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Eye lens SDS-Page electrophoresis and the systematics of Cephalopods

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Résumé : Les protéines du cristallin des Céphalopodes ont été analysées par électrophorèse en milieu dénaturant (SDS-Page), en vue de tester leur pouvoir discriminant à différents niveaux taxonomiques. Tous les individus appartenant à la même espèce (dix espèces, représentant cinq genres) présentaient toujours un certain nombre de bandes communes. La comparaison des images électrophorétiques obtenues pour chaque espèce a permis la discrimination aux différents niveaux taxonomiques, de l'ordre à la population. Le dendrogramme de similarité souligne la puissance de cette technique. Ainsi, l'analyse des protéines cristallines par électrophorèse en milieu dénaturant peut être utilisée comme critère taxonomique supplémentaire et semble pouvoir distinguer aussi des populations non inter-fécondes.

Abstract : Eye lens proteins of cephalopods were tested by SDS-Page electrophoresis, for their discriminating value at different taxonomic levels. All individuals belonging to the same species (ten species representing five genera) always displayed a certain number of identical bands. The comparison of the electrophoretic patterns obtained with each species allowed discrimination at the different taxonomic levels, from the order to the population. The similarity dendrogram illustrates the discriminating power of the technique. Thus, eye lens protein SDS-Page electrophoresis can be used as an additional taxonomic criterion and appears to discriminate non interbreeding populations.

INTRODUCTION

Cephalopod taxonomy is established on classical identification criteria : anatomy, morphometry, observation of beaks, suckers, spermatophores, radula etc. (Roper & Voss, 1983). It allows identification to the species level, but not necessarily to the subspecies or population levels. Voss (1977) pointed out that 50 % of genera are monospecific, and 85 % are represented by 5 species or fewer. On the other hand, some genera, like *Octopus* or *Sepia*, include over 100 species, some of which are probably subspecies or closely related species. New criteria are needed at all taxonomic levels, but especially at the infraspecific level. Roper (1983) pointed out that the 4 major families of Cephalopods (Sepiidae, Loliginidae, Ommastrephidae, Octopodidae), which comprise at least 90 % of the world fishery catches, are in critical need of modern, comprehensive, systematic studies.

The electrophoretic techniques lead to the identification of molecular differences, which in turn reflect genetic variation. They have been used in many instances to study population variations, among others in bivalve tissues (Torigoe & Inaba, 1975), and in fish eye lens (Smith, 1962, 1965, 1966, 1967, 1969a ; Barret and Williams, 1967 ; Bon *et al.*, 1968 ; Cobb *et al.*, 1968). The eye lens is a highly specific organ, better suited for taxonomic purposes than muscle tissue (Boucher-Rodoni *et al.*, 1989). Its transparency requires a high

concentration of proteins, 95 % of which are crystallins, water soluble, structural proteins, stable throughout life and as old as the animal itself (Bon *et al.*, 1966 ; Smith, 1969b ; Delaye and Tardieu, 1983 ; De Jong & Hendriks, 1986).

The cephalopod eye is a classical example of convergent evolution between vertebrates and invertebrates ; their eye lens proteins have often been compared (Bon *et al.*, 1967, Smith, 1969b ; Dohrn, 1970 ; Swanborn, 1971). The results showed no immunological identity between cephalopods and vertebrates (Wollman *et al.*, 1938 ; Halbert and Fitzgerald, 1959). Recent thorough biochemical investigations point out that, although endowed with the same physiological function and often named α, ß and δ as in fish (Bon *et al.*, 1967 ; Swanborn,1971), the cephalopod lens crystallins are different from those of vertebrates (Siezen & Shaw, 1982 ; Chiou, 1984).

Smith (1969b) and Swanborn (1971) showed that electrophoretic techniques can be used on cephalopod lens for taxonomic purposes, octopods and decapods being easily distinguished. Smith (1969b) suggested that the technique might also distinguish non-interbreeding populations.

This study is a first attempt to test the discriminating power of cephalopods eye lens proteins at different taxonomic levels, using electrophoretic separations obtained from various cephalopod species belonging to the three main orders of Coleoidea, as defined by most modern systematists : Sepioidea, Teuthoidea and Octopoda.

MATERIALS AND METHODS

Ten species representing five genera were studied. Most of them were caught off Roscoff (R) and Banyuls-sur-mer (B) by trawling, and *Loligo forbesi* by jigging off Roscoff. They arrived alive at the laboratory. The lenses were dissected out at once and immediatly dipped into liquid nitrogen, then stored at - 20°C. The two species from the Falkland (F) islands area were caught by industrial trawlers, and their conditions of freezing and storing before arriving at the laboratory are unknown.

Different individuals of each species were used for the electrophoretic investigation of eye lens proteins : 17 *Sepia officinalis* (SO) : 8 from Roscoff (SOR), 9 from Banyuls (SOB), 3 *Sepia orbignyana* (SYB), 3 *Sepia elegans* (SEB), 3 *Loligo vulgaris* (LVB), 3 *Loligo forbesi* (LFR), 6 *Loligo patagonica* (LPF), 1 *Moroteuthis ingens* (MIF), 2 *Octopus vulgaris* (OVB), 2 *Eledone moschata* (EMB), 2 *Eledone cirrhosa* (ECB).

Each eye lens was homogenized at 4°C in 0.02M Tris-HCl buffer, pH 7 (9ml buffer for 1g tissue). The supernatant from 30 min centrifugation at 15 000 rpm was recovered. One aliquot was diluted ten times. Both concentrated and diluted samples were stored at -20°C. The entire lens (cortex and nucleus) was used, in order to avoid a source of variability related to the difficulties of dissecting small pieces.

Before running the electrophoresis, the protein concentration of each sample was determined (Bradford, 1976), to choose the optimal quantity of extract for the best resolution.

The same protein concentration was then used for each species, by adjusting the quantity of sample loaded in the electrophoretic wells.

To each extract was added a sample buffer: Tris-HCl 0.625M, 2 mercaptoethanol 5 %, SDS 0.2 %, sucrose 10 %, Bromophenol blue. SDS-polyacrylamide gel electrophoresis was then performed according to the method of Laemmli (1970), using 12.6 % acrylamide (Siezen and Shaw, 1982). A Pharmacia vertical apparatus was used for separation, at 30V per plate for 3 to 5 hours, the temperature being maintained at 7.5 to 8.5°C. In each well, 1.4 (diluted) or 130 (concentrated) µg protein were applied, and the electrophoresis run in the presence of a Tris-Glycine-HCl buffer with 0.1 % SDS, pH 6.8. After migration, the gels were fixed and stained with a Coomassie brilliant blue R250 solution, and electrophoretically destained at 24V for 60 to 90 min. The relative migration of each fraction was measured and the Rf calculated. Molecular weight markers were run in parallel after mixing with the sample buffer, in order to determine the molecular weight of the different fractions.

The presence-absence coefficient of similarity of Sörensen (1948, in Legendre and Legendre, 1979) was calculated to relate species and Rf (concentrated samples). It is a binary similarity coefficient excluding the double absence. It gives a double weight to the double presence :

$$S=2A/(2A+B+C)$$

where A = double presence : B = presence-absence ; C = absence-presence. The intergroup variance was taken as algorithm (Delabre *et al.*, 1973).

RESULTS

After electrophoretic processing of samples at concentrations appropriate to give a good resolution of the minor fractions, a heavily charged zone appeared in the middle region of the electrophoregram of all the species tested. Sample dilution was aimed at giving a better resolution of this region. Concentrated sample electrophoresis revealed a number of polypeptides varying from 30 to 40, according to the species, and their molecular weight ranged from 11 to 173 Kdaltons. Only two bands, 25-30 KD of molecular weight, could be identified in the middle region after dilution.

Prior to a comparative analysis, the eye lens samples were first tested for the reproducibility of their electrophoretic pattern. Left and right eye lens, from different individuals belonging to the same population, show no variability whatever the sex or the maturation stage (Fig. 1). Each population is thus characterized by a constant number of identical bands, as well for concentrated as for diluted sample extracts.

By comparing the results obtained with the different species, all taxonomical levels could be retraced. Teuthoidea and Sepioidea (Decapoda) display many common bands and their electrophoretic pattern is very different from that of Octopoda. Within one family, the electrophoretic technique discriminates the genera, then the species. Figure 2 shows the results obtained with concentrated and diluted samples in the 3 orders. Six main regions (A

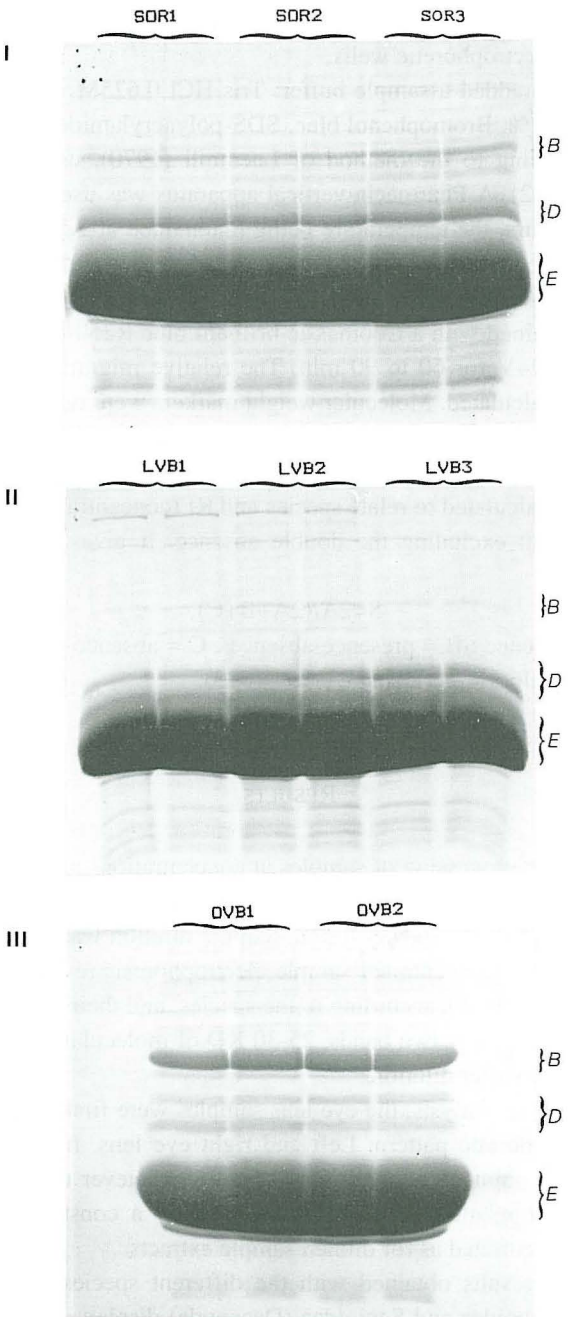

Fig. 1 : Three examples showing the reproducibility of electrophoretic patterns of concentrated samples for a given species. (B, D, E: main regions). I. SOR1, 2, 3 : 3 different individuals of *Sepia officinalis*. II. LVB1, 2,3 : 3 different individuals of *Loligo vulgaris*. III.OVB1,2 : 2 different individuals of *Octopus vulgaris*.

to F) have been arbitrarily recognized in each group. The comparison of the bands in each region confirms the gel observation but this representation gives also a clearer image of the minor variations than the photographic one. In the species *Sepia officinalis*, a further analysis of intraspecific variations is thus possible. If two populations, one from the Mediterranean and the other from the English Channel are compared, they can be identified electrophoretically by variations affecting mainly the bands of the F and D region (Fig. 2). SORA corresponds to an old animal. From this preliminary result, it seems that aging might induce (as in fish) minor changes in the electrophoretic pattern, a few fractions being absent in the D region in SORA.

The dendrogram of similarity illustrates grouping, from the order to the population (Fig. 3).

DISCUSSION AND CONCLUSIONS

The SDS-Page electrophoresis of cephalopod eye lens reveals a large number of polypeptides in all the species. The electrophoretic pattern is identical for all individuals belonging to the same population, regardless of the period of capture, sex, body weight or size of the animal. Cephalopods thus share with fish the congruence of their eye lens electrophoretic patterns (Smith, 1962. Bon *et al.*, 1966). The statistical analysis on the Rf leads to a dendrogram confirming the unaided observation of the gel plates. Its advantage is a rapid classification of rough results from all the species.

Molecular variations with no genetic base can appear, however, as the result of poor material preservation (Smith, 1969b ; Ferguson, 1980). Protein degradation might explain why several authors reported from cephalopod lens many cristallin classes, as in vertebrates (Bon *et al.*, 1967 ; Dohrn, 1970 ; Brahma, 1978 ; Brahma & Lancieri, 1979), whereas only one class was described more recently (Siezen & Shaw, 1982 ; Chiou, 1984). The absence of certain polypeptides in the Falkland Islands material (Fig. 2 :*) might likewise be due to uncontrolled freezing conditions. Immediate processing, or immediate and rapid freezing, is essential for reliable electrophoretic analysis.

The analysis of the results obtained here with the different species tested shows that the electrophoretic analysis of cephalopod eye lens proteins agrees with the classical taxonomy of the class ; moreover it may be adequate for distinguishing separate breeding populations. The similarity is higher between Sepioids and Teuthoids than between Sepioids or Teuthoids and Octopods. This is in agreement with the results obtained by immunoelectrophoretic methods (Brahma & Lancieri, 1979 ; Boucher-Rodoni *et al.*, 1989), and with the phylogenetic position, Young (1977) and Donovan (1977), considering Teuthoids and Sepioids as having a common ancestor, distinct from that of Octopods.

Cephalopods represent an important potential resource ; their systematics, ecology and populations biology should be studied as in all other fisheries species (Roper, 1983 ; Amaratunga, 1987 ; Saville, 1987). Because of rapid growth, cephalopods have life spans

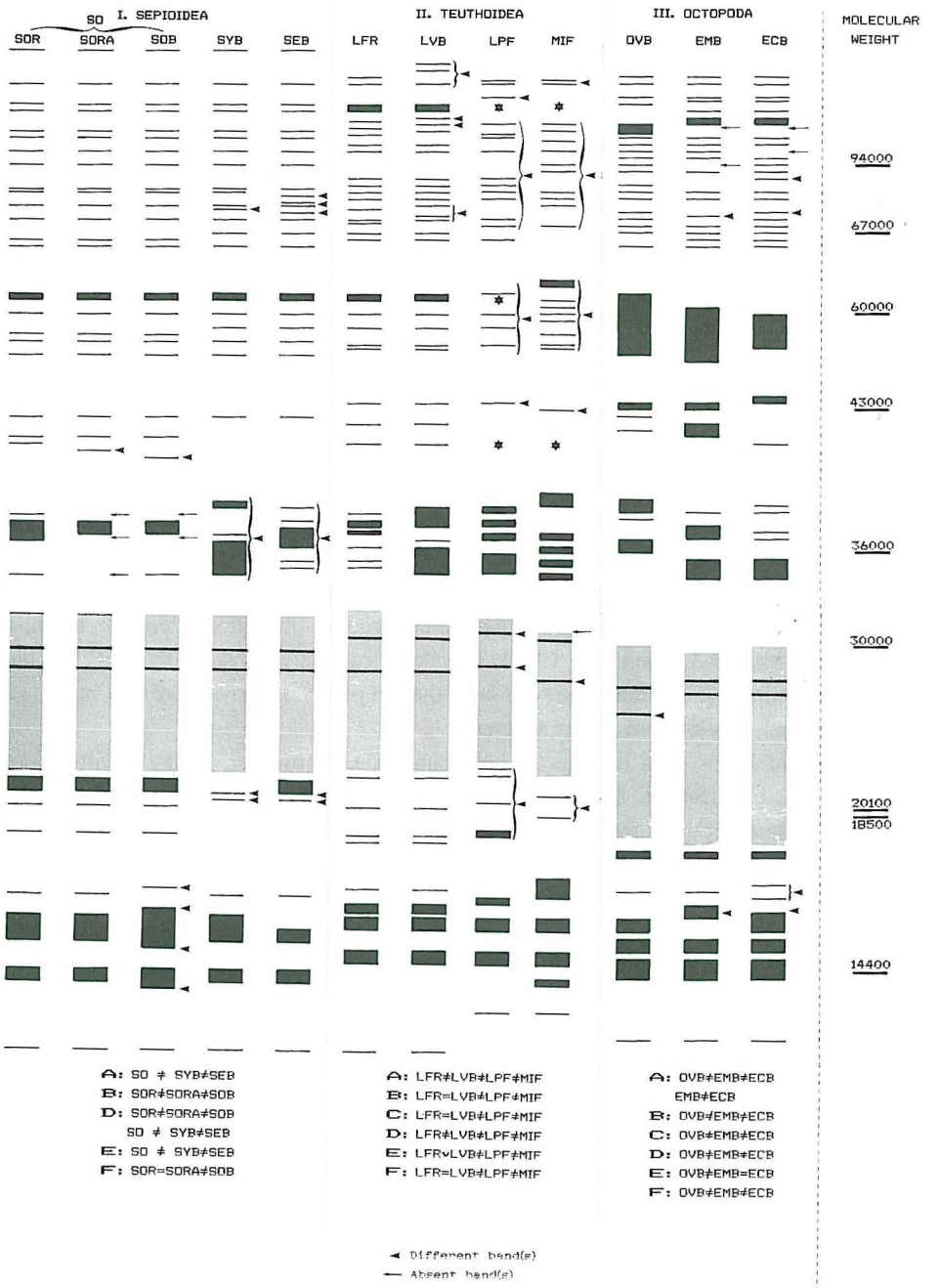

Fig. 2 : Overview of the electrophoretic patterns from different species and populations, allowing an intraspecific and interspecific comparison.

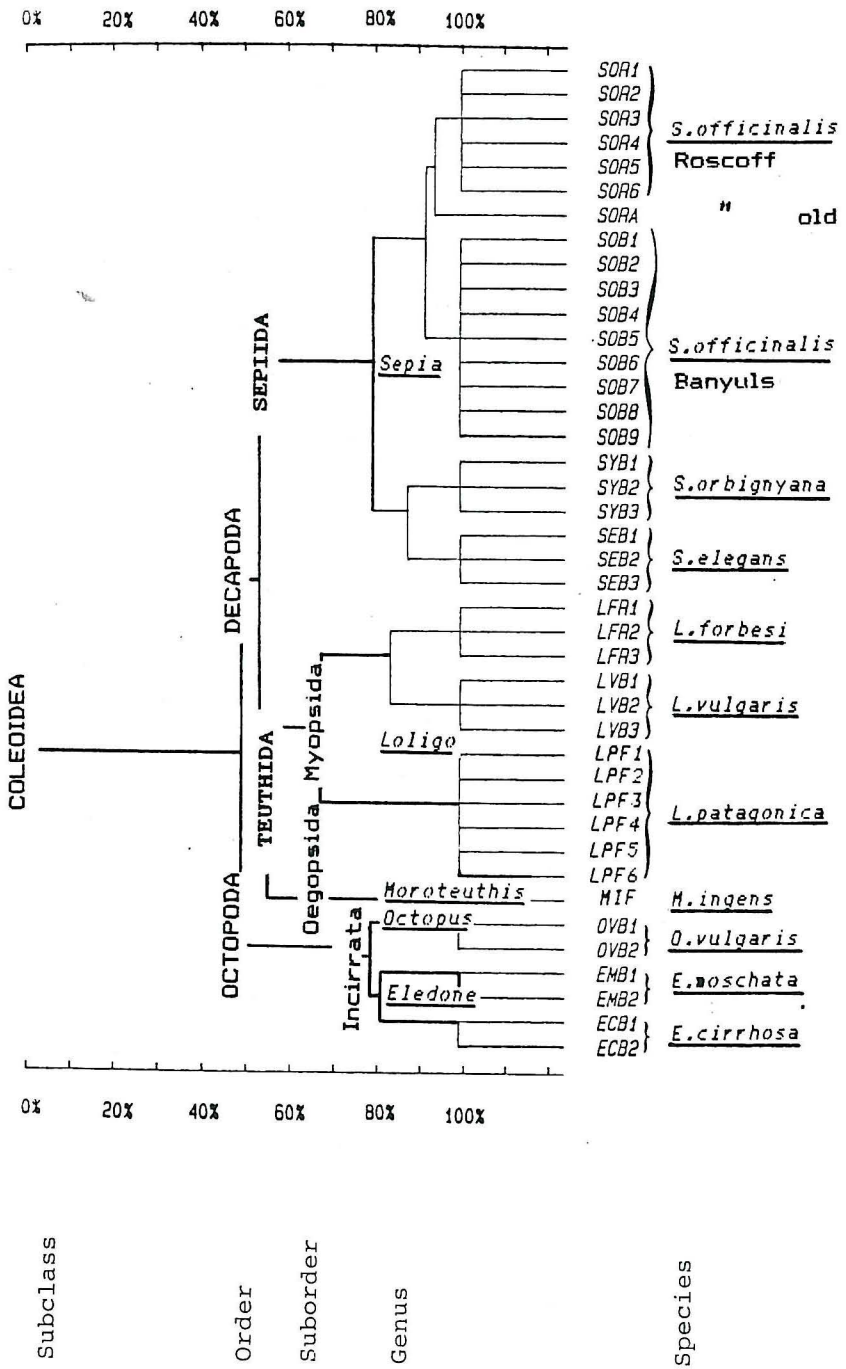

Fig. 3 : Dendrogram of similarity (Sorensen), calculated from the Rf values of all the bands.

that usually are short ; in general the females reproduce only once (Mangold, 1987). Our results confirm that electrophoretic analysis of cephalopod lens proteins is a simple, reproducible and rapid method for both speciation and intraspecific discrimination studies. This could be of importance for cephalopod stock identification, preservation and exploitation. It is thus a method of interest for taxonomists and for fishery biologists.

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