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RESEARCH ARTICLE

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Genetic divergence between east and west Atlantic populations of *Actinia* spp. sea anemones (Cnidaria: Actiniidae)

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Abstract The sea anemone *Actinia equina* was considered a highly variable species with a wide geographical distribution, but molecular systematic studies have shown that this wide distribution may be the result of the lumping of cryptic species. In this work enzyme electrophoresis was used to analyse the genetic variability of *A. equina* from the Atlantic coasts of Europe and Africa, as well as the relationships between those populations and other species of the genus. Samples of *A. equina* from the United Kingdom and France were compared with supposedly conspecific populations from South Africa and a recently described species from Madeira, *Actinia nigropunctata*. The South African and Madeiran populations were genetically very divergent from each other (genetic identity, $I = 0.15$), as well as from the *A. equina* population from the United Kingdom and from the other species studied (I between 0.23 and 0.78), indicating that the African population of "*A. equina*" belongs to a different species. High genetic similarities were found between west Atlantic *A. bermudensis* and east Atlantic *A. sali*, in spite of the great geographical distance between them, and between the European *A. equina* and *A. prasina*. The results confirm the presence of the new species *A. nigropunctata* from Madeira and suggest a new species within the *A. equina* species group, from South Africa.

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Introduction

Species boundaries within the phylum Cnidaria are often difficult to assess because of the small number of diagnostic characters and the large plasticity assumed to occur for many morphological traits. Consequently, many morphological variants have been considered to belong to the same highly variable species (Perrin et al. 1999). The sea anemone *Actinia equina* (Linnaeus, 1758) is a very good example of over-conservative systematics; until the 1980s it was considered a highly variable species with a very wide geographic distribution from the cold and brackish waters of North Russia (Kola peninsula) and the Baltic Sea to the tropical waters of West Africa and the Red Sea, South Africa and the Far East (Stephenson 1935; Schmidt 1972; Manuel 1981; Song and Cha 2002). *A. equina* has been claimed to reproduce sexually or asexually and to display an enormous array of colour morphs, including red, green, brown and yellow, with generally plain, but also striped or spotted columns (for a revision see Perrin et al. 1999).

Many authors have divided the species into several varieties, never assuming, however, the specific status of any morph (e.g. Gosse 1860; Stephenson 1935; Schmidt 1971, 1972; Quick et al. 1983, 1985). Since about 1980, studies with allozyme electrophoresis and other nuclear markers have shown that "*A. equina*", in fact, comprises a number of reproductively isolated sibling species, some showing fixed morphological differences (Carter and Thorpe 1981; Haylor et al. 1984; Solé-Cava and Thorpe 1987, 1992; Monteiro et al. 1997; Douek et al. 2002). For example, in the geographically most extensive study of *A. equina* to date, red morphs from the British Isles, the Mediterranean coast of France and the Cape Verde Islands, all assigned to *A. equina* by previous authors, were found to belong to three biological species (Monteiro et al. 1997). The binomial *A. equina* was retained for the asexually reproducing British samples, *A. sali* was created for the asexual samples from Cape Verde, and *A. schmidti* was erected for the *Actinia* from

the Mediterranean, which reproduces exclusively sexually (Monteiro et al. 1997; Perrin et al. 1999). The discovery that the *Actinia* from Cape Verde were not *A. equina* raised doubts about the presence of that species on other parts of the African coast.

In the present study we used allozyme electrophoresis to compare populations of *A. equina* from Britain, from the Atlantic and Mediterranean coasts of France and from South Africa, covering over 10,000 km of African and European coastline, to verify if they are conspecific. The above populations were also compared with a species recently created, on morphological grounds, for individuals originally identified as *A. equina* in the Madeira Islands (*A. nigropunctata* den Hartog and Ocaña, 2003). Other species of *Actinia* were also included in the study to estimate mean levels of inter-specific genetic divergence within the genus. This was necessary because the populations compared were allopatric, resulting in a difficulty of applying the biological species concept (Mayr 1963) to them (see Klautau et al. 1999 for a discussion of the application of genetic divergence data in the comparison of allopatric populations). We found a high level of divergence between samples morphologically assigned to *A. equina* from South Africa, Britain and the Atlantic coast of France. Also, the population from South Africa was genetically closer to *A. tenebrosa* (from Australia) than to any of the Old World populations. The large level of genetic divergence and the paraphyletism of the South African population of *A. equina* clearly demonstrate that it belongs to a different species of *Actinia*.

Materials and methods

Collection of samples

A total of 166 samples of *Actinia* spp. from Europe, South America, Africa and Oceania were analysed (Table 1). To minimise collecting clone mates, all individuals were collected at least 2 m apart (Solé-Cava 1986; Monteiro et al. 1997). The anemones were individually wrapped in damp paper towels and transported in plastic bags, without refrigeration, to the laboratory. We found that all anemones survived well for at least 3 days under these conditions. Once in the laboratory, the external morphology of the live anemones was observed, and they were then stored in liquid nitrogen until required for analysis.

Electrophoresis

Tissue samples were homogenised with 2 vol of distilled water and analysed by horizontal 12.5% starch gel electrophoresis, using a tris citrate pH 8.0 buffer (Ward and Beardmore 1977). Enzymes were stained according to Manchenko (1994). Fourteen enzymes, coding for 18 loci, gave reproducible results: catalase (CAT, E.C. 1.11.1.6); α-esterase (α-EST, E.C. 3.1.1.1); glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9); glutamate dehydrogenase (GDH, E.C. 1.4.1.3); glutamate-oxaloacetate transaminase (GOT, E.C. 2.6.1.1); isocitrate dehydrogenase (IDH, E.C. 1.1.1.42); leucine aminopeptidase (LAP, E.C. 3.4.11.1); malate dehydrogenase (MDH, E.C. 1.1.1.37); mannose-6-phosphate isomerase (MPI, E.C. 5.3.1.8); octopine dehydrogenase (ONDH, E.C. 1.5.1.11); peptidase (PEP, E.C. 3.4.11); phosphoglucomutase (PGM, E.C. 5.4.2.2); phophogluconate dehydrogenase (PGD, E.C. 1.1.1.4.4); and xanthine oxidase (XOX, E.C. 1.1.3.22). The substrate used for the enzyme peptidase was α-prolyl-phenylalanine.

Data analysis

Allozyme patterns were used to estimate allele frequencies, heterozygosities and pairwise genetic identities and distances (Nei 1978) using the program BIOSYS-2 (Swofford and Selander 1981). Observed genotype frequencies were also compared with Hardy–Weinberg expected values, using a two-tailed exact test (Haldane 1954), corrected with a sequential Bonferroni transformation for multiple tests (Lessios 1992). A permutation test was performed to verify the presence of linkage disequilibrium, using the program GENETIX 4.01

Table 1 *Actinia*spp. Sampling sites, sample sizes, column and pedal disc colouration and presence or absence of the blue ring around the pedal disk or broods in the enteron of the anemones collected

Species	Sampling site	Country	Date	Latitude	Longitude	*N*	Column	Pedal disc	Blue ring	Broods
A.bermudensis	Rio de Janeiro	Brazil (Br 1)	Jul 1996	22°57′S	43°10′W	29	Red	Pink	No	Yes
A.bermudensis	Santa Catarina	Brazil (Br 2)	Jul 1996	27°26′S	48°34′W	22	Red	Pink	No	Yes
A.equina	Isle of Man	UK	Jun 1999	54°11′N	4°29′W	14	Red	Grey/Striped	Yes	Yes
A.equina	Port Alfred	South Africa (SA)	Mar 1998	33°54′N	25°34′E	7	Red	Pink	No	Yes
A.equina	Saint Énongat	France (Fr)	Mar 1998	49°32′N	0°05′E	9	Red	Pink	Only 3	Yes
A.nigropunctata	Porto Santo	Madeira (M 1)	Jan 2001	33°03′N	16°19′W	5	Black spots	Pink	No	No
A.nigropunctata	Madeira	Madeira (M 2)	Jan 2001	32°45′N	16°46′W	10	Black spots	Pink	No	No
A.prasina	Isle of Man	UK	Jun 1999	54°11′N	4°29′W	13	Green	Grey	Yes	Yes
A.schmidti	Thessaloniki	Greece (Gr)	Oct 1999	40°37′N	23°01′E	7	Red	Pink	Yes	No
A.sali	Island of Sal	Cape Verde (CV)	Apr 1999	16°45′N	22°57′W	26	Red	Pink	No	Yes
A.tenebrosa	Sydney	Australia (Aus)	Sep 1998	33°50′S	151°10′E	24	Red	Pink	No	Yes

(Belkhir et al. 2001). Due to the large stochastic errors associated with estimating genetic identities based on few loci, pairwise unbiased genetic distances were used to construct a UPGMA dendrogram (Sneath and Sokal 1973), with the program MEGA 2.1 (Kumar et al. 2001). This algorithm has been shown to perform well in those cases (Nei and Roychoudhury 1974; Nei 1987; Wiens 2000).

Results

Morphological characteristics

The samples of *Actinia equina* varied in pedal disc colour. The samples from the Isle of Man had grey or striped pedal discs, whereas those from Saint-Énongat (France), Port Alfred (South Africa) and the Madeira archipelago all had pink pedal discs (for localities see Table 1). All samples of *A. equina* from the Isle of Man and three of the anemones from Saint-Énongat had a blue ring around the limbus (see Manuel 1981). Preliminary analysis indicates that the presence of the blue ring is not correlated with allozyme differences (Perrin et al. 1999; authors' unpublished results). The individuals from South Africa all had red tentacles, columns and oral discs, with blue acrorhagi. They were morphologically identical to those described by Carlgren (1938) as *A. equina* from South African populations located in Durban and Still Bay, respectively, east and west from our collecting site. The anemones from Madeira had numerous little black spots spread irregularly across the column, making these clearly distinct from any other morph of *A. equina*, as described by den Hartog and Ocaña (2003). No broods were observed in the enteron of the individuals from Madeira (see also Ocaña 1994; den Hartog and Ocaña 2003), indicating that this species may reproduce only sexually, similar to *A. schmidti* and *A. fragacea* (see Perrin et al. 1999). Details of the external morphology of all samples analysed are given in Table 1.

Allozyme variation

Allele frequencies and sample sizes for the 18 loci studied are given in Table 2. Significant deviations from Hardy–Weinberg expectations (heterozygote deficiencies) were found for the *PGM* locus in the *A. prasina* population, for the *IDH* and *XOX* loci in the *A. sali* population and for the *GDH* and *XOX* loci in the *A. tenebrosa* population. Heterozygote deficiencies are common in marine invertebrates (Hare et al. 1996), and have been ascribed to various factors such as gel scoring errors, null alleles, aneuploidy, effects of selection, or Wahlund effect (see e.g. Zouros and Foltz 1984).

Due to differences in enzyme activity not all individuals were analysed for all loci, which could lead to an underestimation of overall heterozygosity. Nevertheless, heterozygosity levels (H) in the species studied ($H = 0.097$ to 0.295; Table 2) were well within the range usually observed in other species of sea anemones (Solé-Cava and Thorpe 1991; Russo et al. 1994; Solé-Cava et al. 1994a; Monteiro et al. 1997) and in other cnidarians (McFadden 1997, 1999; Knowlton 2000; Ridgeway et al. 2001). No individuals were identical over all the polymorphic loci. This shows that clonemates were not present in the samples analysed.

Of the 153 pairwise comparisons of linkage disequilibria, 26 were significant (data not shown). These were never between the same pairs of loci over the populations analysed, indicating that the linkages observed might be artefactual.

An analysis of the gene frequencies (Table 2) and genetic distances (Table 3) indicates that the three populations of *A. equina* are genetically very different from each other. The British and South African samples are the most divergent ($I = 0.23$; Fig. 1), with nine diagnostic loci (sensu Ayala 1983) between them. Diagnostic loci were also found between the samples of *A. equina* from the Isle of Man and *A. nigropunctata* from Madeira; the identity values between these two species were also low ($I = 0.77$). A surprisingly high genetic identity ($I = 0.92$) was found between the samples of *A. bermudensis* from Brazil and *A. sali* from Cape Verde. An even higher value ($I = 0.98$) was found between *A. prasina* and the samples of *A. equina* with grey and striped pedal disc from the Isle of Man.

Discussion

The two most salient findings of this work were the discovery of a new species (from South Africa) within the *Actinia equina* species group and the high genetic similarity between two nominate species that live in different continents (*A. bermudensis* in South America and *A. sali* in Africa). The results also confirm the specific status of *A. nigropunctata* from Madeira.

Samples of sympatric morphs of the same species should (given certain assumptions and allowing for sampling errors) have the same allele frequencies at all loci, except those coding for their morphological differences (Thorpe and Solé-Cava 1994). In allopatry, some genetic divergence is to be expected, because the geographical distance between populations will usually prevent substantial gene flow. In these cases, the finding of significant differences in the gene frequencies alone is not enough to warrant their specific status. Fortunately, it is possible to infer taxonomic relationships in allopatry through the estimation of genetic distances between populations (see e.g. Thorpe 1982; Nei 1987), preferably internally calibrated through the comparison of sympatric species of the same genus (Klautau et al. 1999; Vianna et al. 2003). Many studies have demonstrated the correspondence between genetic distance and taxonomic rank (reviews by Thorpe 1982, 1983; Nei 1987; Thorpe and Solé-Cava 1994; Solé-Cava and Boury-Esnault

Table 2 *Actinia*spp. Allele frequencies at the 18 loci studied (*N*number of individuals scored; $H_o$and $H_e$direct count and Hardy–Weinberg expected mean heterozygosities per locus, respectively). Alleles are named based on their mobility relative to the starting point of the gel (population abbreviations, see Table 1)

Allele	*A.bermudensis*		*A.equina*			*A.nigropunctata*		*A.prasina*	*A.sali*	*A.schmidti*	*A.tenebrosa*
	Br 2	Br 1	UK	Fr	SA	M 2	M 1	UK	CV	Gr	Aus
MDH-1											
(*N*)	22	27	14	9	7	10	5	13	26	7	24
A	0.091	0.093	0	0	0.714	0	0	0	0.038	0	0.146
B	0.909	0.907	0	0.333	0.286	0	0	0	0.962	0.714	0.813
C	0	0	0.143	0	0	0	0	0	0	0.286	0
D	0	0	0.857	0.667	0	1.000	1.000	1.000	0	0	0.042
MDH-2											
(*N*)	22	28	14	8	7	10	5	12	26	7	24
A	1.000	1.000	0	0	1.000	0	0	0	1.000	1.000	1.000
B	0	0	1.000	1.000	0	1.000	1.000	1.000	0	0	0
GOT-1											
(*N*)	15	21	14	7	7	10	5	9	25	7	12
A	0	0	0.143	0.429	0	0	0	0.111	0	0	0
B	0	0	0.857	0.571	0	1.000	1.000	0.889	0	0.429	0
C	1.000	1.000	0	0	1.000	0	0	0	1.000	0.571	1.000
GOT-2											
(*N*)	21	26	14	8	6	10	5	11	24	7	21
A	0	0	0.143	0.063	0	0	0	0.091	0	0	0.929
B	0.929	0.942	0.857	0.938	1.000	0.950	1.000	0.909	1.000	1.000	0.071
C	0.071	0.058	0	0	0	0.050	0	0	0	0	0
LAP											
(*N*)	22	29	14	4	5	10	5	13	25	7	24
A	0.045	0	0.107	0.500	0.400	0	0	0.077	0.260	0.714	0
B	0.955	1.000	0.786	0.500	0.400	0.400	0.200	0.923	0.740	0.286	0
C	0	0	0.107	0	0.200	0.550	0.800	0	0	0	1.000
D	0	0	0	0	0	0.050	0	0	0	0	0
PGM											
(*N*)	22	26	14	7	6	10	5	12	25	7	23
A	0	0	0	0	0	0	0	0	0.020	0	0
B	1.000	0.962	0.643	0.286	1.000	0.050	0.400	0.250	0.740	0.143	1.000
C	0	0.038	0.357	0.714	0	0.950	0.400	0.750	0.240	0.643	0
D	0	0	0	0	0	0	0.100	0	0	0.214	0
E	0	0	0	0	0	0	0.100	0	0	0	0
AEST											
(*N*)	22	29	14	9	7	9	5	13	26	7	24
A	0.045	0.069	0.179	0.444	0.214	0.056	0	0.077	0.154	0.714	0.063
B	0.864	0.862	0.714	0.111	0.643	0.667	0.800	0.462	0.731	0.143	0.833
C	0.091	0.069	0.107	0.333	0.143	0.278	0.200	0.462	0.115	0.143	0.104
CAT											
(*N*)	22	29	14	9	7	10	5	13	25	7	24
A	0	0	0.964	0.556	0	0.800	0.900	0.654	0	0.571	0.042
B	0.864	0.948	0	0.444	0.714	0	0	0.231	0.800	0.429	0.875
C	0.136	0.052	0.036	0	0.286	0.200	0.100	0.115	0.200	0	0.083
IDH											
(*N*)	22	27	14	9	7	10	5	13	25	7	24
A	0	0.093	0	0.111	0	0	0	0	0.160	0	0
B	1.000	0.907	0.571	0.444	1.000	0	0	0.500	0.840	0.786	1.000
C	0	0	0.429	0.444	0	1.000	1.000	0.500	0	0.214	0
PEP-1											
(*N*)	13	23	14	6	7	5	1	10	26	5	12
A	1.000	0.935	0	0.833	0	1.000	1.000	0.100	1.000	1.000	1.000
B	0	0	0	0	1.000	0	0	0	0	0	0
C	0	0.065	1.000	0.167	0	0	0	0.900	0	0	0
PEP-2											
(*N*)	16	15	12	5	4	5	1	5	22	5	18
A	1.000	1.000	1.000	1.000	0	1.000	1.000	1.000	1.000	1.000	0
B	0	0	0	0	1.000	0	0	0	0	0	1.000
XOD											
(*N*)	17	28	14	3	7	8	4	13	24	7	21
A	0	0	0.679	0.500	0	0	0	0.731	0	0.429	0
B	0	0	0.321	0	0	1.000	1.000	0.269	0	0.286	0
C	0	0	0	0.500	0	0	0	0	0.083	0	0.095
D	0.735	0.911	0	0	0.857	0	0	0	0.729	0.286	0.643
E	0.265	0.089	0	0	0.143	0	0	0	0.188	0	0.262

Table 2 (Contd.)

Allele	*A.bermudensis*		*A.equina*			*A.nigropunctata*		*A.prasina*	*A.sali*	*A.schmidti*	*A.tenebrosa*
	Br 2	Br 1	UK	Fr	SA	M 2	M 1	UK	CV	Gr	Aus
MPI											
(*N*)	21	28	13	8	7	10	5	13	26	6	24
A	0.238	0.214	0	0.438	0.929	0	0	0	0.135	0.333	0.979
B	0.762	0.536	1.000	0.563	0.071	1.000	1.000	0.962	0.865	0.667	0.021
C	0	0.232	0	0	0	0	0	0	0	0	0
D	0	0.018	0	0	0	0	0	0.038	0	0	0
GDH											
(*N*)	22	29	14	9	7	10	5	13	26	7	24
A	0.659	0.466	0	0.056	0.214	0	0	0	0.423	0.429	0
B	0.318	0.534	0.071	0.667	0.786	0	0	0	0.577	0.571	0.771
C	0.023	0	0.929	0.278	0	1.000	1.000	1.000	0	0	0.229
ODH											
(*N*)	22	29	14	9	6	10	5	13	25	7	23
A	0	0	0	0	0	0	0	0	0	0	0.065
B	0.909	0.983	0	0	0	0	0	0	0	0.286	0.913
C	0.091	0.017	0	0	1.000	0	0	0	1.000	0	0.022
D	0	0	1.000	1.000	0	1.000	1.000	1.000	0	0.714	0
PGD											
(*N*)	22	29	14	9	6	10	5	13	26	7	24
A	0	0	0	0	0	0	0	0	0	0.286	0
B	0	0	0.929	0.556	0.167	0.950	1.000	0.692	0	0.286	0
C	1.000	0.983	0.071	0.444	0.667	0.050	0	0.308	1.000	0.357	0.500
D	0	0.017	0	0	0.167	0	0	0	0	0.071	0.208
E	0	0	0	0	0	0	0	0	0	0	0.292
PGI-1											
(*N*)	22	29	14	9	7	10	5	12	26	7	23
A	0	0	0	0	0	0.050	0.200	0	0	0	0
B	0	0	0	0	0	0.200	0	0	0	0	0
C	0	0	0	0	0	0.450	0	0	0	0	0
D	0	0	0.143	0.167	0	0.150	0.700	0.083	0	0	0
E	0	0	0.857	0.833	0	0	0	0.917	0.077	0.571	0
F	1.000	1.000	0	0	1.000	0	0	0	0.923	0.429	1.000
G	0	0	0	0	0	0.150	0.100	0	0	0	0
PGI-2											
(*N*)	22	29	14	9	7	10	5	13	26	7	24
A	0	0	0	0	0	0.050	0	0	0	0	0
B	0	0	0	0	0	0.650	0.700	0	0	0	0
C	0	0	0	0	0	0	0.100	0	0	0	0
D	0	0	1.000	1.000	0	0	0	1.000	0	0.571	0
E	0.977	0.983	0	0	1.000	0	0	0	1.000	0.429	0.938
F	0.023	0.017	0	0	0	0.300	0.200	0	0	0	0.063
H_o	0.138	0.099	0.135	0.295	0.193	0.135	0.122	0.147	0.114	0.122	0.097
H_e	0.130	0.134	0.202	0.375	0.193	0.165	0.148	0.213	0.179	0.406	0.157

Table 3 *Actinia*spp. Unbiased genetic identities (*above diagonal*) and distances (*below diagonal*) (Nei 1978) (populations abbreviations, see Table 1)

	Species	Population	1	2	3	4	5	6	7	8	9	10	11
1	*A.bermudensis*	Br 2		0.993	0.352	0.452	0.726	0.312	0.325	0.356	0.934	0.746	0.753
2	*A.bermudensis*	Br 1	0.007		0.340	0.451	0.732	0.295	0.306	0.345	0.922	0.737	0.763
3	*A.equina*	UK	1.043	1.079		0.838	0.229	0.759	0.767	0.978	0.351	0.596	0.172
4	*A.equina*	Fr	0.794	0.796	0.177		0.318	0.719	0.695	0.876	0.497	0.803	0.294
5	*A.equina*	SA	0.320	0.312	1.476	1.146		0.140	0.164	0.211	0.778	0.559	0.758
6	*A.nigropunctata*	M 2	1.164	1.221	0.276	0.330	1.966		0.974	0.778	0.335	0.545	0.175
7	*A.nigropunctata*	M 1	1.125	1.186	0.265	0.364	1.807	0.026		0.748	0.334	0.519	0.217
8	*A.prasina*	UK	1.033	1.063	0.022	0.133	1.557	0.251	0.290		0.368	0.612	0.145
9	*A.sali*	CV	0.068	0.081	1.046	0.698	0.251	1.094	1.095	1.001		0.773	0.687
10	*A.schmidti*	Gr	0.293	0.305	0.518	0.219	0.581	0.607	0.656	0.491	0.257		0.548
11	*A.tenebrosa*	Aus	0.283	0.270	1.761	1.224	0.277	1.744	1.528	1.933	0.375	0.602	

1999). Populations of the same species have pairwise *I*-values usually higher than 0.9 and not lower than 0.85; different species of the same genus have *I*-values generally between about 0.3 and 0.8, and species in different genera have *I*-values between 0 and about 0.4. More specifically, a recent compilation of 384 intraspecific

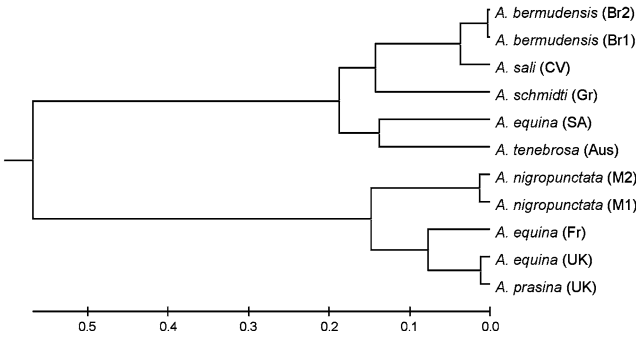

Fig. 1 *Actinia* spp. UPGMA dendrogram of Nei's *D*-values among the populations studied

comparisons within the genus *Actinia* showed that 99% have *I*-values higher than 0.80 (Vianna et al. 2003).

The low genetic similarity ($I = 0.73$) between morphs of *A. equina* from the Isle of Man/France and *A. nigropunctata* from Madeira (Table 3; Fig. 1) indicates that they are, indeed, different species. Besides the genetic differences, morphological differences were found: the samples from Madeira had a unique pattern of numerous black spots distributed irregularly around the column. Additional morphological differences were found by Ocaña (1994) working with sympatric red morphs of *A. equina* from Madeira (with and without black spots).

It has been proposed that the origin and endemism of some Madeiran fauna has been due to the isolation of those Islands during the last glaciation period (Ocaña 1994 and references therein; den Hartog and Ocaña 2003). Nevertheless, the molecular data do not support this hypothesis. The evolutionary rates estimated for allozymes vary from 2 to 18 million years (Nei 1987). Thus, even using the least conservative calibration of the molecular clock available ($1D = 2$ million years), we see that the separation of these species (520,000 years before present) occurred well before the last glaciation (see Salgado-Labouriau 1994).

The genetic differentiation observed between both South African and British ($I = 0.23$) as well as South African and French ($I = 0.32$) *A. equina* is as large as that between species of different genera in other invertebrates (Thorpe and Solé-Cava 1994; Thorpe et al. 2000). The *I*-value found between the South African samples and *A. tenebrosa* from Australia ($I = 0.76$) is similar to that estimated from earlier studies ($I = 0.70$; Ayre 1984), and to that between South African samples and the species *A. sali* ($I = 0.78$). These values are well within the usual range observed between congeneric species. There is, therefore, a high variation in levels of genetic divergence between different species of the genus *Actinia*, particularly if we also consider the very low level of differentiation ($I = 0.98$) observed between *A. equina* and *A. prasina*. These two species live in sympatry, and do not present diagnostic loci, but display significant and consistent differences in allele frequencies over various loci, indicating that they do not interbreed (Haylor et al. 1984; Solé-Cava and Thorpe 1987, 1992; Perrin et al. 1999). Similarly high variance in levels of congeneric differentiation has been found in marine sponges, and could indicate either some hugely variable levels of molecular divergence, or the incorrect generic assignment of some species (Solé-Cava and Boury-Esnault 1999; Boury-Esnault and Solé-Cava 2004). High levels of variance in gene identity have also been found between species of another sea anemone genus, *Anthopleura* (Manchenko et al. 2000), which is polyphyletic (Geller and Walton 2001). However, the monophyly of *Actinia* has been supported in a phylogenetic study of six species of the genus (Solé-Cava et al. 1994b).

The large genetic divergence between the supposedly conspecific populations of *A. equina* suggests that its potential for long-distance dispersal is not as great as it was once thought. In the *A. equina* species group the sexually produced larva, if existent, probably lacks the potential for major dispersal. The asexually produced offspring in these species are not capable of long-distance dispersal either (as in other asexual-reproducing species, Jackson 1986; Russo et al. 1994). However, asexual reproduction is probably important in maintaining *Actinia* populations, and sexually produced larvae may be important for colonising new areas (Ayre 1984; Ayre et al. 1991).

With an apparently low capability for long-distance dispersal, it is reasonable to speculate that an amplified founder effect may have been the main process of speciation in the group (Perrin et al. 1999); however, the generally high levels of heterozygosity found in these species and in other sea anemones (Solé-Cava and Thorpe 1991) indicate otherwise.

The high levels of heterozygosity found could be reasonably explained by the high probability of mutations propagating in the population through clonal reproduction (Muller 1964; Fautin 1997; Vollmer and Palumbi 2002). Moreover, whenever sexual reproduction took place, the great genetic variability might facilitate differentiation, since more variable species generally have higher rates of population differentiation than those which are less variable (Skibinski and Ward 1981, 1982).

We found a high genetic identity (Fig. 1) between the grey/striped pedal disc morph of *A. equina* and *A. prasina* from the United Kingdom. A high genetic similarity between the species *A. prasina* and *A. equina* has already been reported (Haylor et al. 1984; Solé-Cava and Thorpe 1987), but no distinction of morphotypes in *A. equina* was made in these works. In fact, a high similarity between *A. prasina* and the grey/striped pedal disc morph of *A. equina* has also been found on the basis of morphological, physiological and ecological as well as biometric investigations of the cnidae of both species (Quicke and Brace 1983, 1984; Brace and Reynolds 1989; Perrin 1993; Watts et al. 2000). However, since significant biometric differences have been found between *A. equina* with grey/striped

pedal disc and *A. prasina* (Watts et al. 2000), the question of the conspecificity of those two morphs remains unsettled.

The significance of pedal disc coloration has been a subject of considerable debate in *A. equina* taxonomy, and various authors have considered these morphs to be different "varieties", but have never assumed them to have specific status (e.g. Quicke et al. 1983, 1985; Quicke and Brace 1984; Donoghue et al. 1985). The higher genetic identity found between the grey/striped pedal disc morph and *A. prasina* (0.97) when compared to that between these morphs and the pink pedal disc morph from France (0.83), gives further support to the distinction between the two pedal-disc colour morphs of *A. equina*, as suggested earlier by other authors (see Watts et al. 2000).

In comparisons between other species of the genus, an extremely high genetic identity (0.92) was found between the west Atlantic species *A. bermudensis* and the east Atlantic *A. sali*, indicating that these species might be conspecific. When Monteiro et al. (1997) described *A. sali* as a new species from the Cape Verde Islands, no genetic comparisons were made with the west Atlantic tropical species *A. bermudensis*, mainly because the population from Cape Verde had been described earlier as *A. equina* (Carlgren 1949) and *A. bermudensis* was thought to occur exclusively in the west Atlantic. Interestingly, only populations of the genus *Actinia* from Cape Verde (Ocaña 1994) and Brazil (Dube 1975) have been reported to lack holotrichs in the acrorhagi. The acrorhagi themselves have been reported missing in some individuals (Dube 1975; Ocaña 1994). However, this was not the case with any of the samples of either species analysed by us, or with those analysed by Monteiro et al. (1997).

A high genetic identity ($I = 0.82$) has been reported in comparisons of populations of *A. bermudensis* from Brazil and Bermuda, 8,000 km apart (Vianna et al. 2003), which indicates that this species, unlike others of the genus, may be capable of long-range dispersal. It might be possible, thus, that the population from the Cape Verde Islands is, in fact, an African population of the species *A. bermudensis*. The continent on which the species may have originated is unclear. Levels of gene variation were slightly higher in *A. sali* ($H = 0.18$) than in *A. bermudensis* ($H = 0.13$). This, and the prevailing westerly surface currents at the equator, would support an African origin for the species. On the other hand, *A. bermudensis*/*A. sali* are genetically more similar to the Mediterranean *A. schmidti* than they are to *Actinia* sp. 1 from South Africa or to *A. nigropunctata* from Madeira. It is clear that, before any conclusions can be drawn even about the conspecific status of *A. bermudensis* and *A. sali*, a larger set of data, including mitochondrial and nuclear DNA sequences will have to be analysed phylogenetically. The discovery of the new species of *Actinia* from South Africa and the new species from Madeira raises to seven the number of species contained within what was called "*A. equina*" until 1980 and indicates that other citations of this species, in places like the Red Sea, Hong Kong, Korea and Japan (Carlgren 1949; Song and Cha 2002) are likely to also refer to different species of the genus. A formal description of the new species from South Africa is in preparation (Schama et al., unpublished data).

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