Variation in the Ribosomal Internal Transcribed Spacers and 5.8S rDNA Among Five Species of *Acropora* (Cnidaria; Scleractinia): Patterns of Variation Consistent with Reticulate Evolution

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The ITS sequences of *Acropora* spp. are the shortest so far identified in any metazoan and are among the shortest seen in eukaryotes; ITS1 was 70–80 bases, and ITS2 was 100–112 bases. The ITS sequences were also highly variable, but base composition and secondary structure prediction indicate that divergent sequence variants are unlikely to be pseudogenes. The pattern of variation was unusual in several other respects: (1) two distinct ITS2 types were detected in both *A. hyacinthus* and *A. cytherea,* species known to hybridize in vitro with high success rates, and a putative intermediate ITS2 form was also detected in *A. cytherea*; (2) *A. valida* was found to contain highly (29%) diverged ITS1 variants; and (3) *A. longicyathus* contained two distinct 5.8S rDNA types. These data are consistent with a reticulate evolutionary history for the genus *Acropora.*

Introduction

Acropora is the largest extant genus of scleractinian corals, comprising over 370 nominal and at least 150 true species (Wallace and Willis 1994). The possession of specialized axial corallites from which the radial polyps bud, together with its light skeletal characteristics and fast growth rate, have seen *Acropora* become the dominant coral on most Indo-Pacific reefs (Veron 1986, p. 126; Wallace and Willis 1994).

Despite its numerical and ecological importance, systematic relationships within the genus *Acropora* remain unclear. A contributing factor to this is the morphological plasticity, reflected in within-species variability and between-species similarities, that is typical of the genus (Veron and Wallace 1984, p. 134). Three species are placed in the subgenus *Isopora,* and are clearly distinct from the remaining taxa (subgenus *Acropora*). Based on gross skeletal morphology and fundamental similarities in skeletal detail, 14 species groups have been defined within the subgenus *Acropora* (Veron and Wallace 1984, pp. 134–424). However, these groups have no real taxonomic status (Veron 1995, p. 244). Although the application of a wide variety of morphological and biochemical techniques has corroborated or contradicted aspects of this species group scheme (Harrison 1988, pp. 16–119; McMillan et al. 1991; Wallace et al. 1991), overall the contributions that these studies have made to *Acropora* systematics have been relatively minor.

Synchronous mass spawning of corals (Harrison et al. 1984), resulting in the mixing of gametes from a wide range of species, often over long distances, creates widespread opportunities for hybridization. Although naturally occurring hybridization events have been documented in a range of terrestrial and freshwater animals and are thought to be common in plants, there have been few reports of hybridization in marine invertebrates (for reviews, see Arnold 1992; Bullini 1994). Hybridization between taxonomically diverse coral species (including *Acropora* spp.) has been demonstrated in vitro (Wallace and Willis 1994; Willis et al. 1997) and results in viable embryos that can live for several years (Willis et al. 1997) (although the reproductive capacity of hybrid colonies is presently unknown). The extent and significance of hybridization events in the field are unknown, but clearly these experiments have major implications for both the evolution and systematics of corals. Hybridization events create the potential for reticulate evolution or introgression (hybrids backcrossing with parental species; Arnold 1992).

Molecular markers are required to evaluate the possible contribution of hybridization events in the evolutionary history of the genus *Acropora,* as well as to establish subgeneric relationships. The internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA (rRNA) transcription unit (the rDNA) have been shown to be appropriate for addressing relationships at or below the genus level for a wide range of plant and animal groups (e.g., Lee and Taylor 1992; Wesson, Porter, and Collins 1992; Ritland, Ritland, and Straus 1993; Vogler and Desalle 1994), including anthozoan cnidarians (Chen and Miller 1996; Chen, Willis, and Miller 1996). Several recent studies (Sang, Crawford, and Stuessy 1995; Buckler and Holtsford 1996*a,* 1996*b*) demonstrate the usefulness of ITS sequence data in tracing hybridization events and reticulate evolution. Multigene families, such as the rRNA genes, undergo concerted evolution (Dover 1982). A number of mechanisms (unequal crossing over and gene conversion) usually lead to the rapid homogenization of such multicopy sequence families within a (freely interbreeding) species. However, rates of homogenization are dependent on a number of factors, including whether individual repeat units are on the same chromosome (Schlötterer and Tautz 1994). In variable regions such as the ITS, novel variants that arise may rapidly become fixed; thus, in many organisms, the ITS regions differ significantly between genetically discrete units (species or popula-

Abbreviation: ITS, internal transcribed spacer.

Key words: *Acropora,* coral, rDNA, internal transcribed spacer, reticulate evolution.

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Table 1
Size and Nucleotide Composition Data for the ITS1, 5.8S, and ITS2 Regions in Five *Acropora* Species

	ITS1					5.8S					ITS2				
	%A	%T	%C	%C	Length (bp)	%A	%T	%C	%G	Length (bp)	%A	%T	%C	%G	Length (bp)
A. hyacinthus (colony A)															
Aht.1	34.2	27.4	16.4	21.9	73	20.5	23.1	28.2	28.2	156	24.5	27.3	22.7	25.5	110
Aht.9	36.1	25.0	18.1	20.8	72	20.5	23.1	28.2	28.2	156	22.3	24.1	25.0	28.6	112
A. hyacinthus (colony B)															
Ahy.2	36.0	26.7	14.7	22.7	75	20.5	23.1	28.2	28.2	156	22.3	25.0	24.1	28.6	112
Ahy.5	34.2	27.4	17.8	20.5	73	20.5	23.1	28.2	28.2	156	21.4	25.0	24.1	29.5	112
A. cytherea															
Acy.2	36.0	26.7	14.7	22.7	75	20.5	22.4	28.8	28.2	156	22.3	25.0	23.2	29.5	112
Acy.3	35.6	26.0	16.4	21.9	73	20.5	23.1	28.2	28.2	156	22.7	26.4	22.7	28.2	110
Acy.4	35.6	27.4	16.4	20.5	73	20.5	23.1	28.2	28.2	156	24.5	28.2	21.8	25.5	110
Acy.8	36.0	26.7	14.7	22.7	75	20.5	23.1	28.2	28.2	156	22.3	25.0	23.2	29.5	112
A. valida															
Ava.2	33.8	31.3	13.8	21.3	80	20.0	22.6	28.4	29.0	155	24.5	25.5	24.5	25.5	106
Ava.3	31.6	26.3	15.8	26.3	76	20.0	22.6	28.4	29.0	155	25.5	24.5	24.5	25.5	106
Ava.13	33.8	30.0	15.0	21.3	80	20.0	22.6	28.4	29.0	155	24.5	25.5	24.5	25.5	106
A. formosa															
Afo.2	27.4	31.5	21.9	19.2	73	20.0	21.9	29.0	29.0	155	25.2	28.0	23.4	23.4	107
Afo.3	31.1	29.7	18.9	20.3	74	20.0	21.9	29.0	29.0	155	22.9	27.6	23.8	25.7	105
Afo.4	29.7	31.1	17.6	21.6	74	20.0	21.9	29.0	29.0	155	24.0	24.0	26.9	25.0	104
A. longicyathus															
Alo.1	21.9	26.0	30.1	21.9	73	20.9	19.6	31.0	28.5	158	24.0	21.0	27.0	28.0	100
Alo.3	18.6	24.3	28.6	28.6	70	20.3	19.6	31.0	29.1	158	23.0	22.0	27.0	28.0	100
Alo.4	20.5	26.0	30.1	23.3	73	21.8	17.9	32.1	28.2	156	20.2	21.2	28.8	29.8	104
Alo.5	17.8	27.4	30.1	24.7	73	21.8	17.9	32.1	28.2	156	23.0	22.0	27.0	28.0	100

tions). Divergent and reticulate evolution result in very different outcomes with respect to multigene families. If speciation proceeds in a divergent fashion, each species is predicted to be homogenous in ITS sequences but distinct to other species, except where speciation has occurred very recently. Reticulate evolution, on the other hand, may result in a single species harboring several divergent but homologous sequences (originating from the parental species), or classes of repeat that are intermediate between those of the parental species.

Here, we report the results of a preliminary study of ITS sequence variation within and between five *Acropora* species. Two of these species, *A. hyacinthus* and *A. cytherea,* fall within a single species group (the *A. hyacinthus* group) *sensu* Veron and Wallace (1984, pp. 298–318) and are known to hybridize in vitro with high success rates (Willis et al. 1997). The other species each belong to different species groups. The results show that the ITS regions of *Acropora* species are unique in two respects: they are the shortest ITS regions identified to date in any metazoan, and they contain an unprecedented level of variation. The pattern of variation found is consistent with a reticulate evolutionary history for the genus.

Materials and Methods

DNA was prepared from sperm (McMillan et al. 1988) collected from six individual colonies of five species of *Acropora* (order Scleractinia; family Acroporidae; genus *Acropora*; subgenus *Acropora*), *A. hyacinthus* (two colonies), *A. cytherea, A. longicyathus, A. valida,* and *A. formosa* collected from Geoffrey Bay, Magnetic Island (19°10′S, 146°51′E) on the Great Barrier Reef.

The forward primer, GGTACCCTTTGTACACACCGCCCGTCGCT, annealing at positions 1621–1645 in the 18S rDNA of *Anemonia sulcata* (Hendriks et al. 1990; GenBank accession number X53498) was used in conjunction with the reverse primer, GCTTTGGGCTGCAGTCCCAAGCAACCCGACTC (Chen et al. 1995), which anneals to the 28S rDNA to amplify a ~820-bp region spanning the 3′ terminus (~175 bp) of the 18S rDNA through to the 5′ end (~260 bp) of the 28S rDNA. Polymerase chain reaction (PCR) conditions, cloning, and sequencing were as described in Chen et al. (1995), with the exception that products were cloned into pGEM-T (Promega). For each species, a minimum of four clones were sequenced in both directions using universal and the following internal sequencing primers: GGCGACCCGCTGAATTCAAGCATAT and TATGCTTAAATTCAGCGGGT, annealing to the 28S rDNA; GTCGTAACAAGGTTTCCGTA, annealing to the 18S rDNA; and AGCTTGCTGCGTTCTTCATCG, annealing to the 5.8S rDNA.

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Table 2
Pairwise Sequence Comparisons Across All Clones for the ITS1 and ITS2 Regions

Taxon	Aht.9	Ahy.2	Ahy.5	Acy.2	Acy.3	Acy.4	Acy.8	Ava.2	Ava.3	Ava.13	Afo.2	Afo.3	Afo.4	Alo.1	Alo.3	Alo.4	Alo.5
Aht.1	94/85	94/86	95/85	94/86	94/94	95/99	94/86	71/80	60/80	71/80	58/84	57/87	60/80	57/74	62/73	58/69	56/73
Aht.9		95/99	97/98	95/98	95/88	95/85	95/98	70/80	58/81	70/80	56/76	58/78	61/83	59/74	60/73	61/70	55/73
Ahy.2			95/99	100/99	95/89	97/86	100/99	73/81	61/82	73/81	58/77	56/79	59/84	56/75	60/74	57/71	54/74
Ahy.5				95/98	94/88	97/85	95/98	71/80	60/81	71/80	58/76	57/78	60/83	56/75	60/74	57/71	54/74
Acy.2					95/88	97/86	100/100	73/81	61/82	73/81	58/77	56/79	59/83	56/74	60/73	57/70	54/73
Acy.3						95/95	95/88	72/78	60/79	72/78	57/82	56/85	58/81	56/74	60/73	57/70	54/73
Acy.4							97/86	71/80	58/80	71/80	57/84	56/87	58/80	56/74	60/73	57/69	54/73
Acy.8								73/81	61/82	73/81	58/77	56/79	59/83	56/74	60/73	57/70	54/73
Ava.2									71/93	98/100	69/77	68/78	71/84	56/66	58/66	57/64	53/66
Ava.3										71/93	49/83	45/83	48/90	39/63	41/63	39/61	36/63
Ava.13											68/77	67/78	70/84	54/66	57/66	56/64	52/66
Afo.2												84/94	87/87	50/67	52/66	52/65	54/66
Afo.3													97/86	56/70	55/69	57/66	57/69
Afo.4														56/65	55/64	57/61	57/64
Alo.1															92/99	98/96	90/99
Alo.3																94/96	92/100
Alo.4																	91/96
Alo.5																	

NOTE.—Numbers represent percent identity, with numerators and denominators representing ITS1 and ITS2, respectively.

Sequences were aligned using CLUSTAL W 1.6 (Thompson, Higgins, and Gibson 1994), followed by manual editing using SeqApp 1.9 (Gilbert 1992*a*). The boundaries of the various regions were deduced by comparison with data for a range of other cnidarians (Chen, Willis, and Miller 1996; Odorico and Miller 1997). Base composition data were generated using MEGA 1.02 (Kumar, Tamura, and Nei 1993). Pairwise sequence comparisons were conducted with CLUSTAL W 1.6 (Thompson, Higgins, and Gibson 1994) using the default settings (gaps included), based on the alignment submitted to EMBL and available from them (at ftp.ebi.ac.uk in the /pub/databases/embl/align directory) via anonymous ftp as alignment number DS28409. Maximum-likelihood analyses were conducted using DNAML (global rearrangements with 10 random additions) in PHYLIP 3.572c (Felsenstein 1993). Distance analyses were also performed on a bootstrapped data set (1000 replicates; SEQBOOT) using DNADIST (Kimura two-parameter) followed by the neighbor-joining method implemented by NEIGHBOR (PHYLIP 3.572c; Felsenstein 1993). Split decomposition analysis (1,000 bootstrap replicates) based on the Hamming distances was conducted using SplitsTree 2.0d5 (Huson 1996; Dress, Huson, and Moulton 1997). Gaps were included in all phylogenetic analyses.

Folding of the 5.8S rDNA was conducted using MulFold (Gilbert 1990). For the ITS2 regions, secondary structures were predicted using the Mfold server (Zuker 1989) located at http://ibc.wustl.edu/~zuker/rna/, to identify common patterns between species. After common patterns were identified between the five species, the remaining sequences were compared manually for common folding. Structures were drawn with the aid of MulFold (Gilbert 1990) and LoopDloop (Gilbert 1992*b*).

Results

Characteristics of the ITS and 5.8S rDNA

PCR experiments using template DNA from a wide variety of anthozoans implied that the ITS sequences of *Acropora* spp. were unusually short. PCR products given by other anthozoans, including other members of the family Acroporidae, *Montipora digitata* and *Astreopora* sp., were more than 200 bases larger than for any *Acropora* species studied. The compact nature of the ITS regions in *Acropora* spp. was confirmed by DNA sequencing of cloned PCR products. A total of 25 clones, yielding 18 distinct sequence types, were sequenced across the range of species studied. The sequences of the individual clones were submitted to GenBank, under accession numbers U82719–U82736. Overall, the size of the ITS1 varied from 70 to 80 bases, that of the ITS2 varied from 100 to 112 bases, and that of the 5.8S rDNA varied from 155 to 158 bases (table 1). The base composition of each of the three regions did not vary significantly within species.

The sequence data revealed unexpected patterns and levels of diversity. As well as the predicted interspecific heterogeneity, high levels of intraspecific vari-

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ation were also detected. Intraspecific heterogeneity was highest in the ITS1 region of *A. valida* (29% divergence between Ava.2/13 and Ava.3), but was significant in all species for both ITS1 and ITS2. In *A. longicyathus,* intraspecific heterogeneity extended to the 5.8S rDNA. In this species, two distinct 5.8S rDNA types were detected, differing from each other at five (six in one case) positions (i.e., substitutions and indels). By contrast, 5.8S rDNA sequences were identical within each of the other species, with the exception of a single base change in one *A. cytherea* clone (possibly a PCR artifact).

Whereas in *A. longicyathus* ITS variants were clearly more similar to each other than to variants in other species, this distinction was not so marked for the other *Acropora* species studied. In the case of *A. hyacinthus* and *A. cytherea,* inter- versus intraspecific similarities were blurred (table 2). In these species, two distinct ITS variants were detected. Clones Aht.9, Ahy.2, Ahy.5, Acy.2, and Acy.8 were >98% identical in the ITS2 (Ahy.2, Acy.2, and Acy.8 were also identical in the ITS1) and are hereafter referred to as type A. Clones Ahy.1 and Acy.4 appear to represent a second ITS2 variant (type B) which differs by 14% from type A. Clone Acy.3 appears to be intermediate between the types; while being overall more like type B, it has several substitutions in common with type A (at positions 297, 328, and 387 in the alignment). While some of the substitutions present in single clones may be PCR artifacts, the frequent occurrence of common patterns between clones indicates that most of the sequence variation reflects real ITS heterogeneity.

Phylogenetic Analyses of the ITS Regions

Phylogenetic analyses were conducted on individual ITS sequences (ITS1 only, ITS2 only, and combined data sets), using both maximum-likelihood and distance methods, with the aim of evaluating relationships between individual sequences. Because the ITS2 sequences could be aligned with higher confidence than could the ITS1 data, results of analysis of the former region only are shown in figure 1. However, phylogenetic trees based on ITS1 alone and on combined (ITS1 + ITS2) datasets were very similar in overall topology to those based on ITS2 alone. In the resulting trees, *A. longicyathus* was always clearly resolved from the other four species of *Acropora.* Both repetitive sequence (McMillan et al. 1991) and sperm ultrastructure (Harrison 1988, pp. 16–119) data support this distinction. These ITS analyses also resolved *A. formosa* and *A. valida,* but indicated a close relationship between these species. However, clones from *A. cytherea* and *A. hyacinthus* formed a single cluster, resolved from the other species but not from each other. Split decomposition analyses supported most aspects of the topology of the distance tree shown in figure 1*a*; the splits graph shown in figure 1*b* has a splittability index of 60.8%, indicating that most of the splits are significant (Dress, Huson, and Moulton 1997).

Secondary Structure Prediction

The level of variation detected implied that there might be consequences for the secondary structure of the ITS and 5.8S rDNA regions. The size and level of variation within the ITS1 effectively precluded the identification of common folding patterns for this region. However, the lower level of variation and larger size of the ITS2 permitted the identification of common features in predicted secondary structures for this region across the range of species studied. For these analyses, the 3′ end of the 5.8S rDNA was paired with the 5′ end of the 28S rDNA according to the ITS2 model for yeast (Yeh and Lee 1990). With this constraint, all of the individual sequences gave similar predicted secondary structures, each with six helical domains (fig. 2; helix I representing the region of complementarity between the 5.8S and 28S rDNAs). Similarity at the primary-structure level between the two ITS2 sequence types in the *A. hyacinthus* species group was reflected in the inferred secondary structures for this region, which show only minor differences (see fig. 2). For other species, more widespread, though still minor, intraspecific variation was detected. Helix IV was highly conserved across all clones.

The 5.8S rDNA was assessed in terms of the effects of the polymorphic sites on predicted secondary structure. Figure 3 shows the 5.8S rDNAs of *A. hyacinthus* and *A. longicyathus* folded according to the 23S-like rRNA model proposed by Gutell, Gray, and Schnare (1993). When G·T (as well as Watson-Crick) base pairing was permitted, most nucleotide substitutions could be shown to be compensatory. However, at two sites (positions 180 and 245 in the alignment), C↔T transitions resulted in C○A base pairing being required to maintain the secondary structure.

Discussion

The ITS regions of *Acropora* spp. are the shortest characterized to date in the Metazoa and are among the shortest known in eukaryotes (see Hausner, Klassen, and Reid 1993; Katiyar, Visvesvara, and Edlind 1995). *Acropora* spp. appear to be atypical of the Anthozoa (Chen, Willis, and Miller 1996), even other members of the same scleractinian family having significantly larger ITS regions. In yeast, the secondary structure of these spacers is essential for correct processing of the rRNA primary transcript (e.g., Van Nues et al. 1995*a*). However, in a group of amitochondrial protozoans, it has

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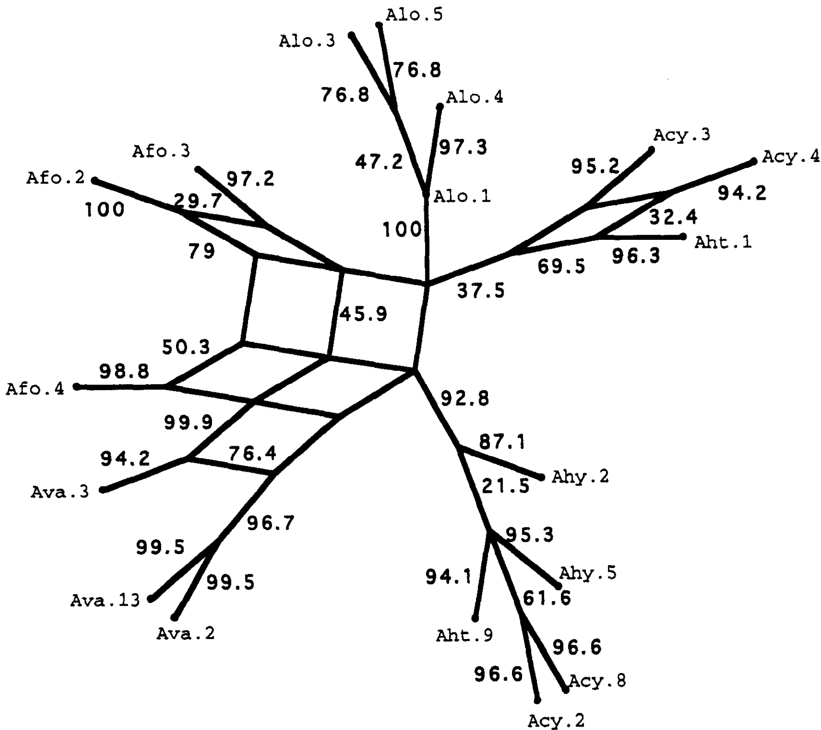
FIG. 1.—*a,* Phylogenetic analyses of the *Acropora* ITS2 sequence data. The unrooted tree on the left is the result of maximum-likelihood analyses, and that on the right is the result of distance analyses. **, branch lengths estimated by DNAML which are significantly positive, $P < 0.01$ (Kishino and Hasegawa 1989). Numbers against branches on the neighbor-joining tree indicate the percentage of 1,000 bootstrap replicates supporting the topology shown. *b,* Phylogenetic analyses of the *Acropora* ITS2 sequence data. For simplicity, the splits graph is drawn with edges having equal lengths. Numbers against the branches indicate the percentage of 1,000 bootstrap replicates supporting the topology shown.

(a)

Alo.4
Alo.3
Alo.5
Alo.1
A. longicyathus
Ahy.2
Acy.2
Acy.8
Ahy.5
Aht.9
A. hyacinthus species group
Acy.3
Acy.4
Aht.1
Afo.3
A. formosa
Afo.2
Afo.4
Ava.3
A. valida
Ava.13
Ava.2
0.1

Alo.3
Alo.5
Alo.4
A. longicyathus
Alo.1
38
69
100
Ahy.5
Ahy.2
Acy.2
Acy.8
Aht.9
65
31
43
90
A. hyacinthus species group
54
52
61
93
Acy.4
Aht.1
Acy.3
50
Afo.4
80
Ava.3
96
100
Afo.3
Afo.2
A. formosa
A. valida
Ava.2
Ava.13

(b)



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Ahy.2
ΔG= -50.3

Acy.3
ΔG= -43.4

Aht.1
ΔG= -43.1

Alo.4
ΔG= -34.9

Ava.2
ΔG= -39.7

Afo.2
ΔG= -32.5

FIG. 2.—Predicted secondary structures for the ITS2 region of a representative range of *Acropora* clones. The figure shows predicted structures for type A (Ahy.2), type B (Aht.1), and the putative intermediate type (Acy.3) clones from *A. hyacinthus* and *A. cytherea,* as well as representative clones from each of the three other *Acropora* species (Ava.2, Alo.4, and Afo.2). Free energy (ΔG) values for each structure are expressed in kcal/mol. Helix I represents 5.8S–28S rDNA complementarity (see text). The 3′ end of the 5.8S rDNA and the 5′ end of the 28S rDNA are identified by "*" and "* *" respectively. Aht = *A. hyacinthus* (colony A); Ahy = *A. hyacinthus* (colony B); Acy = *A. cytherea*; Ava = *A. valida*; Afo = *A. formosa*; Alo = *A. longicyathus.*

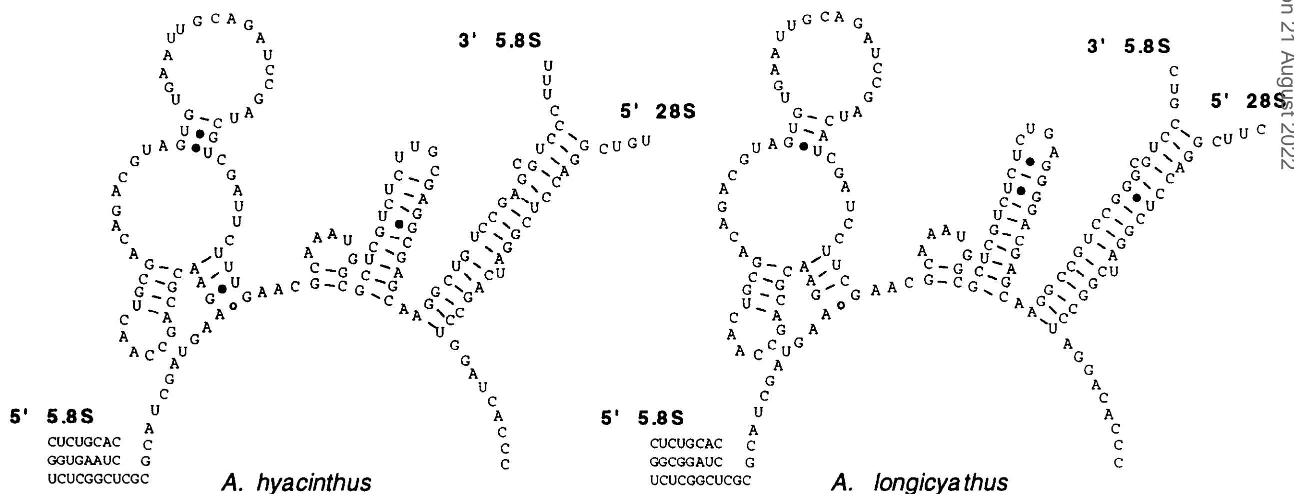

FIG. 3.—5.8S rDNA sequences from *A. hyacinthus* (clone Ahy.2) and *A. longicyathus* (clone Alo.3) folded according to the secondary structure model for the 23S-like rDNA of Gutell, Gray, and Schnare (1993).

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been suggested that it is the lack of secondary structure in the spacers that may facilitate processing (Katiyar, Visvesvara, and Edlind 1995). Like *Acropora* spp., these protists have very short ITS regions. However, whereas in the protists the spacers have little potential for secondary structure formation, our analyses (fig. 2) clearly demonstrate that this is not the case in *Acropora* spp. The compact nature of the *Acropora* ITS regions should facilitate the identification of those features required for processing of the rRNA primary transcript. Highly conserved regions, such as helix IV (fig. 2), are good candidate processing elements (Van Nues et al. 1995*b*). In addition, as in many respects cnidarians are "mainstream" metazoans (see Berghammer et al. 1996), *Acropora* may be highly informative with respect to the general principles of rRNA processing in animals.

While it is not unusual for a single individual to harbor more than one ITS sequence variant, the pattern and level of rDNA heterogeneity in *Acropora* spp. appear to be without precedent. Similarity in base composition within species and in predicted secondary structure argues against any of these variants representing pseudogenes (Buckler and Holtsford 1996*a*, 1996*b*). As the normal range of structural constraints presumably applies, the extreme brevity of these regions in *Acropora* makes the high level of heterogeneity more remarkable. The only other study of cnidarian ITS sequences contrasts markedly with the results for *Acropora* spp.; only minor intraspecific differences in the ITS sequences were detected across wide geographic ranges in tropical corallimorphs (Chen and Miller 1996; Chen, Willis, and Miller 1996), and ITS sequences were more similar between a range of *Rhodactis* spp. than within a single individual of (for example) *A. valida*.

For several other animal and plant species (e.g., Wesson, Porter, and Collins 1992), higher mutation than homogenization rates (Ohta and Dover 1983; Schlötterer et al. 1994) can account for observed levels of sequence heterogeneity. However, estimates for rates of divergence for the same region in *Drosophila* are in the region of 1.2% per Myr (Schlötterer et al. 1994). Therefore, the extreme level of intraindividual variation observed in *Acropora* spp. (up to 29% divergence in the ITS1 in *A. valida*; table 2) is inconsistent with explanations based on simple rate imbalances.

The detection of two ITS2 sequence types common to both *A. cytherea* and *A. hyacinthus* may be explained by the operation of differential maintenance mechanisms (see Ritland, Ritland, and Straus and 1993) if the variants were present before these species diverged from a common ancestor. However, consideration of the known hybridization potential of *A. cytherea* and *A. hyacinthus* (Willis et al. 1997) leads us to believe that the presence of sequence variants that are shared between species is evidence of recent interspecific hybridization. Note that in addition to the sequences included in the alignment shown, two other clones identical with Ahy.2 and one other identical with Acy.2 were detected. Therefore, of the total of 11 clones sequenced for *A. hyacinthus* and *A. cytherea,* eight had type A and two had type B ITS2 sequences, with one clone (Acy.3) being intermediate.

Our interpretation of these results is that the ITS2 is undergoing homogenization in favor of type A at the level of a "metaspecies" (Veron 1995, pp. 210–239) comprising a minimum of *A. cytherea* and *A. hyacinthus,* and that clone Acy.3 represents an intermediate stage in this process. The origin of the type B sequence may be more clear when ITS data are available for other species known to hybridize in vitro with this group (Willis et al. 1997). The presence of highly diverged ITS1 sequence variants in *A. valida* and of distinct 5.8S rDNA types in *A. longicyathus* are both also suggestive of recent hybridization events.

Based on this model, several testable predictions can be made with respect to *Acropora* rDNA data. First, other ITS2 variants intermediate between types A and B may be present in the *A. hyacinthus/A. cytherea* complex. Second, there may be spatial variation in the distribution of ITS types, arising from different distribution ranges of hybridizing species. Third, there may be ITS1 and 5.8S rDNA types in *A. valida* and *A. longicyathus* respectively, that are intermediate between those found here. Fourth, it may be possible to identify other species that contain only one of the sequence variants. Finally, similar phenomena are predicted to occur in other *Acropora* species.

In summary, the ITS and 5.8S rDNA data suggest a reticulate evolutionary history for the genus *Acropora*. In peonies (*Paeonia* spp.), the evidence for reticulate evolution is that some species show patterns of ITS substitutions that are additive with respect to the putative parental species; the hybridization events that gave rise to these species occurred relatively recently (during the late Pleiocene and Pleistocene), and concerted evolution has not completely homogenized the ITS sequences (Sang, Crawford, and Stuessy 1995). Levels of inter- and intraspecific variation in the ITS regions of peonies contrast markedly with those seen in *Acropora* spp. This may reflect the rather limited opportunities for hybridization in peonies compared with corals; the majority of *Paeonia* spp. are allopatric, whereas *Acropora* species capable of hybridizing in vitro frequently occur sympatrically, and fertilization can occur over relatively long ranges. Therefore, in corals, hybridization may occur on biological, rather than geological, time scales.

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LITERATURE CITED

ARNOLD, M. L. 1992. Natural hybridization as an evolutionary process. Annu. Rev. Ecol. Syst. **23**:237–261.

BERGHAMMER, H., D. HAYWARD, P. HARRISON, and D. J. MILLER. 1996. Nucleotide sequence of ub52 from the cnidarian *Acropora millepora* reveals high evolutionary conservation. Gene **178**:195–197.

BUCKLER, E. S., and T. P. HOLTSFORD. 1996*a*. *Zea* systematics: ribosomal ITS evidence. Mol. Biol. Evol. **13**:612–622.

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———. 1996*b*. *Zea* ribosomal repeat evolution and substitution patterns. Mol. Biol. Evol. **13**:623–632.

Bullini, L. 1994. Origin and evolution of animal hybrid species. Trends Ecol. Evol. **9**:422–426.

Chen, C. A., and D. J. Miller. 1996. Analysis of ribosomal ITS1 sequences indicates a deep divergence between morphologically similar *Rhodactis* (Cnidaria: Anthozoa: Corallimorpharia) species from the Caribbean and the Indo-Pacific/Red Sea. Mar. Biol. **126**:423–432.

Chen, C. A., D. M. Odorico, M. Ten Lohuis, J. E. N. Veron, and D. J. Miller. 1995. Systematic relationships within the Anthozoa (Cnidaria: Anthozoa) using the 5'end of the 28S rDNA. Mol. Phylogenet. Evol. **4**:175–183.

Chen, C. A., B. L. Willis, and D. J. Miller. 1996. Systematic relationships of the tropical corallimorpharians (Cnidaria: Anthozoa): utility of the 5.8S and internal transcribed spacers (ITS) of ribosmal DNA units. Bull. Mar. Sci. **59**:269–281.

Dover, G. 1982. Molecular drive: a cohesive mode of species evolution. Nature **299**:111–117.

Dress, A. W. M., D. H. Huson, and V. Moulton. 1997. Analyzing and visualizing sequence and distance data using SplitsTree. Discrete Appl. Math. (in press).

Felsenstein, J. 1993. PHYLIP: phylogeny inference package. Version 3.57c. University of Washington, Seattle.

Gilbert, D. G. 1990. MulFold: a Macintosh version of the MFOLD (V2.0), software for the prediction of RNA secondary structure. Published electronically on the Internet, available via anonymous ftp to ftp.bio.indiana.edu.

———. 1992*a*. SeqApp: a biological sequence editor and analysis program for Macintosh computers. Published electronically on the Internet, available via anonymous ftp to ftp.bio.indiana.edu.

———. 1992*b*. LoopDloop: a Macintosh program for visualizing RNA secondary structure. Published electronically on the Internet, available via anonymous ftp to ftp.bio.indiana.edu.

Gutell, R. R., M. W. Gray, and M. N. Schnare. 1993. A compilation of large subunit (23S- and 23S-like) ribosomal RNA structures. Nucleic Acids Res. **21**:3055–3074.

Harrison, P. L. 1988. Comparative ultrastructure of scleractinian spermatozoa and its evolutionary implications. Ph.D. thesis, James Cook University of North Queensland, Townsville, Australia.

Harrison, P. L., R. C. Babcock, G. D. Bull, J. K. Oliver, C. C. Wallace, and B. L. Willis. 1984. Mass spawning in tropical reef corals. Science **223**:1184–1189.

Hausner, G., G. R. Klassen, and J. Reid. 1993. Unusually compact ribosomal RNA gene cluster in *Sphaeronaemella fimicola*. Curr. Genet. **23**:357–359.

Hendriks, L., Y. Van De Peer, m. Van Herck, J.-M. Neefs, and R. De Wachter. 1990. The 18S ribosomal RNA sequence of the sea anemone *Anemonia sulcata* and its evolutionary position among other eukaryotes. FEBS Lett. **269**: 445–449.

Huson, D. H. 1996. SplitsTree2—a program for analyzing and visualizing evolutionary data. Available from ftp://ftp.unibielefeld.de/pub/math/splits/splitstree2.

Katiyar, S. K., G. S. Visvesvara, and T. D. Edlind. 1995. Comparisons of ribosomal RNA sequences from amitochondrial protozoa: implications for processing, mRNA binding and paromomycin susceptibility. Gene **152**:27–33.

Kishino, H., and M. Hasegawa. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in hominoidea. J. Mol. Evol. **29**:170–179.

Kumar, S., K. Tamura, and M. Nei. 1993. MEGA: molecular evolutionary genetics analysis. Version 1.02. Pennsylvania State University, University Park.

Lee, S. B., and J. W. Taylor. 1992. Phylogeny of five fungus-like protoctistan *Phytophthora* species, inferred from the internal transcribed spacers of ribosomal DNA. Mol. Biol. Evol. **9**:636–653.

McMillan, J., T. Mahony, J. E. N. Veron, and D. J. Miller. 1991. Nucleotide sequencing of highly repetitive DNA from seven species in the coral genus *Acropora* (Cnidaria: Scleractinia) implies a division contrary to morphological criteria. Mar. Biol. **110**:323–327.

McMillan, J., D. Yellowlees, A. Heyward, P. Harrison, and D. J. Miller. 1988. Preparation of high molecular weight DNA from hermatypic corals and its use for DNA hybridization and cloning. Mar. Biol. **98**:271–276.

Odorico, D. M., and D. J. Miller. 1997. Internal and external relationships of the Cnidaria: implications of primary and predicted secondary structure of the 5'-end of the 23S-like rDNA. Proc. R. Soc. Lond. B **264**:77–82.

Ohta, T., and G. A. Dover. 1983. Population genetics of multigene families that are dispersed into two or more chromosomes. Proc. Natl. Acad. Sci. USA **80**:4079–4083.

Ritland, C. E., K. Ritland, and N. A. Straus. 1993. Variation in the ribosomal internal transcribed spacers (ITS1 and ITS2) among eight taxa of the *Mimulus guttatus* species complex. Mol. Biol. Evol. **10**:1273–1288.

Sang, T., D. J. Crawford, and T. F. Stuessy. 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. Proc. Natl. Acad. Sci. USA **92**:6813–6817.

Schlötterer, C., M. T. Hauser, A. Von Haeseler, and D. Tautz. 1994. Comparative evolutionary analysis of rDNA ITS regions in *Drosophila*. Mol. Biol. Evol. **11**:513–522.

Schlötterer, C., and D. Tautz. 1994. Chromosomal homogeneity of *Drosophila* ribosomal DNA arrays suggests intrachromosomal exchanges drive concerted evolution. Curr. Biol. **4**:777–783.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22**:4673–4680.

Van Nues, R. W., J. M. J. Rientjes, S. A. Morre, E. Mollee, R. J. Planta, J. Venema, and H. A. Raue. 1995*b*. Evolutionary conserved structural elements are critical for processing of internal transcribed spacer 2 from *Saccharomyces cerevisiae* precursor ribosomal RNA. J. Mol. Biol. **250**: 24–36.

Van Nues, R. W., J. Venema, J. M. J. Rientjes, A. Dirks-Mulder, and H. A. Raue. 1995*a*. Processing the eukaryotic pre-rRNA: the role of the transcribed spacers. Biochem. Cell Biol. **73**:789–801.

Veron, J. E. N. 1986. Corals of Australia and the Indo-Pacific. Angus and Robertson, Sydney, Australia.

———. 1995. Corals in space and time: the biogeography and evolution of the Scleractinia. University of New South Wales Press, Sydney, Australia.

Veron, J. E. N., and C. C. Wallace. 1984. Scleractinia of Eastern Australia. Part V. Aust. Inst. Mar. Sci. Monogr. Ser. 6.

Vogler, A. P., and R. DeSalle. 1994. Evolution and phylogenetic information content of the ITS-1 region in the tiger beetle *Cicindella dorsalis*. Mol. Biol. Evol. **11**:393–405.

Wallace, C. C., J. M. Pandolfi, A. Young, and J. Wolstenholme. 1991. Indo-Pacific coral biogeography: a case

study from the *Acropora selago* group. Aust. Syst. Bot. **4**: 199–210.

WALLACE, C. C., and B. L. WILLIS. 1994. Systematics of the coral genus *Acropora*: implications of new biological findings for species concepts. Annu. Rev. Ecol. Syst. **25**:237–262.

WESSON, D. M., C. H. PORTER, and F. H. COLLINS. 1992. Sequence and secondary structure comparisons of ITS rDNA in mosquitoes (Diptera: Culicidae). Mol. Phylogenet. Evol. **1**:253–269.

WILLIS, B. L., R. C. BABCOCK, P. L. HARRISON, and C. C. WALLACE. 1997. Hybridization and breeding incompatibilities within the mating systems of mass spawning reef corals. Coral Reefs (in press).

YEH, L.-C. C., and J. C. LEE. 1990. Structural analysis of the internal transcribed spacer 2 of the precursor ribosomal RNA from *Saccharomyces cerevisiae.* J. Mol. Biol. **211**: 699–712.

ZUKER, M. 1989. On finding all suboptimal foldings of an RNA molecule. Science **244**:48–52.

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