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Genetic relationships between ecologically divergent species of talitrid amphipod (Crustacea)

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ABSTRACT: Allozyme electrophoresis was used to investigate the genetic relationships between several species of the amphipod family Talitridae representative of different ecomorphological groups. The analysis of 21 putative gene loci revealed that in general the species studied have relatively low levels of genetic variability when compared with published data for other crustaceans, including other amphipod species. The genetic identities between the species studied were in general accord with existing taxonomy and in good agreement with the broad relationship expected between taxonomic divergence and genetic identity. Dendrograms produced by UPGMA and Wagner clustering procedures differed in the position of 1 species. However, both dendrograms suggested that species of the genera *Orchestia* (beachfleas) and *Talorchestia* (sandhoppers) are more closely related to each other than to *Talitrus saltator* (also a sandhopper), and hence the results did not show a clear genetic divergence between the different ecological groups of talitrids. The analyses supported the taxonomic decision of previous authors to remove the genus *Hyale* from the family Talitridae. However, the levels of genetic divergence found within the family are too low to reconcile readily with the proposed radiation of the group during the Cretaceous period and indicate that the talitrids probably evolved considerably more recently.

KEY WORDS: Talitrids · Genetics · Allozymes · Amphipods

INTRODUCTION

Talitrid amphipods, along with the larvae of seaweed flies, are the dominant invertebrates of macrophytic detritus (wrack) cast up on the strand line of both soft and hard shores. These groups accordingly act as the most important macrofaunal consumers of cast algae on temperate shores of both the northern and southern hemispheres (e.g. Griffiths & Stenton-Dozey 1981, Moore & Francis 1985). Talitrids may be the predominant consumers of algal floating wrack (aevja) (Robertson & Lucas 1983), of *Spartina* spp. litter in saltmarshes (Levinton et al. 1977) and of turtle grass debris in the tropics (Venables 1981). Detritus from the breakdown of the strand line may constitute an important source of food intertidally, for instance for the fauna of sandy beaches, while derived nutrients may contribute to primary production in nearshore areas (e.g. Griffiths & Stenton-Dozey 1981, Koop et al. 1982a, b). Talitrids are early colonisers of strand debris and may reach extremely high population densities (e.g. Griffiths & Stenton-Dozey 1981) and levels of production (Venables 1981). They are exploited by a large range of predators, including fish, birds, mammals and other wrack invertebrates (reviewed by Wildish 1988). The family Talitridae exhibits many adaptations for semi-terrestrial and terrestrial life, and is unique among amphipods in having fully terrestrial representatives (Hurley 1959). In those parts of the world where they occur, euterrestrial talitrids (landhoppers) may play a dominant role in the exploitation of land-plant debris in forest-floor leaf litter communities which parallels that of their maritime counterparts (Bousfield 1984).

The Talitridae, with more than 200 described species, constitute the largest family within the superfam-

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ily Talitroidea (Bousfield 1982). Despite advances in taxonomy and many additional distributional records in recent years, major problems remain with the systematics of the group. From distributional data it has been concluded that the Indo-Pacific region of the southern hemisphere is the epicentre of talitroid species diversity (Bousfield 1984). Bousfield proposed that talitrids evolved during the Cretaceous Period and early Cenozoic Era from aquatic ancestors, allied to primitive genera of the family Hyalidae; the break-up of Pangaea into many continents, providing new coastal regions, and the later evolution of angiosperm forests, providing food resources, stimulated the geographical and evolutionary radiation of the talitrids. Bousfield (1984) also subdivided the family Talitridae into 4 loose systematic-ecological groups: palustral species, which he considered morphologically primitive and which occur in marine, estuarine, and some freshwater habitats; beachfleas, which are more advanced species, occur in supralittoral habitats and coastal rain forests, and are non-substrate-modifying; sandhoppers, which are supralittoral substrate-modifying (burrowing) species found on sandy beaches; and landhoppers, which are specialised terrestrial species occupying forest leaf litter.

These commonly used ecomorphological sub-groups of the family are informal and probably polyphyletic (Bousfield 1982, 1984). For instance, within the landhoppers Bousfield recognised 2 assemblages, simplidactylate and cuspidactylate forms, with separate evolutionary origins. According to Bousfield (1984) the simplidactylate landhoppers evolved from palustral ancestors in the southern hemisphere, and the cuspidactylate landhoppers are thought to be derived from beachflea ancestors of tropical and warm-temperate coastal regions.

Recently some attention has been given to phylogenetic relationships within talitrids, although none of the work has used any molecular techniques. From a cladistic analysis of gammaridean morphology Kim & Kim (1993) concluded that the Talitroidea were monophyletic. Lindeman (1991) used phenetic and cladistic analysis of morphological data in a phylogenetic study of neotropical landhoppers and Moore et al. (1993) used the morphology of the antennary gland exit duct in an ecological series of talitroids to assess whether the differences in form support Bousfield's hypothesis.

The study of enzyme variation has long been used as a tool in population genetics and also to provide taxonomic information on a wide range of organisms including many invertebrates (reviewed by, e.g., Thorpe & Solé-Cava 1994; see Stewart 1993 for review of amphipod data). The few such studies of talitrids (McDonald 1985, 1987, 1991, Bulnheim & Scholl 1986, De Mathaeis et al. 1994) were not concerned with phylogeny. The present study investigates genetic relationships between some of the talitrid ecological groups defined by Bousfield (1984). To put relationships within the family in context, additional comparisons are made within the superfamily Talitroidea and with one non-talitroid amphipod species.

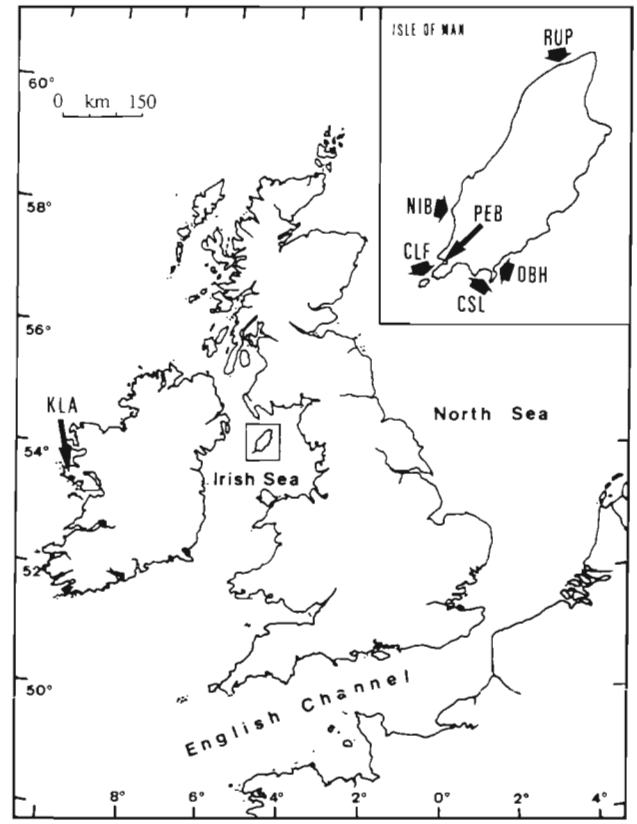

Fig. 1. British Isles: inset shows Isle of Man sampling sites. Site names are given in full in Table 1

MATERIALS AND METHODS

Sampling and collecting sites. The landhopper *Arcitalitrus dorrieni* was collected amongst leaf litter. All other talitroids and *Gammarus crinicornis* were taken from the intertidal and supralittoral regions of sandy or rocky shores, and were collected among algae or sand. Samples were maintained alive in the laboratory until required for electrophoresis. The species studied and the sampling sites (Fig. 1) are listed in Table 1.

The choice of the 2 non-talitrid species for comparisons outside the Talitridae followed from previous work. Thus, an intertidal species of *Hyale* was used for comparison as it is regarded as ecologically analogous and phylogenetically close to the probable ancestor of the Talitridae (Bousfield 1984). The genus *Hyale* has

Table 1. Talitrids and other amphipods used in this study, and the ecological type (for talitrids only; after Bousfield 1984) and sampling locality (see Fig. 1) for each species

Species	Ecological type	Family	Sampling site	Location
Talitrus saltator (Montagu, 1808)	Sandhopper	Talitridae	Derbyhaven (DBH)	Isle of Man
Talorchestia deshayesii (Audouin, 1826)	Sandhopper	Talitridae	Castletown Bay, Langness (CSL)	Isle of Man
Orchestia gammarellus (Pallas, 1766)	Beachflea	Talitridae	Niarbyl (NIB)	Isle of Man
Orchestia mediterranea Costa, 1857	Beachflea	Talitridae	Calf of Man (CLF)	Isle of Man
Arcitalitrus dorrieni (Hunt, 1925)	Simplidactylate landhopper	Talitridae	Kylemore Abbey (KLA)	Co. Galway, Ireland
Hyale nilssoni (Rathke, 1843)	–	Hyalidae	Port Erin Bay (PEB)	Isle of Man
Gammarus crinicornis Stock, 1966	–	Gammaridae	Rue Point (RUP)	Isle of Man

been used to define the plesiomorphic state for the phylogenetic analyses of talitrid morphological characters by Bousfield (1984), Lindeman (1991), and Moore et al. (1993). It was also taken as the starting point in the review of physiological adaptations of talitrids to semi-terrestrial and terrestrial environments by Spicer et al. (1987). The non-talitroid family Gammaridae has been considered to include a pool of species representing the most primitive kind of gammaridean amphipod (Barnard 1974), although this view is by no means universally held (e.g. Bousfield 1984). The well-studied genus *Gammarus* has been the subject of many electrophoretic investigations (see Stewart 1993), and a species of the genus was considered to represent the typical condition of aquatic gammarideans in the comparative study of talitrids by Moore et al. (1993).

Table 2. The 13 enzyme systems scored in all species in this study, with E.C. numbers, buffers used, and number of loci studied. See 'Materials and methods, Electrophoretic analysis' for numbering of buffer systems

Enzyme (abbreviation)	E.C. number	Buffer system	No. of loci
Acid phosphatase (ACP)	3.1.3.2	2	1
Aspartate aminotransferase (AAT)	2.6.1.1	2	2
Diaphorase (DIA)	1.8.1.4	2	3
Fumarase (FUM)	4.2.1.2	1	1
Glucose-6-phosphate isomerase (GPI)	5.3.1.9	1	1
Hexokinase (HK)	2.7.1.1	1	1
Lactate dehydrogenase (LDH)	1.1.1.27	1	1
Leucine aminopeptidase (LAP)	3.4.11.1	3	2
Malate dehydrogenase (MDH)	1.1.1.37	1	3
Malic enzyme (ME)	1.1.1.40	3	2
Mannose-6-phosphate isomerase (MPI)	5.3.1.8	1	1
Peptidase (PEP)	3.4.13.11	3	2
Phosphoglucomutase (PGM)	5.4.2.2	1	1

Electrophoretic analysis. Electrophoresis followed standard methods (Harris & Hopkinson 1976, Murphy et al. 1990). The homogenisation buffer used was 0.2 M Tris-HCl pH 8.0. The homogenates were centrifuged at 3000 × *g* for 3 min. Full details are given in Conceição (1995).

The buffer systems utilised were (1) Tris-citrate, pH 8.0 (Siciliano & Shaw 1976), (2) discontinuous Tris-borate-citrate, pH 8.2–8.7 (Poulik 1957), and (3) lithium hydroxide, pH 8.15–8.30 (Shaklee & Keenam 1986). Thirty-one enzyme systems were tested, of which 13 could be scored in all species (see Table 2). *Talitrus saltator* was used as a standard for the measurement of the relative mobilities of alleles for all species.

The program BIOSYS-1 (Swofford & Selander 1981) was used to estimate the variability within species and genetic differentiation among species. Nei's (1978) unbiased genetic distance was used to construct a UPGMA phenogram (Sneath & Sokal 1973), and the Prevosti distance (Wright 1978) was employed to estimate the phylogenetic relationship through the Wagner procedure (Farris 1972).

Goodness of fit statistics were chosen as the primary criteria for evaluating which tree-building method produced the better tree. The statistics employed were: the *f* of Farris (1972), the *F* of Prager & Wilson (1976), the percent standard deviation (*%SD*) of Fitch & Margoliash (1967), and the cophenetic correlation (*CC*) of Sneath & Sokal (1973). These statistics are intended to estimate the degree to which the output (tree) distances between taxa reflect the corresponding input distances, indicating the amount of homoplasy between a pair of taxa (Farris 1972), and are often used to choose between alternative trees generated by different methods (Swofford 1981).

Table 3. Allele frequencies in the 7 amphipod species used in this study. (1) *Talitrus saltator*, (2) *Talorchestia deshayesii*, (3) *Orchestia gammarellus*, (4) *Orchestia mediterranea*, (5) *Arcitalitrus dorrieni*, (6) *Hyale nilssoni*, (7) *Gammarus crinicornis*. N shows the number of specimens used

Locus, Alleles	Species 1	2	3	4	5	6	7
*GPI**							
(N)	(59)	(70)	(48)	(33)	(62)	(45)	(39)
39	0.000	0.000	0.000	0.000	0.000	0.024	0.000
56	0.000	0.000	0.000	0.000	0.000	0.976	0.000
58	0.000	0.000	0.010	0.045	0.000	0.000	0.000
67	0.000	0.336	0.990	0.955	0.000	0.000	0.000
80	0.000	0.000	0.000	0.000	0.000	0.000	0.064
85	0.008	0.664	0.000	0.000	0.000	0.000	0.000
100	0.898	0.000	0.000	0.000	0.000	0.000	0.910
113	0.000	0.000	0.000	0.000	1.000	0.000	0.026
115	0.093	0.000	0.000	0.000	0.000	0.000	0.000
*PGM**							
(N)	(49)	(62)	(48)	(31)	(65)	(48)	(45)
65	0.000	0.016	0.000	0.000	0.000	0.000	0.000
80	0.000	0.435	0.000	0.000	0.000	0.000	0.000
87	0.020	0.540	0.000	0.000	0.000	0.000	0.000
90	0.112	0.008	0.000	0.032	0.000	0.000	0.000
95	0.000	0.000	0.000	0.000	0.000	1.000	0.000
100	0.837	0.000	0.990	0.839	0.979	0.000	0.000
102	0.000	0.000	0.000	0.000	0.000	0.000	0.100
105	0.000	0.000	0.000	0.000	0.021	0.000	0.000
108	0.000	0.000	0.000	0.000	0.000	0.000	0.900
110	0.031	0.000	0.010	0.129	0.000	0.000	0.000
*MPI**							
(N)	(30)	(42)	(47)	(30)	(62)	(45)	(39)
85	0.000	0.000	0.160	0.050	0.000	0.113	0.000
90	0.000	0.000	0.000	0.000	1.000	0.000	0.000
95	0.000	0.988	0.830	0.950	0.000	0.000	0.000
98	0.000	0.000	0.000	0.000	0.000	0.000	0.038
100	1.000	0.012	0.011	0.000	0.000	0.887	0.000
102	0.000	0.000	0.000	0.000	0.000	0.000	0.115
105	0.000	0.000	0.000	0.000	0.000	0.000	0.692
110	0.000	0.000	0.000	0.000	0.000	0.000	0.154
*AAT-1**							
(N)	(35)	(50)	(48)	(33)	(65)	(48)	(45)
−50	0.000	0.000	1.000	1.000	0.000	0.000	0.000
−20	0.000	1.000	0.000	0.000	0.000	0.000	0.000
−15	0.000	0.000	0.000	0.000	0.000	0.000	0.022
0	0.014	0.000	0.000	0.000	0.000	0.000	0.000
60	0.000	0.000	0.000	0.000	0.000	0.008	0.967
70	0.000	0.000	0.000	0.000	1.000	0.000	0.000
100	0.986	0.000	0.000	0.000	0.000	0.000	0.000
125	0.000	0.000	0.000	0.000	0.000	0.000	0.011
130	0.000	0.000	0.000	0.000	0.000	0.992	0.000
*AAT-2**							
(N)	(35)	(50)	(48)	(33)	(65)	(48)	(44)
90	0.000	0.000	0.000	0.000	0.000	1.000	0.000
100	1.000	1.000	0.000	0.000	0.000	0.000	1.000
105	0.000	0.000	0.000	0.000	1.000	0.000	0.000
110	0.000	0.000	1.000	0.000	0.000	0.000	0.000
115	0.000	0.000	0.000	1.000	0.000	0.000	0.000
*MDH-1**							
(N)	(30)	(30)	(30)	(33)	(45)	(45)	(21)
85	0.000	0.000	0.000	0.000	0.000	1.000	0.000
87	0.000	0.000	0.000	0.000	1.000	0.000	0.000
90	0.000	1.000	0.000	0.000	0.000	0.000	0.000
95	0.000	0.000	1.000	0.000	0.000	0.000	0.000

Locus, Alleles	Species 1	2	3	4	5	6	7
*MDH-1** (continued)							
100	1.000	0.000	0.000	1.000	0.000	0.000	0.000
180	0.000	0.000	0.000	0.000	0.000	0.000	1.000
*MDH-2**							
(N)	(30)	(30)	(30)	(33)	(45)	(45)	(21)
85	0.000	0.000	0.000	0.000	0.000	1.000	0.000
90	0.000	1.000	0.000	0.000	1.000	0.000	0.000
95	0.000	0.000	1.000	0.000	0.000	0.000	0.000
100	1.000	0.000	0.000	1.000	0.000	0.000	0.000
200	0.000	0.000	0.000	0.000	0.000	0.000	1.000
*MDH-3**							
(N)	(30)	(30)	(30)	(33)	(45)	(45)	(21)
70	0.000	0.000	0.000	0.000	0.000	1.000	0.000
80	0.000	0.000	1.000	0.000	0.000	0.000	0.000
90	0.000	1.000	0.000	0.000	1.000	0.000	0.000
95	0.000	0.000	0.000	0.000	0.000	0.000	1.000
98	0.000	0.000	0.000	1.000	0.000	0.000	0.000
100	1.000	0.000	0.000	0.000	0.000	0.000	0.000
*ME-1**							
(N)	(30)	(30)	(30)	(33)	(42)	(48)	(21)
75	0.000	0.000	0.000	0.000	1.000	0.000	0.000
98	0.000	0.000	0.000	1.000	0.000	0.000	0.000
100	1.000	1.000	0.000	0.000	0.000	0.000	0.000
102	0.000	0.000	1.000	0.000	0.000	0.000	0.000
120	0.000	0.000	0.000	0.000	0.000	1.000	1.000
*ME-2**							
(N)	(30)	(30)	(30)	(33)	(42)	(48)	(21)
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*LDH**							
(N)	(30)	(30)	(30)	(33)	(40)	(48)	(21)
90	0.000	0.000	1.000	0.000	0.000	0.000	0.000
95	0.000	0.000	0.000	0.000	0.000	1.000	0.000
100	1.000	1.000	0.000	1.000	1.000	0.000	0.000
120	0.000	0.000	0.000	0.000	0.000	0.000	1.000
*LAP-1**							
(N)	(30)	(30)	(30)	(33)	(40)	(48)	(21)
90	0.000	0.000	0.000	0.000	0.000	1.000	0.000
95	0.000	1.000	1.000	1.000	0.000	0.000	1.000
100	1.000	0.000	0.000	0.000	1.000	0.000	0.000
*LAP-2**							
(N)	(30)	(30)	(30)	(33)	(40)	(48)	(21)
100	1.000	1.000	1.000	1.000	0.000	1.000	0.000
130	0.000	0.000	0.000	0.000	0.104	0.000	0.000
140	0.000	0.000	0.000	0.000	0.896	0.000	0.000
180	0.000	0.000	0.000	0.000	0.000	0.000	1.000
*FUM**							
(N)	(30)	(30)	(30)	(33)	(42)	(40)	(38)
50	0.000	0.000	0.000	0.000	1.000	0.000	0.000
70	0.000	0.000	0.000	1.000	0.000	0.000	0.000
80	0.000	1.000	0.000	0.000	0.000	1.000	0.000
85	0.000	0.000	0.000	0.000	0.000	0.000	0.092
90	0.000	0.000	1.000	0.000	0.000	0.000	0.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.184
100	1.000	0.000	0.000	0.000	0.000	0.000	0.000
105	0.000	0.000	0.000	0.000	0.000	0.000	0.724

Table 3 (continued)

Locus, Alleles	Species 1	2	3	4	5	6	7
*HK**							
(N)	(30)	(30)	(30)	(33)	(42)	(40)	(21)
80	0.000	0.000	0.000	0.000	0.000	1.000	0.000
82	0.000	0.000	0.000	0.017	0.000	0.000	0.000
85	0.000	0.000	0.000	0.000	1.000	0.000	0.000
90	0.000	1.000	1.000	0.983	0.000	0.000	0.000
95	0.000	0.000	0.000	0.000	0.000	0.000	1.000
100	1.000	0.000	0.000	0.000	0.000	0.000	0.000
*DIA-1**							
(N)	(30)	(30)	(30)	(33)	(45)	(40)	(21)
95	0.000	0.000	0.000	0.000	0.000	0.000	1.000
100	1.000	0.000	0.000	0.000	0.000	0.000	0.000
105	0.000	1.000	1.000	1.000	1.000	1.000	0.000
*DIA-2**							
(N)	(30)	(30)	(30)	(33)	(45)	(40)	(21)
80	0.000	0.000	0.000	0.000	0.000	1.000	0.000
90	0.000	0.000	0.000	0.000	0.000	0.000	1.000
95	0.000	0.000	0.000	1.000	0.000	0.000	0.000
100	1.000	0.000	0.000	0.000	1.000	0.000	0.000
105	0.000	0.000	1.000	0.000	0.000	0.000	0.000
110	0.000	1.000	0.000	0.000	0.000	0.000	0.000
*DIA-3**							
(N)	(30)	(30)	(30)	(33)	(45)	(40)	(21)
90	0.000	0.000	0.000	0.000	1.000	0.000	0.000
95	0.000	0.000	0.000	1.000	0.000	0.000	1.000
100	1.000	1.000	1.000	0.000	0.000	1.000	0.000
*ACP**							
(N)	(30)	(30)	(30)	(33)	(45)	(50)	(21)
80	0.000	0.000	0.000	0.000	0.230	0.000	0.000
100	1.000	0.000	0.000	0.000	0.690	1.000	0.000
105	0.000	0.000	1.000	1.000	0.000	0.000	0.000
110	0.000	0.000	0.000	0.000	0.070	0.000	1.000
120	0.000	1.000	0.000	0.000	0.010	0.000	0.000
*PEP-1**							
(N)	(30)	(30)	(30)	(33)	(39)	(45)	(37)
50	0.000	0.000	0.000	0.000	0.000	1.000	0.000
90	0.000	0.000	0.000	0.076	1.000	0.000	0.000
95	0.000	1.000	0.000	0.000	0.000	0.000	0.000
100	1.000	0.000	1.000	0.924	0.000	0.000	0.000
110	0.000	0.000	0.000	0.000	0.000	0.000	1.000
*PEP-2**							
(N)	(30)	(30)	(30)	(33)	(39)	(45)	(37)
75	0.000	0.000	0.000	0.000	1.000	0.000	0.000
80	0.000	0.000	0.000	0.000	0.000	1.000	0.000
85	0.000	0.000	0.000	0.000	0.000	0.000	0.932
95	0.000	0.000	1.000	1.000	0.000	0.000	0.000
100	1.000	1.000	0.000	0.000	0.000	0.000	0.000
105	0.000	0.000	0.000	0.000	0.000	0.000	0.068

All the species were successfully analysed for 13 enzymes coded by a total of 21 loci. Allele frequencies for all species studied are shown in Table 3. Only 4 loci (*GPI**, *PGM**, *MPI**, and *AAT-1**) were consistently polymorphic (0.99 criterion) in several or most of the species. *FUM** and *PEP-2** were also polymorphic in *Gammarus crinicornis*, *HK** and *PEP-1** in *Orchestia mediterranea*, and *LAP-2** and *ACP** in *Arcitalitrus dorrieni*. The remaining loci were monomorphic within species, but were in many cases fixed for alternative alleles in different species.

Table 4. Mean heterozygosities (H) (SE in parentheses), the mean number of alleles per locus, and percentage of polymorphic loci (P) (0.99 criterion) in the 7 amphipod species studied

Species	H Observed	H Expected	Mean no. alleles	P
Talitrus saltator	0.020 (0.014)	0.024 (0.016)	1.3	14.3
Talorchestia deshayesii	0.056 (0.038)	0.047 (0.032)	1.2	14.3
Orchestia gammarellus	0.015 (0.013)	0.016 (0.014)	1.2	14.3
Orchestia mediterranea	0.030 (0.016)	0.031 (0.015)	1.3	23.8
Arcitalitrus dorrieni	0.025 (0.017)	0.033 (0.024)	1.2	14.3
Hyale nilssoni	0.014 (0.011)	0.013 (0.010)	1.1	14.3
Gammarus crinicornis	0.059 (0.027)	0.070 (0.031)	1.5	28.6

Table 5. Estimates of Nei's (1978) genetic identity (above the diagonal) and genetic distance (below the diagonal) between the 7 amphipod species. (1) *Talitrus saltator*, (2) *Talorchestia deshayesii*, (3) *Orchestia gammarellus*, (4) *Orchestia mediterranea*, (5) *Arcitalitrus dorrieni*, (6) *Hyale nilssoni*, (7) *Gammarus crinicornis*.

Species	1	2	3	4	5	6	7
1	·····	0.347	0.235	0.325	0.270	0.237	0.141
2	1.058	·····	0.352	0.359	0.249	0.246	0.152
3	1.448	1.045	·····	0.560	0.145	0.195	0.100
4	1.125	1.025	0.580	·····	0.192	0.146	0.150
5	1.309	1.392	1.931	1.652	·····	0.131	0.055
6	1.439	1.402	1.637	1.922	2.032	·····	0.100
7	1.960	1.885	2.307	1.894	2.900	2.305	·····

Table 4 summarises the results for mean number of alleles per locus, percentage of loci polymorphic, and mean heterozygosity for each species studied. The observed heterozygosity ranged from 0.014 in *Hyale nilssoni* to 0.059 in *Gammarus crinicornis*. The matrix of genetic similarity and distance coefficients (Nei 1978) is shown in Table 5. The values for genetic identity ranged from a very low value of 0.055 between *G. crinicornis* and *Arcitalitrus dorrieni* to 0.560 between the congeneric species *Orchestia gammarellus* and *O. mediterranea*.

Both dendrograms (Fig. 2a, b) suggest that *Talorchestia deshayesii*, a sandhopper, is more closely related to the beachfleas (*Orchestia gammarellus* and *O. mediterranea*) than to *Talitrus saltator*, the other sand-

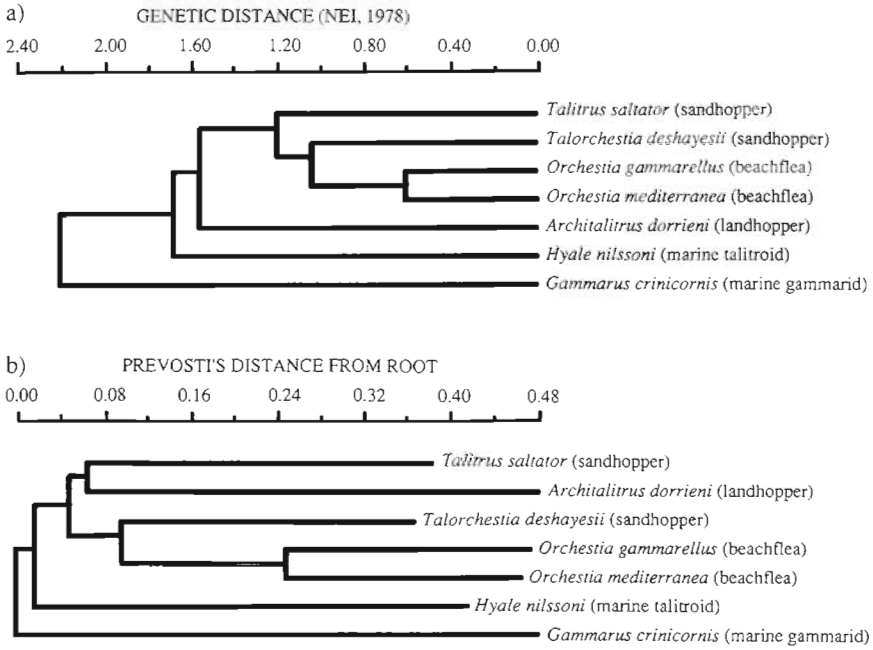

Fig. 2. Dendrograms to indicate interrelationships of the 7 amphipod species studied (a) derived by the UPGMA method using Nei's genetic distance and (b) by the Wagner procedure using Prevosti's distance. Goodness of fit statistics: (a) $f = 4.312$, $\%SD = 14.08$, $F = 12.59$, $CC = 0.870$; (b) $f = 0.404$, $\%SD = 4.74$, $F = 2.46$, $CC = 0.969$. For further information and references to statistical methodology see 'Materials and methods'

hopper species. The main difference between the dendrogram obtained by the Wagner distance procedure (Fig. 2b) and that obtained by the UPGMA analysis is that *Arcitalitrus dorrieni* was grouped with *T. saltator* in the Wagner dendrogram, whereas in the UPGMA dendrogram (Fig. 2a) *A. dorrieni* is the most divergent of the talitrid species studied. The goodness of fit statistics (see Fig. 2a, b) indicate that the Wagner distance procedure produced the better tree.

DISCUSSION

Levels of genetic variation

The heterozygosity values for the talitrid species studied (Table 4) are low when compared with published data for several other amphipod species (e.g. Battaglia et al. 1978, Dickson et al. 1979, Gooch & Hetrick 1979, Siegismund et al. 1985, Kane et al. 1992, Patarnello et al. 1992) and when compared with estimates for other crustaceans (Hedgecock et al. 1982). Bulnheim & Scholl (1986) and De Mathaeis et al. (1994) have also reported low levels of genetic variability for *Talitrus saltator* and *Talorchestia deshayesii*. Thus it appears that talitrids may have generally rather low levels of heterozygosity. Levels of heterozygosity vary greatly over the wide range of organisms surveyed (Nevo 1978, Nevo et al. 1984), but the implications of this variation are far from clear (review by e.g. Zouros & Foltz 1987). Ward et al. (1992) found that for invertebrates about 34% of variance in average protein heterozygosity was attributable to taxon effects and 41% to the effects of protein structure and function.

The landhopper *Arcitalitrus dorrieni* is native to south-east Australia and is thought to have been introduced to the British Isles via imported plants (Richardson 1980). The various isolated colonies of *A. dorrieni* in Britain probably arose from relatively few introduced individuals; hence, a population bottleneck (founder effect) and consequently reduced levels of allozyme heterozygosity are to be expected. Although the heterozygosities of *PGM** and *GPI** are relatively low, the mean level of heterozygosity found for *A. dorrieni* was similar to that of the native species studied. (*A. dorrieni* was polymorphic for the otherwise monomorphic loci *LAP-1** and *ACP**.) However, bottlenecks are believed to have a larger effect on allelic diversity than on heterozygosity, and if population size increases rapidly following a single bottleneck of short duration, the loss in mean heterozygosity may be small (Nei et al. 1975, Leberg 1992). In order to establish the significance of the pattern of genetic variation observed in *A. dorrieni*, it would be necessary to compare the results with genetic variation of putative ancestral Australian populations.

Genetic relationship between species

The distribution of genetic identities between the species studied corresponds with the general relationship expected between taxonomic divergence and genetic identity (Thorpe 1982). The genetic identity for

the 2 *Orchestia* species (Table 5) is within the range proposed for congeneric species (0.35 to 0.85), and the values of genetic identity among the other different genera are around 0.35 or below, also as would be predicted.

However, in a review of genetic data from amphipods, Stewart (1993) suggested that, when identity values fell between 0.45 and 0.85, it may be difficult to make decisions regarding specific status of populations, and additional factors such as evidence of a lack of gene flow and concordant morphological variation should be considered. The poor distinction found by Stewart is possibly due to a taxonomic effect, since most of the data came from the single genus *Gammarus* or other Gammaridae. It appears from the data summarised by Stewart (1993) that some *Gammarus* species are only about as closely related as talitrid genera.

Our genetic results do not indicate a clear relationship between Bousfield's systematic-ecological groups of talitrids and their genetic divergence, since both the UPGMA and the Wagner procedure dendrograms (Fig. 2a, b) suggest that the genera *Orchestia* (beachflea) and *Talorchestia* (sandhopper) are more closely related to each other than to *Talitrus* (sandhopper). This differs from the relationship in the phenogram of Bousfield (1982, 1984). However, the relative isolation of *Talitrus saltator* within the family has also been commented on by Hurley (1975) and Moore et al. (1993), whilst Bousfield (1984) stressed that his groups were not necessarily monophyletic.

As outlined above, the genetic relationships among all the species shown by a UPGMA dendrogram using Nei's genetic distance (Fig 2a) suggest that the landhopper *Arcitalitrus dorrieni* is the most divergent of the talitrid species studied. However, results from the Wagner procedure, which has stronger statistical support than the UPGMA tree (see Fig. 2a, b), show a different branching pattern for *A. dorrieni*. In the Wagner dendrogram *A. dorrieni* was clustered, albeit loosely, with *Talitrus saltator*, suggesting that both species share a direct common ancestor. It should be noted, however, that none of our results indicates a particularly close relationship between *A. dorrieni* and *T. saltator*, the type-species of *Talitrus*, despite the referral of *A. dorrieni* and other simplidactylate landhoppers to *Talitrus* (*sensu lato*) by some authors (e.g. Hurley 1975, Richardson 1980). Bousfield (1984) suggested that simplidactylate landhoppers evolved from an ancestral palustral species, although Morritt (1988) and Moore et al. (1993) hypothesised an evolutionary pathway from the hyalids to the simplidactylate landhoppers without a palustral species as intermediary.

Our results also indicate that *Hyale nilssoni* is more closely related to talitrids than is *Gammarus crinicornis*. *H. nilssoni* was formerly placed in the family Talitridae, but, largely on ecological criteria, has been referred to the family Hyalidae within the superfamily Talitroidea (Bulycheva 1957). This is compatible with the genetic data presented here. The data also provide limited support for the hypothesis that the talitrids may have originated from aquatic ancestors allied to primitive hyalids (e.g. Bousfield 1984).

The degree of genetic divergence reported here between the ecomorphological groups of the Talitridae is unexpectedly small in the light of Bousfield's (1984) evolutionary scenario (see also Hurley 1975), which proposed an ancient origin of simplidactylate landhoppers, with a divergence from other groups around 100 million years before present, during the Cretaceous period. This long divergence is not easily reconciled with the values of Nei's (1978) genetic identity, which indicate far more recent divergence. Calibrations of molecular clocks are controversial and at best inaccurate, but our data would generally be considered to indicate that the major divergence of the Talitridae has probably occurred over about the last 10 million years (see Thorpe 1982, 1989, Nei 1987), and thus almost certainly commenced within Neogene (late Cenozoic) times (ca 2 to 24 million yr before present).

Overall, therefore, our data, when compared with previous hypotheses, indicate some small but significant differences in patterns of divergence of the Talitridae, but also show a degree of general agreement, although they are not compatible with the very long timescale proposed in the major study by Bousfield (1984). To clarify further the phylogeny of the talitrids it would be desirable to include some palustral species and cuspidactylate landhoppers in the analysis and also to increase the number of species in each ecological group.

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