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The extent of asexual reproduction in sponges of the genus *Chondrilla* (Demospongiae: Chondrosida) from the Caribbean and the Brazilian coasts

C. Zilberberg [a], A.M. Solé-Cava [a,*], M. Klautau [b]

[a] *Laboratório de Biodiversidade Molecular, Departamento de Genetica, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941-590-Rio de Janeiro, RJ, Brazil*

[b] *Laboratório de Biologia de Porifera, Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941-590-Rio de Janeiro, RJ, Brazil*

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Abstract

In this study, we estimated the extent of asexual reproduction and genotypic diversity at the intra- and interspecific levels in sponges of the genus *Chondrilla* in the Caribbean and along the coast of Brazil. Allozymes were used to identify the genotypes of specimens of *Chondrilla* in each location. The four species studied displayed a large variation in the extent of clonal reproduction and genotypic diversity, with the two species from the Bahamas having a greater proportion of asexually produced individuals than those along the coast of Brazil. Conspecific Brazilian populations of *Chondrilla* sp. had large differences in clonality: the population from a heterogeneous environment and under the influence of a strong upwelling had little clonality (7%), whereas the population located 350 km south along the coast, in a more homogeneous and temporally stable environment, had a five-fold larger (39%) proportion of asexually derived individuals. Finally, we were able to confirm that, besides fission, the genus *Chondrilla* displays a second mode of asexual reproduction, by fragmentation of teardrop shaped individuals.

Keywords: Allozymes; Clonality; Genotypic diversity; Porifera

1. Introduction

Many benthic marine invertebrates, such as corals, gorgonians and sponges, are known to combine sexual reproduction with asexual propagation (Wulff, 1991; Hunter, 1993; McFadden, 1997). Asexual reproduction is an efficient way to spread multiple copies of well-adapted individuals (Shick and Lamb, 1977; Ayre and Willis, 1988), avoiding the cost of meiosis, which, through recombination, might result in the loss of co-adapted genotypes (Maynard-Smith, 1992). The propagation of clones, in sessile organisms, can also provide an effective way to compete for space and decrease size-dependent mortality rates during recruitment (Hughes et al., 1992; Hall and Hughes, 1996). Evaluating the extent of clonality is important because asexual reproduction may bias estimates of genetic variation, effective population size and genetic identity among populations (Wright, 1978; Stoddart, 1984; Ayre and Willis, 1988). Extensive and detailed studies have examined the relative contribution of sexual and asexual reproduction among populations of many clonal organisms, including

* Corresponding author. Tel.: +55 21 2562 6389; fax: +55 21 2286 9819.

E-mail address: sole@biologia.ufrj.br (A.M. Solé-Cava).

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Table 1
Allele frequencies calculated from the genetically unique individuals (genets)

Locus/population	BAH I	BAH II	AC	UB	SA
ACP					
(*N*)	(0)	(0)	(21)	(0)	(18)
A	–	–	–	–	0.472
B	–	–	0.810	–	0.528
C	–	–	0.190	–	–
CAT					
(*N*)	(38)	(22)	(38)	(18)	(19)
A	0.040	0.341	–	–	–
B	0.303	0.386	–	–	0.421
C	0.368	0.182	–	–	0.579
D	0.263	0.091	0.105	0.556	–
E	0.026	–	0.895	0.444	–
HK					
(*N*)	(38)	(22)	(37)	(32)	(19)
A	0.053	0.159	0.284	–	–
B	0.750	0.727	–	–	–
C	0.145	0.114	0.554	1.000	–
D	0.052	–	–	–	–
E	–	–	0.162	–	1.000
MPI					
(*N*)	(0)	(0)	(35)	(11)	(0)
A	–	–	–	–	–
B	–	–	0.300	0.591	–
C	–	–	0.700	0.409	–
D	–	–	–	–	–
PEP I					
(*N*)	(37)	(21)	(0)	(32)	(19)
A	0.216	0.738	–	–	–
B	0.338	0.214	–	1.000	0.290
C	0.365	0.048	–	–	0.710
D	0.081	–	–	–	–
PEP II					
(*N*)	(37)	(22)	(0)	(32)	(19)
A	0.244	0.773	–	0.625	–
B	0.324	0.227	–	0.375	–
C	0.432	–	–	–	0.263
D	–	–	–	–	0.684
E	–	–	–	–	0.053
PEP III					
(*N*)	(35)	(22)	(0)	(32)	(19)
A	0.143	0.750	–	–	–
B	0.714	0.204	–	–	–
C	0.143	0.046	–	–	0.421
D	–	–	–	0.063	0.579
E	–	–	–	0.359	–
F	–	–	–	0.578	–
PEP V					
(*N*)	(0)	(0)	(31)	(0)	(0)
A	–	–	0.032	–	–
B	–	–	0.339	–	–
C	–	–	0.484	–	–
D	–	–	0.145	–	–
PGI					
(*N*)	(36)	(22)	(38)	(32)	(19)
A	–	0.318	–	–	–
B	–	0.682	–	–	–
C	0.972	–	1.000	1.000	1.000
D	0.028	–	–	–	–
PGM					
(*N*)	(0)	(0)	(21)	(0)	(0)
A	–	–	0.619	–	–
B	–	–	0.228	–	–
C	–	–	0.143	–	–
α-EST I					
(*N*)	(0)	(0)	(28)	(18)	(0)
A	–	–	0.036	–	–
B	–	–	0.964	1.000	–
α-EST II					
(*N*)	(0)	(0)	(0)	(18)	(0)
A	–	–	–	0.056	–
B	–	–	–	0.138	–
C	–	–	–	0.139	–
D	–	–	–	0.667	–
H_e	0.501	0.463	0.279	0.285	0.344
H_o	0.309	0.365	0.212	0.262	0.379

See text for population name codes. Only polymorphic *loci* are shown. PEP IV and PEP VI were monomorphic. PEP I=Gly–Tyr I, PEP II=Gly–Tyr II, PEP III=Phe–Pro, PEP V=Leu–Tyr, *N*=number of alleles sampled per *locus*, H_e=mean expected heterozygosity, H_o=mean observed heterozygosity.

sponges (Neigel and Avise, 1983; Stoddart and Taylor, 1988; Battershill and Bergquist, 1990; Wulff, 1991; Hunter, 1993; Ayre and Dufty, 1994; McFadden, 1997; Ayre and Miller, 2004).

Sponges are important members of benthic marine communities (Witman and Sebens, 1990) and, as other clonal organisms, can reproduce sexually, by gamete production, and asexually, by fission, fragmentation, budding and gemmulae (Bergquist and Sinclair, 1973; Fell, 1993). The presence of asexual reproduction in sponges has been strongly supported by histocompatibility, field observations and population genetic studies (Neigel and Avise, 1983; Wulff, 1991, Davis et al., 1996; Miller et al., 2001, Duran et al., 2004; Whalan et al., 2005). However, there has not been, to date, any direct attempt to genetically estimate the extent of clonality in sponges.

The sponge genus *Chondrilla* Schmidt 1862 can be a good model to determine the extent of sponge asexual propagation both at the inter- and intraspecific levels, due to their world-wide distribution and known asexual reproduction by fission (Gaino and Pronzato, 1983; Fromont, 1999, Usher et al., 2004). Teardrop shaped individuals have also been observed in *Chondrilla australiensis* and *Chondrilla nucula*, where the sponge tissue becomes thin at the point of attachment to the adult sponge and thicker at the edge where it hangs. This was speculated to be a form of asexual reproduction through passive fragmentation (Fromont,

1999; Sidri et al., 2005), as seen in other clonal organisms, like zoanthids (Acosta et al., 2005). However, what remains unknown is whether these teardrop shaped sponges, after detaching, are capable of recruiting elsewhere.

In this study, we compared the genetic variability and the extent of asexual reproduction of four species of the genus *Chondrilla* from the Caribbean and Brazilian coasts (see Klautau et al., 1999 for details), using allozymes. Allozyme electrophoresis has been extensively used in studies of the contribution of asexual reproduction in clonal invertebrates (Hunter, 1993; McFadden, 1997; Ayre and Hughes, 2000; Ayre and Miller, 2004; Miller and Ayre, 2004). This is the first attempt to estimate, using genetic techniques, the relative contribution of asexual reproduction in sponge populations at both the inter- and intraspecific levels.

2. Materials and methods

2.1. Studied species and sites

On account of the several undescribed cryptic species, within what was formally known as "*C. nucula*" (Klautau et al., 1999), we will be using numbers to denominate different species. Most of the species observed correspond to those found by Klautau et al. (1999). Additionally, two further sympatric species, from the Bahamas, were found by the presence of a diagnostic *locus* (Table 1). Four species of *Chondrilla* were sampled: two in the Caribbean and two along the Brazilian coast (Fig. 1). To determine whether there were differences on the extent of asexual reproduction between sympatric species of *Chondrilla*, the relative proportions of ramets and genets (*sensu* Harper, 1977) were estimated for *Chondrilla* sp. 1 (*Chondrilla* sp. 'C', *sensu* Klautau et al., 1999) and *Chondrilla* sp. 2 (Zilberberg, 2006) occurring at 3–5 m of depth at the Tug and Barge reef (Lee Stocking Island, Bahamas; 24°15′N, 76°00′W; Fig. 1).

We also examined the intraspecific differences on the relative contribution of asexual reproduction between localities, comparing a population of *Chondrilla* sp. 3 (*Chondrilla* sp. 'B'; *sensu* Klautau et al., 1999) from Arraial do Cabo (1–2 m depth; Forno beach, Rio de Janeiro State, Brazil; 23°52′S, 42°01′W; Fig. 1) with one from Ubatuba (Picinguaba beach; 1–2 m depth; São Paulo State, Brazil; 23°22′S, 44°50′W; Fig. 1). In Arraial do Cabo, the substrate is characterized by the presence of boulders with a high cover of algal turf and interspaced by sand while, in Ubatuba, the rocky substrate is mostly consolidated and homogeneous, with a few boulders but no sand. Also, Arraial do Cabo is under the influence of a strong upwelling during spring and summer months, so that, even though yearly average temperatures are the same as in Ubatuba (18–23 °C), during the

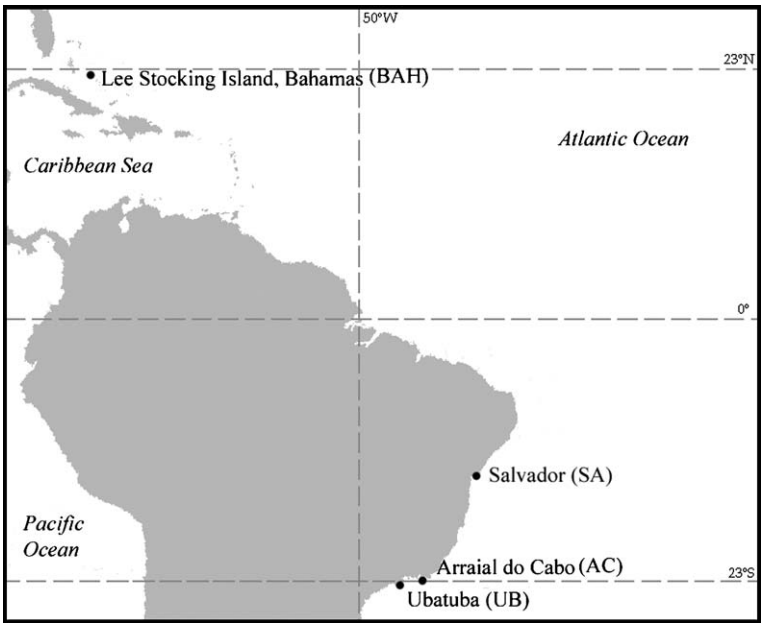

Fig. 1. Study sites: One in the Bahamas and three along the Brazilian coast.

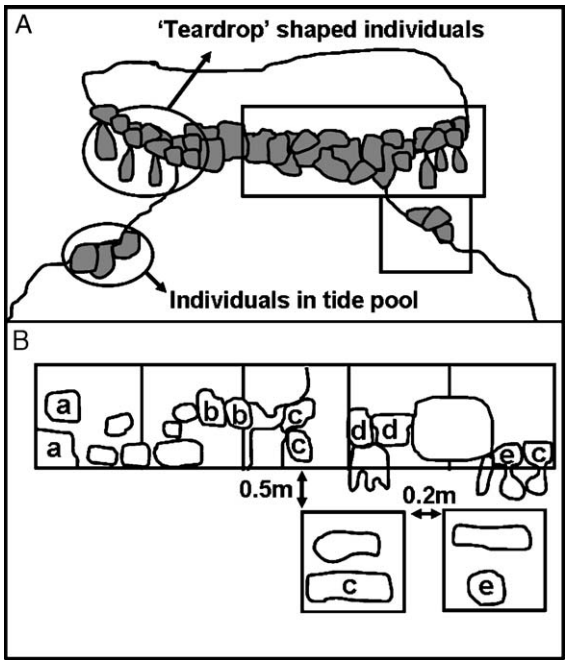

Fig. 2. Schematic representation of the site in Salvador where *Chondrilla* sp. 4 was mapped. (A) Location of individuals found on the overhangs and on tide pools right below. (B) Detailed mapping of individuals within quadrats. Groups of genetically identical ramets are represented by the same letters. The two lower quadrats show two individuals that could be the products of asexual reproduction from teardrop shaped individuals located at the above quadrats.

upwelling season surface temperatures can drop to 12 °C (Yoneshigue, 1985; Yoneshigue-Valentin and Valentin, 1992; Floeter et al., 2001).

Finally, to confirm whether the teardrop shaped sponges were able to detach and settle on the substrate below, a population of *Chondrilla* sp. 4 (Zilberberg, 2006) was mapped at Salvador (Guarajuba beach, Bahia State, Brazil; 12°22′S, 38°30′W; Fig. 1). Individuals of *Chondrilla* sp. 4 occurred at the mid-intertidal area, and were often exposed at low tides, contrasting with the other four populations, where individuals were found subtidally. At the studied site in Salvador, individuals were distributed along boulders, where the rocky substrate had overhangs, and individuals located at their edges were often shaped like small teardrops (5–10 cm; Fig. 2). The sponges on the overhangs were genetically compared to those on the rocks 50 cm below, to verify if they were clone-mates.

2.2. Collection

In the Bahamas, all *Chondrilla* sp. 1 and *Chondrilla* sp. 2 (in this study denominated as BAH I and BAH II, respectively) specimens found within three 1 m^2 quadrats, placed 2 m from each other along a transect line, were collected. In Arraial do Cabo, a transect line was laid parallel to the water line, where *Chondrilla* sp. 3 was common (called here AC), and all individuals within each 1 m^2 quadrat were collected. Five quadrats were placed at intervals of 0 m, 8 m, 16 m, 20 m, and 50 m along the transect line, to estimate the potential dispersal of clone-mates and the overall contribution of asexual reproduction. In the other two Brazilian locations, Ubatuba (*Chondrilla* sp. 3; denominated here as UB) and Salvador (*Chondrilla* sp. 4; denominated SA), 0.25 m^2 quadrats were placed along transect lines and every individual within quadrats was carefully mapped (Figs. 2 and 3). In Ubatuba, three mappings were completed at three different times (March, August, November 2002), totaling 14 quadrats distant between 0.3 m and 300 m from each other, to make sure that no individual sponge was collected more than once. In all cases, a sample of each individual was collected, frozen in dry ice or liquid nitrogen and brought to the laboratory for the genetic analyses.

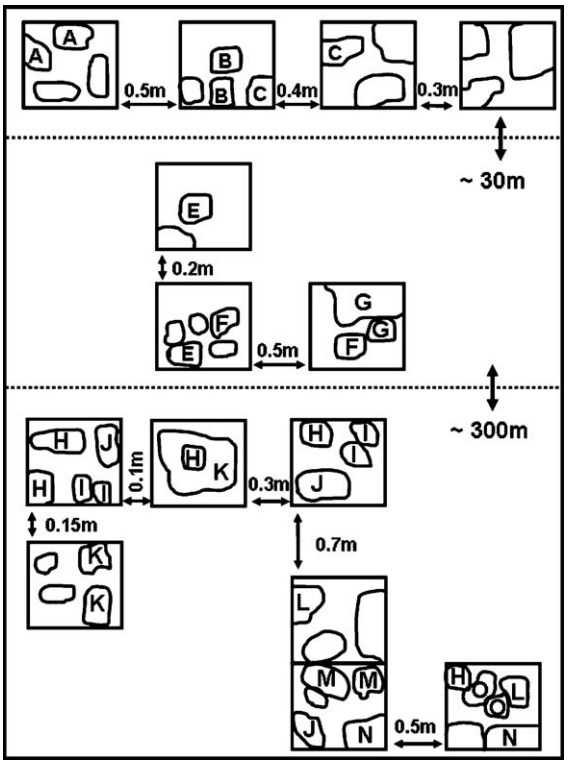

Fig. 3. Detailed mapping of individuals in the Ubatuba population of *Chondrilla* sp. 3. Groups of genetically identical ramets are represented by the same letters. Distances along transect lines are depicted between each quadrat. Three mappings were completed at different times (separated in the figure by dotted lines).

2.3. Allozyme electrophoresis

Horizontal 13% starch gel electrophoresis was carried out as previously described for sponges (Solé-Cava and Thorpe, 1986). Thirty enzyme systems were tested, of which eight gave clear and consistently reproducible results. The buffer system used was the Tris–EDTA–Maleate pH 7.4 (Hillis et al., 1996), and the enzymes used were: Acid Phosphatase (ACP, EC 3.1.3.2), α-Esterase (α-EST, EC 3.1.1.1), Catalase (CAT, EC 1.11.1.6), Hexokinase (HK, EC 2.7.1.1), Mannose 6-Phosphate Isomerase (MPI, EC 5.3.1.8), Phosphoglucomutase (PGM, EC 5.4.2.2), Phosphoglucose Isomerase (PGI, EC 5.3.1.9), and Peptidases (PEP, EC 3.4.1.1) with five different substrates, PEP I and PEP II (Glycine–Tyrosine), PEP III (Phenylalanine–Proline), PEP IV (Proline–Phenylalanine), PEP V (Leucine–Tyrosine) and PEP VI (Proline–Phenylalanine–Alanine). The enzymes PEP IV, PEP V and PEP VI were used only in the Arraial do Cabo population. Enzyme staining followed standard procedures (Manchenko, 1994). All allozyme electrophoresis analyses were performed at least twice for each individual, placing them each time at different positions on the gel, ensuring the accurate assignment of genotypes.

2.4. Data analyses

In this study, specimens with a continuous pinacoderm were treated as ramets, and genetically unique individuals (i.e., products of sexual reproduction) were called genets (*sensu* Harper, 1977). Clone-mates are ramets that belong to the same genet. For measures of genotypic diversity, individuals were considered clone-mates if they possessed the same genotype across all *loci*. Given the observed heterozygosities, and assuming the absence of large deviations from Hardy–Weinberg equilibrium, the probabilities of any two individuals presenting the same multi-locus genotype by chance varied between 10^{-2} and 10^{-6} (Monteiro et al., 1998).

Allele frequencies, heterozygosities, deviations from Hardy–Weinberg equilibrium (F_{IS}; Robertson and Hill, 1984; Weir and Cockerham, 1984) and linkage disequilibria were estimated for each population using the Genetix version 4.02 program (Belkhir et al., 1996). Significance of F_{IS} (H_o: $F_{IS}=0$) was tested by permutation-based statistical inference, with 1000 permutations (Belkhir et al., 1996). All calculations were done either using all individuals analyzed (ramets), or only the genetically unique individuals (genets).

We compared the contribution of asexual reproduction within populations using two measures of genotypic diversity. The first was the proportion of unique genotypes in each population, N_c/N, where N_c is the number of distinct genets observed and N is the total number of ramets (Ellstrand and Roose, 1987; Ayre and Hughes, 2000). Because only a small portion of the genome was analyzed (5–7 gene *loci*), it is possible that genetically different individuals could be electrophoretically identical over the *loci* analyzed. Therefore, $1-(N_c/N)$ provides a maximum estimate of asexual reproduction in the studied populations (Johnson and Threlfall, 1987). The second measure of clonality used was the observed genotypic diversity (G_o, Stoddart and Taylor, 1988):

$$G_o = 1 \Big/ \sum_{i}^{k} g_i^2$$

where k is the number of different compound genotypes in the population, and g_i is the relative frequency of genotype i. g_i is calculated as $g_i=n_i/N$, where n_i is the number of ramets of genotype i in a sample of N individuals. When there is only one genotype in the population, G_o is 1; when the genotypes are distributed uniformly in the population, G_o will have the maximum value of k.

The differences between observed (G_o) and Hardy–Weinberg expected (G_e) genotypic diversities were tested using a *t*-test following Stoddart and Taylor (1988), with a program developed in FORTRAN (J.A. Stoddart, unpublished). A population dominated by few genotypes will have a low G_o/G_e ratio, while in populations with many genotypes and few clones G_o/G_e will approach unity.

Since the number of analyzed *loci* was different among populations, a Spearman rank correlation analysis was performed, using the program Systat 10 (Systat Software, Inc., Point Richmond, CA), to test whether the number of analyzed *loci* was correlated to the proportion of unique genotypes found in each location.

3. Results

Allele frequencies and heterozygosities of the 14 *loci* analyzed were similar in the calculations based only on genets or including all ramets. Therefore, we calculated gene frequencies based on genets only (Table 1). Mean observed heterozygosities were high for all populations (Table 1), which is a common feature of most marine invertebrates, particularly sponges (Solé-Cava and Thorpe, 1991). Significant deficits and excesses of heterozygotes were detected for a number of *loci* in

most populations (Table 2). Linkage disequilibrium was not found when calculations were performed only with genets, but it was evident ($P<0.01$) when calculations were done including all ramets of the BAH I, BAH II and UB species.

The proportion of asexually produced individuals was high for most species (27–52%), with the exception of the Arraial do Cabo population of *Chondrilla* sp. 3 (7%; Table 3). The number of unique genotypes (N_c) varied greatly among populations (Table 3), but it was not significantly correlated to the number of analyzed *loci* (Spearman Rank Correlation: $R=0.671$, $P>0.100$). Additionally, genotype diversities (G_o) were significantly lower than those expected under purely sexual reproduction in all samples, except in AC (Table 3).

In most cases, less than 40% of the genets within each sample were effectively contributing to asexual reproduction. In the AC population, most individuals were genetically unique, with the exception of three genets each with two ramets. One of the genets had ramets in quadrats 8 m apart, while the others were in the same quadrat. On the other hand, in the Bahamas, a few genets had a considerably higher asexual contribution than others. Indeed, for the BAH I species, a single genet had a total of nine ramets and other two had five ramets each. For the BAH II, one genet had eleven ramets and, another, six ramets. Most clone-mates were found within the same quadrats, with one exception for BAH I and one for BAH II. In both cases clone-mates were found in quadrats distant 2 m from each other. In Salvador (Fig. 2) and Ubatuba (Fig. 3), where each sponge was mapped, ramets were usually found living side-by-side. In Salvador, the comparison between overhanging teardrop shaped individuals and individuals below them showed that they were genetically identical (Fig. 2).

Table 3
Genotypic diversity measures for the analyzed species and populations

Population	N	N_c	$1-(N_c/N)$	G_o	S.D.	G_e	G_o/G_e
BAH I	63	40	0.37	20.56	4.37	60.06	0.34*
BAH II	46	22	0.52	9.98	4.74	42.42	0.24*
SA	26	19	0.27	14.70	3.43	23.35	0.63*
UB	54	33	0.39	26.04	5.2	48.05	0.54*
AC	41	38	0.07	35.77	3.34	39.39	0.91

See text for population name codes. N=sample size; N_c=number of unique genotypes; $1-(N_c/N)$=maximum contribution of asexual reproduction; G_o=observed genotypic diversity; G_e=genotypic diversity expected under H–W equilibrium; S.D.=observed genotypic diversity standard deviation; *=significant deviation from expectation ($P<0.05$).

4. Discussion

Species of the genus *Chondrilla* display marked differences on the extent of asexual reproduction and genotypic diversity, with the species from the Bahamas exhibiting lower observed genotypic diversity than those from Brazil. Environmental conditions also seem to be important in determining the extent of asexual reproduction, since the two analyzed populations of *Chondrilla* sp. 3, living in different environments, had marked differences in clonality. Also, our data indicate that the teardrop fragments produced by *Chondrilla* are

Table 2
Estimates of heterozygosity departures (F_{IS}) expected under Hardy–Weinberg equilibrium for the analyzed species and populations, utilizing only the unique compound genotypes (GENETS) or including all individuals (RAMETS), at each allozyme *locus*

	Genets					Ramets				
Locus	BAH I	BAH II	AC	UB	SA	BAH I	BAH II	AC	UB	SA
ACP			−0.212*		0.247			−0.212*		0.134
α-EST I			−0.019*					−0.018*		
α-EST II				0.163					0.057	
CAT	0.000	0.105	−0.105*	−0.097	−0.491*	−0.204*	−0.088	−0.096*	−0.212	−0.625*
HK	0.435	−0.238*	0.503*			0.437*	−0.206*	0.521*		
MPI			0.534*	0.107				0.549*	−0.144	
PEP I	0.660*	0.663*			−0.125	0.447*	0.760*			−0.171
PEP II	0.428*	0.751*		0.215	−0.350*	0.390*	0.670*		0.242*	−0.302*
PEP III	0.502*	0.667*		0.019	0.163	0.601*	0.759*		−0.035	0.299*
PEP V			−0.061					−0.040		
PGI	−0.014	−0.448*				−0.008*	−0.460*			
PGM			0.489*					0.489*		
All *loci*	0.386*	0.216*	0.242*	−0.080	−0.104	0.285*	0.156*	0.252*	−0.017	−0.128

Calculations were done only for the polymorphic *loci*. See text for population name codes. Positive values denote heterozygote deficits and negative values denote heterozygote excesses. Asterisks indicate values significantly different from H–W equilibrium after Bonferonni correction ($P<0.05$).

able to detach and recruit in another substrate, supporting the suggestion of Fromont (1999) and Sidri et al. (2005) that this is another mode of asexual reproduction in the genus *Chondrilla*. Overall, sexual reproduction was the main source of individuals in all populations of *Chondrilla*, given that, on average, more than 60% of individuals were genetically unique. The levels of clonality found in the present study are similar to those observed in previous studies of asexual contribution in sponges, using field observations, histocompatibility techniques and other population genetic studies (Neigel and Avise 1983; Wulff, 1991; Davis et al., 1996; Miller et al., 2001, Duran et al., 2004; Whalan et al., 2005).

Estimates of *locus*-wise Hardy–Weinberg departures showed a mixture of heterozygote deficits and excesses. This was true even when ramets (i.e., clone-mates) were excluded from the analyses. Therefore, the observed deviations could not solely be explained by asexual reproduction as it has often been suggested (Stoddart, 1984; Ayre and Dufty, 1994; Russo et al., 1994; Miller et al., 2001). Deviations from Hardy–Weinberg equilibrium are a common feature of many clonal marine invertebrates, including sponges (Black and Johnson, 1979; Ayre and Dufty, 1994; Miller et al., 2001; Duran et al., 2004; Schama et al., 2005; Whalan et al., 2005) and might result from several factors, including miscoring of heterozygotes (France, 1994), natural selection (Borsa et al., 1992), null alleles (Foltz, 1986), aneuploidy (Zouros and Foltz, 1984), Wahlund effect (Li, 1976), and inbreeding (Wallace, 1981). Miscoring seems highly unlikely in this study, since care was taken to ensure correct identification of genotypes, running all samples at least twice (see Materials and methods for details). Selection is also improbable, since the deviations were observed more or less haphazardly across *loci*, not showing any signature that might result from selective forces. The possibility of aneuploidy, presence of null alleles, Wahlund effect and inbreeding, however, may not be discarded as having some effect on the observed deviations.

The low genotypic diversity found for most *Chondrilla* species is similar to those found in populations of the sponge *Latrunculia* in New Zealand (Miller et al., 2001), and other clonal taxa (Hunter, 1993; McFadden, 1997). Additionally, many studies on clonal organisms have shown that the extent of asexual reproduction can vary across species and populations (Hoffman, 1986; Hunter, 1993; McFadden, 1997; Miller et al., 2001). In this study, we found widely variable (from 7% to 52%) contributions of clonality in *Chondrilla*. Asexual reproduction was important for the two *Chondrilla* species from the Bahamas, which had ramets unevenly distributed among genets. This pattern might be explained by a higher rate of clonality in a few locally adapted genotypes, or to a highly variable survival rate among individuals of different genotypes.

Interestingly, conspecific populations from two areas separated by approximately 350 km along the Brazilian coast displayed contrasting levels of clonality (7% in the Arraial do Cabo and 39% in Ubatuba). Differences in the extent of asexual reproduction among populations of the same species have been demonstrated for other clonal organisms, such as corals and gorgonians (Hoffman, 1986; Hunter, 1993; McFadden, 1997; Ayre and Hughes, 2004). Asexual reproduction seems to be positively correlated with temporal and spatial stability of the environment (Ayre, 1984; Karlson, 1991). Ayre (1984) demonstrated that stable and more homogenous shores were dominated by asexual individuals of the anemone *Actinia tenebrosa* Farquhar 1898, while a more heterogeneous environment sustained a population dominated by sexual recruits. Individuals in Ubatuba are located in an apparently more homogeneous environment than those of Arraial do Cabo. In Ubatuba, the substrate is consolidated and homogeneous, whereas Arraial do Cabo is characterized by boulders covered with algal turfs interspaced by sand. Additionally, Arraial do Cabo differs from the other sites analyzed by the presence of a strong upwelling event during spring and summer months, which might contribute to temporal environmental instability (Valentin, 1984; Yoneshigue-Valentin and Valentin, 1992; Floeter et al., 2001). However, other factors such as predation, competition, wave exposure, substratum stability and sedimentation may account for the disparity in the levels of clonality observed between these two conspecific populations of *Chondrilla* (Ayre, 1984; Karlson, 1991; Hunter, 1993; McFadden, 1997; Ayre and Hughes, 2000, 2004).

Although there were differences in the extent of asexual propagation among localities, all species behaved similarly in presenting a limited dispersal of clone-mates. Similar results have been found in the soft coral *Alcyonium rudyi* Verseveldt and Ofwegen 1992 and other cnidarians, where clonal reproduction was also restricted (Ayre, 1984; McFadden, 1997). Sponges, like many other clonal organisms, are known to have indefinite growth and be long lived (Wörheide et al., 1997). The longevity of clones is an important factor affecting the size and distribution of ramets of a single genet (Tanner, 2001). The longer a ramet survives the wider it is expected to be distributed (Ayres and Ryan, 1997; Tanner, 2001). In the case of encrusting sponges,

which usually grow two dimensionally, processes such as fission and partial mortality allow the formation of smaller individuals that can continue to grow away from the parental individual (Hall and Hughes, 1996; Tanaka, 2002). The restricted spatial distribution of clone-mates in *Chondrilla* populations suggests that either they grow so slow that their dispersal is very limited, or that they were the result of recent asexual proliferations. The *Chondrilla* population from Arraial do Cabo seems to suffer reductions in population size during the fall (Zilberberg, personal observation), although the reason for this reduction it is still unknown. This type of seasonality has been described for other sponge species, such as *Paraleucilla magna* Klautau et al. 2004, which is abundant in Rio de Janeiro, Brazil, during the summer but disappears in the fall to reappear again as small individuals during winter (Klautau et al., 2004). However, this seasonality is not observed in Ubatuba, since individuals were easily found and mapped at three different times along the year.

The hypothesis that teardrop shapes are another type of asexual reproduction in *Chondrilla* (Fromont, 1999) is supported by our data. We observed that two out of the four sampled individuals were genetically identical to teardrop shaped individuals located on the overhang 50 cm above. This phenomenon is not exclusive to *Chondrilla*, since it has been observed in other species, like *Chondrosia reniformis* (Sarà and Vacelet, 1973; Bonasoro et al., 2001). However, the unique characteristic of this *Chondrilla* population is that the teardrop shaped individuals are quiet small (5–10 cm) and located in an exposed intertidal rocky area, contrasting with the long teardrop shapes of *C. reniformis* that occurs subtidally (Bonasoro et al., 2001). Hence, it is very unlikely that the teardrops in the *Chondrilla* specimens reach the substrate 50 cm underneath them before they detach, like observed in *Chondrosia*. The fragmentation in *Chondrilla* is probably passive, such that, as the sponge grows, its own weight might allow the individuals to break-off (Bonasoro et al., 2001).

Asexual reproduction is an important reproductive mechanism in *Chondrilla*, but less frequent than in many clonal organisms, such as plants and gorgonians (Ellstrand and Roose, 1987; McFadden, 1997). It would be interesting to verify if this is a feature of *Chondrilla* or if it is a more general phenomenon in sponges.

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