

Geographic and genetic differentiation of *Echinocardium cordatum* (Pennant) – The current state of a complex question: The status of *Echinocardium fenauxi* (Péquignat)

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ABSTRACT: *Echinocardium cordatum* was taken as an exemplar of indirect development within the Spatangoida. Investigation of its genetic structure showed that there was no significant genetic differentiation in this spatangoid among Atlantic sites, more than 1000 km apart. However, there was strong genetic differentiation between Atlantic sites and Mediterranean ones. In spite of this, morphologists noted that individuals from some Atlantic and Mediterranean sites shared common morphological characters with *Echinocardium fenauxi*. To clarify the status of this species, we have collected specimens in the type area of *E. fenauxi* (vicinity of Marseille). The GPI locus gave 2 very different patterns: 1) a typical one from *E. cordatum*, common to all *E. cordatum* studied from Golfe du Lion, Galicia, Brittany, Scotland; and 2) a totally different one, typical of some *Echinocardium* collected in Marseille. The fact that no individual from Marseille showed a combination of alleles from both patterns strongly suggests reproductive isolation between the two groups. In addition to differences in structure of the protein, this leads us to consider GPI as a diagnostic locus for a species of *Echinocardium* different from *E. cordatum*. However, this new taxon cannot unequivocally be assigned to *E. fenauxi* because of the lack of an unambiguous and well-supported morphological diagnosis.

The present work was done within the framework of a larger study dealing with the consequences of developmental mode and dispersal capacity in the genetic differentiation of marine benthic invertebrates. We are using two models from among the echinoderms. One is *Abatus cordatus*, a brooding direct developer (Poulin & Féral, 1994, 1996 and this volume). The other is *Echinocardium cordatum*, an indirect developer whose planktotrophic larvae may live several weeks in the water column before metamorphosis.

Echinocardium cordatum lives burrowed in different types of fine to coarse sediment, five to twenty cm below its surface. To estimate the scale of geographic differentiation, we collected animals by SCUBA diving in three places lying about 1000 km apart from each other, in the Golfe du Lion (Western Mediterranean), in Galicia (Atlantic) and in Brittany (Atlantic and Channel), respectively. Among the 12 tested enzymatic activities, 4 loci were polymorphic and interpretable:

- GPI, glucose phosphate dehydrogenase (EC 5.3.1.9),
- MDH-1 and MDH-2, malate dehydrogenase (EC 1.1.1.37)
- PGM, phosphoglucomutase (EC 2.7.5.1).

In Féral *et al.* (1995), it was shown that (1) there was no significant genetic differentiation between Atlantic sites (the scale of geographical differentiation of *Echinocardium cordatum* being at least on the order of 1000 km) and (2) there was a strong genetic differentiation between Atlantic and Mediterranean sites.

These results must be contrasted with those of the morphologists (Laurin *et al.* 1994, David & Laurin 1996, David & Laurin, *pers. comm.*), since they supposed the presence of more than one species in the Golfe du Lion and in Galicia. For instance, individuals from both sites showed common morphological characters with *Echinocardium fenauxi*. Some were "more *cordatum*", whereas some were "more *fenauxi*". The existence of such polytypism in *Echinocardium cordatum* was hypothesized in Laurin *et al.* (1994). Considering that each population (from the Atlantic: Galicia and from the Mediterranean: Golfe du Lion) is genetically homogenous, and that intermediary morphologic characters coexist, the question arises: is *Echinocardium fenauxi* a true species?

Péquignat (1963, 1964) described *Echinocardium fenauxi* from the Ligurian Sea. He used specimens

collected at Noli (near Genova, Italy), at Villefranche-sur-mer and Marseille (France). If typical forms of both *E. cordatum* and *E. fenauxi* are easily distinguishable, criteria given by Péquignat are not useful to identify intermediary [non-typical] forms. Among these characters, Péquignat underlined the often-greater size of *E. fenauxi* and the fact that this species could live in very coarse sediment or even in gravel.

We collected *Echinocardium* in the vicinity of Marseille at 3 places at about 11-12 m depth by SCUBA diving:

- *Monastériou* (North of Ile de Riou), rather fine sand, one sampling
- *Anse de l'Orjol* (Baie d'Endoume), gravel between seagrass beds, one sampling
- Off *Calanque Saint Estève* (NorthEast of Ile Ratonneau), fine to coarse sand, two samplings at several hundred meters apart.

Most of the individuals were between 6 cm and 9 cm in length, which is relatively large compared to the 3.5 cm that *Echinocardium* individuals collected in the Golfe du Lion generally attain, and where a maximum of 6.5 cm in length was observed for very few individuals. Therefore, we collected what would generally be considered *Echinocardium fenauxi* by systematists using Péquignat's (1963, 1964) work.

Among the studied loci, GPI was of great interest (Table 1). This locus gave two very different electrophoretic patterns. One was a dimer, typical from *E. cordatum*, showing 3 alleles. This was the common pattern (called *pattern 100*) of all studied *E. cordatum* from Golfe du Lion, Galicia, Brittany and also Scotland. The other (called *pattern 200*) was totally different from the previous one. It was a monomer and migrated much faster. The two patterns were not distributed randomly. *Pattern 100* was found at the 3 sites whereas all individuals collected during the second dive at Calanque Saint-Estève showed *pattern 200*. The differences between these 2 diving sites were very minor. They were 1 m in depth and some hundred meters apart. The granulometry of the sediment was similar. However, the ripple marks were deeper at the second site indicating a possible difference in hydrological characteristics. A unique individual, 3 cm in length, collected during the first dive at Calanque Saint Estève showed *pattern 200* as was also the case for a single individual (4.8 cm in length) collected at Monastériou. It is concluded that the 2 patterns (*100* and *200*) of GPI are sympatric in the vicinity of Marseille. Only *pattern 100* is typical of *E. cordatum*. The fact that no individual showed a combination of alleles from both *pattern 100* and *pattern 200* strongly suggests reproductive isolation. This, in addition to the difference in structure of the protein (monomer / dimer), led us to consider GPI

Table 1. Allele frequencies of GPI in *Echinocardium* collected in the Golfe du Lion (GdL), Galicia (GAL), Brittany (Atlantic -BA, Channel -BC), Scotland (SCO) and in Marseille. Mediterranean sites are in white, Atlantic sites are in gray.

Populations	GPI Allele					N
	90	100	110	170	200	
Camarinas (GAL)	0.000	0.971	0.015	0.000	0.000	34
Costa de Cabra (GAL)	0.081	0.839	0.081	0.000	0.000	31
Morgat (BA)	0.036	0.928	0.036	0.000	0.000	79
Locquémeau (BC)	0.026	0.947	0.026	0.000	0.000	19
Oban (SCO)	0.017	0.958	0.017	0.000	0.000	59
Mull (SCO)	0.071	0.893	0.036	0.000	0.000	14
Paulilles (GdL)	0.098	0.873	0.029	0.000	0.000	51
Racou (GdL)	0.081	0.865	0.054	0.000	0.000	37
Marseille <i>E. cordatum</i>	0.087	0.887	0.025	0.000	0.000	40
Marseille " <i>E. fenauxi</i> ?"	0.000	0.000	0.000	0.075	0.925	20

as a diagnostic locus for a species of *Echinocardium*, different from *E. cordatum*.

However, this genetically differentiated species cannot presently be considered to be *E. fenauxi*. There is a need to re-evaluate the morphological criteria used by Péquignat. Did he describe a large *E. cordatum*? Does *E. cordatum* represent two morphotypes (Atlantic/Channel morphotype and a so-called *E. fenauxi* morphotype) and a number of intermediary ones (Golfe du Lion, Galicia)? This also requires reliable morphological and architectural criteria to easily distinguish the two (or more?) species. Answer to these questions will then permit us to infer phylogenies in order to evaluate the divergence of this *Echinocardium* species from *E. cordatum* and from the other *Echinocardium* species, using both molecular and morphological criteria.

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REFERENCES

- David, B. & B. Laurin 1996. Morphometrics and cladistics: measuring phylogeny in the sea urchin *Echinocardium*. *Evolution*, 50: 348-359.
- Féral, J.-P., E. Poulin, E. Derelle, S. Gallardo & C. Chambon 1995. Genetic differentiation of *Echinocardium cordatum* as revealed by allozymes and rRNA sequencing. In R.H. Emson, A.B. Smith and A.C. Campbell (eds), *Echinoderm Research 1995 - Proc. 4th European Echinoderm Colloquium, London, 10-13 April 1995*: 41-42. Rotterdam : Balkema.
- Laurin, B., J.-P. Féral, B. David & É. Derelle 1994. Phylogeny of the spatangoid echinoid *Echinocardium* : morphological versus molecular approach. In B. David, A. Guille, J.-P. Féral and M. Roux (eds), *Echinoderms through time - Proc. 8th IEC, Dijon, 6-10 Sept. 1993*: 739-745. Rotterdam.: Balkema.
- Péquignat, E. 1963. Sur un nouvel *Echinocardium* de Ligurie et de Provence: *Echinocardium fenauxi* n. sp.. *Doriana* 3 (138), 9 pp.
- Péquignat, E. 1964. Sur les *Echinocardium* d'Europe. Description d'une espèce nouvelle de grande taille, repérée dans trois localités entre Marseille et Gênes: *Echinocardium fenauxi* Péquignat. *Bull. Inst. Océanogr. Monaco* 62 (1291), 22 pp.
- Poulin, É. & J.-P. Féral 1994. The fiction and the facts of Antarctic incubation - population genetics and phylogeny of schizasterid echinoids. In B. David, A. Guille, J.-P. Féral and M. Roux (eds), *Echinoderms through time - Proc. 8th IEC, Dijon, 6-10 Sept. 1993*: 837-843. Rotterdam.: Balkema.
- Poulin, E. & J.-P. Féral, 1996. Why are there so many species of brooding Antarctic echinoids? *Evolution* 50: 820-830.