. diversicolor* located at different spatial scales in four estuaries along the English Channel and the North Sea. The general protein band and non-specific esterase band patterns, can be produced relatively easily and used for direct comparison of different populations of *H. diversicolor*. Moreover, in order to assess intra-population banding pattern variability, high resolution two-dimensional electrophoresis analysis of proteins was performed.

MATERIALS AND METHODS

Collection of individuals and sampling sites: individuals were collected by hand in a restricted area from different localities of North France during Spring of 1998 (28 April to 17 May). Individuals were obtained from 13 sites located along the estuaries of Aa, Canche, Authie and Rance. The sampling sites are shown in figure 1 and their related habitats described in Table I. These estuaries are located along the English Channel and the North Sea. Thirty individuals were collected per site.

Only one sample was collected on the northern side of the Aa estuary near the beach of Grand-Fort-Philippe, 6 stations were sampled along the Canche estuary on the northern and the southern sides. At station 3, a transect was designed and 3 samples collected along this transect. Sites 3a, 3b and 3c correspond respectively to a stagnant pool located at the upper tidal level, an eroding channel located at mid-tide level and an isolated pool located at the lower tidal level. In a similar manner, another transect was designed on the southern side of the Authie estuary near the mouth. Sites a, b and c correspond to samples collected within an isolated pool located at the upper tidal level and in an eroding channel at the mid-tide level, and at the lower tidal level respectively. The last sample was collected onto the mudflat of La Richardais immediately located above the tidal power station of the Rance estuary.

Sample preparation: worms were kept alive in an aquarium at 15 °C, continuously supplied with natural sea water and aeration. Worms were used for preparing samples within a period of two days following the collection.

Assays in polyacrylamide gradient gel electrophoresis were performed on pooled extracts proceeding from 30 individuals. Animals were homogeneized at 4 °C into 4 volumes of ice-cold 0.01 M Tris/HCl, pH 8.0 and then centrifuged at 15 000 g for 30 min at 4 °C. The supernatant was used for the electrophoretic analysis.

In order to analyze abundant proteins of adults by two-dimensional electrophoresis, four adults were sampled from the Aa and the Authie estuaries (site b). The preparation of samples for two-dimensional electrophoresis was carried out on excised metameres. We used the method described by Boyer *et al.* (1993) with minor modifications for the preparation of samples. Metameres were reduced into a powder directly in liquid N2 using a mortar and a pestle. One ml of extraction solution (10% w/v trichloroacetic acid, 0.07% v/v β-mercaptoethanol in cold acetone) was added. Proteins were precipited at – 20 °C for 45 min. After centrifugation at 30 000 × g for 20 min at 4 °C the supernatant was discarded and the pellet washed twice with 1 ml β-mercaptoethanol (0.07%; v/v) in cold acetone at –20 °C for 45 min, in order to remove any residual TCA. After centrifugation at 30 000 × g for 20 min at 4 °C, the β-mercaptoethanol solution was discarded and the pellet dried for at least 2 hours. To solubilize the proteins, the pellet was resuspended for 2 h in a lysis buffer (Damerval *et al.* 1986) (60 ml/mg dry weight pellet) and then centrifuged at 30 000 × g for 20 min at 4 °C. Supernatants were stored at –70 °C for further analysis.

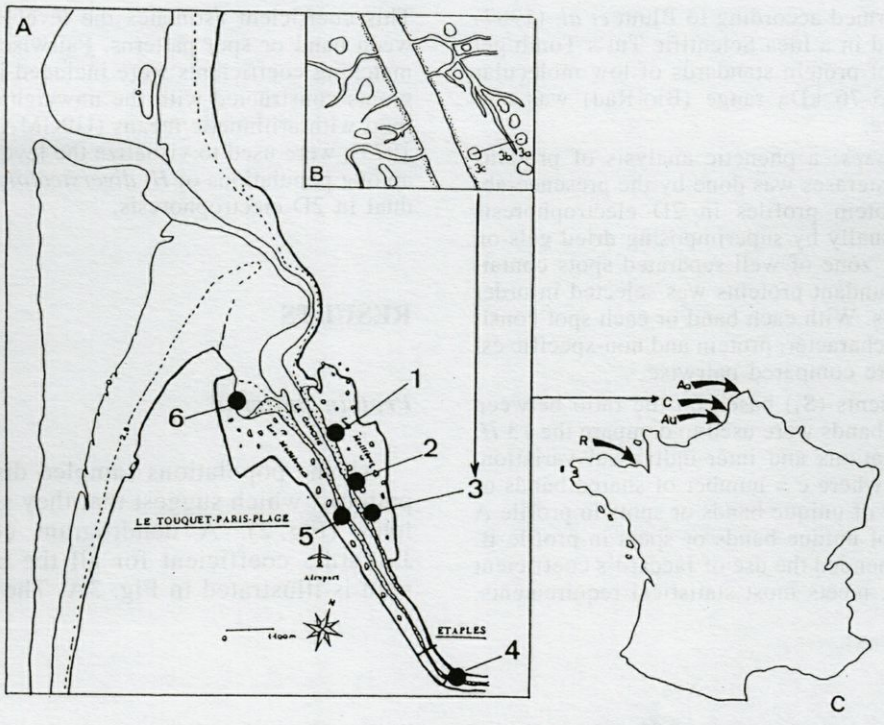


Fig. 1. – A, Sampling sites in the Canche estuary. B, Detail of the transect at station 3. C, Map of France with geographical location of Aa, Canche (C), Authie (Au) and Rance (R) estuaries. See Table I for details of site numbering.

Table I. – Description of sampling sites.

Code	Site	Sediment type
Canche estuary sites		
1	Military cemetery	Soft mud
2	La Pinède camping site	Soft mud
3a	Les Mollières	
	Upper tidal-level	Compact soft mud
3b	Mid tidal-level	Very soft mud
3c	Lower tidal-level	Soft mud
4	Etaples	Mudddy sand
5	Le Touquet airport	Soft mud
6	Le Touquet nautical circle	Sandy mud
Other sites		
Aa	Aa estuary	Soft mud with some pebbles and debris (e.g. cans, fragments of bottles,...)
a	Mouth of Authie estuary	
	Upper tidal-level	Soft mud
b	Mid tidal-level	Soft mud
c	Lower tidal-level	Very soft mud
R	Rance estuary	Organically rich mud

Electrophoretic analysis:

Polyacrylamide Gradient Gel Electrophoresis (PAGGE): Polyacrylamide gradient gel electrophoresis (PAGGE) under non-denaturating conditions was performed on a 5-30% acrylamide gel as described by Scaps *et al.* (1996). Migration was carried out at 4 °C for the appropriate period of time necessary to obtain a stabilized protein pattern, i. e. 20 to 22 hours. After being separated by electrophoresis, protein were stained with a 0.1% solution (w/v) of brillant blue Coomassie isopropanol-acetic acid-water (2.5/1/6.5). Gels were washed with a 10% solution (v/v) of acetic acid. Gels were also stained for non-specific esterases (EST; EC 3.1.1) according to the method of Selander *et al.* (1971).

Two-dimensional electrophoresis (2D-PAGE): two-dimensional electrophoresis was essentially performed as described by O'Farrell (1975) with the modification of Hilbert *et al.* (1992) using the Protean II cell (Bio-Rad, Richmond, CA). Isoelectric focusing gels utilizing diacrylylpiperazine as the cross-linking agent in place of bisacrylamide were prepared. Ampholytes were added to a final concentration of 5.5% and consisted of 90% ampholytes (pH 3-10) and 10% ampholytes (pH 5-7). About 100 mg of protein were loaded on the basic end of the capillary tubes. The gels were 1 mm in diameter and 13.5 cm long. The isolelectric focusing (IEF) was performed at room temperature with a constant voltage of 1 200 V for 17.5 h, followed by 1 500 V for 0.5 h and power was limited to 3 W. IEF capillary gels were extruded from the glass tubes, equilibrated and loaded onto a 12% homogeneous SDS gel. Gels were electrophoresed in the running buffer (24 mM Tris base; 192 mM glycine; 0.1% SDS). Electrophoresis in the second dimension was carried out with a constant voltage of 350 V until the dye front reaches the bottom of the gel (approximately 3 h).

After electrophoresis, gels were fixed overnight in 200 ml of a solution containing 50% ethanol, 12% glacial acetic acid and 200 µl 35% formaldehyde. Silver

staining was performed according to Blum *et al.* (1987) and gels were dried in a Idea Scientific Tut's Tomb gel dryer. A mixture of protein standards of low molecular weight in the 17.5-76 kDa range (Bio-Rad) was employed as reference.

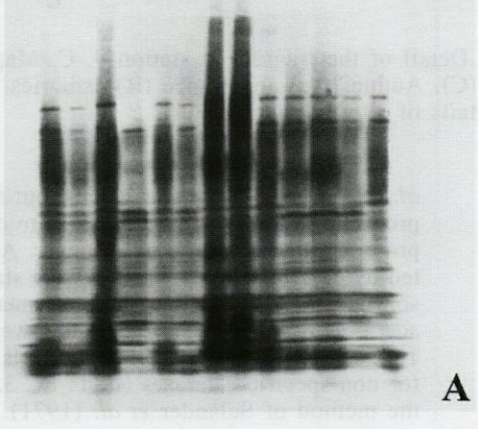
Statistical analyses: a phenetic analysis of proteins and non-specific esterases was done by the presence-absence criteria. Protein profiles in 2D electrophoresis were evaluated visually by superimposing dried gels on a bench viewer. A zone of well separated spots containing about 250 abundant proteins was selected in order to compare the gels. With each band or each spot considered as a distinct character, protein and non-specific esterase patterns were compared pairwise.

Jaccard coefficients (S_J) based on the ratio between shared and unique bands were used to compare the 13 *H. diversicolor* populations and inter-individual variation. $S_J = c/a_u + b_u + c$ where c = number of shared bands or spots, a_u = number of unique bands or spots in profile A and b_u = number of unique bands or spots in profile B. Shi (1993) recommended the use of Jaccard's coefficient because this index meets most statistical requirements. This coefficient estimates the level of divergence between band or spot patterns. Pairwise data of Jaccard's matching coefficients were included in matrix. Dendrograms constructed with the unweighted pair group method with arithmetic means (UPGMA) (Sneath & Sokal 1973), were used to visualize the level of differentiation among populations of *H. diversicolor* and among individual in 2D electrophoresis.

RESULTS

Protein patterns

All the populations sampled displayed common patterns, which suggest that they share common alleles (Fig. 2). A dendrogram constructed from Jaccard's coefficient for all the populations sampled is illustrated in Fig. 3A. The two populations



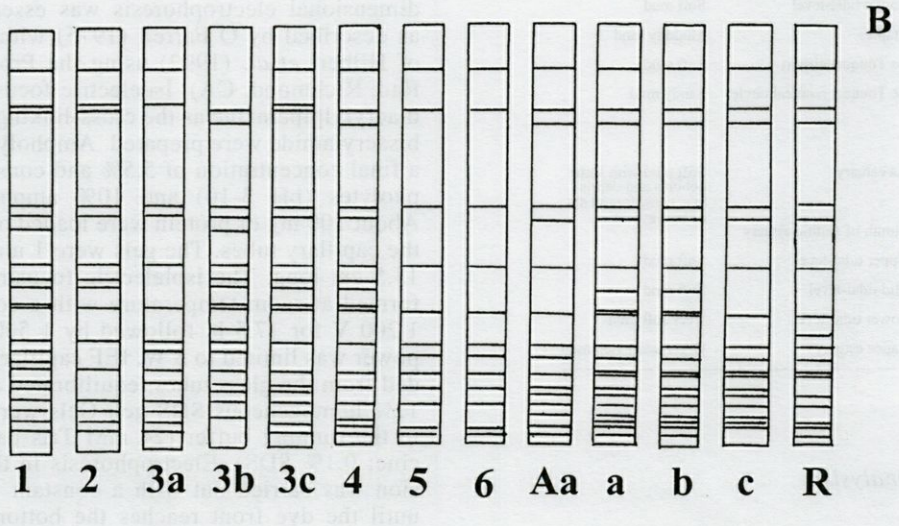


Fig. 2. – PAGGE profiles of general proteins in *Hediste diversicolor* from 13 sites located along the estuaries of Aa, Canche, Authie and Rance (A). Interpretation of general protein profiles (B), not same scale. See Table I for details of site numbering.

from the southern side of the Canche estuary have similar protein patterns (S_j = 1). Similarly, the two populations sampled within the irrigation channel of the Authie estuary (site 8) but from two different tidal levels (a and b) have also similar protein patterns. We observe no clear clustering of populations; nevertheless, there is no apparent correlation between S_j and the geographic distance; in fact, the more distant population (R) is not opposite to the other populations.

If we suppress populations 3a and 3c which are located in very isolated pools and population 3b which is situated at a higher tidal level than the other populations in the Canche estuary, we still do not observe clear clustering of populations (Fig. 3B).

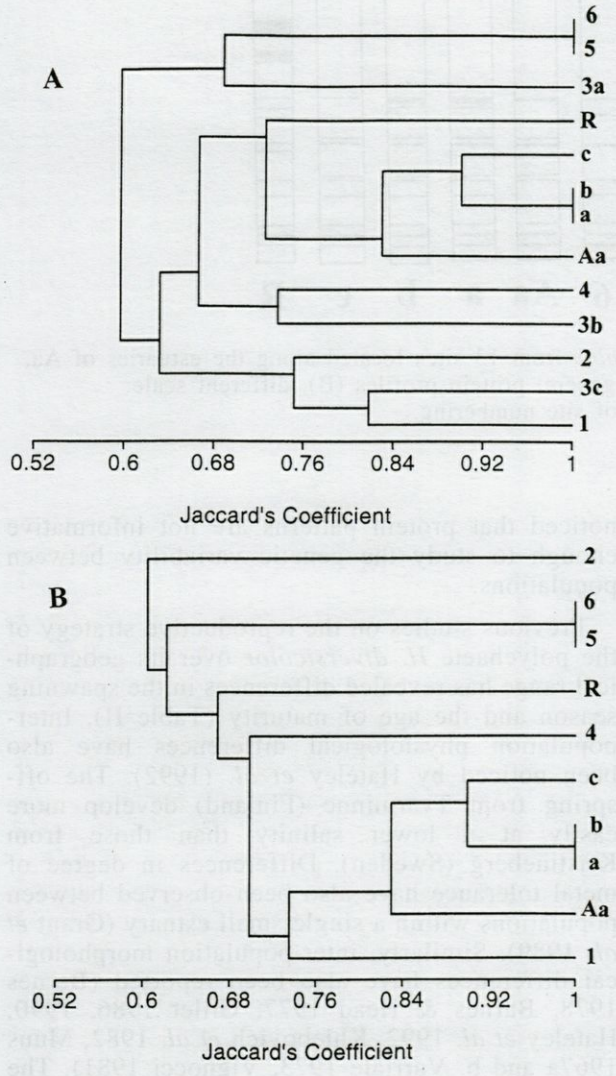


Fig. 3. – UPGMA dendrograms derived from inter-population S_j values based on general proteins with all the populations studied (A) and with the exception of populations at site 3 (B). See Table I for details of site numbering.

Non-specific esterase patterns

All the sampled populations shared the same common bands (Fig. 4). A dendrogram constructed from Jaccard's coefficient for all the populations sampled is illustrated in figure 5A. As for general protein patterns, the two populations from the southern side of the Canche estuary (5 and 6) and the two populations sampled within the irrigation channel of the Authie estuary (site 8) but from two different tidal levels (a and b) have similar patterns (S_j = 1). There is also no apparent correlation between S_j and the geographic distance; in fact, the more distant population (R) is not opposite to the other populations.

If we suppress populations at site 3, we observe a clear discrimination between populations located in different estuaries (Fig. 5B). Moreover, there is an opposition between populations located on the northern (1, 2) and the southern (5, 6) sides of the Canche estuary which cluster differently. Population 4 which is more distant from the mouth of the estuary than the other populations located on the northern side of the Canche estuary cluster with the populations of the southern side.

2D-PAGE

In a preliminary step, the general protein pattern of four individuals from the Aa estuary and four individuals from the Authie estuary (site a) were compared by 2D-PAGE in order to assess inter and intra-population variation.

An example of 2D-gel pattern is presented in Fig 6. Using high resolution two-dimensional electrophoresis, we have separated more than 1 000 protein spots. Spots were observed over most of the molecular weight and pH ranges used. We selected about 100 spots located in the best resolution region of the gels in order to compare intra and inter-individual variability (Fig. 6). The overall pattern of the gels were similar enough to permit direct comparisons and identification of persistent polypeptides (Fig. 7).

A dendrogram constructed from Jaccard's coefficient is illustrated in figure 8. We observe a clustering of individuals from the Authie estuary. Individuals from the Aa estuary cluster but in two different groups and are separated from the individuals of the Authie estuary; this means that inter-individual variability in protein profiles in 2D-PAGE is very high.

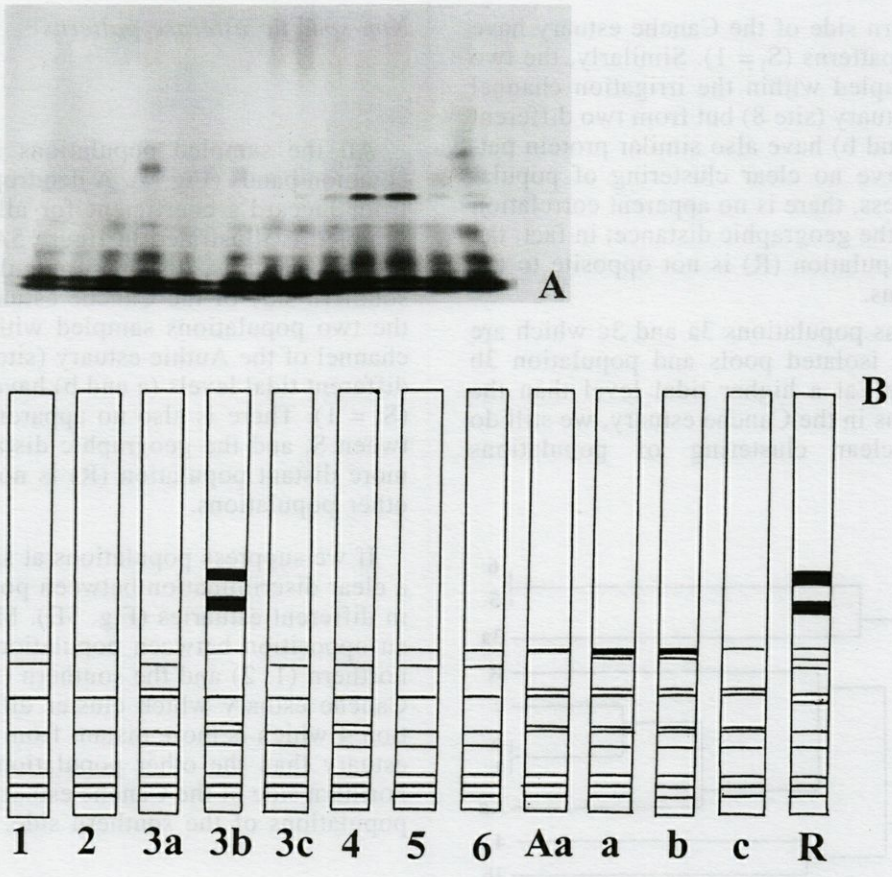


Fig. 4. – PAGGE profiles of esterases in *Hediste diversicolor* from 13 sites located along the estuaries of Aa, Canche, Authie and Rance (A). Interpretation of general protein profiles (B), different scale. See Table I for details of site numbering.

DISCUSSION

Population divergence at different geographical scales

As samples are separated by an average distance of about ten meters, hundred of meters, kilometers and hundred of kilometers, it is possible to test hypotheses relating to the relative contribution of migration in the population genetic differentiation. We have shown for the non-specific esterase and the protein patterns of *H. diversicolor* that bands are shared by all the sampled populations suggesting the monospecificity of this species. In the case of high level of gene flow maintaining genetic similarity, we would predict that interlocality differences over the largest scale of geography would be the greatest. Results obtained from both the general proteins and the non-specific esterases show that the population of the Rance estuary is not more distant from the other populations which are, at a closer geographical scale. So, our results indicate that the gene flow is restricted also at a relatively small scale. However, d'Hondt & Goyffon (1986) noticed that protein patterns are not informative enough to study the genetic variability between populations.

Previous studies on the reproductive strategy of the polychaete *H. diversicolor* over its geographical range has revealed differences in the spawning season and the age of maturity (Table II). Inter-population physiological differences have also been noticed by Hateley *et al.* (1992). The offspring from Tvarminne (Finland) develop more easily at a lower salinity than those from Kristineberg (Sweden). Differences in degree of metal tolerance have also been observed between populations within a single small estuary (Grant *et al.* 1989). Similarly, inter-population morphological differences have also been reported (Barnes 1978, Barnes & Head 1977, Gillet 1986, 1990, Hateley *et al.* 1992, Khlebovich *et al.* 1982, Muus 1967a and b, Varriale 1973, Vignocci 1981). The number of paragnaths showed no relationship with animal size, but the average number in each group varies between populations. Moreover, Hateley *et al.* (1992) showed no relationship between habitat and the number of paragnaths, during the first few months of life and have demonstrated the

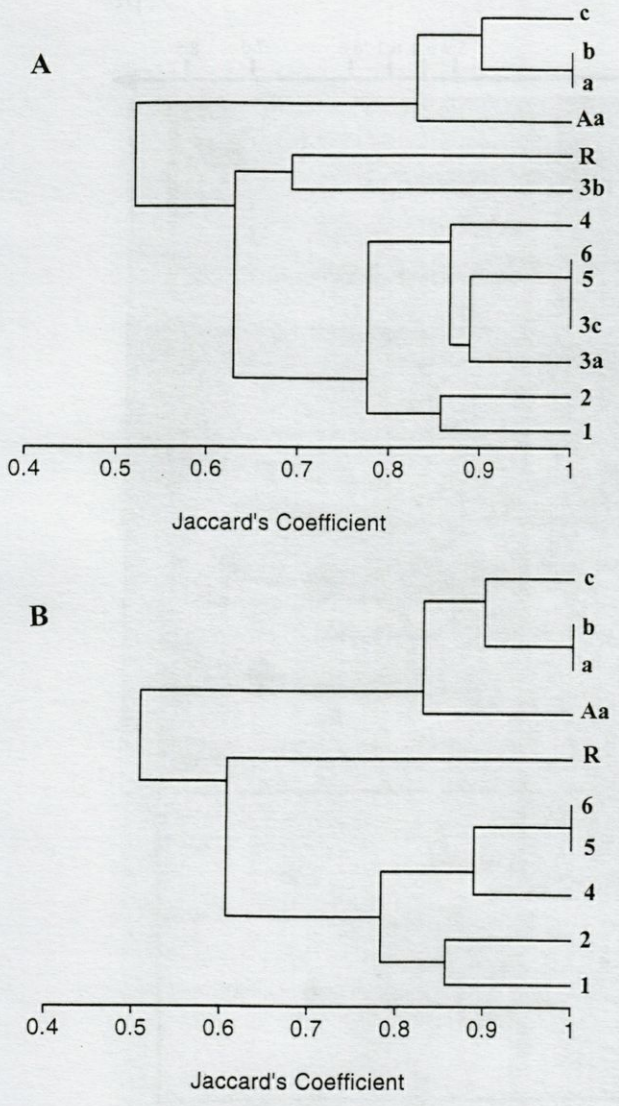


Fig. 5. – UPGMA dendrograms derived from inter-population S_j values based on esterases with all the populations studied (A) and with the exception of populations at site 3 (B). See Table I for details of site numbering.

heritability of the paragnath number by cross-fertilization experiments at least for four of six size groups. However, these authors pointed out that causes for this inter-population paragnath variation are not clear. It is possible that the paragnath number reflects differences in diet or the dominant mode of feeding. The mechanical implications of these methods for paragnaths are likely to be different. Differences in salinity tolerance of larvae (Smith 1964) and in time of reproduction may be entirely explained by plastic responses towards fluctuating environments (Hateley *et al.* 1992, Scaps 1992). Recently, allozyme evidence of genetic differentiation between populations of *H. diversicolor* from the North Sea and the Baltic Sea (Röhner *et al.* 1997) and from the Western Mediterranean (Abbiati & Maltagliati, 1996) was demonstrated.

Our results suggest that there is a substantial genetic heterogeneity between estuaries. According to Hateley *et al.* (1992) the differences in paragnath number between populations and patterns of variation in allozymes at two loci are consistent with a substantial degree of genetic differentiation between estuaries. Moreover, Fong & Garthwaite (1994) have examined populations of *H. diversicolor* which were separated by about 30 km, and suggested that observed differences could be explained either by a restricted gene flow between populations or short-term selection.

Microgeographic variation

Genetic heterogeneity will be discussed on a small spatial-scale, within estuaries and at different levels of the intertidal zone.

Estuarine variation

Our results showed no differentiation in the two populations sampled on the southern side of the Canche estuary. In contrast, inter-populations differences appeared between populations sampled on the northern side of the same estuary although they are located at the same intertidal height (populations 1, 2, and 4). Population 4 is distant from the mouth of the estuary and the channel at this site is narrow; in consequence, this population behaves as a population of the southern side. These results suggest the existence of a basic population heterogeneity within the Canche estuary. It is useful for the following discussion to describe the life cycle of *H. diversicolor*. According to Cazaux (1970), *H. diversicolor* has a "bentho-pelagic" life cycle with a brief semi-pelagic phase. Eggs are large (egg diameter = 300 µm), lecitotrophic, demersal and, according to experimental observations (Bartels-Hardège & Zeeck 1990) are laid by the female inside the burrow before the male ejects its sperm into the burrow's entrance. The female subsequently intensifies its ventilory activity and brings back the sperm into the burrow in a kind of feeding behaviour. Fertilized eggs remain into the maternal burrow, to be brooded by the female. Hatching occurs at the trochophore stage. Larvae exhibit a slightly developped ciliary crown and often crawl on the bottom or remain in the maternal burrow until reaching the 7 or 8 segments stage. Then the female dies. At the end of the "semi-pelagic" phase, animals become sedentary at the 3-setiger erpochaete stage. Erpochaeta lack their ciliary crown and thus are completely benthic. The juvenile, benthic worm of 10 or 11 segments has the same style of life as adult. This kind of life cycle

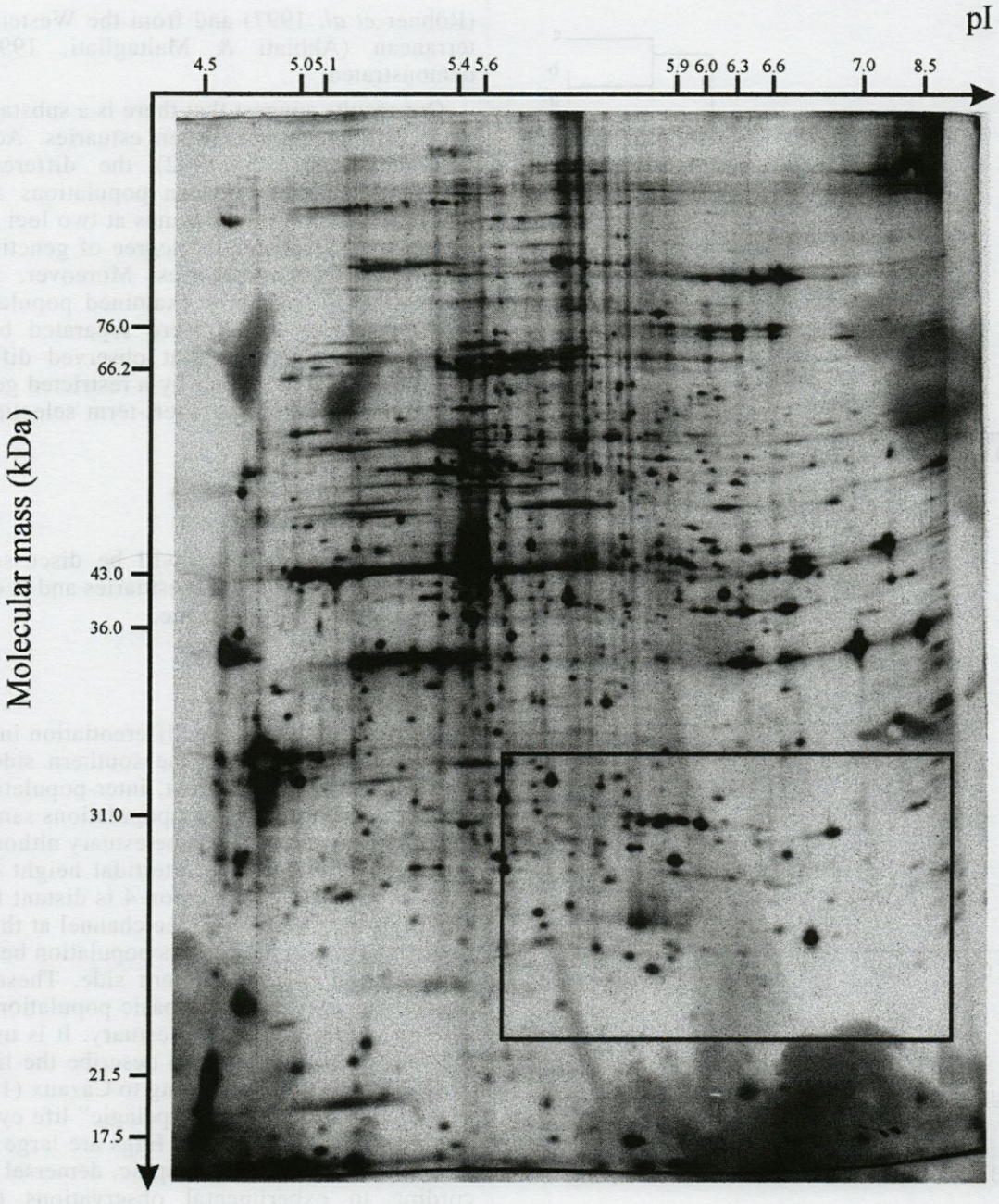


Fig. 6. – Example of an electrophoretic analysis of the water-soluble protein fraction in an extract of metameres of an individual of *Hediste diversicolor* collected in the Aa estuary. Proteins taken into account for the inter-individual comparisons are delineated by a rectangular area.

does not favour a great dissemination of larvae. As a consequence, protein similarity (general protein and non-specific esterase patterns) of the populations from the southern side of the Canche estuary could be explained by local marine currents which are more intense on the southern side of this estuary, favouring an orientated dissemination of larvae to the southern side and isolating populations from the opposite side.

Our results, showing an heterogeneity of populations within the Canche estuary are consistent with those of Hateley *et al.* (1992) who found differences between populations within an estuary and interpreted them as indicating a limited amount of gene flow between sites located 1 km apart. However, patterns of variation at two loci provide only weak evidence for differentiation within estuaries and therefore did not allow these authors to provide an unequivocal conclusion. In other respects, in all breeding populations of *H. diversicolor*, the observed sex ratio is favorable to females. Herpin (1925) reported that the ratio of males to females

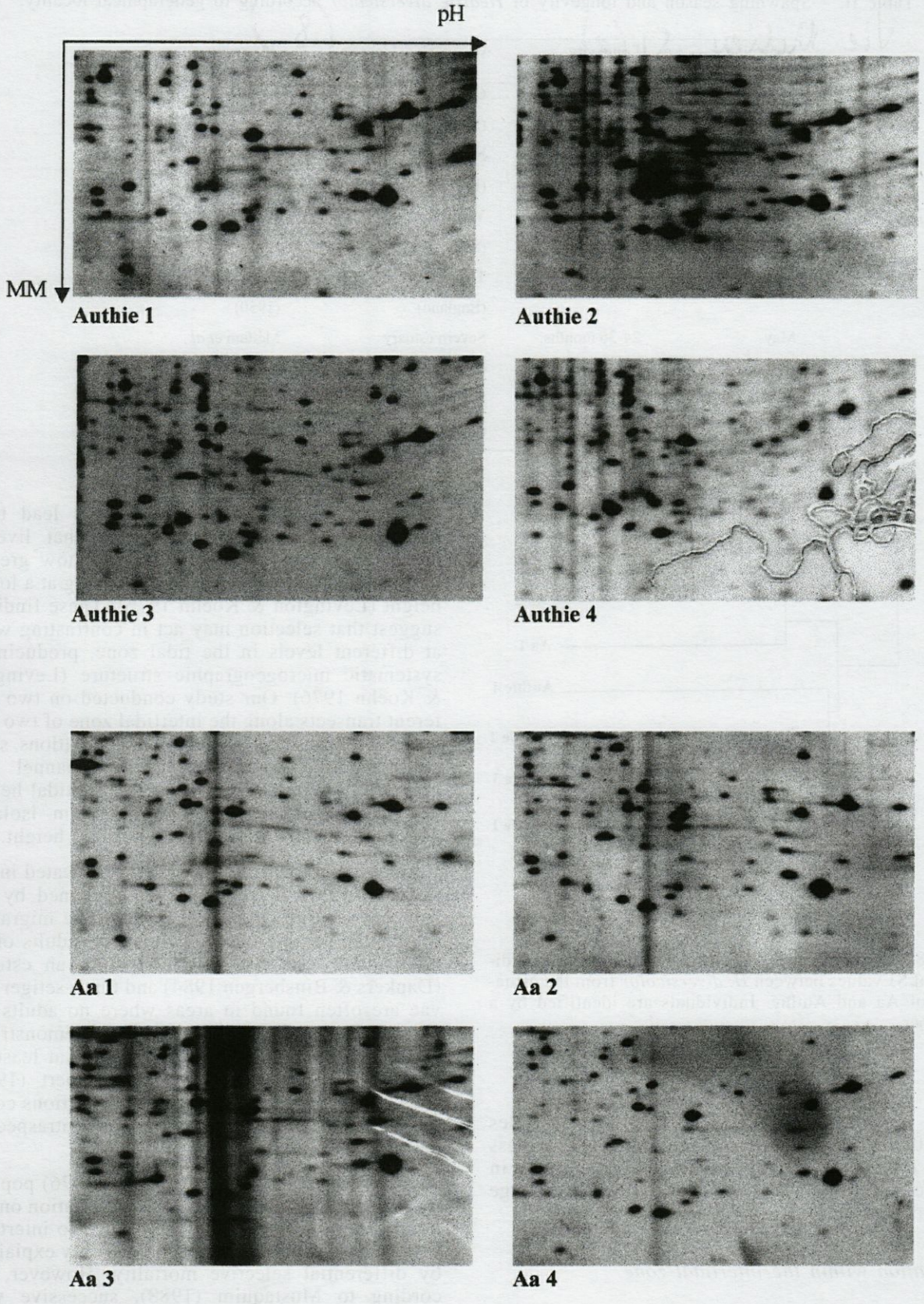


Fig. 7. – Detailed examples of inter-individual variations in the gels for the eight individuals studied.

Table II. – Spawning season and longevity of *Hediste diversicolor* according to geographical locality.

Spawning	Longevity	Geographical locality	Reference
May	24-36 months	Güteborg (Sweden)	Möller (1985)
February and April to August	12-18 months	Norsminde Fjord (Denmark)	Kristensen (1984)
January to March And June to August	18-24 months	Ythan estuary (Scotland)	Chambers and Milne (1975)
February	18 months	Thames estuary (England)	Dales (1950)
May	24-36 months	Severn estuary (England)	Mettam *et al.*, (1982)
March-April	36 months	Blyth estuary (England)	Olive and Garwood (1981)

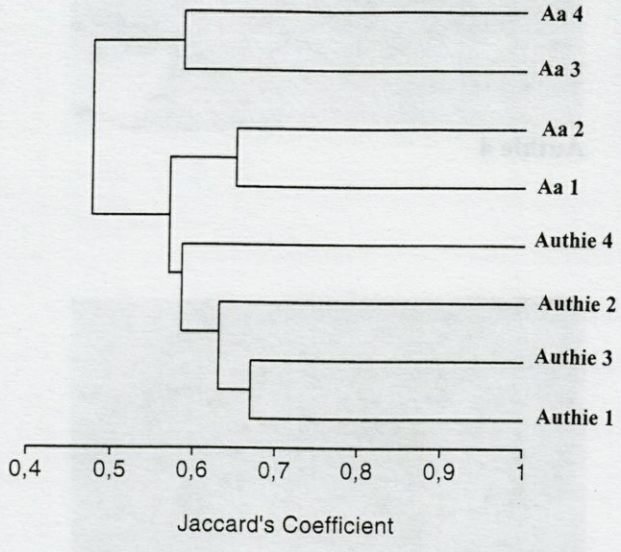


Fig. 8. – UPGMA dendrograms derived from inter-individual SJ values between *H. diversicolor* from the estuaries of Aa and Authie. Individuals are identified by a number.

does not reach 1/7 at Cherbourg (France). Dales (1950) estimated the percentage of males to be less than 10% in the Thames estuary. This too argues in favour of very restricted gene flow and large founder effects.

Variation within the intertidal zone

The intertidal zone differs dramatically in exposure time, temperature, heat transfer, water retention (Newell 1979) and could provide an obvious source of heterogeneity which could lead to a microgeographic variation. Species that live at higher levels in the intertidal zone show greater physiological tolerance than those living at a lower height (Levington & Koehn 1976). These findings suggest that selection may act in contrasting ways at different levels in the tidal zone, producing a systematic microgeographic structure (Levington & Koehn 1976). Our study conducted on two different transects along the intertidal zone of two different estuaries has shown that populations sampled along the same eroding channel are genetically identical whatever the intertidal height is, whereas populations sampled from isolated pools differ according to the intertidal height.

Genetic mixing of the populations located in the same eroding channel could be explained by the fact that eroding channels canalized the migration of "bentho-pelagic" larvae. However, adults of *H. diversicolor* can also migrate within an estuary (Dankers & Binsbergen 1984) and three-setiger larvae are often found in areas where no adults are present (Davey & Goerge 1986). This demonstrates the dispersal ability of *H. diversicolor* at least on small spatial-scales. Moreover, Lambert (1986) suggested that adults of Brittany populations could be displaced in channels when the intraspecific density is too high.

According to Levinton & Koehn (1976) population genetics of mussels reveal that variation on the microgeographic scale may be related to intertidal height, mussel size and age, and possibly explained by differential selective mortality. However, according to Mustaquim (1988), successive year groups of *H. diversicolor* from the Blyth estuary (England) are not genetically different. Moreover, studies conducted on *Mytilus edulis* strongly sug-

gest that environmental factors are the main factor influencing allozyme variations (Levinton & Koehn 1976). According to this point of view, estuaries represent fluctuating environments and thus provide an obvious selective force towards spatial heterogeneity among populations. Moreover, some authors have shown the presence or the absence of some electrophoretic bands in relation to environmental conditions (d'Hondt & Goyffon 1986, Sin & Jones 1983). These authors explain these differences by selective mortality against heterozygotes. Kerambrun & Guérin (1984) noticed biochemical modifications in relation to stress and showed variations in zymograms in marine invertebrates in relation with dietary, pollution and thermic effects. Moreover, d'Hondt & Goyffon (1986) transfered populations from one site to another and showed that some enzyme systems could be inducible. Our results suggest that the inter-population variability of *H. diversicolor* within an estuary could be related to genetic (reduce gene flow) and ecophysiological (adaptation to fluctuant environments) factors. In the present state of our knowledge and in order to distinguish between these two processes, enzyme systems should be studied by reciprocal transplantation of individuals from one site to another to test their inducibility.

Inter-individual variation

Two-dimensional electrophoresis is a powerful tool to study protein variation between and among natural populations. According to Piñeiro *et al.* (1998) 2D-PAGE proves to be a valuable tool for the differential characterization among closely related *Meuluccius spp* (fish). 2D-PAGE was also used by Van der Beek *et al.* (1998) to study genetic variation among parthenogenetic plant-parasitic root-knot nematode species belonging to the genus *Meloidogyne*.

Surprisingly our results show a great inter-individual variability in protein profiles in 2D-PAGE. Theses results seem to corroborate the hypothesis of a restricted gene flow between populations of *H. diversicolor* rather than short term selection because in that case the inter-individual variability would be very low.

Two-dimensional electrophoresis allowed us to determine both the isoelectric points and molecular weights of the major water-soluble proteins and to perform protein characterization in the polychaete annelid *H. diversicolor*. Moreover, this data-base will now be used as a powerful tool to identify proteins in which synthesis is regulated in various experimental situations, in particular in toxicity test, since this species seems to be a good candidate for laboratory studies to test the toxicity of pollutants (Scaps *et al.* 1997).

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