

## CLONALITY AS A TAXONOMIC CHARACTER OF ACTINIAN SPECIES

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## ABSTRACT

Sea anemones of some species have been considered to exist both clonally and as solitary individuals. In two temperate taxa, these alternative forms have been demonstrated through molecular techniques actually to belong to separate species. We have sequenced a variable portion of the cytochrome oxidase I mitochondrial gene from both solitary and clonal individuals of two nominal species of actinians that host anemonefish and that are abundant on Indo-Pacific reefs -- *Entacmaea quadricolor* and *Heteractis magnifica*. Our molecular data support the conclusion based on morphology that *H. magnifica* constitutes a single species from the Red Sea to French Polynesia, and encompasses both solitary and clonal morphs. The sequences from *E. quadricolor* differ from those of *H. magnifica*, but are too preliminary for assessment of whether more than a single species is represented.

## INTRODUCTION

Sea anemones (Class Anthozoa, Order Actiniaria) are simple, asexual animals. Because they have few morphological characters, taxonomic distinctions rely to a greater degree than in other cnidarians on features such as cnidae (Fautin 1988) and sexuality (Fautin and Chia 1986). The extent to which these traits and others, such as reproductive mode, are taxonomically significant has been difficult to assess (Fautin 1991). Molecular characters have the potential to probe whether these traits are of taxonomic value and, as for many other marine invertebrates (Knowlton 1993), to allow discrimination among taxa that do not differ in conventional characters. In an effort to determine whether clonal and solitary individuals of each of two nominal species of tropical sea anemones actually may belong to different species, we compared the sequence of a portion of the mitochondrial genome.

Both *Heteractis magnifica* (family Stichodactylidae) and *Entacmaea quadricolor* (family Actiniidae) occur as solitary individuals and in clonal clusters (Fautin and Allen 1992). Clonal and solitary individuals of each species do not differ consistently in morphology and cnidae (Dunn 1981), but there are some differences. Clonal individuals tend to be but are not invariably smaller than solitary ones (personal observation, DGF). Clonal and solitary individuals of both species may harbor symbiotic anemonefish; fish of some species occur primarily with hosts of one morph whereas others inhabit both (Dunn 1981; Fautin and Allen 1992).

Throughout the range of the species, solitary individuals of *E. quadricolor* tend to protrude from holes on reefs at depths typically greater than several meters, whereas clones occur atop reefs in shallow water; I (as Dunn 1981) regarded these as ecophenotypes in the sense of Mayr (1969).

By contrast, clonality of *H. magnifica* varies geographically, not bathymetrically. Solitary specimens inhabit the core of the species' geographical range, from eastern Indonesia through New Guinea and the Great Barrier Reef. To the east (for example, in French Polynesia) and the west (from Java to the Red Sea), the species is found as clusters of several individuals or beds of tens of them.

The pattern seen in *E. quadricolor* was considered by Hand (1956) to exist in anemones of the species *Metridium senile* (family Metridiidae) in the northeast Pacific. Small, clonal individuals in shallow water gradually give way to larger, solitary ones with depth. In 1990, the solitary morph of *M. senile* was described as a separate

species, *M. giganteum*, by Fautin et al., based on differences in allozymes, in regenerative ability, and some in morphology and cnidae that had been considered correlates of size. The other species of northeast Pacific anemone that reportedly occurs both clonally and solitarily is *Anthopleura elegantissima* (family Actiniidae) (Hand 1955). In a detailed natural history study of the two morphs, Francis (1979) concluded they are sibling species, but Smith and Potts (1987) found them to be virtually identical electrophoretically. A reanalysis of those electrophoretic data has convinced McFadden and Grosberg (personal communication to DGF) that there are, indeed, two species.

Thus, in one temperate actinian species pair the potential taxonomic significance of life history, size, and habitat differences in anemones has been reinforced by molecular data. In another, although the genetic divergence appears less complete (which may correlate with recency of separation), clonal and solitary individuals appear to represent sibling species, too. Since in these two taxa, clonal and solitary morphs have been recognized as distinct species, we reconsidered the status of such morphs in the tropical anemones *Heteractis magnifica* and *Entacmaea quadricolor*.

## MATERIALS AND METHODS

Sequence data are from a clonal specimen of *Heteractis magnifica* (Quoy and Gaimard, 1833) collected off the Sinai Peninsula (Red Sea) and two from Moorea (French Polynesia), plus three solitary individuals from Madang (Papua New Guinea). The specimens from Moorea were inferred to be clonemates, based upon their proximity to one another and identical coloration. DNA from a solitary specimen of *Entacmaea quadricolor* (Rüppell and Leuckart, 1828) from Lizard Island, Great Barrier Reef, and a clonal one from the Red Sea was also sequenced. The specimens had been frozen or placed in 100% EtOH upon collection.

For most specimens, mitochondrial DNA was extracted from a piece of the pedal disc or mesentery to avoid possible amplification of zooxanthella genome. Frozen and alcohol-preserved tissue from the same animal were compared to ascertain that method of preservation did not affect the results. DNA extraction was with Promega Wizard Minipreps. Amplification was with the "universal" primers described by Folmer et al. (1994) for a 710-bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI). The PCR product was cleaned with Wizard PCR Preps before automated sequencing of both strands was done with a Li-Cor 4000L.

Alignment of amino acids was by eye, using the program Esee (Cabot 1995); editing of the sequence data was also by eye. We translated the nucleotide sequence given by Clary and Wolstenholme (1985) for *Drosophila yakuba* using Esee's code for *Drosophila*, then used that amino acid sequence for alignment using Esee's Cnidaria code to translate our data. To assess reliability of our data, we aligned sequences from three pieces of tissue taken from a single individual anemone.

## RESULTS

Extraction of mtDNA from *Entacmaea quadricolor* was much less successful than that from *Heteractis magnifica*. We therefore discuss mainly the latter species.

D. yakuba	ACTTTATATTTCAATTTTGGAGCTTGAGCGGAATAGTAGGAACATCTTTAAGAATTTTAAATTCGAGCAG	70
	T L Y F I F I G A W A G M V G T S L S I L I R A	23
H. magnif.	ACNNTATATTTAGTATTTGGAAATAGGACCGGTATGATAGGCACANNTTAAAGTATGTTAATAGATTTGG	70
	T Y L V F G I G G M I G T L S M L I R L	20
D. yakuba	AATTAGGTCATCCAGGACCATTAATGGAGATGATCAAATTTAATGTAATGTTACTGCACATGCTTT	140
	E L G H G F G A L I G D D Q I Y N V I V T A H A F	47
H. magnif.	AACTATCGCCCTGGACTATGTTGGGGGACACCATCTTAAATGTCATAGTAGCGGCACCGCTT	140
	E L S A P G T M L G D D H L Y N V I V T A H A F	44
D. yakuba	TATTATAATTTTTTATAGTAATACCTATTATAATTTGGGGGTTGGAAATTTGATGCTCCTTAA	210
	I M I F F F M V M P I M I G G F G N W L V P L M	70
H. magnif.	TATTATGATTTTCTTCNGWAATGCCAGTAATGATAGGAGGGTTGGTAATTTGGTGGTACCATAT	210
	I M I F F F L M P V M I G G F G N W L V P L Y	66
D. yakuba	TTAGGAGCTCCTGACATAGCATTCCCACGAATAAATAATAAGATTTTGTACTACCTCCTGCTCTTT	280
	L G A P D M A F P R M N N M S F W L L P P A L	93
H. magnif.	ATTGGCCCTCCCGATATGGCCTTCCCACGACTAAACAATATAGTTTGTACTCCTCCTCGCGGTWA	280
	I G A P D M A F P R L N N I S F W L L P P G	88
D. yakuba	CTTTATTATTAGTAAGAAGAATAGTTGAAAACGGAGCTGGTACAGGTTGAACCTTTACCTCCTTATC	350
	S L L L V S M V E N G A G T G W T V Y P P L S	117
H. magnif.	TACTATNNNNNGCTCGCTTTGTTGAGCAAGGATAGGACNGGGTGACAGTTTATCCTCCTTATC	350
	I L S A F V E Q G V G T G W T V Y P P L S	109
D. yakuba	TTGAGGATCGCTCATGTTGGAGCTTCTGTAGATTAGCTATTTTTCTCTTCATTTAGCTGGAAATTCCT	420
	S T L F A V G G A S V D L A I F S L H L A G I S	140
H. magnif.	CGGCTTCAACAGCCTCGGGCGGGCGGTGATAGCCATCTTTAGCCTTCATTTAGCGGGTGGCTCT	420
	G I Q T H S G G A V D M A I F S L H L A G A S	132
D. yakuba	TCAATTTAGGAGCTGTAATTTTATGACTGTAATTAATACGACTGAACCTGGAATTCATGATAGACC	490
	R M P L F V W S V V I T A L L L L S L P V L A	187
H. magnif.	TCTATTTAGGGCAATGAATTTTATAACAACCATATTTAATATGAGAGCCCGCGGTTAACAGTGA	490
	S I L G A M N F I T T I F N M R A P G L T M D	155
D. yakuba	GAATACCTTATTTGATATGATCAGTACTTATCTGCTTTATACITTTTACTATCTTTACCACTTCTGGC	560
	R M P L F V W S V V I T A L L L L S L P V L A	187
H. magnif.	GACTCCCATTTTGTCTGCTATTTWAACWACTGCCITTTNATTTACTTCTCTACCACTTTTACG	560
	R L P L F V W S I T A F L L L S L P V L A	176
D. yakuba	CGGAGCTATTACTATTTTAAACAGACCGAAATTTAAACTCTCTTTTTGATCCAGCTGGAGGAGGA	630
	G A I T M L L T D R N L N T S F F D P A G G G	210
H. magnif.	AGGTGGAATAACCATGCTTTTAAACAGATAGGAAATTTAACAACCTTTTGGACCC	617
	G G I T M L L T D R N F N T T F F D	194

Fig. 1: Consensus from 12 sequences of the region of the COI gene flanked by primers LC01490 and HCO2198 for *Heteractis magnifica*. The sequence of *Drosophila yakuba* used to align it and the translated amino acid sequences for the two species are also shown.

Figure 1 is the consensus sequence of the region of the COI gene flanked by primers LC01490 and HCO2198 for *Heteractis magnifica* and that of *Drosophila yakuba* used for comparison. Fig. 1 also provides the translated amino acid sequence for the two species.

The sequence of *D. yakuba* consistently differed from that of *H. magnifica*, as did that of *Entacmea quadricolor*. However, within *H. magnifica* there was no clear a priori division of the sequences into two groups. Nor did separate analyses of clonal and solitary individuals reveal differences. In a stretch of 111 bases for which we had data from three pieces of the same individual, two of which had been frozen and one of which had been preserved in EtOH, the sequences differed in three bases. The consensus sequence of these three runs differed in two bases from the sequence of a clonemate. Individuals from three clones differed in two of 224 bases. In a stretch of 167 bases, two solitary anemones differed in four bases.

#### DISCUSSION

Based on the intra-individual comparison, we are confident that our technique produces an error rate of no more than 1% (3 in 3 x 111 bases). This is corroborated by our data from two clonemates. It is similar to the magnitude of the difference in sequences between solitary individuals, and between individuals of different clones.

Even after grouping the sequences by growth form, we detected no differences between clonal and solitary individuals of *H. magnifica*. This portion of the COI gene therefore supports conventional data that, unlike in the temperate sea anemones *Metridium senile* and *Anthopleura elegantissima*, both clonal and solitary morphs exist in the tropical anemone species *H. magnifica*. This conclusion would be strengthened by similar data from this species on a more rapidly evolving gene and by comparative data from a congeneric species. Data from *Entacmea quadricolor* are too few to be conclusive, but the sequences we did obtain are distinct from those of *H. magnifica*. We therefore infer this region of the COI gene may be informative for assessing interspecific differences in actinians, as it is for other taxa (Folmer et al 1994).

#### ACKNOWLEDGMENTS

This research was supported by Career Advancement Award (US National Science Foundation) DEB-9306403, and US National Science Foundation grant DEB-9521819 (PEET) to DGF. We are grateful for logistical support from CEDAM International, the Centre de Recherches Insulaires et Observatoire l'Environnement de Moorea, the Christensen Research Institute, Madang, Papua New Guinea, and the Lizard Island Research Station, Great Barrier Reef.

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