Longitudinal variation in algal symbionts (zooxanthellae) from the Indian Ocean zoanthid *Palythoa caesia*

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ABSTRACT: Single-celled, symbiotic, dinoflagellate algae known as zooxanthellae form associations with many shallow-water tropical marine invertebrates. Local ecological factors, particularly light levels, are known to influence algal type and distribution within hosts. Here I investigate whether biogeographic factors are similarly important in a convenient model, the zoanthid *Palythoa caesia* (Dana, 1846). Algal genotypes from *P. caesia* specimens from the eastern and western Indian Ocean were determined by restriction analysis and sequencing of the small subunit RNA gene, following PCR amplification with algal-specific primers. RFLP results indicate 2 common algal genotypes in the east, but only a single genotype in the west. Results from sequencing suggest further geographic patterning, with restricted geographic distribution of clades from the Seychelles, Sulawesi and Thailand.

KEY WORDS: Zooxanthellae · *Palythoa caesia* · Biodiversity · RFLP

INTRODUCTION

Zooxanthellae are the golden, brown or yellow unicellular algae that form symbiotic relationships with a wide range of marine invertebrates. Among the most important of these are members of the dinoflagellate genus *Symbiodinium* hosted by the Scleractinia (reef building corals) and many other Cnidaria and invertebrates (Freudenthal 1962; reviews by Davies 1992, Trench 1997, Rowan 1998). Zooxanthellae play a major role in the ecology of coral reefs, through their contribution to host nutrition (Muscatine 1973, Muscatine & Porter 1977, Davies 1984, Farrant et al. 1987, Sorokin 1991) and to the calcification process in corals (Goreau 1959, Smith & Douglas 1987, Marshall 1996).

The shortage of unambiguous morphological features led early workers to consider *Symbiodinium* monospecific, as *S. microadriaticum* Freudenthal, 1962. Subsequent work using 'classical' microbiological methods, including ultrastructure and biochemistry (reviewed

by Trench 1997) and the application of molecular genetic methods (reviewed by Rowan 1998) revealed a more diverse range of symbionts. Host-symbiont relationships were initially believed to be highly specific; however, further work revealed not only the occurrence of multiple strains within hosts, but that local environmental conditions drove ecological zonation in the relative abundance of different algal strains among colonies (Rowan & Knowlton 1995) and even over the surface of individual host colonies (Rowan et al. 1997) in response to differing light levels. Ecophysiological plasticity of individual algal strains is limited (Bythell et al. 1997), but multiple strains may provide a degree of flexibility to host:symbiont associations. In corals at least, zooxanthella diversity provides a mechanism for adaptation to changing environmental conditions (Buddemeier & Fautin 1993, Rowan et al. 1997). As a consequence, regional differences in zooxanthella diversity may be important (Baker & Rowan 1997).

There are currently rather little published data on broad-scale biogeographic patterns in zooxanthellae associated with any taxa. Rowan & Powers (1991) sampled the same taxa from multiple locations, but geographic variation was not their prime concern and

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sample sizes were too small to draw strong conclusions in this regard. Baker & Rowan (1997) showed significantly less diversity in coral-associated zooxanthellae from the eastern Pacific than in the Caribbean, but since there are no species common to these 2 areas they studied different hosts in each. Baillie et al. (2000) demonstrated that zooxanthellae isolated from giant clams were similar over a wide latidudinal range in the western Pacific, from the Great Barrier Reef to Japan, but used no more than 3 isolates per species in each locality. To date there are no published large-scale studies of geographic variation in single species with convincing sample sizes. Here, I examine patterns of zooxanthella diversity in an Indian Ocean species, the zoanthid *Palythoa caesia* (Dana, 1846). Specifically, I test the hypothesis that zooxanthella diversity is uniform along an east-west transect across the equatorial Indian Ocean from Indonesia to Tanzania, also taking in 2 south-western populations.

Zoanthids are an order of mostly colonial anthozoans in the subclass Hexacorallia. Their origins are obscure, although ultrastructural and molecular data indicate their closest relatives are probably the Antipatharia (Schmidt 1974, Berntson et al. 1999). *Palythoa caesia* is a geographically widespread zoanthid common throughout the Indian Ocean and the western Pacific (Ryland & Muirhead 1993, Burnett et al. 1995, 1997, Burnett & Ryland 1997, Ryland et al. 2000). Zooxanthellae in this species are environmentally transmitted, presumably after settlement and metamorphosis since neither broadcast-spawned oocytes nor larvae contain algal cells (Ryland et al. 2000). Zooxanthellae present in any one colony, therefore, reflect not only local environmental conditions and innate species preferences, but also local availability of algal strains. The results presented here show an east-west reduction in algal diversity associated with this species.

of 100% ethanol in their own plastic vial. Back in the laboratory, DNA was extracted from each sample using Puregene™ kits (Gentra Ltd) using the manufacturer's suggested protocol for preserved tissue. Extracts were stored frozen prior to use. Polymerase chain reaction (PCR) was used to selectively amplify algal small subunit rRNA genes from DNA extracts using zooxanthella-specific primers (Rowan & Powers 1991) in 50 µl reactions. Residual primers were removed by precipitating PCR products in 0.6 volumes of 20% polyethylene glycol (molecular weight 8000), 2.5 M NaCl and incubating at 37°C for 30 min. Precipitated PCR products were recovered by centrifugation, rinsed once in 70% ethanol, air dried and re-suspended in ultrapure water.

Symbiont type of all specimens was determined using restriction fragment length polymorphism (RFLP) (Rowan Powers 1991). PCR products were digested in a total volume of 20 µl with the restriction enzyme *Taq*1. The entire reaction volume was loaded onto a 2% agarose/Tris-EDTA-acetate gel and run for 2 h at 120 mV/180 mA in a cooled horizontal gel rig. Gels were stained in 500 ml of 0.5 µg ml^{-1} ethidium bromide solution for 20 min, destained in tap water for 2 min and visualised on a UV transilluminator.

Higher resolution data were obtained by DNA sequencing PCR products from a subset of specimens, including 7 Sulawesian, 4 Balinese, 9 Maldive, 4 Thai, 6 Seychelles, 5 Mauritian and 7 Zanzibar specimens. PCR products were direct sequenced using an ABI377 automated sequencer and primer NS21 (5′-AAT ATA CGC TAT TGG AGC TGG-3′) (Simon et al. 1992). Sequences were aligned by eye. A phylogeny was constructed using maximum parsimony, using the PHYLIP package (Felsenstein 1993). The aligned dataset was bootstrapped 1000 times, and nodes with greater than 70% support shown resolved in the resulting trees.

MATERIALS AND METHODS

Specimens of *Palythoa caesia* were collected, using SCUBA or by snorkelling, from the edges of unshaded colonies in shallow water (0 to 2 m deep) around Zanzibar, Seychelles, Mauritius and Indonesia during 1998 and 1999 (Table 1). Specimens from the Maldives and Rodrigues were collected from around 20 m. Five specimens were collected from Thailand with no information about degree of shading other than that they were intertidal. A small piece of tissue (ca. 50 mg) from each specimen was preserved in 2 ml

Table 1. Localities, sample numbers (n) and observed RFLP genotype frequencies of clade C and D algae (f_C and f_D, respectively). Locality numbers correspond to those used in Fig. 1

Locality name	Specific location	Latitude	Longitude	n	f_C	f_D
NE Sulawesi	South reef, Bunaken Island	1° 42′ N	124° 45′ E	49	34	15
SW Sulawesi	Samalona Island, Makassar Strait	5° 10′ S	119° 22′ E	12	9	3
SE Sulawesi	Patch reef, Kendari Bay	3° 58′ S	122° 45′ E	38	30	8
S Bali	Sanur Beach	8° 40′ S	115° 15′ E	20	15	5
Thailand	Fringing reef, Phuket	7° 53′ N	98° 23′ E	5	3	2
Maldives	Malé Atoll	4° 27′ N	73° 09′ E	21	10	11
Seychelles	Southeast reef, Isle St. Anne	4° 37′ S	55° 27′ E	35	35	0
Zanzibar	Bawe Island	6° 10′ S	39° 15′ E	29	29	0
Rodrigues	NE reef slope	19° 40′ S	63° 30′ E	13	13	0
Mauritius	Blue Bay	20° 30′ S	57° 16′ E	22	22	0

RESULTS

RFLP

Each *Palythoa caesia* colony contained only a single detectable algal type during RFLP analysis. *Taq*1 digestion patterns revealed the presence of 2 algal genotypes within *P. caesia*, corresponding to previously published patterns C and D of Rowan & Powers (1991), recently clarified by LaJeunesse et al. (in press; Fig. 1). Type C zooxanthellae were present in high frequency throughout the Indian Ocean, whereas type D was present in all populations east of and including the Maldives, but absent from all populations to the west (Fig. 2).

Fig. 1. *Taq*1 restriction fragment length patterns from type C and D zooxanthellae associated with *Palythoa caesia*. Fragment size is denoted in base pairs

Sequencing

All the individual colonies examined showed single, unambiguous sequences, but variation was observed among individuals at all localities except Zanzibar. From 42 specimens, 424 unambiguously aligned nucleotide sites were used in phylogenetic analysis (Fig. 3). Results show the presence of 2 major clades largely corresponding to RFLP clades C and D. A single specimen with RFLP type C, Mauritius 5, clustered with type D genotypes in the sequence analysis. The tree topology shows evidence of a biogeographic segregation of variability. Within major clade D, samples from Sulawesi and Thailand both form well-supported sub-clades, whilst Seychelles samples form a sub-clade within major clade C. The data were inadequate to resolve robustly a large polytomy at the base of clade C.

The disagreement on the identity of Mauritius 5 between the 2 methods (RFLP and sequencing) probably stems from the much larger number of characters used in sequence analysis. However, since the remaining 41 sequenced individuals fell into the correct RFLP clade, this result is not considered to seriously compromise the results of the RFLP analysis.

DNA sequences are available from GenBank, under the accession numbers AF 398922–398928 and AF 410390–410424

DISCUSSION

The observations presented here clearly demonstrate that, while some zooxanthella genotypes are geographically widespread, populations of western Indian Ocean *Palythoa caesia* have different algal complements from their eastern relatives. Clades C and D, as defined by RFLP patterns, are both present in the east, while only clade C occurs in the west. Phylogenetic analysis revealed well-supported sub-clades unique to Seychelles, Sulawesi and Thailand. The geographic distribution of zooxanthella genotypes associated with *P. caesia* is clearly not homogeneous.

Since vertical transmission (infection of juveniles with symbionts from the parent) does not occur in *Palythoa caesia* (Ryland 1997), zooxanthellae must be drawn from a free-living environmental pool. Species-specific preferences may influence which algae are accepted and which rejected, but these data show that *P. caesia* commonly harbours a diverse range of algae. The failure to observe clade D symbionts in western Indian Ocean samples may reflect either an ecological difference from the east or else reduced environmental availability of this type relative to clade C.

The ecological factor believed to have the greatest influence on symbiont clade within a particular host is irradiance (Rowan & Knowlton 1995, Rowan et al. 1997).
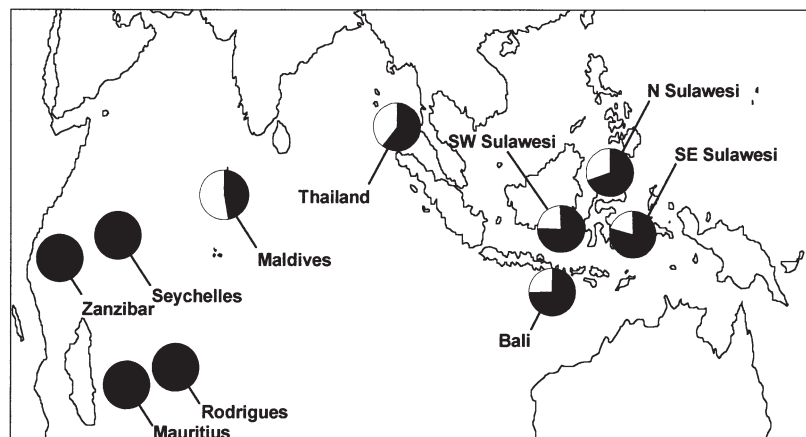

Fig. 2. Frequency distribution of zooxanthella RFLP genotypes from *Palythoa caesia* samples from the Indian Ocean. Within each pie diagram the frequency of type C symbionts is shown in black and type D in white
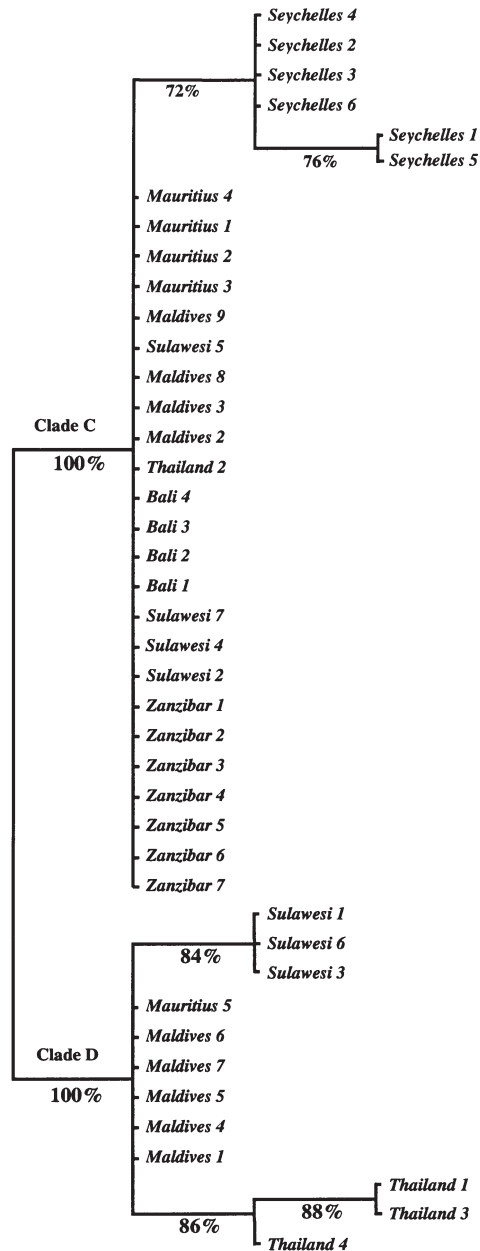
Fig. 3. Maximum parsimony phylogeny based on 424 un-ambiguously aligned nucleotide sites. Sequences fall into 2 major clades, corresponding to RFLP clades C and D for all specimens except Mauritius 5, which has a clade C RFLP pattern but a clade D sequence

absent from 2 high-latitude populations (Mauritius and Rodrigues). Furthermore, the Rodrigues sample was also taken in deeper, relatively turbid water and should have the lowest light levels of any sample. Eco-logical influences, as far as we currently understand them, do not appear to explain the observed distribu-tion of symbiont types in *P. caesia*. Conversely, there are plenty of precedents for a biogeographical expla-nation. Many taxa show a decline in diversity with increasing distance from the central Indo-west Pacific (Veron 1986, Sheppard 1998, Briggs 1999) while others show breaks in their distributions between east and west (Harlin 1996, Randall 1998, Ridgway et al. 1998). Genetic differences within taxa have been observed over the same scales (Williams & Benzie 1998). I con-clude that the failure to observe type D symbionts in western Indian Ocean *P. caesia* is more likely to reflect the limits of its geographic distribution than unfavour-able ecological factors.

Variation in zooxanthella diversity among geo-graphic regions could have major implications for local communities. Zooxanthella diversity may provide hosts with a rapid mechanism for adaptation to change (Buddemeier & Fautin 1993, Rowan et al. 1997); hence hosts in regions with depauperate floras may have few adaptive options (Baker & Rowan 1997). It should be noted, however, that the importance of variation within rRNA defined clades remains to be determined and the link between rRNA diversity and photophysiology clarified.

These data concern only a single host species, in a restricted set of geographic regions. Further wide-spread sampling of diverse taxa is required in order to determine if the geographic variability observed in symbioses of *Palythoa caesia* and zooxanthellae is unusual or the norm.

However, in most cases overall light levels should be similar among samples in this study; all except 3 are at low latitudes with clear shallow water. *Palythoa caesia* colonies are generally small (5 to 10 cm), flat and encrusting, so self-shading is not important. The increased incidence of type D strains in the deeper water sample from the Maldives might suggest that lower light levels favour this strain. However, D is

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