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Genetic differentiation of *Echinocardium cordatum* as revealed by allozymes and RNA sequencing

Jean-Pierre Féral, Élie Poulin, Évelyne Derelle, Sylvie Gallardo & Cécile Chambon
Observatoire Océanologique, Université Pierre et Marie Curie, CNRS URA, Banyuls-sur-Mer, France

This work is a contribution to the study of possible consequences of developmental type and dispersal capacity on the genetic structure and the evolution in echinoids. The Antarctic brood-protecting genus *Abatus* was chosen as a model for non-dispersing species (Poulin & Féral 1994) and the planktotrophic genus *Echinocardium* for disperser species. Although apparently well defined morphologically, two nominal species *E. cordatum* and *E. fenauxi*, described from the Atlantic and the Mediterranean, respectively, present intermediate forms (Laurin et al. 1994). Individuals from the Atlantic (Brittany and Galicia) and from the Mediterranean (Catalunya) were used (Fig. 1), according to a 3 level hierarchical sample (seas / regions / localities) for a pilot study. The aim of this study was to evaluate the geographical scale of genetic differentiation in the case of a planktotrophic species. Individuals from populations exhibiting typical and non-typical morphological characters were used.

Among 12 tested loci, 4 polymorphic loci [PGI, 3 alleles; MDH1 and MDH2, 4 alleles; PGM, 3 alleles] proved useful.

The genetic differentiation was quantified with the F-statistics of Wright. FST were 0.0119 for Brittany / Galicia and 0.2444 for Atlantic / Mediterranean, respectively. Significance was improved by a 1000 permutation test. Results show that firstly there is no significant genetic differentiation between the Atlantic sites. The scale of differentiation is of the order of

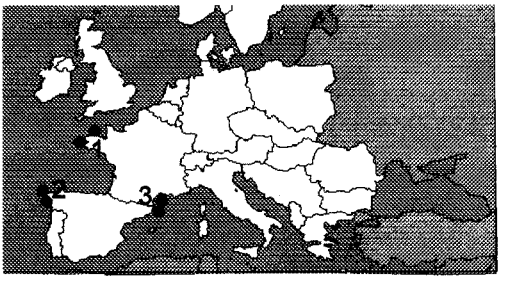

Figure 1. Sampling sites for *Echinocardium cordatum* (1-Britanny, N=89) and for intermediate forms of *E. fenauxi?* (2-Galicia, N=66, 3-Catalunya, N=88).

1000 km, whereas it was of only 10 km in the brooding *Abatus* (Poulin & Féral 1994). The results also show that there is a strong genetic differentiation between Atlantic and Mediterranean populations. Two major groups are discriminated, one in the Mediterranean Sea, the other in the Atlantic Ocean. The samples belong either to very differentiated populations of the same species, or to two very closely related species. This is clearly shown by the tree built with the Nei's genetic distance (Fig.2).

To confirm the status of our samples, we used 28S ribosomal RNA sequencing, which seemed to discriminate at the species level in schizasterids (Féral et al. 1994a) and in annelids (Féral et al.

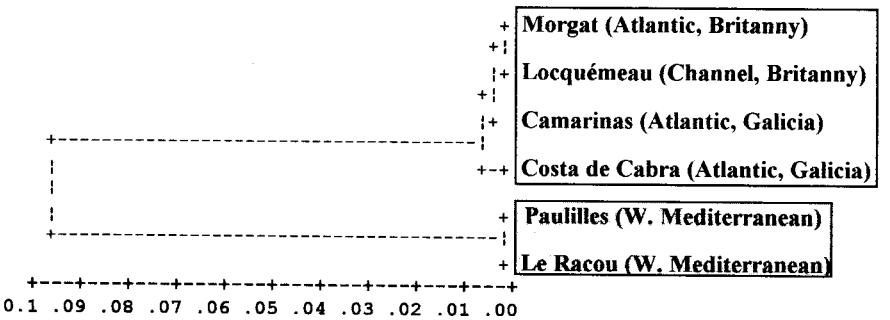

Figure 2. Dendrogram of the six samples of *Echinocardium* separeted by Nei's unbiased minimum genetic distance calculated from the four polymorphic loci.

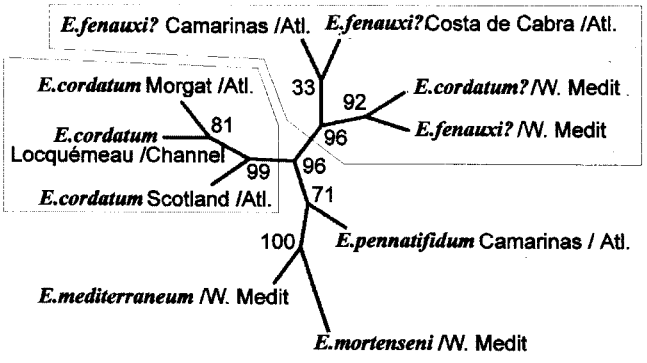

Figure 3. Bootstrapped parsimony tree (DNABOOT) inferred from the aligned sequences of D1, D2 and D8 divergent domains of the 28S rRNA (21 informative positions, 3 equiprobable trees, 25 steps, 1000 bootstrap replicates)

1994b). We used the aligned sequences of 3 diver gent domains (D1, D2, D8) in order to infer phylogenetic trees. We studied one individual of each from the 6 sites considered for genetic purposes. We added one individual from Scotland (a morphologically typical *E. cordatum*) and we used other *Echinocardium* species as an outgroup. Rather small differences in the sequences (1 to 3 nucléotides) of geographically close individuals led us to suspect the possibility of a polymorphism in the rRNA among individuals of the same site. In this case, the used domains of rRNA are not specific markers usable for large disperser species whose effective population sizes are enormous. Besides this polymorphism, there are two clusters:

(1) one with typical *E. cordatum* from Northern Atlantic and from the Channel,

(2) another grouping the Mediterranean and the Galician individuals.

The topology of this tree (Fig.3) is very different from the topology of Nei's tree which demonstrated a strong genetic flow between Britanny and Galicia, excluding the possibility of several species (namely *cordatum* and *fenauxi*) in the tested Atlantic populations as suggested by the rRNA tree. However, individuals from Galicia have a sequence very close to that of the Mediterranean ones, indicating a common origin. In fact, allozymes and rRNA are two very different markers. rRNA is a conservative molecule, both in structure and function. Only a few viable mutations are possible. On the contrary, allozymes evolve faster, therefore they would reflect a more recent change and probably reflect the present situation of an interrupted gene flow at the level of the Strait of Gibraltar. rRNA would keep the trace of earlier events of colonization.

To evaluate the polymorphism, we had to analyse the RNA of several individuals per site. We found one individual from Galicia whose sequence of D8 was similar to the Atlantic specimens. That means that both sequences, from the Atlantic and from the Mediterranean, are present in Galicia. This supports the hypothesis of a continuing reproductive compatibility between two populations which have certainly separated for a period, but not for a sufficiently long time to speciate. Therafter, the gene flow rehomogenized the Atlantic population.

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