Marine Biology 112, 243–252 (1992)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Genetic divergence between colour morphs in populations of the common intertidal sea anemones *Actinia equina* and *A. prasina* (Anthozoa: Actiniaria) in the Isle of Man

A. M. Solé-Cava* and J. P. Thorpe

Department of Environmental and Evolutionary Biology, University of Liverpool, Port Erin Marine Laboratory, Port Erin, Isle of Man, U.K.

Date of final manuscript acceptance: September 24, 1991. Communicated by J. Grassle, New Brunswick

Abstract. A distinctive morph of *Actinia equina* (L.) is found at low frequency among populations on intertidal hard substrata on some shores on the Isle of Man. This morph is red with rows of green oval spots or elongate markings running longitudinally down the column. Starch gel electrophoresis of 21 allozyme loci was used to compare samples of this morph from two localities with other sympatric *Actinia* spp. Collections were made in early summer 1986. For one sampling site the comparison was with red *A. equina* and green *A. prasina* Gosse, whilst at the other site with red *A. equina* only. At both sites significant genetic differences in allele frequencies at several loci were found between the new morph and sympatric *A. equina* and *A. prasina*. The results also confirm the reproductive isolation of *A. prasina*. As with much previous work on sea anemones, levels of mean heterozygosity per locus (H) were found to be high ($H = 0.157$ to 0.342). A surprising feature of the results, although bearing out earlier unpublished data, is that high levels of genetic differentiation are found between populations of what appear to be the same morphs collected from shores only a few km apart. Among the *Actinia* spp. studied, differentiation between populations of the same morph on different shores was of the same order as between sympatric, reproductively isolated populations.

Introduction

In Britain the sea anemone *Actinia equina* (L.) is very common on most rocky shores and other intertidal hard substrata. Its range extends throughout the Atlantic coasts of Europe and North Africa and into the Mediterranean (Stephensen 1935, Schmidt 1971, Manuel 1981, 1988). It is also found in South Africa (Stephenson 1935) and, indeed, the geographical limits of the species are far from clear. As a result of its abundance *A. equina* is of considerable ecological importance in Europe and has been extensively studied, particularly in the British Isles.

For many years *Actinia equina* was considered to be a single, highly variable species with many colour and other variants (for descriptions see e.g. Tugwell 1856, Gosse 1860, Stephenson 1935, Schmidt 1971, Manuel 1988) and in the past this has resulted in taxonomic disagreements. Several early workers gave specific status to a variety of colour morphs (e.g. Johnston 1847, Dalyell 1848, Tugwell 1856, Milne-Edwards 1857), but throughout most of this century all have generally been considered morphs of a single species (see e.g. Stephenson 1935, Schmidt 1971, Campbell 1976).

Stephenson (1935) in his major work considered there to be only two varieties: the highly variable var. *mesembryanthemum* and the generally much larger monotypic (dark red with green spots) var. *fragacea*, which, although common on rocky coasts in western France, in Britain is found only on certain shores in the extreme southwest of England and in the Channel Isles. Until recently most authors (e.g. Schmidt 1971, Cambell 1976) followed the conclusions of Stephenson that there was only one species. Carter and Thorpe (1981) found marked differences in reproduction and ecology between Stephenson's two varieties and used allozyme electrophoresis to demonstrate clear genetic differences indicating reproductive isolation. Therefore, they raised Stephenson's (1935) var. *fragacea* to species level as *Actinia fragacea* Tugwell and Stephenson's var. *mesembryanthemum* then became simply *Actinia equina* (see also Manuel 1981).

In a similar study, Haylor et al. (1984) found significant genetic differentiation between a green morph and other sympatric *Actinia equina* and gave this morph specific status as *Actinia prasina* Gosse. This conclusion was subsequently confirmed by further such work by Solé-Cava and Thorpe (1987 a) comparing *A. prasina* and *A. equina* on a different shore. Another genetically differentiated morph, distinguished morphologically by a pattern of radial lines on the pedal disc, may belong to a

* Present address: Departamento de Genética, Instituto de Biologia, Unversidade Federal do Rio de Janeiro, CCS-Bloco A, Ilha do Fundão, 20000-Rio de Janeiro-RJ, Brazil

different species (Quicke et al. 1985). It appears, therefore, that the original *A. equina* (sensu Stephenson 1935) may be composed of a number of different biological species.

In the present work the taxonomic status of an unusual colour morph of *Actinia equina* (sensu Stephenson 1935) from two localities in the Isle of Man (Irish Sea) was investigated using allozyme electrophoresis. This technique is very useful for systematic studies (reviews by e.g. Avise 1974, Thorpe 1982, Richardson et al. 1986) and has been used to investigate cryptic species in a variety of marine invertebrate groups (e.g. Grassle and Grassle 1976, Thorpe et al. 1978, Solé-Cava and Thorpe 1986). Our objectives were to establish whether there is any genetic divergence of the unusual colour morph from "normal" red *A. equina* and to compare levels of genetic differentiation between sympatric and allopatric populations of *Actinia* spp. A comparison was also made with green *A. prasina* in order to consider the possibility that the new colour morph might be a variant of that species.

Materials and methods

Collection of samples

Samples of three morphs of *Actinia*, the red *A.* cf. *equina* (R), the green *A. prasina* (G) and an unusual morph of *A.* cf. *equina* which was red with green dots (RG), were collected intertidally at Fleshwick Bay, and the red and red/green morphs of *A. equina* at Port St. Mary Ledges (both southwest Isle of Man, Northern Irish Sea). Collections were made in early summer 1986. The two sampling sites are 4 km apart (linear distance), but ca. 13 km along the coast. Samples of *A. prasina* were not collected from Port St. Mary because this is an exposed shore where this species is very rare (*A. prasina* occurs mainly in more sheltered areas; see Haylor et al. 1984). Special care was taken to collect anemones at least 2 m apart from each other, to reduce the possibility of the collection of asexually produced offspring. The presence of clones was checked again by the analysis of individual genotypes. Some anemones were found to be identical over all loci analysed and were, hence, considered to be possible clonemates and in the subsequent calculations were treated as only one individual. Thus, the numbers of anemones typed by electrophoresis were rather larger than indicated in the results.

Characterisation of the different morphs

To assist future taxonomic work on *Actinia* spp. it is desirable that the morphs studied here have clear descriptions. These are given below.

Actina cf. equina (Red morph)

The red anemones used in this work all had plain red columns and were typical *Actinia equina* sensu Manuel (1988). To avoid possible confusion only samples with red pedal discs were used. The pedal disc colour was 10C8 on colour charts (Kornerup and Wanscher 1978) whilst the column colour of the samples used was in the range 10F8 to 10E8.

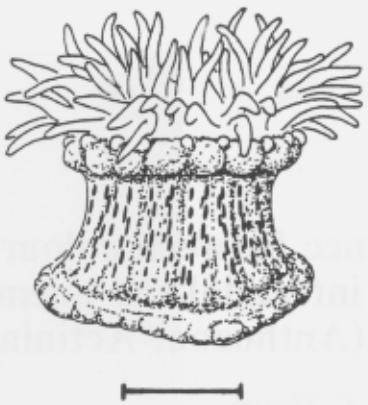

Fig. 1. *Actinia* cf *equina*. Red/Green morph. Note rows of oval spots along the column. Scale bar = 1 cm

Actinia prasina (Green morph)

These anemones had both column and pedal discs plain green. All the morphological characteristics are identical to those described for *Actinia prasina* (Haylor et al. 1984, Solé-Cava and Thorpe 1987a).

Actinia cf. equina (Red/Green morph)

This unusual morph was dinstinguished from the red morph by the presence of tiny green to grey-green oval spots distributed in numerous vertical rows down the column (Fig. 1). Rows of spots have been described before for *Actinia equina* (e.g. Manuel 1980, 1988), and in our specimens they were visible on both small and large anemones. The oval spots were always oriented with the long axis of the spot vertical. In some individual anemones some spots were highly elongate and could be described as short stripes or dashes. The rows of spots often appeared like dashed lines running down the column. The spots fluoresce green when exposed to ultraviolet light. The pedal discs of this morph were pinkish grey to greenish grey, bordered by a blue line (also present on *A. prasina*, but absent on all examples of the red morph used in the present work). The pedal disc colour was 6C3 to 3B2 (Kornerup and Wanscher 1978), the column colour was 10E8, and the spots were 29C7.

It should be noted that, although the description may appear superficially similar, this morph is very different from *Actinia fragacea*. The latter is very much larger and rather darker red, with proportionally larger spots which are fairly evenly scattered over the column and not arranged in rows.

Some anemones of the red morph of *Actinia equina* were found with greyish discolourations on the columns which might be interpreted as "spots". However, these "discolourations" are produced by scarification resulting from abrasion against rocks or by intraspecific aggression, never appearing as distinct rows along the column. The presence of the diagnostic rows of spots vertically aligned along the column allowed the unambiguous identification of all anemones of the red/green morph analysed here.

Specimens of the *Actinia cf. equina* RG morph have been deposited in the British Museum (Natural History) in London, where they have been given numbers BMNH 1986.12.17.6–8.

Electrophoresis

Before electrophoresis, anemones were maintained alive in tanks of circulating seawater. Tissue samples were taken from the oral disc and column, homogenised in not more than an equal volume of distilled water, and analysed by horizontal 12.5% starch-gel elec-

trophoresis (reviews by e.g. Harris and Hopkinson 1978). A total of 21 putative loci were typed, although not all of these could be successfully resolved for all the five populations studied. Several other enzymes were stained for, but this produced no useful results (see Solé-Cava 1986). To avoid possible sampling bias (e.g. heterozygotes being more likely than homozygotes to show inadequate enzyme activity), if any anemones used from a sample did not give useful results for any particular locus on a particular gel, no results for that sample were included for that locus. For further details of electrophoretic methods for *Actinia* spp. see Solé-Cava and Thorpe (1987a).

Nematocyst analysis

Nematocysts for six anemones of each of the three colour morphs from Fleshwick Bay were also analysed. Ten nematocysts of each of the eight types found in the mesenteric filaments, actinopharynx, acrorhagi and tentacles of these individuals were measured. Preparation of nematocysts was in accordance with standard methods and classification followed standard terminology (Carlgren 1949, Hand 1955). Further details of methods are given by Solé-Cava (1986) (see also Solé-Cava et al. 1985).

Data analysis

Banding patterns were used to calculate gene frequencies, heterozygosity levels and pairwise genetic identities (Nei 1972) between the populations, using the BIOSYS-1 genetic analysis programme (Swofford and Selander 1981). Estimates of genetic divergence between populations may be reduced to a single figure by the use of any of several published measures of genetic similarity or identity (measures of similarity) or of genetic distance (measures of dissimilarity) (reviews by Thorpe 1982, Nei 1987). The most commonly used statistic, and that used here, is the genetic identity measure, I, and its converse genetic distance, D, of Nei (1972). The scale of I values ranges from 1 (no difference) to 0 (no genes is common), whilst D ranges from 0 (no difference) to infinity (no genes is common). Also used was the genetic similarity measure S of Thorpe (1979). Values of Nei's I are known to be unduly affected by the numbers of alleles present and if, as in the present work, the populations concerned are highly polymorphic, estimates can be artificially high (for discussion see Thorpe 1979, 1982; Hillis 1984). The simplified measure, S of Thorpe (1979) approximates to I and for monomorphic populations is identical, but S estimates the overlap of allele frequency and hence, unlike I, is unaffected by numbers of alleles per se. Additionally, possible associations at each locus between allele frequencies and different populations were tested by contingency table analysis. Nematocyst data were compared between populations by two-way analysis of variance (Sokal and Rohlf 1981).

Results

Allele frequencies for the allozyme loci studied in the five samples of *Actinia equina* and *A. prasina* are presented in Table 1. This table also given samples sizes and Enzyme Commission (EC) numbers for each locus. Table 2 gives values for genetic identity and genetic distance (Nei 1972) between the samples for each of the two localities. The levels of significance for loci showing significant ($P<0.05$) differences between sympatric samples are indicated in Table 3. Probabilities were calculated by contingency χ^2 analsyis or, where sample sizes were too small, using binomial or Fisher's exact tests (Sokal and Rohlf 1981).

The results for two putative loci require further explanation. Some anemones sampled were found to yield on the gels blue bands which were faintly visible even before any staining mixtures had been used. The substance forming these bands showed properties normally associated with proteins and stained very strongly with protein specific stains and was therefore presumed to be a protein. This protein (possibly a pigment) was unambiguously either present or absent in any given anemone, although it showed apparent polymorphism in the population of the *Actinia equina* red morph from Fleshwick. It was absent in all samples of the RG morph from both Fleshwick and Port St. Mary and from all *A. prasina*. This protein provides a clear difference between various samples, although, of course, the genetic basis of this difference is not known. The protein may well be coded by a single genetic locus which is absent (or at least not functional) in some specimens. Individuals lacking this protein were scored as though homozygous for a "null" allele. Since it stained with a general protein stain, the locus was called the General Protein (*Gp*) locus. A very similar, and probably homologous, locus, based on a apparently identical blue protein, has been described by Solé-Cava et al. (1985) in sea anemones of the related actiniid genus *Urticina*. In *U. felina*, the absence of significant deviations from Hardy-Weinberg expectations over considerable numbers of anemones indicates that this protein was probably coded by a single locus in that species. We assumed, thus, that the blue protein in *Actinia* spp. could also be interpreted as the product of a single gene locus. This interpretation is conservative, and more parsimonious than considering, for example, the existence of two loci, both with null alleles.

The second locus requiring explanation is Octopine Dehydrogenase (*Odh*), which also yielded clear activity in some specimens and a total absence of any staining in others. Again results were interpreted as indicating a null allele. Whether this explanation for the *Odh* banding pattern is reasonable is unclear because the function of the enzyme in sea anemones is open to speculation. ODH is absent in most other animals which have been investigated, its known occurrence being largely limited to certain taxa of marine molluscs. Since its function in sea anemones has not been extensively studied, it is not clear whether ODH is metabolically important in *Actinia* spp. (see Ellington 1980). ODH does not stain and so may be absent in various other sea anemones, although, again, it is present in *Urticina* spp. (authors' unpublished results) and in *Bunodosoma cavernata* (Ellington 1980). For the *Gp* and *Odh* loci banding patterns other explanations are possible, and those suggested here may be incorrect (for a discussion of the problems of interpretation of null alleles see e.g. Utter et al. 1987). However, the overall results are similar with or without the inclusion of these two putative loci which, therefore, have little effect on the main conclusions drawn from the data. In any case the loci are of interest because they provide further indications of differences between *A. equina* (Red morph) and the RG morph described here. The blue protein also provides evidence of a further distinction between the common (red) morph of *A. equina* and the green *A. prasina*.

Table 1. *Actinia equina* and *A. prasina*. Gene frequencies over 21 allozyme loci for five population samples. Dash (–) indicates poor resolution. *n*: number of alleles samples. Enzymes analysed are aconitase (ACON, EC 4.2.1.3), catalase (CAT, EC 1.11.1.6) esterases (EST, EC 3.1.1.1), glutamate dehydrogenase (GDH, EC 1.4.1.3), glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1), general protein (GP), hexokinase (HK, EC 2.7.1.1), leucine aminopeptidase (LAP, EC 3.4.1.1), malate dehydrogenase (MDH, EC 1.1.1.37), mannosephosphate isomerase (MPI, EC 5.3.1.8), octopine dehydrogenase (ODH, EC 1.5.1.11), prolyl-phenylalanine peptidases (PEP, EC 3.4.11.1), phosphogluconate dehydrogenase (PGD, EC 1.1.4.4), phosphoglucose isomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 2.7.5.1), xanthine oxidase (XOD, EC 1.2.3.1)

Locus	Allele	Fleshwick			Port St. Mary	
		Red	Green	Red/ Green	Red	Red/ Green
Acon	1	0.400	0.375	–	–	–
	2	0.567	0.625	–	–	–
	3	0.033	0.000	–	–	–
	n	30	16	–	–	–
Cat	1	0.167	0.500	1.000	0.300	0.500
	2	0.833	0.500	0.000	0.700	0.500
	n	24	30	20	20	20
Est	1	0.125	0.000	0.000	–	–
	2	0.875	1.000	1.000	–	–
	n	48	34	30	–	–
Gdh	1	0.286	0.000	0.000	0.452	0.000
	2	0.571	0.813	1.000	0.262	1.000
	3	0.143	0.188	0.000	0.262	0.000
	n	14	16	10	42	4
Got-1	1	1.000	1.000	1.000	1.000	1.000
	n	30	36	30	32	20
Got-2	1	0.773	0.618	0.633	0.500	0.389
	2	0.227	0.382	0.367	0.500	0.556
	3	0.000	0.000	0.000	0.000	0.056
	n	22	34	30	30	36
Gp	1	0.375	0.000	0.000	0.875	0.000
	2	0.250	0.000	0.000	0.000	0.000
	null	0.375	1.000	1.000	0.125	1.000
	n	8	10	6	46	6
Hk-1	1	0.080	0.176	–	0.000	0.444
	2	0.920	0.824		1.000	0.556
	n	50	34		30	36
Hk-2	1	0.650	0.214	–	–	0.222
	2	0.150	0.571	–	–	0.611
	3	0.200	0.214	–	–	0.167
	n	40	28	–	–	30
Lap	1	0.079	0.083	0.000	0.000	0.083
	2	0.763	0.750	1.000	1.000	0.833
	3	0.105	0.167	0.000	0.000	0.083
	4	0.053	0.000	0.000	0.000	0.000
	n	38	36	30	32	30
Mdh-1	1	0.059	0.000	0.094	0.200	0.000
	2	0.941	1.000	0.906	0.800	1.000
	n	34	36	32	30	30
Mdh-2	1	0.632	1.000	1.000	1.000	1.000
	2	0.316	0.000	0.000	0.000	0.000
	3	0.053	0.000	0.000	0.000	0.000
	n	38	36	32	30	30
Mpi	1	0.125	0.091	0.200	0.200	0.000
	2	0.750	0.750	0.333	0.500	0.722
	3	0.125	0.159	0.400	0.300	0.278
	n	48	44	30	30	36
Odh	1	0.000	0.000	0.000	0.304	0.000
	2	1.000	0.000	0.000	0.478	0.000
	null	0.000	1.000	1.000	0.217	1.000
	n	10	10	10	46	6
Pep	1	1.000	0.972	1.000	0.800	1.000
	2	0.000	0.028	0.000	0.200	0.000
	n	46	36	30	30	34
Pgd	1	0.900	0.810	0.967	0.500	1.000
	2	0.100	0.190	0.033	0.500	0.000
	n	50	42	30	32	36
Pgi-1	1	0.045	0.045	0.500	0.100	0.222
	2	0.955	0.955	0.500	0.900	0.778
	n	44	44	32	30	36
Pgi-2	1	0.938	0.841	1.000	0.600	0.722
	2	0.063	0.159	0.000	0.400	0.278
	n	48	44	32	30	36
Pgm	1	0.478	0.143	0.967	0.000	0.125
	2	0.522	0.857	0.033	1.000	0.875
	n	46	28	30	30	32
Sod	1	0.950	1.000	1.000	0.476	1.000
	2	0.000	0.000	0.000	0.310	0.000
	3	0.050	0.000	0.000	0.214	0.000
	n	40	36	38	42	6
Xod	1	0.762	0.706	0.833	–	–
	2	0.238	0.294	0.167	–	–
	n	42	34	30	–	–

Levels of genetic variation were very high (Table 4) with only one locus (*Got-1*) not being polymorphic in at least one of the five samples used. Values for mean observed heterozygosity per locus ranged from 0.180 to 0.348.

The observed genotypic frequencies were, with one exception, not significantly divergent from Hardy-Weinberg expectations (but note the restrictions of the power of χ^2-tests of fits to Hardy-Weinberg frequencies, especially with small sample sizes; see Fairbairn and Roth 1980, Valenzuela 1985). For the *Pgi* locus a significant ($P<0.05$) excess of heterozygotes was observed for the RG population from Fleshwick Bay. Samples of the same morph from the other collection site (Port. St. Mary) did not show any significant deviation from expectations, and it is likely that the one significant deviation resulted from sampling error, since, based on chance alone, such a result would be expected from ca. 5% of loci studied. Asexual reproduction can be eliminated as an explanation, as none of the results came from individuals genetically identical over all loci.

Nematocyst measurements are summarised in Table 5. No significant differences were found between any of the samples of *Actinia equina* and *A. prasina* for any class of nematocyst.

Table 2. *Actinia equina* and *A. prasina*. Genetic similarities between populations from Fleshwick Bay (Flwk) and Port St. Mary (PSM). Values above diagonal indicated by dashes: Genetic Identity (Nei 1972); values below diagonal: Genetic Distance (Nei 1972). R: red; G: green; RG: red/green

Population		Fleshwick			Port St. Mary	
		A. equina (R)	*A. prasina* (G)	*A. equina* (RG)	*A. equina* (R)	*A. equina* (RG)
Flwk	*A. equina* (R)	–	0.874	0.803	0.877	0.827
Flwk	*A. prasina* (G)	0.135	–	0.908	0.835	0.983
Flwk	*A. equina* (RG)	0.219	0.097	–	0.687	0.905
PSM	*A. equina* (R)	0.131	0.180	0.375	–	0.802
PSM	*A. equina* (RG)	0.190	0.017	0.100	0.221	–

Table 3. *Actinia equina* and *A. prasina*. Contingency table comparisons [χ^2 (DF)] between anemone populations from Fleshwick (Flwk) and Port St. Mary (PSM). R: *A. equina* Red, *G*: *A. prasina* Green; *RG*: *A. equina* Red/Green spots. NS: not significant ($P>0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$; (–): data not available. Only loci with at least one significant difference are shown

Locus	R × G (Flwk)	R × RG (Flwk)	G × RG (Flwk)	R × RG (PSM)
Cat	*	***	*	NS
Hk-1	NS	–	–	***
Hk-2	***	–	–	NS
Mpi	NS	**	**	***
Odh	***	***	NS	***
Pgi-1	NS	***	***	NS
Pgm	*	***	***	NS
Gp	**	*	NS	***
Gdh	NS	NS	NS	***
Mdh-1	NS	NS	NS	***
Mdh-2	***	***	NS	NS
Pgd	NS	NS	NS	***
Sod	NS	NS	NS	*
Lap	NS	**	**	*
Est	NS	*	NS	–

Table 4. *Actinia equina* and *A. prasina*. Levels of genetic variation in five populations in Fleshwick Bay (Flwk) and Port St. Mary (PSM). H_0: Mean observed heterozygosity; H_e: mean expected heterozygosity; $P_{(0.95)}$: proportion of polymorphic loci; *n*: mean effective number of alleles per locus; *N*: mean number of alleles per locus. R: red; G: green; RG: red/green

Location	Morph	H_0	H_e	$P_{(0.95)}$	*n*	*N*
Flwk	*A. equina* (R)	0.258	0.275	0.810	1.379	2.238
Flwk	*A. prasina* (G)	0.242	0.218	0.619	1.279	1.810
Flwk	*A. equina* (RG)	0.180	0.157	0.278	1.186	1.444
PSM	*A. equina* (R)	0.348	0.342	0.706	1.520	1.941
PSM	*A. equina* (RG)	0.262	0.238	0.500	1.312	1.667

Discussion

The two most salient features of the allele frequency data shown in Table 1 are the levels of genetic divergence, unusually high for conspecific populations, between all of the five *Actinia equina* and *A. prasina* samples, and the high levels of genetic polymorphism within each sample. High levels of gene variation seem to be the rule for sea anemone species (Bucklin 1985, Solé-Cava et al. 1985, Shaw et al. 1987, Russo and Solé-Cava 1991). The reasons for this are not clear and, as has been extensively discussed elsewhere (Solé-Cava and Thorpe 1989, 1991), the phenomenon is open to both neutralist and selectionist explanations.

For the five samples studied in the present work, estimates of genetic identity, *I*, and distance, *D* (Nei 1972), for all possible pairwise comparisons are given in Table 2. There is a vast published body of literature giving levels of genetic divergence observed between various populations and species (review by e.g. Thorpe 1982, 1983; Nei 1987). In general conspecific populations have *I* values above ca. 0.9 and rarely as low as 0.85, whilst between congeneric species the usual range is ca. 0.30 to 0.85.

Divergence between conspecific populations is, of course, only likely if these are allopatric and there little or no gene flow between them or if there is strong selective pressure. Between sympatric populations of a single species (e.g. different colour morphs) there should be, apart from possible sampling errors, no differences in gene frequency at any locus since, by definition, conspecific indi-

Table 5. *Actinia* and *A. prasina*. Mean size (μm) and standard deviation (SD) for each nematocyst type (six individuals analysed for each of the three morphs from Fleshwick Bay). Fisher's F^a was calculated from a two-way analysis of variance (Sokal and Rohlf 1981)

Organ nematocyst type	Red		Green		Red/Green	
	Mean	SD	Mean	SD	Mean	SD
Mesenteric filaments						
Basitric	13.4	0.79	12.9	0.62	12.7	0.36
Microbasic p-mastigophore	22.5	1.98	22.7	2.17	23.5	1.19
Microbasic b-mastigophore	35.0	2.80	33.6	4.27	35.0	0.78
Actinopharynx						
Basitric I	24.4	1.91	24.5	2.12	24.4	2.06
Basitric II	13.9	1.43	14.1	1.34	13.5	0.50
Acrorhagi						
Holotrich	50.3	6.09	48.9	3.15	50.7	2.75
Basitric	13.8	0.59	13.3	0.36	13.0	0.58
Tentacles						
Basitric	22.4	1.93	21.7	1.30	22.0	0.31

[a] $F_{[A.\ equina\ (R) \times A.\ prasina \times A.\ equina\ (RG)]} = 2.066$; DF = 2 and 14; $P>0.31$ (not significant)

viduals should be freely interbreeding. Any significant difference in gene frequency between sympatric populations indicates that there is the likelihood of a barrier to gene flow and, therefore, that the two populations may not be conspecific (for a discussion see e.g. Ayala 1983; Solé-Cava and Thorpe 1987b). Allowance must be made for possible distortion of gene frequencies by, for example, asexual reproduction or self-fertilisation, but in the present work neither of these factors is likely to have affected the data. None of the individuals providing the gene frequency data was genetically identical to any other over all loci, and so no two can have been from the same clone. Self-fertilisation can be eliminated as a possibility because as far as is known all *Actinia* species are dioecious. The fact that fewer than 5% of loci differed significantly (at the 0.05 level) from Hardy-Weinberg expectations also argues against the occurrence of any reproductive modes likely to cause large scale distortion of gene frequencies.

In this study the sample sizes for each morph varied considerably among the various loci sampled. For most loci at least 15 anemones of different genotypes were typed from each sample, but, in a few cases, results were for considerably smaller numbers, in one case for only two. However, small sample size have little effect on the accuracy of the estimation of genetic identity or genetic distance and will make only a small contribution to the total sampling errors. This is because small sample sizes will affect only the accuracy of estimates of gene frequencies at polymorphic loci. Errors in estimates of gene frequencies will result only in increased intralocus sampling errors, which contribute only a very small amount (typically $<1\%$) to the total sampling errors of I values. The other component of the sampling errors of I values consists of interlocus errors which are inevitably much larger than the intralocus errors and are effectively a function of the numbers of loci for which comparative gene frequency data are available. Even on impracticably large numbers of loci sampled (e.g. 100+) the interlocus errors will generally remain by far the major source of sampling error. Hence the errors of I or D values are effectively a function of the number of gene loci studied and are little affected by sample sizes. Indeed, for estimation of genetic diversity between species, fairly robust estimates of I or D may be made from samples as small as only one individual as long as a number of loci are studied (see Nei 1978, Gorman and Renzi 1979, Thorpe 1982). Thus for the purposes of the present work many of the sample sizes used for electrophoresis were unnecessarily large, although ideally it would have been preferable to have had rather more samples for the loci with the smallest numbers. The results and conclusions would not be greatly affected if the data for the smallest sample sizes were left out entirely, but these have been included for completeness and because, as pointed out above, they will make little difference to the total sampling errors.

For the samples from Fleshwick Bay, where the three morphs [red (R), and red/green (RG) *Actinia cf. equina*, and green (G) *Actinia prasina*] are sympatric, no fewer than five loci (*Cat*, *Mpi*, *Pgi-1*, *Pgm*, *Lap*) showed significant ($P<0.05$) differences between the RG morph and both the R morph and *Actinia prasina* (Table 3). Four further loci (*Odh*, *Gp*, *Mdh-2*, *Est*) also differed significantly between the R and RG morphs. There were also clear genetic differences between the R morph and *A. prasina*, but this is already known (Haylor et al. 1984, Solé-Cava and Thorpe 1987a), although the data do provide further evidence of the genetic distinctness of *A. prasina*. The R and the RG samples from Port St. Mary also showed significant differences at nine loci (*Hk-1*, *Mpi*, *Odh*, *Gp*, *Gdh*, *Mdh-1*, *Pgd*, *Sod*, *Lap*) thus confirming that these two morphs are genetically isolated at this sampling site also. Comparisons with *A. prasina* were not made because this species is very rare at Port St. Mary (see "Materials and methods"). It is clear, therefore, that although no diagnostic loci (sensu Ayala 1983) were found for sympatric morphs at either Port St. Mary or Fleshwick, the RG morph is extensively genetically differentiated from other sympatric *A. equina*. It follows, therefore, that, given certain assumptions, the RG morph is reproductively isolated and hence possibly should not be considered conspecific. The assumptions made are that allele frequencies are not significantly modified by, for example, the action of postzygotic selection and also that differences do not result from patchy recruitment of genetically diverse larvae. The extensive literature covering to putative effects of selection on allozyme loci (reviewed by Nei 1978) indicates that resultant differences are rarely, if ever, as marked as those described here, particularly over several loci. It is thus improbable that the observed level of genetic differentiation of sympatric morphs could result from selection alone and, even if it did, this would provide evidence that the morphs were "different". Heterogeneous recruitment is probably a more viable explanation for genetic patchiness, but is likely to require the existence of a sexually produced pelagic larva, which is not known in *A. equina*.

Despite the apparent reproductive isolation between the various morphs the genetic identity (Nei 1972) values between sympatric populations are generally high, ranging from 0.802 (between the R and RG morph at Port St. Mary) to 0.908 (between the RG and G morphs at Fleshwick). The I values obtained between the R and RG morphs from Fleshwick ($I=0.802$) and Port St. Mary ($I=0.803$) are very similar and both outside the range of values expected for conspecific populations (for a discussion see Thorpe 1982; Nei 1987). However, in the present work such criteria are not highly relevant to species identification, because, as discussed above, numerous significant differences in allele frequencies indicate that the sympatric populations of these morphs are reproductively isolated.

It should also be noted that in the present work the I values are misleadingly high because they are distorted by the high levels of polymorphism. If S (Thorpe 1979) is used for the present data, the differentiation between sympatric morphs becomes much greater; for example, the particularly high I value of 0.908 for the comparison between the RG and G morphs at Fleshwick gives an S value of only 0.821. Similarly the values for the two comparisons between the sympatric R and RG morphs at Fleshwick ($I=0.803$) and Port St. Mary ($I=0.802$) give S

values of only 0.701 and 0.671, respectively. High levels of heterozygosity are common in sea anemone populations (Manchenko and Balakirev 1984, Solé-Cava and Thorpe 1991), and this consequent problem of the interpretation of unexpectedly high I values has been noted and discussed previously (Solé-Cava et al. 1985).

Most of the genetic identity values found in the present work fall within the upper part of the range generally expected for comparisons of congeneric species of plants and animals (see Thorpe 1982, 1983, Ayala 1983) and are fairly typical of values found between recently diverged species. Similarly high values have been obtained for divergence between *Actinia equina* and *A. prasina* from Port Erin Bay (Haylor et al. 1984) and for that between *Urticina felina* and *U. eques* (Solé-Cava et al. 1985). In the latter study the high genetic identity contrasts with the clear morphological differences and highly significant divergence at some loci found between *U. felina* and *U. eques*. The genetic identity between the R morph at Port St. Mary and the RG morph at Fleshwick is considerably lower ($I=0.687$) and is close to a typical value for congeneric species. Some of the higher I values in Table 2 are within the range usually associated with conspecific populations, but where morphs are sympatric and allele frequencies differ significantly, high genetic identities should not be taken as evidence that the populations are not reproductively isolated. There are several examples in the literature of clearly distinct species with very high identities, a striking example being the early study of Avise and Ayala (1976), who found the very high identity of 0.948 between two clearly distinct minnow species previously considered to be in different genera. Many related bird species show similarly high I values (Avise and Aquadro 1982), which have been considered to indicate rapid speciation (Nei 1987). This may also apply to species to *Actinia*.

In many ways the genetic differentiation of *Actinia equina* (sensu Stephenson 1935) may be considered to mirror, on a much reduced scale, the complexes of numerous species revealed in several important early studies of biochemical genetic differentiation in *Drosophila* spp. (see e.g. Ayala et al. 1974, Avise 1976). These too, showed numbers of reproductively isolated cryptic species with the more similar being genetically differentiated only by significant differences in allele frequencies, but with very few loci which were "diagnostic" (as defined by Ayala 1983). In *Drosophila*, however, reproductive isolation between cryptic species detected by allele frequencies could be confirmed by laboratory breeding experiments.

A further notable feature of our results is the comparatively high genetic differentiation between allopatric populations of what appear to be the same morphs. Genetic identity values between Fleshwick and Port St. Mary populations were 0.877 and 0.905 for the R and the RG morphs of *Actinia cf. equina*, respectively. As can be seem from Table 2 the amount of genetic divergence between allopatric populations of particular morphs is of the same order (i.e., high for congeneric species or low for conspecific populations) as that between the apparently reproductively isolated sympatric populations. Thus the red *A. equina* populations from Fleshwick and Port St. Mary show about the same divergence from each other as each does from (sympatric or allopatric) *A. prasina* or from the RG morph. Likewise the two samples of the RG morph are also genetically differentiated by a similar amount from each other and from the other samples used. This comparatively substantial differentiation of samples of particular morphs of *Actinia* over short geographical distances appears to be a general feature of the genetics of *Actinia* populations, at least around the Isle of Man, since other data (authors unpublished results) indicate similarly substantial genetic divergence and apparent genetic isolation between morphologically similar anemones collected from other sampling areas separated by only small geographical distances (e.g. 2 to 10 km).

The high genetic divergence between allopatric populations of *Actinia* spp. is less surprising given the high levels of heterozygosity in the populations, which will give correspondingly increased scope for the stochastic divergence of gene frequencies (see Skibinski and Ward 1981, Solé-Cava and Thorpe 1989). Such divergence is likely to be enhanced by the probability that effective population sizes are small in *Actinia* spp. (Solé-Cava and Thorpe 1991) and by the potential for the actions of population bottlenecks and founder effect in a low recruiting species. High genetic variability will also give additional scope for the possible divergent effects of selective forces.

The generally high levels of genetic divergence between allopatric populations of most morphs of *Actinia* spp., even when sampled over only short geographical distances, indicate that genetic differentiation takes place on a very small scale and, hence, the likelihood of larval dispersal is, at best, minimal. Unfortunately, practically nothing is known of larval dispersal in *Actinia* spp., and the reason that dispersal is so slight could be simply that dispersive larva may not be produced. In his major review of sea anemone reproduction Chia (1976) concluded that *A. equina* lacked a dispersive larval stage. The only offspring known to be produced in *A. equina* are the small anemones brooded in the enteron and subsequently released through the mouth. It appears that these are always genetically identical to the brooding parent (Orr et al. 1982) and, hence, must be asexually produced. Cloned offspring are known to be similarly reared in the enteron in the Australian *A. tenebrosa* (Black and Johnson 1979) and in Brazilian populations of *A. bermudensis* (authors' unpublished results), but apparently do not occur in *A. fragacea* (Carter and Thorpe 1981). It is clear that sexual reproduction must occur in *A. equina*, because at all times of the year and in all population some individuals can be seen (in histological sections) to have male or female gonads (not both) in the mesenteries, although in many individuals (usually most) gonads are apparently absent (see Carter and Thorpe 1981). Cloned offspring commonly occur in individuals of either sex and in those lacking gonads. Further evidence that sexual reproduction occurs is that populations generally show high levels of genetic variability and allele frequencies approximate to Hardy-Weinberg expectations, but the details concerning mode of sexual reproduction or concerning larval production or dispersal in *A. equina* remain unknown.

The adults are essentially non-dispersive and, as it is difficult to envisage cross fertilisation occurring over other than very short distances, the suggestion that there may be little or no gene flow between shores becomes quite plausible.

In the present work the electrophoretic analysis of the populations of *Actinia equina* and *A. prasina* studied indicates, thus, two important facts about their genetics and ecology: (a) the R, G and RG populations are reproductively isolated and are likely, therefore, to belong to different biological species and (b) the genetic differentiation between geographically isolated populations of the same morphs is about as high as the difference between sympatric populations of the different *Actinia* morphs (i.e., probable species) (Fig. 2).

The lack of any significant difference between the nematocysts of the various morphs studied here is not surprising. Nematocysts are considered important characters in the taxonomic study of sea anemones (Hand 1955, Manuel 1981, 1988), but do not always differ significantly between related species. Although *Urticina felina* and *U. eques* are morphologically fairly distinct, Solé-Cava et al. (1985) could find no nematocyst differences between them.

The "red with green spots" morph described here has been overlooked in the majority of genetic or ecological studies of *Actinia equina*. This may be because most have concentrated on the overall colour of the species or the pedal disc colour or simply because the morph was not present at the localities sampled. The morph, although easily identifiable once seen, is not particularly conspicuous and also, even where found, is far from abundant.

The taxonomy of the species group formerly called *Actinia equina* is far from simple. The successive splits that this species has undergone recently (Carter and Thorpe 1981, Haylor et al. 1984, this paper) suggest that, in the past, taxonomists have treated observed morphological differences in a rather conservative way. Similar observations have been made for other sea anemone genera (e.g. McCommas and Lester 1980, Bucklin and Hedgecock 1982, Solé-Cava et al. 1985, Shaw et al. 1987) and seem to indicate that cryptic species may not be uncommon in sea anemones.

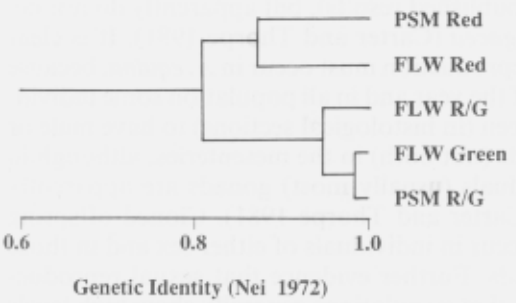

Fig. 2. *Actinia* cf. *equina* [Red and Red/Green (R/G)] and *A. prasina* (Green). Unweighed Pair-Group Mean Analysis (Sneath and Sokal 1973) dendrogram of Genetic Identity (Nei 1972) values between the five samples studied. PSM: Port St. Mary; FLW: Fleshwick

It is not clear why closely related groups of coexisting cryptic species should exist in various sea anemone taxa. Low mobility benthic organisms may "see" the environment as "coarse grained" (Levins 1968) and hence as a heterogeneous assemblage of niche opportunities. In some species the genotype amplification effect of asexual reproduction (Ayre 1984, Bucklin 1985) may also be important. Limited dispersal between allopatric populations established in this way could lead to divergence and speciation on a very local scale, in a way similar to that which is thought to occur among species occupying groups of islands (Yang et al. 1974), or in "species flocks" (Greenwood 1984).

Another factor which may be relevant is the very high level of intraspecific genetic variation in sea anemones (see Solé-Cava and Thorpe 1989, 1991 and references therein). If the rates of divergence of individual loci are related to their levels of heterozygosity (Skibinski and Ward 1981, Ward and Skibinski 1985), then species with high genetic variation may be expected to evolve more rapidly than those which are less polymorphic. This suggestion is compatible with our data since the loci that contribute most to the genetic differentiation between the populations studied (Table 3) present a significantly ($t = 4.128$; DF = 79; $P < 0.001$) higher level of genetic variation (H; mean heterozygosity = 0.377; SD = 0.197; $n = 23$) than the rest of the loci studied ($H = 0.177$; SD = 0.197; $n = 58$). However, correlation found between rates of divergence and heterozygosity can be tentatively explained by either selectionist or neutralist hypotheses (see e.g. Skibinski and Ward 1981).

Sea anemones present a series of unusual features in their population biology: high levels of genetic variation, high differentiation between geographically close populations and, possibly, niche segregation on a local scale, which make this a very interesting group from both ecological and genetic viewpoints.

At present the genetic structure of populations of *Actinia* spp. has been investigated for only a few shores in Britain. If sympatric cryptic species and extensive genetic differences between neighbouring populations of the same species are found to be more general, it will become increasingly difficult to define species of *Actinia*. Allopatric populations inevitably present problems for the definition of species (see Mayr 1987, Van Valen 1988, Templeton 1989), and current ideas of the species concept may not prove adequate for the situation which may exist in the genus *Actinia*.

Acknowledgements. The authors are most grateful to P. Ward for drawing Fig. 1, to Prof. J. A. Beardmore for helpful discussion and to Prof. T. A. Norton for provision of facilities. Financial support was provided by CAPES and FAPERJ (Brazilian Government).

Literature cited

Avise, J. C. (1974). The systematic value of electrophoretic data. Syst. Zool. 23: 465–481

Avise, J. C. (1976). Genetic differentiation during speciation. In: Ayala, F. J. (ed.) Molecular evolution. Sinauer Associates, Sunderland, Massachusetts, p. 106–122

Avise, J. C., Aquadro, C. F. (1982). A comparative summary of genetic distances in the vertebrates: patterns and correlations. Evolutionary Biol. 15: 151–185

Avise, J. C., Ayala, F. J. (1976). Genetic differentiation in speciose versus depauperate phylads: evidence from the California minnows. Evolution 30: 46–58

Ayala, F. J. (1983). Enzymes as taxonomic characters. In: Oxford, G. S., Rollinson, D. (eds.) Protein polymorphism: adaptive and taxonomic significance: Academic Press, London, p. 3–26

Ayala, F. J., Tracey, M. L., Hedgecock, D., Richmond, R. C. (1974). Genetic differentiation during the speciation process in *Drosophila*. Evolution 28: 576–592

Ayre, D. J. (1984). The effects of sexual and asexual reproduction on geogrphic variation in the sea anemone *Actinia tenebrosa*. Oecologia 62: 222–229

Black, R., Johnson, M. S. (1979). Asexual viviparity and population genetics of *Actinia tenebrosa*. Mar. Biol. 53: 27–31

Bucklin, A. (1985). Biochemical genetic variation, growth and regeneration of the sea anemone, *Metridium*, of British shores. J. mar. biol. Ass. U.K. 65: 141–157

Bucklin, A., Hedgecock, D. (1982). Biochemical genetic evidence for a third species of *Metridium* (Coelenterata: Actiniaria). Mar. Biol. 66: 1–7

Campbell, A. C. (1976). Guide to the shallow seas of Britain and Europe. Hamlyn, London

Carlgren, O. (1949). A survey of the Phytodactiaria, Coralimorpharia and Actiniaria. K. svenska Vetensk Akad. Handl. 1: 1–121

Carter, M. A., Thorpe, J. P. (1981). Reproductive, genetic and ecological evidence that *Actinia equina* var. *mesembryanthemum* and var. *fragacea* are not conspecific. J. mar. biol. Ass. U.K. 61: 79–83

Chia, F. S. (1976). Sea anemone reproduction: patterns and adaptive radiation. In: Mackie, G. O. (ed.) Coelenterate ecology and behaviour. Plenum Press, New York, p. 261–270

Dalyell, J. G. (1848). Rare and remarkable animals of Scotland represented from living subjects: with practical observation on their nature. Van Voorst, London

Ellington, W. R. (1980). Partial purification and characterisation of a broadly-specific Octopine dehydrogenase from the tissues of the sea anemone *Bundosoma cavernata* (Bosc). Comp. Biochem. Physiol. 67B: 625–631

Fairbairn, D. J., Roth, D. A. (1980). Testing genetic models of isozyme variability without breeding data: can we depend on the χ^2? Can. J. Fish. aquat. Sciences 37: 1149–1159

Gorman, G. C., Renzi, J. (1979). Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia 1979: 242–249

Gosse, P. H. (1860). A history of the British sea anemones and corals. Van Voorst, London

Grassle, J. P., Grassle, J. F. (1976). Sibling species in the marine pollution indicator, *Capitella capitata* (Polychaeta). Science, N.Y. 192: 567–569

Greenwood, P. H. (1984). African cichlids and evolutionary theories. In: Echelle, A. A., Kornfield, I. (eds.) Evolution of fish species flocks. University of Maine at Orono Press, Orono, Maine, p. 141–154

Hand, C. (1955). The sea anemones of central California, Pt 2. The endomyarian and mesomyarian anemones. Wasman J. Biol. 13: 37–99

Harris, H., Hopkinson, D. A. (1978). Handbook of enzyme electrophoresis in human genetics. North-Holland, Amsterdam

Haylor, G. S., Thorpe, J. P., Carter, M. A. (1984). Genetical and ecological differentiation between sympatric colour morphs of the common intertidal sea anemone *Actinia equina*. Mar. Ecol. Prog. Ser. 16: 281–290

Hillis, D. M. (1984). Misuse and modification of Nei's genetic distance. Syst. Zool. 33: 238–240

Johnston, G. (1847). A history of the British zoophytes. Van Voorst, London

Kornerup, A., Wanscher, J. H. (1978). Methuen handbook of colour. Eyre Methuen, London

Levins, R. (1968). Evolution in changing environments. Princeton University Press, Princeton, New Jersey

Lanchenko, G. P., Balakirev, E. S. (1984). Allozymic variation in *Anthopleura orientalis* from Peter the Great Bay of the Sea of Japan. Genetika (USSR) 10: 2072–2074

Manuel, R. L. (1980). The Anthozoa of the British Isles – a colour guide. Underwater Conservation Society, Manchester

Manuel, R. L. (1981). British Anthozoa. Academic Press, London

Manuel, R. L. (1988). British Anthozoa, rev. edn. E.J. Brill, Leiden

Mayr, E. (1987). The ontological status of species: scientific progress and philosophical terminology. Biol. & Philos. 2: 145–166

McCommas, S. A., Lester, L. J. (1980). Electrophoretic evaluation of the taxonomic status of two species of sea anemone. Biochem. Syst. Ecol. 8: 289–292

Milne-Edwards, H. (1857). Histoire naturelle des coralliaires ou polypes proprement dits, Vol. 1, Roret Paris

Nei, M. (1972). Genetic distance between populations. Am. Nat. 106: 283–292

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, N.Y. 89: 583–590

Nei, M. (1987). Molecular evolutionary genetics. Columbia University Press, New York

Orr, J., Thorpe, J. P., Carter, M. A. (1982). Biochemical genetic confirmation of the asexual reproduction of brooded offspring in the sea anemone *Actinia equina*. Mar. Ecol. Prog. Ser. 7: 227–229

Quicke, D. L. J., Donoghue, A. M., Keeling, T. F., Brace, R. C. (1985). Littoral distributions and evidence for differential post-settlement selection of the morphs of *Actinia equina*. J. mar. biol. Ass. U.K. 65: 1–20

Richardson, B. J., Baverstock, P. R, Adams, M. (1986). Allozyme electrophoresis. Academic Press, Sydney

Russo, C. A. de M., Solé-Cava, A. M. (1991). High levels of gene variation and the population structure of a tropical sea anemone, *Bundosoma caissarum* (Cnidaria, Actiniidae). Revta Biol. trop. (in press).

Schmidt, H. (1971). Taxonomie, Verbreitung und Variabilität von *Actinia equina* Linne 1766 (Actiniaria: Anthozoa). Z. zool. Syst. EvolForsch. 9: 161–169

Shaw, P. W., Beardmore, J. A., Ryland, J. S. (1987). *Sargatia troglodytes* (Anthozoa: Actiniaria) consists of two species. Mar. Ecol. Prog. Ser. 41: 21–28

Skibinski, D. O. F., Ward, R. D. (1981). Relationship between allozyme heterozygosity and rates of divergence. Genet. Res. 38: 71–92

Sokal, R. R., Rohlf, F. J. (1981). Biometry. Freeman, San Francisco

Sneath, P. H. A., Sokal, R. R. (1973). Numerical taxonomy. Freeman, San Francisco

Solé-Cava, A. M. (1986). Studies of biochemical genetics and taxonomy in coelenterates and sponges. PhD thesis, University of Liverpool. Port Erin, Isle of Man

Solé-Cava, A. M., Thorpe, J. P. (1986). Genetic differentiation between morphotypes of the marine sponge *Suberites ficus* (Demospongiae: Hadromerida). Mar. Biol. 93: 247–253

Solé-Cava, A. M., Thorpe, J. P. (1987a). Further genetic evidence for the reproductive isolation of the green colour morph of the common intertidal sea anemone *Actinia equina*. Mar. Ecol. Prog. Ser. 38: 225–229

Solé-Cava, A. M., Thorpe, J. P. (1987b). The use of electrophoresis in sponge taxonomy. In: Vacelet, J., Boury-Esnault, N. (eds.) Taxonomy of Porifera. Springer-Verlag, Berlin, p. 243–258

Solé-Cava, A. M., Thorpe, J. P. (1989). Biochemical correlates of genetic variation in marine lower invertebrates. Biochem. Genet. 27: 303–312

Solé-Cava, A. M., Thorpe, J. P. (1991). High levels of genetic variation in natural populations of marine lower invertebrates. J. Linn. Soc. 44: 65–80

Solé-Cava, A. M., Thorpe, J. P., Kaye, J. G. (1985). Reproductive isolation with little genetic divergence between *Urticina* (= Tealia) *felina* and *U. eques* (Anthozoa: Actiniaria). Mar. Biol. 85: 279–284

Stephenson, T. A. (1935). The British sea anemones, Vol. 2. Ray Society, London

Swofford, D. L., Selander, R. B. (1981). BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72: 281–283

Templeton, A. R. (1989). The meaning of species and speciation: a genetic perspective. In: Otte, D., Endler, J. A. (eds.) Speciation and its consequences. Sinauer Associates Inc., Sunderland, Massachusetts, p. 3–27

Thorpe, J. P. (1979). Enzyme variation and taxonomy: the estimation of sampling errors in estimates of interspecific genetic similarity. J. Linn. Soc. 11: 369–386

Thorpe, J. P. (1982). The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. A. Rev. Ecol. Syst. 13: 139–168

Thorpe, J. P. (1983). Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. In: Oxford, G. S., Rollinson, D. (eds.) Protein polymorphism: adaptive and taxonomic significance. Academic Press, London, p. 131–152

Thorpe, J. P., Beardmore, J. A., Ryland, J. S. (1978). Genetic evidence for cryptic speciation in the marine bryozoan *Alcyonidium gelantinosum*. Mar. Biol. 49: 27–32

Tugwell, G. (1856). A manual of sea-anemones commonly found on the English coast. Van Voorst, London

Utter, F., Aebersold, P., Winans, G. (1978). Interpreting genetic variation detected by electrophoresis. In: Ryman, N., Utter, F. (eds.) Population genetics and fishery management. University of Washington Press, Seattle, Washington, p. 21–46

Valenzuela, C. Y. (1985). Algebraic and epistemological restrictions in studies on Hardy-Weinberg equilibrium. Am. Nat. 125: 744–746

Van Valen, L. M. (1988). Species, sets, and the derivative nature of philosophy. Biol. & Philos. 3: 49–66

Ward, R. D., Skibinski, D. O. F. (1985). Observed relationships between protein heterozygosity and protein genetic distance and comparisons with neutral expectations. Genet. Res. 45: 315–340

Yang, S. Y., Soule, M., Gorman, G. C. (1974). *Anolis* lizards of the Eastern Caribbean: a case study in evolution. I. Genetic relationships, phylogeny and colonisation sequence of the *roquet* group. Syst. Zool. 23: 287–299