

## DNA fingerprints of a gorgonian coral: a method for detecting clonal structure in a vegetative species

Mary Alice Coffroth<sup>1</sup>, Howard R. Lasker<sup>1</sup>, Margaret E. Diamond<sup>1</sup>, Jeremy A. Bruenn<sup>1</sup> and Eldredge Bermingham<sup>2</sup>

<sup>1</sup> Department of Biological Science, State University of New York at Buffalo, Buffalo, New York 14260, USA  
<sup>2</sup> Smithsonian Tropical Research Institute, Apdo 2072 Balboa, Republic of Panama

Date of final manuscript acceptance: June 1, 1992. Communicated by J. Grassle, New Brunswick

**Abstract.** Clonal reproduction, a common life history strategy among sessile marine invertebrates, can lead to high local abundances of one to a few genotypes in a population. Analysis of the clonal structure of such populations can provide insight into the ecological and evolutionary history of the population, but requires markers that can identify individual genets. Forensic and demographic studies have demonstrated that DNA fingerprinting can provide markers that are unique for an individual genotype. We have generated DNA fingerprints for over 70 colonies of the clonal gorgonian, *Plexaura A* (*Plexaura* sp. A) collected from June 1990 through July 1991 in the San Blas Islands, Panama. DNA fingerprints within a single individual were identical and fingerprinting resolved multiple genotypes within and among reefs. On one reef in the San Blas Islands, Panama, 59% of the colonies sampled were of one genotype and this genotype was not found on any other sampled reefs. A previous study using tissue grafts identified 13 putative clones on these reefs, while DNA fingerprints of the same colonies differentiated 17 genotypes. The present study demonstrates the utility of DNA fingerprinting for distinguishing clones and for identifying clonal structure of marine invertebrate populations.

son and Threlfall 1987). The pervasiveness of this strategy implies its evolutionary success, but the importance of clonal growth undoubtedly varies among species. Since the genetic structure of the population reflects the relative contributions of sexual and clonal reproductive strategies, analyses which measure genotypic diversity and the extent and size of clones can be used to deduce the commonness of sexual and vegetative reproduction (Neigel and Avise 1983a, Willis and Ayre 1985, Carvalho et al. 1991).

Analyses of the genetic structure of a population requires the accurate identification of individual genotypes. This entails the use of genetic markers which, ideally, provide a unique representation for each genotype. Past histocompatibility and electrophoretic studies have characterized clonal structure among several species of invertebrates (Hoffmann 1976, 1986, 1987, Ayre 1983, 1984, Neigel and Avise 1983a, b, Stoddart 1984, Hunter 1985, Willis and Ayre 1985, Wulff 1986, Ayre and Willis 1988, Hebert et al. 1988, 1989). However, for many species, these techniques do not resolve genet-specific genotypes (Curtis et al. 1982, Stoddart et al. 1985, Willis and Ayre 1985). Genetic techniques that identify variation at the level of the individual's DNA (restriction fragment analysis, sequencing) provide the most accurate measure of genetic similarity. For example, minisatellite DNA is highly variable in most vertebrate species (Jeffreys et al. 1985a, Jeffreys and Morton 1987) and can provide genetic markers that are unique for an individual genotype. Minisatellite DNA probes have been used to generate DNA fingerprints used in demographic studies to distinguish between sibs and parent/offspring in birds and humans (Jeffreys et al. 1985b, Burke and Bruford 1987, Wetton et al. 1987, Burke et al. 1989, Westneat 1990), cultivars of plants (Nybom and Schaal 1990), and clones of aphids (Carvalho et al. 1991), and fishes (Turner et al. 1990). These latter studies have shown that DNA fingerprinting can differentiate clones that have been indistinguishable using other markers. This makes DNA fingerprinting a powerful tool for understanding population structure. To date the use of DNA fingerprinting has

### Introduction

Clonal growth is a common reproductive strategy among taxonomically diverse group of sessile marine invertebrates (Highsmith 1982, Jackson 1985). Asexual reproduction allows established genotypes to dominate space and can play an important role in the recolonization of disturbed areas (Williams 1975, Ayre 1984). In many species clonal propagation is the most important source of recruitment into established populations (Williams 1975, Ayre 1983, 1984, Lasker 1984, 1990, Stoddart 1984, John-

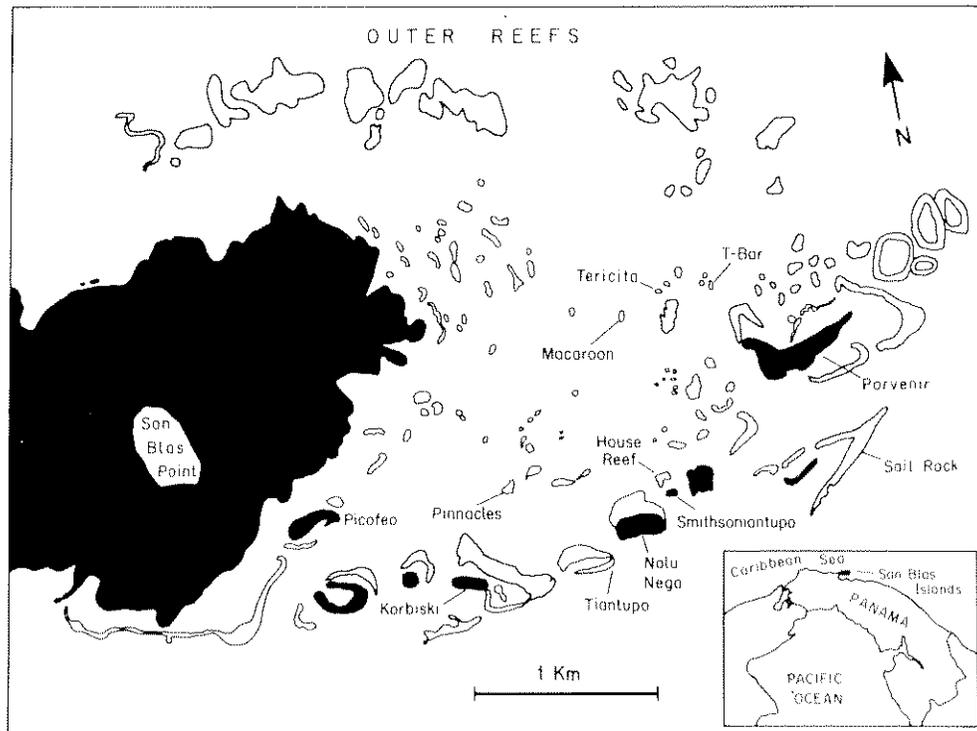


Fig. 1. *Plexaura A.* San Blas Point region of the San Blas Islands, Panama. Collections made from Korbiski, Pinnacles, Tiantupo, Sail Rock, T-Bar, Tericita and Macaroon Reefs.

been mainly limited to vertebrate, plant and a few terrestrial invertebrate species. In this paper we describe the use of DNA fingerprinting to investigate clonal structure and population relatedness in a clonal marine invertebrate, the gorgonian *Plexaura A* (*Plexaura* sp. A).

*Plexaura A*, an undescribed gorgonian coral (see Lasker 1984), is found throughout the Caribbean and has a life history strategy which relies largely on vegetative propagation. Asexual reproduction occurs via fragments which form when branches are broken off a colony and reattach to the substrate (Lasker 1984, 1990). Long-term monitoring (7 yr) of three different reefs in the San Blas Islands, Panama, show that all successful recruits of *Plexaura A* to 230 m<sup>2</sup> of reef were initiated from fragments (Lasker 1990, unpublished data). Only sexually produced larvae, however, can disperse between reefs, and because *Plexaura A* larvae develop in the water column (Brazeau and Lasker 1989, Lasker unpublished data), we can expect a distribution of genotypes of varying relatedness between reefs. Thus *Plexaura A* can be expected to have a highly clonal population structure within reefs, but genets should be restricted to single reefs. An examination of *Plexaura A* fingerprints from within and between reefs should provide a good test for the utility of DNA fingerprinting in delineating clones.

In an organism such as *Plexaura A* that is colonial as well as clonal, it is necessary to define what is meant by the term "individual". In this paper we refer to individuals as those colonies which are either the result of a successful settlement event by a larva or the result of the reattachment and subsequent growth of a fragment. All individual colonies that share a common genotype are termed the genet.

## Methods

### Sample collection and storage

Samples of *Plexaura A* for DNA fingerprint analyses were collected from seven reefs in the San Blas Islands off the Caribbean coast, Panama between June 1990 and July 1991 (Fig. 1). Small (5–10 cm) branch pieces were clipped from each colony, placed in labelled bags and transferred to the Smithsonian Tropical Research Institute's (STRI) San Blas field station. Branches were subsampled and replicate 2 cm pieces were frozen in liquid nitrogen and transferred to the laboratory either in liquid nitrogen or on dry ice where they were stored at -80°C until analyzed. Live samples were always kept submerged, and handling during sampling and subsampling was kept to a minimum.

### Detailed fingerprinting protocol

DNA was extracted from 0.5 to 1.0 cm pieces of adult gorgonian branches (approximately 250 mg wet wt) by grinding the tissue in 0.5 ml of CTAB (hexadecyltrimethyl ammonium bromide) buffer [1.4 M NaCl, 20 mM EDTA (ethylenediaminetetraacetic acid), 100 mM Tris-HCl pH 8.0, 2% CTAB and 0.2% 2-mercaptoethanol] following a modification of the methods of Saghai-Maroul et al. (1984). Proteinase K was added to the samples to a final concentration of 0.1 mg ml<sup>-1</sup> and the samples incubated at 65°C for 1 h. An equal volume of chloroform was added to each sample, the samples mixed well, spun at high speed on a microcentrifuge for 10 min and the aqueous layer was transferred to a clean tube. The samples were then extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). DNA was precipitated with 2 volumes of cold 95% ethanol (ETOH) without the addition of additional salt. The pellet was washed twice with 70% ETOH, dried and resuspended in the appropriate volume of TE (10 mM Tris-HCl pH 8.0 and 1 mM EDTA), depending on the pellet size. RNA was digested by adding 1 µl of RNase (10 µg µl<sup>-1</sup>) and incubating the samples at 37°C for 30 min.

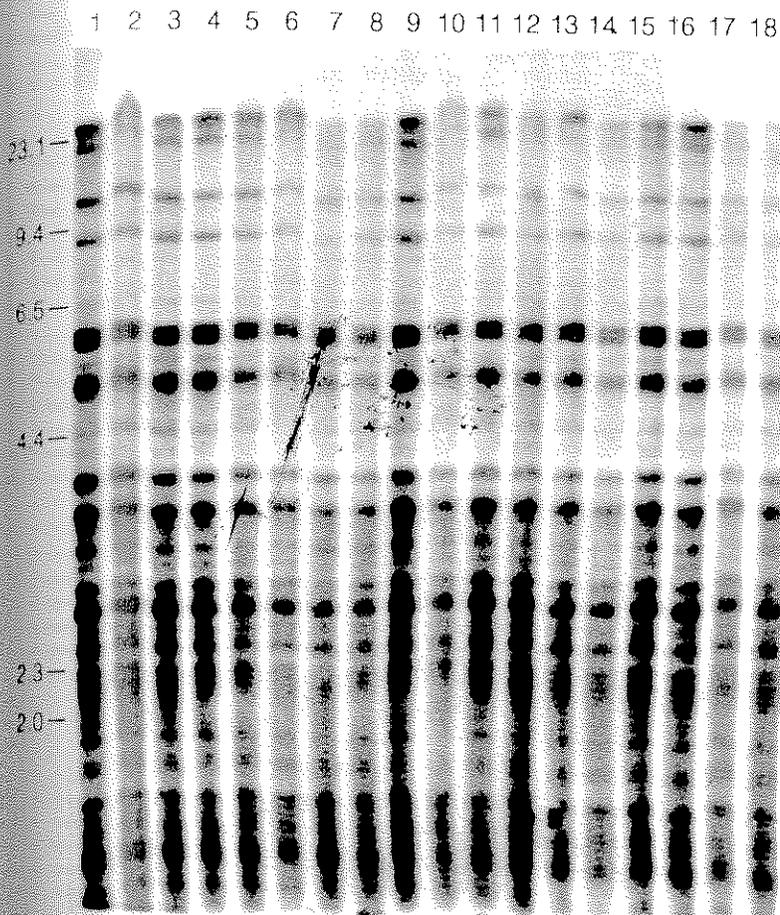


Fig. 2. *Plexaura A.* Autoradiograph showing DNA fingerprints of 18 branches from a single colony of *Plexaura A.* DNA was digested with the endonuclease *Hae*III and probed with the bacteriophage, M13 DNA. Size markers (kb) are *Hind*III digests of lambda DNA

DNA (ca. 2 µg) was digested overnight at 37°C in 30 µl reaction mixtures with 20 units of a restriction endonuclease. Replicate digestions of DNA extracted from the same individual yield identical fingerprints, indicating consistent and complete digestions. Seven endonucleases [*Aha*I, *Bgl*II, *Eco*RI, *Hae*III, *Hinc*II, *Hin*II and *Msp*I (New England Biolabs and Bethesda Research Laboratories)] were initially screened, and two (*Hae*III and *Hinc*II) were selected for use in the majority of the analyses. Fragments were separated by electrophoresis through a 0.8% agarose gel in TBE (0.09 M Tris-borate and 0.002 M EDTA) for 36 to 48 h at 30 to 40 V, with recirculating buffer. DNA was transferred to nylon membranes (Zetabind, Cuno) which were hybridized for 24 h at 60°C (Westneat et al. 1988). The probe, DNA from the bacteriophage M13, Jeffreys probe 33.6 or Jeffreys probe 33.15, was labelled with radioactive [<sup>32</sup>P]dCTP (deoxycytidine 5'-triphosphate) by the random priming method (Feinberg and Vogelstein 1983) using a BRL Random Priming Labelling kit. Each of these probes contain a repeat region [15, 33 and 37 bp (base pairs), respectively, for M13 DNA, Jeffreys probe 33.15 and 33.6] that detects variability in a range of species (Jeffreys et al. 1985a, Jeffreys and Morton 1987, Vassart et al. 1987, Westneat et al. 1988, Ryskov et al. 1988, Westneat 1990). Membranes were washed according to Westneat et al. (1988) except that an additional wash in 2 × sodium chloride, sodium citrate (SSC, 1 × SSC = 0.15 M NaCl and 0.015 M Na-citrate) and 0.1% sodium dodecyl sulfate (SDS) at 60°C and a wash with 1 × SSC, 0.1% SDS at 60°C were added when background counts were high. Membranes were placed on X-ray film with an intensifying screen at -80°C for 7 d, depending on the radiation level of the membrane.

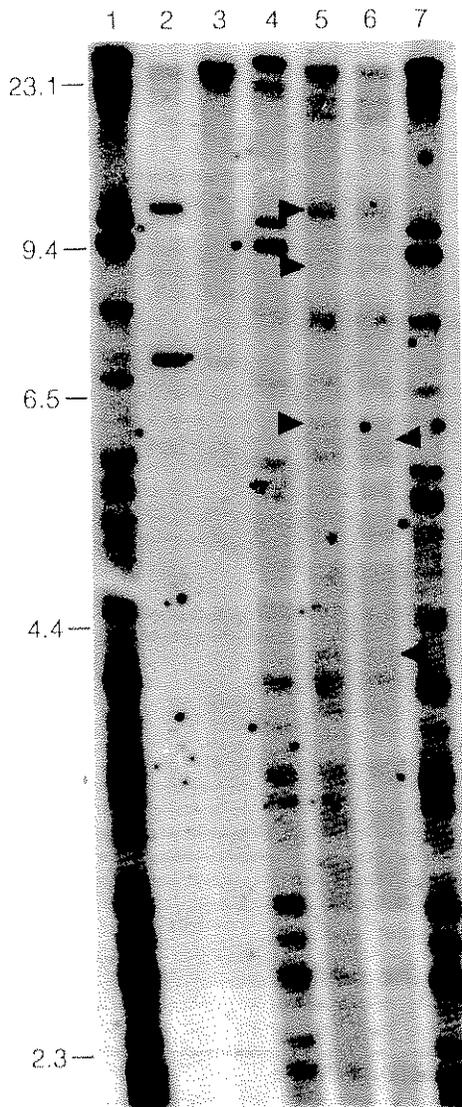
#### Experimental approach

Two sets of fingerprint analyses were conducted aimed at verifying the accuracy and utility of DNA fingerprinting of *Plexaura A.*

colonies. Firstly, it was necessary to document that DNA fingerprints are somatically stable throughout an individual colony. Secondly, as *Plexaura A.* adults contain intracellular algal symbionts, it was necessary to test for possible contamination of the gorgonian DNA with zooxanthellae DNA.

To assay within colony variation, DNA was isolated from 18 branches from a single colony following the protocols described above. The DNA was divided into two aliquots and digested with either *Hae*III or *Hinc*II. Samples from a single enzyme digestion were run in adjacent lanes on a single gel. These gels were transferred to nylon membranes and probed with M13 DNA as described above.

To assay for contamination of gorgonian DNA from zooxanthellae DNA, two experiments were conducted. In the first experiment, gorgonian planulae DNA (the planulae are aposymbiotic) and DNA from both the parental colonies and their zooxanthellae were isolated and their DNA fingerprints compared. In the second experiment, gorgonian DNA and zooxanthellae DNA were isolated from the same colonies and again fingerprints were generated and compared. Gorgonian adult and planulae DNAs were purified following the protocols outlined above. Two protocols were used to isolate zooxanthellae DNA. Initially zooxanthellae DNA was isolated following a modification of Rowan and Powers (1991). Small pieces of gorgonian (1 to 2 cm) were ground in Isolation Buffer (IB = 0.4 M NaCl, 50 mM EDTA, 10 mM Dithiothreitol, 10 mM Tris-HCl and pH 7.6) and strained through a 250 µm mesh net with a total of 5 ml IB. Samples were spun at 500 rpm for 5 min and the supernatant discarded. The pellet was resuspended in 5 ml IB and spun again for 5 min. This was repeated two more times and the pellet transferred to a microfuge tube with 0.5 ml of IB and SDS added to a final concentration of 1% (v/v). The samples were incubated at 65°C for 20 to 60 min and then Proteinase K added to a final concentration of 0.1 mg ml<sup>-1</sup> and the samples incubated at 37°C overnight. The samples were spun to pellet spicules and the



**Fig. 3.** *Plexaura* A. Autoradiograph of DNA fingerprints of gorgonian DNA and DNA from zooxanthellae isolated from the same gorgonian colony. DNA was digested with *Hae*III and probed with M13 DNA. Lanes 1, 4 and 7 contain DNA from a single gorgonian colony. Lanes 5 and 6 contain DNA from zooxanthellae isolated from the same gorgonian colony. Arrows indicate bands unique to the zooxanthellae lanes. Size markers (kb) are *Hind*III digests of lambda DNA

supernatant transferred to a clean tube. NaCl and CTAB were added to a final concentration of 0.8 M and 1%, respectively, and the samples were incubated at 65°C for 20 min. They were then extracted with chloroform and phenol:chloroform:isoamyl alcohol (25:24:1) and the procedure previously described followed. A similar protocol was used for isolation of zooxanthellae DNA in the second experiment, but in this case the zooxanthellae pellet was passed through 74 and 20 µm mesh netting after the third wash, the suspension was spun at 800 rpm for 10 min and the pellet transferred to 0.3 ml CTAB buffer (described in the previous section). The pellet was vortexed with glass beads for 5 min to help rupture cell walls. Proteinase K was added to a final concentration of 0.1 mg ml<sup>-1</sup> and the protocol described above for isolation of gorgonian DNA was followed. In these experiments the DNAs were digested with either *Eco*RI or *Hae*III and run in adjacent lanes on a gel. In both experiments, the DNA was transferred to nylon membranes.

probed with M13 DNA as described above and the banding patterns compared.

Following these analyses, fingerprints from 73 colonies from seven reefs (Fig. 1) in the San Blas Islands, Panama, were compared. Since colonies from different reefs developed from different larvae, inter-reef comparisons tested the resolving power of fingerprinting in detecting different genotypes. Within-reef comparisons were conducted to determine clonal structure.

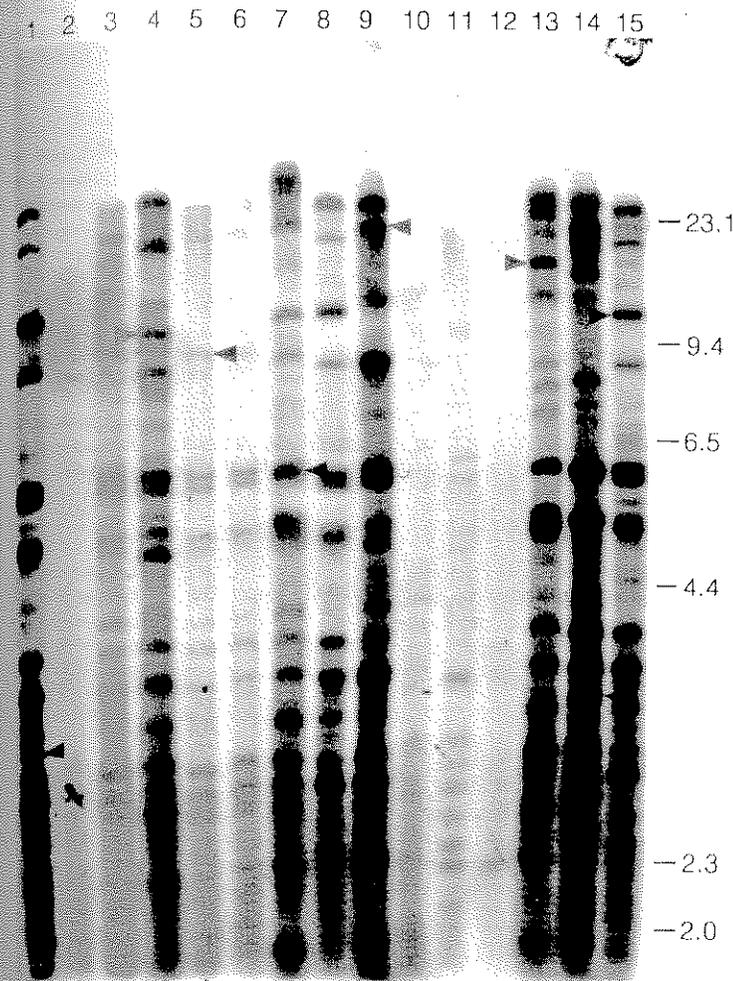
#### Data analysis – scoring and interpretation of autoradiographs

Each autoradiograph was scored using a lightbox and rulers at three locations across the gel to standardize band position across the gel. DNA from a single individual was loaded at three locations on the gel as a control for different migration rates across the gel. For each position where a fragment band was present in at least one lane, the presence or absence of bands in all other lanes at this position was noted. The bands were coded as follows: (1) definitely present, (2) definitely absent, (3) ambiguous. This latter category was used when the quantity of DNA in a particular lane was such that only faint bands were present. In these cases absence of a band could have been caused by either absence of the fragment or by the presence of insufficient amounts of the fragment to be visualized. In such cases the position was not scored. Likewise, the code of "ambiguous" was used when a position had high levels of background that made it difficult to conclude if a fragment was present, and again the position was not scored.

Gorgonian clonemates were identified by pairwise comparison of fragment patterns among the individual colonies sampled. Comparisons were only made among colonies run on the same gel and positions coded as ambiguous were omitted from these comparisons. Similarity coefficients *D* (Wetton et al. 1987) were calculated for all pairwise comparisons of genotypes identified on a gel. *D* is  $2 N_{AB} / (N_A + N_B)$  where  $N_A$  and  $N_B$  are the number of bands in individuals A and B and  $N_{AB}$  is the number of bands shared by A and B. (*D* is the same as the similarity index, *S*, defined by Lynch 1990). Using this statistic, if two individuals share all bands, *D* = 1, and if no bands are shared, *D* = 0. Only individuals with a similarity coefficient of 1 were classed as having the same genotype.

#### Results

Eighteen branches collected from a single colony were compared to assay within colony variation. No within-colony fingerprint variation was detected with either *Hae*III (Fig. 2) or *Hinc*II digests. DNA fingerprints from zooxanthellae, aposymbiotic planulae, and adult gorgonian DNA were compared to test for contamination of gorgonian DNA with zooxanthellae DNA. All bands in the fingerprints of adult DNA were also present in DNA fingerprints of its aposymbiotic planulae, indicating that all bands in the lane of gorgonian DNA were of gorgonian origin. There were no bands unique to the zooxanthellae lanes, suggesting that despite washing the zooxanthellae pellet three to four times, the zooxanthellae DNA preparation contain mostly gorgonian DNA and very little zooxanthellae DNA. A second protocol used glass beads to disrupt the zooxanthellar cell wall in an attempt to isolate more zooxanthellae DNA. In this experiment zooxanthellae unique bands were present in the zooxanthellae lanes, but as in the initial comparison, gorgonian DNA had contaminated the zooxanthellae DNA (i.e., some bands were shared between gorgonian and zooxan-



**Fig. 4.** *Plexaura A.* Autoradiograph of DNA fingerprints from colonies from five different reefs in the San Blas Point area. DNA digested with the restriction enzyme, *Hae*III and probed with M13 DNA. Lanes 1, 8, and 15 contain DNA from a single colony from Korbiski Reef. Lanes 3 to 6 contain DNA from colonies from Macaroon Reef. Lane 7: Tiantupo Reef; Lane 9: Tericita Reef. Lanes 13 and 14: T-Bar Reef. Lanes 2, 10 11 and 12 not scored. Lanes 3, 5 and 6 are members of a single Macaroon clone. Arrows indicate some of the bands that were used to differentiate clones. Size markers (kb) are *Hind*III digests of lambda DNA

thellae lanes, Fig. 3). These experiments verify that our extraction protocols did not introduce measurable contamination from zooxanthellae DNA into the extracted gorgonian DNA. Thus, we are examining fingerprints of gorgonian DNA.

In the survey of 73 colonies from seven different reefs, we generated fingerprints using two different restriction enzymes and three probes. We did not test each colony with all probe-enzyme combinations. In combination we produced a total of 185 fingerprints from the 73 different colonies.

All fragments between ca. 3 and 23 kilobases in size were scored as described above (present, absent, or ambiguous). The complexity of the fingerprint and the number of informative bands (bands that were definitely present in one but not all lanes) varied with the endonucleases and probes used. The number of scorable bands  $\text{ind.}^{-1}$  ranged from 7 to 26 with a mean of 16.8 bands  $\text{ind.}^{-1}$  ( $\text{SD} = 3.8$ ) for all enzyme-probe combinations combined. Higher molecular weight bands were more variable between individuals. Within the same individual, different enzyme-probe combinations produced a different fingerprint. In 97.3% of the colonies scored, the absolute fingerprints (i.e. clone vs non-clonemate) were identical regard-

**Table 1.** *Plexaura A.* Distribution of clones of *Plexaura A* within and among reefs in the San Blas Point region, San Blas Island, Panama

Reef	No. of colonies examined	No. of reef-specific genotypes	No. of genotypes shared between reefs	No. of colonies in the two dominant reef-specific genotypes
Korbiski	32	8	0	19, 5
Macaroon	14	3	0	11, 2
Pinnacles	2	2	0	1, 1
Sail Rock	12	8	0	3, 2
T-Bar	10	5	0	5, 2
Tericita	2	2	0	1, 1
Tiantupo	1	1	0	1
Totals	73	29	0	-

less of the enzyme-probe combination used. However, in two cases, one enzyme-probe combination distinguished closely related non-clonemates (i.e., one fragment difference) which had been scored as identical using a different enzyme-probe combination.

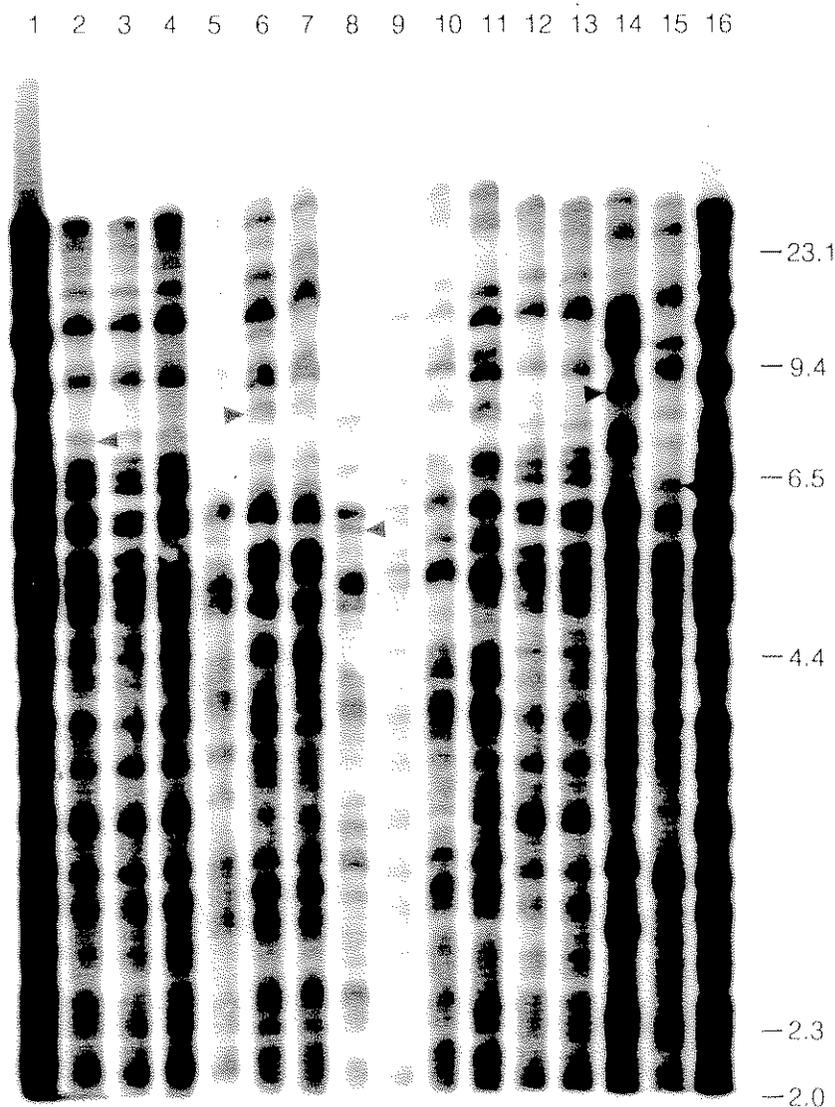


Fig. 5. *Plexaura A.* Autoradiograph of DNA fingerprints of *Plexaura A.* The DNA was digested with the endonuclease *Hae*III and probed with M13 DNA. Lanes 1, 9 and 16 contain DNA from a single Korbiski colony. Lanes 1 to 4, 9, 12, 13 and 16 contain DNA from colonies that are members of the largest Korbiski clone. Lanes 5; 6 and 7; 8; 10 and 11; 14; and 15 are representatives of six additional clones. Arrows indicate some of the bands that were used to differentiate clones. Size markers (kb) are *Hind*III digests of lambda DNA

Comparisons of individuals from different reefs (between reef comparisons) did not detect any common genotypes (Table 1 and Fig. 4). In Fig. 4 each of the eleven fingerprints contained on average 17.4 bands ( $SD=2.7$ , range 14 to 21). Only individuals within a given reef shared a genotype (i.e., were clonemates) (Table 1). On Korbiski Reef, 19 of 32 tested colonies had identical fingerprints. Six of the colonies in Fig. 5 belong to this large Korbiski clone. In Fig. 5 there were 18.9 scorable fragments colony<sup>-1</sup> ( $SD=0.6$ , range = 18 to 20) and a total of 28 different fragments. With the exception of the two cases of single fragment differences noted above, different clones within reefs differed by three or more fragments. Mean similarity coefficient of the different genotypes on Korbiski Reef was 0.75 ( $SD=0.15$ ,  $n=28$ ) and on Macaroon,  $D$  was 0.58 ( $SD=0.20$ ,  $n=7$ ). The mean similarity ( $D$ ) for all reefs was 0.53 ( $SD=0.11$ ,  $n=68$ ).

## Discussion

DNA fingerprinting of colonies of the gorgonian *Plexaura A.* has demonstrated the ability of the minisatellite probes to detect polymorphisms among gorgonian DNAs. DNA fingerprints from 73 colonies distributed over seven reefs in the San Blas Islands, Panama, identified 29 different genotypes. The mean number of fragments ind.<sup>-1</sup> (16.8) and the overall band sharing among these populations (0.53) is similar to that observed in other organisms (Burke and Bruford 1987, Jeffreys and Morton 1987, Wetton et al. 1987, Birkhead et al. 1990, Gilbert et al. 1990, Nybom and Rogstad 1990, Nybom and Schaal 1990, Turner et al. 1990, Carvalho et al. 1991). Fragment pattern complexity varied depending on the restriction endonuclease utilized. This finding is similar to that reported for several other invertebrate species (*Caenorhabditis elegans*: Uitterlinden et al. 1989, aphids:

Carvalho et al. 1991) and contrasts with most human studies (Jeffreys et al. 1985a, b, 1988). This implies that it is necessary to screen a series of different enzymes to find those which are most useful in separating genotypes. Once the appropriate enzyme-probe combinations have been identified, DNA fingerprinting has the sensitivity to detect genotypes with a small number of enzyme-probe combinations. In only two cases did the addition of a second enzyme or probe detect a new genotype not previously identified among the 73 gorgonian colonies of this study.

These data are among the first fingerprints of a marine invertebrate and one of the first uses of DNA fingerprinting to delineate clonal structure in any organism (also see Nybom and Schaal 1990, Turner et al. 1990, Carvalho et al. 1991, Rogstad et al. 1991). The utility of DNA fingerprinting in analyses of clonal structure depends on satisfying several assumptions. Firstly, the markers must be somatically stable within an individual colony. Secondly, the markers must display Mendelian inheritance, and thirdly, the markers must be specific to an individual genet. DNA extracted from 18 branches collected from a single colony gave identical fingerprints, demonstrating that these markers are identical within a gorgonian colony. A detailed segregation analysis was not part of the present study, so Mendelian inheritance of fragment-based alleles remains to be established. However, DNA isolated from gorgonian planulae, which were the products of controlled matings, was run adjacent to their parental colonies and all planulae bands could be assigned to one of the two parents.

DNA fingerprinting of gorgonian DNA resolved genotypes between and within reefs. Given that *Plexaura A* has dispersed planktonic larvae (Brazeau and Lasker 1989), there is no *a priori* reason to believe that genets are more related within a reef than between reefs. [Earlier analyses suggesting commonplace parthenogenesis in *Plexaura A* (Brazeau and Lasker 1989) now appear to be incorrect (Lasker unpublished data)]. However, the only cases of identical fingerprints which we observed were from colonies located on the same reef and, often, spatially close to one another. Members of a population may have similar fingerprints if minisatellite alleles become fixed at some loci by inbreeding due to population bottlenecks or mating systems (Jeffreys et al. 1987, Faulkes et al. 1990, Kuhnlein et al. 1990, Reeve et al. 1990, Bellamy et al. 1991). However, inbreeding is not likely in *Plexaura A*, since most populations of *Plexaura A* are founded by sexually produced larvae which float in the water column for periods of 12 to 48 h allowing larval dispersal of distances up to several kilometers (Lasker unpublished data). Unless individuals were vegetatively derived, it is highly unlikely that 19 highly related (i.e., with identical fingerprints) individuals would have made up 59% of the 32 colonies sampled at Korbiski reef and be totally absent from the 41 colonies sampled on other reefs ( $\chi^2 = 33.09$ ,  $df = 1$ ,  $p < < 0.001$ ). Taken together, these findings establish DNA fingerprinting as a valuable technique for distinguishing clones and for identifying clonal structure.

The DNA fingerprinting data characterize Korbiski and Macaroon as reefs which are dominated by a few

clones. Seventy-five percent and 92% of the individuals examined on Korbiski and Macaroon, respectively, belonged to one of two clones. The shared fingerprints commonly found among colonies located on the same reef most likely reflect low genotypic diversity within the reef due to a low level of larval recruitment and subsequent vegetative reproduction. Across these same populations, histocompatibility assays identified 13 putative clones while DNA fingerprinting analyses of the same colonies distinguished 17 clones (Lasker and Coffroth 1985, Coffroth, Lessios, Brazeau, Lasker and Bermingham in preparation).

The data presented here show that DNA fingerprinting is capable of resolving closely related individuals. Thus DNA fingerprinting should yield unambiguous measures of clonal diversity and allow accurate assessments of clonal structure. The degree of band sharing may also be used to characterize relatedness of genets and thus trace the origin and differentiation of clones (Carvalho et al. 1991), though as Carvalho et al. (1991) caution, one must know the allelic distribution within the population as a whole as well as among the individuals sampled (Lynch 1988).

Clonal structure has been previously assessed in marine invertebrates using techniques such as allozymes (Hoffmann 1976, 1986, 1987, Ayre 1983, 1984, Stoddart 1984, Hunter 1985, Willis and Ayre 1985, Ayre and Willis 1988) histocompatibility (Neigel and Avise 1983a, b, Hunter 1985, Lasker and Coffroth 1985, Willis and Ayre 1985, Wulff 1986), and morphology (Hunter 1985, Wulff 1986). We have shown that DNA fingerprinting can also be used to make such assessments. Furthermore, DNA fingerprinting directly assays the genome and surveys a greater number of loci than the other techniques. Thus DNA fingerprinting (or other DNA-based methodologies) may prove to be the technique of choice when other techniques yield poor resolution or results which are subject to debate (i.e., Curtis et al. 1982, Stoddart et al. 1985, Willis and Ayre 1985).

*Acknowledgements.* We thank the Republic of Panama and the Kuna people for permission to work in the San Blas Islands, Panama. We also thank M. Walker for laboratory assistance. This work was supported by NSF grants OCE-8915089 (MAC), OCE-9012168 (HRL) and the NOAA Office of Sea Grant, U.S. Department of Commerce, under Grant No. NA90AA-D-SG078 (HRL and JAB) to the New York Sea Grant Institute. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies.

#### Literature cited

- Ayre, D. J. (1983). The effects of asexual reproduction and inter-genotypic aggression on the genotypic structure of populations of the sea anemone *Actinia tenebrosa*. *Oecologia* 57: 158-165
- Ayre, D. J. (1984). The effect of sexual and asexual reproduction on geographic variation in the sea anemone *Actinia tenebrosa*. *Oecologia* 62: 222-229
- Ayre, D. J., Willis, B. L. (1988). Population structure in the coral *Pavona cactus*: clonal genotypes show little phenotypic plasticity. *Mar. Biol.* 99: 494-505

- Bellamy, R. J., Inglehearn, C. F., Jalili, I. K., Jeffreys, A. J., Bhattacharya, S. S. (1991). Increased band sharing in DNA fingerprints in an inbred human population. *Hum. Genet.* 87: 341-347
- Birkhead, T. R., Burke, T., Zann, R., Hunter, F. M., Krupa, A. P. (1990). Extra-pair paternity and intraspecific brood parasitism in wild zebra finches *Taeniopygia guttata*, revealed by DNA fingerprinting. *Behav. Ecol. Sociobiol.* 27: 315-324
- Brazeau, D. A., Lasker, H. R. (1989). The reproductive cycle and spawning in a Caribbean gorgonian. *Biol. Bull. mar. biol. Lab., Woods Hole* 176: 1-7
- Burke, J., Bruford, M. W. (1987). DNA fingerprinting in birds. *Nature, Lond.* 327: 149-152
- Burke, T., Davies, N. B., Bruford, M. W., Hatchwell, B. J. (1989). Parental care and mating behavior of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. *Nature, Lond.* 338: 249-251
- Carvalho, G. R., Maclean, N., Wratten, S. D., Carter, R. E., Thurston, J. P. (1991). Differentiation of aphid clones using DNA fingerprints from individual aphids. *Proc. R. Soc. (Ser. B)* 243: 109-114
- Curtis, A. S. G., Kerr, J., Knowlton, N. (1982). Graft rejection in sponges. *Transplantation* 33: 127-133
- Faulkes, C. G., Abbott, D. H., Mellor, A. L. (1990). Investigation of genetic diversity in wild colonies of naked mole-rats (*Heterocephalus glaber*) by DNA fingerprinting. *J. Zool., Lond.* 221: 87-97
- Feinberg, A. P., Vogelstein, B. (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analyt. Biochem.* 132: 6-13
- Gilbert, D. A., Lehman, N., O'Brien, S. J., Wayne, R. K. (1990). Genetic fingerprinting reflects population differentiation in the California Channel Island fox. *Nature, Lond.* 344: 764-767
- Hebert, P. D. N., Beaton, M. J., Schwartz, S. S., Stanton, D. J. (1989). Polyphyletic origins of asexuality in *Daphnia pulex*. I. Breeding-system variation and levels of clonal diversity. *Evolution, Lawrence Kansas* 43: 1004-1015
- Hebert, P. D. N., Ward, R. D., Weider, L. J. (1988). Clonal-diversity patterns and breeding-system variation in *Daphnia pulex*, an asexual-sexual complex. *Evolution, Lawrence, Kansas*, 42(1): 147-159
- Highsmith, R. C. (1982). Reproduction by fragmentation in corals. *Mar. Biol. Prog. Ser.* 7: 207-226
- Hoffmann, R. J. (1976). Genetics and asexual reproduction of the sea anemone *Metridium senile*. *Biol. Bull. mar. biol. Lab., Woods Hole* 151: 478-488
- Hoffmann, R. J. (1986). Variation in contributions of asexual reproduction to the genetic structure of populations of the sea anemone *Metridium senile*. *Evolution, Lawrence, Kansas*, 40(2): 357-365
- Hoffmann, R. J. (1987). Short-term stability of genetic structure in populations of the sea anemone *Metridium senile*. *Mar. Biol.* 93: 499-507
- Hunter, C. U. (1985). Assessment of clonal diversity and population structure of *Porites compressa* (Cnidaria, Scleractinia). *Proc. 5th int. coral Reef Congr.* 6: 69-74 [Gabri , C. et al. (eds.) Antenne Museum - EPHE, Moorea, French Polynesia]
- Jackson, J. B. C. (1985). Distribution and ecology of clonal and asexual benthic invertebrates. In: Jackson, J. B. C., Buss, L. W., Cook, R. F. (eds.) *Population biology and evolution of clonal organisms*. Yale University Press, New Haven, Connecticut, U.S.A. p. 297-355
- Jeffreys, A. J., Morton, D. B. (1987). DNA fingerprinting of dogs and cats. *Anim. Genet.* 18: 1-16
- Jeffreys, A. J., Royle, N. J., Wilson, V., Wong, Z. (1988). Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. *Nature, Lond.* 322: 278-281
- Jeffreys, A. J., Wilson, V., Kelly, R., Taylor, B. A., Bulfield, G. (1987). Mouse DNA 'fingerprints': analysis of chromosome localisation and germ-line stability of hypervariable loci in recombinant inbred strains. *Nucleic Acids Res.* 15: 2823-2836
- M. A. Coffroth et al.: DNA fingerprinting of a gorgonian coral
- Jeffreys, A. J., Wilson, V., Thein, S. L. (1985a). Hypervariable 'minisatellite' regions in human DNA. *Nature, Lond.* 314: 67-71
- Jeffreys, A. J., Wilson, V., Thein, S. L. (1985b). Individual-specific 'fingerprints' of human DNA. *Nature, Lond.* 316: 76-79
- Johnson, M. S., Threlfall, T. J. (1987). Fissiparity and population genetics of *Coccinasterias calamaria*. *Mar. Biol.* 93: 517-525
- Kuhnlein, U., Zadworny, D., Dawe, Y., Fairfull, R. W., Gavora, J. S. (1990). Assessment of inbreeding by DNA fingerprinting: development of a calibration curve using defined strains of chicken. *Genetics, Baltimore, Md* 125: 161-165
- Lasker, H. R. (1984). Asexual reproduction, fragmentation, and skeletal morphology of a plexaurid gorgonian. *Mar. Ecol. Prog. Ser.* 19: 261-268
- Lasker, H. R. (1990). Clonal propagation and population dynamics of a gorgonian coral. *Ecology* 71: 1578-1589
- Lasker, H. R., Coffroth, M. A. (1985). Vegetative reproduction, clonal spread, and histocompatibility in a Caribbean gorgonian. *Proc. 5th int. coral Reef Congr.* 4: 331-336. [Gabri , C. et al. (eds.) Antenne Museum - EPHE, Moorea, French Polynesia]
- Lynch, M. (1988). Estimation of relatedness by DNA fingerprinting. *Molec. Biol. Evolut.* 5(5): 584-599
- Lynch, M. (1990). The similarity index and DNA fingerprinting. *Molec. Biol. Evolut.* 7: 478-484
- Neigel, J. E., Avise, J. C. (1983a). Clonal diversity and population structure in a reef-building coral, *Acropora cervicornis* self-recognition analysis and demographic interpretation. *Evolution, Lawrence, Kansas* 37: 437-453
- Neigel, J. E., Avise, J. C. (1983b). Histocompatibility bioassays of population structure in marine sponges. *J. Hered.* 74: 134-140
- Nyblom, H., Rogstad, S. H. (1990). DNA "fingerprints" detect genetic variation in *Acer negundo* (Aceraceae). *Pl. Syst. Evol.* 173: 49-56
- Nyblom, H., Schaal, B. A. (1990). DNA "fingerprints" reveal genotypic distributions in natural populations of blackberries and raspberries (*Rubus, Rosaceae*). *Am. J. Bot.* 77: 883-888
- Reeve, H. K., Westneat, D. F., Noon, W. A., Sherman, P. W., Aquadro, C. F. (1990). DNA "fingerprinting" reveals high levels of inbreeding in colonies of the eusocial naked mole-rat. *Proc. natn. Acad. Sci. U.S.A.* 87: 2496-2500
- Rogstad, S. H., Nyblom, H., Schaal, B. A. (1991). The tetrapod DNA fingerprinting M13 repeat probe reveals genetic diversity and clonal growth in quaking aspen (*Populus tremuloides* Salicaceae). *Pl. Syst. Evol.* 175: 115-123
- Rogstad, S. H., Patton, J. C., Schaal, B. A. (1988). M13 repeat probe detects DNA minisatellite-like sequences in gymnosperms and angiosperms. *Proc. natn. Acad. Sci. U.S.A.* 85: 9176-9178
- Rowan, R., Powers, D. A. (1991). Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). *Mar. Biol.* 71: 65-73
- Ryskov, A. P., Jinchardze, A. G., Proshnyak, M. I., Ivanov, P. L., Limborska, S. A. (1988). M13 phage DNA as a universal marker for DNA fingerprinting of animals, plants and microorganisms. *Fedn. eur. biochem. Soc. (FEBS) Lett.* 233(2): 388-392
- Saghai-Maroof, M. A., Sollman, K. M., Jorgensen, R. A., Allard, R. W. (1984). Ribosomal DNA spacer-length polymorphisms in barley. Mendelian inheritance, chromosomal location and population dynamics. *Proc. natn. Acad. Sci. U.S.A.* 81: 8014-8018
- Stoddart, J. A. (1984). Genetical structure within populations of the coral *Pocillopora damicornis*. *Mar. Biol.* 81: 19-30
- Stoddart, J. A., Ayre, D. J., Willis, B., Heyward, A. J. (1985). Self-recognition in sponges and corals? *Evolution, Lawrence, Kansas* 39: 461-463
- Turner, B. J., Elder, J. F., Laughlin, T. F., Davis, W. P. (1990). Genetic variation in clonal vertebrates detected by simple-sequence DNA fingerprinting. *Proc. natn. Acad. Sci. U.S.A.* 87: 5653-5657
- Uitterlinden, A. G., Slagboom, P. E., Johnson, T. E., Vug, V. (1989). The *Caenorhabditis elegans* genome contains monomorphic minisatellites and simple sequences. *Nucleic Acids Res.* 17: 9527
- Vassart, G., Georges, M., Monsieur, R., Brocas, H., Lequarte, A. S., Christophe, D. (1987). A sequence in the M13 phage detects

- hypervariable minisatellite in human and animal DNA. *Science*, N.Y. 235: 683-684
- Reinert, D. F. (1990). Genetic parentage in the indigo bunting: a study using DNA fingerprinting. *Behav. Ecol. Sociobiol.* 27: 67-76
- Reinert, D. F., Noon, W. A., Reeve, H. K., Aquadro, C. F. (1988). Improved hybridization conditions for DNA "fingerprinting" probed with M13. *Nucleic Acids Res.* 16: 4161
- Retton, J. H., Carter, R. E., Parkin, D. T., Walters, D. (1987). Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature, Lond.* 327: 147-149
- Williams, G. C. (1975). *Sex and evolution*. Princeton University Press, Princeton
- Willis, B. L., Ayre, D. J. (1985). Asexual reproduction and genetic determination of growth form in the coral *Pavona cactus*: biochemical, genetic and immunogenic evidence. *Oecologia* 65: 516-525
- Wulff, J. L. (1986). Variation in clone structure of fragmenting coral reef sponges. *Biol. J. Linn. Soc.* 27: 311-330