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Variation in clone structure of fragmenting coral reef sponges

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Populations of three branching Caribbean demosponge species are composed of clones produced by asexual fragmentation. Dispersal of the fragments before they become established as independent individuals scatters clone members widely and intermixes members of different clones, complicating study of the clone structure of these populations and contrasting with many other sessile clonal organisms. Clone structures of these populations were inferred using a combination of tissue-compatibility relationships and an analysis of variations in morphology and colour. Although tissue compatibility cannot be used for precise identification of sponge clones, in general, patterns of variation in morphological characters influencing fragmentation and patterns of fragment dispersal and recruitment suggest that, in these populations, tissue-compatibility relationships closely reflect clone structure. Conditions that must be met in order to use tissue compatibility for study of sponge clones are discussed, and previous results, from which conflicting conclusions have been drawn, reconciled in this context. Variations among clones in numbers of physiologically independent members and in size and shape of areal extent are discussed in the context of processes that may affect evolution of clonal characters in these populations and in other species that propagate by dispersing asexual fragments.

KEY WORDS:—Sponges – tissue compatibility – clone structure – morphological variation – asexual fragmentation.

CONTENTS

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INTRODUCTION

Variation in morphological and life history characters plays a central role in the ecology and evolution of a species. Understanding this role depends on accurate methods of describing and quantifying variation among conspecific individuals. Thus, an important recent focus of botanists and invertebrate zoologists has been extension of the theoretical framework and experimental techniques developed for describing and studying population structures of sexually reproducing organisms to include clonal organisms (e.g. Harper, 1977; Jackson, Buss & Cook, in press).

Two aspects of variation are unique to populations of clonal organisms. One involves traits that can only be described on the level of entire clones. For example, clones may vary in areal extent, rate of expansion, pattern of spatial distribution and number of physiologically independent members. This is not qualitatively different from variation among genetically different individuals for sexually propagating organisms. However, variation in clonal characters may be difficult to recognize and the possibility for partial mortality of a clone may make the outcomes of selection on variants difficult to predict.

The other special aspect of variation involves the transmission of variable traits among physiologically independent individuals. Individuals may share a trait because they are derived from each other either by asexual fragmentation (clone-mates) or by sexual reproduction (kin). To understand a trait in an evolutionary context therefore requires that the proportions of the population that share that trait by sexual or asexual means must be known.

Variation among clones can only be studied once the clones are accurately identified. Unambiguous distinction of clones is possible for plants that spread by runners or rhizomes, as long as connections remain (e.g. Kershaw, 1962; Harberd, 1963; Hartnett & Bazzaz, 1983), or for corals that retain the history of connection in their carbonate skeletons (Hughes & Jackson, 1980); but when connections are severed, indirect methods must be used. Clone structure has been inferred in plant populations from distinctive morphological characters, quantitative genetics, cross-incompatibility and electrophoresis (reviewed by Silander, in press). For invertebrate populations, attempts have been made to use somatic tissue-grafting experiments to infer clone structure (e.g. Jokiel, Hildemann & Bigger, 1982; Neigel & Avise, 1983a,b). All of these methods may be subject to technical and interpretational problems. Although no one technique can unambiguously identify clones, accuracy of interpretations can be improved by using a combination of several indirect evaluations of clone structure and by understanding the processes by which clone structure develops in a population. This is particularly important for marine invertebrates and other organisms that produce dispersing asexual fragments.

This study focuses on population structure of three species of coral reef sponge that propagate extensively by dispersing asexual fragments. In particular, I set out to describe and understand variations in clonal characters in the context of variations which can be described among individual sponges. To do this, I

determined the approximate clone structure of these populations using a combination of tissue-compatibility relationships and patterns of variation in morphological characters. I discuss (1) conditions that must be met for tissue compatibility to be an appropriate tool for studying clone structures of sponge populations; and (2) variations in clonal characters in species that propagate by dispersing asexual fragments, and how these differ from variations among clones in many terrestrial plants.

EXPERIMENTAL METHODS

Natural history of fragmenting sponges

Demosponges are unusually homogeneous organisms, not generally divisible into subunits comparable to the polyps or zooids of colonial invertebrates. A spong individual is, therefore, defined by an unbroken surface pinacoderm (Hartman & Reiswig, 1973), and a 'ramet' (*sensu* Harper, 1977) can only be defined once it is completely severed from the rest of the sponge.

Branching growth forms are characteristic for coral reef populations of the three species chosen for this study: *Iotrochota birotulata* Higgin, *Haliclona rubens* (Pallas) [= *H.* (*Amphimedon*) *compressa* D & M, see Wiedenmayer (1977)], and *Aplysina* (= *Verongia*) *fulva* (Pallas) [see systematic discussions by Laubenfels (1936) and Soest (1978)]. Individuals in each species vary in branching patterns, branch diameters, and extents to which branches grow adherent to the substratum or up into the water column. Branches range from 0.5 to 3.0 cm in diameter, and can be fragmented by rough water movement, fish bites or weakening of diseased or abraded sections. Repent branches can also be fragmented by starfish grazing or by smothering under sediment. Fragmentation is important in the life histories of these sponges, fragments survive well (Wulff, 1985), and virtually all recruitment into established populations is by asexually produced fragments (Wulff, unpubl. data). These demosponges do not have dense skeletons and, therefore, fragments can be easily dispersed by water movements or gravity before adhering to solid substrata and becoming established as independent individuals (Wulff, 1985).

Site description

The sponge populations studied live on a shallow plane and on the slopes of a channel to the leeward of Guigala Tupo, an island near the San Blas Field Station of the Smithsonian Tropical Research Institute in Panama. The substratum is a mixture of rubble from ramose coral species, especially *Acropora cervicornis* (Lamarck) and *Porites furcata* Lamarck, dotted with small to medium-sized (up to 1 m in diameter) massive corals.

The sponge fauna is characteristic of lagoon channels, shallow back-reef areas, steep slopes within bays and some deep fore-reef habitats, throughout the San Blas Islands and elsewhere in the Caribbean (Laubenfels, 1936; Wiedenmayer, 1977; Bonem & Stanley, 1977; and pers. obs. in Jamaica, Belize and Panama). The demosponges of the Guigala Tupo community are densely distributed (87.2 individual sponges/m^2, from a complete census of 16 m^2) and diverse (42 species in 16 m^2). In this habitat sponges exceed all other sessile invertebrates in

biomass and the morphological types represented include vases, clusters of tubes, vines, encrusters, excavaters, massive balls and branching forms.

The three branching species chosen for this study are among the most abundant sponges, in both biomass and numbers of individuals. Individual sponges range from 1 cm to 2 m in largest dimension. Populations of all three species were studied within a quadrat of 10×20 m, naturally bounded on the short sides by large mounds of live and dead corals. The substratum slopes at an average of about 16 degrees, from a flat plane at 2.3 m. The sponges live attached to dead and live coral heads and especially to the ubiquitous rubble.

Tissue-compatibility relationships

For each species, the 60 largest individuals within the 200 m^2 were tagged, and pieces of tissue were transferred among them to determine tissue-compatibility relationships. Pieces, 3–6 cm long, were cut and attached to branches of conspecific individuals with plastic ties, such that surface pinacoderms of host and donor individuals were in contact. Sponges were never removed from the water. Grafts were labelled with tags of coded plastic, so that they could be scored as accepted or rejected without the scorer knowing the identity of the donor sponge. Grafting was initiated in December 1981, and continued at a rate of several new grafts every week onto each sponge until, by June 1982, every sponge could be unambiguously assigned to one 'fusion group'. Fusion groups are defined such that all grafts among members of a fusion group are accepted, and all grafts between members of different fusion groups are rejected.

Only the largest individuals could be used for these detailed grafting experiments, because of the large amount of tissue required for making grafts, but many smaller individuals also lived within the quadrat. To determine if the fusion group diversity recognized among the large individuals was representative of their entire populations, 30 smaller individuals of each species were chosen randomly for grafting onto representatives of several recognized fusion groups.

Populations of these species also live in a similar habitat, 250 m across a deep channel. Pieces of 12 individuals of each species (18 for *I. birotulata*) from these populations were grafted onto the representative hosts. Exchange of fragments between these populations is precluded by the fine sediment that floors the channel. Tissue compatibility between individuals from these two populations would, therefore, suggest that fusion does not necessarily imply genetic identity.

Variation in morphology and surface colour

Each of the individuals used in the fusion experiments was characterized morphologically. Because sponge morphology may be extremely plastic (e.g. Bergquist, 1978; Wilkinson & Vacelet, 1979; Palumbi, 1984), many characters vary as much within individuals as among individuals. Traits that do not vary within individuals are therefore rare. However, such traits may also be more likely to be genetically determined in these plastic creatures and, therefore, be useful for evaluating genetic similarity. If tissue compatibility provides an

accurate reflection of clone structure in these populations, then such characters would be predicted to be consistent among individuals in the same fusion group.

Characters observed in the field and laboratory included surface colour, branching pattern, branch diameter and spicule (megasclere) length. All morphological characters were assessed before results of the grafting experiments were known. Characterizations based on division into categories rather than direct measurement were made on two occasions to ensure consistency.

EXPERIMENTAL RESULTS

Tissue-compatibility relationships

Each graft was scored for acceptance/rejection 2–10 times over 3–6 months. When tissue of host and donor fused, tissue and skeletal material began to grow between them and oscules often formed in this new tissue. Fluorescein dye injected into host or graft was able to exit by oscular openings on either, demonstrating confluence of aquiferous systems of fused sponges. By contrast with the clear responses of accepted grafts, a variety of reactions, detailed below, to incompatible tissue from a conspecific sponge were observed. Although these rejection reactions to incompatible grafts differed on a macroscopic scale, the end result of all appeared to be stable (up to 2 years) coexistence of clearly demarcated host and donor tissue.

Most grafts onto *I. birotulata* could be scored unambiguously after 4–5 days, but a few, apparently fused by the first scoring, later separated into distinct tissues and remained separated. In some cases incompatible tissues adhered together, while still maintaining a strict line of demarcation. In a few cases the donor tissue shrank, but later re-expanded. No tissue death, on either host or donor, could be seen in the field.

Many grafts onto *H. rubens* were confusing initially, and had to be scored repeatedly for up to 4 weeks before results stabilized. Some incompatible grafts adhered firmly to their host with no evidence of tissue damage; but on others a dead strip, 1–3 mm wide, appeared. In a few cases, host or donor tissue began to grow into the skeleton of the other, preceded by a narrow dead strip. In all cases which eventually showed tissue damage, the host and donor sponges at first appeared healthy and compatible and dead tissue did not develop until after a week. After a few weeks, the macerated surfaces were sloughed and surfaces of live tissue remained in contact with no further damage, but without fusing.

Incompatible grafts in *A. fulva* almost invariably adhered firmly to the host, while maintaining a clear line of demarcation. One or both sponges often developed a tissue extension of 1–3 mm towards the other. Grafts in this species could be scored unambiguously 3–4 days after initiation. No tissue death could be seen in the field.

Although 1770 different pairwise combinations are possible among the 60 large individuals in each species, the number of different grafts actually made for *I. birotulata*, *H. rubens* and *A. fulva* were 374, 268 and 292, respectively. If it is assumed that sponges which fuse with each other will accept and reject grafts from other individuals identically, then the number of grafts made were, respectively, 1.20, 2.35 and 2.34 times the minimum required to define the relationships discovered. Corroborating the above assumption, all tested tissue-

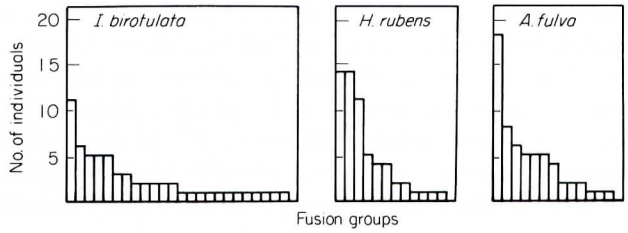

Figure 1. Relative proportions of physiologically independent individuals in each fusion group. Fusion groups are ranked in order of decreasing numbers of members along the horizontal axis. All individuals within a fusion group are tissue-compatible with each other, but reject tissue grafts from members of all other groups.

compatibility relationships of every sponge were consistent with those of fellow fusion group members. All replicated grafts gave identical results. Patterns of acceptance and rejection of grafts remained consistent for all sponges throughout the 6 months during which grafting continued and were not altered by other grafts on the host sponge or by the passage of time.

The 60 largest *I. birotulata* within the 200 m^2 quadrat sorted into 24 fusion groups, the 60 *H. rubens* sorted into 12, and the 60 *A. fulva* into 13. The number of members in fusion groups varied from as few as one individual to up to 30% of the population (Fig. 1). Spatial distributions of different fusion groups overlap widely (Fig. 2) and members of different groups are interspersed with each other to the extent that nearest conspecific neighbours of only half of the individuals in each species (30/60 = 50% for *I. birotulata*, 35/60 = 56% for *H. rubens* and 30/60 = 50% for *A. fulva*) are members of the same fusion group. Fusion groups are spread over distances of up to 13 m, and these distances are independent of the numbers of fusion group members ($\chi^2 = 1.2$, $P > 0.25$, for categories of 0–6 m vs. 7–13 m and 2–4 individuals vs. 5–18 individuals).

The numbers of the 30 smaller individuals of *I. birotulata* that fused with hosts representing fusion groups with 11, 6, 5, 3, 3, 2, 1 members were 4, 1, 0, 1, 0, 0, 0, respectively; for *H. rubens*, numbers of smaller sponges fusing with hosts representing fusion groups with 14, 11, 5, 4, 1, 1 members were 5, 4, 3, 2, 0, 0, respectively; and for *A. fulva*, numbers of smaller sponges fusing with hosts representing fusion groups with 18, 8, 6, 4, 2, 1 members were 7, 5, 5, 1, 1, 1, respectively. Thus, the numbers of small and large individuals that are members of the same fusion group are consistent. These data also show that the proportion of small individuals which fused with representative hosts is not significantly different from the proportion of large individuals represented by those hosts for *H. rubens* (14/30 vs. 36/60; $\chi^2 = 2.2$, $0.1 < P < 0.25$) and *A. fulva* (20/30 vs. 39/60; $\chi^2 = 0.03$, $P > 0.25$), but is significantly lower for *I. birotulata* (6/30 vs. 31/60; $\chi^2 = 12.31$, $P < 0.001$).

These data demonstrate that these populations of hundreds of individual sponges are predominantly composed of 12 fusion groups for *H. rubens*, 13 for *A. fulva*, and more than 24 for *I. birotulata*. Individuals are distributed unequally among fusion groups in each species, in approximately the proportions represented in Fig. 1. The objection may be made that fusion groups might not actually vary in numbers of individuals, but rather that groups with many members appear that way solely because the centres of their

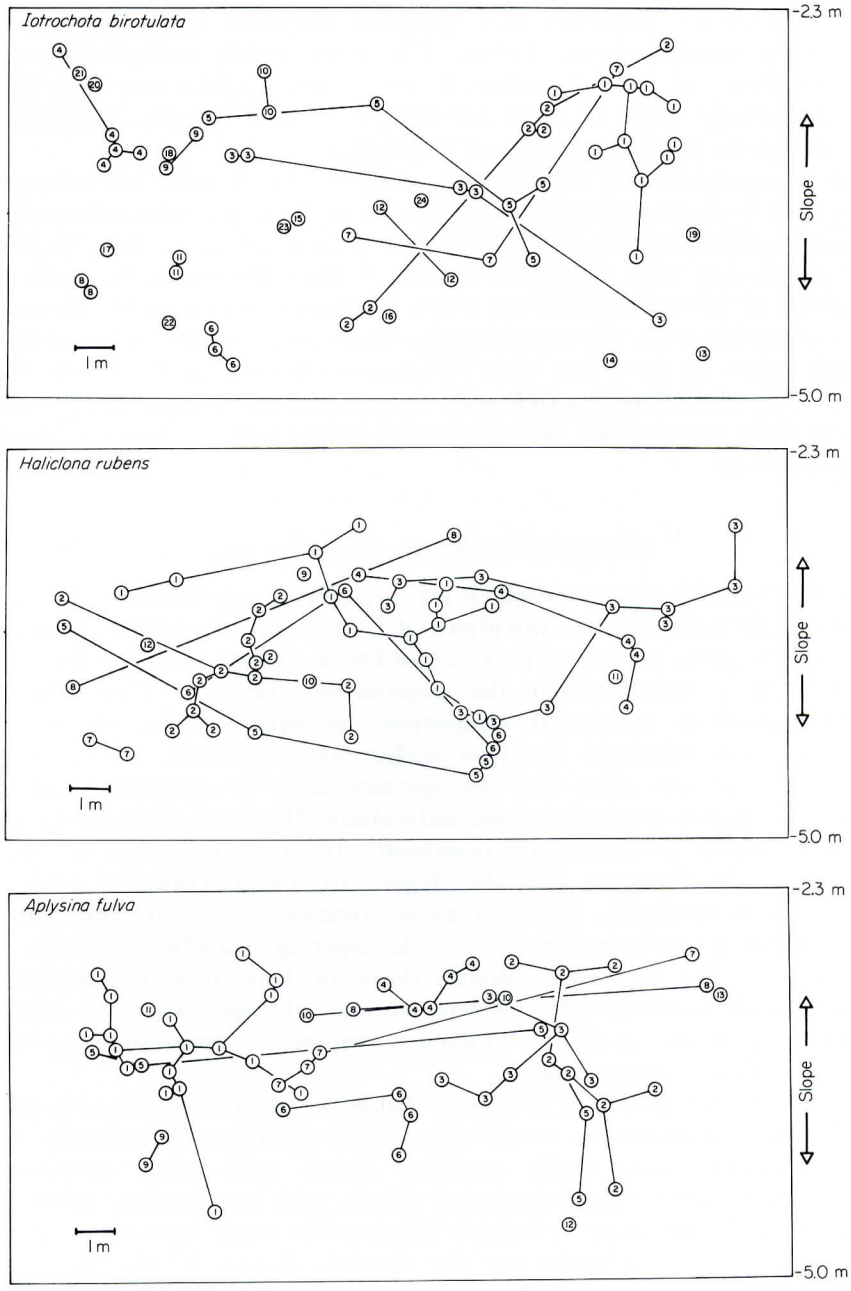

Figure 2. Spatial distributions of fusion groups. Grafts to determine tissue compatibility relationships were made among the 60 largest individuals within the 10 × 20 m quadrat, for each of the three species. Each circle marks the location of one physiologically independent individual. Circles representing individuals which are members of the same fusion group bear the same number and the lines connect each with the circle representing the nearest tissue-compatible individual. Groups are numbered in order of decreasing numbers of members. The same quadrat is here reproduced three times so that tissue-compatibility relationships can be represented for each species separately.

spatial distributions were sampled, whereas only the edges of groups which appear to have few members were sampled. However, the individuals in the centre of the quadrat do not belong disproportionately to fusion groups with many members (χ^2 for *I. birotulata*, *H. rubens* and *A. fulva* are 1.13, 0.75 and 0.31, respectively; none of these are significant at $P > 0.25$), suggesting that fusion groups do vary in numbers of individuals.

If the two populations that are separated by the channel are homogeneous with respect to tissue-compatibility types, then the expected numbers of tissue-compatible individuals are (18) (31)/60 = 9.3 for *I. birotulata*, (12) (36)/60 = 7.2 for *H. rubens*, and (12) (39)/60 = 7.8 for *A. fulva*. However, for each species, none of the 12–18 individuals from across the fragment-impassable channel were tissue compatible with the representative hosts (significantly different from the expected values by the *G*-test with Williams correction, at $P < 0.0001$ for each species). This result is compatible with the use of tissue compatibility to identify clones in these populations, but might also reflect limited dispersal of sexually produced larvae.

Variation in morphology and surface colour

The only variable character which was unambiguously characteristic of entire physiologically independent individuals of *I. birotulata* was surface colour, which can be bright green, olive green, or purple-black. Colour did not vary obviously among *H. rubens* individuals in this population, and lack of branches on all smaller individuals precluded separation according to branching pattern. Spicule lengths, relatively constant within an individual, did vary among individuals. *Aplysina fulva* has no spicules and is extremely variable in morphology, both within and among individuals. However, all individuals of this population could be described consistently by a combination of branching pattern, branch diameter and the degree to which branches are oriented horizontally or vertically. Morphological categories were, therefore, described by: (1) dichotomous branching; (2) thick, tapering branches sprouting from a single base; (3) (4) (5) narrow, intermediate, or thick branches (depending on the individual) coming off a repent runner at long intervals and in an alternating pattern; and (6) narrow, frequently anastomosing branches sprouting from a common base.

Special attention was paid to branch diameter, which, because of its potential for affecting fragmentation, could affect clone structure. Specifically, sponges which fragment more easily produce clones with more physiologically independent members. Environmental effects, such as abrasion, breakage and fish bites, cause the range of branch diameter in many individual sponges to span the range of variation for the species. Range is, therefore, a poor characterization and branch diameter of a sponge is better represented as a relative overall average (narrow, medium-narrow, medium, medium-wide, wide) based on comparisons among conspecifics in the field. Independent evaluations of average branch diameters for each sponge were always consistent.

Branch diameter varies with other characters in these populations. Branches of green *I. birotulata* are narrower than those of olive or black individuals. When branch diameter averages are scored (narrow = 1, medium-narrow = 1.5, medium = 2, medium-wide = 2.5, wide = 3) the mean scores for all green, olive

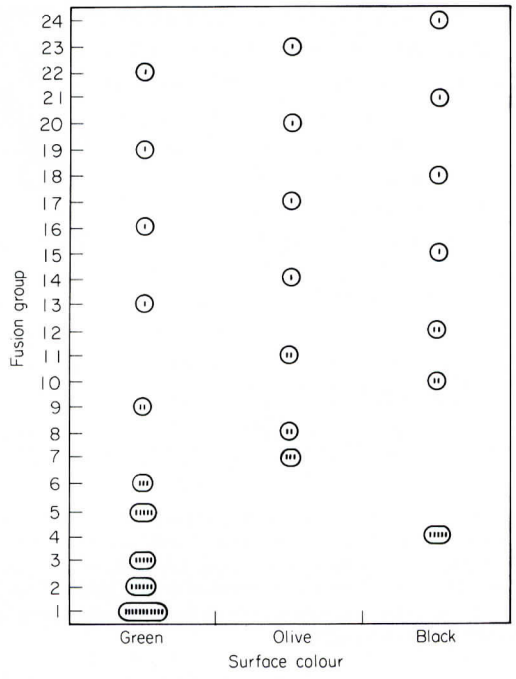

Figure 3. Tissue-compatibility relationships and variation in surface colour among *I. birotulata* individuals. Fusion groups are ranked on the vertical axis according to decreasing number of members. Each dot represents one physiologically independent sponge individual. Dots representing all individuals that are both tissue-compatible with each other and also indistinguishable by surface coloration are encircled.

and black individuals are 1.5, 2.8 and 2.4, respectively (branches of both olive and black sponges are significantly wider than those of green sponges by the Wilcoxon–Mann–Whitney test at $P < 0.001$, but not different from each other, $P > 0.05$). Diameter measurements taken at 4 cm intervals throughout several randomly chosen individuals of each colour corroborate this pattern. The mean cross-sectional areas of, green, olive and black sponges are 1.4 cm^2 (181 diameters), 2.2 cm^2 (120 diameters) and 2.6 cm^2 (97 diameters), respectively. Branch diameter increases with spicule length in *H. rubens*. The diameters (measured 5 cm below an undamaged tip) of sponges with spicules of 140–160, 160–180 and 180–200 μm are 1.2, 1.5 and 1.9 cm respectively (all comparisons are significantly different by the Wilcoxon–Mann–Whitney test, $P < 0.002$). Branch diameters were included in the morphological type descriptions of *A. fulva*.

These characters did not vary among individuals in the same fusion group in 28 of the 30 groups with more than one member. *Iotrochota birotulata* surface colour did not vary within fusion groups (Fig. 3). Spicule length did not vary within fusion groups of *H. rubens* (Fig. 4), with the exception of one group (fusion group 6 in Figs 2 & 4). Branching morphology did not vary within fusion groups in *A. fulva* (Fig. 5), again with the exception of one group (fusion group 8 in Figs 2 & 5). This result is even more striking when reported in terms of numbers of individual sponges. Of the 161 individuals that were tissue compatible with at least one other individual, only six (= 3.7%) differed in

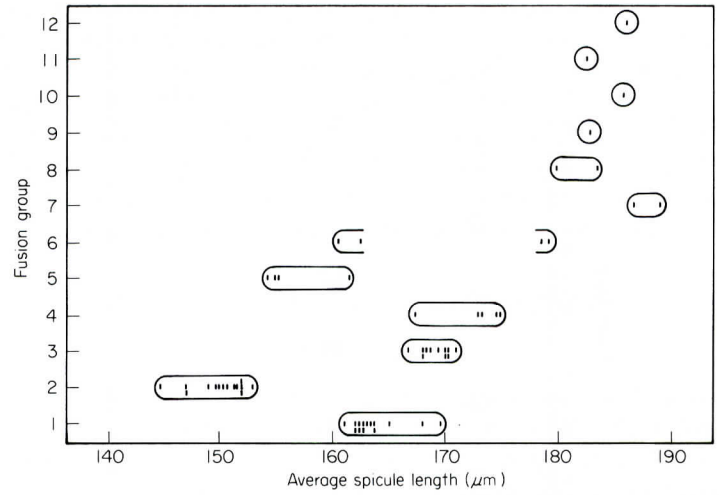

Figure 4. Tissue-compatibility relationships and variation in spicule length among *H. rubens* individuals. Fusion groups are ranked on the vertical axis according to decreasing number of members. Each dot represents the mean spicule length ($N = 40$) for one sponge individual. Dots representing individuals which were both tissue-compatible and also indistinguishable on the basis of spicule length are encircled. The four individuals in fusion group 6 were tissue-compatible, but divisable by spicule length into two groups.

morphology or surface colour from other fusion group members. This contrasts with the expected number of 109 (109/161 = 67.7%) individuals that would differ in these characters from fusion group members if morphological and colour characters were distributed among individuals randomly with respect to

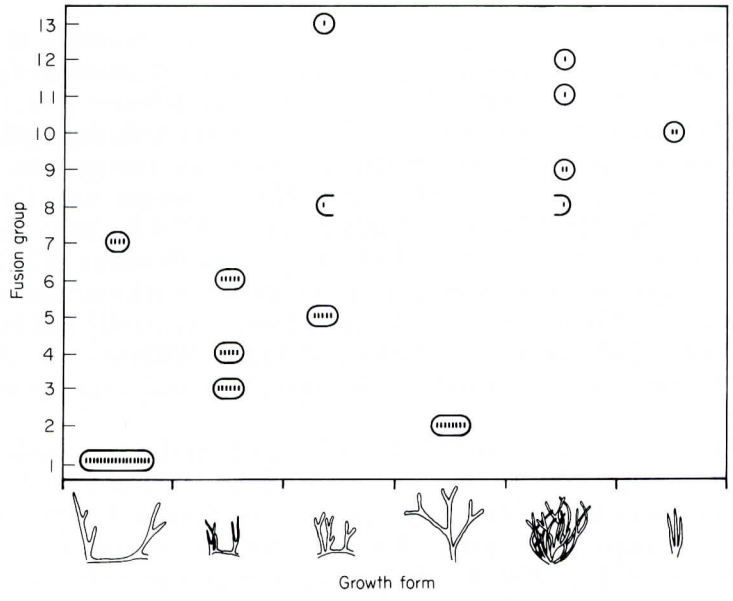

Figure 5. Tissue-compatibility relationships and variation in branching pattern among *A. fulva* individuals. Fusion groups are ranked on the vertical axis according to decreasing number of members. Each dot represent one sponge individual. Dots representing individuals which were both tissue-compatible and classified into the same morphological category are encircled. The two individuals in fusion group 8 were tissue-compatible, but differed in branching pattern.

fusion groups. If these characters are indeed genetically determined, then at least two of the 30 fusion groups represent more than one clone each. Although a third assay of genetic similarity might reveal that some of the groups defined by this combination of tissue compatibility and morphology represent more than one clone, these combined data are in accord with the suggestion that tissue-compatibility relationships closely reflect clone structure in these populations. Exactly how accurate this reflection is will have to be determined when other techniques for determining genetic similarity are applied to sponges.

WHEN DOES FUSION IDENTIFY CLONE-MATES?

Introduction

Sponges have become popular subjects of tissue fusion experiments because grafts can be made easily and scored quickly. Marine sponges have, however, been particularly intractable subjects for breeding experiments and other more direct approaches to understanding the genetic basis for tissue compatibility. Consequently, the genetic basis for fusion remains unknown. Compatibility of sponge somatic tissue has been equated with clonal identity by some investigators (e.g. Hildemann, Johnson & Jokiel, 1979; Jokiel *et al.*, 1982; Neigel & Avise, 1983b; Neigel & Schmahl, 1984) but others (e.g. Van de Vyver, 1970; Kaye & Ortiz, 1981; Curtis, Kerr & Knowlton, 1982) have not considered an inference of clonal identity from tissue compatibility to be necessarily appropriate. For this reason, I will discuss the types of evidence that have been used to evaluate tissue-compatibility data and attempt to reconcile some of the apparently divergent conclusions reported from previous studies. I will also describe the circumstances under which tissue compatibility is most likely to reflect clone structure and discuss my own data in that context.

Genetic models for tissue compatibility

Two possible requirements for tissue compatibility between sponge individuals are that (1) they share all alleles at loci governing tissue compatibility, or (2) they share a subset of those alleles. Distinction between these two possible conditions has been attempted (Neigel & Avise, 1983b; Neigel & Schmahl, 1984) by demonstrations that tissue-compatibility relationships among members of a population obey a transitive law (i.e. for any three individuals, a, b, c, if $a = b$ and $a = c$, then $b = c$). If transitivity is demonstrated among all trios of individuals in a population, it can be accepted that all alleles at loci governing tissue compatibility must be shared for sponges to fuse. However, the sample size required in order to be confident of the negative result 'no intransitivities' may be difficult to determine, and impracticably large.

For example, consider the simple case of a tissue-compatibility system of two loci, each with two alleles, only one of which must be shared between individuals for fusion to occur (the intransitive case). The probability, calculated under the assumptions discussed below, that two individuals picked at random are tissue compatible is then 0.77 (Curtis, *et al.*, 1982). The probability that a third sponge will fuse with one, but not with the other of this tissue-compatible pair is 0.17, and thus the probability of observing an intransitive trio ($a = b$, $a = c$, but $b \neq c$), is $(0.17)\ (0.77) = 0.13$.

In practical terms then, for this example, all pairwise grafts must be made for at least eight trios to expect to observe one intransitivity, distinguishing this from a transitive case. However, violation of each of the assumptions on which these calculations are based increases the number of trios that must be examined if a hypothesis of transitivity is not to be accepted unduly. First, the actual genetic system underlying tissue compatibility may be more complex than the two locus, two allele model used for this example. Secondly, for convenience, Curtis *et al.* (1982) based their calculations of fusion probabilities on the assumptions that (1) all alleles are present in equal proportions, (2) alleles are in linkage equilibrium, and (3) all reproduction is sexual. Because current abilities to manipulate sponges do not allow tests of any but the last assumption, tests of transitivity cannot now distinguish genetic models for tissue compatibility. This last assumption will surely be violated by populations for which clone structure, generated by asexual propagation, is being studied.

No intransitivities have been found among trios of individuals in branching sponge species (Kaye & Ortiz, 1981; Neigel & Avise, 1983b; Neigel & Schmahl, 1984; this study). None were found for one tube-shaped species, but a clearly insufficient sample of only three trios was examined (Neigel & Schmahl, 1984). However, even an adequate demonstration that all alleles at all tissue compatibility loci must be shared for sponges to fuse would not indicate that tissue-compatible sponges are necessarily clone-mates. This inference rests on the assumption that the genetic basis of tissue-compatibility type determination is sufficiently complex to uniquely identify each individual. Whereas this assumption may be generally realistic for mammals, current data do not support it for invertebrates. Tissue compatibility has been demonstrated between individuals which are known to differ genetically for the compound ascidian *Botryllus* (e.g. Bancroft, 1903; Oka, 1970; Sabbadin, 1977; Scofield *et al.*, 1982) and for the hydroid *Hydractinia* (e.g. Hauenschild, 1954; Ivker, 1972). Fusion has been demonstrated between electrophoretically distinct colonies of the scleractinian coral *Montipora* (Heyward & Stoddart, 1985). Until the genetic bases for tissue compatibility in sponges are understood, these results from ascidians and cnidarians must caution against glib interpretation of tissue-compatible sponges as clone-mates.

Interpretations of spatial distributions

Spatial distributions of groups of sponges that accept or reject grafts from each other have been interpreted in a variety of ways. Presumed limited vegetative spread has been used to support both a conclusion that fusion groups are clones (Neigel & Avise, 1983b) and also a conclusion that fusion groups are not clones (Kaye & Ortiz, 1981). Conversely, a suggestion that larvae have limited dispersal has been used to corroborate a conclusion that tissue-compatible sponges are not clone-mates (Curtis *et al.*, 1982). Little information is available on larval dispersal in sponges, though it is known to be extremely limited in at least some species (Bergquist, 1978). Experiments investigating dispersal of asexual fragments of *I. birotulata, H. rubens* and *A. fulva* in the same quadrat in which the tissue-compatibility experiments reported on here were performed demonstrated that dispersal of fragments can be far (up to 18.75 m in 4 weeks) and that fragments survive well (30% over a year),

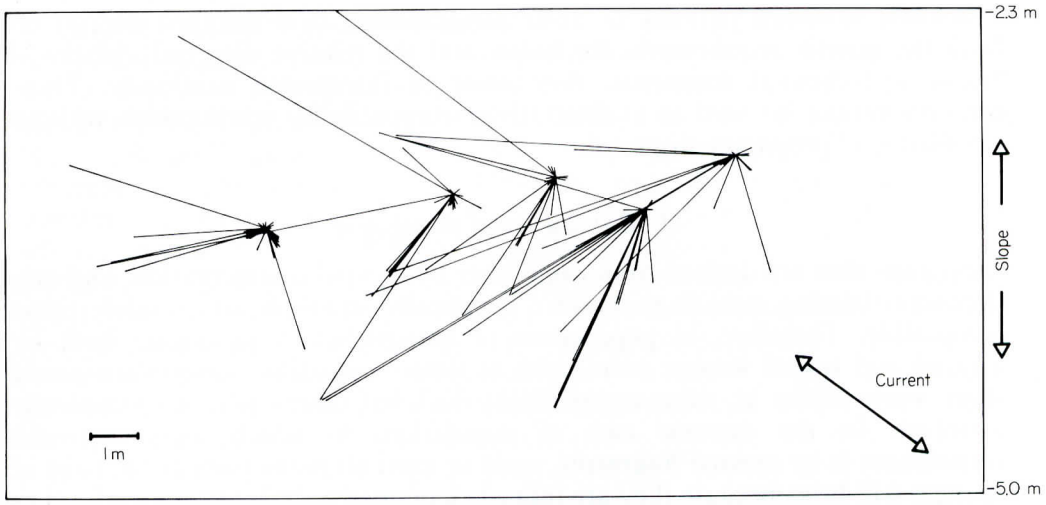

Figure 6. Experimental dispersal of fragments. Map of the same 10 × 20 m quadrat represented in Fig. 2. Lines represent dispersal trajectories of all tagged, experimental fragments that could be relocated 4 weeks after they had been placed at the five origin points. The trajectory of one fragment continued for an additional 3.8 m beyond the boundary of the area represented in this map (from Wulff, 1985).

depending on the weather in the first weeks after fragment generation (Fig. 6; Wulff, 1985). If these fragment-dispersal experiments are representative, distance *per se* appears to be no barrier to fragment dispersal across suitable terrain and a clone could be spread throughout a continuous reef system.

Tissue compatibility between populations separated by substratum that is inhospitable to fragments would constitute evidence that fusion does not imply clone membership, but this has not been reported for marine sponges in this study or in previous studies. The negative result of no compatibility between populations that are unable to exchange fragments is less easily interpreted. This pattern has been interpreted as support for the use of tissue compatibility for unique identification of sponge genotypes (Hildemann *et al.*, 1979; Jokiel *et al.*, 1982). The same pattern could also be generated by limited larval dispersal, as also pointed out by Curtis *et al.* (1982).

Changes in tissue compatibility with distance within acceptable habitat are also difficult to interpret. Clustering of tissue-compatible sponges has been interpreted as limited spread of clones in *Iotrochota birotulata* (Neigel & Avise, 1983b). However, this pattern could have also resulted from the scouring of all substratum between isolated coral heads during a hurricane (pers. obs., 1980). Lack of clustering of fusion group members in *Verongia* (= *Aplysina*) *longissima* was interpreted by Kay & Ortiz (1981) to be evidence that tissue compatibility does not identify clones. However, the same pattern could have been generated by scrambling of asexual fragments during violent storms (pers. obs., 1980). In the populations of *I. birotulata, H. rubens* and *A. fulva* reported on here, lack of clustering of fusion group members may reflect, at least in part, the extremely facile dispersal of asexual fragments throughout the appropriate habitat (Wulff, 1985). This may also be the case for populations reported on previously, but no other data have been reported with which a comparison of dispersal of asexual fragments can be made.

Because observed patterns of tissue compatibility over distance depend on both the genetic requirements for fusion and the relative dispersal abilities of larvae and asexual fragments, they must be interpreted cautiously. These patterns cannot be used to evaluate tissue-compatibility relationships without knowledge of propagule dispersal.

Asexual and sexual propagation

Sponges that are derived from each other by asexual fragmentation, and are, therefore (barring somatic mutation), genetically identical, are certainly tissue compatible. Therefore, in populations of sponges which propagate both by asexual and sexual means, some pairs of tissue-compatible sponges may only share some alleles at tissue-compatibility loci but others may be genetically identical. In the extreme case of populations in which most successful recruitment is by asexual fragments, most or even all tissue-compatible pairs of sponges will be so because they are indeed clone-mates, derived from each other by fragmentation. Among clonal marine invertebrates, high rates of propagation by fragmentation relative to sexual reproduction have been associated with branching morphology (Bothwell, 1981; Tunnicliffe, 1981; Highsmith, 1982; Rylaarsdam, 1983; Lasker, 1983; Wulff, unpubl. data). It is for populations of such species that tissue compatibility is most likely to help to define clone structure. Conversely, as also pointed out by Stoddart *et al.* (1985), tissue-compatible sponges cannot reasonably be inferred to be clone-mates in populations in which no asexual propagation is demonstrated.

With this in mind, partial resolution may be made among some previously reported results. Morphological variation within fusion groups of a tube-shaped sponge, *Aplysina fistularis*, has been attributed to phenotypic plasticity on the basis of presumed clonal identity of tissue-compatible sponges (Neigel & Schmahl, 1984). In the same study, no morphological variation was found in fusion groups of the congeneric sponge, *A. cauliformis*, a branching species. If asexual fragmentation is more prevalent in the branching sponge species, these results may be interpreted very differently. Most or all of the fusion groups may indeed represent clones in the branching species, as corroborated by their morphological homogeneity. However, in the species with less fragmentable morphology, morphological variation among tissue-compatible sponges may actually constitute evidence for genetic differences among tissue-compatible sponges.

For another species, *Ectyoplasia ferox*, Curtis *et al.* (1982) concluded that tissue-compatible sponges can differ genetically, after finding variation in plasmalemma proteins among members of the same fusion group. Tissue-compatible sponges would also not be expected to be clone-mates in this species if its thickly encrusting or fistulose growth form is not conductive to asexual fragmentation.

Van de Vyver (1970) has explicitly considered both sexual and asexual propagation with respect to tissue compatibility. She found that all small sponges that hatched from gemmules (asexual propagules) from a single individual of *Ephydatia fluviatilis* were able to fuse with each other. Of the small sponges that grew from larvae (sexually produced) from a single individual of *Crambe crambe*, 75% were tissue compatible. All apparently sexually produced

larvae from a strain of *E. fluviatilis* were able to fuse with each other (Van de Vyver & Willenz, 1975). Grafts were also accepted among individuals in a population of *Axinella verrucosa* in which no asexual propagation is known to occur (Buscema & Van de Vyver, 1984).

All these results are compatible with a scheme in which tissue compatibility does not imply clone membership for sponges, in general (additional discussions in Buscema & Van de Vyver, 1984; Stoddart *et al.*, 1985). However, in populations in which asexual fragmentation is known to be an important mode of propagation, as in easily fragmented branching species, many sponge individuals will be derived from each other by asexual fragmentation and be tissue compatible for that reason. Under these circumstances, tissue compatibility can be a useful tool for approximating clone structure.

Conclusions for this study

In the Guigala Tupo populations of *Iotrochota birotulata, Haliclona rubens* and *Aplysina fulva*, several independent lines of evidence suggest that tissue-compatibility relationships closely reflect clone structure. (1) High rates of recruitment by asexual fragments in these populations (Wulff, unpubl. data), and branching species in general, increase the probability that tissue-compatible sponges are derived from each other by fragmentation. In such populations, tissue compatibility is more likely to identify clone-mates, whatever the genetic basis for tissue compatibility. (2) Asexual fragments of these sponges are able to disperse throughout the study quadrat (Fig. 6) and they survive well in this habitat (Wulff, 1985). Dispersal of experimental fragments from origin points can be used as a model of the development of spatial distributions of clone-mates, demonstrating that spatial distributions of fusion groups are similar to those that would be predicted for clones in this habitat (Figs 2 & 6). (3) All grafts between sponges on opposite sides of a fragment-impassable channel were rejected. This result is in accordance with use of tissue compatibility to identify clones, but could also be due to limited larval dispersal. (4) Variable morphological and colour characters do not vary among sponges in 28 of the 30 fusion groups with more than one member. If these characters are genetically determined, then at least two fusion groups represent more than one clone each. However, these groups represent only 3.7% of the individuals in these populations, whereas with random distribution of morphological and colour characters with respect to fusion groups an expected 67.7% of the individuals would differ from fusion group members. Morphological homogeneity within most fusion groups therefore further supports the suggestion that fusion groups closely reflect clone structure. (5) Branch morphologies favouring fragmentation are characteristic of sponges in fusion groups with many members, as predicted if fusion groups represent clones. This final corroboration is discussed in the following section.

None of these data alone constitute sufficient evidence by which to accept the approximation of clone structure by tissue-compatibility relationships in these populations. In general, no single technique can unambiguously identify clone-mates. Genetic identity can only be demonstrated for the specific aspect of the genotype assayed by a technique, be that tissue compatibility, morphological analysis or protein electrophoresis. A combination of morphological and

ecological data suggests that tissue-compatibility relationships very closely, though clearly not exactly, represent the clone structures of these populations. Therefore, in the following discussion I will refer to clones and clone structure, with the understanding that this is an approximation, but one that is sufficient for comparative purposes.

VARIATION IN CLONE STRUCTURE

Variation in numbers of clone members: morphological correlates of fragmentation in sponges

Fusion groups in these populations vary widely in numbers of physiologically independent members (Fig. 1). If fusion groups closely reflect the clone structure of these populations, then the numbers of members in fusion groups must be related to the rates at which asexual fragments become established as independent individuals. Thus, variation among fusion groups in numbers of individuals may result from variation in morphological attributes affecting fragmentation, with sponges that fragment more easily generating clones with more members.

Characters that increase fragmentation are associated, as predicted, with fusion groups with more members in these sponge populations. Branch width is one character that may affect fragmentation in species of ramose growth form. For example, the narrow-branched Caribbean coral species *Acropora cervicornis* is fragmented more readily than its robustly branched congener *A. palmata* (e.g. Woodley *et al.*, 1981). For two of these sponge species, narrow branches are indeed characteristic of sponges in fusion groups with many members. For *I. birotulata*, individuals in four of the five fusion groups that have more than three members are green and have narrow branches (Fig. 3). Similarly for *H. rubens*, all five of the fusion groups that are morphologically homogeneous and have more than three members have short spicules and narrow branches (Fig. 4). In contrast to the skeletons of *I. birotulata* and *H. rubens*, which are made of siliceous spicules joined by the protein spongin, the skeleton of *A. fulva* is made only of spongin. Perhaps because of this, branches of any width in this species are highly elastic. However, dense tissue of sponges of this genus may make their repent branches especially susceptible to smothering under fine sediments. The morphologies of individuals in six of the seven fusion groups with more than three members are dominated by repent branches (Fig. 5). That morphological attributes favouring fragmentation are characteristic of sponges in fusion groups with many physiologically independent members further corroborates the suggestion that tissue-compatibility relationships closely reflect clone structure in these sponge populations.

If fragmentation rate depends on morphological characters that are, at least in part, genetically based, then the extent to which a sponge splits into fragments may be subject to selection. Variation in this clonal character may, therefore, be influenced by the balance of circumstances favouring fragmentation into two or more pieces versus remaining in one piece.

Sponges that produce dispersing fragments are able to sample many situations simultaneously and also cover a large area quickly. Because impact of storms may be unpredictably patchy (e.g. Stoddart, 1963; Woodley *et al.*, 1981), chances of survival are increased for a genotype that is scattered over a large

area and in a variety of different situations. Fragmentation is not a foolproof method of propagation, however. Mortality sources that act more stringently upon smaller individuals may hurt fragmented sponges more. For example, small individuals of the plate-shaped coral *Agaricia agaricites* suffer higher mortality than large individuals (Hughes & Jackson, 1980), and portions of plants that have been severed from their 'parent' plant are less likely to become established in *Solidago canadensis* (Hartnett & Bazzaz, 1983). During massive storms, easily fragmented sponges may be fragmented and dispersed to the extent that many are killed, rather than propagated (e.g. Woodley *et al.*, 1981). In addition, species that rely heavily on propagation by fragmentation may be particularly slow to repopulate large or isolated areas devastated by storms, because recruitment from distant populations may be rare. For example, the easily fragmented, branching coral *Acropora cervicornis* was damaged more and also repopulated reefs more slowly than other species after a hurricane in Belize (Stoddart, 1974). On the other hand, individuals that are not easily fragmented may be the champion survivors of epic storms (examples in Woodley *et al.*, 1981). Thus, in populations of fragmenting sponges, variation in morphological characters affecting fragmentation, such as branch width and orientation, may depend on the rates of propagule exchange among populations and the frequency and magnitude of fragment-generating disturbances experienced by different populations. Variation in these morphological characters would, in turn, be reflected in variation among clones in numbers of members.

Variation in size and shape of clones: comparison of sponges with terrestrial plants

Fusion groups in these sponge populations also vary widely in the sizes and shapes of the areas over which they are spread (Fig. 2). Because fragments of these species can be dispersed, the size and shape of the area over which members of a clone are spread is independent of the numbers of members (see Results). Dispersal trajectories varied greatly in distance and direction for marked experimental fragments that were of the same length and shape and released at the same time (Fig. 6). Fragment dispersal trajectories and, therefore, the areal extents and shapes of clones, cannot be controlled by the sponges, but depend instead on details of substratum slope, small-scale topography, and the strength and direction of the current at the time a fragment is produced (Wulff, 1985).

Lack of control over the areal extent and shape of a clone and lack of dependence of the area covered on the number of ramets in these sponges provides a striking contrast to processes of clone formation and the resultant clone structures of many terrestrial plants. Because most clonal land plants spread by extension of vegetative parts, the overall shape and size of plant clones may depend, in large part, on morphological characters that have a genetic basis. Characters such as angle of branching and internode length can affect the rate and density of spread of a clone (e.g. Silander & Antonovics, 1979; Lovett Doust, 1981; Angevine & Handel, 1985). Genetic determination of some of the morphological characters affecting clone morphology has been demonstrated by common garden experiments (e.g. Turkington & Harper, 1979; Silander & Antonovics, 1979; Hancock & Bringhurst, 1979; Burdon, 1980; Turkington & Maze, 1983). Size and shape of a clone may also depend upon its ability to

'track' a favourable habitat as it grows (e.g. Kershaw, 1962; Turkington & Harper, 1979) or to alter growth in response to resource abundance or limitation (e.g. Hartnett & Bazzaz, 1983). The sizes and shapes of clones in many well-studied terrestrial plants appear to reflect genetically based morphological and 'tracking' abilities as well as environmental effects, and thus may be subject to selection.

For clones of sessile species that are able to track a favourable habitat, variation in adaptation to particular types of microhabitats may be predicted, and this is the theoretical context in which variation in plant clonal characters has often been interpreted. Variation in size and shape of the area infiltrated by a clone has been related to the resource structure and competitive neighbours a clone may expect to encounter (e.g. Harberd, 1961; Hancock & Bringhurst, 1978, 1979; Turkington & Harper, 1979; Burdon, 1980; Lovett Doust, 1981).

In contrast, sponges and other organisms that produce dispersing fragments may have little or no control over where portions of a clone scatter. However, from the viewpoint of non-encrusting sponges, such as these branching species, the resource structure of the environment may also make impossible the prediction of whether a particular location is, and will be, favourable or not. Mortality for these sponges may be more likely to come from unpredictable external disturbances than from inappropriate nutrient conditions or hostile neighbours (e.g. Reiswig, 1973). If this is generally true, then clones of branching sponges may not only be unable to expand in a particular direction or rate, but the ability to do so may have no selective advantage. Under such circumstances, adaptation to a variety of microhabitat types is unlikely to account for variation among clones.

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