

C. S. McFadden

## Genetic and taxonomic relationships among Northeastern Atlantic and Mediterranean populations of the soft coral *Alcyonium coralloides*

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**Abstract** Species boundaries among taxa of colonial marine organisms are often obscured by intraspecific morphological and ecological variation; genetic comparisons of recognized “ecotypes” frequently reveal them to be reproductively isolated species. Based on morphological similarities, it has been proposed that the Mediterranean soft coral *Alcyonium* (= *Parerythropodium*) *coralloides* Pallas, 1766 and its Atlantic congener *A. hibernicum* belong to one highly variable and geographically widespread species, *A. coralloides*. I collected *A. coralloides* from ten Atlantic and three Mediterranean locations in 1990 and 1994, and used differences in colony form, substrate use and color to separate them into five distinct morphotypes. Two occur sympatrically in the Mediterranean (M1, M2) and three have overlapping distributions in the Atlantic (A1, A2, A3). I used allozyme electrophoresis to compare morphotypes genetically at 14 enzyme loci. Where two morphotypes occurred sympatrically, fixed allelic differences at 4 to 6 loci indicated reproductive isolation. In all but one pairwise comparison (M1 and A2), morphotypes whose ranges did not overlap were also separated by large genetic distances. From these results I suggest that the five morphotypes represent four distinct species. *A. coralloides* comprises two morphotypes (M1, A2) with relatively high genetic identity. *A. hibernicum* (= A1) is reproductively isolated from *A. coralloides* and should be retained as a valid species; levels of genetic diversity and heterozygosity within populations support the absence of outcrossing in this reportedly asexual species. Morphotypes M2 and A3 are also reproductively isolated from *A. coralloides*; they are taxonomically distinct from

but belong to the same phylogenetic clade as *A. hibernicum*. Although preliminary observations suggest that differences in reproductive timing maintain species boundaries in sympatry, wider geographic sampling will be required to elucidate the events leading to speciation within this species complex.

### Introduction

Recognition of taxonomic boundaries is a necessary first step to understanding the biogeographical and ecological distributions of organisms, as well as the mechanisms by which speciation occurs. Genetic and ecological studies of marine organisms have frequently exposed the existence of cryptic or sibling species [i.e. species that are difficult or impossible to distinguish morphologically (Knowlton 1993)], revealing many species to be more ecologically and evolutionarily specialized or to have narrower geographic ranges than previously supposed (Knowlton and Jackson 1994). Study of the factors that reproductively isolate morphologically similar taxa can contribute significantly to our understanding of the speciation process in marine environments (Knowlton 1993; Palumbi 1994).

Morphological species boundaries are often especially difficult to assess in cnidarians. Because the growth form of some species has been demonstrated to be influenced by the environment (Foster 1979; Willis 1985; West et al. 1993; Bruno and Edmunds 1997), morphological variants associated with distinct microhabitats often are assumed to represent ecotypes of a single, environmentally plastic species (e.g. Knowlton et al. 1992). Recent studies of genetic differences among morphological variants of corals, however, have concluded that many “ecotypes” are indeed reproductively isolated [Ayre et al. 1991; Knowlton et al. 1992, 1997 (but see Van Veghel and Bak 1993); Brazeau and Harvell 1994; Stobart and Benzie 1994; Lasker et al. 1996]. Likewise, genetic comparisons of color or life-history variants have revealed numerous cryptic species complexes in sea

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C.S. McFadden  
Department of Biology,  
Harvey Mudd College,  
Claremont, California 91711, USA  
Fax: 001 (0) 909 607-7172  
e-mail: Catherine\_McFadden@hmc.edu

anemones (Carter and Thorpe 1981; Bucklin and Hedgecock 1982; Solé-Cava et al. 1985; Shaw et al. 1987; Solé-Cava and Thorpe 1987; McFadden et al. 1997; Monteiro et al. 1997).

The alcyonacean soft coral *Alcyonium* (= *Parerythropodium*) *coralloides* Pallas, 1766 exhibits considerable variation in colony growth form, color, habitat and life history across a broad geographic range. Although the recognized variants of this species have been compared morphologically and ecologically (Weinberg 1975; Groot and Weinberg 1982), their genetic relationships have never been examined. Throughout the western basin of the Mediterranean, *A. coralloides* is most commonly found as an epibiont on dead gorgonian skeletons, but it also may form dense aggregations on vertical or overhanging rock surfaces and in shallow caves (Weinberg 1979, 1980; Van Soest and Weinberg 1980; Groot and Weinberg 1982). Its growth form varies greatly, from thinly encrusting ( $\leq 2$  mm thick) to upright lobate and digitate colonies up to 50 mm in height (Groot and Weinberg 1982). Colony color is also highly variable, ranging from deep red through various shades of pink to white and yellow (Weinberg 1975; Groot and Weinberg 1982). *A. coralloides* is a sexually-reproducing gonochore that broods planula larvae to a late developmental stage, and as a result probably has relatively limited dispersal abilities (de Lacaze-Duthiers 1900).

A very similar soft coral, originally described as *Alcyonium* (= *Parerythropodium*) *hibernicum* Renouf, 1931, occurs on shaded vertical or overhanging rock surfaces in the shallow subtidal throughout the British Isles and Atlantic coast of France (Hartnoll 1977; Van Soest and Weinberg 1980; Groot and Weinberg 1982). Colonies of *A. hibernicum* are typically lobate or digitate rather than thinly encrusting, and exhibit relatively little color polymorphism (Van Soest and Weinberg 1980; Groot and Weinberg 1982). Like *A. coralloides*, this species also broods planula larvae, but it is believed to reproduce parthenogenetically rather than sexually (Hartnoll 1977). Despite these differences, Van Soest and Weinberg (1980) concluded on the basis of sclerite morphology that *A. hibernicum* was a geographic variant of the Mediterranean species and synonymized them under the name *A. coralloides*. Groot and Weinberg (1982) subsequently compared the different morphological and color variants from both geographical regions and also concluded that they belonged to one, highly variable, species. The absence of sexual reproduction in the Atlantic form should, however, reproductively isolate it from sexual Mediterranean populations, in which case they should continue to be considered separate species (Mayr 1963).

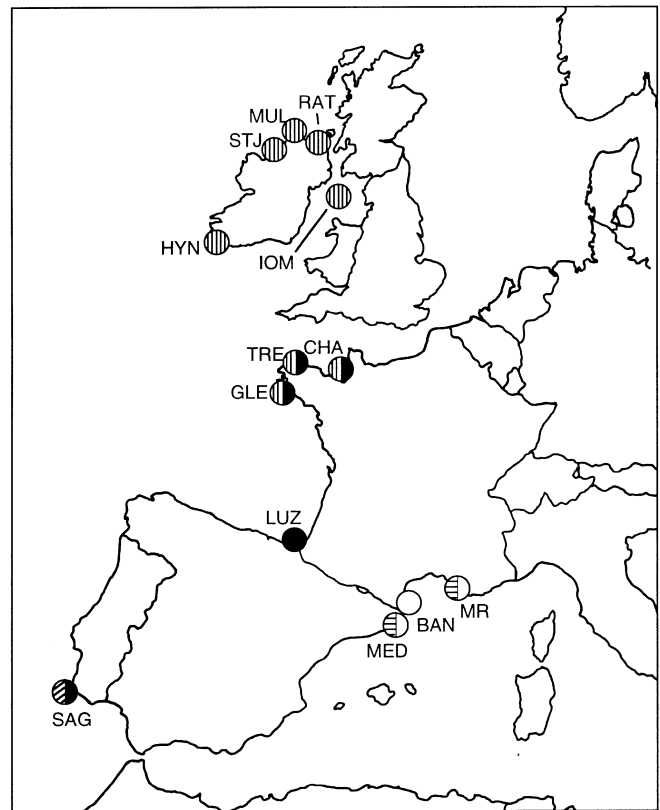
I used allozyme electrophoresis to examine the genetic relationships among (1) Atlantic and Mediterranean populations of *Alcyonium coralloides* and (2) the different morphological and color variants ("morphotypes") found within populations. In cases where sympatrically occurring morphotypes exhibit fixed allelic differences, I have concluded that they do not interbreed

and should therefore be recognized as distinct species. In comparisons of allopatric forms, I have used the degree of genetic and morphological differentiation among morphotypes to evaluate their taxonomic relationship.

## Materials and methods

### Collection

I collected *Alcyonium coralloides* Pallas, 1766 in 1990 from five locations in Ireland and the Isle of Man and in 1994 from eight locations in France, Spain and Portugal (Fig. 1). Using SCUBA, I removed entire colonies from the rock substrate with a knife, or used wire cutters to clip branches of gorgonians occupied by *A. coralloides*. *A. coralloides* can reproduce by vegetative propagation (own personal observation); therefore, to minimize the possibility of collecting multiple samples of the same clone, I sampled only a single colony from any group of colonies that appeared to be clonemates (based on proximity, stolonal connections or similarity of color). At most locations I collected colonies from several different dive sites separated by a few hundred meters to several kilometers.



**Fig. 1** *Alcyonium coralloides*. Map of western Europe showing locations at which five morphotypes were collected (*Split circles* denote presence of two morphotypes; *vertical stripes* Type A1; *black A2*; *diagonal stripes* A3; *open M1*; *horizontal stripes* M2. RAT Rathlin Island, Northern Ireland; MUL Mulroy Bay, Republic of Ireland; STJ St. John's Point, Republic of Ireland; HYN Lough Hyne, Republic of Ireland; IOM Isle of Man; CHA Iles Chausey, France; TRE Trébeurden, France; GLE Iles de Glénan, France; LUZ St. Jean-de-Luz, France; SAG Sagres, Portugal; MED Illes Medes, Spain; BAN Banyuls-sur-Mer, France; MR Marseille, France)

For all samples collected in 1994, I used Vernier calipers to measure the maximum height or thickness ( $H$ ) of each colony when fully contracted, the basal length ( $L$ ) (maximum diameter of the base where it was attached to the substrate) and the basal width ( $W$ ) (maximum dimension of the base perpendicular to  $L$ ). I estimated basal area ( $A$ ) as an ellipse using the formula  $A = \pi(L/2)(W/2)$ . For encrusting colonies with very irregular shapes, I traced the outline onto waterproof paper and later estimated basal area by superimposing a 1 mm<sup>2</sup> grid and counting grid squares. I used the ratio of (height)<sup>2</sup> to basal area as a non-dimensional measure of colony morphology ( $H^2:A$ ). For a perfectly hemispherical colony ( $L = W = 2H$ ),  $H^2:A = 0.32$ ; values less than this correspond to flat colonies, values greater than this to erect colonies. I classified colony morphology as lobate (height  $\geq$  basal diameter), digitate (multiple discrete lobes arising from the same basal tissue), or encrusting (basal diameter much greater than height), and recorded the substrate to which each colony was attached (rock, gorgonian coral, worm tube or "other").

Color was judged by visual inspection of the colony surface (the coenenchyme) and polyps with a hand lens. For a majority of the colonies collected in color-polymorphic populations, I also preserved in 70% EtOH a small slice of tissue containing polyps and coenenchyme: colony color is due primarily to alcohol-insoluble pigments in microscopic calcareous sclerites embedded in the soft tissues (Tixier-Durivault 1940). Later, I examined these preserved samples under a dissecting microscope and determined the color of sclerites in the (a) tentacles, (b) distal (crown and points) and (c) proximal regions of the polyp, and (d) colony surface (calyx and coenenchyme) (alphabetic designation of anatomical regions follows Weinberg 1975, 1977).

As soon as possible following collection, living polypoid tissue was excised from each colony, blotted to eliminate excess water and mucus, cleaned of attached debris, and frozen in individual vials in liquid nitrogen. Tissue samples were kept frozen in liquid nitrogen or in a -80 °C freezer until used for electrophoresis.

#### Allozyme electrophoresis

Frozen tissue was diced into small pieces, then crushed with a glass rod in 22  $\mu$ l grinding buffer [10% sucrose, 0.1%  $\beta$ -mercaptoethanol, 1 mg/ml NADP, bromphenol blue (Stoddart 1983a)]. The resulting homogenate was centrifuged at 12000 rpm for 2.5 min, and filter-paper wicks soaked in the supernatant were loaded onto 12% (w/v) starch gels. I ran gels using Selander et al.'s (1971) Buffer Systems 2 (discontinuous LiOH) and 5 (tris-citrate, pH 8.0) at 4 °C. System 2 gels were stained for adenylate kinase (*AK*, E.C. 2.7.4.3), esterase (*EST*, E.C. 3.1.1.1), glucosylphosphate isomerase (*GPI*, E.C. 5.3.1.9), hexokinase (*HK*, E.C. 2.7.1.1), leucine amino-peptidase (*LAP-1*, *LAP-2*, E.C. 3.4.11.1), L-leucyl-tyrosine peptidase (*LTP-1*, *LTP-2*, E.C. 3.4.11/13), and nucleoside phosphorylase (*NP*, E.C. 2.4.2.1). System 5 gels were stained for malate dehydrogenase (*MDH-1*, *MDH-2*, E.C. 1.1.1.37), octanol dehydrogenase (*ODH*, E.C. 1.1.1.73), 6-phosphogluconate dehydrogenase (*6PGD*, E.C. 1.1.1.44) and triose-phosphate isomerase (*TPI*, E.C. 5.3.1.1). I added 20 mg NADP to the System 5 gel buffer to improve resolution at the *6PGD* locus. Standard staining recipes were followed (e.g. Shaw and Prasad 1970). I numbered loci and alleles in order of decreasing electrophoretic mobility.

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#### Genetic data analysis

Allele and genotype frequencies, observed heterozygosity and percentage of polymorphic loci were determined separately for each morphotype at each location. I tested the genotype frequencies of polymorphic loci for conformance to Hardy-Weinberg expectations, and compared allele frequencies between different morphotypes within populations using the pseudoproability  $\chi^2$  tests of Zaykin and Pudovkin (1993). Unlike conventional  $\chi^2$  tests, this method gives reliable probability estimates even when expected cell frequencies are small. A sequential Bonferroni procedure was used to adjust probability levels for multiple tests (Hochberg 1988).

I used Biosys-1 (Swofford and Selander 1981) to estimate pairwise genetic distance measures [Nei's (1978) unbiased  $D$ ] among all populations and morphotypes and to construct an UPGMA (unweighted pair-group method using unweighted averages) dendrogram of  $D$  values. In addition, I used Phylip Version 3.5c (Felsenstein 1993) to construct a neighbor-joining tree of pairwise genetic distance values with Cavalli-Sforza and Edwards' (1967) chord distance. Unlike Nei's  $D$ , Cavalli-Sforza and Edwards' chord distance does not assume constant population size over time (Felsenstein 1993); and, unlike UPGMA, the neighbor-joining method of tree construction allows evolutionary rates to vary among lineages (Swofford et al. 1996). Allozyme frequencies for two additional nominate species of *Alcyonium*, *A. acaule* (a Mediterranean species collected at Marseille, MR) and *A. glomeratum* (an Atlantic species collected at Trébeurden, TRE), were included in the analysis. *A. acaule* was used to root the neighbor-joining tree.

## Results

### Colony morphology and color

On the basis of colony growth form, substrate use and color, I recognized three distinct morphotypes of *Alcyonium coralloides* in Atlantic populations and two in the Mediterranean (Table 1). Hereafter, I refer to these forms as Atlantic morphotypes A1, A2 and A3 and Mediterranean Morphotypes M1 and M2. Because

**Table 1** *Alcyonium coralloides*. Summary of general features of three Atlantic (A1, A2, A3) and two Mediterranean (M1, M2) morphotypes (r red; p pink; w white; y yellow)

Morphotype	Colony morphology	Substrate	Most common sclerite colors of:			
			tentacles	distal polyp	proximal polyp	coenenchyme
A1	lobate, digitate	rock	w, p	p	p	p
A2	encrusting, lobate	rock, worm tubes, other organisms	y	r, p	r, y	r
A3	lobate, digitate	rock	w, y	r, p	r, p	w
M1 <sup>a</sup>	encrusting	gorgonians	y	r	y	r
M2	lobate, digitate, encrusting	rock, other organisms	y	r	r	r, p, w

<sup>a</sup>Populations found below 20 m depth are often highly color-polymorphic (Table 2)

I observed relatively little variation in either growth form, color or allozyme frequencies among colonies collected from the different dive sites at most locations, I pooled sites for the analyses. At Mediterranean location MR, however, there was significant variation among colonies from five different dive sites; these were not pooled, and are denoted throughout the analyses as MR-1 to MR-5.

### Morphotype A1

I found colonies of Morphotype A1 at all Atlantic locations north of and excluding St. Jean-de-Luz (LUZ); throughout the British Isles this was the only Form I

encountered (Fig. 1). Type A1 corresponds to the original and subsequent descriptions of *Alcyonium hibernicum* (the Lough Hyne site sampled in this study is the type location of that species) (Renouf 1931; Hartnoll 1977). A1 colonies were typically lobate or digitate, and up to 30 mm in height when fully contracted (Table 2). Overall colony coloration ranged from very light pink (typical of Irish populations) to a somewhat darker pink in some French populations [described by Groot and Weinberg (1982) as “pink-2”]. All colonies had pink sclerites in both regions of the polyp and in the coenenchyme, while tentacular sclerites were pink, white, or a mixture of those two colors. Colonies were always attached directly to rock or other hard, inorganic surfaces (Table 2).

**Table 2** *Alcyonium coralloides*. Morphology, substrate use and color of colonies collected from NE Atlantic and Mediterranean populations in 1994 [A1, A2, A3 Atlantic morphotypes; M1, M2 Mediterranean morphotypes; N number of colonies collected; H colony height (cm);  $H^2:A$  non-dimensional measure of overall colony morphology; L % lobate; D % digitate; E % encrusting; R % on rock (or other inorganic surfaces such as metal ship hulls); G

% on gorgonians; W % on worm tubes; O % on other organic substrata (e.g. barnacles, bryozoans, sponges); a tentacles; b distal polyp region; c proximal polyp region; d coenenchyme; r red; p pink; w white or colorless; y yellow; double letter codes sclerites of both colors present in similar proportions (only those color varieties that were found in > 15% of individuals sampled are shown)]

Type, location	[N]	Colony morphology				Substrate use				Sclerite colors				
		H (SD)	$H^2:A$ (SD)	L	D	E	R	G	W	O	a	b	c	d
<b>A1</b>														
CHA	[62]	1.46 (0.69)	8.25 (8.19)	34	56	10	100	0	0	0	pw	p	p	p
TRE	[39]	0.84 (0.36)	6.34 (5.52)	69	13	18	100	0	0	0	pw	p	p	p
GLE	[37]	1.73 (0.53)	12.16 (4.72)	54	46	0	100	0	0	0	pw	p	p	p
<b>A2</b>														
CHA	[48]	0.95 (0.61)	2.20 (2.89)	44	10	46	100	0	0	0	y	ry	ry	r
TRE	[41]	0.27 (0.11)	0.68 (1.04)	2	0	98	100	0	0	0	y	r	ry	r
GLE	[11]	0.50 (0.33)	0.52 (0.42) <sup>a</sup>	0	0	100	45	0	10	45	y	r	ry	r
LUZ	[44]	0.67 (0.31)	1.29 (1.29) <sup>b</sup>	27	0	73	0	0	2	98	r	r	r	r
SAG	[36]	0.47 (0.34)	0.70 (1.14) <sup>c</sup>	22	11	67	65	0	35	0	y	r	y	r
<b>A3</b>														
SAG	[55]	1.28 (0.50)	5.61 (4.91)	85	13	2	100	0	0	0	y	r	r	w
											y	r	ry	w
											w	r	r	w
<b>M1</b>														
MED	[45]	0.39 (0.07) <sup>d</sup>	–	0	0	100	0	98	2	0	y	r	ry	r
											y	p	y	p
BAN	[45]	0.53 (0.19)	–	0	0	100	0	100	0	0	y	r	y	r
											w	w	w	w
MR2	[26]	0.22 (0.46)	–	0	0	100	0	100	0	0	y	r	y	r
MR3	[21]	0.40 (0.08)	–	0	0	100	0	100	0	0	y	r	y	r
MR4	[36]	0.50 (0.16)	–	0	0	100	0	100	0	0	y	y	y	w
											y	r	y	r
											y	y	y	pw
<b>M2</b>														
MED	[34]	0.29 (0.17)	0.76 (1.24)	0	9	91	34	0	3	63	y	r	r	r
											y	r	y	r
MR1 <sup>e</sup>	[34]	1.00 (0.46)	8.31 (6.81)	9	76	15	?	0	0	?	y	r	r	r
MR2	[10]	0.10 (0.0)	0.03 (0.01)	0	0	100	30	0	0	70	y	r	r	r
MR3	[15]	0.13 (0.07)	0.63 (0.28)	0	0	100	7	0	0	93	y	r	r	r
MR5	[63]	1.45 (0.51)	9.47 (10.42)	0	98	2	100	0	0	0	y	r	r	rw
											y	r	r	pr

<sup>a</sup> Mean excludes four colonies with elongated, stalked morphology. When these individuals are included, mean  $H^2:A$  = 8.85 (14.34 SD)

<sup>b</sup> Mean H and  $H^2:A$  exclude one colony found encrusting a worm tube

<sup>c</sup> Mean H and  $H^2:A$  exclude 19 colonies found encrusting worm tubes [mean H = 0.26 (= 0.14 SD)]

<sup>d</sup> Height of Type M1 colonies is thickness of largest “knot” of polyps in a colony.  $H^2:A$  not calculated

<sup>e</sup> Substrate use not recorded, but all colonies were on either rock or other (bryozoans, sponges)

### Morphotype A2

Colonies of Morphotype A2 occurred at all Atlantic locations from Iles Chausey (CHA) southward, but were not found in the British Isles (Fig. 1). A2 colonies were typically smaller and more thinly encrusting than either Types A1 or A3 (Table 2); at the four Atlantic locations at which two morphotypes co-occurred, A1 or A3 colonies were always significantly taller than A2 colonies, and had significantly greater  $H^2:A$  ratios, indicating a more upright colony morphology (unpaired Student's  $t$ -tests,  $p < 0.001$ ) (Fig. 2). Many of the A2 colonies I found at Sagres (SAG) were thinly encrusting epibionts on the tubes of polychaete worms, and those in other populations were often overgrowing barnacles or other sessile organisms (Table 2). Type A2 colonies were generally red or very dark pink, with some yellow in the polyps (the "pink-4" of Groot and Weinberg 1982). Coenenchymal sclerites were always red or a mixture of red and pink; tentacular sclerites were almost always

yellow (occasionally red, and very rarely white), and sclerites in the polyps were usually red or pink, often mixed with yellow, especially in the proximal region (Table 2).

### Morphotype A3

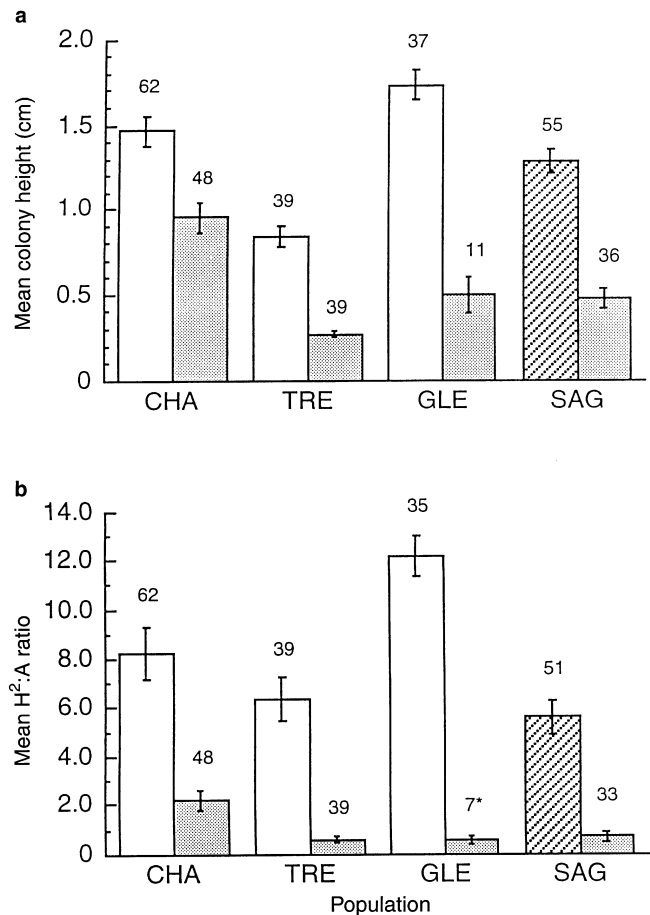
I found colonies of Morphotype A3 only in Portugal (SAG). These colonies were very similar in morphology and substrate use to Type A1, but differed in color (Table 2). The majority of A3 colonies had white coenenchymal sclerites, and both polyp regions usually contained predominantly red or pink sclerites, although some colonies had white, yellow or a mixture of red with either white or yellow sclerites in the proximal region. Tentacular sclerites were white or yellow. I found no colonies of the "pink-2" type in this population.

### Morphotype M1

Mediterranean Morphotype M1 colonies were always found as epibionts growing on the skeletons of gorgonian corals, and fit published descriptions of *Alcyonium* (= *Parerythropodium*) *coralloides* (Weinberg 1975, 1977). M1 colonies were very thinly encrusting ( $\leq 2$  mm thick) over most of the gorgonian axis (Table 2), but at irregular intervals high densities of polyps occurred on raised areas of coenenchyme up to 20 mm thick. In shallow water ( $< 25$  m), the predominant colony color was a deep wine-red with yellow polyps, produced by the combination of red sclerites in the coenenchyme, yellow (sometimes mixed with red) in the proximal and red in the distal polyp regions, and yellow (or, rarely, white) in the tentacles (Table 2). In deeper water ( $> 25$  m), additional color morphs were found, including pure white; pink coenenchyme with either yellow or white sclerites in the tentacles and/or polyps [Groot and Weinberg's (1982) "pink-3" and "pink-2", respectively]; and white coenenchyme with yellow in the tentacles and/or polyps (Table 2).

### Morphotype M2

Morphotype M2 colonies were always found on hard substrata or overgrowing organisms such as barnacles, bryozoans or sponges (Table 2). Colony morphology ranged from small, thinly encrusting forms 1 to 2 mm thick to lobate or digitate forms up to 35 mm in height. Color varied, although in overall appearance colonies were usually a shade of red or dark pink. Coenenchymal sclerites were usually either red or various mixtures of red, pink and white, tentacular sclerites were almost invariably yellow, and sclerites in the distal region of the polyp were always deep red; sclerites in the proximal region of the polyp were also usually red, although white or yellow occurred in some colonies (Table 2).



**Fig. 2** *Alcyonium coralloides*. Comparisons of **a** mean colony height (when fully contracted) and **b**  $H^2:A$  ratio (measure of colony morphology) between colonies of Morphotype A1 (open bars) or A3 (hatched bars) and A2 (shaded bars) at the four NE Atlantic locations where two morphotypes co-occurred (error bars standard errors of mean; numbers above bars sample sizes). Differences in mean height and  $H^2:A$  ratio were significant at all locations (unpaired Student's  $t$ -tests,  $p < 0.0001$ ) (Location abbreviations as in Fig. 1)

**Table 3** *Alcyonium coralloides*. Allele frequencies in NE Atlantic and Mediterranean populations; alleles numbered in order of decreasing mobility [*A1*, *A2*, *A3* Atlantic morphotypes; *M1*, *M2* Mediterranean morphotypes; *location abbreviations* as in Fig. 1; (*N*) number of individuals analyzed for each locus; *H<sub>o</sub>* observed heterozygosity (direct count); *H<sub>ave</sub>* observed heterozygosity averaged over all loci (including monomorphic loci); *P* percent of loci polymorphic]

Locus, allele	Location and morphotype																						
	RAT A1	MUL A1	STJ A1	HYN A1	IOM A1	CHA A1	TRE A1	GLE A1	LUZ A2	SAG A3	MED M1	BAN M1	MRI M2	MR2 M1	MR3 M1	MR4 M1	MR5 M2						
<i>AK</i>	(27)	(24)	(25)	(24)	(78)	(44)	(26)	(06)	(26)	(00)	(18)	(48)	(22)	(07)	(13)	(02)	(20)	(00)	(16)	(00)	(26)	(03)	
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.927	1.000	0.114	0.286	0.192	1.000	0.000	0.000	0.125	0.038	1.000	
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.886	0.286	0.654	0.000	0.875	0.000	0.875	0.000	0.904	0.000	
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.429	0.077	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000	0.125	0.000	0.000	0.000	0.058	0.000	
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.073	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
<i>H<sub>o</sub></i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.063	0.000	0.000	0.077	0.000	0.150	0.000	0.000	0.000	0.115	0.000	
<i>GPI</i>	(28)	(24)	(25)	(24)	(73)	(44)	(33)	(17)	(25)	(05)	(32)	(08)	(22)	(36)	(21)	(31)	(32)	(25)	(06)	(20)	(13)	(35)	(54)
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.417	0.000	0.081	0.000	0.220	0.000	0.025	0.000	0.057	0.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.136	0.000	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.341	0.000	0.000	0.371	0.000	0.371	0.000	0.720	0.000	0.725	0.000	0.471	0.000
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.386	0.000	0.000	0.069	0.194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.095	0.000	0.344	0.000	0.000	0.000	0.000	0.577	0.000	0.000
7	0.000	0.000	0.000	0.000	0.000	0.000	0.758	0.941	0.000	1.000	0.000	0.000	0.000	0.905	0.000	0.453	0.000	0.000	0.000	0.000	0.385	0.000	0.370
8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.139	0.000	0.290	0.000	0.290	0.000	0.060	0.000	0.250	0.000	0.400	0.000
9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.056
10	1.000	1.000	1.000	1.000	1.000	1.000	0.242	0.941	0.059	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>H<sub>o</sub></i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.409	0.528	0.190	0.677	0.250	0.250	0.280	0.167	0.300	0.462	0.600	0.600	0.426
<i>HK</i>	(28)	(24)	(25)	(24)	(78)	(44)	(34)	(34)	(26)	(08)	(37)	(50)	(49)	(40)	(27)	(33)	(31)	(26)	(09)	(20)	(15)	(36)	(62)
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.500	1.000	0.010	0.959	0.913	0.926	0.742	0.984	0.519	1.000	0.550	1.000	0.458	1.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.490	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.020	0.087	0.074	0.212	0.016	0.154	0.000	0.125	0.000	0.278	0.000	0.000
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.490	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.327	0.000	0.325	0.000	0.264	0.000	0.000
6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>H<sub>o</sub></i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.082	0.175	0.148	0.485	0.032	0.808	0.000	0.600	0.000	0.583	0.000	0.000
<i>LAP-1</i>	(24)	(20)	(19)	(14)	(56)	(06)	(12)	(18)	(12)	(02)	(30)	(45)	(37)	(36)	(14)	(18)	(19)	(19)	(05)	(15)	(11)	(23)	(47)
1	1.000	1.000	1.000	1.000	1.000	1.000	0.167	1.000	0.000	0.000	0.000	0.422	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.833	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.578	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>H<sub>o</sub></i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.489	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>LAP-2</i>	(17)	(16)	(17)	(17)	(19)	(40)	(30)	(27)	(15)	(20)	(05)	(28)	(45)	(38)	(25)	(34)	(25)	(16)	(04)	(14)	(07)	(25)	(58)
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.660	0.000	0.160	0.000	0.250	0.000	0.571	0.000	0.500
2	1.000	1.000	1.000	1.000	1.000	1.000	0.133	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.340	0.000	0.000	0.000	0.000	0.000	0.840	0.000	0.750	0.000	0.429	0.000	0.500



## Allozymes

*EST*, *LTP-1*, and *MDH-2* were monomorphic across all populations. The remaining 11 loci varied within or among populations (Table 3). As in some other cnidarians (Carter and Thorpe 1981; Solé-Cava et al. 1985; Smith and Potts 1987), *GPI* homozygotes displayed three bands of equal intensity, while heterozygotes exhibited complex patterns ranging from five to nine distinct bands. Most individuals of Morphotypes A1 and A3 exhibited a poorly resolved five-banded pattern for *GPI*, which I scored as a unique allele, *GPI-10* (Table 3). This pattern may represent a fixed heterozygous condition (the lower three bands appeared to correspond to allele *GPI-7*), or the additional bands may have resulted from enzyme degradation or other post-translational modifications (e.g. Richardson et al. 1986).

Individuals of Type A1 also had an atypical banding pattern at the *6PGD* locus, an invariant double band that I scored as a unique allele (*6PGD-5*). This double band, which was not seen in the other morphotypes, could represent a duplication of the *6PGD* locus, or could be the result of a post-translational modification (Richardson et al. 1986). Activity and resolution of *6PGD* was very poor in A3 individuals, and therefore was not scored for that morphotype. A1 and A3 also differed from the other morphotypes in the level of enzyme activity at the *AK* locus. The *AK-1* allele always stained very darkly in A1 and A3; a band of the same mobility that was present in some individuals of the other morphotypes was always very faint, and many individuals of A2, M1 and M2 had no detectable *AK* activity.

### Genetic variation and heterozygosity within morphotypes

Populations of Type A1 exhibited no genetic variation or heterozygosity at any of the 14 enzyme loci: every individual in every population was genetically identical, with the sole exception of one individual from the TRE population that appeared to have a different *GPI* genotype (Table 3). Levels of genetic variation and heterozygosity within most populations of Type A2 were also low; percentage of polymorphic loci averaged 14.7%. All A2 individuals collected at LUZ were genetically identical and homozygous at all loci, and the average heterozygosity observed in three of the other four populations was 0.04 or lower (Table 3). The eight individuals of Type A2 collected at Iles de Glénan (GLE) were an exception, as they were all heterozygous at both *HK* and *TPI*. Because they were collected from the same boulder, and because they all shared a rare *HK* allele, it is likely that they belonged to a single clone.

Levels of genetic variation and heterozygosity in populations of Types A3, M1 and M2 were higher than for A1 and A2. Average observed heterozygosity ranged from 0.060 to 0.088 in M1, 0.056 to 0.111 in M2, and

was 0.109 in the one population of A3 (Table 3). The percentage of polymorphic loci averaged 22.6% in M1, 37.3% in M2 and 33.3% in A3 (Table 3). These values fall within the range typical of most sexually reproducing marine invertebrates (Nevo et al. 1984).

Genotype frequencies at polymorphic loci differed significantly from Hardy–Weinberg expectations in only 3 of 61 cases, although most populations had slight heterozygote deficiencies at the *AK*, *GPI* and *LAP-2* loci (Table 4). Because sample sizes and expected allele frequencies were low in most populations, the power to detect significant differences is also low, so these results should be interpreted cautiously.

### Genetic differentiation among morphotypes

In all cases where two morphotypes were present at the same location, they could be distinguished by fixed allelic differences. M1 and M2 occurred together at Illes Medes (MED) and Marseilles (MR-2 and MR-3). These two morphotypes showed fixed allelic differences at six loci (*GPI*, *LAP-1*, *LAP-2*, *LTP-2*, *6PGD* and *TPI*), and M2 populations had alleles at the *MDH-1* and *ODH* loci that were not found in M1 (Table 3). Allele frequencies also differed significantly between the two types at the *AK* locus in the MED population ( $\chi^2$ ,  $p < 0.001$ ), and at *HK* in the MR-2 and MR-3 populations ( $\chi^2$ ,  $p < 0.01$ ).

Morphotypes A2 and A3 co-occurred at SAG. They were distinguished by fixed allelic differences at four loci (*GPI*, *HK*, *LAP-1* and *LAP-2*), and by significant differences in allele frequencies at *NP* and *TPI* ( $\chi^2$ ,  $p < 0.001$ ) (Table 3).

Morphotypes A1 and A2 co-occurred at CHA, TRE and GLE. At all three sites they exhibited fixed or nearly fixed allelic differences at five loci (*GPI*, *LAP-1*, *LAP-2*, *LTP-2* and *6PGD*), significant differences in allele frequencies at *TPI* ( $\chi^2$ ,  $p < 0.001$ ) and consistent differences in *AK* activity (Table 3). At CHA, several A2 individuals appeared to have *LAP-1* or *LAP-2* alleles that were characteristic of A1, and at both CHA and TRE several A2 individuals exhibited the five-banded *GPI-10* pattern characteristic of A1; conversely, one individual of A1 at TRE appeared to have the Type A2 *GPI-7* genotype (Table 3).

The average pairwise genetic distances (Nei's *D*) among four of the five morphotypes ranged from  $D = 0.281$  to  $0.612$  (Table 5). These values are considerably greater than those typically found among conspecific populations (Thorpe 1983; Thorpe and Solé-Cava 1994), and are comparable to the value ( $D = 0.601$ ) separating the nominate species *Alcyonium acaule* and *A. glomeratum*. In contrast, average *D* values among populations of each morphotype were all  $< 0.065$ , indicating relatively little geographic differentiation within morphotypes (Table 5).

The average genetic distance among A2 and M1 ( $D = 0.133$ ) was considerably less than that among all other pairs of morphotypes (Table 5). Although there

**Table 4** *Alcyonium coralloides*. Heterozygote deficiencies,  $D[(H_0 - H_e) : H_e]$  within populations of three Atlantic (*A1*, *A2*, *A3*) and two Mediterranean (*M1*, *M2*) morphotypes. Significance values are shown for loci at which genotype frequencies deviated from

Hardy–Weinberg expectations: (\*  $p < 0.05$ ) (*Location abbreviations* as in Fig. 1; – loci not polymorphic in that population; populations with no polymorphic loci are not included)

Location, Type	Locus										
	<i>AK</i>	<i>GPI</i>	<i>HK</i>	<i>LAP-1</i>	<i>LAP-2</i>	<i>LTP-2</i>	<i>MDH-1</i>	<i>NP</i>	<i>ODH</i>	<i>6PGD</i>	<i>TPI</i>
CHA											
A2	–	–1.000*	–	–1.000	–1.000*	–	–	–	–	–	0.188
TRE											
A1	–	–1.000	–	–	–	–	–	–	–	–	–
A2	–	–1.000	–	–	–	–	–	–	–	–	0.289
GLE											
A2	–	–	1.000	–	–	–	–	–	–	–	1.000
SAG											
A3	–0.538	–	–	0.002	–	–0.656	–	0.079	–	–	–
A2	–	–0.421	0.032	–	–	–	–	–	–	–	0.055
MED											
M1	–0.774	–0.202	0.096	–	–	–0.319	–	–	–	–	–
M2	–1.000	0.105	0.080	–	–0.198	0.156	0.029	–	0.050	0.062	–
BAN											
M1	–	–0.072	0.207	–	–	0.046	–	–	–	–	–
MR1											
M2	–	–0.606*	0.016	–	–0.107	–	–0.460	–	0.024	–0.108	–
MR2											
M1	–0.314	–0.348	0.346	–	–	–	–	–	–	–	–
M2	–	0.091	–	–	–1.000	–	0.091	–	–	0.029	–
MR3											
M1	–1.000	–0.271	0.041	–	–	–	–	–	–	–	–
M2	–	–0.109	–	–	–0.417	–	0.091	–	0.286	–0.200	–
MR4											
M1	–0.353	–0.015	–0.093	–	–	–	–	–	–	–	–
MR5											
M2	–	–0.197	–	–	–0.103	–	–0.394	–	0.037	0.123	–

were significant differences in allele frequencies among populations of A2 and M1 at several loci (*AK*, *GPI*, *HK*, *TPI*), no fixed allelic differences distinguished these morphotypes (Table 3). Pairwise genetic distances between individual populations of A2 and M1 ranged from  $D = 0.045$  to  $0.251$ ; these values overlap those measured among populations of A2 only ( $D = 0.003$  to  $0.157$ ). There was no overlap in the ranges of pairwise

$D$  values within vs between morphotypes for any of the other morphotypes (Table 5).

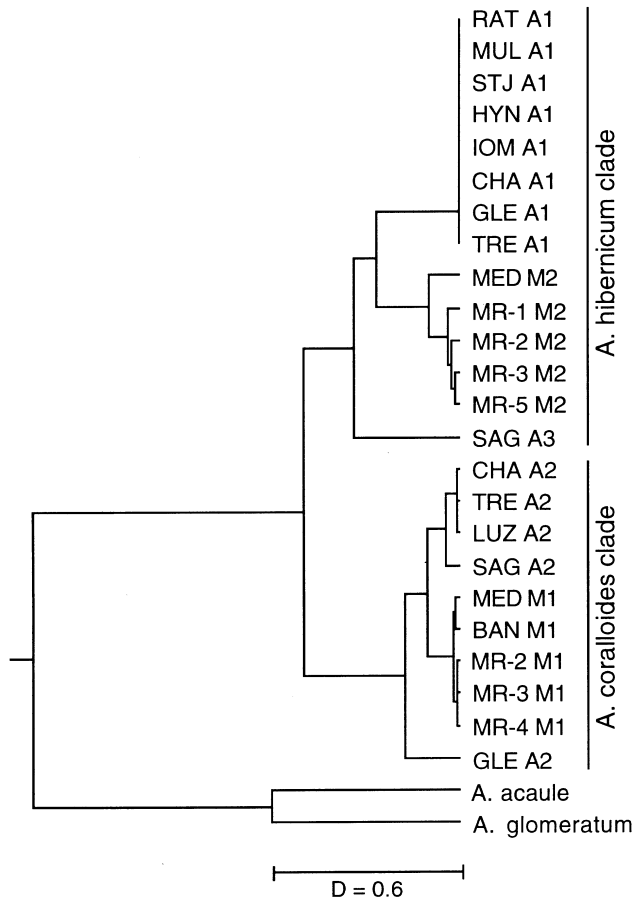
#### Phylogenetic relationship of morphotypes

Both the UPGMA dendrogram of Nei's  $D$  values (Fig. 3) and the neighbor-joining tree of Cavalli-Sforza

**Table 5** *Alcyonium coralloides*. Nei's (1978) genetic distance,  $D$ , averaged among populations of each morphotype; range of pairwise  $D$  values in parentheses. (*A1*, *A2*, *A3* Atlantic morphotypes;

*M1*, *M2* Mediterranean morphotypes; *AA* *A. acaule*; *AG* *A. glomeratum*; numbers in brackets no. of populations of each morphotype)

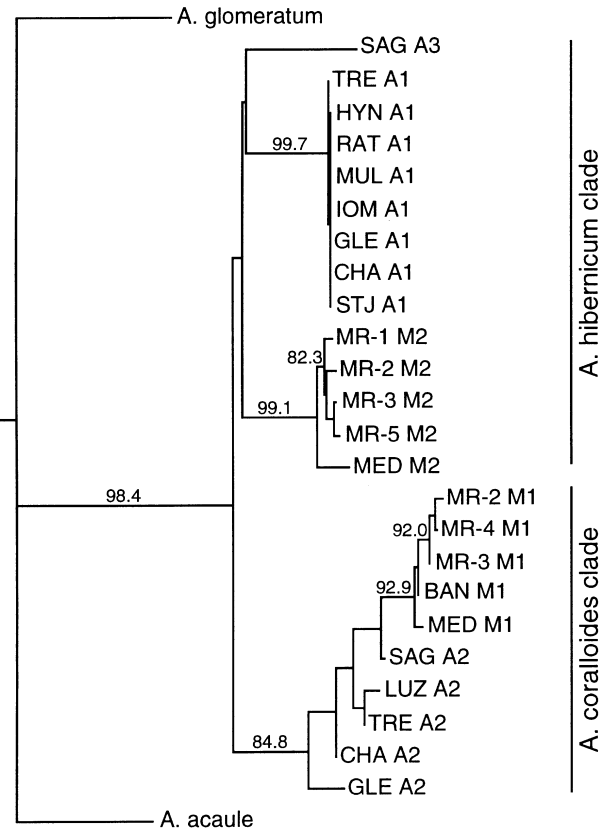
	Morphotype or species						
	A1 [8]	A2 [5]	A3 [1]	M1 [5]	M2 [5]	AA [1]	AG [1]
A1	0.000 (0.000–0.000)	0.442 (0.359–0.511)	0.338 (0.338–0.340)	0.612 (0.570–0.646)	0.281 (0.261–0.320)	1.007 (1.004–1.007)	1.260 (1.257–1.261)
A2		0.061 (0.003–0.157)	0.438 (0.399–0.492)	0.133 (0.045–0.251)	0.417 (0.357–0.507)	1.217 (1.109–1.361)	1.248 (1.226–1.277)
A3			–	0.552 (0.525–0.573)	0.368 (0.342–0.379)	1.228	1.380
M1				0.012 (0.002–0.023)	0.574 (0.498–0.637)	1.176 (1.162–1.195)	1.484 (1.439–1.520)
M2					0.052 (0.006–0.119)	1.147 (1.138–1.164)	1.081 (1.035–1.220)
AA						–	0.601
AG							–



**Fig. 3** *Alcyonium coralloides*. UPGMA dendrogram of Nei's  $D$  values among three Atlantic ( $A1$ ,  $A2$ ,  $A3$ ) and two Mediterranean ( $M1$ ,  $M2$ ) morphotypes. *A. acaule* and *A. glomeratum* are included as outgroups (Location abbreviations as in Fig. 1)

and Edwards' (1967) chord distances (Fig. 4) divide the five morphotypes into two distinct, monophyletic clades: an "*Alcyonium coralloides*" clade comprising morphotypes  $A2$  and  $M1$  and an "*A. hibernicum*" clade that includes  $A1$ ,  $A3$  and  $M2$ . Within the "*A. hibernicum*" clade,  $A1$  and  $M2$  fall into distinct monophyletic groups supported by very high bootstrap values (Fig. 4). In both trees,  $A3$  is distinct from and falls outside the  $A1$  and  $M2$  clades, but low bootstrap values indicate uncertain placement of this group: the neighbor-joining tree places  $A3$  as a sister group to  $A1$ , while the UPGMA dendrogram suggests that  $A1$  and  $M2$  are sister taxa. This latter topology is strongly supported by DNA sequence data obtained from the internal transcribed spacer regions (ITS) of the nuclear ribosomal gene complex (McFadden et al. in preparation).

Both trees unite populations of Morphotype  $M1$  monophyletically, but indicate that  $A2$  is paraphyletic with respect to  $M1$ . This relationship is consistent with the conclusion that  $A2$  and  $M1$  are geographically differentiated forms of the same species, and suggests that the Mediterranean populations may have been derived from an Atlantic progenitor (Fig. 4).



**Fig. 4** *Alcyonium coralloides*. Neighbor-joining tree of Cavalli-Sforza and Edwards' chord distances among three Atlantic ( $A1$ ,  $A2$ ,  $A3$ ) and two Mediterranean ( $M1$ ,  $M2$ ) morphotypes. *A. acaule* and *A. glomeratum* are included as outgroups; *A. acaule* was used to root the tree (Numbers above branches bootstrap percentages from 1000 iterations, only bootstrap values  $> 70\%$  are shown; location abbreviations as in Fig. 1)

## Discussion

### Genetic and taxonomic relationships among morphotypes

The results of this genetic study suggest that the soft coral known as *Alcyonium coralloides* is not one highly variable species, but rather a complex of at least four distinct species that differ in colony growth form, color, microhabitat and geographic range. The original description of *A. coralloides* referred to a specimen found growing on the dead axis of a gorgonian in the Mediterranean (Pallas 1766: cited in Weinberg 1977), and subsequent revisions of that species describe it as occurring primarily as an encrusting epibiont on gorgonians (Weinberg 1977). Morphotype  $M1$  clearly fits these original descriptions of *A. coralloides*.

The second morphotype found in the Mediterranean,  $M2$ , is genetically distinct and reproductively isolated from *Alcyonium coralloides* ( $M1$ ): fixed allelic differences at 6 of 14 enzyme loci indicate that they do not interbreed, despite the fact that they often live immediately adjacent to one another. Species  $M2$  grows attached to

rocks and benthic organisms other than gorgonians, and I found it most commonly on the undersides of rock ledges and on the walls and roofs of shallow caves. Populations matching the description of M2 have been noted throughout the western Mediterranean, from the Banyuls-sur-Mer region, the Côte d'Azur and Costa Liguri, and in Corsica and Sardinia (Weinberg 1979, 1980; Groot and Weinberg 1982). M2 closely resembles *A.* (= *Parerythropodium*) *bosphorensis* Tixier-Durivault, 1961, described from the eastern Mediterranean (Groot and Weinberg 1982), and further morphological and genetic comparisons will be necessary to determine their taxonomic relationship.

The three morphotypes attributed to *Alcyonium coralloides* in the NE Atlantic are also genetically distinct, and fixed allelic differences where they occur sympatrically indicate that A2 is reproductively isolated from both A1 and A3. Morphotype A2 belongs to the same clade as *A. coralloides* (M1), and the average genetic distance between them is considerably less than that among any of the other morphotypes (Table 5). Because *A. coralloides* (M1) and Morphotype A2 are geographically separated, however, reproductive isolation in sympatry cannot be used as the criterion for determining if they belong to the same or different species. Because no fixed allelic differences distinguish A2 from M1, and because the pairwise distances among some A2 populations are greater than the distances separating some Mediterranean (M1) and Atlantic (A2) pairs, I suggest, in the absence of direct evidence of reproductive isolation, that Morphotype A2 continue to be considered a geographic variant of *A. coralloides*.

Atlantic Morphotype A1 was originally described as *Parerythropodium hibernicum* Renouf, 1931, but was later synonymized with *Alcyonium coralloides* on the basis of morphological similarities (Van Soest and Weinberg 1980). The genetic data suggest strongly, however, that *A. hibernicum* is a valid species, reproductively isolated from *A. coralloides* (A2) where their ranges overlap in the English Channel. Although these two species are distinguished by fixed or nearly fixed allelic differences at five enzyme loci, the presence of small numbers of individuals with the "wrong" genotype at particular loci (i.e. an allele characteristic of the other species: Table 3) could indicate a small amount of hybridization between them. This hypothesis is supported by the observation that some individuals appear to have copies of ribosomal ITS sequences from both species (McFadden et al. unpublished data). Despite these few irregularities, the conclusion that *A. hibernicum* (A1) and *A. coralloides* (A2) are reproductively isolated species is well supported by the genotypes of the majority of individuals sampled in their range of sympatry.

The third Atlantic morphotype, A3, is also reproductively isolated from *Alcyonium coralloides* (A2), as demonstrated by fixed allelic differences between the two forms where they co-occur at SAG. Although all three members of the "*A. hibernicum*" clade (A1, A3, M2) are clearly reproductively isolated from *A. coralloides* (A2,

M1), the taxonomic relationships among them are more difficult to evaluate. Fixed allelic differences distinguish A1 and A3 at three loci, A1 and M2 at 4 loci, and A3 and M2 at 3 loci (Table 3). Because none of these morphotypes occur sympatrically, these genetic differences could simply indicate geographic differentiation of populations rather than biological barriers to interbreeding. The number of fixed differences and the magnitude of the genetic distances separating the three morphotypes ( $D = 0.281$  to  $0.368$ ) suggest, however, that genetic exchange rarely, if ever, occurs among them. I propose, therefore, that *A. hibernicum* (A1), A3 and M2 all be recognized as distinct species.

At least three other described species of *Alcyonium* appear to be very similar morphologically and ecologically to *A. coralloides*, and may be synonymous with that species or one of the others distinguished in this paper. Alternatively, they may represent additional members of the *A. coralloides*/*A. hibernicum* species complex. *A.* (= *Parerythropodium*) *maris-tenebrosi* Stiasny, 1937 and *A. senegalense* Verseveldt and van Ofwegen, 1992, both from the Atlantic coast of NW Africa, and *A.* (= *Parerythropodium*) *grandiflorum* Tixier-Durivault and d'Hondt, 1974 from the Azores all occur as epibionts on gorgonians, like Mediterranean populations of *A. coralloides* (M1). The taxonomic relationship of these three species, as well as *A. bosphorensis*, to the four species distinguished here needs to be examined further, preferably using genetic techniques.

#### Genetic variation and mode of reproduction in *Alcyonium hibernicum*

The results of a histological study of *Alcyonium hibernicum* suggest that this species reproduces parthenogenetically (Hartnoll 1977). Samples taken throughout the year in a population from the Isle of Man revealed that all colonies produced ova that developed into brooded planula larvae, but no trace of sperm or testicular tissue was ever detected. To date, however, there has been no genetic confirmation of asexual reproduction in this species, and the underlying cytological mechanism of parthenogenesis remains unidentified.

The complete lack of genetic variation observed within populations of *Alcyonium hibernicum* is consistent with asexual reproduction. Populations of asexual species are often dominated locally by individuals belonging to one or a few clones (Wright 1969; Jain 1976; Suomaleinen et al. 1976; Baur and Klemm 1989). The complete lack of allozyme variation throughout the entire geographic range of *A. hibernicum* is, however, highly unusual; most parthenogens exhibit relatively high clonal diversity among populations due to the accumulation of mutations locally (Suomaleinen et al. 1976; Parker 1979; Stoddart 1983b; Ellstrand and Roose 1987). The lack of differentiation among *A. hibernicum*

populations could indicate that the species evolved only recently (or has recently been through a genetic bottleneck) and not enough time has passed since its origin for local variants to have arisen by mutation (Lokki 1976; Ellstrand and Roose 1987).

The complete homozygosity exhibited by *Alcyonium hibernicum* is also uncharacteristic of species that reproduce by the most common type of parthenogenesis, apomixis (Suomaleinen et al. 1976). Apomicts lack meiosis and therefore are expected to accumulate mutations in heterozygous form: in the absence of recombination, homozygosity can only be restored by rare complementary or backmutations (Lokki 1976). Several other cnidarians that produce larvae asexually exhibit high levels of fixed heterozygosity, consistent with apomictic parthenogenesis (Ayre and Resing 1986; Shaw et al. 1987). In contrast, *A. hibernicum* appears to be homozygous at all loci, with the possible exception of *GPI*.

High homozygosity is characteristic of automictic parthenogens, in which diploidy is restored by nuclear fusion following meiosis (Suomaleinen et al. 1976), and of obligately self-fertilizing hermaphrodites (Wright 1969; Jain 1976). It is possible, therefore, that *Alcyonium hibernicum* is an automictic (rather than an apomictic) parthenogen. Alternatively, it could be a self-fertilizing hermaphrodite in which sperm production is so low that it cannot easily be detected by standard histological methods (e.g. O'Foighil and Eernisse 1988). Further cytological and genetic studies will be necessary to confirm the mechanism of reproduction in *A. hibernicum*, but so far the genetic data are consistent with a lack of outcrossing in this species.

#### Reproductive isolation and origin of species

Throughout the portion of its geographic range sampled in this study, *Alcyonium coralloides* almost always occurred sympatrically with a species from the *A. hibernicum* clade. Although sympatric species typically differed slightly in substrate use (Table 2), it was not unusual to find them growing in very close proximity or even in direct contact with each other. For instance, at MED, colonies of species M2 occurred around and on the bases of gorgonians occupied by *A. coralloides* (M1), and at CHA, I found colonies of *A. coralloides* (A2) and *A. hibernicum* in direct physical contact within a dense mixed-species population occupying the hull of a shipwreck. These observations suggest that ecological divergence (which may be necessary for their coexistence) does not contribute significantly to reproductive isolation among these species, as sperm could easily travel the short distances separating individuals.

Differences in reproductive timing or in the gamete recognition systems that control fertilization (e.g. Palumbi 1994) are the most likely barriers to reproduction among this group of species. At present little information is available on the timing of reproduction in any species other than *Alcyonium hibernicum* (Hartnoll

1977). I observed fully developed planula larvae in ~40% of the colonies of *A. coralloides* (A2) collected at SAG in May 1994. In contrast, <10% of colonies of Species A3 had embryos at that time, and they appeared to be at an earlier developmental stage, indicating a possible difference in reproductive timing between these two species. Approximately 50% of the *A. hibernicum* colonies I sampled at CHA, TRE and GLE in late July 1994 contained fully developed planulae, compared to only ~10% of *A. coralloides* (A2) colonies at CHA and none at TRE or GLE. Regardless of the timing of larval production, however, the absence of outcrossing in *A. hibernicum* should reproductively isolate it from *A. coralloides*. Neither *A. coralloides* (M1) nor Species M2 were reproductive when I sampled Mediterranean populations in early July 1994.

While the evolution of parthenogenesis or obligate selfing, such as has occurred in *Alcyonium hibernicum*, would result in instantaneous reproductive isolation even in sympatry (Mayr 1963), changes in reproductive timing or gamete compatibility are more likely to have evolved allopatrically. The recent history of the Mediterranean region has provided ample opportunities for allopatric speciation followed by range expansion and secondary contact. The Mediterranean underwent an extreme salinity crisis  $\approx$  5.5 million years ago, and has been recolonized largely since then (Hsü et al. 1977; Pérès 1985). Climatic fluctuations throughout the Quaternary have repeatedly altered its temperature, salinity and circulation patterns, alternately favoring the immigration and survival of different faunas (Pérès 1985), and periodic isolation of its major basins have promoted rapid genetic diversification and high speciation rates (Sarà 1985; Tortonese 1985). Present-day circulation patterns may continue to restrict genetic exchange between Atlantic and Mediterranean populations: genetic discontinuities reported in several species appear to coincide with the location of an anticyclonic gyre off the SE coast of Spain, which may represent a significant barrier to larval dispersal (Tintore et al. 1988; Quesada et al. 1995a, b; Borsa et al. 1997; Pannacciulli et al. 1997).

The initial divergence of the *Alcyonium coralloides* and *A. hibernicum* clades could have occurred as a result of the isolation of an ancestral population of *A. coralloides* within the Mediterranean. Differences in the observed genetic diversity of *A. coralloides* populations are consistent with a Mediterranean origin and subsequent spread into the Atlantic. The greater allelic diversity and higher heterozygosity of Mediterranean (M1) compared to Atlantic (A2) populations suggest that Mediterranean populations have had a longer time to accumulate genetic diversity. Moreover, the very low genetic diversity of A2 populations at the northern end of their range in France is consistent with these populations having experienced a recent genetic bottleneck. As suggested by Groot and Weinberg (1982), a founder effect associated with the colonization of the Atlantic from the Mediterranean is one likely bottleneck.

A Mediterranean origin of *Alcyonium coralloides* is not, however, well supported by the dendrograms, both of which suggest that Mediterranean (M1) populations originated subsequent to the diversification of Atlantic (A2) populations. It is possible, therefore, that the *A. coralloides* and *A. hibernicum* clades diverged within the Atlantic and that members of both clades (M1, M2) have colonized the Mediterranean subsequently. To confirm the geographic origin of *A. coralloides*, it will be necessary to sample populations from a variety of additional locations, especially in the extreme western Mediterranean (Alboran Sea) and Straits of Gibraltar.

Several other species of *Alcyonium* are sympatric with members of the species complex described here; these include *A. acaule* and *A. palmatum* in the Mediterranean, and *A. glomeratum*, *A. digitatum* and another undescribed species in the NE Atlantic (McFadden unpublished data). Although morphological and life-history differences clearly distinguish these species, they are ecologically very similar and, with the possible exception of *A. palmatum*, can all be found living immediately adjacent to members of the *A. coralloides* and *A. hibernicum* clades. Although they diverged from one another at different times in the past, the mechanisms by which all these *Alcyonium* species maintain reproductive isolation in sympatry must be similar. Further phylogenetic and reproductive analysis of the genus *Alcyonium* will contribute greatly to our understanding of the role reproductive characters play in the origin and maintenance of species boundaries in the marine environment.

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