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HIGH GENETIC SIMILARITY BETWEEN GEOGRAPHICALLY DISTANT POPULATIONS IN A SEA ANEMONE WITH LOW DISPERSAL CAPABILITIES

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Samples of the large sublittoral sea anemone *Urticina eques* (Gosse) were collected from three localities in the northern North Sea and from one locality in the northern Irish Sea. Around the coast the total distance between sampling sites is approximately 1,200 km. The species has a large lecithotrophic larva which may not be planktonic. All samples were screened genetically for 13 loci coding for 11 different enzymes. Results overall indicated a high degree of genetic uniformity over the four populations sampled (F_{ST} = 0·025). The data are discussed in relation to current ideas of larval dispersal and results from other similar studies. It is concluded that the lack of genetic differentiation shown by *Urticina eques* is surprising given the apparently poor dispersive powers of the larva.

INTRODUCTION

The large actiniid sea anemone *Urticina eques* is widespread on subtidal hard substrata to depths of 400 m (Manuel, 1988). It occurs in many areas of the North Atlantic and may be circumpolar in distribution, although the precise range of the species is unclear because older records are complicated by earlier taxonomic difficulties. The larva of *Urticina eques* has been studied in detail by Carlgren (1906, 1924) and also by Stephenson (1935) (as *Tealia felina* var. *lofotensis*). The larvae are described as 'relatively large, unwieldy larvae, rich in yolk' and as being uniformly ciliated. Stephenson (1935) also considered that the larvae 'remain near the bottom and are not truly planktonic'. More recent reviews of sea anemone reproduction (Chia, 1976; Shick, 1991) give no new information on the larva of *U. eques*. Chia & Spaulding (1972) provide a detailed study of larval development in the North American species *Tealia* (= *Urticina*) *crassicornis*, which has a larva apparently similar to that of *U. eques*.

Although it has long been implicitly assumed or explicitly stated that the primary role of marine larvae was for dispersal (reviewed by *e.g.* Todd, 1985) this assertion has been questioned (Strathmann, 1985; Knowlton & Keller, 1986). However, it is to be expected that species with longer-lived larvae will be generally more widely dispersed, and hence will show reduced genetic variation with distance (*e.g.* Hedgecock, 1986).

Urticina eques is unusual in being widely distributed, but having a larva with relatively weak powers of dispersal. It was therefore considered that a study of genetic differentiation of populations of *U. eques* would provide potentially valuable comparative data. Published data for other actiniid sea anemones lacking planktotrophic larvae suggest that genetic differentiation is often large, even, in some cases, over very short distances (*e.g.* Ayre *et al.*, 1991; Solé-Cava & Thorpe, 1992; Russo *et al.*, 1994). The technique chosen for the present work was enzyme electrophoresis, a method which has been extensively used over a number of years for studying various aspects of the genetic structure of natural populations in a wide range of organisms (reviewed by *e.g.* Avise, 1994) including a systematic investigation of *Urticina* species (Solé-Cava *et al.*, 1985).

MATERIALS AND METHODS

Samples of *Urticina eques* were collected from four localities (Figure 1). Three of these were in the North Sea spread over about 110 km, approximately 140-190 km off the east coast of Scotland. Depths (north to south) were 75, 85 and 81 m respectively. The fourth sample was from about 10 m in Port Erin Bay (south-west Isle of Man) in the northern Irish Sea. The minimum distances by sea between the North Sea sampling areas and Port Erin Bay are about 1,200 km around the north of Scotland or about 1,800 km around the south of England. Anemones were collected either by dredging (North Sea) or by SCUBA diving (Port Erin). Since *U. eques* is a fairly large and delicate anemone prone to

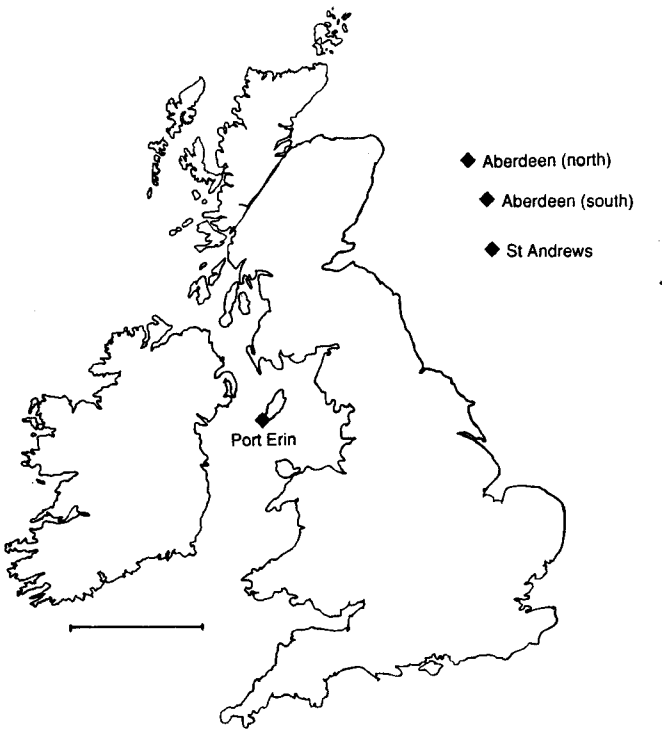

Figure 1. Sites from which *Urticina eques* were collected. Scale: 200 km.

die in transit, those collected in the North Sea were frozen instantly and stored for two weeks in liquid nitrogen, then transferred to the Port Erin Marine Laboratory, where they were used immediately for electrophoresis. Because of potential storage and transport problems of samples kept in liquid nitrogen these North Sea samples had to be restricted to 12 anemones from each site. Samples from Port Erin were kept alive in aquaria until required.

Electrophoretic methods were as previously described for *Urticina* (Solé-Cava *et al.*, 1985). A total of 13 putative loci coding for 11 different enzymes were typed for each sample. The genetic calculations were carried out using the program BIOSYS (version 1·7) (Swofford & Selander, 1981). For small sample sizes probabilities were estimated using Fisher's exact test, which unlike χ^2 is independent of sample size.

RESULTS

A remarkable degree of genetic uniformity was observed between the allele frequencies (Table 1) of all four samples of *Urticina eques* with none of these differing significantly (P=0·05) from any other. Estimates of Genetic Identity (I) and Distance (D) (Nei, 1978) for all possible pairwise comparisons between the four samples are given in Table 2. Divergence was very small, with D values ranging from 0·014 to 0·063. I values were used to construct a dendrogram (Figure 2) using the UPGMA method of Sneath & Sokal (1973). Over the four samples the Wright's (1978) F_{ST} value, 0·025, was very low, again indicating minimal genetic differentiation.

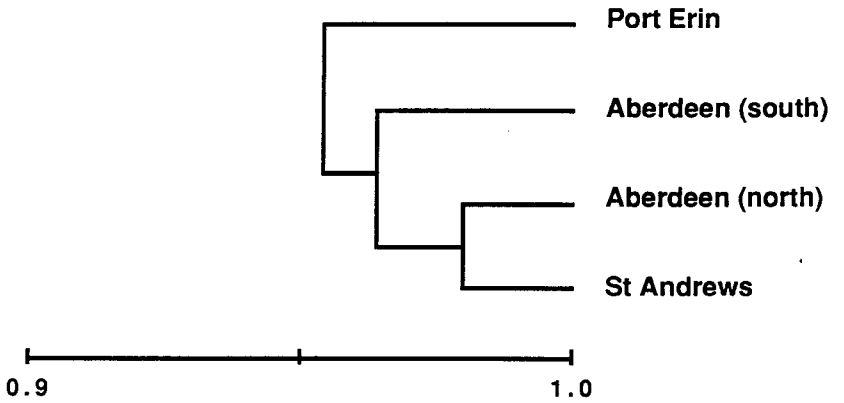

Figure 2. UPGMA (Sneath & Sokal, 1973) dendrogram of Genetic Identity (Nei, 1978) values between the four populations of *Urticina eques* studied.

At polymorphic loci fits of genotype frequencies to Hardy-Weinberg expectations were generally good and only one significant (P<0·05) deviation from Hardy-Weinberg equilibrium (Fisher's exact test) was found: this was for the locus *Pgi-1*, which showed a heterozygote deficiency in the sample from Port Erin Bay. Since a 5% frequency of 'significant' results is to be expected by chance alone, one significant result over the number of loci studied does not indicate that genotype ratios are other than as expected for outbreeding.

Table 1. *Gene frequencies in four populations of* Urticina eques. *Sampling localities are as shown in Figure 1.*

Locus	Allele	Port Erin	Aberdeen South	Aberdeen North	St Andrews
Acon	A	0.42	0.00	0.00	0.00
	B	0.58	1.00	1.00	1.00
Ada	A	0.00	0.00	0.10	0.19
	B	0.25	0.35	0.30	0.44
	C	0.75	0.65	0.60	0.37
Got-1	A	1.00	1.00	1.00	1.00
Got-2	A	0.46	0.46	0.41	0.46
	B	0.50	0.50	0.54	0.54
	C	0.04	0.04	0.05	0.00
Hk	A	0.12	0.30	0.40	0.30
	B	0.27	0.50	0.10	0.50
	C	0.21	0.00	0.00	0.10
	D	0.40	0.20	0.50	0.10
Me	A	0.04	0.00	0.05	0.00
	B	0.90	1.00	0.88	0.92
	C	0.04	0.00	0.07	0.08
	D	0.02	0.00	0.00	0.00
Odh	A	0.09	0.45	0.29	0.13
	B	0.81	0.46	0.71	0.67
	C	0.10	0.09	0.00	0.20
Pgd	A	0.12	0.20	0.08	0.13
	B	0.60	0.55	0.75	0.66
	C	0.24	0.20	0.17	0.21
	D	0.04	0.05	0.00	0.00
Pgi-1	A	0.00	0.00	0.04	0.04
	B	0.35	0.42	0.46	0.35
	C	0.65	0.58	0.50	0.61
Pgi-2	A	0.75	0.36	0.60	0.50
	B	0.25	0.64	0.40	0.50
Pgm	A	0.28	0.04	0.43	0.60
	B	0.37	0.38	0.43	0.30
	C	0.35	0.58	0.14	0.10
Sod-1	A	1.00	1.00	1.00	1.00
Xod	A	1.00	0.70	0.90	0.80
	B	0.00	0.30	0.10	0.20
Ho		0.39	0.40	0.35	0.39
He		0.40	0.35	0.35	0.37
N		58	24	24	24

Ho, mean observed heterozygosity per locus; He, mean expected heterozygosity per locus; N, mean number of alleles sampled per locus for each population.

Table 2. *Unbiased genetic identities (above diagonal) and distances (below diagonal) (Nei, 1978) between populations of* Urticina eques. *Sampling localities are as shown in Figure 1.*

	Port Erin	Aberdeen South	Aberdeen North	St Andrews
Port Erin	*	0.939	0.972	0.952
Aberdeen South	0.063	*	0.963	0.965
Aberdeen North	0.028	0.038	*	0.986
St Andrews	0.050	0.036	0.014	*

DISCUSSION

The main feature of note is that there is little overall divergence between samples of *Urticina eques* from any of the four sites sampled (Tables 1 and 2). This is very surprising in a species with a weakly dispersive larva where genetic differentiation on a small scale is to be expected (Hedgecock, 1986). The low values for D and F_{ST} indicate that there is substantial genetic uniformity, not only between geographically separated subpopulations of *U. eques* from the North Sea, but also between these and the sample from the Irish Sea. The sample sizes used in the present work were of necessity small (12-29 anemones per site), but these will have a minimal effect on the sampling errors of measures of genetic divergence (*e.g.* I or D of Nei, 1978), which are for practical purposes effectively a function of the numbers of loci studied and are largely independent of the numbers of animals used (see Nei, 1978, 1987; Thorpe, 1979, 1982).

The most detailed study of genetic divergence in relation to larval dispersal for a species with relatively short-lived non-planktotrophic larvae is probably that of Todd *et al.* (1988, 1994) on the nudibranch *Adalaria proxima*. This species has a larva which is thought to be longer-lived and hence is apparently better suited to dispersal than that of *U. eques*, but the adults show marked and temporally stable disjunctions in gene frequencies over distances as small as a few kilometres. From this and other work genetic differentiation might have been expected between the three North Sea samples of *U. eques* which were separated by distances of about 50 to 110 km.

The results for *U. eques* showing very low genetic differentiation with distance despite a non-planktotrophic larva are also much at variance with those for other sea anemone species. The levels of genetic identity (Table 2 and Figure 1) observed between all the samples of *U. eques* are substantially higher than those commonly found between adjacent populations or even sympatric morphs of the related actiniid species *Actinia equina* (*e.g.* Solé-Cava & Thorpe, 1992). In *A. equina* the extent of larval dispersal is unclear (Carter & Miles, 1989). The Australian *A. tenebrosa* is thought, like *U. eques*, to have a non-feeding larva and with distance shows marked genetic discontinuities in population structure (Ayre, 1984; Ayre *et al.*, 1991). Hunt & Ayre (1989) found a very low F_{ST} value of 0·03 for populations of the actiniid anemone *Oulactis mucosa* over 735 km in south-east Australia, and from this concluded that the species must have a planktotrophic larva capable of dispersal over at least this distance. Over about 1050 km of the coastline of Brazil Russo *et al.* (1994) found a predictably low F_{ST} value of 0·042 for several populations of another actiniid anemone, *Bunodosoma caissarum*, which is known to have a long-lived planktotrophic larva. Russo *et al.* (1994) also studied *Actinia bermudensis*, which has a non-feeding planktonic larva, and found a very large F_{ST} value of 0·32 over the slightly larger distance of 1150 km. Our data show *U. eques* to have a lower F_{ST} value (0·025) over a larger distance (at least 1200 km) than any of these species. Thus *U. eques* appears to have the most genetically uniform population structure of any sea anemone studied to date. This is particularly remarkable for a species with a short-lived non-feeding larva. It is even more surprising given the high heterozygosity (Table 1), because variable loci are more likely to diverge rapidly than monomorphic loci (Skibinski & Ward, 1981).

The shortest distance around the coast between the Irish Sea and North Sea sampling areas is about 1200 km. There is a net northerly flow of water through both the Irish Sea and to a lesser extent the North Sea, but there are also incursions of oceanic water around the north of Scotland into the northern North Sea (see Anon., 1981). Such flow patterns would make it extremely difficult for even very long-lived larvae to progress from the Irish Sea to the North Sea or *vice versa* through the English Channel or from the North Sea to the Irish Sea around the north of Scotland; all these routes would require an ability to travel against the prevailing current. Over an appropriate time scale transport from the Irish Sea to the North Sea around the north of Scotland could be possible for a long-lived planktotrophic larva. As discussed above *U. eques* has a large lecithotrophic larva, which, if planktonic at all, is unlikely to travel large distances. Even in situations where larval transport would be straightforward, gene flow over distances in excess of 1000 km is likely to be achieved among sessile invertebrates generally only by species with long-lived planktotrophic larvae (but see Jokiel, 1987, 1989 for 'rafting' on coral fragments). In sessile species with short-lived non-feeding larvae it is to be expected that dispersal will be far more limited (see also Todd *et al.*, 1988). Endler (1973) considered that species with high gene flow between geographically distant populations may be uncommon.

The reasons for such low levels of genetic heterogeneity between widely geographically separated populations of *U. eques* are unclear. Models of gene flow between populations vary greatly in their assumptions, but, almost irrespective of this, a clear conclusion is that (in the absence of extreme or abnormal selective forces), practically any level of migration, however small, will be sufficient to preclude genetic divergence (for brief discussion see *e.g.* Maynard Smith, 1989; detailed reviews by Slatkin, 1985, 1987). A possible interpretation of the main results of the present work is that there is little genetic differentiation because there is likely to be gene flow (*i.e.* larval dispersal) between the four populations of *U. eques*. Various marine invertebrates show little genetic differentiation over substantial distances, but these are mostly species known to have long-lived planktotrophic larvae. Low F_{ST} values, as found here, have often been considered to be evidence for gene flow and hence for pelagic larval transport between populations (*e.g.* Hunt & Ayre, 1989; Todd *et al.*, 1991), but we know of no other cases of similarly low values over long distances in species with non-feeding larvae. Alternatively the high genetic identities between populations of *U. eques* in the Irish Sea and North Sea may be a consequence of the geologically recent nature of the two sea areas (McCave *et al.*, 1977) with more substantial divergence yet to occur.

However, in interpreting the results it should be borne in mind that most available data on the genetic effects of larval dispersal are derived from studies of intertidal species, some of which have fairly restrictive requirements for substratum or other ecological parameters. In *U. eques* the substratum requirements of the species (hard rock or gravel) are not restrictive and in many areas any larva would have a good chance of encountering substrata suitable for settlement. Also, being mainly a sublittoral species, the possibility of successful larval transport is increased, because this occurs in two dimensions. Many intertidal species are effectively restricted to linear dispersal and any larvae dispersing outside the intertidal zone are likely to perish. If more data become

available it may be found that larvae are generally able to disperse more effectively in subtidal than in intertidal species.

Whatever the reason, high levels of genetic similarity over long distances in a species with apparently poor dispersal capabilities, are of considerable interest because very few such cases are recorded in the literature. Species where the genetic population structure deviates from that expected from a knowledge of larval dispersal potential are generally those where expected dispersal is not realized (see *e.g.* Hedgecock, 1986; Todd *et al.*, 1988) and not as in *Urticina eques*, where the converse is true.

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