

A MOLECULAR PHYLOGENETIC ANALYSIS OF REPRODUCTIVE TRAIT EVOLUTION IN THE SOFT CORAL GENUS *ALCYONIUM*

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Abstract.—The soft coral genus *Alcyonium* is among the most reproductively diverse invertebrate taxa known: The genus includes species that vary both in mode of reproduction (including broadcast spawners, internal brooders, and external brooders) and sexual expression (gonochores, hermaphrodites, and a unisexual parthenogen). Such diversity offers a unique opportunity to examine associations between reproductive and morphological traits in a phylogenetic context. We used an approximately 900-bp sequence of the nuclear ribosomal gene complex spanning the internal transcribed spacer (ITS) regions to construct a molecular phylogeny for 14 European and North American species of *Alcyonium* onto which we mapped the known distribution of reproductive and morphological traits. The phylogeny suggests that hermaphroditism or parthenogenesis has evolved independently at least twice in this genus, and always in internally brooding species. Broadcast spawning and external brooding only occur in species with large colony size, whereas all species with small colony size brood their larvae internally. Internal brooding and small size appear to be ancestral in this genus; if this is the case, an association between broadcast spawning and large colony size has evolved independently in at least two clades. This tendency of small adults to brood their larvae while large adults broadcast spawn them into the plankton has been observed in a variety of solitary invertebrate taxa, but to date has not been documented in any other colonial invertebrates. Moreover, it has been suggested that organisms with a colonial growth form should not experience the allometric constraints on brood space that have been proposed to explain the association between adult size and mode of reproduction in solitary organisms. Unlike many other colonial groups, however, module (polyp) size is strongly correlated with colony size in *Alcyonium*, and constraints on brooding may be imposed by module, rather than colony, allometry. The very close genetic relationship (< 1% sequence divergence) and shared polymorphisms among *A. digitatum* (a large, gonochoric broadcast spawner), *A. siderium*, and *A. sp. A* (intermediate-sized and small hermaphroditic, internal brooders) suggest that evolutionary transitions between broadcast spawning and brooding and between gonochorism and hermaphroditism can occur easily and rapidly in this group.

Key words.—*Alcyonium*, cnidarian, internal transcribed spacer, larval development, life-history evolution, molecular systematics, ribosomal DNA, sexual expression, soft coral.

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Our understanding of life-history evolution continues to be illuminated by comparative studies of reproductive traits in sessile and sedentary marine invertebrates (e.g., Menge 1975; Jablonski 1986; Janson 1987; Emler 1995; Wray 1996; Hart et al. 1997). Occupying one end of a continuous spectrum of reproductive strategies are species with no brood care that broadcast spawn their gametes directly into the water column. The resulting embryos often reside in the plankton for many weeks prior to metamorphosis, during which time they may suffer high mortality but have the potential to disperse long distances (Strathmann 1985; Scheltema 1986). At the other end are species that internally brood their embryos, releasing them into the environment as already-metamorphosed juveniles with high survival but limited dispersal potential (Jackson 1986). Species that can be found living side-by-side (Menge 1975), closely related sister taxa (Arndt et al. 1996; Hart et al. 1997), and, in some cases, individuals within a species (Levin 1984; Levin et al. 1991; Gibson and Chia 1995) may exhibit reproductive strategies that fall at opposite ends of this wide spectrum.

A variety of adaptive explanations have been proposed to explain the differences in developmental mode that can be

observed among otherwise similar taxa. One widely supported hypothesis links brood care to adult body size (Strathmann and Strathmann 1982). As organisms increase in size, their surface area:volume ratio typically decreases. If brood space is proportional to surface area but fecundity increases volumetrically, the fraction of its embryos that an individual can brood successfully will decrease as adult body size increases (Strathmann and Strathmann 1982; Strathmann et al. 1984); allometric constraints may therefore limit the ability of large adults to brood their offspring. Large individuals may be able to achieve higher reproductive success by producing large numbers of planktonic offspring, a strategy that may not be feasible for small individuals because they are unable to produce enough embryos to counter high rates of mortality in the plankton (Menge 1975). The pattern of large individuals broadcast spawning large clutches of planktonic offspring, while small individuals brood small clutches of nonplanktonic offspring is supported by observations across a wide variety of noncolonial invertebrate taxa (Strathmann 1985, 1990; but see Hess 1993).

Associations between size and developmental mode, however, have not been well documented to date in any group of colonial organisms (Jackson 1986; Harrison and Wallace 1990). Colonial invertebrates are typically composed of individual modules (e.g., polyps or zooids) of uniform size. Colonies increase in size by adding modules, and if module size and the number of embryos produced per module remain constant as colony size increases, colony fecundity should

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increase linearly with surface area. Consequently, the ability of colonial organisms to successfully brood their offspring should not diminish with colony size (Strathmann and Strathmann 1982). Allometric constraints could, however, operate at the level of modules, in which case large-moduled colonies should be less likely to brood than colonies with small modules (Strathmann and Strathmann 1982; Jackson 1986).

The anthozoan cnidarians (corals and sea anemones) are a large group of mostly colonial organisms whose members vary greatly in mode of larval development. Anthozoans may broadcast spawn gametes into the plankton, brood embryos internally to varying stages of larval development, or brood embryos externally. Although developmental mode is frequently conserved within families (Alino and Coll 1989; Benayahu et al. 1990) or genera (Richmond and Hunter 1990), examples of congeners with contrasting modes of development are not uncommon (e.g., Hartnoll 1977; Szmant 1986; Edmonds 1995).

Among the best-studied group of colonial anthozoans, the scleractinian corals, developmental mode does not appear to be correlated with module size (Jackson 1986; Szmant 1986; Harrison and Wallace 1990; Shlesinger et al. 1998). However, correlations between developmental mode and average colony size have been documented for some groups and geographic regions (Stimson 1978; Szmant 1986). In these cases, brooding and small colony size have been postulated to be indirectly associated because both may be adaptations to frequent physical disturbance of the environment (Stimson 1978; Van Moorsel 1983; Szmant 1986). In general, however, adaptive explanations for the observed distribution of scleractinian reproductive patterns have not been well supported (Harrison and Wallace 1990; Shlesinger et al. 1998).

The reproductively diverse alcyonacean soft coral genus *Alcyonium* offers excellent opportunities for further examination of possible adaptive patterns of anthozoan life-history evolution. This genus includes species that broadcast spawn planktonic larvae, as well as species that brood larvae either internally or externally. In addition, sexual expression, a trait that is phylogenetically conserved within most higher taxa of anthozoans (Harrison and Wallace 1990; Shlesinger et al. 1998) varies within this genus, which includes gonochoric, hermaphroditic, and putatively parthenogenetic species. The genus *Alcyonium* also encompasses a range of growth morphologies, from thinly encrusting to massive digitate or lobate forms, and average colony size varies over four orders of magnitude among species. However, most *Alcyonium* species are very similar ecologically, and species with different life histories frequently co-occur both sympatrically and syntopically (Hartnoll 1977; McFadden 1999).

Using a subset of the species in this genus we address two questions. First, are there morphological correlates of mode of development in *Alcyonium* that support an adaptive explanation for the observed variation? In particular, is mode of development associated with either module or colony size? If broadcast spawning evolves as a solution to allometric constraints on brood care, we would predict that species with large module size would be more likely to broadcast spawn than those with small modules. Second, are there associations between mode of development and sexual expression in this genus? In particular, it has been proposed that internal brood-

ing may precede and facilitate the evolution of both hermaphroditism (Strathmann and Strathmann 1982; Strathmann et al. 1984) and parthenogenesis (Lively and Johnson 1994) in invertebrates. Has the evolution of hermaphroditism and parthenogenesis in *Alcyonium* been limited to brooding lineages?

We address these questions using a comparative phylogenetic approach. We use nuclear ribosomal RNA gene sequences to infer the phylogeny of the genus *Alcyonium* and subsequently map the distribution of known reproductive and morphological traits onto this phylogeny. The recurrence of particular suites of traits (large module or colony size and broadcast spawning, small module or colony size and brooding, brooding and hermaphroditism or parthenogenesis) in phylogenetically independent lineages supports adaptive explanations for the patterns of life-history variation observed within this genus.

The Genus Alcyonium

The alcyonacean soft coral genus *Alcyonium* has a circum-global distribution, with representatives in most of the major tropical, temperate, and polar regions of the world; although between 75 and 135 species have been described worldwide, the majority remain very poorly known and little or no life-history information is available for them. The North American and European members of the genus, however, include several species whose ecology and reproductive biology have been well studied (*A. acaule*: Garrabou 1999; *A. coralloides*: Lacaze-Duthiers 1900; Groot and Weinberg 1982; *A. digitatum*: Hickson 1895; Matthews 1917; Hartnoll 1975; *A. hibernicum*: Hartnoll 1977; *A. rudyi*: McFadden 1986, 1991, 1996; *A. siderium*: Sebens 1983a,b,c, 1984; Sebens and Koehl 1984). We have included in our study the eight nominate species from these two geographic regions, as well as six species that are currently either undescribed or of uncertain taxonomic affinity and for which reproductive information is incomplete (Table 1). These include three new species from the northeastern Pacific (*A. sp. B*, *A. sp. C*, and *A. sp. D*) whose formal descriptions are pending (C. S. McFadden and F. G. Hochberg, unpubl. ms.), as well as three European species whose taxonomic status is uncertain. Two of these species (*A. sp. M2* and *A. sp. A3*) have recently been separated from *A. coralloides* (McFadden 1999), while a third (*A. sp. A*) can be distinguished from *A. digitatum* by allozyme and life-history differences (C. S. McFadden, unpubl. data).

These 14 *Alcyonium* species are all very similar to one another ecologically. All are passive suspension feeders that use arrays of eight-tentacled feeding polyps to filter small plankton from the water. Morphologically, species differ from one another primarily in average colony size and growth form, which ranges from thinly encrusting to upright lobate or digitate forms (Table 1). Although they grow indeterminate, most species of *Alcyonium* reach a characteristic maximum colony size. Colony size limits may be imposed by longevity, food availability, or hydrodynamic forces (Sebens 1984); in addition, upon reaching a particular colony size, some species undergo fission to form new, physiologically distinct colonies (ramets; e.g., McFadden 1986, 1991). Although growth morphology and colony size may vary intra-

TABLE 1. Life-history characteristics of European and North American members of the soft coral genus *Alcyonium*. Geographic range: NEAtl, northeast Atlantic; NWAtl, northwest Atlantic; Med, Mediterranean; NEPac, northeast Pacific. Habitat: IR, intertidal, rock; SR, shallow subtidal, rock; SE, shallow subtidal, epifaunal; DS, deep subtidal, soft substrate. Colony morphology: Di, digitate; En, encrusting; Lo, lobate. Colony size: Lg, large (mean colony wet weight > 10 g); Int, intermediate (2–10 g); Sm, small (< 2 g). Sexual expression: G, gonochoric; H, hermaphroditic; P, parthenogenetic. Larval development: Sp, broadcast spawner; Br-I, internal brooder; Br-E, external brooder; ?, character state unknown. Clade: as designated in text and Figure 3.

Species ^a	Geographic range	Habitat	Colony morphology	Colony size	Sexual expression	Larval development	Clade
<i>A. digitatum</i> ^{1,2}	NEAtl	SR	Di	Lg	G	Sp	II
<i>A. glomeratum</i> ³	NEAtl	SR	Di	Lg	G	Sp	IV
<i>A. palmatum</i> ^{4,5}	Med	DS	Di	Int	G	Sp	IV
<i>A. acaule</i> ⁶	Med	SR	Di	Lg	G	Br-E	IV
<i>A. coralloides</i> ⁵	Med,NEAtl	SE	En,Lo	Int	G	Br-I	III
<i>A. rudyi</i> ^{7,8}	NEPac	IR	En	Sm	G	Br-I	I
<i>A. sp. B</i> ⁹	NEPac	IR,SR	En	Sm	G	?	I
<i>A. sp. M2</i> ¹⁰	Med	SR	En,Lo	Sm	?	?	III
<i>A. sp. A3</i> ¹⁰	NEAtl	SR	Lo	Sm	?	Br-I	III
<i>A. sp. C</i> ³	NEPac	DS	Lo	Sm	?	Br-I	II
<i>A. sp. D</i> ⁹	NEPac	SR	En	Sm	?	Br-I	I
<i>A. siderium</i> ^{11,12}	NWAtl	SR	Lo	Int	H	Br-I	II
<i>A. sp. A</i> ³	NEAtl	SR	En	Sm	H ^b	Br-I	II
<i>A. hibernicum</i> ²	NEAtl	SR	Lo	Sm	P ^c	Br-I	III

^a References: (1) Hartnoll 1975; (2) Hartnoll 1977; (3) McFadden, unpubl. data; (4) Frenzel 1937; (5) Lacaze-Duthiers 1900; (6) Garrabou 1999, pers. comm.; (7) McFadden 1991; (8) McFadden 1996; (9) McFadden and Hochberg, unpubl. ms.; (10) McFadden 1999; (11) Sebens 1983a; (12) Sebens 1983b.

^b All colonies brood larvae; allozyme frequencies suggest outcrossing (McFadden, unpubl. data).

^c Selfing hermaphroditism not ruled out (McFadden 1999).

specifically, the range of variation within species is typically less than that observed among species (e.g., McFadden 1999).

Alcyonium colonies typically grow attached to rock substrata (usually vertical walls or overhanging ledges) in shallow areas (0–40 m depth) of moderate to high water movement; the only notable exceptions are *A. palmatum* and *A. sp. C*, which both live in deeper, soft-sediment habitats, attached to hard objects such as bivalve shells. In many places three or four species occur sympatrically, and it is not unusual to find congeners living together in dense, mixed-species assemblages (McFadden 1999).

All *Alcyonium* produce a similar, nonfeeding planula larva, but the mode of development varies among species (Table 1). At least three species broadcast spawn their gametes directly into the water column and the larvae develop planktonically. Most other species brood their embryos internally, releasing fully developed larvae that are competent to settle and metamorphose immediately (e.g., Sebens 1983c). *Alcyonium acaule* broods its embryos externally—fertilized eggs are released to the surface of the female colony, where they remain trapped in mucus during embryonic development (Garrabou 1999). Unlike other invertebrate groups, there is no correlation between mode of development and egg size in alcyonaceans, that is, brooding and broadcast spawning species produce eggs of similar size (Benayahu and Loya 1986; Kruger et al. 1998).

Most of the *Alcyonium* species for which sexuality has been determined are gonochoric (the entire colony produces only ova or testes). *Alcyonium siderium*, however, is a protandrous hermaphrodite (Sebens 1983b), and *A. sp. A* also appears to be hermaphroditic (C. S. McFadden, unpubl. data). *Alcyonium hibernicum* is believed to be a unisexual parthenogen (Hartnoll 1977), although genetic studies do not rule out the possibility that it could be a selfing hermaphrodite with very low sperm production (McFadden 1999). Species that brood in-

ternally include gonochores, hermaphrodites, and the putative parthenogen; in contrast, the broadcast spawning species and external brooder are all gonochores (Table 1).

MATERIALS AND METHODS

Collection of Material

Specimens of *Alcyonium* were obtained between 1989 and 1996 from locations in the north Atlantic, Mediterranean, and northeast Pacific (Table 2). Most species were collected from depths of 5–40 m using scuba; specimens of *A. sp. C* and *A. palmatum* were obtained from depths of about 100 m by dredging, and *A. rudyi* was collected intertidally. As soon as possible following collection, a small amount of polypoid tissue was removed from each colony, blotted to eliminate excess water and mucus, cleaned of attached debris, and frozen in liquid nitrogen. Tissue samples were kept frozen in a –80°C freezer. Samples of *A. palmatum* were preserved in 95% ethanol and stored at 4°C.

Determination of Reproductive and Morphological Characteristics

When possible, we bisected freshly collected colonies and examined them with a hand lens or dissecting microscope to determine reproductive status. Presence of mature ova, testes, or developing planula larvae was recorded and used to infer or confirm mode of development and sexuality. Additional specimens were relaxed in 3.75% MgSO₄, fixed in 10% formalin, and preserved in 70% EtOH. Preserved specimens were cleaned of any adhering debris, blotted dry, and weighed to the nearest 0.01 g. In specimens that had been preserved with polyps fully extended, we used an ocular micrometer to measure the length of the anthocodial column (oral surface of polyp to colony surface, tentacles excluded). We used

TABLE 2. Collection sites of *Alcyonium* species used for DNA sequencing. ATL, Atlantic Ocean; MED, Mediterranean Sea; PAC, Pacific Ocean.

Species	Collection site	Depth	No. of sequences
<i>A. digitatum</i>	Isle of Man (ATL)	10 m	5
	Iles Chausey, France (ATL)	13 m	2
	Iles des Glenans, France (ATL)	10 m	3
<i>A. glomeratum</i>	Trébeurden, France (ATL)	20 m	2
	St. Jean-de-Luz, France (ATL)	25 m	1
<i>A. palmatum</i>	Banyuls-sur-Mer, France (MED)	100 m	4
<i>A. acaule</i>	Marseille, France (MED)	12–25 m	2
	Banyuls-sur-Mer, France (MED)	30 m	2
<i>A. coralloides</i>	Trébeurden, France (ATL)	20 m	2
	Banyuls-sur-Mer, France (MED)	30 m	2
	Marseille, France (MED)	30–45 m	2
<i>A. rudyi</i>	Tatoosh Island, WA, USA (PAC)	intertidal	3
<i>A. sidereum</i>	Nahant, MA, USA (ATL)	6–9 m	2
<i>A. sp. B</i>	San Juan Islands, WA, USA (PAC)	0–20 m	3
<i>A. sp. M2</i>	Marseille, France (MED)	13–25 m	4
<i>A. sp. A3</i>	Sagres, Portugal (ATL)	7–13 m	2
<i>A. sp. C</i>	San Juan Islands, WA, USA (PAC)	110 m	2
<i>A. sp. D</i>	Santa Catalina Island, CA, USA (PAC)	20 m	3
<i>A. sp. A</i>	Isle of Man (ATL)	10 m	5
<i>A. hibernicum</i>	Iles Chausey, France (ATL)	13 m	2

Vernier calipers to measure colony height (h), length (L), and width (w) of the widest part of the colony and length and width of the basal attachment disc. Basal area and cross-sectional area at the widest part of the colony were estimated as ellipses ($A = \pi r_1 r_2$, where $r_1 = L/2$ and $r_2 = w/2$) and averaged to calculate a mean cross-sectional area, A_m . We then estimated colony volume as a cylinder, $V = A_m h$. Volume of *A. coralloides* colonies, which encrust dead gorgonian corals, was determined by measuring total volumetric displacement in water and subtracting the volume of the underlying gorgonian skeleton (estimated from the diameter and length of the cylindrical branches).

DNA Extraction, Polymerase-Chain-Reaction Amplification, and Sequencing

DNA was extracted from frozen or ethanol-preserved tissue using the method of Coffroth et al. (1992). We modified this procedure by grinding an approximately 1-cm³ piece of polypoid tissue to a fine powder in liquid nitrogen before adding it to cold 2× CTAB buffer and by extracting samples twice with an equal volume of chloroform:isoamyl alcohol (24:1) prior to extraction with phenol:chloroform:isoamyl alcohol (25:24:1). DNA concentration was quantified using a TKO 100 fluorometer (Hoefer Scientific Instruments, San Francisco, CA.).

An approximately 1050-bp region of the nuclear ribosomal gene complex spanning the 3' end of the 18S subunit, internal transcribed spacer 1 (ITS-1), 5.8S subunit, ITS-2, and the 5' end of the 28S subunit was amplified with a polymerase chain reaction (PCR) using primers 1s (5'-GGTACCCTTTGTACACACCGCCCGTCGCT-3') and 2ss (5'-GCTTTGGGCTGCA-GTCCCAAGCAACCCGACTC-3') of Chen et al. (1996). Although they typically exhibit low levels of intraspecific polymorphism, the two ITS regions have been shown to be useful for resolving intrageneric relationships in other cnidarians (Chen and Miller 1996; Chen et al. 1996; Odorico and Miller 1997a,b).

For each 50-μL PCR we used 10–50 ng DNA, 1.25 units Taq DNA polymerase (Gibco BRL, Life Technologies, Rockville, MD), 5 μL 10× PCR buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 2 mM MgCl₂, 2 mM each dNTP, and 25 pmol each primer. The amplification conditions consisted of a hotstart (DNA and water incubated at 94°C for 4 min prior to addition of the remaining reaction ingredients) followed by 30 cycles of 94°C for 30 sec, 52°C for 60 sec, and 72°C for 90 sec and a final extension step at 72°C for 5 min. PCR products that yielded fragments of the expected size were purified by centrifugation using Ultrafree[®]-MC Millipore Corp. (Bedford, MA) 100,000 NMWL filtration units, and 200–225 ng was then used in a cycle sequencing reaction (ABI PRISM[™] Dye Terminator Cycle Sequencing Ready Reaction Kit, Perkin Elmer, Foster City, CA), following the manufacturer's recommended protocol. The products were sequenced using an ABI 373A automated DNA sequencer.

The ITS-1 region was sequenced in both directions using the universal primer pair ITS-2 (5'-GCTACGTTCTTCATCG-ATGC-3') and ITS-5 (5'-GGAAGGAGAAGTCGTAAC-AAGG-3'; White et al. 1990); the ITS-2 region was sequenced using primer 5s (5'-AAGTAGTGTGAATTGCAG-3'; ten Lohuis 1992) in the forward direction and 2w (5'-ATTGCCACGT-ACGGGGTTGTC-3') in the reverse direction. We obtained sequences from a minimum of two individuals per species (Table 2); consensus sequences have been deposited in GenBank under accession numbers AF262337–AF262355. A sequence for one member of a related alcyonacean genus, *Dendronephthya* sp., was obtained from GenBank (Odorico and Miller 1997a).

Sequence Alignment

Sequences were aligned using ClustalW version 1.4 (Thompson et al. 1994). The sequences we obtained ranged from 842 bp to 928 bp. All sequences included 114 bp of the 3' terminus of the 18S, the entire 157 bp of 5.8S, and 194 bp of the 5' end of the 28S coding regions; these regions

TABLE 3. Variation in length and base composition of ribosomal internal transcribed spacer regions (ITS-1, ITS-2) among soft coral species in the genus *Alcyonium*. *Dendronephthya* sp. included as an outgroup.

Abbr.	Species	ITS-1		ITS-2	
		Length (bp)	% (G + C) ¹	Length (bp)	% (G + C) ¹
(Aa)	<i>A. acaule</i>	232	42.2	169	53.9
(Ag)	<i>A. glomeratum</i>	228	43.4	186	50.5
(Ap)	<i>A. palmatum</i>	228	43.4	186	50.5
(Ac)	<i>A. coralloides</i>	197–199	44.4	163–167	47.0
(AcA)	<i>A. coralloides</i> (ATL)	196	43.9	163	46.6
(Ah)	<i>A. hibernicum</i>	209	39.2	171	48.0
(M2)	<i>A. sp. M2</i>	209	39.7	171	47.4
(A3)	<i>A. sp. A3</i>	207	40.6	163	44.8
(Ad)	<i>A. digitatum</i>	185–188	39.4	198	53.0
(spA)	<i>A. sp. A</i>	185–193	39.4	198	53.0
(As)	<i>A. siderium</i>	185	40.0	200	52.5
(spC)	<i>A. sp. C</i>	213	40.4	203	53.7
(Ar)	<i>A. rudyi</i>	229	41.9	174	50.6
(spB)	<i>A. sp. B</i>	244	43.4	192	50.5
(spD)	<i>A. sp. D</i>	244	44.7	193–196	53.4
(Den)	<i>Dendronephthya</i> sp.	216	46.3	198	58.6

¹ Average G + C content if more than one sequence was detected for a species.

could be aligned unambiguously. Both of the ITS regions, however, varied greatly in length among species (ITS-1: 185–244 bp; ITS-2: 163–200 bp; Table 3), resulting in considerable alignment ambiguity. In addition to using ClustalW's default gap weighting parameters (gap opening penalty [GOP] = 10.0; gap extension penalty [GEP] = 5.0), we generated nine alternative alignments of the ITS-1 and ITS-2 regions using all possible combinations of three GOP values (\log_2 GOP = 0, 2, 5) and three GEP values (\log_2 GEP = -4, 0, 2). These values were chosen as representative of the range shown by Morrison and Ellis (1997) to affect alignment length and, subsequently, inferred tree topology. Lengths of the alternative alignments ranged from 939 bp to 1100 bp.

TABLE 4. Mean colony size (\pm SD) of European and North American *Alcyonium* species. Biomass is blotted wet weight; height is measured for contracted colonies; volume and polyp length estimated as described in text. *N*, number of colonies measured; ?, data unavailable.

Species	<i>N</i>	Mean volume (cm ³)	Max. volume (cm ³)	Mean biomass (g)	Max. biomass (g)	Mean height (cm)	Polyp length (mm)
<i>A. digitatum</i>	46	?	?	43.1 \pm 47.4	236.1	?	6.8 \pm 1.6
<i>A. glomeratum</i>	6	43.2 \pm 52.2	156.1	18.0 \pm 14.5	45.3	5.5 \pm 1.5	3.8 \pm 0.4
<i>A. palmatum</i>	32	18.1 \pm 24.0	119.6	7.6 \pm 9.3	43.7	6.3 \pm 3.2	3.0 \pm 0.5
<i>A. acaule</i>	5	65.1 \pm 26.1	98.6	32.2 \pm 15.1	52.8	6.4 \pm 0.9	2.8 \pm 0.4
<i>A. coralloides</i> ¹	25	2.9 \pm 2.9	9.4	3.8 \pm 3.3	11.3	0.4 \pm 0.1	1.6 \pm 0.3
<i>A. rudyi</i>	23	0.07 \pm 0.02	0.13	0.05 \pm 0.02	0.10	0.2 \pm 0.04	1.1 \pm 0.2
<i>A. sp. B</i>	13	0.5 \pm 0.3	1.0	0.5 \pm 0.0	0.5	0.3 \pm 0.0	1.5 \pm 0.3
<i>A. sp. M2</i>	33	0.6 \pm 1.0	4.4	0.4 \pm 0.4	1.6	1.2 \pm 0.4	?
<i>A. sp. A3</i>	11	1.1 \pm 1.8	6.7	0.7 \pm 1.1	3.7	1.3 \pm 0.3	?
<i>A. sp. C</i>	14	1.0 \pm 0.9	3.2	0.5 \pm 0.4	1.2	1.1 \pm 0.5	1.8 \pm 0.3
<i>A. sp. D</i>	6	1.3 \pm 0.7	2.3	0.7 \pm 0.4	1.2	0.7 \pm 0.2	?
<i>A. siderium</i> ²	51	4.8	?	6.7	?	5.6 \pm 2.3	7.2 \pm 1.2 ³
<i>A. sp. nov. A</i>	53	0.6 \pm 0.6	2.8	0.4 \pm 0.4	1.7	0.5 \pm 0.2	2.5 \pm 0.8
<i>A. hibernicum</i>	82	1.9 \pm 2.3	15.3	1.4 \pm 1.8	10.6	1.5 \pm 0.8	2.7 \pm 0.5

¹ Mean colony height for *A. coralloides* is the height of the thickest raised area in a colony (see McFadden 1999).

² Biomass and volume from Sebens (1984), Halfway Rock population; polyp length from Sebens and Koehl (1984).

³ Polyp lengths measured from photographs of live specimens in the field; may not be comparable to measurements for other species, which were taken from preserved specimens.

Phylogenetic Analysis

We used the maximum likelihood (DNAML, with global rearrangement option) and neighbor-joining (DNADIST; NEIGHBOR) methods in PHYLIP version 3.5 (Felsenstein 1995) as well as maximum parsimony (PAUP* version 4.0; Swofford 1999) to generate phylogenies from each of the 10 alternative alignments of the complete sequence. For maximum parsimony we did bootstrap analyses (100 or 1000 replicates) using the heuristic-search option with simple addition of sequences and TBR branch swapping. Genetic distances used for the neighbor-joining analyses were calculated using Kimura's (1980) two-parameter model of nucleotide substitution with a transition:transversion ratio of 2.0; use of either Jukes and Cantor's (1969) model or Felsenstein's (1981) maximum-likelihood model did not alter tree topology. For all neighbor-joining analyses the dataset was resampled 100 or 1000 times using a nonparametric bootstrap (SEQBOOT). For the maximum-likelihood analysis, data were bootstrapped 100 times for one representative alignment (alignment parameters: \log_2 GOP = 2, \log_2 GEP = 0). This alignment was also used to construct phylogenies from the combined ITS regions (549 bp) and the combined 18S, 5.8S, and 28S regions (466 bp) alone. Gap sites were treated as missing in the maximum-likelihood and neighbor-joining analyses; treatment of gaps as fifth characters in the maximum-parsimony analysis did not alter tree topology. *Dendronephthya* sp., the only alcyonacean sequence outside of the genus *Alcyonium* that was available, was specified as the outgroup root for all trees.

RESULTS

Colony Size and Morphology

The average size of reproductively mature *Alcyonium* colonies varied over three orders of magnitude (Table 4). The smallest species, *A. rudyi*, had a mean colony volume of 0.07 cm³ and mean wet weight of 0.05 g, whereas the largest, *A.*

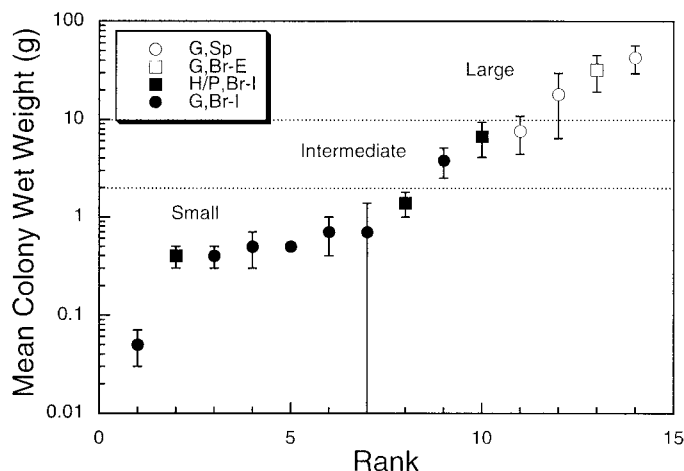


FIG. 1. Relationship between colony size (mean wet weight) and reproductive mode in *Alcyonium*. Species are ranked from smallest (1) to largest (14). Dotted lines separate size classes: large species, >10 g; intermediate species, 2–10 g; small species, <2 g. Colony volume, thickness, and anthocodial length are all significantly positively correlated with wet weight and show a similar pattern. G,Sp (open circle), gonochoric broadcast spawner; G,Br-E (open square), gonochoric external brooder; H/P,Br-I (closed square), hermaphroditic or parthenogenetic internal brooder; G,Br-I (closed circle), gonochoric internal brooder. Error bars are 95% confidence limits of the mean.

digitatum, averaged 43.1 g wet weight and has been reported to grow to 1 kg (Hartnoll 1977). Three species had mean colony volumes > 20 cm³ and mean wet weights > 10 g (large species), three species had mean volumes 3–20 cm³ and mean wet weights 2–10 g (intermediate species), and the remaining eight species averaged less than 3 cm³ and 2 g (small species; Table 4). Because these species are not phylogenetically independent of one another, a statistical test for significant differences among size classes was not done. The 95% confidence intervals for mean wet weight of small species, however, do not overlap those of intermediate or large species (Fig. 1), and the only large species whose lower 95% confidence limit overlaps that of an intermediate species is *A. glomeratum*, for which sample size was very small. The three large species include two broadcast spawners and the external brooder, whereas all of the small species brood their embryos internally (Fig. 1); the species of intermediate size include a broadcast spawner and two internal brooders.

Log mean colony volume and log mean wet weight were significantly correlated ($r = 0.99$, $P < 0.05$), and there was also a significant positive relationship between log mean wet weight and log polyp (anthocodia) length ($r = 0.65$, $P < 0.05$; Fig. 2). Log colony height (a maximum estimate of the length of the gastrovascular cavities within which embryos are brooded) was also significantly positively correlated with both log wet weight ($r = 0.86$, $P < 0.05$; Fig. 2) and log polyp length ($r = 0.69$, $P < 0.05$).

Sequence Variation and Genetic Distances among Taxa

The 18S, 5.8S, and 28S regions varied little among *Alcyonium* species. Six of 114 nucleotide sites varied in the 18S region, five of 157 in the 5.8S region, and 13 of 194 in the 28S region. Genetic distances among species ranged from

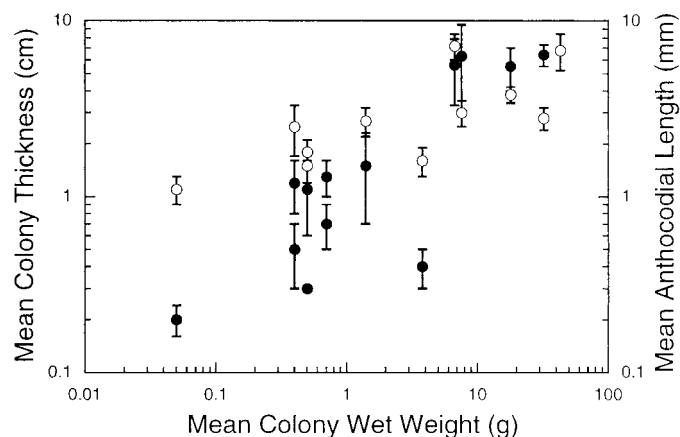


FIG. 2. Relationship between mean colony height or thickness (open symbols), anthocodial length (closed symbols), and colony wet weight in soft corals of the genus *Alcyonium*. The anthocodia is the extensible region of a polyp, and colony thickness is a measure of the length of the nonextensible gastrovascular region. Both measures of polyp length are significantly correlated with colony wet weight ($P < 0.05$). Error bars are standard deviations.

0% to 3.6% for these relatively invariant regions (Table 5). Both ITS regions, however, varied greatly among species (Table 3). Some pairs of species differed by as much as 48% (excluding gap regions), whereas others exhibited almost no sequence divergence (< 1%; Table 5). For instance, *A. hibernicum* and *A. sp. M2* differed by only a single substitution within each ITS region, and *A. glomeratum* and *A. palmatum* by only two nucleotide substitutions in each region. There was also <1.0% sequence divergence among *A. digitatum*, *A. siderium*, and *A. sp. A*: *A. digitatum* and *A. sp. A* had identical ITS-2 sequences, whereas *A. siderium* differed from them only by a 2-bp indel, and some (but not all, see below) individuals of *A. digitatum* and *A. sp. A* shared the same ITS-1 sequence as *A. siderium*.

Several species exhibited sequence polymorphism in the ITS regions. A 3-bp insertion in ITS-2 was found in one of three *A. sp. D* individuals. Mediterranean populations of *A. coralloides* were polymorphic for two distinct sequences that differed by two short indels (3 bp, 1 bp) plus two substitutions in ITS-1 and two substitutions in ITS-2. Although these differences are greater than those found among some species pairs (e.g., *A. acaule* and *A. glomeratum*), allozyme analysis suggests that all of the *A. coralloides* individuals sequenced belong to the same gene pool (McFadden 1999).

Because the rRNA genes are multicopy, it is possible for an individual to have copies of more than one sequence (Caranza et al. 1996; Odorico and Miller 1997b). Four of 10 individuals of *A. digitatum* exhibited intraindividual polymorphism for a 3-bp insertion with two flanking substitutions in the ITS-1 region. Two of five individuals of *A. sp. A* had an ITS-1 sequence that was identical to the shorter of the two *A. digitatum* sequences, whereas the other three individuals of *A. sp. A* had an ITS-1 that was 8 bp longer due to three 2–3-bp insertions. All of the unique sequences found in species that exhibited either intra- or interindividual polymorphisms were included in the phylogeny (Fig. 3).

TABLE 5. Genetic distances among *Alcyonium* species (Kimura two-parameter method). Distances calculated for combined ITS-1 and ITS-2 DNA sequences are above the diagonal; distances for combined partial 18S, 5.8S, and partial 28S sequences are below the diagonal. ITS sequences are aligned as in Figure 3. Species abbreviations are defined in Table 3.

Sp.	Aa	Ag	Ap	Ac1	Ac2	AcA	Ah	M2	A3	Ad1	Ad2	spA1	spA2	As	Ar	spB	spD1	spD2	spC	Den
Aa		.039	.036	.254	.247	.245	.280	.280	.251	.287	.295	.296	.289	.294	.349	.339	.337	.348	.311	.473
Ag	.002		.007	.266	.260	.258	.279	.279	.258	.274	.273	.279	.276	.281	.350	.321	.320	.330	.318	.470
Ap	.000	.002		.266	.260	.258	.284	.284	.258	.274	.273	.279	.276	.281	.342	.309	.308	.318	.311	.451
Ac1	.015	.020	.018		.011	.011	.082	.076	.057	.299	.299	.305	.301	.297	.399	.389	.398	.410	.287	.473
Ac2	.015	.020	.018	.000		.000	.070	.064	.051	.283	.284	.286	.286	.282	.386	.371	.380	.392	.281	.469
AcA	.015	.020	.018	.000	.000		.070	.064	.051	.283	.284	.286	.286	.282	.382	.367	.376	.388	.281	.465
Ah	.011	.015	.013	.007	.007	.007		.005	.030	.272	.276	.278	.274	.271	.405	.390	.404	.416	.289	.483
M2	.011	.015	.013	.007	.007	.007	.000		.030	.272	.276	.278	.274	.271	.395	.390	.404	.416	.289	.473
A3	.015	.020	.018	.009	.009	.009	.007	.007		.268	.272	.269	.269	.267	.367	.376	.390	.402	.261	.475
Ad1	.009	.011	.009	.024	.024	.024	.020	.020	.020		.008	.008	.000	.000	.359	.361	.367	.373	.091	.445
Ad2	.009	.011	.009	.024	.024	.024	.020	.020	.020	.000		.005	.008	.008	.362	.364	.369	.375	.099	.438
spA1	.009	.011	.009	.024	.024	.024	.020	.020	.020	.000	.000		.008	.008	.364	.370	.376	.382	.099	.452
spA2	.009	.011	.009	.024	.024	.024	.020	.020	.020	.000	.000	.000		.000	.356	.364	.370	.376	.091	.443
As	.009	.011	.009	.024	.024	.024	.020	.020	.020	.000	.000	.000	.000		.362	.363	.369	.375	.093	.441
Ar	.024	.026	.024	.033	.033	.033	.033	.033	.033	.024	.024	.024	.024	.024		1.94	.193	.193	.390	.477
spB	.020	.018	.020	.026	.026	.026	.027	.026	.031	.024	.024	.024	.024	.024	.018		.036	.036	.372	.443
spD1	.020	.018	.020	.029	.029	.029	.029	.029	.033	.024	.024	.024	.024	.024	.017	.000		.000	.368	.445
spD2	.020	.018	.020	.029	.029	.029	.029	.029	.033	.024	.024	.024	.024	.024	.017	.000	.000		.373	.450
spC	.013	.013	.013	.027	.027	.027	.022	.022	.022	.009	.009	.009	.009	.009	.002	.020	.020	.020		.462
Den	.014	.026	.024	.031	.031	.031	.036	.035	.036	.029	.029	.029	.029	.029	.026	.018	.017	.017	.024	

Phylogenetic Relationships

All of the phylogenetic analyses divided the genus into four distinct clades (Fig. 3). Although the inferred relationship of these clades to one another and the relationships of the species within each clade varied slightly depending on the alignment parameters and phylogenetic method used, clade membership did not vary among analyses, and the bootstrap values on the basal nodes of each of the four clades were >95% in all but one case. Within each of the clades genetic distances among species were low. The combined 18S, 5.8S, and 28S regions differed by <1% among members of the same clade, and by 1.0–3.3% among clades. The ITS regions varied < 10% within clades, with the exception of the clade containing *A. rudyi*, which differed from its closest sister taxa by >19% (Table 5). In contrast, sequence differences among members of different clades ranged from 25% to 42% for the ITS regions. This wide range of ITS sequence divergence is comparable to that reported among species in several other anthozoan genera (Chen et al. 1996; Odorico and Miller 1997b).

Clade I includes three of the four northeast Pacific species, all of which form small, encrusting colonies. *Alcyonium rudyi* is a gonochoric brooder. *Alcyonium* sp. B is also gonochoric, but its mode of development remains unknown; *A. sp. D* is an internal brooder whose sexual expression is unknown. All but two of the analyses positioned clade I basal to the monophyletic group comprising the other three *Alcyonium* clades; recently obtained mitochondrial gene sequences also support this topology (C. S. McFadden, unpubl. data).

Clade II comprises three north Atlantic species, *A. digitatum*, *A. siderium*, and *A. sp. A*, as well as the northeast Pacific *A. sp. C*. *Alcyonium digitatum* is a large, gonochoric broadcast spawner; *A. siderium* and *A. sp. A* are both hermaphroditic brooders of intermediate and small size, respectively; and *A. sp. C* is a small brooder whose sexual expression is not known. Whereas all analyses clearly placed *A. sp.*

C basal to the three Atlantic species, the relationship among the latter cannot be resolved. All three species had identical 18S, 5.8S, and 28S sequences, and <1% sequence divergence in the ITS regions (Table 5). Moreover, one of the two ITS-1 variants found in *A. digitatum* is identical to one of two *A. sp. A* sequences and to *A. siderium*; the other two ITS-1 sequences found in this clade (*A. digitatum*-2, *A. sp. A*-1) are more similar to one another than to the other shared variant (Fig. 3), suggesting that the polymorphism predates speciation.

Clade III includes four northeast Atlantic and Mediterranean species that were previously considered to belong to one highly variable species, but have recently been separated based on allozyme and morphological differences (McFadden 1999). All four species form encrusting or lobate colonies of either intermediate or small size, and three of four are known to brood their larvae. *Alcyonium coralloides* is gonochoric, *A. hibernicum* is putatively parthenogenetic, and sexual expression is not known for the other two species.

Clade IV comprises two Mediterranean species, *A. acaule* and *A. palmatum*, and the northeast Atlantic *A. glomeratum*. All three are gonochoric species of intermediate to large size. *Alcyonium palmatum* and *A. glomeratum* both broadcast spawn, whereas *A. acaule* broods its embryos externally. All of the analyses support a very close relationship between *A. glomeratum* and *A. palmatum* and position *A. acaule* basally.

The only major feature of the inferred phylogeny that varied among analyses was the relationship among the four clades. For all three phylogenetic methods, eight of 10 alternative alignments of the full sequence as well as the analyses of the ITS regions alone and the combined 18S, 5.8S, and 28S regions produced the majority-rule consensus tree depicted in Figure 3. In this phylogeny clades II, III, and IV form a monophyletic group, with clades III and IV as sister taxa; clade I is basal to the other three clades. Bootstrap support for the node uniting clades II, III, and IV was gen-

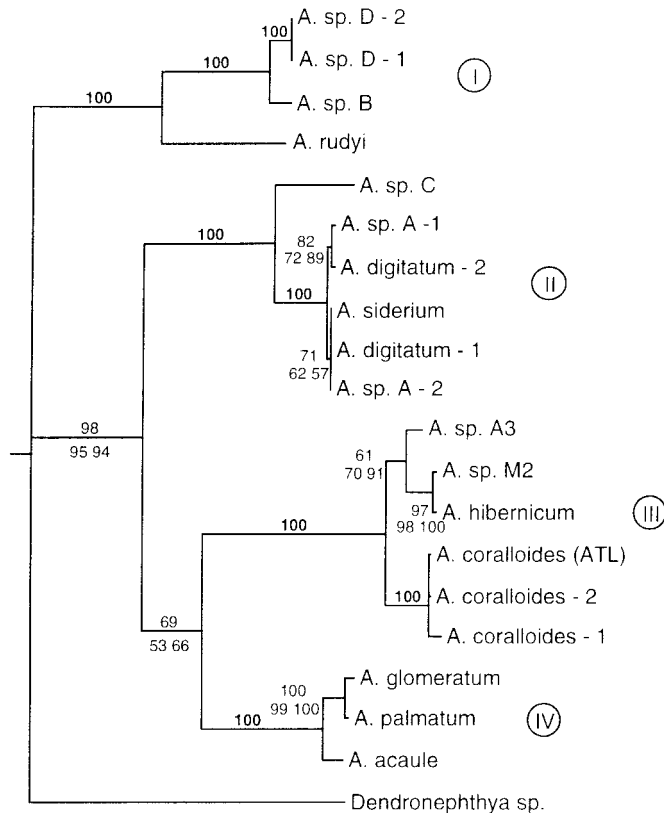


FIG. 3. Phylogeny of the European and North American members of the soft coral genus *Alcyonium* based on ~900-bp nuclear ribosomal sequence spanning both internal transcribed spacer regions. Circled roman numerals identify the four clades discussed in the text. The topology and branch lengths shown are based on maximum-likelihood analysis of one of 10 alternative sequence alignments; this topology is identical to that of the majority-rule consensus tree. Numbers above each node are nonparametric bootstrap percentages (100 replicates) for the maximum-likelihood analysis; numbers below nodes are bootstrap percentages (1000 replicates) for maximum-parsimony (left) and neighbor-joining (right) analyses of the same alignment. A single value shown in boldface indicates that all three methods yielded a bootstrap value of 100%. *Dendronephthya* sp. was specified as the outgroup.

erally high (> 75% in eight of 10 neighbor-joining trees and six of 10 maximum-parsimony trees). Support for the node uniting clades III and IV was weak to moderate in most of the analyses, however, exceeding 70% in only four neighbor-joining trees and six parsimony trees. Because the evolutionary steps required to explain the distribution of life-history traits within the genus are the same for all three possible relationships among clades II, III, and IV, the conclusions reached below are unaffected by the uncertainty in this node.

Evolution of Life-History Traits

The phylogenetic reconstruction indicates that an association between large colony size and broadcast spawning or external brooding occurs in two separate clades within the genus, clades II (*A. digitatum*) and IV (*A. glomeratum*, *A. acaule*; Fig. 4a). Because the true outgroup of the genus is unknown and taxon sampling is incomplete, we cannot reliably infer from these data whether this association of char-

acter states is ancestral or derived within either clade. Within the tree shown in Figure 4, however, the evolution of large, broadcast spawners and external brooders from small, internal brooders is a more parsimonious explanation for the observed distribution of traits, requiring a minimum of three evolutionary steps. A minimum of five steps is required to explain the trait distribution if broadcast spawning and large size are assumed to be the plesiomorphic character states within the genus. Inclusion of additional taxa within the tree could, however, alter this interpretation—because broadcast spawning is the most common mode of reproduction among other members of the family Alcyoniidae (Alino and Coll 1989; Benayahu et al. 1990), we might expect it to be the true ancestral state in the genus *Alcyonium*. Moreover, in most other invertebrate groups broadcast spawning is ancestral and the evolution of brooding frequently represents an irreversible character state change (Strathmann 1978; Reid 1990; Lieberman et al. 1993; Strathmann and Eernisse 1994; Wray 1996; Hart et al. 1997; but see Rouse and Fitzhugh 1994). Ignorant of the correct polarity of character state change, we can conclude nonetheless that either an association between broadcast spawning and large colony size or one between internal brooding and small colony size has arisen independently more than once within the genus *Alcyonium*.

The predominance of gonochorism among alcyonaceans (Alino and Coll 1989; Benayahu et al. 1990; Benayahu 1991) suggests that it is probably the plesiomorphic character state within the genus *Alcyonium*; the most parsimonious explanation for the distribution of sexuality in the phylogeny shown in Figure 4b also requires gonochorism to be ancestral (although only one additional evolutionary step is required to explain the tree if gonochorism is a derived state). Assuming gonochorism is plesiomorphic, the phylogeny suggests that the trait has been lost independently at least twice within *Alcyonium*, once in the parthenogenetic *A. hibernicum* found in clade III and at least once in clade II, which includes the hermaphrodites *A. siderium* and *A. sp. A* (Fig. 4b). Because the relationship among the latter two species and *A. digitatum* remains unresolved, we cannot distinguish among three possibilities: (1) hermaphroditism evolved twice independently, once in *A. siderium* and once in *A. sp. A*; (2) hermaphroditism evolved once in the ancestor of *A. siderium* and *A. sp. A*, after it diverged from *A. digitatum*; (3) hermaphroditism evolved once in the ancestor of all three species, but *A. digitatum* has subsequently reverted to gonochorism. If *A. siderium* and *A. sp. A* are sister taxa, explanation 2 is the most parsimonious.

The phylogeny provides only weak support for an ordered transition series from gonochoric brooder to brooding hermaphrodite or parthenogen. Regardless of the true ancestral state of the genus, the parthenogenetic *A. hibernicum* has evolved within a clade in which brooding appears to be plesiomorphic (Fig. 4b). If the ancestral state of the genus is broadcast spawning, brooding and broadcasting are equally parsimonious ancestral states for the clade containing the hermaphrodites *A. siderium* and *A. sp. A*; whether these species evolved from a gonochoric ancestor also remains uncertain.

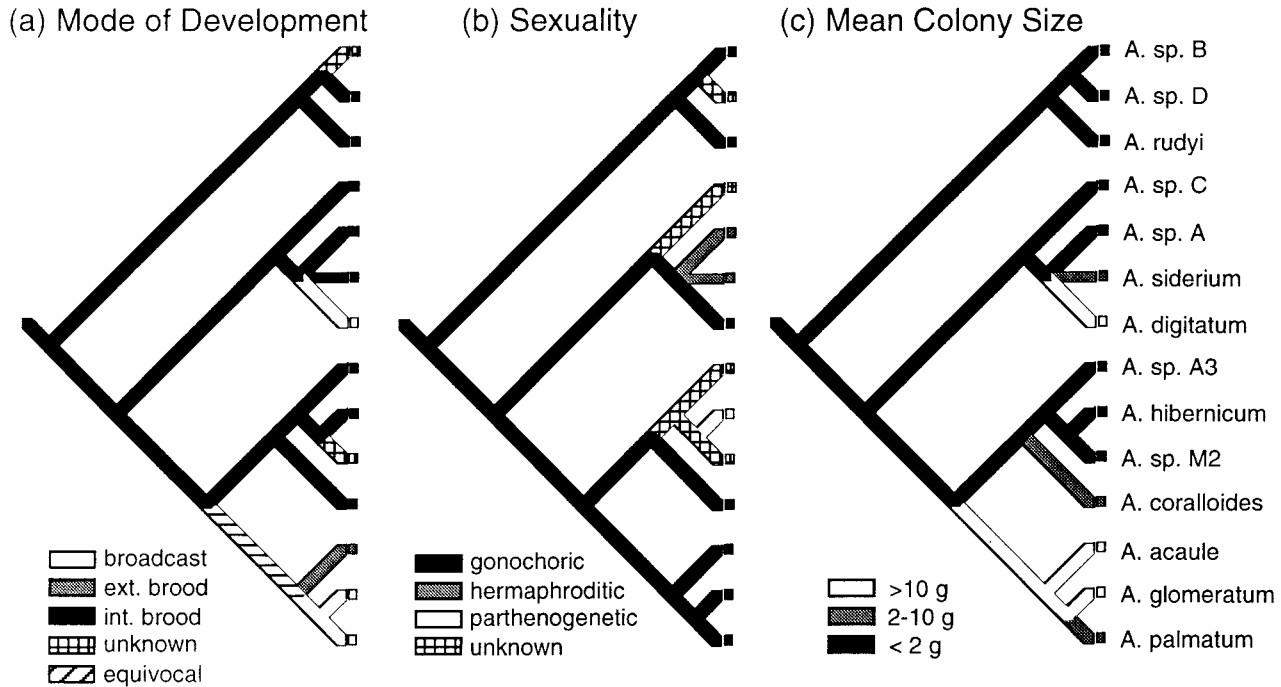


FIG. 4. Phylogenetic distribution of reproductive traits and colony size in the soft coral genus *Alcyonium*: (a) mode of larval development; (b) sexual expression; (c) mean colony wet weight. The phylogeny is that shown in Figure 3 with duplicate sequences for some species eliminated and the unresolved relationship among *A. digitatum*, *A. siderium*, and *A. sp. A* reduced to a trichotomy.

DISCUSSION

Phylogenetic Distribution of Life-History Traits

In three of the four *Alcyonium* clades studied here, the distribution of life-history traits appears to be phylogenetically conservative. All of the species in clades I and III for which information is currently available are gonochoric, brood internally, and have small to intermediate body size, with the exception of the parthenogenetic *A. hibernicum* that appears on a terminal branch in clade III. Much remains to be learned about the reproduction of species in these two clades, however, and it is possible that other life-history patterns will be found. Clade IV, in contrast, consists solely of intermediate to large, gonochoric broadcast spawners and the externally brooding *A. acaule*. Although the life-history patterns observed within each of these three clades are consistent with adaptive explanations for the association of traits (e.g., small size and brooding, large size and broadcast spawning), they could also be the result of phylogenetic conservation of traits within each clade.

The distribution of traits within clade II, however, argues more strongly for adaptive explanations of life-history evolution in *Alcyonium*. Almost the complete range of life-history variation documented within the genus occurs among the three terminal taxa in this clade. *Alcyonium digitatum* is a gonochoric broadcast spawner that grows to large colony size; *A. siderium* is an internally brooding hermaphrodite of intermediate size; and *A. sp. A* is a small, internally brooding hermaphrodite. In the same clade and basal to these three species is *A. sp. C*, a small, internal brooder whose mode of sexual expression has not yet been determined.

Alcyonium digitatum, *A. siderium*, and *A. sp. A* appear to

have diverged from one another very recently. Schlötterer et al. (1994) have estimated the rate of substitution in the ITS regions to be 1.1–1.2% per million years in *Drosophila*, whereas Chen and Miller (1996) suggest that anthozoan ITS sequences may evolve even faster. If *Alcyonium* and *Drosophila* ITS regions evolve at similar rates (an untested assumption), the 0.5–0.8% sequence differences observed among these three species (Table 5) suggest they have speciated within the last 400,000–700,000 years. Pairwise genetic distances (Nei's [1978] unbiased *D*) calculated from allozyme data are also less among these three species than among pairs of sister species in any of the other three clades (C. S. McFadden, unpubl. data). Finally, all three species are extremely similar morphologically (Verseveldt 1973; C. S. McFadden, unpubl. data), differing primarily only in mean colony size and life history.

Because the multicopy nuclear RNA genes evolve by concerted evolution, gene homogenization should occur rapidly (Hillis and Davis 1988), and the presence of a shared polymorphism in the ITS-1 of *A. digitatum* and *A. sp. A* also supports recent divergence of these two species. An alternative explanation for the shared ITS sequences, however, is hybridization—indeed, allozyme and morphological data suggest that these two species may occasionally hybridize despite their different life histories (C. S. McFadden, unpubl. data).

The occurrence of different suites of life-history traits in recently diverged taxa suggests that these traits can evolve rapidly and, perhaps, reversibly in *Alcyonium*. Unlike other invertebrate groups, in which the evolution of brooded larvae from planktonic forms often involves a loss of complex structures (for feeding or swimming) that makes evolutionary re-

versals unlikely (Strathmann 1978; Wray 1996), the larvae of most anthozoans are very similar morphologically regardless of whether they develop internally or in the plankton (Harrison and Wallace 1990). In both brooding and broadcast spawning alcyonaceans, the gametes develop on mesenteries within the gastrovascular cavities of the polyps (Benayahu and Loya 1983, 1984b). In broadcast spawners the mature ova are released from the mesenteries into the main polyp cavity and subsequently exit the polyp via the mouth; usually, the ova are fertilized after they have entered the water column (Hartnoll 1975; Benayahu and Loya 1986). In internally brooding species, the mature ova are retained within the polyp cavity, and it is there that fertilization and embryonic development take place (Lacaze-Duthiers 1900; Feldman 1976; Benayahu et al. 1989); presumably sperm enter the polyp cavity through the mouth (Fautin et al. 1989). Although one family of alcyonaceans has evolved specialized brood chambers within the polyp cavities (Benayahu 1991), the alcyoniids have not. The transition from broadcast spawning to brooding (and vice versa) can therefore be accomplished solely by a shift in the timing of release of ova (or embryos) from the polyp cavity and an accompanying change in the site of fertilization.

External brooding may represent an intermediate transitional stage between internal brooding and broadcast spawning. In externally brooding alcyonaceans, fertilization may occur within the polyp cavity, followed by expulsion of the developing embryos onto the colony surface (Benayahu and Loya 1983; Benayahu et al. 1989) or ova may be fertilized externally, after they are on the colony surface (Farrant 1986; Benayahu 1989). In some species, such as *A. acaule* and *A. (= Parerythropodium) fulvum fulvum*, the embryos remain attached to the colony by mucus (Benayahu and Loya 1983; Garrabou 1999), whereas other external brooders have no special mechanism for embryo retention (Alino and Coll 1989).

Similarly, the evolutionary transition between gonochorism and hermaphroditism in alcyonaceans does not require the loss or gain of any specialized organs or structures. Sex determination in alcyonaceans is unknown, but in some other cnidarians it appears to have an epigenetic rather than a chromosomal basis (Fautin 1992). Male and female gametes develop in similar positions on the gastrovascular mesenteries, and in at least some hermaphrodites male and female gametes develop within the same mesentery (Hartnoll 1975; Benayahu et al. 1989). Low levels of hermaphroditism are not unusual in gonochoric alcyonaceans (Benayahu and Loya 1984a; Farrant 1985; Kruger et al. 1998). Lacaze-Duthiers (1900) states that hermaphrodites are of relatively frequent occurrence in the gonochoric *A. coralloides*, and Hartnoll (1975) reported a 0.5% frequency of hermaphroditism in *A. digitatum*. We found one hermaphrodite (0.3%) among 333 sexually mature individuals of *A. digitatum* and one (0.4%) among 231 reproductive *A. rudyi* sampled during this and related studies. This observation of naturally occurring (albeit rare) hermaphrodites in gonochoric populations suggests that the trait is already present in some *Alcyonium* populations, and, assuming it is genetically based, available to increase in frequency should conditions favor hermaphroditism (for in-

stance when population densities are low and mates are scarce; Ghiselin 1987).

Adaptive Explanations for Mode of Development

The phylogenetic distribution of reproductive traits and the relative ease with which transitions between different modes of larval development and sexuality should be able to occur suggest that the variation observed within the genus *Alcyonium* is likely to be adaptive. There do not, however, appear to be any obvious associations between habitat or other environmental variables and *Alcyonium* reproductive traits. Species with very different life histories can be found living side by side in a variety of locations. For instance, along the Atlantic coast of France *A. digitatum*, *A. glomeratum* (both gonochoric broadcast spawners), *A. coralloides* (gonochoric brooder), and *A. hibernicum* (parthenogenetic brooder) can all be found living in close contact with one another; *A. digitatum*, *A. hibernicum*, and *A. sp. A* (hermaphroditic brooder) occur syntopically in the Isle of Man; and throughout much of the western basin of the Mediterranean, *A. coralloides* co-occurs with *A. acaule* (gonochoric external brooder; McFadden 1999). If either sexuality or mode of development were selected for by some aspect of the present-day physical environment, we might expect co-occurring species to exhibit similar rather than divergent life-history patterns. We cannot, however, rule out the possibility that reproductive traits evolved in response to past environmental conditions and at times when currently sympatric species may have been geographically isolated.

Conversely, if either sexuality or mode of development are adaptations to the present physical environment, we might expect the reproductive traits of species living in different habitats to differ. This contrast is difficult to make for *Alcyonium* because the majority of species examined here occupy the same substrate types (overhanging or vertical rock surfaces) at similar depths and in similar flow environments. *Alcyonium palmatum* and *A. sp. C* both live attached to bivalve shells in deeper, soft substrate habitats, yet they differ from one another in colony size and mode of development: *A. palmatum* is a broadcast spawner of intermediate size, whereas *A. sp. C* is a small brooder. The two species with intertidal distributions (*A. rudyi* and *A. sp. B*) are both small brooders, like the majority of subtidal species examined.

Although there appears to be no association between present physical environment and life history, it is possible that biotic factors such as interspecific competition may select for divergence of reproductive and size characters in sympatric species. For instance, Hartnoll (1977) suggested that the small, brooding *A. hibernicum* and the large, broadcast spawning *A. digitatum* have evolved those traits to minimize interspecific competition. Differences in timing of reproduction and larval dispersal ability (a direct consequence of reproductive mode) may allow otherwise similar species to coexist by colonizing seasonally available microhabitats or substrate patches that differ in age or successional state (e.g., Sutherland and Karlson 1977).

The association between colony size and mode of development in *Alcyonium* does, however, suggest another adaptive explanation for the observed reproductive patterns and

supports a trend that has been well documented in solitary taxa (e.g., echinoderms, bivalves; Strathmann 1985, 1990) and in some scleractinian corals (Szmant 1986). Like these other groups, those *Alcyonium* species that grow to large colony size broadcast spawn or brood externally, whereas all of the species with small colony size brood internally. Among the species in clade II, the one clade with a mixture of broadcast spawners and internal brooders (Fig. 4a), the largest species (*A. digitatum*) broadcast spawns and the other three smaller species brood.

The allometric constraints on ability to brood that have been hypothesized to arise from a decrease in surface area: volume ratio with increasing adult size are not, however, expected to apply to colonial organisms in which module size does not vary with colony size. A large colony composed of many small modules should maintain the same surface area: volume ratio, and therefore the same relative brood capacity, as a small colony with similarly sized modules. Allometric constraints on brooding could operate at the level of modules, however, in which case large-moduled colonies should be less likely to brood than colonies with small module size (Strathmann and Strathmann 1982; Jackson 1986).

In alcyonaceans the lengths of the polyp cavities in which gametes develop are determined by colony thickness (Benayahu 1991). In thin, encrusting colonies, the gastrovascular cavities may be only a few millimeters long (Benayahu and Loya 1983), whereas in some large digitate or lobate colonies they may extend 10–15 cm (Benayahu and Loya 1986). The number of ova produced per polyp is a function of polyp cavity length; species with longer polyp cavities produce more ova per polyp, and polyps in thicker regions of a colony have higher fecundity than those in thinner regions (Benayahu and Loya 1983, 1984b, 1986; Benayahu 1991). Large colonies with long gastrovascular cavities might be unable to brood as many embryos as they are capable of producing, if, for instance, embryos in the bases of polyps do not receive adequate ventilation (Strathmann and Chaffee 1984). In *Alcyonium*, both colony thickness (a measure of the length of the internal polyp cavity) and length of the anthocodia (the extensible region of the polyp) are strongly correlated with colony size (volume and wet weight; Fig. 2). The observed association between colony size and reproductive mode may, therefore, reflect an allometric constraint on brooding imposed on colonies with large modules.

Caribbean scleractinian corals are the only other colonial group for which an association has been found between mode of development and size (Szmant 1986). In this case, brooding is associated with small colony (rather than module) size and has been suggested to be an adaptation to environment rather than a reflection of allometric constraints. In shallow reef flat areas that are frequently disturbed, coral colonies are simply too short lived to grow to large size, yet they must maintain high larval recruitment rates to continually repopulate disturbed substrate. Szmant (1986) suggests that brooding facilitates local recruitment, thus the observed indirect association between small colony size and brooding in habitats subject to frequent disturbance.

Associations between either colony or module size and mode of development have not been found in other scleractinian corals (Jackson 1986; Harrison and Wallace 1990),

and some pairs of congeners with similar module size are known to exhibit different modes of development (Harrison and Wallace 1990). In most scleractinian corals, as well as gorgonians, cheilostome bryozoans, and hydroids, relatively size-invariant modules form a thin, lamellar sheet over a skeleton that may reach massive proportions (Jackson 1979). Such organisms maintain high surface area:volume ratios and should not encounter allometric constraints on brooding as a function of colony size (Jackson 1979, 1986; Strathmann and Strathmann 1982). In contrast, in the alcyonaceans, as well as colonial ascidians and sponges with massive growth morphologies, living tissue is not restricted to the colony surface and module size may vary with colony thickness (Jackson 1979). It is in these groups that decreases in surface area:volume ratios with colony size are most likely to impose allometric constraints on brooding. With only one exception, however, all colonial ascidians brood their larvae (Cloney 1990), as do the majority of sponges (Sar  1992).

Hermaphroditism, Parthenogenesis, and Brooding

In *Alcyonium*, hermaphroditism and parthenogenesis have evolved only in brooding species, supporting similar patterns observed in other invertebrate groups (Strathmann et al. 1984; Lively and Johnson 1994). Strathmann et al. (1984) suggested that brooding facilitates the evolution of hermaphroditism because the relatively restricted dispersal of brooded larvae will lead to inbreeding within populations. In such populations selfing hermaphrodites with reduced male allocation will have a reproductive advantage, and because the population is already highly homozygous the costs of inbreeding depression associated with selfing will be greatly reduced (Knowlton and Jackson 1993; Carlon 1999). Parthenogenesis by automixis is genetically equivalent to selfing, and represents an even further reduction in allocation to male function. Lively and Johnson (1994) propose that parthenogenesis will evolve more easily in brooders not because of a predisposition to selfing, but because internal retention of embryos allows developmentally defective embryos to be selectively aborted and replaced. The putatively parthenogenetic *A. hibermicum* evolved in a clade for which the ancestral state appears to be brooding. The ancestral state of the clade containing the two hermaphroditic species (*A. siderium*, *A. sp. A*) is unclear and depends on whether we presume brooding (the most parsimonious explanation) or broadcast spawning to be the ancestral state of the genus. Although hermaphroditic brooders exist in other genera and families, hermaphroditic broadcast spawning is not known to exist in any alcyonaceans (Benayahu et al. 1990), suggesting that evolution of brooding must either precede or accompany evolution of hermaphroditism in this group.

In support of Strathmann et al.'s (1984) hypothesis that inbreeding facilitates the evolution of hermaphroditism, both hermaphroditic *Alcyonium* species exhibit levels of heterozygosity at allozyme loci that are considerably lower than those of most of the species in the other three clades (McFadden 1996, 1999, unpubl. data). *Alcyonium digitatum*, the gonochoric broadcast spawner that is a sister species to the two hermaphrodites, exhibits similarly low heterozygosity (C. S. McFadden, unpubl. data). Because the ancestral

state of sexual expression for this clade is unclear, however, it is possible that heterozygosity has been reduced subsequent to the evolution of hermaphroditism (if selfing is possible) rather than preceding it. The gonochoric *A. digitatum* might lack heterozygosity as a result of having evolved from an inbred, hermaphroditic ancestor. Alternatively, lack of heterozygosity among the clade ancestors due to other factors (e.g., a population bottleneck) could have facilitated the evolution of hermaphroditism. Confirmation of sexual expression in *A. sp. nov. C*, the basal species in this clade, will help distinguish between these alternative hypotheses.

How representative are the patterns of reproduction observed within the genus *Alcyonium*? Among the species examined here, gonochorism (seven of 10 species) and internal brooding (eight of 12) predominate, a pattern that also holds for members of the xeniid family of alcyonaceans (Benayahu 1991). In contrast, gonochoric broadcast spawning seems to be the rule among tropical reef-dwelling alcyoniid genera (Benayahu et al. 1990). Among scleractinian corals, hermaphroditic broadcast spawning, the one pattern that is missing among the alcyonaceans, is the most common mode of reproduction (Harrison and Wallace 1990; Veron 1995; Shlesinger et al. 1998). Understanding the evolutionary basis for such fundamental differences in reproductive mode among groups that are ecologically and morphologically similar will require additional phylogenetic and functional analyses of reproductive trait distribution.

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