Marine Biology (1997) 129: 425–433

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Extensive genetic divergence between populations of the common intertidal sea anemone *Actinia equina* from Britain, the Mediterranean and the Cape Verde Islands

Received: 18 January 1997 / Accepted: 21 February 1997

Abstract Samples of apparently similar red morphs of the common beadlet sea anemone *Actinia equina* (L.) were collected from rocky shores on the Isle of Man (Irish Sea), on the French Mediterranean coast near Marseille and on the Cape Verde Island of Sal (off West Africa). For additional comparison an orange morph and the green *A. prasina* were also collected from the Isle of Man. Morphological descriptions were made and the samples were compared by nematocyst analysis and enzyme electrophoresis. The three British samples showed little genetic divergence ($I > 0.90$) but the Mediterranean sample was hugely divergent ($I < 0.20$) from the British ones. The Cape Verde Island anemones were also very different ($I < 0.60$) from all other samples. It is concluded that the red morph samples from the Cape Verde Islands, the Mediterranean and the Isle of Man belong to three different species. For the new species from the Mediterranean and Cape Verde Islands formal descriptions are given, and the names *Actinia schmidti* sp.n. and *Actinia sali* sp.n. are proposed.

Introduction

The common intertidal beadlet sea anemone *Actinia equina* (L.) is generally considered to be found on rocky shores over a wide geographical area. *A. equina* has been identified from the subpolar coasts of north Russia (Kola Peninsula) down the Atlantic coast of Europe as far south as West Africa, including the British Isles and many islands along the African coast (Azores, Madeira, Canaries, Cape Verde and St. Thomas) as well as in the Mediteranean, Adriatic and Black Seas (Stephenson 1953; Schmidt 1972; Manuel 1988). It is also considered to occur in South Africa (Manuel 1988), although whether there is continuous distribution down the African coast is unclear. Over this very wide geographical distribution *A. equina* is considered to be highly variable in colour pattern, reproductive biology, morphology and habitat choice. Not surprisingly this anemone has been the subject of much taxonomic debate. The high morphological variability led Gosse (1860) to separate the species into several varieties, which were later reduced by Stephenson (1935) to just two varieties: the large and essentially monotypic *A. equina* var. *fragacea* and the highly polytypic *A. equina* var. *mesembryanthemum*. Schmidt (1971, 1972) in a major study of morphology, anatomy, nematocysts and reproductive biology of European *Actinia equina* divided the species into four subspecies: *A. equina equina*, *A. equina atlantica*, *A. equina mediterranea* and *A. equina fragacea* with most of these subspecies further subdivided into distinct morphs.

The reluctance of these authors to assign specific status to the different morphotypes was understandable because the number of different morphs was very great with several of these often occurring sympatrically on any given shore. Problems were confounded by reports of the existence of intermediates between some of the varieties, and the obvious problems of delimitating species boundaries when the morphs present often varied greatly between adjacent shores. The availability of more powerful genetic methods, like allozyme electrophoresis (reviewed by e.g. Thorpe and Solé-Cava 1994), presented the objective tools to tackle the systematics of *Actinia*. In 1981, Carter and Thorpe, using genetic, ecological and reproductive differences, were able to find clear diagnostic characteristics that led them to give specific status to Stephenson's (1935) varieties *fragacea*

Communicated by O. Kinne, Oldendorf/Luhe

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and *mesembryanthemum*, thus named *A. fragacea* and *A. equina*, respectively. Later the green morph of *A. equina* (possibly Schmidt's *A. equina equina* morph II) was found to be reproductively isolated from the sympatric red morph in the Isle of Man, and was therefore also given specific status, as *A. prasina* (Haylor et al. 1984; later confirmed using samples from a different shore, by Solé-Cava and Thorpe 1987). Further work, with other colour morphs from the British Isles, has suggested that even in that region the number of species may be larger still (Solé-Cava and Thorpe 1992; Perrin et al. 1997).

Due to their ubiquity and ecological importance, a great deal of work has been carried out on *Actinia equina* sensu *latu*) populations from British and Mediterranean shores. This includes ecological (Rees 1984; Chintiroglou and Koukouras 1992; Perrin 1993), phylogenetic (Solé-Cava et al. 1994a), physiological (Boury-Esnault and Doumenc 1979; Young et al. 1988), behavioral (Brace and Quicke 1986) and more recently toxicological and pharmacological (Macek et al. 1994) studies. For these studies to be of wider applicability it is important that individuals are correctly assigned to a biological species. Studies of British populations of *A.* "*equina*" indicate that the species may be a complex, but, to date, there is little genetic information available on samples from elsewhere within its range of distribution.

In the present work we used allozyme electrophoresis to compare samples of British, Mediterranean and West African (Cape Verde Islands) populations of the red *A. equina*. We have also included in this study individuals of the green *A. prasina*, and of an orange morph from Britain. The technique of allozyme electrophoresis was chosen because it has proved successful in resolving problems of cryptic speciation in *Actinia*, as well as within several other sea anemone genera (e.g. *Bunodosoma*, McCommas and Lester 1980; *Metridium*, Bucklin and Hedgecock 1982; *Urticina*, Solé-Cava et al. 1985; *Sagartia*, Shaw et al. 1987; *Anthopleura*, Smith and Potts 1987).

Materials and methods

Collection of samples

Samples of adult red morph *Actinia equina* were collected from rocky intertidal areas of Fleshwick Bay (Isle of Man, northern Irish Sea) and Marseille (France, Mediterranean Sea) during June 1995, and from the island of Sal (Cape Verde Islands, off West Africa) in October 1994 (Fig. 1). The green *A. prasina* and a hitherto unstudied orange morph of *A. equina* were also collected at Fleshwick Bay in June 1995 to provide further comparisons with the Mediterranean and Cape Verde samples. The red *Actinia* collected in Marseille corresponded closely to Schmidt's (1971, 1972) *Actinia equina mediterranea* type I. The samples from the Cape Verde Islands were very similar to Schmidt's (1971, 1972) description of *Actinia equina atlantica* type II.

After collection the anemones were transferred to plastic bags with wet paper towels and put in an insulated container for transport to the laboratory. No anemones died during transportation, which took up to 30 h. In the laboratory the anemones were kept alive in a sea water aquarium for 1 week, during which wet weight and the precise shade of colour (Kornerup and Wanscher 1978) were determined and nematocyst analysis (Manuel 1988) was carried out. Subsequently the anemones were cut open to search for brooded juveniles and then stored frozen until required for electrophoresis.

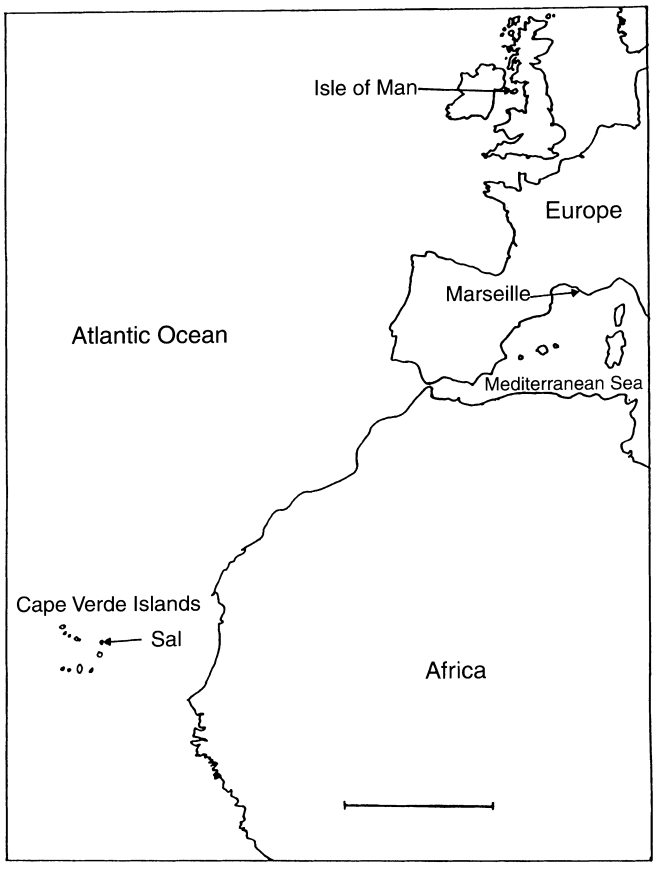

Fig. 1 Location of the three sampling sites in western Europe and northwest Africa. *Scale*: 1000 km

Electrophoresis

Tissue samples taken from the oral disc and column of the anemones were homogenised with not more than an equal volume of distilled water, and analysed by horizontal 12.5% starch gel electrophoresis, using a 0.05 *M* Tris-citrate buffer, pH 8.0 (for further details see Solé-Cava et al. 1985). Thirty enzyme systems were assayed, of which 16 (coding for a total of 19 loci) gave reproducible results.

Nematocyst analysis

Twenty each of the eight types of nematocysts found in the mesenteric filaments, actinopharynx, acrorhagi, and tentacles were measured in three individuals of each morph (a total of 60 nematocysts of each type for each tissue). Nematocyst preparation and classification followed standard methods (Carlgren 1940; Manuel 1988; Shick 1991). Nematocyst samples were taken only from adult anemones because some authors (Dunn 1981; Fautin 1988) have argued that the size of the capsules may be related to the size or age of the anemones. Spirocysts were not measured because they are considered to be of little taxonomic value (Manuel 1988).

Data analysis

Allozyme data were analysed using the programme BIOSYS-1 (Swofford and Selander 1981). Levels of heterozygosity and genetic distance (Nei 1978) were estimated for all populations analysed. The genetic distances were then used to build neighbour-joining trees (Saitou and Nei 1987), using the programme MEGA (Kumar et al. 1993).

Results

Morphological characteristics

The three red morph samples had red columns, tentacles and pedal disc. Some red morphs (at least in Britain) have a grey or green pedal disc, but we did not sample these in this work. The individuals of *Actinia prasina*, the Mediterranean sample of *A. equina* and the orange morph from Britain showed a bright blue ring around the limbus (Table 1; see also Manuel 1988; Solé-Cava and Thorpe 1992). This was not found in the other specimens. For all samples mean wet weight and diameter of the pedal disc are shown in Table 1. The Mediterranean specimens were much larger, both in wet weight and pedal disc diameter, than the British and Cape Verde anemones.

Brooded young

For each sample the numbers of anemones brooding young in the enteron are shown in Table 2. Brooding anemones were common in all populations studied, except the one from the Mediterranean, for which none of the ten individuals analysed was found to be incubating young. Over all the other samples the proportion of anemones brooding young varied from 57 to 70% (Table 2). Also, it was noted that, unlike their British counterparts, the Mediterranean anemones did not form aggregations on the shore (Monteiro personal observation) a possible further indication that they do not reproduce asexually.

Nematocyst analysis

Significant differences ($P < 0.0001$) were found in the average length of some capsule types between the populations studied (Table 3). The microbasic b-mastigophores of the mesenteric filaments, one of the classes of type 1 basitrics of the actynopharynx, and the basitrics of the tentacles appear to be the most useful as diagnostic characters.

Enzyme variation

Allele frequencies and sample sizes for all the loci studied for each sample are given in Table 4. Estimates of unbiased genetic identity, I, and distance, D (Nei 1972, 1978) for all possible pairwise comparisons between the five samples are given in Table 5. From Tables 4 and 5 it is clear that the three samples of red anemones are all genetically very different from each other, with the British and Mediterranean anemones being the most distinct ($I = 0.23$). Between these two samples 8 out of the 19 gene loci analysed were diagnostic (sensu Ayala 1983) for their separation. Between Mediterranean and Cape Verde Islands populations and between the Cape Verde Islands and Britain there is a lower divergence ($I = 0.54$ and 0.50, respectively). The three British samples are far more closely related, with a surprisingly high genetic identity of 0.97 between *A. prasina* and the orange morph of *A. equina*. The interrelationships of the five samples as indicated by the allozyme data are shown diagrammatically in Fig. 2.

Table 1 *Actinia* spp. Colour (codes from Kornerup and Wanscher 1978) and morphological data from the samples analysed. Wet weight and pedal disc diameter are mean values for the samples assayed electrophoretically (– coloured ring around limbus was absent)

	A. equina Isle of Man (Red)	*A. equina* Isle of Man (Orange)	*A. prasina* Isle of Man (Green)	*A. equina* France (Red)	*A. equina* Cape Verde (Red)
Colour					
Column	9 C8	6 D8	3 E8	10 D8	10 D8
Tentacles	8–10 C8	6 C8	3 D7	10 C8	10 D7
Pedal disc	9 C8	6 C7	1 E7	10 B8	10 D7
Limbus	–	23 A8	23 A8	23 A8	–
Size and weight					
Wet weight (g)	5.3	5.9	4.4	12.3	5.0
Pedal disc diameter (cm)	2.4	2.9	2.7	3.7	2.6

Table 2 *Actinia* spp. Numbers of adults brooding juveniles

	A. equina Isle of Man (Red)	*A. equina* Isle of Man (Orange)	*A. prasina* Isle of Man (Green)	*A. equina* France (Red)	*A. equina* Cape Verde (Red)
Total	10	5	10	10	30
Brooding	7	3	6	0	17
% brooding	70	60	60	0	57

Table 3 *Actinia* spp. Nematocyst length measurements (μm) (*SD* standard deviation)

Tissue	Nematocyst type	*A. equina* Isle of Man (red)		*A. equina* Isle of Man (orange)		*A. prasina* Isle of Man		*A. equina* France		*A. equina* Cape Verde Isles	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Mesenteric filaments	Basitric	15.16	1.80	13.78	2.03	13.66	2.03	14.71	2.66	13.26	1.22
	Microbasic p-mastigophore	24.28	4.98	21.76	1.65	23.05	1.54	20.55	2.72	19.41	1.20
	Microbasic b-mastigophore	32.05	3.60	32.76	3.65	33.00	4.54	23.76	3.29	24.14	1.18
Actinopharynx	Basitric I	29.81	2.68	24.92	1.70	27.81	2.40	28.47	2.30	21.46	1.79
	Basitric II	14.53	2.48	13.00	1.70	12.76	1.98	14.97	2.56	12.94	1.84
Acrorhagi	Holotrich	53.89	5.16	48.46	3.77	51.03	4.18	51.76	4.77	47.97	2.73
	Basitric	15.36	1.88	12.87	1.56	12.24	1.71	14.16	2.95	14.36	1.44
Tentacles	Basitric	19.06	5.16	16.51	3.31	16.99	4.13	23.49	3.80	15.78	1.53

Mean observed heterozygosity per locus ranged from 0.17 to 0.27 (Table 4), values which are high by general standards, but well within the range usually found for coelenterates (Solé-Cava and Thorpe 1991). No significant deviations from Hardy–Weinberg expectations (Fisher's exact test with Bonferroni transformation of significance levels for multiple tests, Lessios 1992) were observed at any locus of the populations studied.

Discussion

The two most salient features of this study are the very low genetic identities found between the three allopatric samples of the red morph of *Actinia equina* (from Britain, Mediterranean and Cape Verde Islands) and the particularly high identity (0.97) between the orange morph of *A. equina* and the green *A. prasina* (Table 5). The low identity values between the allopatric samples indicate large genetic divergence.

Divergence between conspecific populations is, of course, only likely if these are allopatric and there is little or no gene flow between them or if there is strong selective pressure. From conventional definitions under the biological species concept (and also for the phylogenetic species concept; Cracraft 1983; see also Templeton 1989), two sympatric morphs should, if conspecific, be part of the same evolutionary unit. Therefore they should have (given certain assumptions and within sampling errors) the same gene frequencies at each gene locus. Clearly, sympatric samples of different morphs that are conspecific should be freely interbreeding and therefore be different only at the loci coding for their morphological difference and perhaps some closely linked loci. Even if these morphs were subject to some sort of assortative mating or strong selection pressure, the gene frequencies over all the other gene loci should, because of recombination, remain similar (Wright 1978).

When populations are geographically separated there will often be little or no gene flow between them and consequently, even if they are of the same species, some genetic differentiation is to be expected. However, the level of genetic differentiation as indicated by genetic identity values can be used as an indicator of taxonomic relatedness. There is a very large published literature giving levels of genetic divergence observed between various populations and species (reviews by e.g. Thorpe 1982, 1983; Nei 1987; Thorpe and Solé-Cava 1994). In general conspecific populations have *I* values above about 0.90 and rarely as low as 0.85, whilst between congeneric species the usual range is about 0.30 to 0.85; between genera *I* values are usually in the range from 0 to about 0.40.

From Table 5 it can be seen that the British and Mediterranean populations of the red morph of *Actinia equina* have an *I* value of 0.23 and also that these two differ from the Cape Verde Islands sample with *I* values of 0.50 and 0.54, respectively. Clearly all these values fall well below the range expected for conspecific populations

Table 4 *Actinia* spp. Gene frequencies of the five populations analysed (*n* number of individuals scored; *nd* not determined; H_{obs} and H_{exp} direct count and Hardy–Weinberg expected mean heterozygosities per locus, respectively)

Locus	Allele	*A. equina* Isle of Man (red)	*A. equina* Isle of Man (orange)	*A. prasina* Isle of Man (green)	*A. equina* France (red)	*A. equina* Cape Verde (red)
Cat	1	0.45	0.80	0.85	0.00	0.25
	2	0.00	0.00	0.00	1.00	0.65
	3	0.55	0.20	0.15	0.00	0.10
	(*n*)	(10)	(5)	(10)	(10)	(10)
Est-2	1	0.00	0.00	0.00	0.25	0.00
	2	0.20	0.00	0.11	0.00	0.13
	3	0.00	0.00	0.00	0.70	0.00
	4	0.00	0.00	0.00	0.00	0.87
	5	0.80	1.00	0.89	0.05	0.00
	(*n*)	(10)	(4)	(9)	(10)	(15)
Est-3	1	1.00	1.00	1.00	1.00	1.00
	(*n*)	(10)	(5)	(10)	(10)	(10)
Gdh	1	0.00	0.00	0.00	1.00	1.00
	2	0.00	1.00	1.00	1.00	0.00
	(*n*)	(10)	(5)	(10)	(8)	(4)
Got	1	0.44	0.40	0.56	0.00	0.00
	2	0.39	0.60	0.38	0.00	0.04
	3	0.17	0.00	0.06	1.00	0.86
	4	0.00	0.00	0.00	0.00	0.11
	(*n*)	(9)	(5)	(8)	(8)	(28)
Hk	1	0.75	1.00	1.00	0.00	0.00
	2	0.00	0.00	0.00	0.50	0.00
	3	0.00	0.00	0.00	0.30	0.23
	4	0.25	0.00	0.00	0.20	0.77
	(*n*)	(10)	(5)	(10)	(10)	(13)
Lap	1	0.19	0.50	0.00	0.50	0.70
	2	0.81	0.50	1.00	0.50	0.30
	(*n*)	(8)	(1)	(9)	(9)	(5)
Mdh	1	0.30	0.00	0.00	1.00	1.00
	2	0.70	1.00	1.00	0.00	0.00
	(*n*)	(10)	(5)	(10)	(10)	(24)
Me	1	0.19	0.00	0.06	0.00	0.00
	2	0.00	0.38	0.28	0.00	0.00
	3	0.00	0.00	0.00	0.67	0.00
	4	0.00	0.00	0.00	0.33	0.00
	5	0.81	0.63	0.67	0.00	1.00
	(*n*)	(8)	(4)	(9)	(9)	(9)
Mpi	1	0.00	0.00	0.00	0.95	0.00
	2	0.30	0.00	0.00	0.00	0.05
	3	0.70	1.00	1.00	0.05	0.95
	(*n*)	(10)	(5)	(10)	(10)	(29)
Odh	1	0.00	0.00	0.00	1.00	0.00
	2	1.00	1.00	1.00	0.00	1.00
	(*n*)	(5)	(4)	(5)	(9)	(9)
Pep-1	1	0.29	0.00	0.00	0.67	0.50
	2	0.00	0.00	0.00	0.08	0.10
	3	0.07	0.00	0.36	0.25	0.40
	4	0.07	0.00	0.21	0.00	0.00
	5	0.21	0.50	0.07	0.00	0.00
	6	0.00	0.00	0.21	0.00	0.00
	7	0.36	0.50	0.14	0.00	0.00
	(*n*)	(7)	(2)	(7)	(6)	(5)
Pep-2	1	0.00	0.00	0.00	0.00	0.25
	2	0.57	0.00	0.00	1.00	0.00
	3	0.07	1.00	0.79	0.00	0.72
	4	0.36	0.00	0.07	0.00	0.00
	5	0.00	0.00	0.14	0.00	0.00
	(*n*)	(7)	(2)	(7)	(7)	(6)

(continued overleaf)

Table 4 (Contd.)

Locus	Allele	*A. equina* Isle of Man (red)	*A. equina* Isle of Man (orange)	*A. prasina* Isle of Man (green)	*A. equina* France (red)	*A. equina* Cape Verde (red)
Pgd	1	0.70	1.00	0.80	0.00	nd
	2	0.30	0.00	0.20	0.90	
	3	0.00	0.00	0.00	0.10	
	(*n*)	(10)	(5)	(10)	(10)	
Pgi-1	1	1.00	1.00	1.00	0.00	nd
	2	0.00	0.00	0.00	0.67	
	3	0.00	0.00	0.00	0.33	
	(*n*)	(9)	(4)	(9)	(9)	
Pgi-2	1	1.00	1.00	1.00	0.00	nd
	2	0.00	0.00	0.00	0.78	
	3	0.00	0.00	0.00	0.22	
	(*n*)	(9)	(4)	(9)	(9)	
Pgm	1	0.06	0.00	0.00	0.00	nd
	2	0.50	0.38	0.83	0.00	
	3	0.06	0.00	0.00	0.94	
	4	0.39	0.63	0.17	0.06	
	(*n*)	(9)	(4)	(9)	(9)	
Sod-2	1	1.00	1.00	1.00	0.00	nd
	2	0.00	0.00	0.00	1.00	
	(*n*)	(10)	(5)	(10)	(10)	
Xod	1	1.00	1.00	1.00	0.00	nd
	2	0.00	0.00	0.00	1.00	
	(*n*)	(10)	(5)	(10)	(10)	
H_{obs}		0.27	0.20	0.17	0.21	0.14
H_{exp}		0.31	0.19	0.18	0.21	0.16

Table 5 *Actinia* spp. Unbiased genetic identities (*above diagonal*) and distances (Nei 1972, 1978) (*below diagonal*)

		1	2	3	4	5
(1) *A. equina*	Isle of Man (red)		0.93	0.94	0.23	0.50
(2) *A. equina*	Isle of Man (orange)	0.08		0.97	0.11	0.47
(3) *A. prasina*	Isle of Man (green)	0.07	0.04		0.13	0.46
(4) *A. equina*	France (red)	1.48	2.25	2.07		0.54
(5) *A. equina*	Cape Verde (red)	0.70	0.76	0.77	0.62	

and strongly indicate that all three should be regarded as separate species. Indeed the differentiation between the British and Mediterranean samples of the red morph is so great that the *I* value is well within the range typical of species in different genera. The *I* values between the other two samples from Britain (*A. prasina* and the orange morph of *A. equina*) and the Mediterranean and Cape Verde Islands samples are even lower (Table 4), so it is clear that the Mediterranean and Cape Verde Islands samples are not conspecific with these either. These *I* values may be compared with genetic identities around 0.50 between various British *Actinia* populations and South American populations of the very distinct *Actinia bermudensis* (Solé-Cava et al. 1994a).

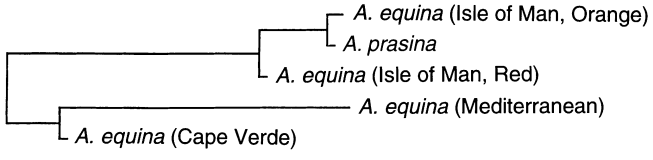

Fig. 2 *Actinia* spp. Neighbour-joining tree showing unbiased genetic distances (Nei 1978) between the populations studied

Values of genetic distance may, given certain assumptions, be stochastically proportional to time of evolutionary divergence. This suggestion constitutes the basis of what has become known as the molecular clock hypothesis (Nei 1972, 1987; Thorpe 1982).

Using a very approximate calibration factor for the molecular clock of 1 D unit = 5 million years (Nei 1987) it is possible to speculate about a correlation between the recolonisation of the Mediterranean Sea, after it became a desert (during the Miocene), by the Atlantic waters reentering through the Strait of Gibraltar (about

4 million years ago) with the genetic distance of Cape Verde Islands and Mediterranean populations of *Actinia* spp. The *D* value of 0.62 between these populations corresponds to a separation of about 3.1 million years, which approximates to the geological age of the Mediterranean. Therefore it is tempting to speculate that the Cape Verde and Mediterranean populations of *Actinia* which we sampled may have been separated since soon after the formation of the Mediterranean Sea. The separation of the British populations from the more southerly populations of Cape Verde (and the Mediterranean) could be maintained by the bifurcation of the Gulf Stream, to form the (northward) North Atlantic Current and the (southward) Canaries Current. However it should be borne in mind that these are ad hoc biogeographical hypotheses that should be considered with caution.

Any detailed discussion of the likelihood of gene flow between *Actinia equina* populations is severely limited by our lack of knowledge of its reproductive biology. Published studies to date indicate that almost all populations of *A. equina* brood young in the enteron (the same is observed in the congeneric *A. tenebrosa* (e.g. Ottaway and Kirby 1975) and *A. bermudensis* (e.g. Russo et al. 1994). These brooded young are presumed to be eventually released through the mouth as fully formed small anemones, and, as far as is known, are always asexually reproduced (Orr et al. 1982). Individuals with either male or female gonads are common, but many individuals of adult size have no apparent gonads. In sexual individuals gametogenic cycles occur (Carter and Miles 1989) and these plus the evidence of genetic polymorphism and genotype frequencies generally compatible with Hardy–Weinberg expectations lead to the assumption that the species reproduces sexually. However it is still unknown whether there is a dispersive (or indeed any) larval stage that might transport genes between populations. Long-distance dispersal by rafting is possible in some marine invertebrates (e.g. Jokiel 1989; Helmuth et al. 1994), but is unlikely in *A. equina* which attaches mainly to rocks. If there is no effective dispersive phase to the life cycle, genetic differentiation over even moderate geographical distances is quite plausible, although the subtidal *Urticina eques*, which probably has only a short-lived, non-planktonic larva, shows little apparent genetic differentiation over long distances (1000+ km; Solé-Cava et al. 1994b).

In contrast to the allopatric populations, high values of genetic identity (Table 5) were observed between the three sympatric British samples. Those values, although within the range normally considered more typical of conspecific populations, are similar to those found between various cryptic species, reproductively isolated morphs or gene pools, in earlier work on *Actinia* (reviewed in Perrin et al. 1997). When sympatric, such entities generally have significant gene frequency differences at various loci, but high overall identity values. Thus, for example the value of 0.94 between the red morph and *A. prasina* from Port Erin is similar to that found in earlier studies (Haylor et al. 1984; Solé-Cava and Thorpe 1987) and between other morphs (Solé-Cava and Thorpe 1992). The higher value of 0.97, with no significant genetic differences at any locus, between *A. prasina* and the orange morph of *A. equina* is more surprising and, allowing for sampling errors in gene frequencies, may indicate that the two are genetically identical. Further study of the orange morph is needed.

The allozyme data indicating great genetic differentiation between the Mediterranean sample and other *Actinia equina*, and hence that the Mediterranean anemones are not conspecific, are reinforced by the observation that these anemones do not reproduce by asexual brooding and are much larger than other *A. equina*. The size of the Mediterranean anemones is possibly a function of their sexual reproduction, since there seems to be a strong association between body size and mode of reproduction in many marine invertebrates (Coates and Jackson 1985). These differences precisely mirror those found in *A. fragacea*, which also is much larger than *A. equina* and never broods young (Carter and Thorpe 1981). However it is clear that the Mediterranean anemones we sampled are not *A. fragacea*. The latter has a very restricted distribution, being found only at low density on certain shores in southwest England and Brittany (Carter and Thorpe 1981), and is very distinctive morphologically with a wine red column covered in large, regularly spaced, almost-irridescent, turquoise spots (Schmidt 1971). It is also considerably larger, on average, than our Mediterranean samples and genetically is far more closely related to British populations of *A. equina* (Carter and Thorpe 1981). In geographical location, sexual reproduction, size and nematocyst types and sizes, our red *Actinia* samples from the Mediterranean appear indistinguishable from what Schmidt (1971, 1972) described as *Actinia equina mediterranea* type I. Furthermore, Schmidt's (1971, 1972) *Actinia equina mediterranea* I is also reported to reproduce exclusively by sexual means (Schmidt 1971; Schäfer 1981).

For the two cryptic species of *Actinia* which we have identified here to be useful to ecologists and other future workers it is desirable that they should be formally described and given scientific names. Unfortunately most *Actinia* species are essentially very similar, and within the *A. equina* complex taxonomically useful characters are a particular problem. In this case the three samples of red morph were selected because they were morphologically very similar since our aim was to study geographical genetic divergence within a single morph. All three samples were red all over (column, tentacles and pedal disc) with no clear morphological features to distinguish them from each other (apart from size). In *Actinia equina* generally tentacles are hexamerously arranged and number increases with the size of the anemone (Manuel 1988). Because of this, the (larger) Mediterranean samples did have, on average, more tentacles than the Cape Verde or British samples, but the difference does not apply to individuals of similar size.

Thus we come down only to differences in mean size, brooding of young, geographical location and possibly nematocyst sizes. However, the former character should be regarded with care since some smaller individuals will be present in any population and, at least in Britain, the mean size of *A. equina* appears to vary between shores (authors' unpublished observation). In our opinion nematocyst differences also should be confirmed on more individuals from other populations.

We propose the names *Actinia schmidti* sp.n. for our Mediterranean species and *Actinia sali* sp.n. for our new species from Cape Verde Islands.

Actinia schmidti sp. nov.
Actinia equina var. *mesembryanthemum* Stephenson 1935 (part)
Actinia equina mediterranea type I Schmidt 1971
Actinia equina Manuel 1988 (part)
not *Actinia equina mediterranea* type II Schmidt 1971

Red column, tentacles and pedal disc, with blue acrorhagii and a blue rim on the edge of the pedal disc (limbus). Size considerably larger than that found in most populations of *Actinia equina*, with mean pedal disc diameter of 3.7 cm (see Table 1). Nematocysts significantly different from red morph *A. equina* sampled from Britain or from the Cape Verde Islands. This species, unlike any of the others studied, has the microbasic b-mastigophores of the mesenteric filaments smaller than the basitrics of the actinopharynx (Table 2). This feature was also observed by Schmidt (1971) for his *A. equina mediterranea* I as being a distinguishing feature in relation to the other varieties that he studied. Also the basitrics of the tentacles are significantly larger (23.5 μm in our samples, 24 μm in Schmidt's *A. equina mediterranea* I) than those in the British *Actinia* (19.1 μm in our samples, 18 μm in Schmidt's *A. equina equina* II). *Actinia schmidti* sp.n. does not brood young. The species occurs on rocky shores on the north coast of the Mediterranean Sea around Marseilles (southern France), but it appears to correspond to *Actinia equina mediterranea* type I of Schmidt (1971) for which he gives a distribution covering most of the Mediterranean and northern Portugal.

The name *Actinia schmidti* was chosen partly in honour of H. Schmidt for his major study of European *Actinia* (1971, 1972) and particularly to draw attention to his description of *Actinia equina mediterranea* I which appears to be the first detailed and unambiguous description of our Mediterranean species. Type material will be deposited at the Natural History Museum, London.

Actinia sali sp. nov.
Actinia equina var. *mesembryanthemum* Stephenson 1935 (part)
Actinia equina atlantica type II Schimidt 1971 (part)
Actinia equina Manuel 1988 (part)

Actinia with red column, tentacles and pedal disc. Acrorhagii are blue, but no blue rim can be observed on the edge of the pedal disc. On gross morphology, this species appears to be, for all practical purposes, indistinguishable from the red-footed red morph of *A. equina* (L.). Apparent nematocyst differences may be useful, but until further confirmed should be regarded with caution. Main diagnostic feature is geographical location. *A. sali* is only known from rocky shores on Sal (Cape Verde Islands). Presumably it is likely to occur on other islands in the group and possibly further afield. The species name *A. sali* is derived from the Cape Verde Island of Sal on which it was collected. Type material will be deposited at the Natural History Museum, London.

Acknowledgements We thank N. Boury-Esnault for help in the collection of some samples, and C. Lazoski for help during the electrophoresis. This work was supported by the Brazilian National Research Council, CNPq.

References

Ayala FJ (1983) Enzymes as taxonomic characters. In: Oxford GS, Rollinson D (eds) Protein polymorphism: adaptive and taxonomic significance. Academic Press, London, pp 3–26

Boury-Esnault N, Doumenc D (1979) Glycogen storage and transfer in primitive invertebrates, Demospongiae and Actiniaria. In: Levi C, Boury-Esnault N (eds) Biologie des Spongiaires. Coloques Internationales du CNRS, Paris, pp 181–192

Brace RC, Quicke DLJ (1986) Seasonal changes in dispersion within an aggregation of the anemone, *Actinia equina*, with a reappraisal of the role of intraspecific aggression. J mar biol Ass UK 66: 49–70

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

Carlgren O (1940) A contribution to the knowledge of the structure and distribution of the cnidae in the Anthozoa. Acta Univ Lund 36: 1–62

Carter MA, Miles J (1989) Gametogenic cycles and reproduction in the beadlet anemone *Actinia equina* (Cnidaria: Anthozoa). Biol J Linn Soc 36: 129–155

Carter MA, Thorpe JP (1981) Reproductive, genetic and ecological evidence that *Actinia equina* var. *mesembryanthemum* and var. *fragacea* are not conspecific. J mar biol Ass UK 61: 71–93

Chintiroglou CH, Koukouras A (1992) The feeding habits of three Mediterranean sea anemone species, *Anemonia viridis* (Forskall), *Actinia equina* (Linnaeus) and *Cereus pedunculatus* (Pennant). Helgoländer Meeresunters 46: 53–68

Coates AG, Jackson JBC (1985) Morphological themes in the evolution of clonal and aclonal marine invertebrates. In: Jackson JBC, Buss LW, Cook RE (eds) Population biology and evolution of clonal organisms. Yale University Press, New Haven

Cracraft J (1983) Cladistic analysis and vicariance biogeography. Am Scient 71: 273–281

Dunn DF (1981) The clownfish sea anemones: Stichodactylidae (Coelenterata: Actiniaria). Trans Am phil Soc 71: 1–115

Fautin DG (1988) Importance of nematocysts to actinian systematics. In: Hessinger DA, Lenhof HM (eds) The biology of nematocysts. Academic Press, London

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

Haylor GS, Thorpe JP, Carter MA (1984) Genetic and ecological differentiation between sympatric colour morphs of the common intertidal sea anemone *Actinia equina*. Mar Ecol Prog Ser 16: 281–289

Helmuth B, Veit RR, Holberton R (1994) Long distance dispersal of a subantarctic brooding bivalve (*Gaimardia trapesina*) by kelp-rafting. Mar Biol 120: 421–426

Jokiel PL (1989) Rafting of reef corals and other organisms at Kwajelein Atoll. Mar Biol 101: 483–493

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

Kumar S, Tamura K, Nei M (1993) MEGA: molecular evolutionary genetics analysis. Pennsylvania State University, State College, Pennsylvania

Lessios HA (1992) Testing electrophoretic data for agreement with Hardy–Weinberg expectations. Mar Biol 112: 517–523

Macek P, Belmonte G, Pederzoni C, Menestrina G (1994) Mechanism of action of equinatoxin II, a cytocysin from the sea anemone *Actinia equina* L. belonging to the family of actinoporins. Toxicology (Amsterdam) 87: 205–227

Manuel RL (1988) British Anthozoa (revised). EJ Brill, Leiden

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

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 89: 583–590

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

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

Ottaway JR, Kirby GC (1975) Genetic relationships between brooding and brooded *Actinia tenebrosa*. Nature, Lond 255: 221–223

Perrin MC (1993) Aspects of the ecology and genetics of *Actinia* colour morphs. Unpublished thesis, University of Liverpool, Port Erin, Isle of Man

Perrin MC, Thorpe JP, Solé-Cava AM (1997) *Actinia equina*: a genetic role model and reproductive enigma. In: Hartnoll RG, Hawkins SJ (eds) Marine biology: a Port Erin perspective (centenary volume of the Port Erin Marine Laboratory). Immel Publishing, London (in press)

Rees TD (1984) The population ecology and behaviour of *Actinia equina*. Ph.D. thesis, University of Nottingham, Nottingham

Russo CAM, Solé-Cava AM, Thorpe JP (1994) Population structure and genetic variation in two tropical sea anemones (Cnidaria: Actiniidae) with differing reproductive strategies. Mar Biol 119: 267–276

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molec Biol Evolut 4: 406–425

Schäfer W (1981) Fortpflanzung und Sexualität von *Cereus pedunculatus* und *Actinia equina* (Anthozoa: Actiniaria). Helgoländer wiss Meeresunters 34: 451–461

Schmidt H (1971) Taxonomie, Verbreitung and Variabilität von *Actinia equina* Linné 1766 (Actiniaria; Anthozoa). Z zool Syst Evol Forsch 9: 161–169

Schmidt H (1972) Prodromus zu einer Monographie der mediterranen Aktinien. Zoologica, Stuttg 42: 1–120

Shaw PW, Beardmore JA, Ryland JS (1987) *Sagartia troglodytes* (Anthozoa: Actiniaria) consists of two species. Mar Ecol Prog Ser 41: 21–28

Shick JM (1991) A functional biology of sea anemones. Chapman and Hall, London

Smith BL, Potts DC (1987) Clonal and solitary sea anemones (*Anthopleura*) of western North America: population genetics and systematics. Mar Biol 94: 537–546

Solé-Cava AM, Russo CAM, Araujo ME, Thorpe JP (1994a) Cladistic and phenetic analysis of allozyme data for nine species of sea anemones of the family Actiniidae (Cnidaria: Anthozoa). Biol J Linn Soc 52: 225–239

Solé-Cava AM, Thorpe JP (1987) 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: 225–230

Solé-Cava AM, Thorpe JP (1991) High levels of genetic variation in natural populations of marine invertebrates. Biol J Linn Soc 44: 65–80

Solé-Cava AM, Thorpe JP (1992) Genetic divergence between colour morphs in populations of the common intertidal sea anemones *Actinia equina* and *Actinia prasina* (Anthozoa: Actiniaria) in the Isle of Man. Mar Biol 112: 243–252

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

Solé-Cava AM, Thorpe JP, Todd CD (1994b) High genetic similarity between geographically distant populations in a sexually reproducing sea anemone species with low dispersal capabilities. J mar biol Ass UK 74: 895–902

Stephenson TA (1935) The British sea anemones. Vol. II. Ray Society, London

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

Templeton AR (1989) The meaning of species and speciation: a genetic perspective. In: Otte D, Endler JA (eds) Speciation and its consequences. Sinauer Associates, Sunderland, Massachusetts, pp 3–27

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

Thorpe JP (1982) Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. In: Oxford GS, Rollison D (eds) Protein polymorphism: adaptive and taxonomic significance. Academic Press, London, pp 131–152

Thorpe JP, Solé-Cava AM (1994) The use of electrophoresis in invertebrate systematics. Zool Scr 23: 3–18

Young GA, Yule AB, Walker G (1988) Adhesion in the sea anemones *Actinia equina* L. and *Metridium senile* (L.). Biofouling 1: 137–146

Wright S (1978) Evolution and the genetics of populations. Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago