Hydrobiologia **216/217**: 509–512, 1991.
R. B. Williams, P. F. S. Cornelius, R. G. Hughes & E. A. Robson (eds),
Coelenterate Biology: Recent Research on Cnidaria and Ctenophora.

Relationships within the family Actiniidae (Cnidaria, Actiniaria) based on molecular characters

Steven A. McCommas
Department of Biological Sciences, Southern Illinois University at Edwardsville, Edwardsville, Illinois 62026, USA

Key words: phylogeny, electrophoresis, isozymes, sea anemones, cladistics, acrorhagi, vesicles, verrucae

Abstract

Current sea anemone systematics is based on relatively few morphological characters, and potentially could benefit from the use of molecular characters. In this paper, the phylogenetic relationships of 12 species from 6 genera in the family Actiniidae have been investigated using electrophoretically separated isozymes. A numerical cladistic analysis has produced an estimated phylogeny. The implications of this phylogeny for the taxonomic use of certain morphological characters are discussed.

Introduction

The taxonomy of sea anemones (Order Actiniaria) is currently based on a rather small number of morphological characters, such as the arrangement and number of tentacles, shapes of certain muscles, column protuberances, and sizes and kinds of nematocysts. Use of more morphological characters is hindered by the structural simplicity of these animals, the plasticity of body size and shape which actiniarians may show resulting from developmental and nutritional state, and the variation caused by some forms of asexual reproduction.

It is clearly desirable to try to find more characters, particularly for elucidating the phylogenetic relationships among the genera and species of this order. Potential candidates are molecular characters, such as isozymes revealed by electrophoresis of enzymes under nondenaturing conditions and DNA or amino acid sequences. These would not only greatly expand the number of taxonomically valuable characters available, but also lend themselves readily to analysis by computer programs designed to process large numbers of characters and to yield estimates of the most likely evolutionary relationships of a group of organisms. The utility of isozymes as characters to clarify taxonomic relationships in anemones has already been demonstrated to some extent by McCommas & Lester (1980).

The Actiniidae is the largest and most varied family of anemones. In a study of this family (McCommas, 1982a), frequencies of isozymes were estimated for 12 species in 6 genera, including 2 population groups (Atlantic and Gulf of Mexico) of *Bunodosoma cavernata* (Bosc) which have been separated for several million years (McCommas, 1982b). In this paper these data are analyzed using numerical cladistic techniques to investigate systematic relationships among these 13 Operational Taxonomic Units (OTUs). Clustering OTUs by their apomorphies (shared derived traits) is an attempt to eliminate the

assumption of homogeneity of evolutionary rates and produce valid estimates of phylogenetic relationships (Lundberg, 1972).

Material and methods

The species and collection sites were as follows. *Bunodosoma cavernata* (Bosc), Atlantic group: Beaufort, North Carolina; Charleston, South Carolina; Ft Pierce Inlet, Florida; La Parguera, Puerto Rico. *Bunodosoma cavernata* (Bosc), Gulf of Mexico group: Cameron, Louisiana; Galveston, Port Aransas and Port Isabel, Texas. *Bunodosoma granulifera* (Lesueur): Cabo Rojo, Puerto Rico; Buenaventura, Panama; Awa Blanku, Curaçao; and Grand Cayman. *Bunodosoma californica* Carlgren: Puerto Penasco, Gulf of California. *Actinia tenebrosa* Farquhar: Whale Beach, Colloroy Beach and Harbord Beach, Sydney, Australia. *Anthopleura carneola* (Verrill): Sannibel Island, Islamorada, Ft Pierce Inlet, Deerfield Beach and Cross Key, Florida. *Anthopleura stellula* (Ehrenberg): Puerto Rico. *Anthopleura pallida* Duchassaing & Michelotti: Islamorada and Cross Key, Florida. *Bunodactis texaensis* Carlgren & Hedgpeth: Dung Beach, Louisiana. *Bunodactis stelloides* (McMurrich): Missouri Key, Florida. *Bunodactis* spp.: Buenaventura, Panama. *Epiactis prolifera* Verrill: Bodega Bay, California. *Phyllactis conquilega* (Duchassaing & Michelotti): La Gata Reef, Puerto Rico.

Each species was assayed under standard conditions for isozymes encoded by 12 loci: peptidase-1, peptidase-2, malic enzyme, esterase-1, esterase-2, octanol dehydrogenase, glycerate-2-dehydrogenase, phosphoglucomutase, isocitrate dehydrogenase, octopine dehydrogenase, glutamate dehydrogenase and superoxide dismutase. Details of processing individuals, electrophoretic conditions, and staining recipes have been given previously (McCommas & Lester, 1980; McCommas, 1982b). Some species failed to show any enzymatic activity for one or more loci. While it might have been possible to find alternative electrophoretic and staining procedures which would have produced activity for these species at these loci, it was more important to keep electrophoretic conditions constant for all species so that loci and isozymes could be properly homologized. Each isozyme was given a number, based on its mobility relative to an arbitrary isozyme chosen from *B. cavernata*. The isozymes and their population frequencies for each species are given by McCommas (1982a).

The numerical cladistic technique chosen was the Wagner parsimony method as implemented by the MIX program of the PHYLIP package of programs for inferring phylogenies (available from J. Felsenstein, Dept. of Genetics SK-50, Univ. of Washington, Seattle, WA 98195, USA). The principles used in constructing Wagner trees have been shown to be formally equivalent to the principles underlying Hennigian cladistic analysis (Farris *et al.*, 1970). This program uses discrete character state data, and assumes that ancestral states are unknown and that a state change from ancestral to derived is just as likely as a change from derived to ancestral. These assumptions are the most appropriate ones for electrophoretic characters, which may differ by only one or two amino acids, and for which information is lacking on the probable pathways of character state evolution. The program uses a 'branch-swapping' algorithm for finding the most parsimonious trees for a given set of data, in which it sequentially adds OTUs to the tree and then tries local rearrangements to see if the tree is improved. In this process, it examines several hundred alternative trees. By parsimonious is meant having the fewest 'steps', or character state changes, necessary to connect the OTUs in the tree. The total number of steps is also referred to as the length of the tree; hence the most parsimonious tree is the shortest.

Each isozyme was treated as a character and each OTU was scored as either having that character (character state '1') or not having that character ('0'). When an OTU failed to show any activity for a particular locus, all isozymes for that locus for that OTU were given the character state '?', meaning unknown. This produced a matrix of character states for 130 characters (the total number of different isozymes) for the 13 OTUs exam-

ined. The algorithm used in this program for finding the most parsimonious tree is influenced to an extent by the order in which the OTUs are presented: it is possible to produce a poor initial topology which affects all subsequant parts of the search. For this reason, the data were presented to the program with the OTUs ordered in 10 different ways, yielding trees with very similar topologies and only slightly different lengths. This gave a good indication of the major features of the topology which were important. The program analyzed a large number of minor variations on this consensus tree, slightly rearranging OTUs, in order to produce even shorter ones.

Results

The shortest tree found was one of 176 steps, and is shown as an unrooted cladogram in Fig. 1. In this type of diagram it is only the branching pattern which is important. The lengths of the branches are not proportional to time, degree of evolution or phenetic difference between OTUs. The nodes, except for the primary bifurcation at the base of the unrooted tree, may be regarded as hypothetical ancestors for all the OTUs extending from them higher up into the tree.

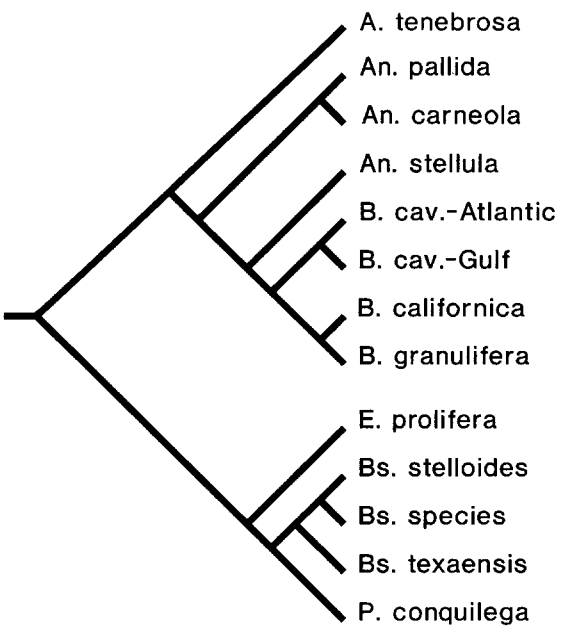

Fig. 1. Unrooted cladogram of 12 species of actiniid anemones. 'B. cav. – Gulf' and 'B. cav. – Atlantic' are the Gulf of Mexico and Atlantic coast populations, respectively, of *Bunodosoma cavernata*, 'Bs. species' is an unidentified species of *Bunodactis*.

Discussion

With the exception of *A. stellula*, all species clustered with their congeners, increasing confidence in the method and therefore in the rest of the phylogeny. Furthermore, the pairs of OTUs known *a priori* to be closely related (*B. cavernata* Gulf and Atlantic populations, and the geminate species *B. granulifera* and *B. californica*; McCommas, 1982b) are also found here to be more closely related to each other than to any other OTUs. The single discrepancy of *A. stellula* joining the *Bunodosoma* species before it joins with the other *Anthopleura* species can be resolved by adding only a single step to the tree. Since trees of 176 steps versus 177 steps are not significantly different, we may regard this alternative tree as a legitimate alternative phylogeny and perhaps even a more likely one, since it would accord with classical taxonomy.

Indeed, the ambiguity in distinguishing between these 2 genera on the basis of molecular characters is mirrored to some extent by morphological characters. One of the chief differences between them is that *Anthopleura* possesses adhesive verrucae on the column whereas *Bunodosoma* possesses nonadhesive vesicles. The specimens of *B. granulifera*, *B. californica* and Atlantic *B. cavernata* used in this study all had nonadhesive vesicles, as expected. However, the Gulf of Mexico specimens of *B. cavernata* very strongly attach pieces of broken shell to their 'vesicles' (strong enough that a large anemone may be picked up by a shell fragment). Moreover, thin sections of these 'vesicles' show them to have the structure expected of verrucae (R. Seaton, *in litt.*). The Gulf specimens are in every other respect morphologically identical to their Atlantic counterparts (coloration, tentacles, cnidome, and retractor muscles; R. Seaton, *in litt.*).

The Gulf specimens are also very similar to the Atlantic specimens genetically, based on values of Nei's genetic distance. The genetic distance between pooled Gulf and Atlantic *B. cavernata* populations is 0.284. This is slightly larger than the value for *B. granulifera* – *B. californica* (0.188), but below the mean value for intrageneric species comparisons (1.661) and far lower than mean intergeneric values (2.592). Thus, the genetic data do not support placing the Gulf specimens in a separate genus from the Atlantic specimens.

This implies that column protuberances in this family may change rather readily from adhesive to nonadhesive and/or *vice versa* within evolutionary lines, thus making poor characters for defining genera (or species, to judge from *B. cavernata*). This conclusion is supported by examination of the occurrence of vesicles and verrucae among the genera examined here in the framework of the phylogeny given. Vesicles are characteristic of *Bunodosoma*, verrucae are characteristic of *Anthopleura*, *Bunodactis* and *Phyllactis*, while *Actinia* and *Epiactis* have neither. Thus, verrucae or vesicles would have had to have been independently gained or lost several times in the evolution of these taxa in order to give the phylogenetic pattern seen here.

The situation with respect to acrorhagi is quite different. Acrorhagi are specialized structures around the oral disc, with characteristic types of nematocysts, used for agonistic encounters with other anemones (Bigger, 1980). These structures are found only in the family Actiniidae, but not among all actiniids. Francis (1973) suggested that these structures and the aggressive behavior associated with them might be monophyletic in origin. The phylogeny presented here is in agreement with this idea. All the species on one side of the major bifurcation possess acrorhagi, while those on the other do not, giving further confidence to this phylogeny. Since acrorhagi are unknown outside the family, lack of them can be regarded as ancestral, and thus possession of acrorhagi is a synapomorphy for *Actinia*, *Anthopleura* and *Bunodosoma*. Given the complexity and ecological importance of acrorhagi and their associated behavior, it is unlikely that they would be independently gained, or frequently lost, among phyletic lines.

A study of 12 species in 6 genera of a family having 43 genera and about 220 species (approximately half of the species in the order) is a small start towards better understanding the phylogenetic relationships of its members, but this study demonstrates the potential utility of molecular characters to augment the use of more traditional characters. However, the genetic divergence among species and genera of anemones is so great that the use of isozymes for systematics is pushed close to its limits. Sophisticated numerical procedures will not help if 2 species are different from each other and all other species at every isozyme analysed. But other types of molecular characters, especially ribosomal DNA sequences, should be ideally suited for furthering understanding of the phylogeny of the Actiniidae and other anemone families.

Acknowledgements

I thank those who made, or assisted me in making, species identifications, especially Charles Cutress and Robert Seaton, and Dr Leo Minasian, Jr for many helpful discussions about anemone systematics. I also thank Dr E.H. Bryant and Dr L.J. Lester for their help and encouragement.

References

Bigger, C. H., 1980. Interspecific and intraspecific acrorhagial aggressive behavior among sea anemones: a recognition of self and not-self. Biol. Bull. 159: 117–134.

Farris, J. S., A. G. Kluge & M. J. Eckardt, 1970. A numerical approach to phylogenetic systematics. Syst. Zool. 19: 172–191.

Francis, L., 1973. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related sea anemones. Biol. Bull. 144: 73–92.

Lundberg, J. G., 1972. Wagner networks and ancestors. Syst. Zool. 21: 398–413.

McCommas, S. A., 1982a. Application of electrophoretic techniques to some systematic problems in the family Actiniidae. Ph.D. thesis, Univ. of Houston, Texas.

McCommas, S. A., 1982b. Biochemical genetics of the sea anemone *Bunodosoma cavernata* and the zoogeography of the Gulf of Mexico. Mar. Biol. 68: 169–173.

McCommas, S. A. & L. J. Lester, 1980. Electrophoretic evaluation of the taxonomic status of two species of sea anemone. Biochem. Syst. Ecol. 8: 289–292.