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Molecular time scales for evolution in *Mysis* and *Pontoporeia*

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The results of recent allozyme studies addressing the controversial hypotheses on the origin of the 'glacial relict' crustacean species in Europe and North America are reviewed. The data suggest that the time since the divergence of the lineages leading to each of the three currently recognized *Pontoporeia* species (*P. affinis*, *P. hoyi*, *P. femorata*) is of the order of tens of million years; this is in sharp contrast to recent suggestions of a late Pleistocene descent of both the European and North American freshwater species from the marine *P. femorata*. The divergence of the *Mysis relicta* lineage from its present marine congeners is probably younger but also dates back to the Tertiary. *M. relicta* itself comprises a group of several distinct species, two of which coexist in the Baltic Sea.

1. Introduction

The use of the term 'glacial relict crustacean' would seem to involve a reference to a certain fixed view on the zoogeographical or evolutionary history of the organisms concerned. However, views on this history have varied substantially through decades, and have hardly ever been uncontroversial. The unclear situation persists even today; contradictory hypotheses on the topic have most recently been presented by Holmquist (1966, 1970), Dadswell (1974), Segerstråle (1977, 1982) and Salemaa (1984). With the changing views and controversies, the meaning of the term 'relict' has naturally also varied and become obscured, although it seems to have survived as a conventional label for this zoogeographical group.

In all events, most authors now seem to agree that most present freshwater (and Baltic) populations have descended from refugial stocks living along the southern margins of the continental ice sheets of the last glaciation (e.g. Segerstråle 1957, 1962, 1982, Ricker 1959, Dadswell 1974); the geographical details of the dispersal of the species may be in doubt, however (Holmquist 1966). The more severe controversy concerns the origin of the 'relict' species: their degree of relationship to their marine congeners, and the relationship between European and North American freshwater populations (Holmquist 1959, 1966, Segerstråle 1962, 1977). The suggested time scales involved in these relationships range from tens of thousands to tens of millions of years.

We have set up a project to elucidate the history of the 'glacial relict' crustaceans using current methods of population genetics and molecular taxonomy. Through comparisons of the lacustrine and marine species, and of the freshwater populations of the two continents by means of enzyme electrophoresis, an insight into the age of the lineages can be obtained and the various hypotheses on their origin evaluated. On the other hand, the patterns of gene frequency variation within the 'relict' species, as related to the knowledge on the geological history of lake networks, shed light on the more recent dispersal history, potentially revealing stocks derived from disjunct late glacial refugia. Such indications have in fact been unexpectedly strong, as the traditional *Mysis relicta* has turned out to be a complex of several genetically distinct and distributionally different species (Väinölä 1986; R. Väinölä, B. J. Riddoch, R. D. Ward & R. I. Jones, unpubl.).

Here I shall summarize the results obtained by Väinölä (1986) and Väinölä & Varvio (1989) regarding the level of genetic divergence among species of *Mysis* and *Pontoporeia*, and their bearings on the views on the origin of the 'glacial relict' species.

2. Earlier hypotheses on the origin of the 'relict' species

The original treatment of these crustaceans as relicts ('marine glacial relicts') was linked to the idea that the freshwater populations were remnants of a marine fauna in an arctic sea which extended over the present environments at the end of the glacial period (Lovén 1862). For *Mysis*, this meant postulating a recurrent descent of *M. relicta* from the marine species *M. oculata* in various newly isolated freshwater bodies, including the Ancylus Lake stage of the Baltic (e.g. Jägerskiöld 1912, Thienemann 1950). As regards *Pontoporeia*, it was early recognized that the immediate ancestors of lacustrine populations were the arctic estuarine *P. affinis*, even though a relatively recent evolution of *P. affinis* from *P. femorata* in the northern seas was also assumed (e.g. Jägerskiöld 1912, Ekman 1918, Lomakina 1952).

With the increasing knowledge on evolutionary biology, zoogeography and Quaternary geology, the original relict hypotheses gradually proved untenable. The theory of dispersal in (and from) proglacial lakes was adopted to explain the wide distribution of the crustaceans in noninundated areas as well (e.g. Segerstråle 1957, Ricker 1959). Holmquist (1959, 1966, 1970), who revised the taxonomy of *Mysis*, disputed the ideas of ancestor-descendant relationships among extant species, and concluded that the 'relicts' are actually ancient and stable species with a freshwater history probably dating back to the late Oligocene (ca. 25 million years ago). However, a more recent, Pleistocene or Pliocene, origin in arctic seas has still been conjectured by e.g. Ricker (1959) and Dadswell (1974). Both Holmquist (1959, 1966) and Dadswell (1974) also rejected the use of the relict concept in this connection.

As regards the relationship between the North American and Eurasian freshwater populations, the *Pontoporeia*s were regarded up to 1977 as conspecific (*P. affinis*), while *Mysis relicta* still remains formally unsubdivided. Originally the conspecificity did not mean an assumption of a common origin, but Segerstråle (1957, 1962) initially regarded the Pleistocene descent of Old and New World 'relicts' from arctic Siberian ancestors as monophyletic. However, upon the reestablishment of the North American *P. hoyi* as specifically distinct from the European *P. affinis*, Segerstråle (1977) regressed towards the early relict hypotheses, and suggested a recent parallel derivation of the New and Old World freshwater *Pontoporeia* species from the marine *P. femorata*; the date of origin was moved back only the minimum possible time necessary to permit the freshwater distributions to be explained as due to sluicing up into proglacial lakes, i.e., to the beginning of the latest glaciation (some tens of thousand years ago). This view of a recent evolution was also adopted by Salemaa (1984), who from karyological evidence suggested that *P. affinis* (chromosome number n=26) arose from *P. femorata* (n=14) through polyploidization.

3. Allozyme studies on genetic and evolutionary divergence

The phenograms in Fig. 1 illustrate the main features of interspecific relationships in *Mysis* and

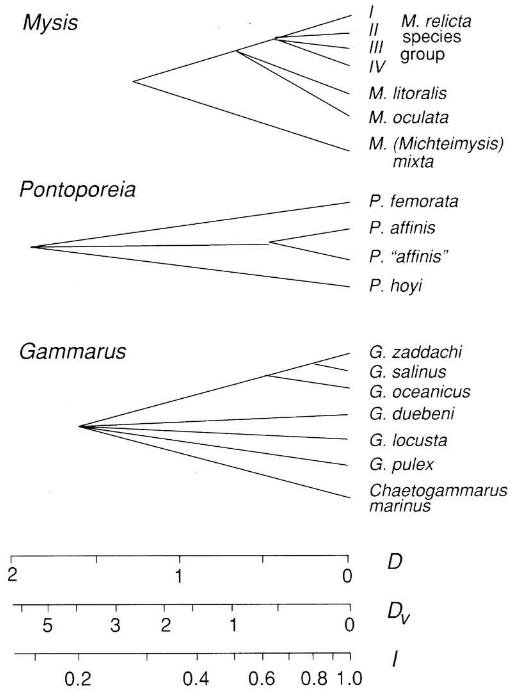

Fig. 1. Schematic presentation of evolutionary relationships in three crustacean groups, as suggested by allozyme studies. — *Mysis* spp.; data from 20 loci (Väinölä 1986 and unpubl. data); *Pontoporeia* spp.; 19 loci (Väinölä & Varvio 1989); Gammaridae; 15–18 loci (Siegismund et al. 1985; Skadsheim & Siegismund 1986). *I* denotes genetic identity, *D* and D_v are measures of genetic distance (see text).

Pontoporeia as suggested by allozyme studies. Three scales of divergence are provided. *I* measures the observed genetic identity (roughly, the proportion of nondifferentiated enzymes among about 20 examined). Distances *D* and D_v are estimates of the true number of electrophoretically observable substitutions per gene, *D* given that the rate of change is the same for all loci, D_v assuming (more realistically) a certain among-locus variance in the substitution rate (Nei 1987). From observations on other animal taxa, a roughly linear relationship between genetic distance and evolutionary time has been suggested, with unit distance, according to various authors, corresponding to 5–20 million years (e.g. Thorpe 1982, Nei 1987). Despite several sources of uncertainty associated with the estimates, the distances probably give a reasonable guide to the order of magnitude of divergence times in crustaceans too. Above $D=1$ the resolution will be poor, however (Nei 1987).

The degree of differentiation between the *M. relicta* group and its closest marine relatives (*M. oculata* and *M. litoralis*) suggests that their divergence took place several million years ago in the Tertiary. Even the four species of the *M. relicta* group may have diverged from one another before the beginning of the Pleistocene ca. 2 million years ago (Väinölä 1986; Väinölä et al. unpubl.).

In *Pontoporeia*, all the three currently recognized nominal species probably diverged tens of millions of years ago (Väinölä & Varvio 1989). Thus, the idea of a recent relationship between the marine *P. femorata* and the lacustrine species (Segerstråle 1977) must be abandoned; a close affinity of *P. affinis* and *P. hoyi*, until recently regarded as conspecific, is also conclusively rejected. A similarly distant relationship was also found between *P. hoyi* and a so far undescribed North American coastal species, *P. "affinis"* (cf. Bousfield 1958, 1989). On the other hand, the latter turned out to be a close relative of the European *P. affinis* (Fig. 1).

Furthermore, no support for the hypothesis of a recent polyploidization event in the *P. affinis* lineage, as put forward by Salemaa (1984), could be found at the level of enzyme gene expression (Väinölä & Varvio 1989). In all, the results of allozyme studies seem to rule out the role of Pleistocene Ice Age phenomena in generating the systematic structure of the genera examined, at least as far as concerns the relationships among the currently formally recognized species.

To illustrate the systematic significance of the observed genetic differentiation, a phenogram based on allozyme studies of the family Gammaridae (Siegismund et al. 1985, Skadsheim & Siegismund 1986) is also included for comparison (Fig. 1). The distances among the nominal *Pontoporeia* spp. are similar to those among genera, but also among many congeneric species in Gammaridae; they are somewhat higher than the distance between two subgenera of *Mysis* (*Mysis* s.str. and *Michteimysis*, Fig. 1).

On the other hand, the divergence between the European *P. affinis* and the North American coastal

P. "affinis", and that among the biochemically identified sibling species in the *Mysis relicta* group, is comparable to that among the morphologically recognized sibling species *G. zaddachi*, *G.oceanicus*, and *G. salinus*, clearly indicating the specific rank of the thus far formally undescribed taxa. The evaluation of the status of these species is not based on the degree of divergence only, however; two species of the *M. relicta* group commonly coexist in the Baltic and also in one lake, with no natural interbreeding, thus meeting the biological species criterion (Väinölä 1986). These systematic interpretations are also in line with comparative data on genetic divergence among taxa in other groups of organisms (e.g. Thorpe 1982).

Finally, the degree and structure of genetic subdivision in *Pontoporeia*, as far as reviewed here, also concur with the new morphological data of Bousfield (1989) presented in this symposium; the three main branches (lineages) of *Pontoporeia* in Fig. 1 correspond with his suggested genera *Pontoporeia*, *Monoporeia* and *Diporeia*.

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