Coral Reefs (2006) 25: 297–301
DOI 10.1007/s00338-006-0094-9

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Assessment of the relative contribution of asexual propagation in a population of the coral-excavating sponge *Cliona delitrix* from the Bahamas

Received: 20 September 2005 / Accepted: 25 January 2006 / Published online: 4 March 2006

Abstract *Cliona delitrix* is a very destructive coral-excavating sponge in Caribbean coral reef systems, particularly for *Montastraea* species. Little is known about how these excavating sponges propagate across coral reefs. In this study a hypothesis was tested that coral breakage caused by the bioeroding activity facilitates the asexual propagation of this sponge and in turn favors the spread of the most aggressive sponge genotypes. An allozyme analysis, involving 12 loci systems of 52 sponge individuals from a total of 13 *Montastraea* heads, found that no two sponges possessed identical multi-locus genotypes. Contrary to the pattern expected for fragmenting species, the incidence of clonality and asexual propagation at the population level was minimal. The lack of correlation between genetic and physical distances for the studied sponges also suggests that population maintenance appears to derive from larval dispersal, with a spatial range of dispersal larger than the average distance between the coral heads (10–10^2 m).

Keywords Allozymes · Porifera · Asexual reproduction · Genotype

Communicated by Biology Editor H.R. Lasker

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Introduction

Some demosponges have the ability to excavate calcium carbonate substrata, such as the skeletons of scleractinian corals, producing galleries in which most of the sponge body is lodged (e.g., Rützler 2002). Because only inhalant papillae and exhalant oscules extend outside the galleries, the degree to which a calcareous substrate is internally invaded by excavating sponges is hard to estimate from external observation. As the sponge bores and grows inside a coral head, the skeletal structure progressively weakens to the stage at which it breaks into pieces. Fragmentation and disintegration of coral skeletons invaded by bioeroding sponges is accelerated by heavy storms, hurricanes, and any other source of physical stress (Tunnicliffe 1981; Highsmith 1982; Rützler 2002). This breakage and dispersal of coral fragments may spread copies of not only the coral genotype but also the sponges living inside the coral skeleton (Tunnicliffe 1981; Schönberg and Wilkinson 2001). Processes facilitating coral fragmentation, therefore, should also favor population maintenance of excavating sponges by an asexual propagation.

Although the activity of boring sponges has long been recognized as important to the ecological economy of coral reefs, very little is known about population dynamics of these sponges and the processes by which they propagate across coral reef systems. In this study, we provide a preliminary assessment of the relative contribution of asexual and sexual propagation to population maintenance in *Cliona delitrix* Pang 1973, a very destructive demosponge that is common in Caribbean reefs (Pang 1973; Rose and Risk 1985).

Cliona delitrix often inhabits massive scleractinians, such as *Diploria labyrinthiformis*, *Montastraea cavernosa*, and *Siderastrea siderea*, where it has been reported to excavate large galleries extending 10–12 cm deep into the coral skeleton (Pang 1973) or deeper (M. Maldonado, personal observation). The real extent of the

gallery system of a sponge individual and the limits within the galleries of genetically different individuals are difficult to estimate by any direct, non-destructive method (Schönberg 2001). It remains unclear, therefore, whether the inhalant and exhalant orifices found on different areas of large coral heads belong to several different *C. delitrix* individuals or just to a single, highly branched individual.

The ability to reproduce asexually appears to be ecologically advantageous to sessile organisms (Maynard-Smith 1992), and is a common process among marine invertebrates. Asexual propagation is expected to be particularly efficient in sponges, because many of their cell types are totipotent and able to regenerate as new small individuals (e.g., Simpson 1984; Maldonado and Uriz 1999a). Furthermore, asexual reproduction may interact synergistically with sexual reproduction to increase the dispersal efficiency and the chances of successfully colonizing distant habitats (Maldonado and Uriz 1999b). The asexual spread of coral-excavating sponges through coral fragmentation could have a relevant role on the population dynamics and genetic evolution of these sponges. This is particularly true if only one sponge genotype is able to recruit on each coral head, as shown for *Pione vastifica* boring on North-Atlantic scallop shells (Barthel et al. 1994). If this is the case in Caribbean *C. delitrix*, then those genotypes that bore more efficiently are predicted to cause coral fragmentation often, obtaining enhanced dispersal and higher frequencies in the reef population at the expense of a decrease in genetic diversity. In this study, allozymes were used to examine whether *Montastraea* coral heads are inhabited by one or more genotypes of *C. delitrix* and estimate the incidence of clonality at the population level.

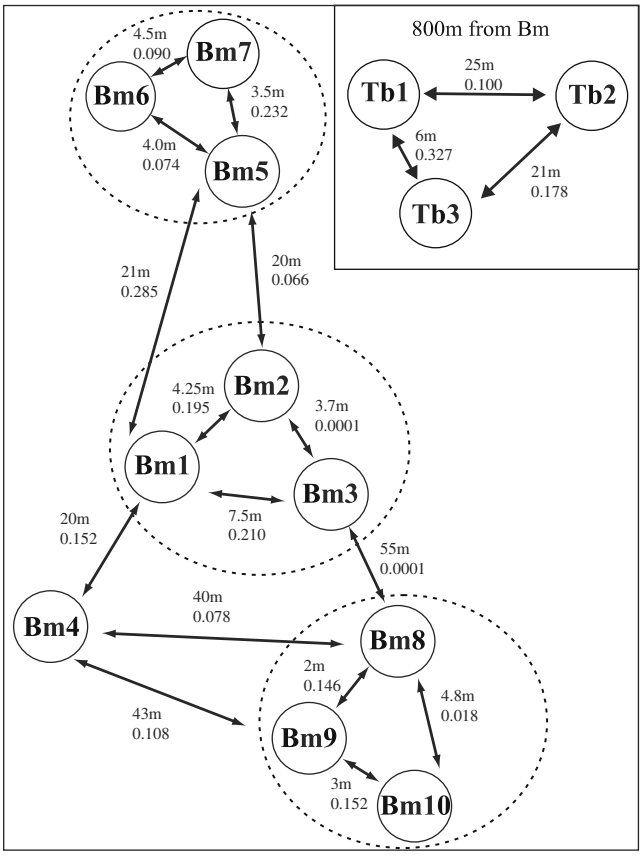

Fig. 1 Schematic representation of the 13 coral heads and boulders of *Montastraea cavernosa* and *Montastraea annularis*, showing the geographic (*above*) and genetic (*below*) distances. Only some of the measured distances are depicted; *Bm* Boomerang Point and *Tb* Tug and Barge reef. Some groups of coral boulders are fragments of old, larger coral heads that could be recognized based on their shape and relative position. Those coral groups are: Head I = Bm1, Bm2, and Bm3; Head II = Bm5, Bm6, and Bm7; Head III = Bm8, Bm9, and Bm10

Materials and methods

Sampling

During October and November 2001, this study investigated a *C. delitrix* population established in a patch reef characterized by large heads of *Montastraea annularis* and *M. cavernosa* scattered over a bed of *Thalassia testudinum* located at the lee side of Lee Stocking Island (Exuma Cays, The Bahamas). A total of 13 large coral colonies were selected, which were spread over a total study area of approximately 1 km^2 (Fig. 1). Ten coral colonies were located within a 100 m×100 m subarea (Boomerang site = Bm site), while the remaining three were close to each other, forming a group (Tug-and-Barge site = Tb site) about 800 m apart from the former (Fig. 1).

Allozyme analyses

To verify whether each coral head was excavated by one or more sponge genets, pieces of the sponge choanosomal tissue were sampled from the larger galleries directly underneath the inhalant papillae and oscules. Because *Montastraea* colonies around Lee Stocking Island were heavily infested by *C. delitrix*, it was possible to select 13 heads, in which the sponge was visible at several points of the coral head. Four samples were collected per head, making a total of 52 samples. Minimum between-sample distance on each coral head was 0.25 m, with sponge samples located on different sides of head and boulders whenever possible. Distances between coral heads were estimated (±50 cm) by direct measurement using an underwater metric line.

Immediately after collection, tissue samples were carefully dissected to avoid accidental contamination by small invertebrates which might have been living inside the sponges. Cleaned samples were then frozen on dry ice and stored in liquid nitrogen. Allozymes of *C. delitrix* were analyzed through horizontal 12.5% starch gel electrophoresis, using a Tris–EDTA–maleate pH 7.4 as buffer (Hillis et al. 1996) and following the standard methodology described elsewhere (Solé-Cava and

Thorpe 1986). Enzyme staining procedures followed Manchenko (1994). Thirty enzyme systems were tested, of which nine gave clear resolution and reproducibility: acid phosphatase (ACP, EC 3.1.3.2), adenilate kinase (AK, EC 2.7.1.20), catalase (CAT, EC 1.11.1.6), α-esterase (α-EST, EC 3.1.1.1), hexokinase (HK, EC 2.7.1.1), malate dehydrogenase (MDH, EC 1.1.1.37), peptidases (PEP, EC 3.4.1.1) with two different substrates, PEP I (Phe-Pro), PEP II (Leu-Ala), phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), and phosphoglucose isomerase (PGI, EC 5.3.1.9). Banding patterns were interpreted as the expression of 12 putative gene loci. All analyses were performed at least three times for each individual, placing them at different positions on the gel to ensure a reliable scoring of the putative alleles.

It was considered that two sponges were clone mates, whenever they yielded a banding pattern indicating identical genotypes over all loci. Allele frequencies, heterozygosities, deviations from Hardy–Weinberg equilibrium (F_{IS}; Weir and Cockerham 1984), and linkage disequilibria (Weir correlation coefficient) were calculated using Genetix 4.02 software (Belkhir et al. 1996), applying Bonferonni corrections when needed. The relationships among the sponge subpopulations were investigated by comparing metric and genetic (Nei 1978) distances between groups of sponges from different coral heads, using the Mantel test available through the IBD-SW Software (Bohonak 2002).

Results and discussion

From a total of 52 analyzed tissue samples, 47 provided interpretable results (36 from Bm site and 11 from Tb site, Table 1). No two individuals possessed identical multi-locus genotypes. The most parsimonious explanation for this result is that the 47 assayed sponges recruited from sexual propagules (i.e., free-swimming larvae), with no occurrence of clonality due to asexual processes (Table 1). For those coral heads in which the four tissue samples gave interpretable results, the consistent pattern was that each sample corresponded to a different sponge genotype, even when samples were only 25 cm apart from each other. In the case of *C. delitrix*, the bioeroding process leading to the potential fragmentation of a coral head appears to favor neither the asexual spread of some particular genotypes nor the population maintenance by asexual propagation. The absence of clonality in *C. delitrix* within the spatial range of our study does not necessarily mean that asexual reproduction is irrelevant in all excavating sponges. Recent studies suggest that boring sponges appear to be more conspicuous after storms or during periods of stressful conditions in the reef (López-Victoria and Zea 2004). Dispersal of boring sponges can be higher in those colonizing branching corals, which break and disperse more readily in storms (Schönberg and Wilkinson 2001), although, it is not clear if this is the direct result of dispersal or simply results from differential mortality.

The occurrence of multiple genotypes of *C. delitrix* per head strongly contrasts with the pattern shown by the sponge *Pione vastifica* when infesting scallop shells, since each shell was found to be occupied by just one sponge individual (Barthel et al. 1994). This could result from ecological differences between temperate areas, where *P vastifica* occurs, and the tropical *C. delitrix*. However, the observed difference could also be easily explained as a result of host size differences. In the small scallop shells (< 10 cm) spatial competition is expected to be intense and the development of mechanism facilitating pioneering recruits to prevent settlement of subsequent recruits cannot be ruled out (Hardin 1960). In contrast, coral boulders offer substrate areas far larger (> 2 m in diameter) which reduces the chances of direct spatial interaction when several genotypically distinct sponges co-occur.

C. delitrix exhibited high mean heterozygosities (Bm:$H_e = 0.444$, $H_o = 0.381$; Tb:$H_e = 0.425$, $H_o = 0.325$) at the two studied sites, which, in turn, is a common feature among invertebrates, particularly in sponges (Solé-Cava and Thorpe 1991; Boury-Esnault and Solé-Cava 2004). Additionally, sponges from both sites displayed significant heterozygote deficits ($F_{IS} = 0.150$ and 0.244 for Bm and Tb sites, respectively). Such deficits also seem to be common in populations of sponges and other marine invertebrates (Ayre and Dufty 1994; Miller et al. 2001). Observed heterozygote deficits could also result from miscoring of heterozygotes (France 1994), but this is highly unlikely, since we analyzed each individual at least three times to ensure reliable allele identification. However, we cannot discount the possibility that selection against heterozygotes (Borsa et al. 1992), the presence of null alleles (Foltz 1986), aneuploidy (Zouros and Foltz 1984), Wahlund effect, and/or inbreeding (Wallace 1981) have some role in causing the observed patterns of heterozygote deficits. We found no evidence of linkage disequilibrium between loci ($P > 0.05$).

Genetic distances between coral heads varied between 0.0001 and 0.330. This latter value is quite high for comparisons among conspecific populations (Thorpe and Solé-Cava 1994), although intraspecific levels of genetic distances in sponge populations tend to be higher than for other organisms (Solé-Cava and Boury-Esnault 1999). More likely, the high distances result from the high variances of gene distances when using small numbers of individuals (2–4 per coral head). Genetic distances were not significantly correlated with geographical distances (Mantel test; $r = 0.201$, $P \geq 0.05$; Fig. 1) among heads. This pattern suggests that larval settlement is unlikely to be philopatric and that the average spatial range of larval dispersal is larger than the average distance between coral heads within the range of this study.

Table 1 Genotypes of all analyzed individuals of *Cliona delitrix* from the two sites in the Bahamas

Samples	ACP	AK	PGD	MDH1	CAT	PGI	PEP1	PEP2	EST1	EST2
Bm1-1	cc	ab	bb	bb	aa	aa	bb	bb	ab	dd
Bm1-3	cc	bd	bb	bb	–	aa	–	–	ab	bd
Bm2-1	cc	bc	ab	ab	–	ab	ab	aa	ab	dd
Bm2-2	cc	bc	–	ab	–	aa	ab	–	ab	dd
Bm2-3	–	bc	bb	–	ab	aa	–	–	bb	dd
Bm2-4	cd	bc	bb	aa	–	bb	ab	–	bb	dd
Bm3-1	dd	bb	ab	ab	aa	ab	ab	aa	ab	dd
Bm3-2	cc	–	ab	bb	bc	ab	aa	bb	–	cd
Bm3-3	cd	bb	aa	ab	bc	ab	ab	aa	ab	cd
Bm3-4	cc	bb	bb	aa	bb	aa	–	cc	–	dd
Bm4-1	cd	ab	aa	aa	ab	ab	bc	aa	ab	dd
Bm4-2	cd	bc	ab	ab	ab	bb	bc	bb	ab	dd
Bm4-3	cd	ab	bb	ab	aa	ab	bc	bb	aa	dd
Bm5-1	cc	ac	aa	bb	aa	aa	bc	aa	aa	dd
Bm5-2	cc	ac	ab	aa	ab	aa	ab	aa	aa	cd
Bm5-3	cd	ac	aa	aa	ab	aa	aa	ab	bb	cc
Bm6-1	cd	bc	aa	aa	ab	ab	cc	ab	ab	ad
Bm6-2	cd	ab	ab	bb	ab	ab	bc	ab	ab	ad
Bm6-3	cc	bc	aa	aa	bb	ab	bc	ac	ab	ad
Bm6-4	dd	bc	ab	ab	ab	aa	bb	bc	bb	aa
Bm7-1	cd	bb	aa	ab	ab	bb	ab	bc	aa	bd
Bm7-2	cd	bb	ab	ab	bb	aa	bb	cc	ab	dd
Bm7-3	dd	bb	bb	aa	cc	aa	ab	cc	bb	dd
Bm7-4	dd	bc	ab	bb	bb	aa	ab	dd	aa	dd
Bm8-1	cd	cd	bb	bb	cc	aa	bb	–	bb	dd
Bm8-2	cd	bc	–	bb	ab	bb	–	–	bb	dd
Bm8-3	cd	bb	bb	aa	–	aa	–	–	ab	dd
Bm8-4	cc	bb	aa	bb	cc	aa	ab	bc	bb	dd
Bm9-1	cc	–	aa	aa	–	ab	–	–	ab	dd
Bm9-2	cc	bb	aa	ab	aa	ab	ab	–	ab	dd
Bm9-3	cd	bb	ab	ab	aa	ab	ab	dd	aa	dd
Bm9-4	cd	bb	bb	ab	–	aa	–	–	bb	dd
Bm10-1	cd	bb	aa	aa	bb	aa	bc	–	ab	cd
Bm10-2	cd	cc	aa	ab	–	ab	bc	–	ab	dd
Bm10-3	cd	bb	bb	aa	aa	ab	bb	bc	ab	cc
Bm10-4	cd	bb	bb	bb	bb	ab	bc	bc	ab	cd
Tb1-1	cc	ab	bb	aa	bb	bb	bc	cc	ab	dd
Tb1-2	cc	bd	ab	aa	ab	bb	bb	ab	ab	dd
Tb1-3	cc	ab	bb	aa	ab	bb	bb	ab	ab	bd
Tb1-4	cc	ab	ab	aa	ab	bb	bb	bb	bb	dd
Tb2-1	–	bb	bb	bb	aa	bb	–	–	bb	dd
Tb2-2	cd	cd	cc	ab	bb	bb	ab	bb	bb	dd
Tb2-3	cd	bc	bc	ab	ab	ab	ac	bc	ab	dd
Tb3-1	dd	bc	bb	aa	aa	aa	ac	ac	bb	dd
Tb3-2	–	cc	cc	aa	–	aa	ab	–	aa	dd
Tb3-3	dd	bb	ab	aa	aa	ab	ac	ac	ab	dd
Tb3-4	dd	bb	bb	–	aa	–	–	–	–	bb

Bm Boomerang Point; *Tb* Tug and Barge reef. Samples are numbered according to the coral head numbers depicted in Fig. 1, such that, Bm 1-1 and Bm1-2 are two samples from coral head Bm1. Monomorphic loci MDH 2 and HK are not shown

Acknowledgments The authors thank Dr. C. Lazoski for help with the allozyme analyses, E. N. Calderon for help with the figure, Dr. C. M. Young for logistic support to travel and work at Lee Stocking Island, and Jeff Watanabe and Shawn Arellano for help in sample collection. The authors also thank C. Schönberg and one anonymous reviewer for their valuable comments on the manuscript. This manuscript was submitted as partial fulfillment of the PhD degree to C. Zilberberg at Universidade Federal do Rio de Janeiro, Brazil. These investigations have been funded by CNPq and FAPERJ, as well as by a NOAA-NURP grant through the Perry Institute—Caribbean Marine Research Center (CMRC-00-NRCY-02-01A) and a grant from Spanish Ministry of Sciences and Education (BMC2002-01228).

References

Ayre DJ, Dufty S (1994) Evidence for restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia's Great Barrier Reef. Evolution 48:1183–1201

Barthel D, Sundet J, Barthel KG (1994) The boring sponge *Cliona vastifica* in a subartic population of *Chlamys islandica*—an example of balanced commensalism? In: van Soest RWM, van Kempen TMG, Braekman JC (eds) Sponge in time and space. Balkema, Rotterdam, pp 289–295

Belkhir K, Borsa P, Goudet J, Chikhi L, Bonhomme F (1996) Génétix, logiciel sous Windows pour la génétique des

populations. Laboratoire Génome, France, Populations et Interactions, CNRS UPR 9060. Université Montpellier 2
Bohonak AJ (2002) IBD (Isolation By Distance): a program for analyses of isolation by distance. J Hered 93:153–154
Borsa P, Jousselin Y, Delay B (1992) Relationship between allozymic heterozygosity, body size and survival to natural anoxic stress in the palourde, *Ruditapes decussatus* L. (Bivalvia: Veneridae). J Exp Mar Biol Ecol 155:169–181
Boury-Esnault N, Solé-Cava AM (2004) Recent contributions of genetics to the study of sponge systematics and biology. Bolletino dei Musei de l'Istituto di Biologia della Universita di Genova 68:3–18
Foltz DW (1986) Null alleles as a possible cause of heterozygote deficiency in the oyster *Crassostrea virginica*. Biochem Genet 24:941–956
France SC (1994) Genetic population structure and gene flow among deep-sea amphipods, *Abyssorchomene* spp., from six California Continental Borderland basins. Mar Biol 118:67–77
Hardin G (1960) The competitive exclusion principle. Science 131:1292–1297
Highsmith RC (1982) Reproduction by fragmentation in corals. Mar Ecol-Prog Ser 7:207–226
Hillis DM, Moritz C, Mable BK (1996) Molecular systematics, 2nd edn. Sinauer, Sunderland
López-Victoria M, Zea S (2004) Storm-mediated coral colonization by an excavating Caribbean sponge. Climate Res 26:251–256
Maldonado M, Uriz MJ (1999a) An experimental approach to the ecological significance of microhabitat-scale movement in an encrusting sponge. Mar Ecol Prog Ser 185:239–255
Maldonado M, Uriz MJ (1999b) Sexual propagation by sponge fragments. Nature 398:476
Manchenko GP (1994) Handbook of detection of enzymes on electrophoresis gels. CRC Press Inc, Ann Arbor
Maynard-Smith J (1992) Age and the unisexual lineage. Nature 356:661–662
Miller K, Alvarez B, Battershill C, Northcote P, Parthasarathy H (2001) Genetic, morphological, and chemical divergence in the sponge genus *Latrunculia* (Porifera: Demospongiae) from New Zealand. Mar Biol 139:235–250
Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590
Pang RK (1973) The ecology of some Jamaican excavating sponges. B Mar Sci 23:227–243
Rose CS, Risk MJ (1985). Increase in *Cliona delitrix* infestation of *Montastrea cavernosa* heads on an organically polluted portion of the Grand Cayman fringing reef. PSZNI Mar Ecol 6:345–363
Rützler K (2002) Impact of crustose clionid sponges on Caribbean Reef corals. Acta Geologica Hispanica 37:61–72
Schönberg CHL (2001) Estimating the extent of endolithic tissue of a clionid Great Barrier Reef sponge. Senckenbergiana maritima 31:29–39
Schönberg CHL, Wilkinson CR (2001) Induced colonization of corals by a clionid bioeroding sponge. Coral Reefs 20:69–76
Simpson TL (1984) The cell biology of sponges. Springer, Berlin Heidelberg New York
Solé-Cava AM, Thorpe JP (1986) Genetic differentiation between morphotypes of the marine sponge *Suberites ficus* (Demospongiae: Hadromerida). Mar Biol 93:247–253
Solé-Cava AM, Thorpe JP (1991) High levels of genetic variation in natural populations of marine lower invertebrates. Biol J Linn Soc 44:65–80
Solé-Cava AM, Boury-Esnault N (1999) Patterns of intra and interspecific genetic divergence in marine sponges. Mem Queensl Mus 44:591–601
Thorpe JP, Solé-Cava AM (1994) The use of electrophoresis in invertebrate systematics. Zool Scr 23:3–18
Tunnicliffe V (1981) Breakage and propagation of the stony coral, *Acropora cervicornis*. P Nat Acad Sci USA 78:2427–2431
Wallace B (1981) Basic population genetics. Columbia University Press, NY
Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370
Zouros E, Foltz DW (1984) Possible explanation of heterozygote deficiency in bivalve mollusks. Malacologia 25:583–591