CHAPTER 4. HISTOCOMPATIBILITY AND ELECTROPHORETIC STUDIES: RELATIONSHIP BETWEEN CLONAL POPULATION STRUCTURE AND MORPHOLOGICAL VARIATION

4.1 Introduction

In the past, most discussions on the nature of morphological variation in corals, have assumed that at least part of the high phenotypic variability exhibited by many species must be genetic in origin (Yonge, 1968). Indirect lines of reasoning have been developed to support this assumption (eg. Brakel, 1977; Potts, 1978, 1984a). However, the first electrophoretic assessment of genetic variation within a coral population (*Pocillopora damicornis*) has recently been completed, and genetic variation was not found to be particularly high in this species (Stoddart, 1984a, 1984b). Despite the paucity of genetic information for coral populations, Potts (1983, 1984, 1985) has presented a model to explain the extreme levels of intraspecific morphological variation in many corals, which is based on the assumption that much of this variation is genetic in origin. Before such models can be evaluated, much more research into the levels of genetic variation within coral species and the relationship between morphological variation and genotypic variation is required.

The role of sexual and asexual reproduction in the life histories of organisms has attracted much attention (Williams, 1975; Maynard Smith, 1978; Bell, 1982). It has been suggested that asexual reproduction, particularly through fragmentation, contributes significantly to the genotypic structure of many coral populations (Bak and Engel, 1979; Highsmith, 1982). The role of boring sponges in facilitating fragmentation has also been discussed (Tunnicliffe, 1979). However, at the inception of this study, there were no published accounts of the levels of asexual reproduction within coral populations. The number of recent studies which have concluded that populations of corals have a clonal structure, either

through electrophoresis (Stoddart, 1984a, 1984b), histocompatibility studies (Bak and Criens, 1982; Bothwell, 1982; Jokiel *et al.*, 1983; Neigel and Avise, 1983; Heyward and Collins, 1985a; Logan, 1985), or both techniques combined (Heyward and Stoddart, 1985; Willis and Ayre, 1985; Resing and Ayre, 1985; Hunter, 1985), suggests that asexual reproduction occurs widely in coral species. However, a major weakness of early tissue grafting studies (Bak and Criens, 1982; Bothwell, 1982; Jokiel *et al.*, 1983; Neigel and Avise, 1983; Heyward and Collins, 1985a; Logan, 1985) was the failure to provide an independent test of the assumption that tissue fusion occurred only when grafted pairs were genetically identical. The histocompatibility bioassay is an extension of earlier work (Hildemann *et al.*, 1977) which suggested that corals had immunological specificity and memory, and it depends on precision in the self-recognition response (ie. fusion with 'self' and rejection of 'non-self'). Although differences in the geographic distances between colonies fusing versus those rejecting, and limited demonstrations of the transitivity[1] of these responses, were two lines of evidence used to support the precision of the self-recognition response in these studies, neither of these methods is independent of the bioassay itself.

The actual genetic basis for the 'immune' response of corals is unknown. Hildemann *et al.* (1977, 1979, 1980) and Jokiel *et al.* (1983) have proposed that corals possess a group of highly polymorphic histocompatibility loci, and that tissue fusion is possible only between individuals which share all histocompatibility alleles. Similar assumptions concerning the genetic basis of the histocompatibility response in sponges (Hildemann *et al.*, 1977) have been criticized by Curtis *et al.* (1982). Recently, Neigel and Schmahl (1984) extrapolated even further beyond the already tenuous interpretations of the bioassay for clonal population structures, to conclude that morphological variation within histocompatibly-defined clones of a sponge represented phenotypic plasticity. Clarification

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[1]Transitivity, in this context, denotes consistency in the histocompatibility response in interactions involving 3 individuals such that, if A=B and A=C, then B fuses with C; or, if A=B and A≠C, then B rejects C.

of the precision of the self-recognition response in corals and sponges is clearly required (Stoddart *et al.*, 1985). Electrophoretic techniques provide a means of testing the self-recognition response independently (Heyward and Stoddart, 1985).

Buss *et al.* (1984) suggested that the totipotency of cell lines in the Cnidaria first led to the need for historecognition, and then secondly provided the means for the evolution of specialized competitive mechanisms. They also suggested that the large number of different mechanisms that have evolved to deal with foreign tissue attests to the chronic occurrence of intra- and interspecific competition within the phylum. There have been numerous studies of interspecific competitition between scleractinian species (cited in Sheppard, 1985) since Lang's (1973) work on aggressive hierarchies in Jamaican corals, although conclusions regarding the role of competition in structuring coral (Bradbury and Young, 1983) or other communities (reviewed by Welden and Slauson, 1986) have been conflicting. However, with the exception of studies by Potts (1976) and Rinkevich and Loya (1983), intraspecific competition in coral populations has largely been ignored. Connell (1983) recently reviewed published field experiments of competition and found that intraspecific competition was as strong as, or stronger than, interspecific competition in three-quarters of studies that included both types of interactions. In asexually reproducing species, the importance of intraspecific competition to the genetic structure of populations is potentially great. This has been demonstrated for sea anemones (Francis, 1973; Ayre 1983), where intraspecific aggression may determine the spatial distribution of clones. Given the evidence for asexual reproduction in scleractinian corals, further research is required to determine the role of intraspecific competition in structuring coral populations.

The results of a reciprocal transplant study (Chapter 2) indicated that adult colonies of *Pavona cactus* did not respond phenotypically (with respect to colony morphology) to different habitats. Also, observations of naturally occurring fragments suggested that high levels of asexual reproduction were present

within the Eclipse Island population. Therefore the histocompatibility bioassay was applied, first, to test for the presence of clones within the population, and secondly, to test if clones were morphologically variable. Histocompatibility responses were interpreted in the light of an electrophoretic study, which tested the sensitivity of the tissue grafting bioassay and provided more detailed information on the genotypic structure of the population. It was assumed that if clonemates displayed only one growth form, despite being widely separated, then morphological differences between clones would be primarily genetically determined.

Reciprocal transplant studies demonstrated that genotypes of *Turbinaria mesenterina* were phenotypically plastic (Chapter 3). The lack of naturally occurring fragments suggested that asexual reproduction did not contribute significantly to the population structure at Nelly Bay. Therefore histocompatibility studies and an electrophoretic survey were undertaken, primarily to test the precision of the histocompatibility response in this species, but also to test predictions concerning asexual reproduction and to provide evidence with which to evaluate the degree of genetic similarity between morphs.

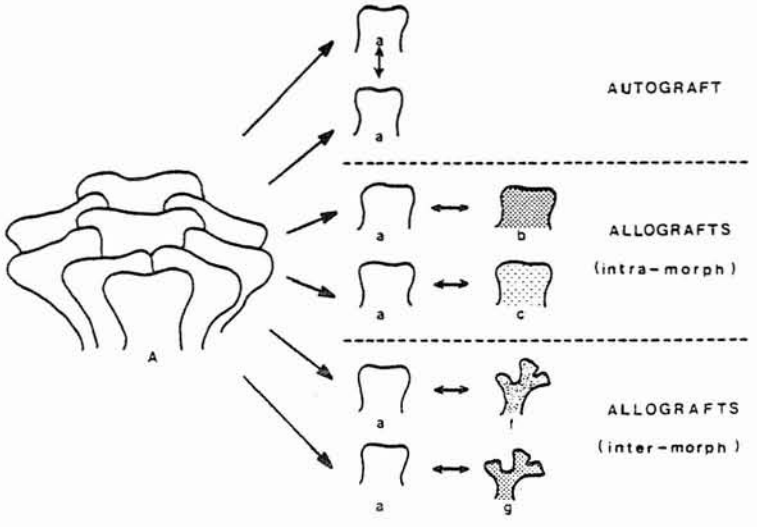
Records of intraspecific interactions were analysed to provide additional information on the extent of intraspecific competiton in coral populations. Rejection responses were considered to be the initial step in the development of a competitive interaction. Therefore grafts were maintained for a longer time period than required for histocompatibility testing, so that the ecological resolutions of such interactions could be analysed for the presence of intraspecific competition.

4.2 Materials and Methods

4.2.1 Histocompatibility Tests

Pavona cactus: To test for evidence of asexual reproduction and genetic similarity between morphs of *Pavona cactus*, histocompatibility responses between 120 pairs of colonies were monitored (90 different combinations of colonies). The histocompatibility bioassay consisted of placing two healthy fragments of equal size (between 50 and 150mm in length) in tissue contact. Fragments of *Pavona cactus* were embedded to a depth of 2cm in a pot of quick-setting cement in order to maintain tissue contact and the normal morphological orientation for the length of the grafting trial. The cement base was then firmly wedged into a steel mesh grid attached to the substratum. The whole procedure was carried out using SCUBA, so that fragments were never out of water. Fragments were paired from the same colony (autografts), between colonies having the same morph (intra-morph allografts), and between colonies of different morphology (inter-morph allografts).

FIGURE 20. Schematic representation of fragment pairing in histocompatibility bioassays. Colonies are identified by letters. The convoluted morph is represented as a single frond and the columnar morph by a branched frond.



Grafting trials were performed over two time periods. During the first period (April 1981 - February 1982), the effect of grafting site on the histocompatibility response of inter-morph combinations, involving morphs which do not naturally co-occur, was tested. Thirty grafts involving the convoluted and columnar morphs were duplicated, and one set assigned to the shallow station, site D, and the other to the deep station, site C (refer Figure 10). Five colonies of each morph were split into 6 fragments and paired with other fragments according to the scheme shown in Figure 20.

Duplication of all pair-wise combinations involving 10 colonies would have been unwieldy. However, autografts for each of the 10 colonies, and representative allografts for all intra- and inter-morph combinations were included in the trials, as summarized in Figure 21.

FIGURE 21. Summary of autografts and allografts established involving the convoluted and columnar morphs of *Pavona cactus* during the first grafting period. Symbols represent: (X), autograft; (0), allograft (intra-morph); (*), allograft (inter-morph); (-), pair not established.

	CONVOLUTED					COLUMNAR				
COLONY NO:	A	B	C	D	E	F	G	H	I	J
A	X	0	-	-	0	*	*	-	-	-
B		X	0	-	-	-	*	*	-	-
C			X	0	-	-	-	*	*	-
D				X	0	-	-	-	*	*
E					X	*	-	-	-	*
F						X	0	-	-	0
G							X	0	-	-
H								X	0	-
I									X	0
J										X

Results from the first grafting period indicated that all surviving replicated pairs performed comparably at the 2 sites (section 4.3.1). As site-dependent effects were not significant, grafts were not duplicated during the second grafting period (May 1982 - March 1983). Thus a further 60 pairs were established and divided equally between the two sites. Autografts were maintained at their site of origin, but allografts were randomly assigned to the two stations. Grafts during this period included all possible combinations of 4 convoluted colonies, 4 columnar colonies and 3 intermediate colonies.

Grafts were examined macroscopically for evidence of mesenterial digestion in the two days immediately following their establishment, and then monitored at monthly to bimonthly intervals. Contacts between fragments were examined microscopically when collected 10 months later and scored as either tissue fusions or rejections. In cases of apparent fusion, tissue on one side of the graft was mechanically stimulated, and tissue on the opposing side observed for evidence of contraction. Although tentacles are rudimentary in *Pavona cactus*, the combined polyp and coenosarc tissue is able to inflate 1-2mm away from the skeleton and provides a useful means of assessing tissue fusion. In some cases, such fusion tests were inconclusive, so the paired fragments were bleached in dilute calcium hypochlorite solution to allow microscopic examination of skeletal contacts. Fusion was considered to have occurred if new skeletal elements had been deposited in the contact zone, so that septo-costae between adjacent corallites became continuous across the fragment interface (Figure 22A). Both unidirectional and bilateral cases of tissue necrosis were scored as rejections. However, if the zone of necrosis extended more than 20mm from the contact interface, the origin of the necrosis was considered ambiguous, and the contact was not scored. Also, no score was recorded if no reaction was apparent (i.e. neither fusion nor necrosis occurred).

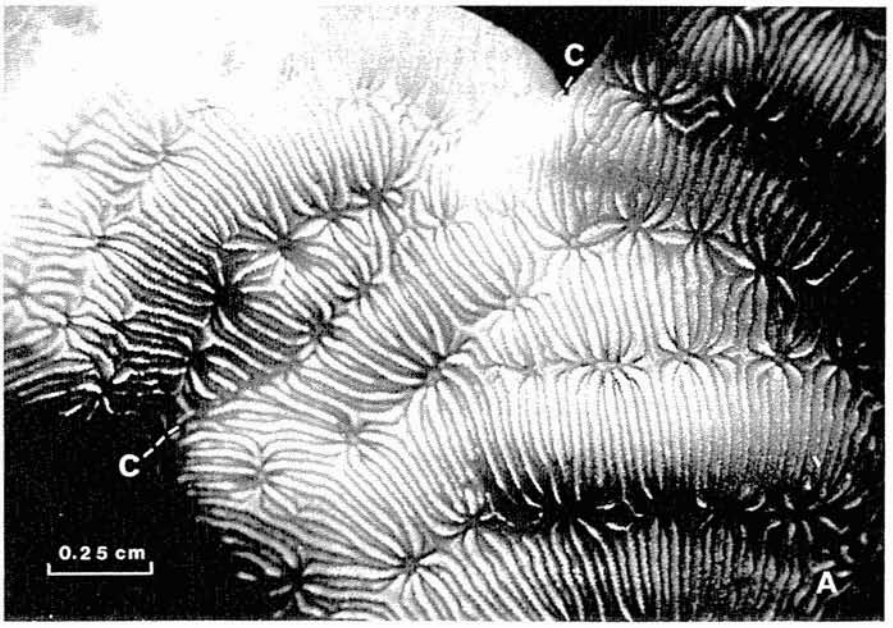
Turbinaria mesenterina: Using procedures identical to those described above, 36 fragment pairs were established. Pairings included all autografts, intra-morph allografts, and inter-morph

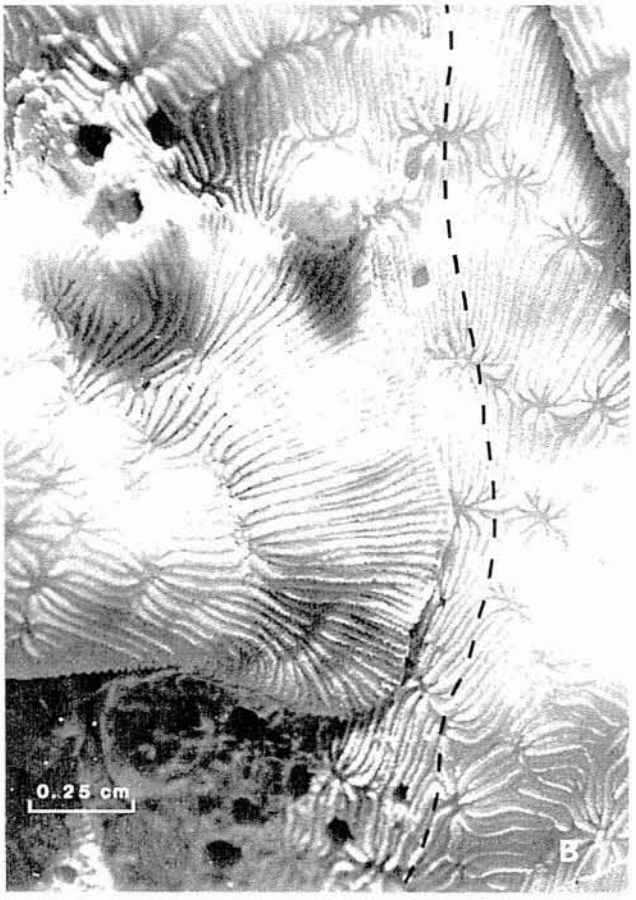
FIGURE 22

Skeletal records of histocompatibility and competitive interactions between fragments of *Pavona cactus*, Eclipse Island.

A Fusion: showing continuity and alignment of septo-costae between two intermediate fragments and the insertion of new corallites at the graft interface.
C - - C: Position of contact interface.

B Rejection followed by overgrowth: showing a columnar fragment overgrowing a convoluted fragment. Line marks extent of overlying tissue necrosis on the convoluted fragment. Fragments were paired so that the mid portions of each frond were in contact. The columnar overgrowth represents the initiation of a new branch at the point of contact.


C
C
0.25 cm
A


0.25 cm
B

allografts possible for 4 convoluted colonies and 4 plate colonies. Autografts and intra-morph allografts were maintained at their site of origin, and inter-morph allografts were divided equally between the deep and shallow stations. Grafts were established in April 1983 and collected in February 1984.

Fusion and rejection responses were monitored during monthly visits to the site. Following collection at 10 months, all contacts were examined microscopically while alive. Responses were identified as fusions if polyp retraction occurred when tissue on the opposing side of the contact interface was stimulated. When fused pairs were bleached, microscopic examination revealed that new corallites had been inserted at the graft interface, and coenosteal structures were confluent. Rejections were identified by unilateral or bilateral tissue necrosis, or the production of undifferentiated coenosteum at the interface.

4.2.2 Electrophoresis

Pavona cactus: Fragments were collected from 80 colonies and frozen under dry ice prior to electrophoresis. Colonies sampled included all those used in the grafting tests, and representatives of all growth forms within the area.

Tissue extracts were prepared by gently crushing samples of frozen coral in an equal volume of an extractant solution (10g sucrose, 0.1g bromophenol blue and 0.1 ml 2-mercaptoethanol/100 ml distilled water). Electrophoresis was carried out on horizontal starch (12%w/v) gels. All enzyme assays were modified from Harris and Hopkinson (1976). Details of enzymes found to be polymorphic, their electrophoretic buffers, and inferred quaternary structures are given in Table 12A. All isozymes were labelled alphabetically in order of decreasing electrophoretic mobility.

The enzymes MDH, MPI, PGI and LPP were variable with isozyme patterns similar to those described for other spec th normal

TABLE 12. Descriptions of polymorphic enzymes assayed for 80 colonies of *Pavona cactus*, Eclipse Island and 21 colonies of *Turbinaria mesenterina*, Magnetic Island. Quaternary structures inferred from banding patterns of apparently heterozygous individuals. Electrophoretic buffers (Selander *et al.*, 1971).

Enzyme	E.C. No.	Locus	Quaternary Structure	Electrophoretic Buffer
		A. *PAVONA CACTUS*:		
malate dehydrogenase	1.1.1.3.7	MDH	dimer	5
mannose phosphate isomerase	5.3.1.8	MPI	monomer	5
leucyl proline peptidase	3.4.11/13	LPP	dimer	6
phospho-glucose isomerase	5.3.9.1	PGI	dimer	9
phospho-gluco-mutase	2.7.5.1	PGM	monomer*	9
		B. *TURBINARIA MESENTERINA*:		
leucyl-glycylgycine peptidase	3.4.11/13	LGG	monomer	6
phospho-glucose isomerase	5.3.9.1	PGI	dimer	9
malate dehydrogenase	1.1.1.3.7	MDH	dimer	5

*Interpreted as 2 loci (see text for explanation).

Mendelian inheritance (Richardson *et al.*, 1985). Specimens were considered to be non-clonemates if they displayed detectably different phenotypes for one or more of these 4 variable loci. Electrophoretically indistinguishable individuals are not necessarily clonemates, as not all genotypic variation can be detected by electrophoretic examination. In addition, six complex 2-, 3-, and 4-banded phenotypes were detected for the enzyme PGM. Although no simple genetic interpretation could be made for this enzyme, colonies were also considered to be non-clonemates if they displayed identical 4-locus genotypes but had distinct phenotypes for PGM.

***Turbinaria mesenterina*:** Fragments were collected from all colonies involved in the reciprocal transplant study which were still alive in November 1984. This subset represented 21 source colonies, and included 4 of the 8 colonies involved in histocompatibility tests. Tissue extracts were prepared as described for *Pavona cactus*. The enzymes LGG, PGI, and MDH were variable with isozyme patterns similar to those described for other species with normal Mendelian inheritance. Further details are given in Table 12B. Specimens were considered to be non-clonemates if they displayed detectably different phenotypes for one or more of these 3 variable loci.

4.2.3 Intraspecific Competition

Interactions which developed between fragments paired in histocompatibility tests were analysed for the presence of an intraspecific dominance hierarchy. Histoincompatibility was considered to be the first step in the development of a competitive interaction. Outcomes were categorized according to the ecological resolution of the interaction. Interactions were classified as standoffs if both members of the pair produced undifferentiated skeleton in the region of the contact, such that the interface was approximately maintained in its original position. Alternatively, interactions were classified as overgrowths if one member deposited skeletal elements on the surface of the other (Figure 22B). Underwater examination at 2 to 4 month intervals monitored the consistency and direction of the interaction through time.

A competitive dominance score was calculated for each clone which was identified through histocompatibility tests and verified electrophoretically. The dominant clone in an interaction was assigned a score of 1, and the subordinate clone 0. Both were assigned 0.5 in standoff interactions. Because the number of contacts varied for each pair of fragments, and each clone was paired with either 4 or 5 other clones, dominance was expressed as a percentage of a potential score. A fragment which overgrew the other member of the pair at all contact sites would therefore score

100 for that clonal pairing. The maximum potential score was 500 for clones paired with 5 others, or 400 for clones paired with 4 others. In order to compare dominance between clones, maximum potential scores were converted to a score out of 100.

4.2.4 Fragment Survival

The ability of the convoluted, intermediate, and columnar morphs of *Pavona cactus* to survive fragmentation was assessed by comparing the relative extent of tissue mortality among fragments used in the histocompatibility tests. The percentage of each fragment's surface area exhibiting mortality was estimated visually. The consistency of visual estimates was tested by repeating the procedure for each fragment in two independent surveys and comparing the results. The mean of the two estimates was used in inter-morph comparisons of fragment survival. Mortality resulting from the grafting procedure was subtracted from the total estimate. This included mortality within 20mm of either the cement interface or an inter-fragment contact. Corrected estimates were therefore representative of the mortality which naturally-produced fragments would experience, due to factors such as infection of broken intra-colony connections or fish grazing.

4.3 Results

4.3.1 Histocompatibility Tests

Pavona cactus: No evidence of mesenterial filament digestion was noted in the two days following graft establishment, or at any time thereafter, suggesting that a histocompatibility response

rather than a competitive response was involved in the initial interaction. Rejections were readily assessed on live grafts, due to the growth of algae and the accumulation of debris at the graft interface (Figure 23B). Live assessment of fusion was less conclusive. In all cases where soft tissues were inflated sufficiently to permit tests of apparent fusion, physical stimulation of one fragment caused tissue contraction in the adjacent fragment across the interface. In general though, the permanent record of the fusion recorded through skeletogenesis proved the clearest way of verifying fusion. Where contraction occurred across the graft interface, the underlying septo-costae were found to be continuous. Deposition of skeletal elements resulting in continuity of septo-costae was therefore considered to indicate fusion. Microscopic examination of living grafts revealed the mechanism by which such continuity was accomplished. The coenosarc in the contact zone of both fragments was observed to vary the direction of its inflation, so that tissues depositing the underlying septo-costae became aligned. Re-alignment of tissues permitted adjustments in the direction of growth of septo-costae, and resulted in their continuity after prolonged contact.

Histocompatibility responses were identical in the 20 cases where both replicated pairs survived to the end of the first grafting period. Surviving replicated pairs included representatives of all autograft, and all intra- and inter-morph allograft combinations. It was concluded that grafting site had no apparent effect on the histocompatibility responses of morphs not naturally occurring at the grafting sites, and results from both sites were pooled in subsequent analyses. As outlined in the methods, it was also assumed that site replication was not necessary for a further 60 grafts established during the second grafting period.

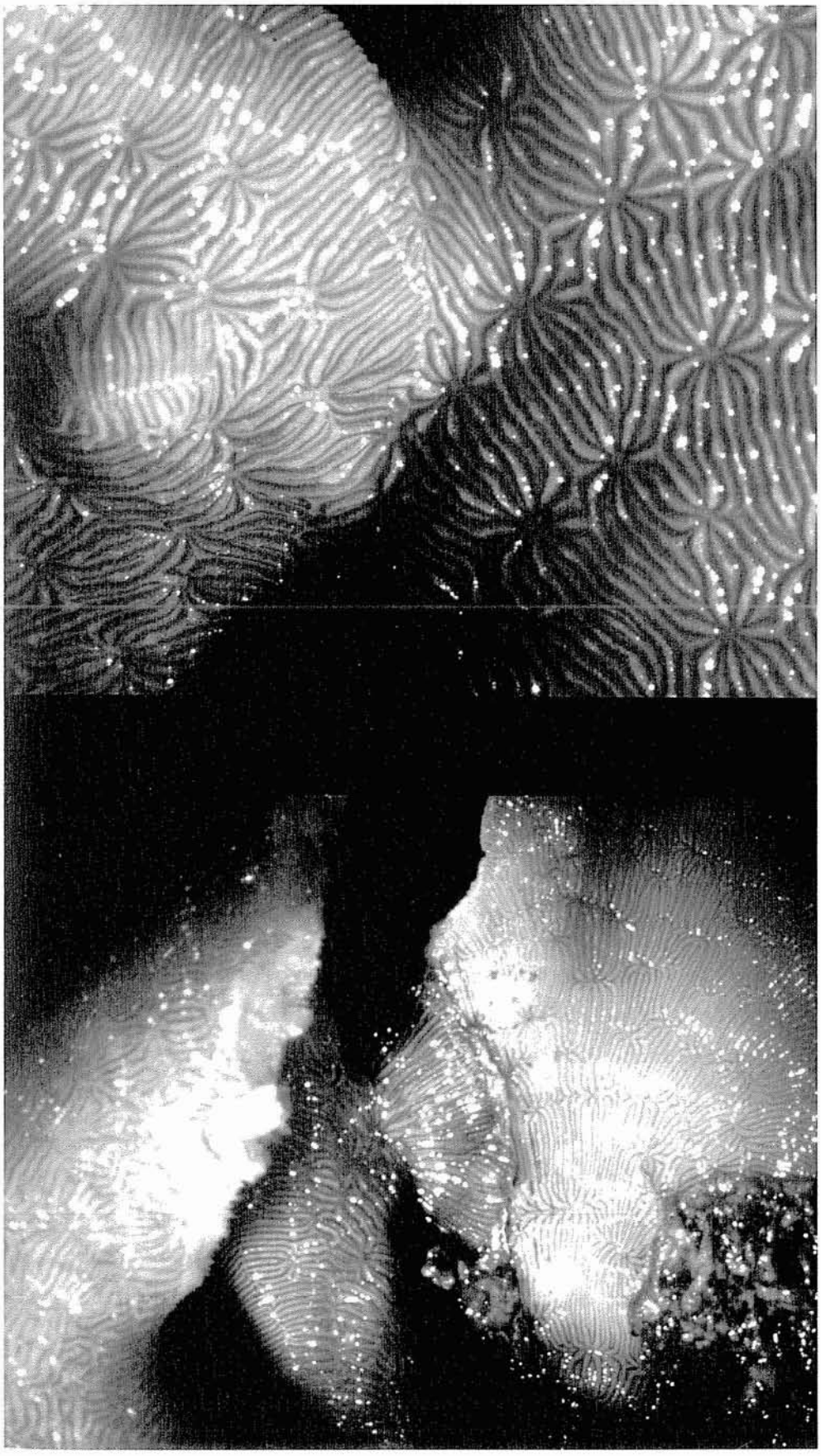
Combining results from both grafting periods, a total of 105 pairs of fragments (80 different combinations) survived the two 10-month periods. Acceptance or rejection responses were scored at 329 contact points. Histocompatibility responses within pairs involving multiple contact points were consistent in all cases. All 16 autografts fused, as did 12 of 20 intra-morph grafts, but only 1

FIGURE 23

Tissue responses in histocompatibility tests between colonies of *Pavona cactus*, Eclipse Island.

Top photograph (A): Fusion: showing tissue confluence between two columnar fragments and alignment of coenosarc overlying septo-costae. Magnification: x 10

Bottom photograph (B): Rejection: showing tissue necrosis on the surface of the convoluted fragment extending for 2mm in front of the encroaching lip produced by the columnar fragment. Algae and debris have accumulated at the graft interface. Magnification: x 10



of 44 inter-morph grafts (Table 13). Fusions of the intra-morph allografts included all 10 combinations of columnar colonies (source colonies for the two fragments in each pair separated by 6.5 to 22m), and 2 of 8 pairs of convoluted colonies (each pair separated by 5 to 7m). There were no fusions in the 3 intermediate intra-morph allografts (all pairs separated by more than 30m). The single case of inter-morph fusion involved fragments of a convoluted and an intermediate colony separated by 60m. These data permit the identification of a minimum of 5 immunological entities (Table 13), including a single widespread group of compatible columnar colonies. They also imply the occurrence of localized asexual recruitment within groups of morphologically similar colonies. Relatively high mortality among the convoluted colonies precluded further conclusions regarding whether convoluted colonies were distinct genotypes or clones.

The 80 histocompatibility tests permitted 51 predictive tests of transitivity. All 3-way networks were found to be transitive, including 6 cases of fusion amongst all three colonies (i.e. A=B=C=A), and 45 cases of rejection of a third colony, by both members of a compatible pair (i.e. A=B≠C≠A). Two of the latter transitive networks included the single inter-morph fusion. 3-way rejections were not considered to be predictive tests of transitivity.

Turbinaria mesenterina: All 72 fragments survived the 10-month period with minimal evidence of tissue mortality. Histocompatibility responses for the 36 paired combinations are shown in Table 14. Responses were scored at 128 contacts and were consistent within all pairs involving multiple contacts. All autografts fused, whereas all intra- and inter-morph grafts showed rejection responses. Thus results of histocompatibility tests were consistent with predictions that asexual reproduction did not contribute to the genotypic population structure of this species.

TABLE 13. Outcome of 80 histocompatibility tests, involving 17 colonies of *Pavona cactus* (including 3 growth forms) and a minimum of 5 immunological entities (Histo. Clone No.). After 10 months grafts were scored as fusions (F) or rejections (R) at all visible zones of contact or as not scoreable (0) due to mortality of one or both fragments.

Colony No.	Histo. Clone No.	COLUMNAR						INTERMEDIATE			CONVOLUTED							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1	F	F	F	F		F	R	0	R	0		R	R	R	R	R	
2	1		F				F					0				R		
3	1			F	F	F	F	R	R	0	R	R		R	R		R	
4	1				F		F	R	R	R	R			R	R		R	
5	1					F	F										R	R
6	1						F	R	R	R	R		R	R	R		R	R
7	2							F	R	R	R			R	R		R	
8	3								F	R	R			R	0		F*	
9	4									F	R			R	R		R	
10	5										F			F	F		R	
11	–											F	0				R	
12	–												F			R		
13	5													F	0		R	
14	5														0			
15	–															F		R
16	3																F	0
17	–																	F

* Fusion between electrophoretically distinct fragments. Colonies 8 and 16 are clonemates according to histocompatibility tests, but are electrophoretically distinct.

TABLE 14. Outcome of 36 histocompatibility tests, involving 8 colonies and 2 growth forms of *Turbinaria mesenterina*. Grafts were scored as fusions (F) or rejections (R).

Colony No.	CONVOLUTED				PLATE			
	1	2	3	4	5	6	7	8
1	F	R	R	R	R	R	R	R
2		F	R	R	R	R	R	R
3			F	R	R	R	R	R
4				F	R	R	R	R
5					F	R	R	R
6						F	R	R
7							F	R
8								F

4.3.2 Electrophoresis

Pavona cactus: Table 15 presents a summary of genotypes recorded in an electrophoretic survey of 80 colonies of *Pavona cactus*, including all colonies used in histocompatibility tests. Electrophoresis revealed evidence of extensive asexual reproduction and a striking association of genotype and growth form. All colonies with the same genotype displayed the same growth form. This included 29 columnar colonies which were electrophoretically indistinguishable, and 9 other replicated genotypes which were represented by 2 or more colonies. Colonies of five replicated genotypes were separated by more than 20m, and three of these were separated by more than 50m.

When clonemates identified by grafting tests were compared with those identified electrophoretically, one discrepancy was noted. Colonies 8 and 16 (=electrophoretic samples 47 and 72) fused, yet they had electrophoretically distinct genotypes. The survey showed that the 64 intra- and inter-morph allografts were equivalent to 18 pairings of electrophoretically distinct colonies. Thus 1 histocompatibility test out of 18 did not match electrophoretic

TABLE 15. The 4-locus genotypes and growth forms of 80 colonies of *Pavona cactus*, Eclipse Island, Great Barrier Reef.

GENOTYPES				COLONY NUMBERS	FREQUENCY	GROWTH FORM
MDH	MPI	LPP	PGI			
AA	AA	AA	CC	1 - 29	29	Columnar
AA	CD	AA	AC	30	1	Intermediate
AA	CC	AA	AB	31	1	Intermediate
AA	CC	AA	CC	32, 38	2	Intermediate
AB	CD	AA	CC	33	1	Intermediate
AA	AA	AA	BC	34	1	Intermediate
AA	BB	AB	AC	35	1	Intermediate
AA	CE	AA	AC	37	1	Intermediate
AA	CC	AA	AC	36, 39-41, 43, 44	6	Intermediate
"	"	"	"	71*	1	Convoluted*
AB	CC	AA	CC	42	1	Intermediate
AA	CE	AB	BC	45, 46	2	Intermediate
AA	CE	AA	BC	49, 54	2	Intermediate
AA	CE	AA	CC	50	1	Intermediate
AA	AE	AA	BC	51	1	Intermediate
AA	EE	BB	BC	52, 53	2	Intermediate
AA	DD	AA	CC	55, 57-61, 63, 69, 76-79	12	Convoluted
"	"	"	"	47*	1	Intermediate*
AA	CD	AA	CC	56	1	Convoluted
AB	DE	BB	BC	62, 65-68, 70	6	Convoluted
AA	CE	AB	BB	64, 80	2	Convoluted
AA	CE	AB	CC	72-75	4	Convoluted
"	"	"	"	48*	1	Intermediate*

* Colonies distinguished from other colonies having the same 4-locus genotype (indicated by: " " " "), by their PGM phenotypes.

predictions. This discrepancy occurred despite the fact that the pair in question was part of 2 transitive identity relationships.

Turbinaria mesenterina: Table 16 presents the 3-locus genotypes for 21 colonies used in reciprocal transplant studies of *T. mesenterina* (Chapter 3). No replicated genotypes were found within the Nelly Bay population, providing further evidence that asexual reproduction does not contribute significantly to the genotypic structure of this population. As fusion occurred between all isografts and rejection between all allografts, the results of histocompatibility tests were consistent with these findings and suggests that the self-recognition response is precise for the 36 pairings tested in this species. There was a significant deficit of heterozygotes at the MDH locus (X^2=18.25, P<0.001). Also, 12 of 14 plate colonies were BB homozygotes whereas 8 of 10 convoluted colonies were CC homozygotes, suggesting that colonies did not represent random samples from a single panmictic population.

4.3.3 Intraspecific Competition

Pavona cactus: Resolution of competitive interactions between 6 clones are summarized in Table 17. Three of these clones could be defined from results of the histocompatibility tests, but the other 3 required additional electrophoretic data for indentification. Colonies 11 and 15 were not included in the summary due to the low survival of pairings involving these colonies and other clones.

Overgrowths developed from 60% of rejection responses (197 contacts). Underwater examination indicated that once overgrowth was initiated, reversal of dominance did not occur. During the 10-month period, dominant clones overgrew subordinate clones by up to 10mm. Dominance scores were calculated for the 6 clones (Table 17), and a dominance hierarchy constructed. One of the intermediate colonies and the columnar clone ranked highest in the hierarchy. The two convoluted clones were clearly subordinate in their competitive abilities. Competition between the columnar and convoluted clones was highly asymmetrical, with the columnar clone

TABLE 16. The 3-locus genotypes of 21 colonies of *Turbinaria mesenterina* involved in reciprocal transplant and histocompatibility studies at Nelly Bay, Magnetic Island. (SAME COLONY): indicates that control and transplant fragments were collected from the same source colony and therefore have the same genotype. (--): electromorph indistinct and therefore not interpreted.

GROWTH FORM	PAIR NO.	GENOTYPES					
		CONTROLS			TRANSPLANTS		
		LGG	PGI	MDH	LGG	PGI	MDH
PLATE MORPH	1	BB	AB	BB	BD	BD	BB
	2	CD	CC	BB	BB	--	BB
	3	BE	CF	BB	DD	DD	BB
	4	BF	DD	BB	DF	AE	BB
	5	BD	CC	AB	EE	CD	CC
	6	DD	BD	BB	SAME COLONY		
	7	BD	GG	BB	SAME COLONY		
	8	AA	AA	BB	DD	DD	BB
CONVOLUTED MORPH	10	BB	FF	CC	SAME COLONY		
	11	AC	CC	CC	SAME COLONY		
	12	BE	GG	--	SAME COLONY		
	13	FF	BD	CC	SAME COLONY		
	14	BB	BD	CC	SAME COLONY		
	15	CF	AD	CC	SAME COLONY		
	16	CF	CD	CC	SAME COLONY		
	17	BB	CF	BC	SAME COLONY		
	18	BB	CD	CC	BD	BF	CC

TABLE 17. Outcome of competitive interactions among 6 clones of *Pavona cactus*. (x): the number of cases where clone X dominated (overgrew) the interaction between clone X and clone Y; (y): number of cases where clone Y dominated; (s): number of standoffs; (D): the overall dominance score for each clone; (F): fusion.

		CLONE Y																
	CLONE X	1			2			3			4			5			6			D
		x	s	y	x	s	y	x	s	y	x	s	y	x	s	y	x	s	y	
COL	1	-	F	-	8	5	2	4	1	4	1	4	4	58	0	0*	14	1	7	64
INT	2				-	F	-	0	7	0	6	1	0	9	0	0*	1	0	0	57
INT	3							-	F	-	7	0	0*	8	4	6	-	F	-	39
INT	4										-	F	-	9	0	4	1	0	2	72
CON	5													-	F	-	3	0	5	28
CON	6																-	F	-	35

COL Columnar morph
INT Intermediate morph
CON Convoluted morph
* Asymmetrical competition

Clone 1: colonies 1, 2, 3, 4, 5, 6
Clone 2: colony 7
Clone 3: colony 8
Clone 4: colony 9
Clone 5: 10, 12, 13, 14
Clone 6: colonies 16, 17

dominating in all 58 interactions which involved the 2 morphs. As equal numbers of inter-morph grafts were assigned to each grafting site, competitive outcomes between the columnar and convoluted clones were not affected by variation in sites of experiments.

Turbinaria mesenterina: Of the 128 contacts scored, 108 involved rejection responses. The competitive resolutions of these rejections are summarized in Table 19. In this species, competitive standoffs involved either the production of undifferentiated coenosteum in the contact zone by both members of the pair, or, a change in the direction of growth of at least 90° away from the contact interface by both members. Thus the contact interface remained in approximately the same position (symmetrical competition). Asymmetrical competitive interactions involved a change in the position of the contact interface, such that a dominant colony could be identified. Dominance was established if one member of the pair overgrew the other, or, if one colony (subordinate) changed its direction of growth so that the colony edge receded from the interface. Interactions where one colony died along the interface but the other remained healthy, were also classified as asymmetrical.

Standoffs occurred relatively more frequently in intra-morph competitive interactions than in inter-morph interactions (Table 18). In asymmetrical competitions, plate colonies dominated in 83% of interactions involving both morphs.

TABLE 18: Summary of the types of intraspecific competitive interactions recorded in intra- and inter-morph pairings of *Turbinaria mesenterina* at Nelly Bay, Magnetic Island.

PAIR TYPE	NO. STANDOFFS	NO. OF ASYMMETRICAL INTERACTIONS:		
		Convoluted Dominates	Plate Dominates	Total
CON-CON	19	8	-	8
PLATE-PLATE	3	-	7	7
CON-PLATE	5	7	59	66

CON Convoluted fragment
PLATE Plate fragment

TABLE 19. Outcome of competitive interactions among 8 colonies of *Turbinaria mesenterina*. (x): the number of cases where colony X dominated (overgrew or caused colony Y to reverse direction of growth); (y): number of cases where colony Y dominated; (s): number of standoffs; (F): fusion.

	COLONY Y																								
	1			2			3			4			5			6			7			8			D
X	x	s	y	x	s	y	x	s	y	x	s	y	x	s	y	x	s	y	x	s	y	x	s	y	
1	-	F	-	0	3	2	1	4	1	0	2	0	2	0	0	2	1	5	0	0	3	1	0	2	42
2				-	F	-	2	3	0	0	3	0	0	1	1	0	0	4	0	1	4	0	1	9	33
3							-	F	-	0	4	2	2	0	5	0	0	4	0	0	3	0	0	3	20
4										-	F	-	0	0	2	0	0	3	0	1	6	0	0	5	24
5													-	F	-	1	1	0	1	0	1	0	0	1	53
6																-	F	-	0	2	0	1	0	0	63
7																			-	F	-	0	0	2	69
8																						-	F	-	80

Colonies 1 to 4: Convoluted morph
Colonies 5 to 8: Plate morph

4.3.4 Fragment Survival

Mortality estimates for 112 fragments of *Pavona cactus* from the two surveys differed by: <5% for 101 fragments; 5-15% for 5 fragments; and 20-25% for 6 fragments. Therefore visual estimations were consistent enough to allow comparisons of mortality to be made between morphs. Mortality did not differ significantly between the convoluted and intermediate fragments (P>0.05, Table 20), but tissue mortality was recorded over a significantly lower proportion of the surface area of columnar fragments (P<0.05, Table 20). There was no detectable effect of grafting site location on fragment survival of the three morphs at the 0.01 level of significance. Exposure to cement produced negligible tissue mortality, with the fragment often overgrowing the cement base.

TABLE 20. Comparison of numbers of grafted fragments showing negligible (<5%) or significant (5-100%) tissue mortality for 3 morphs of *Pavona cactus*.

% MORTALITY	GROWTH FORM		
	Columnar	Intermediate	Convoluted
<5	86	24	53
5 - 20	7	6	13
20 - 40	2	2	8
40 - 60	2	0	8
60 - 80	0	3	2
80 -100	5	1	24
N	102	36	108

Homogeneity chi-squared tests:

Columnar-Intermediate-Convoluted:	$X^2 = 29.1$	P<0.001
Columnar-Intermediate:	$X^2 = 4.1$	P<0.05
Columnar-Convoluted:	$X^2 = 27.6$	P<0.001
Intermediate-Convoluted:	$X^2 = 2.7$	P>0.05

The greater survival rates of the columnar morph may be related to the number of broken surfaces exposed to infection, which characterize fragments of each morph. Fragments from convoluted colonies generally have many exposed edges because numerous intra-colony connections must be severed to produce even a small fragment. In contrast, columnar fragments were generally damaged at only one breakage point. As outlined in section 2.2.2, intermediate colonies are closer to convoluted colonies than to columnar colonies in the degree of anastomosis between fronds. Naturally produced fragments would sustain similar numbers of exposed edges for each morph.

Observations of a high incidence of infection by boring sponges in natural populations of all three morphs, suggests that sponges may contribute significantly to the clonal population structure of *Pavona cactus*. At least two different species of Clionid sponges were present (C. Wilkinson, pers. comm.). One species excavated throughout the living portions of colonies (Figure 24A, 24B). It was particularly common in the convoluted morph, perhaps because the flattened nature of these fronds meant that sponge tissue was never far from the outside environment. The other species (Figure 24C) was restricted to the dead bases of colonies and was most common in the columnar morph.

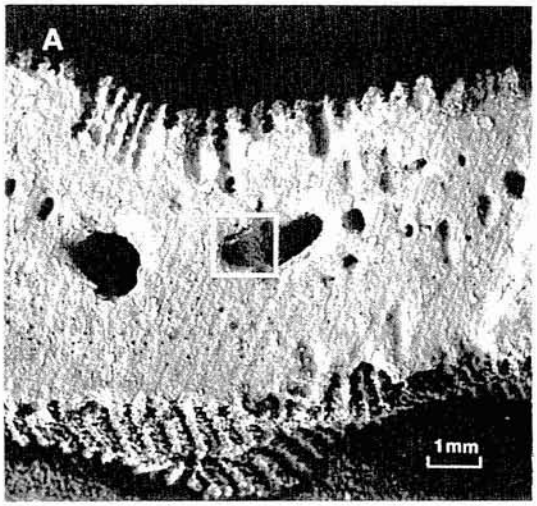
FIGURE 24

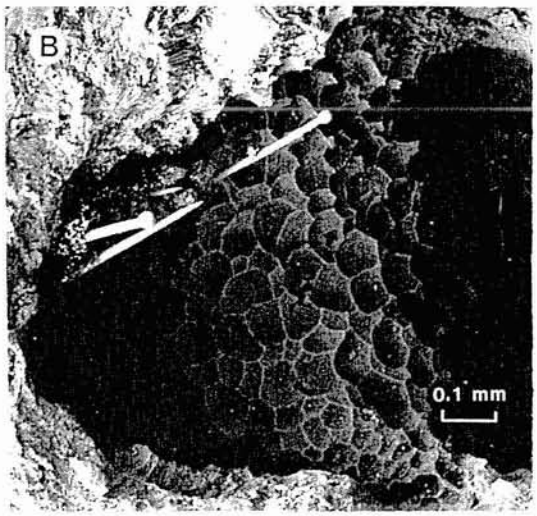
Scanning electron micrographs of boring sponge excavations in skeletons of *Pavona cactus*, Eclipse Island.

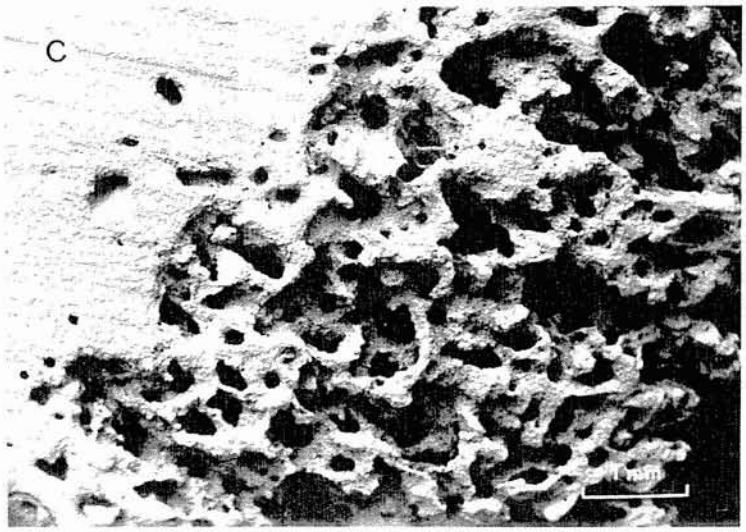
A Cross section of a convoluted frond 4cm from the growing edge of the colony, showing excavations by *Cliona sp.* Morphologically, the sponge is a dark green network of filaments. It penetrates through skeleton adjacent to living tissue, to within 2cm of the edge of the colony.

B Close-up of an excavation in **A** (indicated by the box), showing the typical convex chips removed by the sponge through a combination of chemical and mechanical processes. Several typholsole (pin-like) spicules from the sponge remain within the cavity.

C Excavations from a second species of *Cliona* in the basal regions of a columnar colony. This sponge is orangish-yellow in colouration and has only been observed in dead portions of colonies.


A
1mm


B
0.1 mm


C

4.4 Discussion

When asexual reproduction occurs in the life histories of modular organisms, it has the potential to prolong the lifespan of clonal genotypes. Although individual ramets[1] may be short-lived, the genet[1] itself is maintained, commonly through fragmentation. Vasek's (1980) estimate that the largest clone of the creosote bush may approach 11,700 years, exemplifies the longevities that may be achieved in this way. Potts (1984b) has suggested that genetic input from individual long-lived colonies has retarded the rate of evolutionary change within coral species. As documented for the creosote bush, asexually replicating genotypes may be similar to long-lived colonies in their potential to maintain old genotypes within populations, and thus retard directional selection. It must be pointed out that such reasoning implicitly assumes that rates of somatic mutation (Whitham and Slobodchikof, 1981) are low and insignificant over the generation times involved. Although predictions concerning the contribution of long-lived genotypes to rates of speciation cannot be substantiated, at the very least it can be assumed that successful genotypes of asexually reproducing species will influence local population structures on an ecological time scale. If an individual asexually-replicating genotype is competitively superior in intraspecific interactions and has high fitness as measured in other terms (eg. survival, growth and fecundity), then the potential of that genotype to influence localized population structures may be very great. Stoddart (1984a) has documented populations of *Pocillopora damicornis* with low genotypic diversities, which illustrate how successful asexually reproducing genotypes can dominate local populations and reduce their effective size.

The results of the present histocompatibility and electrophoretic studies indicate that asexual reproduction is an

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[1]Harper (1977) used the term 'ramet' to distinguish asexually produced individuals from the 'genet', the collective group of ramets having the same genotype.

important process in the life history of *Pavona cactus* at Eclipse Island. In contrast, neither technique revealed any evidence of asexual reproduction in the Nelly Bay population of *Turbinaria mesenterina*. Eighty colonies of *P. cactus* were found to represent only 23 electrophoretically distinct clones, and one columnar genotype was replicated 29 times. Clones tended to be spatially clustered, particularly the large columnar clone which covered an area 30m by 11m, excluding other morphs of *P. cactus* and most other coral species from this area of the reef slope. The size of the columnar clone of *Pavona cactus* indicates that it is probably one of the oldest clones within the population.

Asexual reproduction in *P. cactus* probably reflects fragmentation facilitated by boring clionid sponges. As suggested by Tunnicliffe (1979), skeletons weakened by sponge infestations are undoubtedly easily broken, although this need not imply that a simple relationship exists between the degree of infestation and asexual reproductive success. Within the Eclipse Island population of *P. cactus*, the effects of asexual reproduction were most pronounced in the sample of columnar colonies, but this morph was least affected by boring sponges. Only the dead bases of columnar colonies were infested. In contrast, intermediate, and in particular convoluted colonies, were frequently infested throughout the living portions of the colony as well as the dead bases. Following fragmentation, morphological considerations appeared to influence the survival rate of fragments, demonstrating that a second rate-limiting step existed before the contribution of this process to the clonal population structure could be realized. Disproportionately more of the columnar colonies survived the process of artificial fragmentation and tissue grafting. It seems likely that when fragmentation does occur, the greater structural simplicity (i.e. reduced anastomosis between fronds) of the columnar morph, leads to the production of larger fragments, with fewer broken colony connections requiring tissue regeneration. Both of these factors would favour the survival of natural fragments of this morph.

The electrophoretic survey of the Eclipse Island population of *Pavona cactus* revealed a striking association between genotype

and growth form. All colonies having the same 4-locus genotype and electrophoretic phenotype at a fifth locus (PGM), consistently displayed the same growth form. Thus, the distributions of the three morphs of *Pavona cactus* at Eclipse Island reflected the distributions of morphologically distinct clones. This occurred despite the fact that 3 of the 10 clonal genotypes identified had members which were separated by over 40m and spanned 2 sites (i.e. sites C and D, and sites D and E, Figure 10, Chapter 2). As discussed in Chapter 2 the widespread distribution of intermediate clones throughout all sites and depths, and the co-occurrence of intermediate morphs with the other two morphs in localized areas (often side by side), suggested that the observed distribution patterns were not simple associations of habitat patch and growth form. Although there was variation within the intermediate morph itself, it seems likely that consistent differences in the morphology of clones in close proximity reflect the direct effects of genotype on growth form, rather than differing patterns of microhabitat usage. Given the close association between genotype and growth form and the above empirical observations, it is suggested that genetic differences are of greatest significance in determining the observed morphological variation within this population of *Pavona cactus*.

The lack of asexual reproduction within the Nelly Bay population of *T. mesenterina* meant that it was not possible to use replicated genotypes to assess whether there was a specific association between genotype and growth form. The finding that genotypes of this species were phenotypically plastic (Chapter 3), suggested that genetic differences did not play a significant role in determining morphological variation. However, the electrophoretic survey indicated that there were consistent differences in allelic frequencies between plate and convoluted colonies. This result was most pronounced for the MDH locus, where 12 of 14 plate colonies were BB homozygotes and 10 of 12 convoluted colonies were CC homozygotes. Such an extreme heterozygote deficit (only one BC heterozygote was found versus the 12.4 that would be predicted in normal Mendelian segregation) suggests that samples did not come from a single panmictic population. A more extensive

electrophoretic survey has been initiated (Ayre and Willis, in prep.) to clarify the results of this small sample.

Comparison of the results of grafting tests and the electrophoretic survey showed that the self-recognition response was not precise in *Pavona cactus*. One discrepancy was found in 20 pairings between electrophoretically dissimilar fragments. The discrepancy involved a fusion between two fragments which shared no alleles at one locus. However, the histocompatibility bioassay matched predictions based on electrophoretic results for 79 of the 80 different pairings and effectively distinguished non-clonemates in 95% of tests involving electrophoretically distinct fragments, suggesting that the bioassay could be used to elucidate most of the clonal structure of this population.

In histocompatibility tests between colonies of *Turbinaria mesenterina* at Nelly Bay, all 28 pairings between 8 different colonies resulted in rejection responses, with fusions only occurring between autografts. Similarly, no genotypic replication was found in colonies surveyed electrophoretically, which included 4 of the 8 colonies involved in histocompatibility tests. As all pairings between colonies resulted in rejections, and this was consistent with the findings of the electrophoretic survey, it is likely that the histocompatibility response is reasonably precise in *Turbinaria mesenterina*, though further testing involving a greater number of pairings would be necessary to substantiate this conclusion.

These results, plus those of an earlier study of *Montipora verrucosa* and *M. dilatata* by Heyward and Stoddart (1985), and a later study of *Porites cylindrica*, *P. nigrescens*, *Seriatopora hystrix*, and *Stylophora pistillata* by Resing and Ayre (1985), have shown that the precision of the self-recognition response is variable among coral species. Tests discriminated between clones in 50% (*Montipora dilitata*; Heyward and Stoddart, 1985) to 95% (*Pavona cactus*: this study) of pairings for each species, with only two of the nine species tested so far (*Stylophora pistillata*: Resing and Ayre, 1985; *Turbinaria mesenterina*, this study), demonstrating complete accuracy in the response. Thus, the histocompatibility

bioassay may be a useful field technique for determining clonal population structures in large scale studies of selected species, because it requires minimal equipment and expenditure, but only if the precision of the response is tested independently in a subset of individuals and found to be high. Given that it is only possible to sample a subset of the genome through electrophoresis, the histocompatibility bioassay is also useful to distinguish between electrophoretically similar colonies (Willis and Ayre, 1985; Resing and Ayre, 1985). As 51 predictive tests of transitivity, including 2 which contained the inconsistent pair, failed to reveal the discrepancy in the histocompatibility response of *P. cactus*, it is apparent that large numbers of such tests would have to be undertaken to detect low rates of inconsistency. Transitivity tests are therefore of limited use as an independent assessment of the histocompatibility bioassay.

In this study, intraspecific competitive interactions developed between clones of *P. cactus* following tissue incompatibility. The overgrowth of one fragment by another represented significant aquisition of space when compared to normal rates of linear extension (Chapter 6). A competitive hierarchy involving 6 clones was constructed using a point-scoring system to differentiate between overgrowths, standoffs, and tissue retreat. Such dominance scores revealed that no one clone was consistently superior in pairings with other clones (i.e. asymmetrical competition; Connell, 1983), but that competition between clones tended to be symmetrical. Based on dominance scores, one intermediate clone and the columnar clone ranked highest and the two convoluted clones ranked lowest. Alternating ranks between the intermediate and columnar clones suggests that intraspecific dominance is a clonal character rather than being directly linked to a particular growth form. The common occurrence of one genotype exhibiting both dominant and subordinate reactions at different contacts involving a second genotype (symmetrical competiton), suggests that intraspecific interactions are as complex as interspecific interactions have been shown to be (Bak *et al.*, 1982), and may be affected by external factors such as surface symbionts or corallivores. It seems likely that clearly defined linear hierarchies or competitive networks do not normally occur in natural

field situations. However, there were three clonal pairings that were clearly assymetrical, and each involved one of the three top-scoring clones as the aggressor in the interaction. In particular, fragments from the columnar clone overgrew fragments from a convoluted clone in all 58 interactions involving the two genotypes.

Intraspecific dominance and high fragment survival undoubtedly contribute to the overall fitness of the columnar clone and increase the likelihood of the proliferation of this genotype. Histocompatibility tests, and the more extensive electrophoretic survey revealed that this clone was the most abundant and widespread of those identified. Conversely, the more restricted distributions of convoluted clones correlated well with both the low fragment survival and the subordinate response recorded in intraspecific interactions for the two genotypes of this morph. These data illustrate that competitive ability and success in asexual reproduction through fragmentation, are two life history attributes that vary in a consistent manner between genotypes. Also, they provide corroborative evidence that intraspecific dominance and high fragment survival through morphological specializations, are important life history attributes at the genotypic level in asexually reproducing species. Assuming average success in sexual reproduction, and thus in contributing progeny to subsequent generations, the columnar genotype has the potential to exert significant influence on the genotypic structure of the Eclipse Island population, at least on an ecological timescale.

Intraspecific competition was documented between genotypes of *T. mesenterina*, but varied in outcome between intra- and inter-morph pairings. Competitive standoffs were much more frequent in intra-morph genotypic pairings (59%, N=37) than in inter-morph pairings (7%, N=71), suggesting greater equality in competitive interactions between colonies having the same growth form. Conversely, competition was more asymmetrical between genotypes which had different growth forms. The direction of this asymmetry was reasonably consistent, with plate fragments overgrowing convoluted fragments in 89% of interactions involving the two (N=66). Assuming that growth forms and genotypes are not linked and

that genotypes are not associated with specific environments, these data suggest that genotypes surviving in deep water tend to be stronger in intraspecific interactions. The significance of these results is unclear, unless the lower angles of growth (Chapters 3 and 5) of genotypes growing in deep water offers an advantage in competitive interactions. It was demonstrated (Chapter 3) that genotypes were phenotypically plastic and that genotypes transplanted from the deep station could survive and grow equally well throughout the depth range encompassing the morphological variation in this species. Therefore unless subsequent developmental history affects competitive ability, it is hard to reconcile the prediction that genotypes of phenotypically plastic species should be able to survive equally well throughout the distribution of the species, with the finding that there were differences in competitive ability between genotypes which settled at the two different depths. More information on the genotypic structure of the Nelly Bay population of *Turbinaria mesenterina* is required before this inconsistency can be resolved.

In summary, *Pavona cactus* had an extensive clonal population structure at Eclipse Island, whereas colonies of *Turbinaria mesenterina* at Nelly Bay were founded primarily, if not exclusively, through sexual reproduction. Asexual reproduction in *P. cactus* occurred through fragmentation, undoubtedly facilitated by boring sponges. The consistent association between genotype and growth form in *P. cactus*, such that no genotype exhibited more than one growth form even when separated by extensive distances, strongly suggests that morphological variation is primarily genetically determined in this species. Further electrophoretic studies of *T. mesenterina* are required to clarify the relationship between genotype and growth form in this species. Histocompatibility tests interpreted in light of electrophoretic surveys have shown that the self-recognition response is not precise in *P. cactus* but is precise within the limits of the sample size for *T. mesenterina*. The results for *P. cactus* demonstrate that an independent test of the precision of the response is required before the histocompatibility bioassay can be used to detect clonal structures in coral populations. Despite this qualification, the self-recognition response was accurate enough in both *P. cactus* and *T. mesenterina* to

give a good approximation of the clonal structure. Intraspecific competition occurred between genotypes in both species and dominance was associated with the plate morph of *T. mesenterina* and the intermediate and columnar morphs of *P. cactus*. The large area of reef slope dominated by the columnar clone of *P. cactus* may be correlated to its dominance in competitive interactions and higher survival rate of fragments due to its morphology.