Genetic Systems and Hereditary Structures of Reef Corals

Thesis submitted by

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A.J. Heyward

20 March, 1987

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Abstract

Processes involved in the generation of new coral colonies were investigated with a view to elucidating their consequences for population genetic structure.

Electrophoretic and histocompatibility methods of estimating clonal structure in populations were evaluated. Spatial patterns of graft acceptance and rejection were different for two species of *Montipora* on a patch reef in Kaneohe Bay, Hawaii. Electrophoretically distinct tissues were capable of fusing in both species, indicating that clonal identity is not nescessarily inferred by acceptance of grafts. Tissue fusion between graft pairs of dissimilar genotypes occurred in 18 of 40 cases for *M. dilatata* and in 3 of 7 cases for *M. verrucosa*. In the majority of these allogeneic fusions, the donor and recipient colonies had one allele in common at the single polymorphic locus assayed. In one case, fusion occurred between genotypes which shared no alleles. The specificity of the self-recognition response, although different for the two species, was constant through time. It is suggested that self-recognition systems have species-specific levels of genotypic discrimination. A greater understanding of the genetics of invertebrate immunology is required before histocompatibility criteria can be used independently to assess population genetic structure with confidence.

The mode and timing of sexual reproduction was investigated in the genus *Montipora*. During late spring and summer *M. verrucosa*, *M. dilatata*, *M. verrili*, *M. studeri* and *M. flabellata* were studied in Kaneohe Bay, Hawaii. Two populations of *M. digitata* were studied in the central region of the Great Barrier Reef, Australia. All species were

simultaneous hermaphrodites, bearing male and female gonads in the same polyp. In each case, there were four male and four female mesenteries arranged as alternating pairs. An annual gametogenic cycle was apparent for *M. digitata* and inferred for the Hawaiian species. Oocytes mature over approximately six months, exhibiting a steady increase in mean diameter. The most rapid increase in oocyte size occurs in the last month prior to spawning. Testes may also be present at the onset of oocyte development, although almost all development takes place in the final two months prior to spawning. Spawning, which followed a lunar rhythm, was observed for the first time in *M. digitata*, *M. verrucosa* and *M. dilatata*. These species packaged eggs and sperm into a single cluster in each polyp. The gamete clusters, which were shed through the mouth, were highly buoyant and broke up on the surface of the sea. Fertilization and development were external.

The mating patterns of four species which broadcast gametes were compared. All species were simultaneous hermaphrodites, from the central region of the Great Barrier Reef, which shed eggs and sperm into the water during brief annual spawning events. Gametes were collected from colonies which had been isolated in separate containers and combined in controlled fertilization trials. *Montipora digitata* cross-fertilized exclusively. *Acropora tenuis*, *Goniastrea aspera* and *G. favulus* were capable of self-fertilization, but to varying degrees. In all species, cross-fertilization was the dominant mating pattern. Studies of gamete viability indicated that cross-fertilizations were possible at least 6

hours after spawning, although fertilization rates suggest that most fertilization will occur within 3 hours at high gamete concentrations.

Embryogenesis and larval development were studied in detail in the broadcast spawners *Montipora digitata*, *Acropora pulchra*, *Lobophyllia hemprichii* and *Favites abdita*. Similar developmental patterns and rates were observed in all species. Cleavage was initiated 1.5 to 2.5 hours after spawning. Cell division proceeded by progressive furrow formation. Hollow blastulae were formed within 5 to 6 hours. Endoderm formation appeared to be by delamination after the blastulae had flattened into a disk shape. All species were mobile after 24 hours. Highly mobile planulae were formed after 48 hours. Earliest settlement was after 3 days by *Acropora pulchra*. The remaining 3 species first settled on day 4. Approximately 45 % of *L. hemprichii* and *F. abdita* larvae settled on day 4. In all species the majority of larvae had settled within 7 days. These results suggest that broadcast spawning species have less probability of settling on the parental reef than brooders.

Rapidly dividing embryonic tissue was used to investigated coral cytogenetics. A comparison of chromosome number and morphology was made for 4 species of broadcast spawning corals. All species were diploid, with 28 (n = 14) mostly metacentric chromosomes. Differences between the species were largely restricted to alterations in the position of the centromeres on particular chromosomes. Fusion and fission type chromosomal rearrangements, or large quantitatative changes in DNA do not appear to have occurred in the evolution of these broadcast spawning species.

Foreword

At the commencement of this study in 1983, a genetic perspective of reef coral populations was not part of the general coral reef vista. Since fundamental demographic parameters, such as sexual reproduction, remained poorly studied in most scleractinians, this was not surprising. Mode of reproduction was considered a conservative characteristic and, in the absence of experimental data, assumptions of random mating, self-seeding populations (eg. Done, 1982) were the rule. Yet extreme differences in life history patterns between species eg. *Styllophora pistillata* and *Porites* spp. (Loya, 1976) and (Potts, 1983), would suggest a diversity of genetic systems among the Scleractinia.. It was the aim of this study to probe some of the elimentary biological processes influencing the hereditary structure of coral populations. Particularly interesting were species which maintained populations through both sexual and asexual means of colony propagation. The genus *Montipora* was selected as a starting point.

Vivipary and planulation had been observed in numerous common species and were regarded as the reproductive processes giving rise to new coral colonies. In 1983, however, the assumptions of planulation as a sexual process were challenged (Stoddart, 1983a) and two studies using reciprocal grafting (Jokiel et al, 1983; Neigle and Avise, 1983a) described coral populations with significant clonal structure. These epochal studies introduced a quantitative, genetic viewpoint to studies of coral demography. Central to this change in perspective was the application of techniques

for distinguishing individuals on a genetic basis. Histocompatibility systems (Jokiel et al, 1983; Niegle and Avise, 1983a) and gel electrophoresis (Stoddart, 1983a) were proposed as means for identifying unique genotypes.

The possibility that recruitment via fragmentation was extensive in corals (Highsmith, 1982) suggested that in such populations, separate individuals might be of identical genotype. Self-recognition systems appeared to have a broad application in elucidating possible clonal structures. The genetic basis of histocompatibility systems were not known, however, which created uncertainty over the results derived from reciprocal grafting studies. In contrast, there was substantial evidence to support the genetic models upon which interpretation of electrophoresis was based. The present study compared histocompatibility and electrophoretic systems as techniques for distinguishing genotypes, then considered the consequences for analyses of clonal structures.

While acknowledging the potential significance of clonal proliferation, the concept of mating systems and hereditary relationships within coral populations was pursued essentially in relation to sexual processes. Interestingly, in the *Montipora* species (Jokiel *et al.*, 1983) and *Acropora cervicornis* (Neigle and Avise, 1983a) where fragmentation appeared to contribute to populations, nothing was known of sexual reproduction. In a population of *Montipora digitata* in Australia (Heyward, 1981) there was similar evidence of fragmentation, but also suspicion of an annual sexual cycle.

In the genus *Montipora*, the available data (Marshall and Stephenson, 1933; Stimson, 1978; Robertson, 1981;

Heyward, 1981) suggested that the traditional view (eg. Hyman, 1940), of vivipary followed by the release of planulae, did not apply. Intensive sampling regimes were combined with histological analysis to study gametogenesis in Hawaiian and Australian species of *Montipora*.

Successful observation that *Montipora* species released eggs and sperm provided an opportunity to work directly with coral gametes and hence analyse the mating system. The discovery of multi-specific coral spawnings on the Great Barrier Reef (Harrison *et al.*, 1984; Babcock *et al.*, 1986) offered the chance to compare mating systems among several species. This predictable access to coral gametes was unprecedented. Developmental studies and controlled breeding trials were now within reach. The techniques used were refined by taking advantage of the summer spawning period in both hemispheres. Chapters 3 and 4 document the mating systems and embryology of broadcast spawning species. These studies pointed to fundamental differences in the genetic systems operating among various species. The possibility that some species differences may be reflected in variation in their karyotypes had not been investigated due to technical difficulties (Wijsman and Wijsman-Best, 1973). The embryo studies of the present investigation suggested that it might be possible to elucidate at least some basic aspects of coral cytogenetics. The final investigative chapter provides the first look at coral chromosomes and considers the evidence for cytogenetic changes in Scleractinian evolution.

Chapter 1

Assessment of Clonal Structure in Coral Populations.

INTRODUCTION.

In species which reproduce annually, eg. *Montipora digitata* (Heyward and Collins, 1985a), the synchronous release of gametes is a spectacular example of individuals sharing in a common gene pool. Nonetheless, the Mendelian properties of coral populations have been assumed rather than demonstrated. Indeed, the possibility of clonal proliferation via colony fragmentation , rather than the consequences of sexual reproduction, stimulated recent investigations of coral population genetics.

Clones are separate individuals derived asexually and having identical genotypes. The separation of a coral colony into smaller groups of polyps, either by partial tissue loss destroying cytoplasmic continuity or by physical separation due to skeletal breakage, is a well known natural phenomenon. The resulting parts have independent potentials for growth, integration between polyps and mortality and can be regarded as distinct individuals.

Branching morphs in particular have been noted for their regenerative powers following fragmentation (Stephenson and Stephenson, 1933). Several accounts relate the passage of severe storms and the subsequent survival and growth of fragments (see Rinkevich and Loya, 1983b and references therein).

Highsmith (1980) noted that with many long-lived coral species the importance of storm derived fragmentation, leading to asexual (vegetative) colony multiplication, may

have been previously underestimated. Subsequent observations indicated that this form of clonal proliferation could be extensive (Highsmith, 1982) and could also occur , in less robust morphs, under conditions more typical than the severe storms associated with infrequent cyclones or hurricanes (Tunnicliffe, 1981; Heyward and Collins, 1985b). Bull (1982) hypothesized that fragmentation may be the major means by which some populations of *Montipora digitata* maintain themselves.

Several independent studies done in different parts of the world (Jokiel *et al.*, 1983; Neigle and Avise, 1983a; Heyward and Collins, 1985b), have attempted to demonstrate and quantify this type of asexual propagation of coral colonies for branching species in the family Acroporidae. All of these studies have utilised the discriminatory immune response, demonstrated to exist in many reef building corals (Johnston *et al.*, 1981), as an assay for clones. Each study has indicated some clonal structure. These and other studies (Stoddart, 1983a, 1984) have given currency to the hypothesis that clonal structure is a significant feature of some coral populations.

The use of immunogenetic recognition phenomena to denote clonal structure in coral populations derives from an assumption that only identical genotypes will fuse when their living tissues come into contact (Hildemann *et al.*, 1977). The working hypothesis has been that fusion of an intercolony graft-pair indicates isogenic clonemates, while rejection of grafted tissue is symptomatic of allogeneity. On this basis, a program of intercolony grafts can be used to investigate the genetic structure of a population. Neigle and

Avise (1983 a, b) suggest that self-recognition phenomena have great potential for similar application in many invertebrate populations. Nevertheless, all these workers include a caveat concerning the specificity of coral self-recognition phenomena. Both the genetic basis and evolutionary context of histocompatibility remain areas of speculation. Indeed, one of the few attempts to investigate these phenomena empirically suggests that the working hypothesis of individual uniqueness may be incorrect (Curtis *et al.*, 1982). Such a finding for corals would seriously question the extent of clonal structure inferred to date from grafting studies.

The present study attempts to provide a limited test of the validity of the hypothesis that fusion occurs only between genotypically identical coral tissues. In this regard it attempts to replicate the grafting study of Jokiel *et al.* (1983), which showed a substantial clonal structure in patches of *Montipora dilatata* and a lesser developed clonal structure in *M. verrucosa*. As an independent test of genotypic identity, the tissues of grafted pairs were examined electrophoretically. For each of the two species, a single polymorphic locus was assayed. Any fusion between electrophoretically distinct colonies refutes the hypothesis that fusion occurs only between clonemates.

MATERIALS AND METHODS

Location:

Field work was conducted on a single patch reef in Kaneohe Bay, Hawaii (Figure 1.1, see Ch. 2 for map of Kaneohe Bay), using the same areas as for Jokiel *et al.* (1983).

Montipora verrucosa was common and well distributed along the patch reef, while *M. dilatata* occurred in distinct patches of several meters diameter. Four patches of *M. dilatata* were located (Figure 1.1) and a tape measure laid through the middle of each. The position of colonies occurring within a meter either side of the tape was recorded. Colonies smaller than a handspan in diameter were ignored to preclude recently derived fragments. An identical number of *M. verrucosa* colonies were mapped in areas immediately adjacent to the four *M. dilatata* patches. These areas were selected to mimic the size and shape of their corresponding *M. dilatata* patch. All mapped colonies were numbered and tagged.

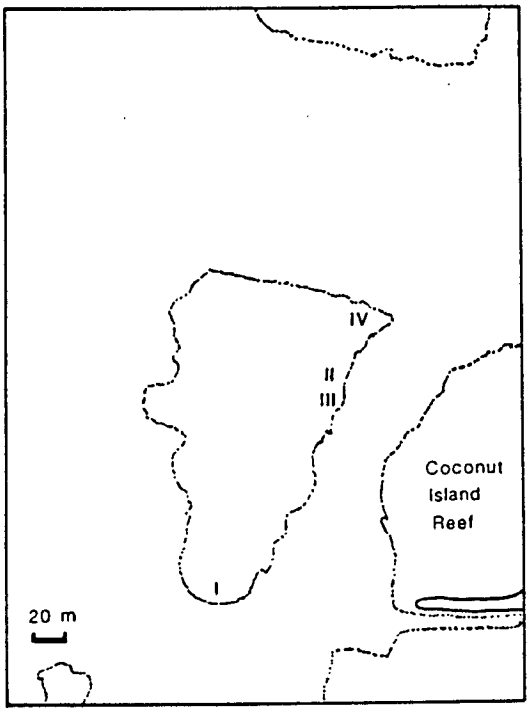


Figure 1.1
Map of Sample Sites I-IV of *Montipora dilatata* and *M. verrucosa* on the patch reef adjacent to Coconut Is., Kaneohe Bay, Hawaii.

Grafting:

Grafts were made using the technique of Heyward and Collins (1985b). A healthy terminal branch-piece, 2 to 5 cm long, was snapped off the donor colony with finger pressure and applied laterally to a branch of the recipient. The graft was made secure with a 3 mm wide plastic-cable tie bearing the donor's number. In addition, a piece of the recipient

colony was grafted to itself, acting as an isogenic control. Within each coral patch, graft combinations were chosen to maximise the modal distance between donor and recipient, i.e. between colonies of the ends and the center. A few combinations were also made between colonies at the extremes of each patch. Four colonies of *Montipora dilatata* in Patch IV were donors for grafts with colonies in Patches I, II and III.

Interpretation of the outcome of each graft was based on the condition of tissue at the graft interface and skeletal morphology with reference to the control graft. Fusions were typically complete after two weeks, but the grafts were not scored until the interaction had progressed for a further three weeks. Each graft was recovered from the field and examined under a stereo-dissector microscope.

Electrophoresis:

Branch tips were homogenized with two volumes of an indicator extractant (Stoddart, 1983 a) and the resultant solution was absorbed by 3 mm squares of Whatman No.3 filter paper. A small section of tissue paper (Kimwipes) was placed between the sample and filter paper to reduce mucus adsorption. The filter paper wicks containing the tissue extracts were loaded onto starch gels prepared from a Tris-EDTA-borate buffer. Electrophoretic techniques were those described by Stoddart (1983 a). Following electrophoresis, the gels were stained for either leucyl proline peptidase (LP) for *Montipora dilatata*, or leucylglycylglycine peptidase (LGG) for *M. verrucosa*. The ratio of the distance traversed by a protein "band" to the distance traversed by the

advancing buffer front was calculated. This value was designated as R_f.

Statistics.

The observed genotypic diversity (Go) and the expected genotypic diversity (Ge) were calculated from genotype and gene frequencies using the formulae of Stoddart (1983 b).

The concordance of observed phenotypic frequencies with the predictions of Hardy-Weinberg equilibria were tested using the chi-squared statistic. To avoid an excess of low expected values, the two rarer alleles for LGG were pooled.

RESULTS.

Grafting:

Fusion of tissues in autographs of both *Montipora dilatata* and *M. verrucosa* often occurred within a week. All fusions, regardless of the type of pairing, were well advanced after 5 weeks, many with new polyps developing in the old graft-interface zone. Both species showed radial branch thickening around the graft ties, occasionally completely overgrowing the tie.

The two species showed distinctly different patterns of graft acceptance and rejection (Table 1.1). All intra-patch grafts of *Montipora dilatata* fused, while for *M. verrucosa* fusion within the arbitrary patches was related to distance between heads from which the grafts originated. In the latter species, no fusions occurred for heads separated by over 2 m and, at 1.2 m distance, 74 % of grafts were not compatible. All pairings of *M. dilatata* from Patch IV were incompatible in the other three patches. Essentially, these are the same results as those of Jokiel *et al.* (1983).

Table 1.1 *Montipora dilatata* and *M. verrucosa*. Results of intra-patch graft combinations for both species. Data pooled from all four patches.

Distance apart (m)	*M. dilatata*		*M. verrucosa*	
	Fusions	Rejections	Fusions	Rejections
<1	80	0	2	1
1.0-1.9	14	0	5	14
2.0-2.9	5	0	0	13
3.0-4.9	12	0	0	2
5.0-8.9	1	0	0	5
Total	40	0	7	35

Electrophoresis.

Montipora dilatata: Two distinct zones of activity were apparent for LP. The zone of greatest anodal activity (R_f=0.7) showed no variation. The slower zone contained two discrete bands (R_f=0.4, R_f=0.2) in varying combinations. The banding pattern in this zone is best described by a model of one locus with two alleles coding for a monomeric enzyme (Figure 1.2). Every patch of *Montipora dilatata* contained at least two of the three possible genotypes (Table 1.2). The Go:Ge ratio for this species was 1.07. The distribution of genotypes, pooled for all patches, does not fit the expected phenotypic ratio of Hardy-Weinberg equilibrium ($p<0.01$) (Table 1.2).

Figure 1.2

Montipora dilatata. Electrophoretic banding patterns observed for leucyl proline peptidase (LP).

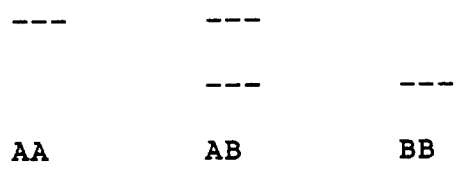


Table 1.2

Montipora dilatata: Frequency of LP phenotypes in the four patches examined.

Patch no.	No. of colonies	No. of phenotypes		
		AA	AB	BB
I	13	2	7	4
II	28	0	9	19
III	17	12	4	1
IV	13	2	3	8

Montipora verrucosa: Similarly to LP, LGG showed a faster monomorphic band (R_f=0.05). A slower region of activity (R_f=0.35 to R_f=0.20) contained five discrete bands in various combinations, with never more than two bands per individual. Both homozygote- and heterozygote-type banding were present and were consistent with a monomeric enzyme coded by a single locus with five alleles (Figure 1.3). Genotypic frequencies are presented in Table 1.3. The Go:Ge ratio for this species was 0.80. The distribution of genotypes, pooled for all patches, does not fit the expected phenotypic ratio of Hardy-Weinburg equilibrium ($p<0.001$) (Table 1.3).

Figure 1.3

Montipora verrucosa. Electrophoretic banding patterns observed for leucylglycylglycine peptidase (LGG).

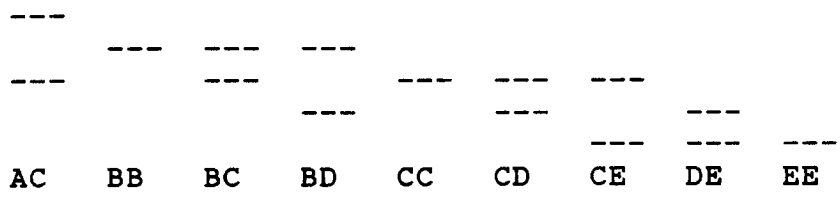


Table 1.3

Montipora verrucosa. Frequency of LGG phenotypes in the four patches examined.

Patch no.	No. of colonies	No. of phenotypes								
		AC	BB	BC	BD	CC	CD	CE	DE	EE
I	14		2	1		3	1	3	3	1
II	29	1	2	11	3	12				
III	17	1	1	3		2		10		
IV	15			3		2		3	7	

Grafting versus electrophoresis.

Both species showed a number of instances of fusion between pairs of branches with differing electromorphs (Tables 1.4 and 1.5). Within patches of *Montipora dilatata*, fused grafts were divided almost equally between identical and distinct pairs. It was only when the data from all the patches were pooled that any trend appeared (Table 1.6). In this case, significantly fewer identical electromorphs were involved in rejections than would have been predicted and, similarly, fewer distinct pairs were involved in fusions.

Table 1.4

Montipora dilatata. Phenotypes of grafted branch pairs and the results of histocompatibility trials.

Combination	Within patches (all fused)	Between patches (all rejected)
AA-AA	8	0
AA-AB	3	1
AA-BB	1	4
AB-AB	4	1
AB-BB	14	2
BB-BB	10	4

Table 1.5

Montipora verrucosa. Phenotypes of grafted branch pairs and results of histocompatibility trials. Data from all patches were combined.

Combination	Accept	Reject
AC-BC	0	1
AC-CC	0	2
BB-CC	0	3
BB-CD	0	1
BB-CE	0	1
BC-BC	2	2
BC-BD	2	0
BC-CC	0	6
BD-CC	0	1
CC-CC	0	3
CC-CD	0	1
CC-CE	1	3
CC-DE	0	3
CE-CE	1	1
DE-CE	0	4
DE-DE	1	0
DE-EE	0	1
EE-CC	0	1
EE-CE	0	1

Table 1.6.

Montipora dilatata and *M. verrucosa*. Comparison of results of graft acceptance or rejection with phenotypes of the graft pairs for both species separately and for pooled results. Chi-squares have one degree of freedom. *M. verrucosa* data incorporate Yates correction for low expected values.

Species	Phenotypes		Statistics	
	Same	Different	Chi-square	*P*
M. dilatata				
Reject	5	7	0.62	>0.05
Accept	22	18		
M. verrucosa				
Reject	7	28	2.59	>0.05
Accept	4	3		
Pooled				
Reject	12	35	8.66	<0.001
Accept	26	21		

DISCUSSION

The results of this study indicate that the hypothesis of fusion occurring only between genetically identical individuals should be rejected for these populations of the corals *Montipora dilatata* and *M. verrucosa*. The frequency of graft acceptance between tissues having different electromorphs is incompatible with the postulate that histocompatibility criteria allow precise discrimination of clones.

It may be argued that the differences in the position of the bands of enzyme activity on the starch gels do not

reflect differences between the genotypes of individuals. In contrast to invertebrate immunology, however, the genetics of the assumptions underlying electrophoretic interpretation have been the subject of extensive empirical research. Although post-transcriptional modifications of electrophoretic significance can occur in some enzymes (eg. Suzuki *et al.*, 1979), these usually produce electrophoretic banding patterns which cannot be interpreted by simple locus allele models (Stoddart, pers. comm.). The ready incorporation of the data into simple Mendelian models suggests the robustness of the assumed genetic basis for these isoenzymes (Heyward and Stoddart, 1985). The possibility that the symbiotic zooxanthellae are responsible for differing electrophoretic patterns in fusing pairs is unlikely since (a) the techniques should not rupture zooxanthellae cells (Dustan, 1979; Stoddart, 1984), and (b) clonemates should inherit the same strain. Using the same techniques, Ayre and Resing (1986) compared the electrophoretic patterns of zooxanthellate and non-zooxanthellate coral species. They conclude that the presence of symbionts did not influence the banding patterns.

The data do not question the nature of the histocompatibility response as part of a complex immune system (cf. Hildemann *et al.*, 1980). Rather, it suggests that different species have reconition systems which vary in their level of discrimination. If, as suggested by Rinkevich and Loya (1983a), the histocompatibility system mediates a colonies' response to interaction, then the specificity of recognition may vary with life history characteristics. The

tissue grafting assay of population structure is in excellent agreement with the results of Jokiel *et al.* (1983), suggesting that the level of recognition specificity is stable in these populations. *Montipora dilatata* displays complete histocompatibility within patches, yet clearly this is not a total lack of response since the interpatch grafts consistently rejected. In Jokiel *et al.*'s (1983) study, branches from any patch rejected branches from all other patches. Two explanations of this spatially restricted compatibility are consistent with the findings of other studies.

Firstly, it has been suggested that other anthozoans may become habituated to contact with allogeneic tissue and cease to respond in further interaction (Purcell and Kitting, 1982). This may be especially so if the initial contact occurs before the coral is immuno-competent (Hidaka, 1985) as in situations of aggregated settlement. Under these conditions, members of patches of *Montipora dilatata* may have had similar developmental histories, being exposed to contact with each other or the same third party. Fragments of the original habituated pairs may retain their habituation when they meet again, giving rise to a number of apparently unconnected corals which can fuse with each other. However, habituation has not been demonstrated in corals. Allografts of adult *M. dilatata* and *M. verrucosa* colonies have not shown evidence of habituation after one year's contact (Jokiel, pers. comm.).

Secondly, it has been suggested that some invertebrates are compatible if they share a common parent (Van de Vyver, 1970; Scofield *et al.*, 1982). In the present study, graft

acceptance occurred in only one case where the pair did not share a common allele. This and the disproportionate number of electrophoretically identical pairs among the fusions suggest that some shared ancestry allows compatibility. In this case, *Montipora dilatata* patches may represent an original aggregative settlement of half-siblings resulting from the proximity of eggs of a single parent.

The finding that the histocompatibility response cannot be relied upon as a discriminator of self is in agreement with studies of sponges (Curtis *et al.*, 1982; Van de Vyver, 1984) and has been supported by subsequent work on corals (Willis and Ayre, 1985; Resing and Ayre, 1985). The estimates of clonal proliferation based on pioneering work using tissue grafting (Jokiel *et al.*, 1983; Neigle and Avise, 1983a; Heyward and Collins, 1985) can no longer be relied upon. This is not to say that no clonal structure is present in the populations studied.

The analysis of a single locus in the present study is obviously inadequate in assessing a population for clonal structure. Field observations, however, suggested that many colonies of both species were growing from fragments. Willis and Ayre (1985) have demonstrated significant localized asexual reproduction in the coral *Pavona cactus*, presumably caused by fragmentation, using multiple electrophoretic loci. In their study, although tissue grafting did not reliably identify all clones, it matched predictions based on electrophoretic results in more than 95 % of the pairings. The specificity of tissue recognition would appear to be far greater in *P. cactus* than in *Montipora dilatata*. While an

analysis of population genetic structure using tissue grafting would provide a very good estimate of asexual colony propagation in *P. cactus*, a similar study of *M. dilatata* could be grossly misleading. The data of Resing and Ayre (1985) highlight this problem. In an extension of the present study they compared, for *Seriatopora hystrix*, an analysis of population structure inferred from grafting with that derived from a multi-locus electrophoretic survey. Grafting implied extensive clonal replication, while electrophoresis indicated a population genetic structure dominated by sexual reproduction.

In conclusion, the use of histocompatibility responses is of little value without an independent test of genetic specificity. Asexual colony proliferation is a feature of many coral populations, but quantitative analyses require a different approach. The development of techniques such as electrophoresis would appear to offer much broader application in determining the dynamics of genetic structure in coral populations. Nevertheless, since electrophoresis samples only a fraction of the genotype, histocompatibility can be useful in testing for differences between electrophoretically identical colonies.

Chapter 2

Sexual Reproduction in the Genus *Montipora*.

INTRODUCTION

Descriptive studies of reproduction in corals appear to be undergoing a renaissance. Since 1980 the literature has blossomed with data on sexual or asexual reproduction by scleractinians in nearly all parts of the tropical world. An heightened awareness of the potential role for vegetative colony propagation has resulted (see Chapter 1), nevertheless, the contribution of sexual reproduction continues to be considered of major importance to nearly all populations eg. Pichon (1978).

Vivipary, leading to the release of planula larvae, has been considered the typical sexual process (Hyman, 1940) and acceptance of the planulation paradigm characterizes early studies of coral reproduction. Stimson (1978) notes that in many earlier observations, little or no inference was made from a particular species failure to planulate. Marshall and Stephenson (1933) observed planulation of several species on the Great Barrier Reef (GBR), but also noted the presence of mature eggs in species eg. *Montipora digitata* (syn. *M. ramosa*), without detecting planula release. In explaining the lack of observed planulation in species such as *M. digitata*, hypotheses suggesting semilparity (Marshall and Stephenson, 1933) or the possibility of some species not breeding in regular or annual cycles (Connell, 1973), attribute the observations to a shortfall in the sampling rather than question the mode of reproduction.

In Hawaii, Stimson (1978) noted developing gonads in many

species of corals, particularly in spring and summer, yet did not detect planulation in the majority. He hypothesized that many of these species may not brood larvae and suggested that the generalization of planulation for coral reproduction might need to be abandoned.

Ease of detection probably accounts for the historical bias towards observations of species which release planulae. Frequently studied corals such as the archtypical species *Pocillopora damicornis*, show a marked lunar periodicity in their release of relatively large, robust planulae and, depending on location, reproduce throughout the year (Harrigan, 1972) or for several consecutive months (Stoddart, 1984). In contrast, the release of sperm and relatively small eggs by broadcast spawning species is often restricted to one or two nights per year (Babcock *et al.*, 1986) and is consequently an highly elusive event. Fortuitous observation of gamete release has occurred on rare occasions eg. Krupp (1983), but in earlier works a collected colony seen releasing gametes would most likely have been regarded as suffering from stress eg. Wilson (1888) and the phenomenon not pursued. Detailed studies of gametogenesis, which have provided forewarning of imminent spawning, characterize recent studies in which broadcast spawning by corals has been recorded (Szmant-Froelich *et al.*, 1980; Kojis and Quinn, 1981; Babcock, 1984; Heyward and Collins, 1985a). While vivipary is the typical mode of reproduction for many species, Harrison *et al.* (1984) have shown that at least as many species are now known to utilize external fertilization and development.

Reassessment of vivipary's generalized role in

scleractinian reproduction has received further impetus from recent work showing that the formation of brooded planulae need not involve syngamy (Stoddart, 1983a; Ayre and Resing, 1986). The release of such asexual planulae has strongly influenced the genetic structure of populations of *Pocillopora damicornis* in Australia and Hawaii (Stoddart, 1983a, 1984) and must be considered as a possible contributing factor to the clonal structure recently observed in Hawaiian and Australian populations of *Montipora* species (Jokiel *et al.*, 1983: Heyward and Collins, 1985b).

The genus *Montipora* has been represented in two major works on coral reproduction (Marshall and Stephenson, 1933; Stimson, 1978). Both studies noted the presence of eggs in *Montipora* species but did not record the mode of sexual reproduction. A more recent study of *M. digitata* on the GBR (Heyward, 1981) demonstrated a protracted period of gametogenesis. Oocytes steadily increased in size for six months then disappeared from colonies in late spring.

The presence of mature oocytes in *Montipora digitata* on the GBR and in *Montipora verrucosa* from Hawaii only during the warmer months of the year (Stimson, 1978; Heyward, 1981) is notable. The present study seeks to document the mode and timing of sexual reproduction for several *Montipora* species in Hawaii and for *M. digitata* on the GBR. No spawning has been recorded for the genus and the data from previous studies suggest that rapid external development of planulae is more likely than vivipary.

MATERIALS AND METHODS.

Location:

In Hawaii five species of *Montipora* were investigated, with the two dominant species near the Hawaii Institute of Marine Biology, *Montipora verrucosa* and *M. dilatata*, being of key interest. All samples were collected from populations in Kaneohe Bay, Oahu, 157° 50' W, 21° 30' N (Figure 2.1). *M. flabellata* and *M. studeri* were found in the area of high wave energy along the barrier reef crest, *M. verrucosa*, *M. dilatata* and *M. verrili* were sampled from the more sheltered patch reefs within the bay (Figure 2.1).

Investigations of *Montipora digitata* on the GBR, Australia, focused on the populations at Geoffrey Bay, Magnetic Island (146° 52'E, 19° 9'S) and Pioneer Bay, Orpheus Island (146° 29' E, 18° 37' S) in the central region near Townsville (Figure 2.2). Both bays contain fringing reefs associated with granitic high islands. Geoffrey Bay is exposed to the predominant south-east trade winds and has more wave action than Pioneer Bay. At both locations *M. digitata* is abundant and dominates the upper inter-tidal reef flat.

Gametogenesis.

Hawaii:

The initial study occurred during the spring and summer of 1983. An extensive field survey on the 26th May, using hammer and chisel to fracture colonies for internal examination, revealed that every colony of all *Montipora* species greater than 20 cm in diameter contained oocytes which were visible to the trained eye.

In order to determine subsequent sampling procedure, 5 colonies of each species were collected and investigated for synchronous gamete maturation. Following fixation and decalcification, 10 polyps were dissected from the edge, middle and center of each colony. Mean oocyte diameters showed considerable but non-significant ($p>0.05$) differences within and between colonies of each species. All sterile zone of varying width (5-30 mm) was present at the plate edges and branch tips. Bearing in mind the apparent population synchrony, 5 large (>50 cm diameter) colonies of *Montipora verrucosa* and *M. dilatata* and 2 colonies of the less abundant *M. verrili* were tagged and sampled each week. Ten additional colonies of these three species were monitored at random each week in situ by inspecting fractured pieces. *M. studeri* and *M. flabellata* occurred in a waveswept environment where relocation of individuals was difficult. Consequently 5 untagged colonies of each were sampled at random each week.

Pieces of each colony were broken off with hammer and chisel, transported to the laboratory in seawater and immediately fixed in 10 % (v/v) formalin-seawater for 24 h. The arrangement and size of the gonads were studied using a stereo-dissector microscope with ocular micrometer, originally by dissecting soft tissues following decalcification in 5 % formalin - 10 % formic acid solution (v/v). The identification of oocytes and spermatogonia was confirmed from histological sections. In treatment for histology, fixed tissue was decalcified in 5 % formalin - 2 % formic acid solution (v/v), processed to parafin, sectioned on a rotary microtome at 5 um and stained with Haemotoxylin

and Eosin as per Winsor (1984).

After the initial observations had provided an understanding of gonad arrangement, fixed and decalcified specimens were used only for histology. Numbers of eggs per mesentery and egg size were recorded from live material which was fractured using wire cutters and supported in seawater under the stereo-dissector. This avoided fixation artifacts when measuring oocytes and gave a better feeling for macroscopic changes in the gonads. Thirty oocytes per species were measured on two perpendicular diameters each week.

Australia:

The steady progression of gametogenesis had already been documented in a previous study (Heyward, 1981). Results of that work indicated that gametes disappeared from the Geoffrey Bay population in late spring shortly after full moon. Samples for the present study were collected at random from 10 colonies per week, commencing on 22nd September, 1983. Histological and stereoscopic examination of the samples was performed as for the Hawaiian species.

Spawning.

Hawaii:

When fresh squashes of testes from the weekly samples revealed motile spermatids, portions of at least 3 colonies per species were collected and placed in flow-through aquaria for close observation. Individual colonies were isolated in buckets or aquaria when spawning appeared imminent. Spawning was either observed directly or inferred form the presence of gametes in the isolated containers. When a colony in the aquaria was observed to spawn, an immediate dive was

organised to check for synchrony with colonies in the field.

Following the observation of spawning in 1983, it was predicted that *M. verrucosa* and *M. dilatata* would spawn again in the summer of 1984. During 1984, commencing on 28th June, 50 colonies of these two species were sampled at random every 3 days. An additional 10 colonies of each were maintained in aquaria for closer monitoring.

Australia:

At Magnetic Island, the gonads of *Montipora digitata* colonies matured rapidly in October, 1983. The data from Geoffrey Bay suggested that spawning might occur in that month and sampling frequency was increased to consecutive collections every 3 days. Samples and night dives were organised on a daily basis commencing one day prior to the full moon of October, 1983.

The population of *M. digitata* in Pioneer Bay lagged behind the Geoffrey Bay population in development of gonads. The gonads matured rapidly during November and sampling frequency was intensified as above for the period around the November full moon. In addition to random samples, 50 colonies were tagged and sampled on a daily basis, commencing on November 20th, in order to test for synchrony of the spawning event.

In 1984 it was predicted that the Geoffrey and Pioneer Bay populations would again be out of phase with regard to spawning and the sampling procedure was repeated. Sampling commenced on a fortnightly basis beginning on September 20th and intensified as above at the October full moon.

Embryology.

Hawaii and Australia, 1983:

The following methodology was employed in the preliminary study of embryogenesis. A complete discussion of coral embryology and modified methodology is provided in Chapter 4.

Spawned gametes were collected, by suction into plastic squeeze bottles, from 4 colonies in the separate aquaria and buckets. This was possible for *M. verrucosa* and *M. dilatata* in Hawaii and both populations of *M. digitata* on the GBR. In 4 petri dishes, gametes of individual colonies were isolated to test for self-fertilization. Four other dishes contained a mixture of gametes from several colonies, providing the opportunity for cross-fertilization.

RESULTS.

Gametogenesis.

Hawaii and Australia:

All 6 species of *Montipora* are simultaneous hermaphrodites, with male and female reproductive structures on separate mesenteries in the same polyp. Gonads were arranged on eight mesenteries, two male alternating two female (Figure 2.3). On each mesentery the oocytes or spermatogonia were arranged along the oral-aboral axis behind the mesenterial filament (Figure 2.4). The general appearance of the gonads as gametogenesis progressed was very similar for all species. In Hawaii, *M. verrucosa* was always more advanced in development and similarly, in Australia the Geoffrey Bay population of *M. digitata* matured earlier than the population at Pioneer Bay.

In Hawaii, using *Montipora verrucosa* as an example, the

progression of gametogenesis was observed as follows. On the 25th May, 1983, ovaries were prominent, with 3-5 oocytes along the oral-aboral axis of each mesentery. Testes had also developed within the mesenteries but were translucent, strap-like bodies and of smaller volume than the ovaries. The oocytes showed a steady increase in size until spawning (Figure 2.7). Maturation of the male gonads was more dramatic. Testes increased markedly in volume in the month prior to spawning, when both male and female gonads became similar in volume and cream to white in colour. Three weeks prior to spawning the density of zooxanthellae in the mesenteries surrounding the gonads increased, particularly around the ovaries. Some oocytes of *M. verrucosa* contained zooxanthellae a week prior to spawning and in one colony of *M. dilatata* zooxanthellae had infected oocytes 12 days prior to spawning. However, infection prior to spawning was not observed consistently and the majority of oocytes had no intracellular zooxanthellae prior to spawning.

One week prior to spawning the surrounding mesenteries were packed with zooxanthellae, causing the ovaries to appear brown. During the rapid maturation of the testes in the month prior to spawning the original strap-like structures became increasingly locular. Testes were distinctly locular 2 weeks prior to spawning (Figure 2.5), with spermatids developing tails as the lumens of the locules developed. A week prior to spawning, squashes of live testes revealed very active spermatids.

This pattern of gametogenesis was repeated in both populations of *Montipora digitata* on the GBR, although

zooxanthellae were not observed in the oocytes until they were released at spawning (Figure 2.6). The final maturation of gametes in the week prior to spawning was again characterized by highly locular testes containing motile spermatids and oocytes surrounded by dense accumulations of zooxanthellae.

Spawning.

Hawaii: *Montipora verrucosa* broadcast gametes on the 10th, 11th and 12th of July, 1983, following the new moon of the 10th (Figures 2.8, 2.9). *Montipora dilatata*, *M. verrili* and *M. studeri* spawned two weeks later, during the week following the full moon of July 25th (Figures 2.8, 2.10). The release of gametes by *M. verrucosa* and *M. dilatata* was directly observed in the aquaria and the field. The time of spawning of *M. verrili* and *M. studeri* was not directly observed, but could be inferred from the disappearance of gonads from the sequential weekly samples. Both these species lost their gametes during the week after the July full moon. One colony of *M. verrili* released gametes in the aquaria on the night of July 28th. The short interval between samples and the record of shed gametes suggested that gametes, rather than planulae, were released in the field at the same time as *M. dilatata*. *Montipora verrili* and *M. studeri* and *M. flabellata* were easily stressed in the aquaria, which may account for the general lack of directly observed spawning. Many colonies of *M. flabellata* still contained gonads after this period and some colonies were mature in August and early September. Not all colonies were gravid, however, after the July full moon. This suggests that *M. flabellata* may have

multiple spawnings during the late summer and fall.

Montipora verrucosa and *M. dilatata* released their gametes in the same manner. Colonies began to exhibit pre-spawning behaviour approximately 30 minutes after sunset. Each polyp elongated away from the skeleton, gathered the gonads under the oral disc, then contracted the body wall. The gametes were formed into a sphere which distended the oral disc. This posture was maintained for approximately 30 minutes before the gametes were ejected through the mouth. The release of eggs and sperm was simultaneous, each polyp releasing a single cluster of eggs packed tightly around the sperm (Figure 2.6). Usually each gamete cluster represented the entire reproductive complement of the poly, containing 12 to 20 ova. At release all ova contained zooxanthellae. The inheritance of maternal zooxanthellae was also noted for the dioecious Porites compressa, which was fortuitously observed spawning gametes on the new and full moons throughout the summer. The presence of zooxanthellae was checked with fluorescence microscopy.

During spawning the gamete cluster was normally propelled clear of the colony and floated quickly to the surface. In the calm conditions of the aquaria the clusters broke apart, releasing the encapsulated sperm and separating the individual ova, within 30 minutes. In *Montipora verrucosa* and *M. dilatata* the release of gametes commenced between 2045 h and 2100 h. Individual colonies shed most of the gametes in the first hour after commencing release.

Each colony released most of its gametes during one evening. In observations of 8 colonies in aquaria however,

small clusters of polyps retained their gametes and spawned on successive nights. Spawnings on successive nights always commenced close to 2100 h. The major population spawning occurred on the 11th of July for *Montipora verrucosa* and the 27th and 28th July for *M. dilatata*. Two days after these major spawnings, 50 colonies of each species were sampled at random in the field and no gravid colonies were found.

In 1984, the population of *Montipora verrucosa* spawned entirely on the 29th of June, the night of the full moon. The spawning of *M. dilatata* was less precise. Sixty percent of the sampled colonies spawned on July 14th and 15th, after the new moon on the 13th. Nearly all the remaining gravid colonies in the population spawned following the next full moon. Nevertheless, 3 % of colonies contained oocytes and testes in the first week of August.

Australia:

The exact moment and nature of gamete release was observed in both populations of *Montipora digitata*. The spawning behaviour was identical to that observed for the Hawaiian species. Eggs and sperm were released as tightly packed clusters. There were 10 to 16 ova per cluster and all contained zooxanthellae.

In 1983, the Geoffrey Bay population released gametes between 1900 and 2100 h, on the first, second and third nights after the full moon of October 22nd (Figure 2.13). The Pioneer Bay population spawned one month later, during the three nights after the full moon of November 20th. A survey of the 50 tagged colonies at Pioneer Bay each night of spawning showed that 6 % of colonies spawned on the first

night after the full moon, 70 % on the second and 60 % on the third night. Each colony shed most of its gametes during the first night on which it spawned, although a minor spawning of the remaining gravid polyps often occurred over successive nights as in Hawaii. In the Pioneer Bay sample, 36 % of the colonies spawned on successive nights. In 1984, spawning occurred again one month earlier at Geoffrey Bay than at Pioneer Bay.

In both 1983 and 1984, a group of researchers (Babcock *et al.*, 1986) observed many species of coral broadcasting gametes at both study sites. The multi-specific spawning events occurred several days after the spawning of *Montipora digitata* (Figures 2.13 to 2.15) during the neap tidal cycles. Over 100 species of scleractinia were recorded in the study which covered a very wide area of the GBR.

Embryogenesis.

Hawaii:

After the spawning in aquaria, for both *Montipora verrucosa* and *M. dilatata*, gametes were collected from separate colonies and mixed together at 2230 h. The first cell divisions were noted at 2345 h, although by 0045 h less than 1 % were at the 2 or 4 cell stage. Some embryos at the 2 cell stage were isolated for observation. Early cell divisions occurred every 45 to 60 minutes in irregular radial development (sensu Giese and Pierce, 1974, pp. 176). Cell divisions were complete and equal , but not always synchronous beyond the 8 cell stage. Seven hours after spawning morulae were common and gastrulae were apparent within 12 h. After 14 h many gastrulae were ciliated, but the

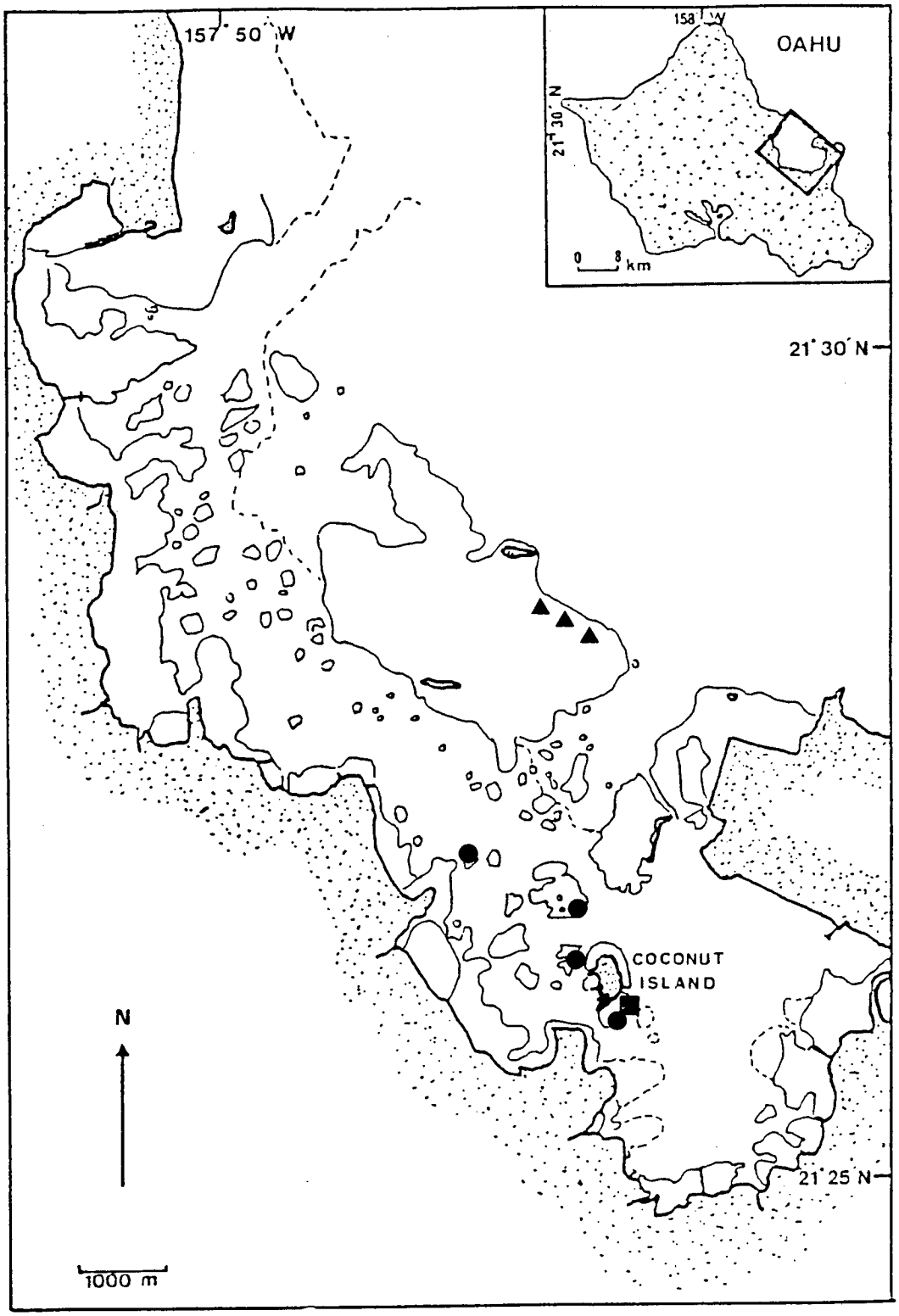


Figure 2.1 *Montipora* spp. study site in Hawaii. Map shows the position of Kaneohe Bay on Oahu and a detailed map of collection sites for Montipora species within the bay.

Symbols ▲ , ● and ■ denote collection sites

▲ - *M. flabellata* and *M. studeri*

● - *M. verrucosa* and *M. dilatata*

■ - *M. verrili*

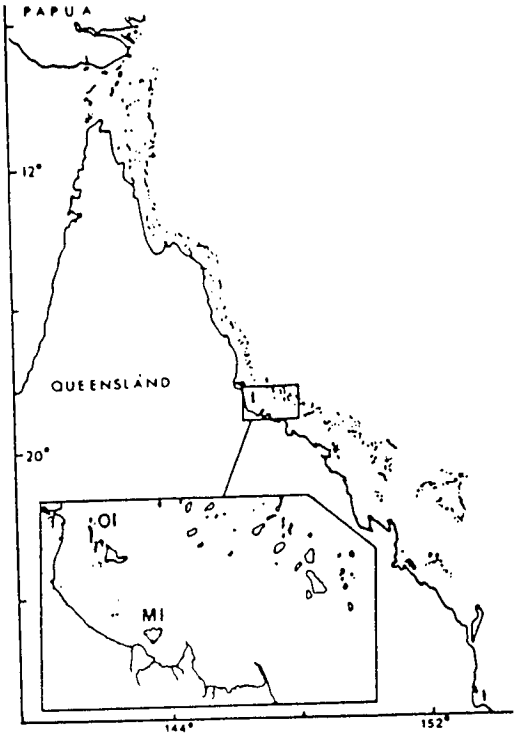
Figure 2.2 *Montipora digitata*. Magnetic and Orpheus Islands' study sites on the Great Barrier Reef.

MI - Magnetic Island.

● - Geoffrey Bay, MI.

OI - Orpheus Island.

▲ - Pioneer Bay, OI.



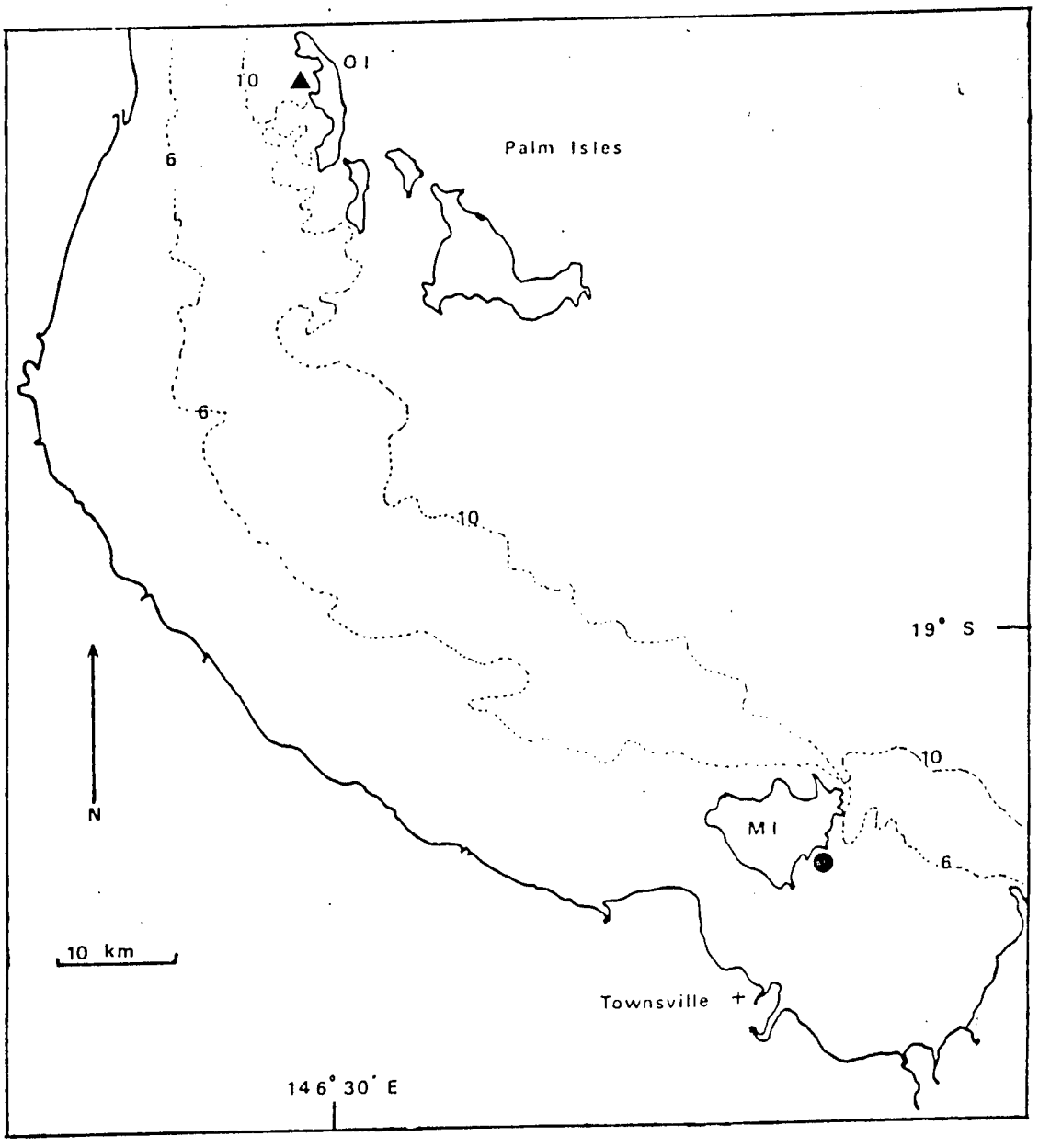


Figure 2.3

Montipora dilatata:
transverse section through a mature polyp. In the genus *Montipora* the gonads are arranged as alternating pairs of testes and ovaries. There are a total of 8 gonad-bearing mesenteries. O - ovary, T - testis, n - nucleus. Scale bar = 300 µm.

Figure 2.4

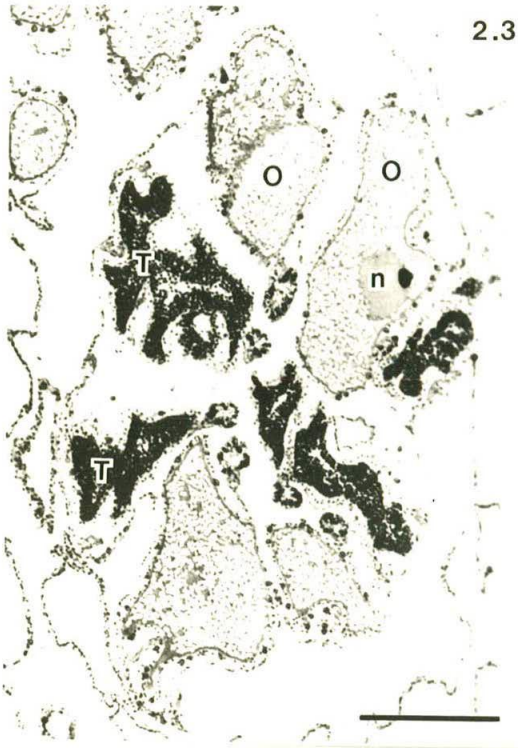
Montipora digitata:
longitudinal section through a mature polyp. The gonads lie within the mesenteries behind the mesenterial filaments. Both testes and oocytes extend in strings along the oral-aboral axis. The testes tend more towards the oral end of the polyp. The mean number of oocytes/polyp was 12. M - mouth, T - testis, O - oocyte. Scale bar = 500 µm.

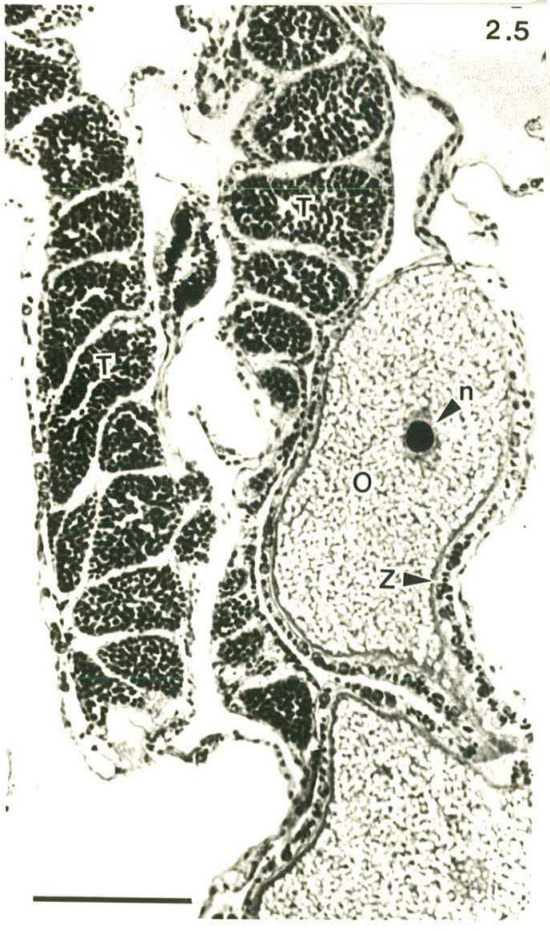
Figure 2.5

Montipora digitata:
a magnified view of the gonads. The testes (T) are composed of many locules. As the sperm mature their tails develop and extend into the central lumen of each locule. The oocytes (O) in this species were surrounded by increasingly dense accumulations of zooxanthellae (Z) in the final few weeks prior to spawning. Unlike *M. dilatata*, however, there was no evidence of the symbionts in the eggs until the moment of spawning. n - nucleolus. Scale bar =220 µm.

Figure 2.6

Montipora digitata:
transverse section through a gamete cluster released from a polyp at spawning. The entire complement of eggs (O) and sperm (S) is packaged into a ball while under the oral disk. The cluster is released through the mouth and floats quickly to the surface, where the gametes separate. All the eggs contain zooxanthellae (Z) at release. Scale bar = 350 µm.





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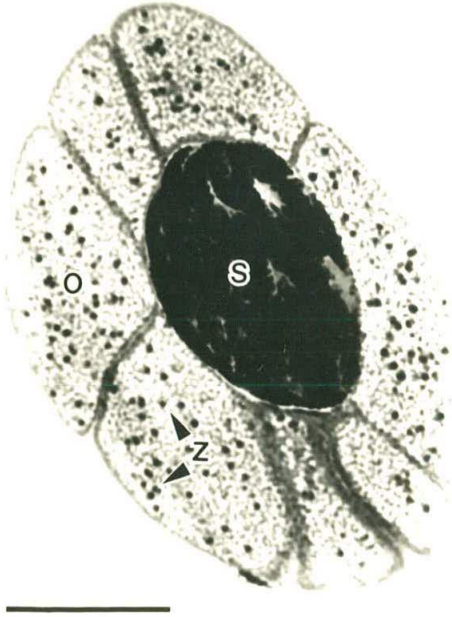


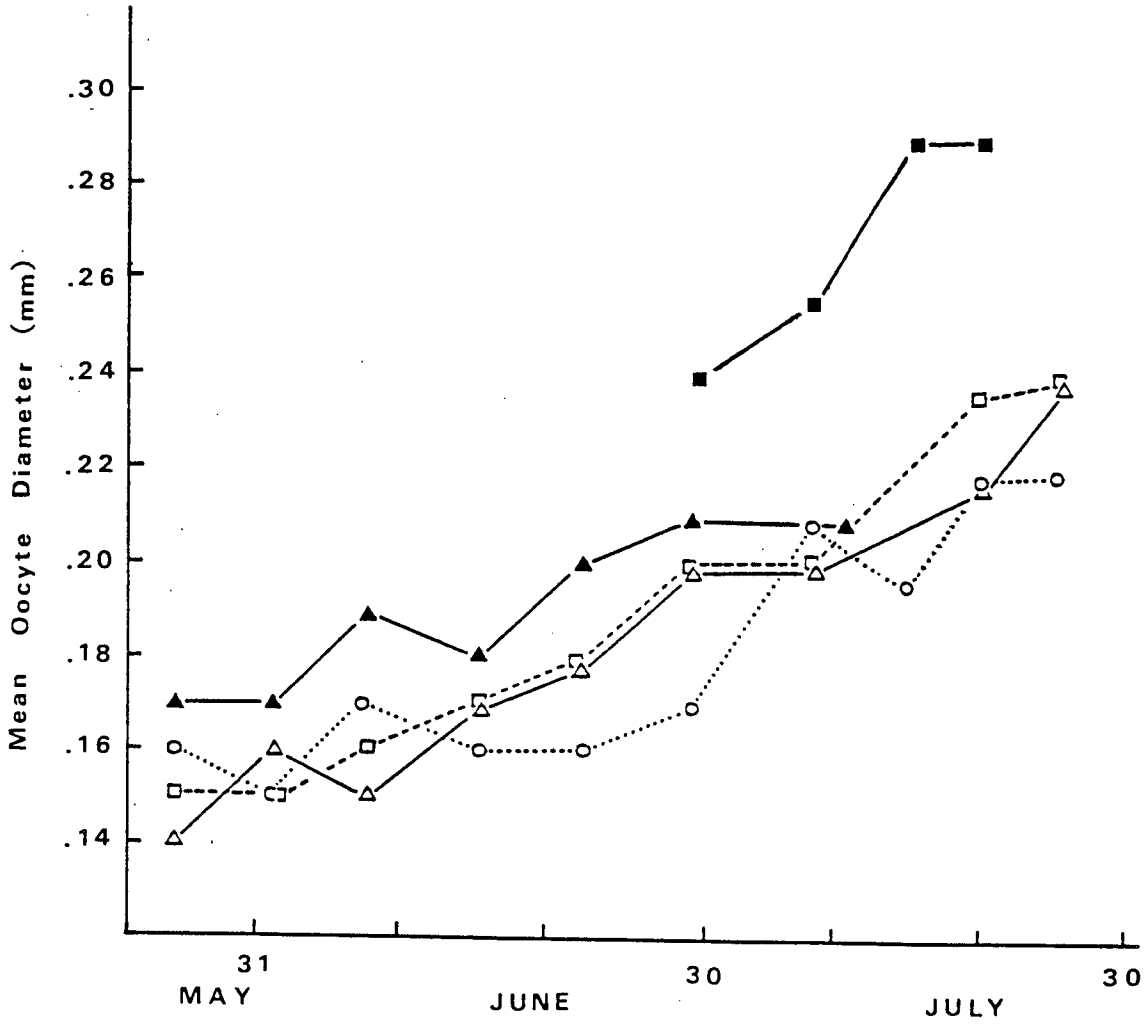


Figure 2.7 *Montipora* spp. in Hawaii. Maturation of oocytes in the summer of 1983. Symbols :- ▲ - *M. verrucosa*, △ - *M. dilatata* ○ - *M. flabellata*, □ - *M. verrilli*, ■ - *M. studeri*.

Table 2.1

Montipora species in Hawaii. Mean Oocyte diameters (as above) and 95 % confidence limits throughout the summer.

Species	Date 25-5-83	1-6-83	8-6-83	16-6-83	23-6-83
M. verrucosa	.17 ±.07	.17±.04	.19 ±.06	.18 ±.04	.20 ±.05
M. flabellata	.16 ±.07	.15±.05	.17 ±.04	.16 ±.03	.16 ±.02
M. verrilli	.15 ±.05	.15±.04	.16 ±.05	.17 ±.04	.18 ±.04
M. dilatata	.14 ±.05	.16±.05	.15 ±.04	.17 ±.04	.20 ±.06

	Date 31-6-83	9-7-83	16-7-83	21-7-83	26-7-83
M. verrucosa	.21±.04	.20 ±.06			
M. flabellata	.17±.07	.21 ±.05	.19±.07	.22 ±.05	.22 ±.06
M. verrilli	.20±.06	.20 ±.05	---	.25±.06	---
M. dilatata	.20±.05	.20 ±.05	---	.22±.05	.25 ±.04
M. studeri	.24±.11	.26 ±.06	.29±.05	.29±.07	---

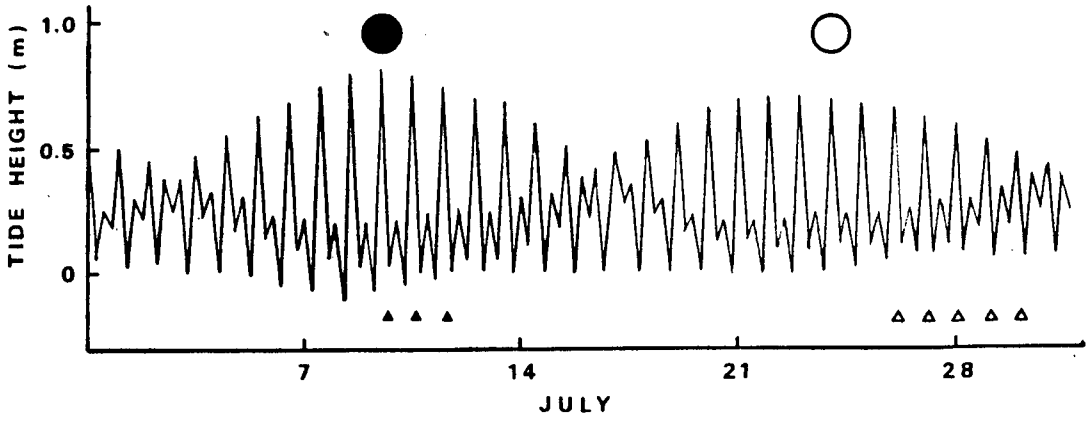


Figure 2.8 Tide cycle and spawning in Hawaii, 1983.

▲ - *M. verrucosa* at new moon

△ - *M. dilatata* at full moon.

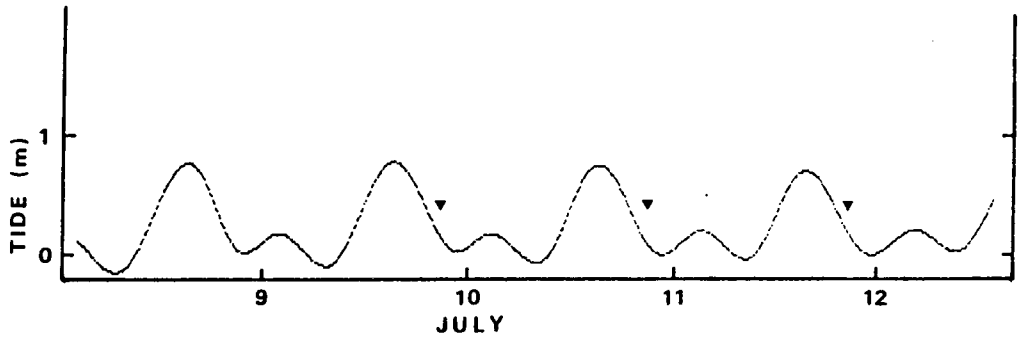


Figure 2.9 *M. verrucosa:* detailed relationship between gamete release and tide cycle in Kaneohe Bay. ▼ - time of release.

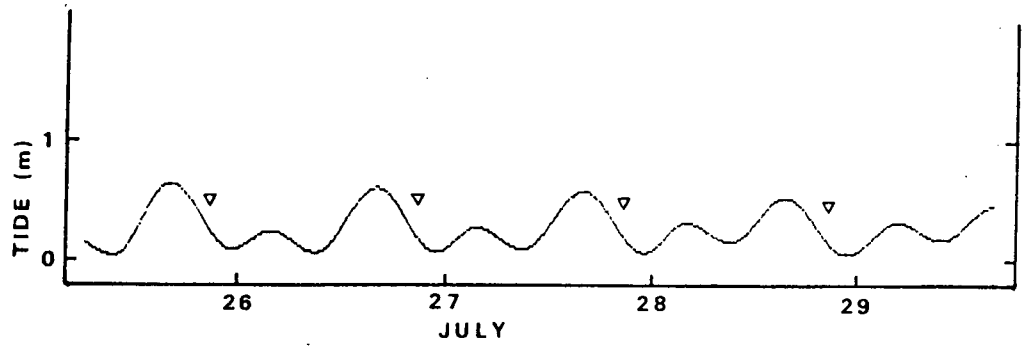


Figure 2.10 *M. dilatata:* detailed relationship between gamete release and tide cycle in Kaneohe Bay. ▽ - time of release.

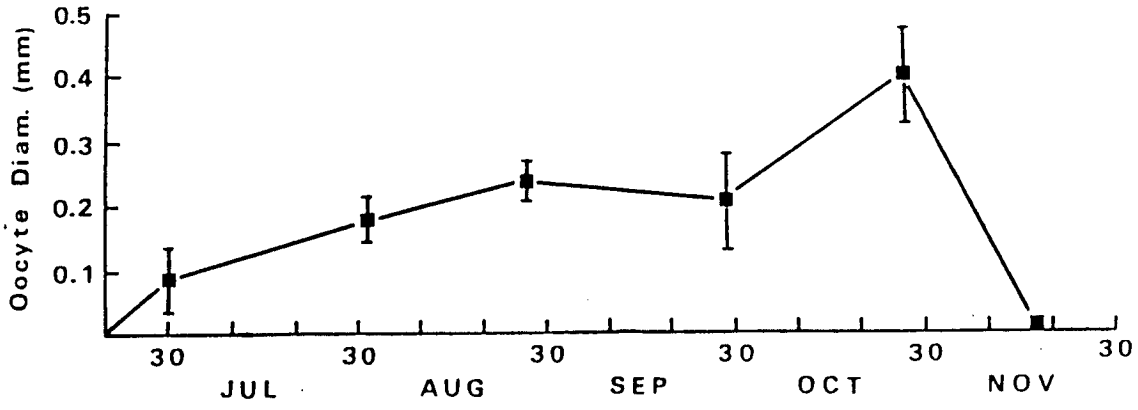


Figure 2.11 *M. digitata:* annual development of oocytes at Geoffrey Bay (after Heyward and Collins, 1985).

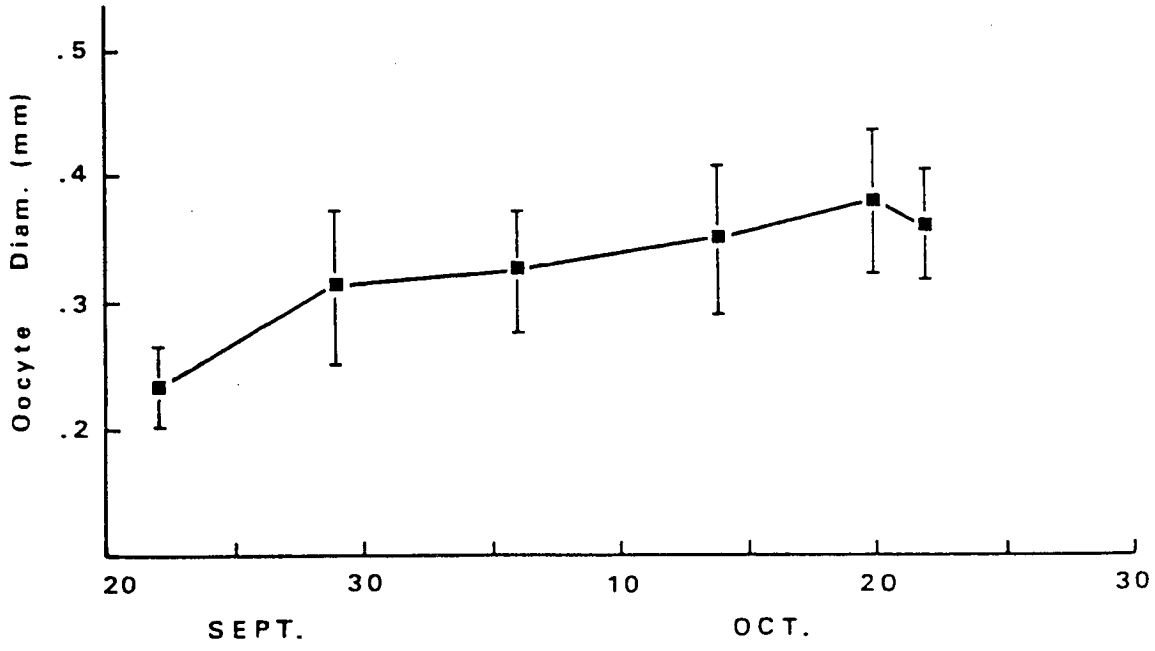


Figure 2.12 *M. digitata:* final maturation of oocytes in the Geoffrey Bay population during 1983.

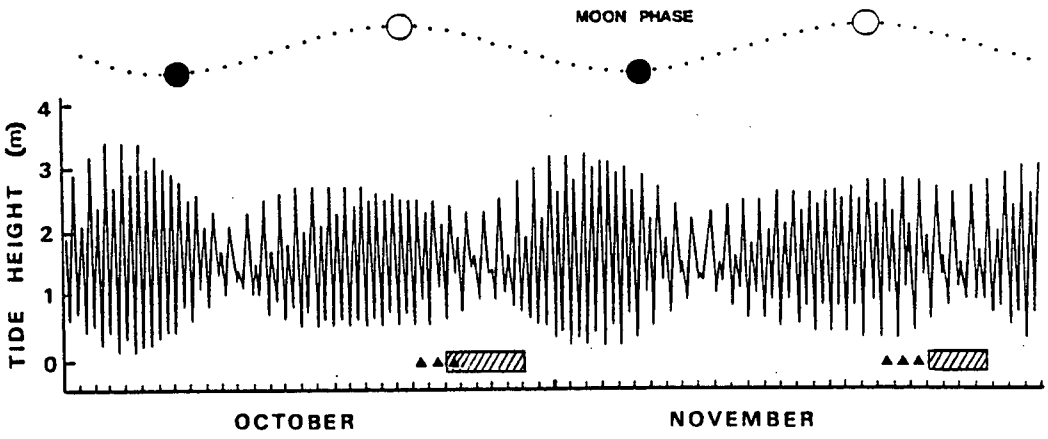


Figure 2.13 *M. digitata:* Triangles (▲) represent spawning in October at Magnetic Island & November at Orpheus Island. Hatched areas represent days of multi-specific spawning.

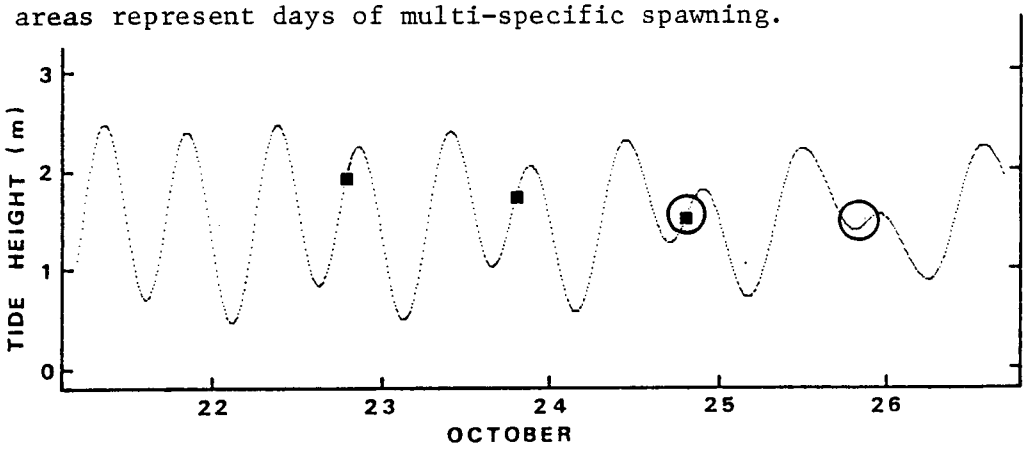


Figure 2.14 *M. digitata:* detail of the tidal cycle and times of spawning at Magnetic Island. ■ - *M. digitata* spawning, ◯ - multi-specific coral spawning

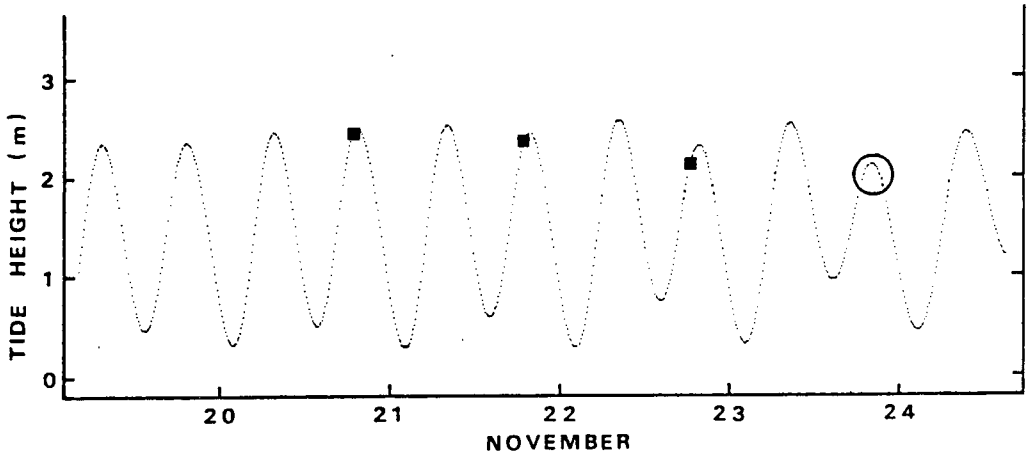


Figure 2.15 *M. digitata:* detail of the tidal cycle and times of spawning at Orpheus Island. ■ - *M. digitata* spawning, ◯ - multi-specific coral spawning

cultures in the petri dishes deteriorated rapidly. Many embryos developing at the air-water interface formed irregular shapes and ceased to divide.

In those dishes where the gametes of individual colonies had been isolated to test for self-fertilization, only 1 egg showed any suggestion of undergoing the first cleavage. All the eggs in these cultures had lysed within 10 h of being spawned.

Australia:

Following the spawning of *Montipora digitata*, gametes were collected from aquaria as above and placed into petri dishes. No self-fertilization trials were successful. In the cross-fertilization trials the first cell divisions were noted 2 h after spawning. Despite its much larger eggs, *M. digitata*'s rate of cell division and early development were indistinguishable from the Hawaiian species studied. Most cultures failed after 12 h, but cultures placed on a shaker bath lasted 24h. In these cultures mortality was very high but surviving individuals at 24 h were ciliated, mobile and of spherical shape..

DISCUSSION

The observed and inferred broadcasting of gametes on only a few nights each year explains earlier workers'(Marshall and Stephenson, 1933; Stimson, 1978) failure to record planulation in these species of *Montipora*. Gametogenesis in *Montipora digitata* proceeds for at least 6 months and culminates in a synchronous and complete

population spawning during 2 to 3 nights (Heyward and Collins, 1985a). An annual reproductive cycle is apparent for *M. digitata* on the G.B.R.and the observed gametogenesis and spawning of the Hawaiian species fit the same pattern. There was no evidence of a second gametogenic cycle commencing following the spawning of *Montipora verrucosa* or *M. dilatata*. Stimson (1978) most frequently encountered gravid colonies of *Montipora verrucosa* in July, but found no gametes during the winter months. His record of a single gravid colony collected in October may be a consequence of taxonomic confusions (Stimson ,pers. comm.), or the result of asynchronous and protracted reproduction during the sample period.

Montipora dilatata demonstrated a high level of population synchrony in 1983 by shedding all gametes during a single period, while in 1984 spawning occurred over a protracted period during the warmer months. The consecutive semi-lunar spawnings of *M. dilatata* are at variance with the synchronous and complete release of gametes over at most a few consecutive days seen in *M. verrucosa* and indeed most other corals (eg. Babcock *et al.*, 1986). There are, however, recent records of other species in Australia (Willis *et al.*, 1985), the Red Sea (Shlesinger and Loya, 1985) and Japan (author, unpublished data) where the broadcasting of gametes within a population occurs with a lunar periodicity over consecutive months. Harrison (pers. comm.) has conjectured that year to year variation in the juxtaposition of sea temperature and lunar cycles could give rise to particular years where such split spawning occurs. Such an hypothesis assumes that sea temperature is critical for gamete

maturation and that there is a staggered response to temperature within some populations. The metabolic cost of reproduction in corals is not known, but the annual cycles of water temperature and insolation are likely to affect reproductive effort (Jokiel and Gunther, 1978; Szmant-Froelich *et al.*, 1980). The earlier maturation of *Montipora digitata* at the Magnetic Island site corresponds to the earlier annual rise in sea temperature compared with Orpheus Island (Babcock *et al.*, 1986) and supports Harriot's (1983) hypothesis that sea temperature is an important trigger for gonad maturation and spawning. In Hawaii, either the new or full moon are appropriate cues for the release of reproductive products (this study; Harrigan, 1972; Krupp, 1983). *Montipora verrucosa* developed mature gametes earlier than *M. dilatata* and spawned first in both 1983 and 1984 after new and full moons respectively. The timing of spawning by the *Montipora* species in Hawaii demonstrates a similar flexibility in calendar dates to *M. digitata* and again supports the model of spawning occurring at the first appropriate period following maturation. Seasonality of spawning would be maintained by such a link with the annual sea temperature cycle.

The sequence of gametogenesis and the mode of reproduction are strikingly similar between all the species of *Montipora* investigated. Simultaneous hermaphroditism and the arrangement of gonads as alternate pairs of male and female mesenteries, a feature of all *Montipora* species investigated to date (this study; Robertson, 1981), is also consistent throughout the Acroporidae (Harrison, 1985). However the presence of zooxanthellae in the *Montipora* eggs

is a feature not observed in Acropora species which spawn eggs. Initial biochemical analysis of *M. digitata* eggs has shown that the zooxanthellae are fixing carbon (Mc Neil, pers. comm.) and that direct translocation of metabolites between the zooxanthellae and eggs or embryonic tissues takes place.

The marked lunar periodicity correlated with the release of gametes is a behaviour now seen as typical of hermatypic corals (Babcock *et al.*, 1986; Fadlallah, 1983; Simpson, 1985) and is known for many other marine organisms (Korringa, 1957). In Hawaii, *Pocillopora damicornis* releases planulae throughout the year on the new and full moons (Harrigan, 1972), but further study has shown this population contains two races, each of which spawns with a different lunar period. Jokiel (1985) has demonstrated the ability of *P. damicornis* to track moonlight intensity and it is likely that other hermatypic species possess the same sensitivity. Lunar rhythms provide a means of synchronizing the release of gametes at a time favourable for fertilization. The Kaneohe Bay population of either *Montipora verrucosa* or *M. dilatata*, however, is capable of spawning on either new or full moon, demonstrating a plasticity of response to lunar light cycles. Lunar and tidal rhythms are, of course, very closely linked; the gametes released at either moon phase in Hawaii will encounter similar tidal conditions (Figure 2.8 to 2.10). Most commonly spawning concurs with reduced tidal movements. The multispecific spawning on the GBR and in the Dampier Archipelago, Western Australia, are associated with the days of neap tides following the full moon (Simpson, 1986; Babcock

et al., 1986). Intuitively the reduced water movement associated with neap tides would enhance cross-fertilization by minimizing gamete dilution. Such an hypothesis gains support from the spawning times of *Montipora digitata*. Although this species spawns a few days before the neaps associated with the multi-specific event (Figure 2.13), the gametes are shed very close to the time of slack water (Figures 2.14, 2.15). Similarly, seven of nine coral species observed spawning in Japan following the full moon of June, 1986, broadcast their gametes close to the time of slack water (author, unpublished data). The tidal range is much smaller in Hawaii (Figure 2.8) but spawning commences as the ebb tide is slackening markedly (Figures 2.9, 2.10) and the dioecious *Porites compressa* was observed spawning on the slack water.

Most corals are known to spawn between dusk and dawn. The hours of darkness provide a period of minimal tidal flow in both Hawaii and the central GBR, during summer, due to a marked diurnal inequality of tidal amplitude. In the present study it was noted that colonies of *Montipora* species which spawned on consecutive nights, commenced spawning at the same time each evening. In addition, spawning was inhibited in colonies kept in an illuminated aquarium. This suggested that a specific dark period was required prior to spawning and such a mechanism could act a fine scale synchronizer for the population. Species specific darkness requirements have now been documented for many other coral species (Babcock *et al.*, 1986). The phenomenon has marked application for controlled spawning studies since it is possible to alter the hour of spawning by manipulating the light/ dark regime. This effect

requires more study, but recently R. Miller (pers. comm.) was able to delay spawning in *Montipora digitata* for several days by maintaining colonies in constant illumination.

The timing of spawning of the *Montipora* species investigated fits a general pattern seen among species which have recently been documented as broadcast spawners. Gametes are shed, following a final maturation, in a population event synchronised by moonlight and daylight. These spawnings are highly predictable on the basis of gamete maturation and lunar calendar. The consistent association with the lunar calendar will cause the actual calendar date to vary from year to year depending on where the moon falls in relation to gamete maturation (Willis *et al.*, 1985). It seems probable that spawning will occur at the first appropriate opportunity after gamete maturation. In Hawaii either the new or full moon provide appropriate cues, while in the central region of the GBR *Montipora digitata* and the majority of coral species release gametes only after full moons. Processes of fertilization, early development and dispersal, which occur after release are major components of the reproductive cycle remain as yet poorly understood.

Chapter 3

Mating Systems.

INTRODUCTION

In coral species which spawn gametes it is possible to directly observe fertilization and cleavage. This contrasts sharply with viviparous species where the few insights into the breeding systems have been inferred by comparing parental and larval genotypes (Ayre and Resing, 1986; Stoddart, 1983a). Broadcast spawning in coral populations is highly synchronous (this study; Babcock *et al.*, 1986), which maximizes gamete concentration and presumably allows for genetic exchange through cross-fertilization. The predictability of the spawning events allows a fleeting but invaluable opportunity to study coral mating systems.

Regardless of the mode of fertilization (internal vs external) apparent in the Scleractinia, the overwhelming majority of taxa adopt hermaphroditic sexuality in their adult colonies (Harrison, 1985). In hermaphroditic species which utilize external development, simultaneous release of eggs and sperm is the common pattern (Kojis and Quinn, 1981 ; Babcock, 1984; Babcock *et al.*, 1986; this study). A notable feature of gamete release in many species is the packaging of the eggs and sperm together in a cluster eg. *Montipora digitata*. Frequently the mature eggs and sperm maintain this close association until the gamete cluster disaggregates at the surface.

Such a spawning mechanism provides an opportunity for high levels of fertilization if the gametes are self-fertile. Nevertheless, the relative potentials for self- and cross-

fertilization, processes which markedly affect the genetic diversity of populations, have never been studied in any scleractinian corals. Self-fertilization has been observed among other marine invertebrate groups in which simultaneous hermaphroditism occurs (Pianka, 1974; Reeve and Cosper, 1975), although its importance may vary with the species and population (Morgan, 1945). In this study the potential for self- and cross-fertilization and the duration of gamete viability after spawning is compared as a preliminary investigation into the mating systems of broadcast spawning corals.

MATERIALS AND METHODS

All the experiments were performed at the Orpheus Island Research Station, 70 km north of Townsville, Australia (Figure 2.2). Four hermaphroditic species of scleractinian corals, *Montipora digitata*, *Acropora tenuis*, *Goniastrea aspera* and *G. favulus*, were collected from Pioneer Bay, immediately seaward of the Research Station. Colonies of each species were collected at least 24 hours prior to the predicted annual spawning event (Harrison *et al.*, 1984) and separated into individual buckets and aquaria. Care was taken to avoid sampling possible clonemates, derived from fragments, by collecting widely separated colonies. A large reservoir of seawater, collected two days prior to any observed spawning of the coral populations in Pioneer Bay, was used as a source of gamete-free water to maintain each isolated colony.

In each species, spawning of the separate colonies occurred within half an hour of each other and direct field

observations confirmed their synchrony with the natural populations. Processing of the gametes commenced once at least two colonies of a species had spawned in their separate containers, ensuring that cross-fertilization trials could be attempted. All the trials for self-fertilization were prepared first, consequently there was a 1 to 2 hours time lag until the cross-fertilization cultures were commenced.

Each colony was assigned a complete set of apparatus to prevent cross-contamination. Following spawning the gametes of each colony were gathered using plastic squeeze bottles and transferred to plankton splitters. In each splitter the gametes were divided into two equal portions. Each portion was transferred to a 1 litre watertight bottle which was then filled with gamete-free water, sealed and gently agitated. When a cross-fertilization trial was desired, half volumes of the gametes from each colony were mixed in a plankton splitter, then the total volume was divided. In this way, replicate trials were created for each test of self- and cross-fertilization.

Fertilization was monitored by regularly subsampling the 1 litre culture vessels. The bottles were removed from agitation and submerged in freshwater to destroy any foreign gametes on their external surfaces. Each bottle was shaken by inverting it five times, then opened and a 30 ml aliquot was removed before the culture was sealed again and returned to agitation. The 30 ml subsample was inspected with the aid of a stereo dissector microscope and a Bogorov sorting tray. Eggs were scored as either 1-cell (unfertilized), 2,4,8 or >8-cell stages.

The duration of gamete viability after spawning was

investigated by taking aliquots from the self-fertilization trials and cross them at progressively longer time intervals after spawning. The gametes were mixed, sealed in a watertight bottle and agitated. The bottles were subsequently inspected 2 and 4 hours after the gametes had been mixed.

RESULTS

Goniastrea favulus released negatively buoyant eggs and jets of sperm simultaneously. Typically the eggs were entangled in a mucous mass and stuck to the surface of the colony (see Kojis and Quinn, 1981). *Montipora digitata*, *Acropora tenuis* and *G. aspera* all released their eggs and sperm packaged together into spherical, buoyant clusters. In the calm conditions of the aquaria, the gametes in these clusters remained clumped together at the water surface for up to 30 minutes. After disaggregating, the eggs were all highly buoyant and remained on the surface while the sperm tended to diffuse throughout the container, clouding the water.

In every trial, the percentage of cleaving embryos increased with time. After 5 to 7 h, when the final quantitative subsample was taken, the cross-fertilization cultures were often approaching 100 % fertilization. Table 3.1 presents the comparative levels of fertilization in the self and cross cultures when the final subsamples were taken. The replicates of each trial showed the same trends and, in most cases, produced almost identical data. The mean percentage of cleaving embryos with time is plotted for each species in Figure 3.1. *Montipora digitata* showed absolutely

no tendency to self-fertilize in any of 12 trials, while *Acropora tenuis* had a very low level (2 to 3 %) of self-fertilized embryos appearing in the cultures between 4 and 7 hours after spawning (Figure 3.1). However the subsequent fate of these embryos was not determined. In both species all the cross-fertilization trials were successful.

In contrast, there was a significant degree of self-fertilization by both species of Goniastrea (Figure 3.1). In *G. aspera* cross-fertilization was close to 100 % successful, nevertheless, self-fertilization trials had approximately 10 % of the eggs undergoing cleavage after 5 hours (Figure 3.1). Self-fertilization was of much greater significance in *G. favulus*. Some self-fertilization was noted for this species in the first 2 to 3 hours after spawning, although at 5 hours these cultures were less successful than the cross-fertilization trials. However, 7 hours after spawning some of the self-fertilization trials had achieved levels of fertilization on par with the the cross-fertilization cultures (Figure 3.1). Self-fertilized embryos of both *G. aspera* and *G. favulus* were raised through to settlement. Their development and settlement appeared to be identical to similarly reared larvae derived from cross-fertilization. Every cross-fertilization trial was also successful in these two species. However the contribution of self-fertilization to the "cross-fertilization" percentages in Figure 3.1 is unknown.

Gamete viability.

The duration of viability, as determined by the ability of a cross-fertilization trial to produce apparently healthy,

Figure 3.1

Montipora digitata, Acropora tenuis, Goniastrea aspera and *G. favulus*. Percentage fertilization with time after spawning. Sample size of eggs/embryos counted from each subsample of culture ranged from 130 to 220 ($\bar{x}$ = 150). Solid symbols represent self-fertilization trials. Open symbols represent cross-fertilization trials. Different shaped symbols (squares or triangles) distinguish trials made using gametes from different colonies.

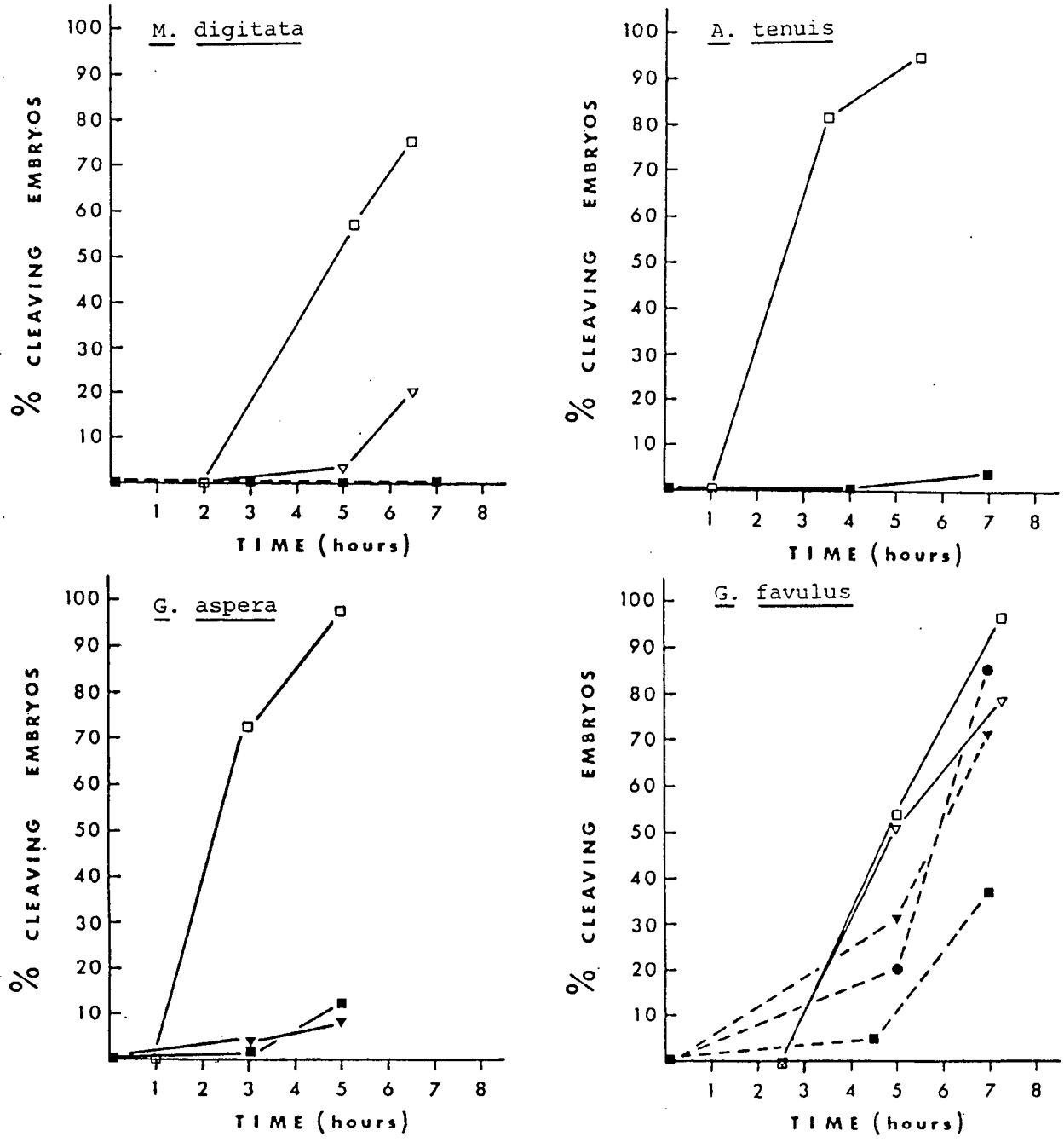
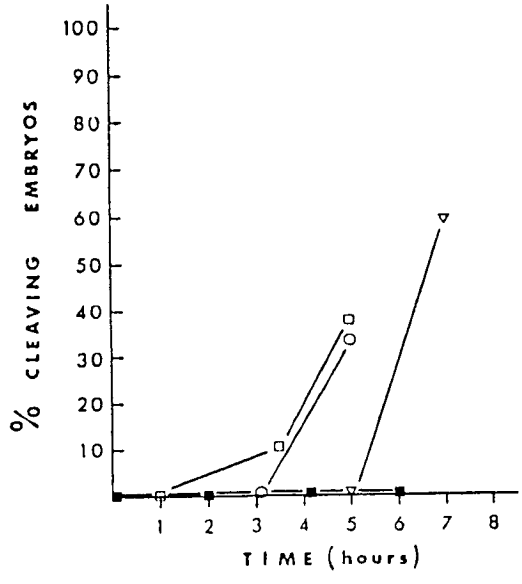


Table 3.1 *Montipora digitata, Acropora tenuis, Goniastrea aspera* and *G. favulus*. Results of the self- and cross-fertilization trials. Data for percentage cleaving embryos are those derived from the final subsampling of the gamete cultures. The data for each replicated trial pair is presented as a pair of numbers divided by a colon. (*n*)= number of colonies.

Species	Time of spawning (1984)	*n*	% cleaving embryos in:	
			Selfs	Crosses
M. digitata	19.00 hrs, 10 Nov.	4	0:0, 0:0 0:0, 0:0	74:77, 19:22
	19.30 hrs, 11 Nov.	2	0:0, 0:0	25:46
A. tenuis	19.30 hrs, 12 Nov.	2	1.5:2.5, 3.5:4.0	95:95
G. aspera	21.45 hrs, 13 Nov.	2	8:16, 6:11	96:98
G. favulus	19.30 hrs, 13 Nov.	3	82:89, 26:53, 67:78	93:97, 81:75

Figure 3.2

Montipora digitata: gamete viability through time. Attempts at self-fertilization (■) were all unsuccessful. The cross-fertilization trials are represented by hollow symbols. □ - cross-fertilization trial commenced 1 hour after spawning; ○ - trial commenced 3 hours after spawning; ▽ - trial commenced 5 hours after spawning.



cleaving embryos, was quite similar among the four species. *Acropora tenuis* gametes produced cleavages when crossed up to 5 hours after spawning. The other three species yielded high levels of fertilization for 6 to 7 hours after spawning. Figure 3.2 illustrates results of delayed cross-fertilization trials with *Montipora dilatata*. In every species there was a noticeable decline in the condition of unfertilized gamete cultures after 7 h. Beyond this time, lysis of unfertilized eggs became increasingly common and consequently the viability trials were concluded at this time. Collections of large numbers of coral embryos from dense windrow aggregations in Pioneer Bay on the mornings following spawning also contained no unfertilized eggs.

DISCUSSION

This study has provided the first data for fertilization rates in corals and demonstrates that different species may utilize different mating systems. While the cross-fertilization rates were uniformly high and the potential for self- fertilization could be demonstrated, the data nevertheless, relate to a system which is quite different from an openwater situation. Direct field observation suggested that the concentration of gametes at the sea surface following spawning was quickly reduced by moderate wind and wave action. Consequently the fertilization rates observed in all species investigated, while demonstrating potentials, may be over-estimates of natural frequencies. The experimental cultures maintained gametes at a high concentration. Dilution of the gametes in the sea means that the rate at which successful egg-sperm encounters occur is

likely to be lower than in the experimental cultures. On the other hand, during 1986 at Magnetic Island very calm conditions prevailed allowing field fertilization to exceed 85 % for *Montipora digitata* 4 hours after spawning (Sample of labelled water mass by surface plankton tow; Heyward and Collins, unpublished data).

The tests for gamete viability indicate that all of the species can achieve cross-fertilizations until at least 5 to 6 hours after spawning. Backround or residual surface currents in the Great Barrier Reef region range from 0.06 to 0.24 m/s (Collins and Walker, 1985), while local effects due to tide and wind can be much greater (Williams *et al.*, 1984). Given suitable environmental conditions, cross-fertilizations could occur between adults separated by many kilometers. In the present experiment, however, more than 50 % of the eggs were fertilized and cleaving within 3 hours of being mixed together. The gametes of synchronously spawning nearest neighbours are most likely to cross-fertilize. As dilution factors come into play, the probability of widely separated, perhaps even inter-reefal, cross-fertilizations becomes remote. Nevertheless, such possibilities should be borne in mind when considering the boundaries of a breeding population.

Observations on several faviid species, including *Goniastrea favulus*, indicated that the final maturation division of the oocytes and the release of polar bodies occurred 15 to 30 minutes after spawning. Consequently, it seems unlikely that the eggs are fertile until some time after release and additionally, that fertilization does not

take place inside gamete clusters.

Clusters of gametes from *Goniastrea aspera* colonies float to the surface of the sea, then break apart. Since the population spawns synchronously, gametes from many colonies are concentrated on the surface of the sea simultaneously. This factor, in combination with the release of polar bodies only after spawning and a delay in the capability to self-fertilize, increases the probability of cross-fertilization in *G. aspera*.

Goniastrea favulus limits the dispersal of its eggs, and consequently decreases the relative chances of encounter between gametes from different colonies. The results of this study show that self-fertilization can be extremely effective in this species. However, it is not yet known how successful sibling sperm are in competition for eggs against sperm from other colonies. The recent application of electrophoretic techniques to scleractinians (Stoddart, 1983a) provides the means to test for competitive self-fertilization by analysis of larval genotypes derived from "cross-fertilization" cultures. In the case of *G. favulus*, the retention of fertilized eggs on the surface of the parent colony should facilitate electrophoretic analysis of parent-progeny relationships which occur under field conditions.

Different patterns of self- and cross-fertilization were expressed by each of the species investigated. Nevertheless, cross-fertilization proceeded at a faster rate than self-fertilization and produced the higher percentage of developing embryos in every case. The obligate out-breeding of *Montipora digitata* indicates that a mechanism for distinguishing sibling gametes exists. Scleractinian corals

sometimes demonstrate a self-recognition response when adult colonies are grafted together in histocompatibility trials (Hildemann *et al.*, 1977). However, the degree of specificity of the response may vary with the species (Stoddart *et al.*, 1985). Scofield *et al.* (1982) have demonstrated a link between histocompatibility in colonial ascidians and the ability to self-fertilize. Their evidence points to a self-fertilization barrier which helps maintain levels of heterozygosity at the histocompatibility locus. Interestingly, the effectiveness of the barrier decreases several hours after spawning. Similar processes may be responsible for the increased rates of fertilization in selfing trials of *Goniastrea favulus* between 4 and 7 hours after spawning (Figure 1). In all four species, cross-fertilization is favoured and self-fertilization may be advantageous by adding to the progeny already obtained from cross-fertilization. This could be especially important where population densities are relatively low, eg., *Spirorbis* spp. (Gee and Brinly Williams, 1965) and barnacles (Barnes and Barnes, 1958). The high frequency of self fertilization possible in *G. favulus* could provide for the retention of locally adapted genotypes, since inbreeding reduces the disruption of coadapted genes present in the successful parent (Shields, 1982). If inbreeding is extensive in *G. favulus*, reduced allelic diversity would be expected in a self-seeding population. However, if the larvae are usually dispersed away from the parental reef, the genetic structure of a population will be influenced greatly by recruitment from the plankton.

The present study has demonstrated the possibility of controlled breeding trials and hence the long-term potential for genetic experimentation. *Montipora digitata* is a poor competitor as an adult and reproduces asexually through fragmentation, while sexually producing larvae with high dispersal potential (Heyward and Collins, 1985a, b). *Goniastrea favulus* is more competitive in interactions for space (Babcock, 1980), does not fragment, and restricts the dispersal of its eggs (Kojis and Quinn, 1981). The contrast between *M. digitata*, which is an obligate outbreeder, and *G. favulus*, a facultative inbreeder, may reflect a phylogenetic trend (Acroporidae vs Faviidae) or be correlated with morphology/life-history pattern. These observations suggest that scleractinian corals could be very useful organisms for the testing of mating system models.

Chapter 4

Embryogenesis and Potential Dispersal of Coral Larvae.

INTRODUCTION.

The extent of genetic exchange, via larval dispersal, between coral populations is not known. Analysis of population genetic structures in *Pocillopora damicornis*, which releases asexual planulae (Stoddart, 1983a, 1984), supports a model of dispersal where at least some larvae settle on the parental reef. At the same time, Richmond (1981) suggests that *P. damicornis* larvae may, via nutritional support from their symbiotic zooxanthellae, remain planktonic for months and be capable of trans-oceanic passages. While the maximum distance such planulae may travel is hypothetical, there is an increasing amount of data to show that brooded planulae often settle soon after release (Szmant, 1986; Shlesinger and Loya, 1985; Stoddart, in press). Brooded larvae are released at an advanced stage of development, while settlement by corals which spawn gametes must, at the least, be delayed while embryogenesis takes place.

The rate of development, leading to planula formation, and the time of earliest settlement has been observed in very few corals. Even when coral gametes have been available, the laboratory culture of embryos has proved fickle. Recent studies of broadcast spawning species (Szmant-Froelich *et al.*, 1980; Kojis and Quinn, 1981; Krupp, 1983; Harrison *et al.* , 1984) have all noted difficulty in raising larvae through to settlement under laboratory conditions. Shlesinger and Loya (1985) noted a typical free-swimming period of six

days for Red Sea corals with external development. In contrast the most rapid settlement observed for larvae from broadcast spawners on the Great Barrier Reef (GBR) was 14 days after spawning (Harrison *et al.*, 1984). If a minimum dispersal period of 2 weeks is representative, then species which broadcast have ample opportunity for inter-reefal gene flow in systems like the GBR on an annual basis.

Summer spawning of gametes is now quite predictable for many species of coral (Shlesinger and Loya, 1985; Babcock *et al.*, 1986; this study). Since the majority of species utilize this mode of sexual reproduction, information on the larval biology of corals represents a large gap in our knowledge of coral demography.

In this study techniques were developed to allow thousands of larvae from any broadcast spawning species to be raised through to settlement. Embryogenesis and settlement were studied in great detail for *Montipora digitata*, *Acropora pulchra*, *Lobophyllia hemprichii* and *Favites abdita*. These four species represent three taxonomic families and *in toto* produce oocytes representative, by size and the presence or absence of zooxanthellae, of the great majority of broadcast spawning corals yet observed on the Great Barrier Reef. These findings are placed into more general perspective by a collaborative study of an additional 17 species from the G.B.R (Heyward and Babcock, 1985; Babcock and Heyward, 1986) and two species of *Montipora* from Hawaii.

MATERIALS AND METHODS.

Coral gametes were obtained from the majority of species during the predictable multispecific spawning periods in

1983, 1984, 1985 and 1986 on the Great Barrier Reef (Harrison *et al.*, 1984; Willis *et al.*, 1985; Babcock *et al.*, 1986) and for *Montipora verrucosa* and *M. dilatata* in Hawaii during 1984 (this study, Chapter 2).

Several colonies of each species were collected 1 to 2 days prior to spawning and maintained in aquaria and buckets. During spawning all hermaphroditic species, with the exception of *Goniastrea favulus*, released gametes as buoyant clusters of eggs and sperm in similar fashion to that described for the Montipora species. As gamete clusters accumulated at the surface of each aquarium they were collected by suction into plastic squirt bottles. *Goniastrea favulus* spawns it's eggs and sperm as separate entities. When ejected the eggs are contained in sticky mucous coats, while the sperm are jetted from the colony in clouds. The eggs are not buoyant and sink to the bottom or remain attached to the parent colony where they can be collected along with sperm which colonies have released into the aquaria (Babcock and Heyward, 1986).

The preliminary study of embryology using petri dishes as culture vessels (Chapter 2) was clearly inappropriate as a technique for successful larval rearing. Lack of water circulation seemed to be an obvious problem. To avoid the deleterious surface tension effects associated with small static containers several means of providing agitation for the embryo cultures were used.

Cultures were attempted in a variety of vessels which were placed in shaker baths for agitation. Both 500 ml glass flasks and 9 litre plastic buckets used in such fashion

proved more successful than the petri dishes. Nevertheless, the high buoyancy of the embryos and regular backwards and forwards motion of the shaker baths caused the embryos to aggregate and many were lost through lysis on the walls of the containers. This demonstrated the need for a less regular agitation pattern simulating wind and wave generated mixing. Consequently, a means was sought with which to culture embryos under more natural conditions. Floating vessels moored in the sea proved extremely successful.

Gametes of all species were transferred into 4 litre plastic jars full of seawater which were sealed by placing plankton mesh, 60 to 200 µm depending on oocyte size of the species, across their mouths. The mesh was held in place by screwing on jar lids which had the centers removed to allow water to pass through. The jars were then transferred to the sea and attached by line to an anchored buoy, where they floated semi-submerged at the sea surface. As the jars bobbed, the gametes were provided with continuous, multi-directional agitation by wave action and experienced ambient sea temperature throughout development. Continuous agitation in many directions, ventilation with seawater and ambient temperature are factors likely to have contributed to the success of this method, since the same containers placed on shaker baths produced markedly inferior results.

Large numbers of all species attempted were raised equally well, provided gametes from at least two colonies were mixed in each jar. Cultures using eggs and sperm from a single colony of an hermaphroditic species were frequently unsuccessful. The progression of embryogenesis was monitored by retrieving the culture jars periodically and pipetting out

subsamples of several hundred embryos. The live embryos from such subsamples were examined immediately using a stereodissector microscope prior to fixing for histology. Fixatives used were Susa, Bouins, buffered 10 % formalin-seawater, acetic ethanol (1:3 acetic acid: absolute ethanol) and for S.E.M., 2 % glutaraldehyde buffered with 0.1 M Sodium Cacodylate.

Specimens fixed for microtomy were firstly mounted and oriented for sectioning in 2 % agar. The agar blocks were trimmed to 3 mm thickness, processed through to parafin wax and sectioned on a rotary microtome at 5 um. Several staining techniques described in Winsor (1984) were evaluated. Haematoxylin and Eosin, a modified Shoobridge polychrome, Gomori's trichrome, Azan trichrome and a progressive Ferrous sulphate-Haematoxylin sequence (Lillie and Fulmer, 1976) all produced adequate results. The progressive Ferrous sulphate-Haematoxylin yielded the best resolution overall and was ultimately used for most of the material examined.

Settlement:

In order to study settlement behaviour a percentage of the larvae were transferred from the culture vessels to aquaria in the laboratory. This transfer occurred when the larvae were highly mobile and generally looked like typical planulae (sensu Hyman, 1940). In 1983 and 1984 the principal objective was to raise significant numbers of larvae through to settlement and investigate substrate preferences. Consequently, several thousand larvae were maintained in the aquaria and provided with several types of substrate

simultaneously. Ceramic tiles, sawn coral blocks, plastic sheet and freshly collected natural substrate in the form of small (5 to 8 cm) shells from dead reef bivalves were provided as possible settlement surfaces. The aquaria were aerated and their water fully exchanged with fresh seawater twice a day.

In order to quantify settlement through time two species, *Favites abdita* and *Lobophyllia hemprichii* were selected for detailed study. Each was cultured through to planula stage as above then six replicates of 30 larvae were each placed in plastic buckets of seawater. The buckets were aerated and provided with a selection of substrates as above. These experiments were commenced with larvae that were two days old. Each day the number of larvae settled was determined by direct counts on the substrate surface and a tally of the larvae still free-swimming.

RESULTS.

Eggs of *Montipora digitata*, *Acropora pulchra*, *Lobophyllia hemprichii* and *Favites abdita* were collected immediately after spawning and examined with a high power compound microscope. Initially all species' eggs were irregular shapes, presumably distorted when packaged for release. After 15 to 30 minutes the eggs became spherical and smooth. The release of polar bodies could not be confirmed for these species, although small protruberances seen on some eggs within the first 60 minutes may have been related to this event. In a collaborative study of 17 additional species (Babcock and Heyward, 1986) polar body release was detected

in only one species. A single polar body was observed within the mucous envelope surrounding eggs of *Goniastrea favulus* less than 10 minutes after spawning. The second polar body was extruded adjacent to the first within 30 minutes of spawning.

The most obvious change noted during the first hour after spawning was the formation of dense aggregations or clots (sensu Miller, 1981) of sperm on the egg surfaces. These clots consisted of very large numbers of sperm clustered head first onto the surface of the egg, all seemingly attracted to a very localized area. Miller (pers. comm.) suggested such sperm behaviour may be related to breakage in the egg membrane. The relationship of sperm clots to the site of cleavage initiation was not determined.

Although the four species studied in detail represent three taxonomic families and vary greatly in mean egg size, the pattern and rate of progress of their embryogeneses are very similar (Table 4.1). The major changes in external morphology as embryogenesis progresses, from first cleavage to planula, are presented in a series of scanning electron micrographs (SEM) (Figures 4.1 A to 4.1 O). The initiation of cleavage is seen as a slight indentation on one side of the egg and is first apparent approximately 90 minutes after spawning (Figure 4.1 A). The indentation progresses across the cell until cleavage is complete (Figure 4.1 B). Such cleavage by progressive furrow formation (Campbell, 1974) was also observed in all species studied by Babcock and Heyward (1986). Second cleavage was in the same plane but perpendicular to the first (Figure 4.1 C). These first two cleavages were equal, complete and typically required 45 to

60 minutes each. Histological sections through these early divisions suggest that the cleavage furrow proceeds from the site of fertilization (Figure 4.2 A) once the synkaryon has formed. Separation of the daughter nuclei occurs prior to cleavage of the remaining cytoplasm (Figures 4.2 B to F). The third cleavage to the 8-cell stage occurred in the horizontal plane and resulted in the upper and lower 4-cell groups being offset in spiral fashion (Figure 4.1 D). Although synchronous, this division was slightly unequal in some embryos of *A. pulchra*, *L. hemprichii* and *F. abdita*. Beyond this stage of development, cleavage followed a pseudospiral pattern (Mergner, 1971) and became asynchronous. In embryos of 8+ cells a central space developed which continued to enlarge until at 4.5 to 5.5 hours after spawning the embryos were hollow, spherical blastulae, with a single layer of cells (Figures 4.3 A,B).

In all species a hollow blastula formed within 6 hours. The embryos then undergo their first major change in shape by thinning into a flattened disk which is two cells thick (Figure 4.3 C). As the two cell layers approach one another the blastocoel is lost and the embryo curves into a concave-convex dish shape (Figures 4.1 I, 4.3 D). At this stage of development the embryos have reached their maximum size, with diameters across the disk up to 1.5 times that of the mean egg size. At 8.5 hours the embryos are pronounced dish shapes of more regular outline. Subsequently the dish contracts along its two major axes and thickens in the center, until it is several cells thick (Figure 4.3 E). Some cellular differentiation is apparent in the surface layer.

At 9 to 10 hours after spawning such differentiated cells become increasingly common as the disk continues to thicken (Figure 4.3 F). It is only the ectodermal layer which becomes increasingly differentiated as the thickening embryo becomes more spherical. Histological sections of such embryos reveal a differentiated ectoderm surrounding a mass of lipid rich, spherical cells. Invagination and differentiation of the ectoderm continue and within 20 hours the oral pore and cilia become apparent (Figure 4.1 M). The four species produced mobile, spherical larvae within 24 hours (Table 4.1, Figure 4.1 N). These development rates agree with those for larvae collected from the field in plankton samples from surface slicks. Subsequent development involves further differentiation of the ectoderm and enlargement of the gastric cavity as the lipids in the mostly undifferentiated endodermal cells are used up. Approximately 2.5 days after spawning a pear-shaped, highly mobile planulae has developed (Figures 4.1 O, 4.4 C,D).

In addition to following the above developmental pattern *Montipora digitata*, *M. verrucosa* and *M. dilatata* embryos developed while containing maternally inherited zooxanthellae. All cells up until the blastula stage contained the symbionts. The cellular differentiation associated with flattening and invagination at 6 to 8 hours, resulted in the zooxanthellae persisting only in undifferentiated, lipid rich cells. Ultimately this resulted in the columnar ectoderm being free of zooxanthellae and all the symbionts being associated with the lipid rich presumptive endoderm (Figure 4.4 A to D). As the lipid reserves were used up over a period of days the zooxanthellae

Species	Egg size (µm)	First cleavage	16-Cell	Morula/Blastula	Flat disk	Dish shape	Thicker dish	Spherical	Ciliated	Mobile
M. digitata	357	1.5-2.5 h	3-4 h	5 h	5+ h	7-10 h	10-14 h	14-16 h	16-20 h	18-24 h
A. pulchra	550	1.5-2.0	4	5	6+	7-10	9-12	12-14	12-18	18-24
L. hemprichii	650	1.5-2.5	4	5	6+	6.5-11	9-12	12-14	12-18	18
F. abdita	402	2	4	5	6+	8-11	10-12	12	12-18	24

Table 4.1 *Montipora digitata*, *Acropora pulchra*, *Lobophyllia hemprichii* and *Favites abdita*. Change in the external morphology of live embryos during the first 24 hours after spawning.

retreated in association with the endodermal cells to line the developing gut cavity (Figure 4.4 C,D).

Settlement:

Larvae 24 to 36 hours old were essentially spherical and although often highly mobile, showed no tendency to search or settle on the surfaces of substrate provided in their containers. The earliest settlement was recorded for *Acropora pulchra* at 72 hours after spawning. Prior to settlement these larvae tended towards a pear-shaped appearance and swam close to the substrate with the aboral end forward. Sometimes the larvae made several stops on the surface over the period of several hours before making a final attachment by the aboral end. Initial attachments were rather tenuous, with many planulae being shifted if a pipette of seawater was squirted at them. If left undisturbed however, the attachment became increasingly secure until by 80 hours the larvae were broadbased disks on the substrate, with a central mouth and mesenteries. While this pattern was repeated for all species studied, the first settlement by larvae of *Montipora digitata*, *Lobophyllia hemprichii* and *Favites abdita* was not observed until day four after spawning.

The majority of larvae from all species had settled within seven days of spawning, which was also true for 16 of the additional species studied by Babcock and Heyward (1986) (Table 4.3). Settlement was frequently aggregative despite an apparent abundance of substrate. Larvae showed a marked preference for rugose substrate, in particular that which had

been 'preconditioned' by one month's prior immersion in the sea. Some larvae from each species were still free swimming ten days after spawning. In practice it proved to be very difficult to quantify larval settlement through time. The settlement trials using *Lobophyllia hemprichii* and *Favites abdita* in aerated containers showed early promise (Table 4.2), but proved unsatisfactory after 5 to 6 days.

Table 4.2 *Lobophyllia hemprichii* and *Favites abdita*: settlement of larvae through time. (Mean percentage ± 95 % confidence limits)

Species	Settlement on day 4	Settlement on day 5
L. hemprichii	46.9 ± 38.9	51.3 ± 21.0
F. abdita	44.7 ± 42.4	----------

Both species had an average of approximately 45 % of their larvae attaching to substrate by the time they were four days old. This level of settlement agreed well with observations made of larvae which were being kept in much larger glass aquaria for future studies of juvenile growth and interactions. On days five and six, however, the larvae in the quantitative experiment were often de-attaching and returning to free swimming status; the majority still attached had not metamorphosed. In sharp contrast, an increasing percentage of those larvae in the large aquaria were continuing to settle and were then metamorphosing. These observations suggested that conditions in the quantitative experiment were not appropriate for settlement and the experiment was discontinued. It seemed possible that the

larvae were very sensitive to water quality and that a flow through system of fresh seawater might overcome water quality problems. In a field situation, however, the technical problems associated with maintaining such a water supply, whilst also retaining and minimizing disturbance to the larvae, could not be overcome during the course of the present research.

Despite these problems, it was possible to study aspects of post-settlement behaviour using larvae from the larger glass tanks. All species which released eggs without zooxanthellae were able to settle and metamorphose free from symbionts. Such settled juveniles were returned to the reef and re-examined 7 and 13 days after settlement. No zooxanthellae could be seen in live polyps one week after settlement using a stereo-dissector microsope. Polyps at this stage had often developed small tentacles. Zooxanthellae were found in polyps 13 days after settlement, appearing as brownish spheres and colouring the, by then, well developed tentacles and oral disk. Some juveniles at this stage were also observed capturing small crustacea.

Figure 4.1 A – O. *Acropora pulchra*: Scanning electron micrographs (SEM) of embryogenesis. All scale bars = 100 µm.

4.1 A - Midway through the first cleavage 1.5 hours after spawning. Cleavage is by progressive furrow formation.

4.1 B - Cytokinesis almost complete in the first cleavage, 2.0 hours after spawning.

4.1 C - A three hour old embryo commencing the second cleavage perpendicular to the first.

4.1 D - An 8-cell stage four hours after spawning. Note the rotation of the blastomeres into a spiral formation.

4.1 E - A 16-cell stage four hours after spawning. Cell arrangement has reverted to that more characteristic of radial cleavage.

4.1 F - Early blastula 5.5 hours after spawning. Note that cell division is asynchronous.

4.1 G - Well developed blastula 7 hours old.

4.1 H - Blastula 7 hours old which has started to flatten. The blastocoel is reduced as the embryo flattens.

4.1 I - Early dish-shaped embryo 8.5 hours old.

4.1 J - Advanced dish-shaped embryo 10 hours old. At this stage the blastocoel has been lost.

4.1 K - A 12.5 hour old embryo which has thickened in the center and reduced its diameter. Such embryos contain differentiated cells as an outer layer. These cells are the early ectoderm.

4.1 L - Fifteen hours after spawning. The embryo becomes increasingly spherical.

4.1 M - A spherical shape has been attained 18.5 hours after spawning. The central primitive oral pore is obvious and the rough surface texture is due to fine cilia. Such larvae are able to spin on their central axis.

4.1 N - A 24 hour old larvae. Such larvae are well ciliated. The primitive mouth has closed, but the site of the oral pore is apparent.

4.1 O - A typical pear-shaped planulae 60 hours old. Cilia have enlarged and such planulae are capable of exhibiting strong movement.

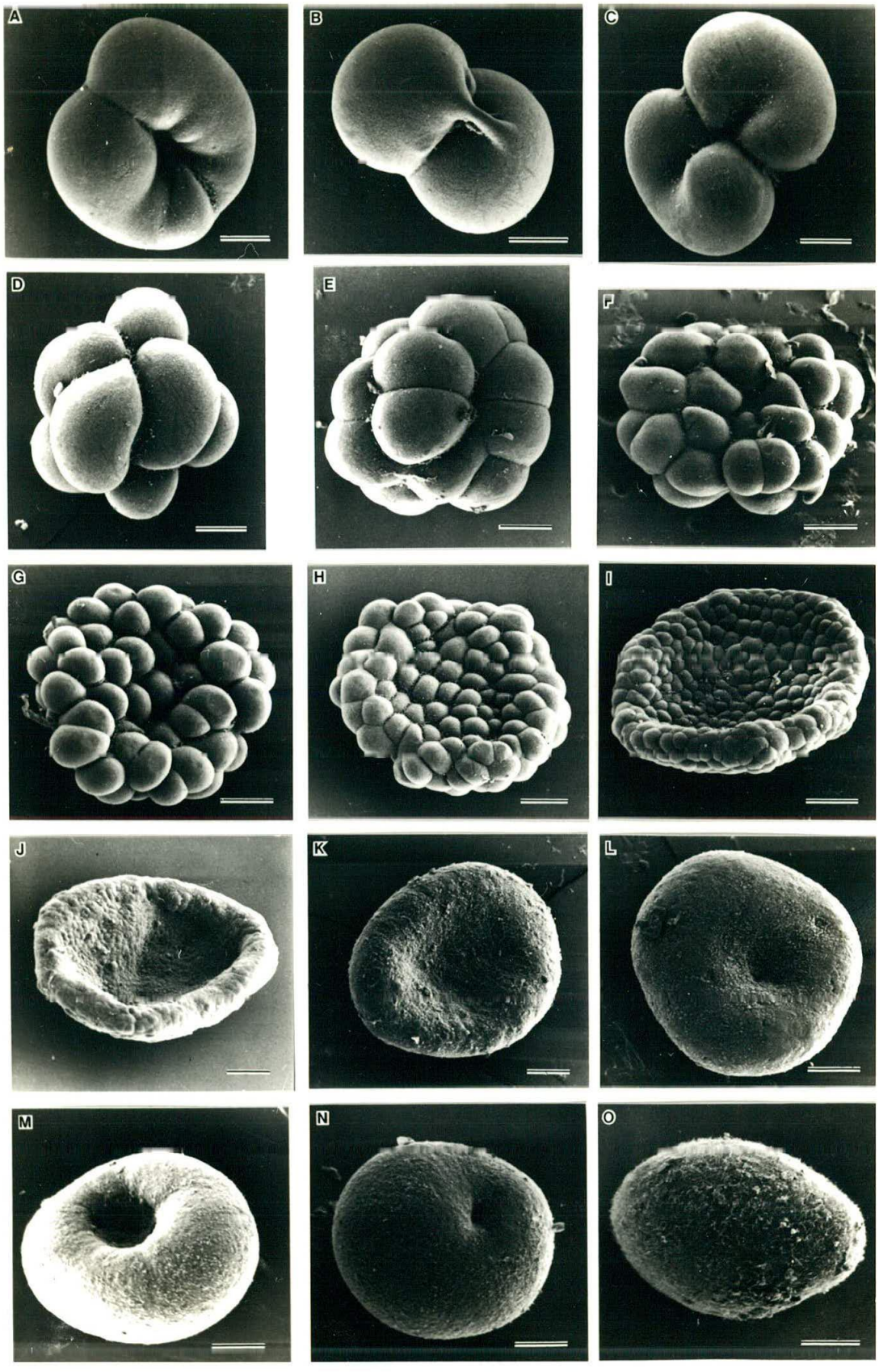

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Figure 4.2 A-F. Histological sections of early developmental stages from single egg to 4-cell stage. All scale bars = 100 µm.

4.2 A - *Acropora pulchra*: single cell. Arrow indicates the dark staining nuclear material adjacent to the cell membrane at the time of fertilization.

4.2 B - *Acropora pulchra*: initiation of the first cleavage. Arrow indicates the direction in which the furrow will head. n - the two daughter nuclei.

4.2 C - *Montipora digitata*: Late first cleavage, at the same stage as Figure 4.1 A. Arrow indicates the direction in which the furrow will progress. n - daughter nuclei. Small dark circles throughout the cytoplasm are zooxanthellae.

4.2 D - *Montipora digitata*: Two cell stage, analagous to a section through the embryo in Figure 4.1 B. Note that cleavage is complete and equal. z - zooxanthellae.

4.2 E,F - *Montipora digitata*: Early and late 4-cell stages. The nuclear replication and division precedes cytokinesis, which is equal and complete. Analagous to sections through embryos such as that in Figure 4.1 C.

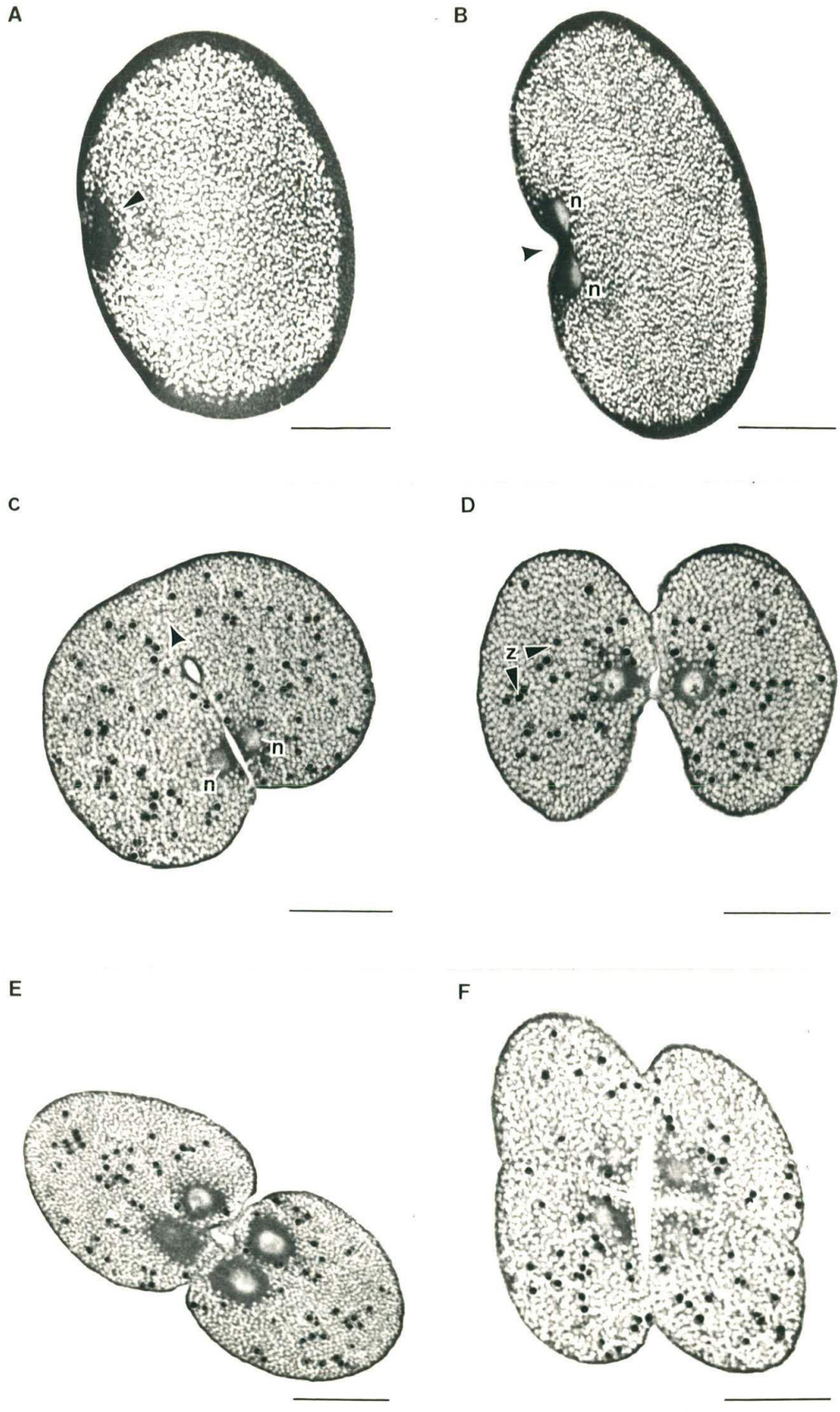
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Figure 4.3 A-F. Histological sections of embryogenesis between blastula formation and early germ layer formation. All scale bars = 100 µm.

4.3 A - *Montipora digitata*: 16-cell stage, showing the early formation of the blastocoel (b). z - zooxanthellae, n - nucleus. This stage is equivalent to the 16-cell, 4 hour old embryo shown in Figure 4.1 E.

4.3 B - *Acropora pulchra*: a well developed blastula 7 hours old. b - blastocoel.

4.3 C - *Acropora pulchra*: a flattened, late blastula equivalent to the embryo in SEM Figure 4.1 H. Note the marked reduction of the blastocoel (b) as the two sides of the embryo come together.

4.3 D - *Acropora pulchra*: early dish shaped stage in which the blastocoel has been lost. Such developmental stages were noted as early as 7 hours after spawning. Embryos maintain this concave-convex shape for several hours, while mitotic divisions reduce cell size (See Figures 4.1 H,I and J).

4.3 E - *Acropora pulchra*: early delamination 12.5 hours after spawning. This embryo is several cells thick following paratangential mitotic divisions from the two-cell thick dish shape into the center. The cells are undifferentiated and lipid rich, which results in poor resolution of the cytoplasm using normal histological technique.

4.3 F - *Acropora pulchra*: fifteen hours after spawning the outermost layer of cells is differentiating into an ectodermal layer (e). The lipid rich, undifferentiated cells in the center (L) maintain a nutritive function and ultimately give rise to the endoderm.

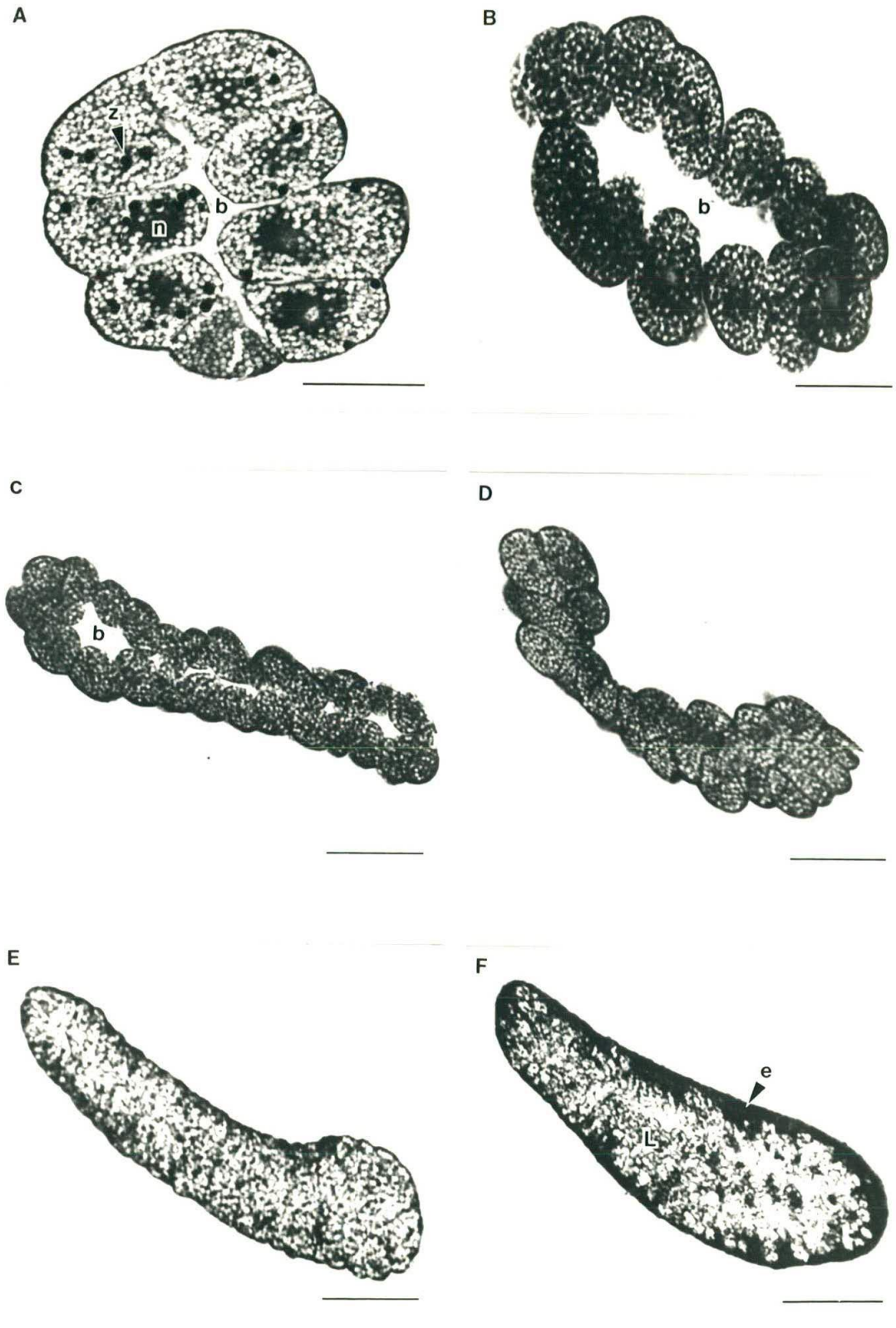
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Figure 4.4 A-D. *Montipora digitata*: primitive and advanced planulae. All scale bars = 100 µm.

4.4 A - *Montipora digitata*: slightly oblique L.S. through a 36 hour planula. The darkly staining ectoderm (e) surrounds presumptive endoderm and zooxanthellae (z). m -mouth.

4.4 B - *Montipora digitata*: median T.S. of a 36 hour planula. The ectoderm (e) is columnar and differentiated, while the endoderm and zooxanthellae (z) are much less well organised and fill the center of the larvae.

4.4 C - *Montipora digitata*: L.S. of a 60 hour planula. The level of cellular differentiation and organisation is much greater than the 36 hour larva. The base of the ectoderm can clearly be distinguished from the endoderm. The mouth (m) now opens into a gut cavity (gc) which has formed as the lipid reserves and some of the prospective endoderm have been consumed. Most of the zooxanthellae (z) and presumptive endoderm have attained an epithelial organization and now line the developing gut cavity.

4.4 D - *Montipora digitata*: T.S section through a 60 hour planula, again showing the zooxanthellae (z) lining the developing gut cavity. e - ectoderm.

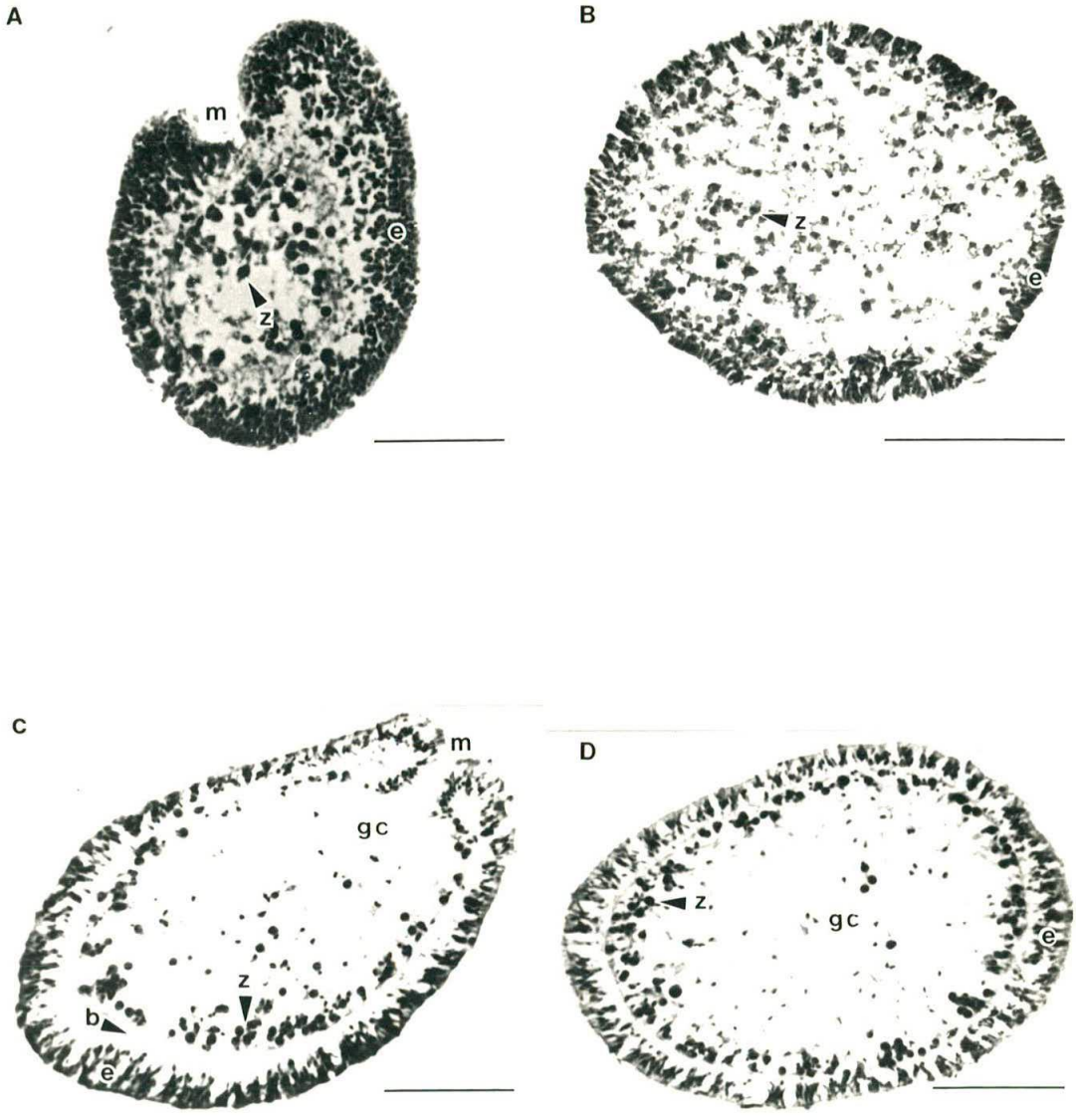
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Table 4.3 Embryonic and larval developmental rates of certain species participating in the mass-spawning event on the GBR. Observations were made too infrequently to provide much information on developmental patterns during the first 24 hours. Studies of the planulae, however, do reveal the general similarities in later development and settling behaviour for a broad group of species. Vacant spaces mean no observation was made. (After Babcock and Heyward, 1986).

Species	Egg size (micrometres)	2 h	12 h	24 h	36 h	48 h	3 days	4 days	4.5 days	5 days	6 days	7 days	8 days	10 days
Platygyra sinensis	395	First cell divisions	Blastosphere or concave disc	Oral pore formed, mobile	Differentiated pharynx	Highly mobile	Pear shaped, half of larvae on bottom	Mesenteries formed	Settling and and meta-morphosing in culture jars	Settling and and meta-morphosing in culture jars		Majority of larvae settled in aquaria		Settled corals placed on reef
Goniastrea favulus	500	First cell divisions	Blastosphere or concave disc	Oral pore formed, mobile	Mobile	Highly mobile	Larvae elongate			Swimming near bottom and meta-morphosing	Some larvae settled		Majority of larvae settled	
Goniastrea aspera	350	First cell divisions	Blastosphere or concave disc	Oral pore formed, mobile		Highly mobile	Pear shaped, half of larvae on bottom	Mesenteries formed	Settled and meta-morphosed in aquaria	Settled and meta-morphosed in aquaria	Majority of larvae settled			Settled corals placed on reef
Favia pallida	430		Blastosphere or concave disc	Mobile		Highly mobile	Pear shaped		Settled and meta-morphosed in aquaria	Settled and meta-morphosed in aquaria	Majority of larvae settled			
Montipora digitata	350	First cell divisions		Mobile		Highly mobile		Elongate with well form-mesenteries		Settled and meta-morphosed in aquaria	Majority of larvae settled			
Acropora formosa	–				Early planula, mobile									
Acropora millepora	540				Early planula, mobile	Highly mobile			Meta-morphosing in culture jars			Majority of larvae settled	Settled corals placed on reef	
Clavarina triangularis	350				Mobile									
Echinopora gemmacea	340				Elongate highly mobile									
Favites complanata	390					Mobile								
Galaxea fascicularis	500				Mobile	Some elongate larvae	Highly mobile	Pear shaped, mesenteries forming	Settled and meta-morphosed in culture jar					
Goniastrea retiformis	345					Pharynx formed	Highly mobile		Majority of larvae elongate	Settled and meta-morphosed in culture jar				
Goniopora lobata	500				Mobile, pharynx formed		Highly mobile			Elongate larvae				
Lobophyllia corymbosa	600				Mobile									
Lobophyllia hemprichii	810						Pear shaped larvae, highly mobile			Crawling on bottom				
Montipora tuberculosa	350				Mobile									
Mycedium elephantotus	430				Mobile	Elongate larva		Mesenteries formed	Meta-morphosed in culture jar					
Pectinia alcicornis	350				Mobile			Pear shaped larva		Meta-morphosed in culture jar				
Pectinia peonia	350				Mobile			Pear shaped larva						

DISCUSSION.

The ability to raise large numbers of larvae through to settlement is a significant technical achievement, which lays a foundation for future studies of larval biology. In the present detailed study of 4 species and the more general observations of a further 17 broadcast spawners (Heyward and Babcock, 1985; Babcock and Heyward, 1986) the large amount of variation in development seen among the anemones (Spaulding, 1974) is absent. This study has demonstrated a high degree of uniformity in the developmental patterns and rates seen amongst broadcast spawning corals.

Despite a rigorous investigation of early development, the formation of the germ layers is not fully resolved. In anemones with yolky eggs, Spaulding (1974, p 518) notes that gastrulation has been reported to occur by "invagination, ingression, epiboly, nuclear rearrangement prior to cytokinesis, or a combination of these". Reviewing general cnidarian development however, Mergner (1971) finds that with few exceptions, endoderm formation in anthozoans is by invagination. The detailed study of external morphology (Figure 4.1) and supporting histological evidence (Figures 4.2 to 4.4) strongly suggest a pattern of embryogenesis in which germ layer formation is the product of primary or mixed delamination, while subsequent invagination gives rise to the archenteron. The process by which the hollow blastula flattens to the curved dish would appear to be the onset of a classic invagination sequence. Subsequent thickening of the embryo and associated differentiation of the ectoderm, however, suggest that the material in the center of the thickening embryo is presumptive endoderm derived as daughter

cells by mitotic division from the blastoderm. The material in the center of the embryo appears to be cellular and eventually assumes an epithelial organisation as the gastrodermis. The best interpretation of these observations is that germ layer formation occurs via a form of delamination. Chia and Spaulding (1972) while studying gastrulation in the anemone *Tealia crassicornis* noted that, as in the present study, the presence of yolk made the endoderm difficult to define in histological preparations. In a close parrallel to this work, they found that the endodermal cells do not form a continuous layer until the planula stage, but rather are scattered with ill-defined bounderies. The early association of the zooxanthellae in *Montipora digitata* with the presumptive endoderm provides a very useful marker. It can be seen that in the very early stage of larval organisation (Figure 4.4 B) the center of the larva is a solid mixture of endoderm and zooxanthellae. Only as the gut forms in the planula larva do both the endodermal cells and the symbionts move towards an epithelial organization (Figures 4.4 C,D). This pattern closely resembles the late development of some hydrozoan sterrogastrula described by Mergner (1971). Unfortunately, it must be concluded that traditional, wax-block based microtomy is limited for studying the lipid rich, largely undifferentiated cells which make up coral embryos at the onset of germ layer formation. Direct cryotomy can be used to avoid the lipid loss associated with parafin based processing, however for future studies the Transmission Electron Microscope offers the best possibilities.

Settlement :

Embryogenesis leads to the development of spherical, mobile planulae, often within 24 hours of spawning, which initially show no tendency to search the substrate or to settle. These early larvae differ from the edwardsiae and halcampoides stage planulae released by brooding species most noticeably in their level of cellular and structural differentiation. The mouth, pharyngeal tube and well differentiated ectoderm characteristic of brooded planulae do develop, however, within two to four days after fertilization. Vandermuelen (1974) suggests that the well differentiated ectoderm of such larvae, particularly at the aboral end, is rich in receptor sites which allow testing of the substrate for appropriate settlement cues. Observations in the present study support this view. In addition it would appear that insertion of mesenteries as the gastric cavity develops seems time related rather than specifically required for competency. Mergner (1971) calls such highly developed planulae secondary larvae. They are active swimmers with demonstrable behavioural responses to basic physical parameters. Preliminary field studies by Babcock (pers. comm.), using larvae of umbrophilic *Oxypora sp.*, have shown negative phototaxis commencing in larvae 2 to 3 days old. Once this stage of development is reached, the searching behaviour noted in this study and the distinct preference shown towards substrate with some live fouling on it, support the notion of specific sensory switches activating a settlement response.

Settlement between three and seven days after spawning for the majority of species studied (this study; Babcock and

Heyward, 1986), is a shorter minimum dispersal period for externally developed larvae than has previously been reported for these species (Kojis and Quinn, 1981; Babcock, 1984; Harrison *et al.*, 1984). The time periods of development and settlement are very close to those demonstrated for species which broadcast gametes in the Red Sea (Shlesinger and Loya, 1985). Recently (author, unpublished data) a minimum free-swimming period of 3 to 4 days was also observed for several species, including *Montipora digitata* and *Goniastrea aspera*, in Okinawa, Japan. These observed rates of larval settlement are supported by studies of larval abundance in the plankton following spawning. It was noted (G. Moore, pers comm.) that live coral planulae, collected from a labelled water mass into which many coral species broadcast gametes on the GBR in 1986, would crawl over the bottom of containers and begin attaching four days after the spawning event. Similarly Bull (1986; pers. comm.) found comparable developmental rates to the present study and, perhaps not coincidentally, noted a rapid decrease in the abundance of planktonic larvae beyond seven days after spawning.

A pre-settlement period of 3 to 7 days permits considerably greater minimum dispersal than has been observed in brooding species (see Szmant, 1986; Shlesinger and Loya, 1985). Nevertheless, the degree of dispersal possible in this period will clearly depend to a large degree on the prevailing weather. Done's (1982) suggestion that reefs are primarily self-seeded stemmed from observations of brooded larvae, but did not appear to apply to broadcast spawning species which required two weeks to settle (Harrison *et al.*,

1984). It is now clear that minimum dispersal times are much closer between broadcasters and brooders. Brooded larvae have greater opportunity to recruit to the parental reef simply because they may be able to settle within minutes or hours of release. The larvae of broadcast spawning species are more likely to disperse away from the parental reef unless local current patterns, such as topographic eddies (Williams *et al.*, 1984), result in prolonged retention of the water moving over a reef. Somewhat surprisingly, a very recent hydrodynamic model of advection and dispersion around some reefs of the GBR (Black and Gay, 1986, abstract only) predicts that in some areas of a reef water residence times may frequently exceed 14 days. Obviously under such a flow regime the species in this study would recruit mostly to the parental reef.

Gene flow depends on larvae which are flushed clear of the parental reef. Sightings of large slicks of coral embryos drifting well clear of adjacent reefs in the central GBR (Willis and Oliver, in press) demonstrate that dispersal is significant even in moderate winds. Even in calm weather and with minimal tidal effects, the residual currents in the GBR region would carry larvae 20 to 80 kilometers in four days (Collins and Walker, 1985). These distances represent absolute minimums for inter-population gene flow. It is now apparent that most local populations release reproductive propagules at least once per year (see Fadlallah, 1983; Shlesinger and Loya, 1985; Szmant, 1986; Babcock *et al.*, 1986; and references therein). In the GBR region this results in the almost certain connectivity between reefs within one weeks travel by current of each other. The annual arrival of

migrant larvae must alter the genetic structure of the population. Subsequent addition of the migrant alleles to the gene pool will result in a cohort of offspring which has a genetic structure intermediate between the connected populations. Ayala (1982) has shown that unless such gene flow stops, the allelic frequencies will become the same in the local population as in the surrounding populations. Therefore, significant differences in the genetic structure of neighbouring coral populations in the central GBR are unlikely unless their is strong local selection. The genetic structure of populations may be strongly influenced by internal effects operating over very small (10 m) spatial scales. Preliminary data for *Acropora* species which broadcast gametes on the GBR (Stoddart, pers. comm.), suggests that intra-reefal genetic variation is of at least the same order of magnitude as inter-reefal differences.

Recent reports of annual larval recruitment independent of the presence of a species (Wallace, 1985; Harriot, 1986), confirm that inter-reef dispersal is common in the GBR region. Such data however, provide no insight into where the larvae originated, although nearest neighbour reefs might be the most probable. In the present experimental approach, larvae were brought into close proximity to suitable substrate; a situation difficult to envisage in the open sea. (although Jokiel (1984) notes the possibility of settlement on floating objects). Consequently, this study provides little insight into maximum dispersal potential. Histological evidence indicates that most of the lipid stores provided by the egg are used up in larvae which are preparing to settle

after one week. The possibility exists that nutritional status influences settlement responses, but the sources of larval nutrition could extend beyond maternal yolk products. While feeding by planulae on plankton has not been demonstrated (although stated otherwise by Szmant, 1986), energetic input from symbionts may enhance the dispersal potential of some coral species (Richmond, 1981). A small number of species in the genera *Montipora* and *Porites* (this study; Kojis and Quinn, 1982a; Heyward and Collins, 1985a; Babcock *et al.*, 1986) are known to release eggs which contain zooxanthellae. The *Montipora digitata* larvae raised in this study received translocated products from their symbionts (Mc Neil, pers. comm.) and it is reasonable to assume that other species with maternal zooxanthellae also receive nutritional benefit. The observation that in Kaneohe Bay, Hawaii, the coral community is dominated by *P. damicornis*, *Montipora spp.* and *Porites compressa* all of which produce larvae with zooxanthellae (Richmond, 1981; this study), while *Fungia scutaria* larvae in Hawaii also become infected prior to settlement (Krupp, 1983), does suggest that the symbionts may assist long distance dispersal. In contrast the majority of broadcast spawning coral species, without symbionts, will depend on the small percentage which can extend their yolk reserves, or those larvae which can obtain supplementary nutrition whilst in the plankton. It is premature, however, to suggest whether or not this uptake is significant to total larval nutrition.

In conclusion, the larvae of broadcast spawning species are likely to achieve some migration away from the parental reef. This annual dispersal phase will tend to distribute

genetic novelties among connected populations. Consequently the genetic structures of populations, which are closely linked by the same current patterns, are expected to have a major common component. In reef systems where mean inter-reefal distances are less than approximately one week's travel by current apart, panmixis is to be expected throughout the system. The level of self-seeding occurring in such populations will vary from year to year, principally due to local weather effects. In contrast, brooding species will nearly always achieve a higher level of self-seeding than broadcasters. This will maintain a high frequency of locally adapted alleles in brooding populations. Due to their facility at self-maintenance, populations of brooders have a greater chance of developing unique genetic structures.

Chapter 5

Coral Cytogenetics

INTRODUCTION.

The simple differences demonstrated for mating systems (Chapter 3), or the variation in the specificity of histocompatibility responses (Chapter 1), show that alternative genetic dynamics are operating within populations of different species. These are likely to be species specific and hence independent genetic systems, as there is no data demonstrating hybridization among the Scleractinia. The mechanisms effecting reproductive isolation of coral species are not known. Synchronous multi-specific spawning (Babcock *et al.*, 1986) points to pre-zygotic rather than temporal (Shlesinger and Loya, 1985) reproductive isolation. The genetic basis of species differences is also unknown. White (1978) argues that in many cases chromosomal alterations are a primary factor in reproductive isolation. This view regarding chromosomal change as causative in speciation may be extreme (Zouros, 1982), nevertheless it is a fact that detectable alterations to chromosomes are often found between closely related species (Arthur, 1984).

There is very little information of any kind about anthozoan cytogenetics. In a preliminary study of scleractinian coral chromosomes (Wijsman and Wijsman-Best, 1973), the use of somatic tissue from adult colonies to establish mitotic figures was only partially successful. No exact chromosome counts were established. Subsequently no further work on coral chromosomes has been reported. The above authors' estimates of chromosome numbers from four

Mediterranean species represent the total data available. This is completely inadequate for even the most rudimentary analysis of the role of cytogenetic change in scleractinian evolution. Furthermore, the inferred difficulties encountered in working with coral karyotypes and the suggestion that very large numbers of very small chromosomes might be involved (Wijsman and Wijsman-Best, 1973) did little to encourage further attempts.

The study of early embryogenesis following broadcast spawning (Chapter 4) suggested that the rapidly dividing cells of coral embryos might be useful for chromosomal studies. Once techniques for raising large numbers of embryos were perfected, a program of experimentation was embarked upon in a search for usable coral karyotypes. Initial results with species from the Great Barrier Reef were encouraging. Subsequent technical improvements were accelerated by taking advantage of the predictable broadcast spawning in both northern and southern hemispheres. In the present study the karyotypes of tropical reef corals, from Hawaii and Australia, are described in terms of chromosome numbers and their shapes and sizes.

MATERIALS AND METHODS.

Field work was performed in Hawaii and Australia, taking advantage of the predictable coral spawning events (Chapter 2). *Montipora dilatata* was collected from Kaneohe Bay and spawned at the Hawaii Institute of Marine Biology. The species in Australia were spawned at Magnetic and Orpheus Islands. The species *Montipora digitata*, *Lobophyllia hemprichii* and *Goniopora lobata*, representing three taxonomic

families, were focused on. Preliminary chromosome studies were also made of additional species which were being cultured for embryological investigations (Chapter 4).

At each location, colonies were collected and placed in aquaria until they spawned in synchrony with the field populations. The released gametes were cultured in 4 litre plastic jars in the sea as for the embryological studies in Chapter 4. Once cell divisions commenced the embryonic tissue was subjected to treatment designed to fix the condensed chromatin present in the dividing cells. Developing embryos at all stages from 8-512 cells were pipetted from the culture jars for treatment.

Treatment:

The chemical colchicine was used to inhibit the completion of mitosis and hence increase the number of cells likely to contain metaphase plates. The colchicine-seawater solutions were prepared immediately prior to use. Embryos were treated in 500 ml plastic bottles with either 0.01, 0.02, 0.05 or 0.1 % (w/v) colchicine-seawater with continual agitation. Treatment times of 15, 30, 60, 90, 120 and 180 minutes were tried at each concentration.

Following colchicine treatment the embryos were initially fixed directly in glacial acetic acid-absolute ethanol (1:3, v/v). Further work, however, showed that soaking the embryos in an hypotonic solution prior to fixation gave superior results. The particular osmotic strength of the hypotonic solution needed to be varied, depending on the developmental stage of the embryos being treated. Three hypotonic solutions were tried; seawater-

freshwater in the ratios 50:50, 65:35 and 80:20 (v/v). Immersion times ranging from 1 to 20 minutes were experimented with. After hypotonic treatment the embryos were fixed in either glacial acetic acid- absolute ethanol (1:3, v/v) or absolute ethanol-50 % acetic acid (1:1, v/v). All fixed material was subsequently stored at 4° C.

Squash preparation:

Prior to staining the fixed embryos were soaked in an organic solvent. This removed most of the lipid reserves in the cells which otherwise made light microscopy difficult. Diethyl ether proved superior to chloroform or acetone. Following a minimum period of 2 hours in the solvent, the embryos were placed in a drop of lacto-acetic orcein on a coverslip. After 15 minutes to allow adequate staining, a glass slide was carefully applied over the droplet and the preparation squashed firmly. To provide a semi-permanent mount the edges of the coverslip were sealed with nail varnish. The squash preparations were photographed using transmitted green light and a 100X oil immersion objective (Olympus BHS 312). Micrographs were recorded on Kodak technical pan 2415 film developed for maximum contrast.

Advanced techniques for chromosome banding.

At the conclusion of this study attempts were made to improve on the squashing techniques and also demonstrate the presence of banding patterns within the coral chromosomes. The techniques developed need refinement before a general analysis of chromosome banding patterns can be undertaken. Nevertheless, C-banding was demonstrated in some species

and the methods are included here as a foundation for future work.

Cell monolayer preparation.

Squashes suffered from depth of field problems due to their multi-layered nature. In order to improve on this the fixed embryos were broken up into pieces which could be spread into a thin layer and allowed to dry on glass slides. The most effective way of breaking up the embryos was to continually re-pipette them in a small vial with several drops of 60 % acetic acid. Approximately 2 minutes pipetting produced a cell suspension which was spread onto a glass slide. Additional 60 % acetic acid was added if nescessary to spread the suspension thinly over the slide, which was then placed on a hotplate (45° C) to dry. These dried preparations were able to be stored for at least several days prior to staining.

C-banding stain.

Staining for C-bands is essentially a modification of Giemsa staining. Dried slide preparations are etched in supersaturated barium hydroxide at room temperature (22° to 25° C) for 2 to 8 mins. The slides are placed in a Coplin jar which is then filled to the brim and covered with a glass slide to seal out oxygen in the air. Seven minutes proved successful for two *Acropora* species. The hydroxide is rinsed off with tapwater then 3 changes of distilled water. The slides are then placed in heated (65° C) sodium chloride-sodium citrate solution for 45 minutes, rinsed in tapwater and twice in distilled water, then layed out on racks for

Giemsa staining. The Giemsa stain is diluted to 10 % in phosphate buffer and poured over the horizontal slides. After 10 to 15 minutes the Giemsa is rinsed off with tap water and the slides dried and mounted in DPX. The formulae for all solutions used in this protocol are listed in Appendix 1.

RESULTS.

General observations:

Embryos at all stages of development provided some evidence of chromosomes. The more advanced stages were the most likely to contain metaphase plates because of their greater cell numbers and the asynchronous nature of cleavage beyond the 16-cell stage. The flattened disk stage (7 to 10 hours old) was the best for squashes, since it provided many cells yet was frequently only two cells thick. In the preparations of *Montipora dilatata* and *M. digitata* the chromatin of both the coral cells and their maternally inherited zooxanthellae was visible. The zooxanthellae appear to also be undergoing quite rapid replication (Figure 5.1).

Treatment:

No differences were detected in chromosome appearance from material treated with different colchicine concentrations. The only effect noted was after one treatment vial was overlooked for several hours. The prolonged treatment (6 hours at 0.02 % w/v) induced polyploidy in cells of the species *Favia pallida*.

The hypotonic treatment caused the cell volumes to increase, which in some cases provided better separation of chromosomes in the karyotype. The particular treatment needed

to be varied depending on the developmental stage used. Embryos showed greater osmotic stress tolerance as their cell numbers increased. Four-cell stages autolysed after 5 minutes in 50 % seawater, whereas 256-cell stages could often tolerate 20 minutes. The optimal treatment time for 10 hour old embryos in 65 % or 80 % seawater was 15 to 20 minutes. Longer times might not cause the cells to lyse, but could cause chromosomes to spread too far from the complement.

Successful hypotonic treatment was also dependent on the type of fixation which followed. Glacial acetic acid-absolute ethanol (1:3, v/v) typically caused cell shrinkage in cells taken directly from seawater. In embryos which had been treated in the hypotonic solutions this effect was exaggerated, resulting in significant cell lysis. The alternative fixative, absolute ethanol-50 % acetic acid (1:1, v/v), avoided these osmotic problems and appeared to give adequate fixation. The fixed material used to provide the karyotypes presented in this study was derived from 10 hour old embryos placed in 0.01 % colchicine for 20 to 30 minutes, treated in 80 % seawater for 20 minutes then fixed in absolute ethanol-50 % acetic acid (1:1, v/v).

Squashes and orcein staining:

Cells were found at all stages of the mitotic cycle. In some embryos very large numbers of metaphase stages were seen. Most commonly the chromosomes were contracted and appeared as a group of dots or small crosses (Figure 5.2). When these chromosomes were well separated such plates were used for counts of chromosome number. The rarer preparations containing elongated chromosomes enabled both counts and

ploidy to be determined (Figure 5.3). Using 20 such plates a complement of 28 (n=14) was determined for *Montipora dilatata*, *M. digitata*, *Lobophyllia hemprichii* and *Goniopora lobata*. Fewer countable plates were found for other species, but the number of chromosomes was consistently 28 (n=14) for *Acropora valida*, *A. millepora*, *Goniastrea aspera* and *Favia pallida*. In one cell of *Acropora formosa* a complement of 30 chromosomes was counted, but the typical complement was again 28 (n=14). The presence of unique pairs in the karyotypes was presumed to reflect the diploid nature of each species.

While the maximum counts typically totalled 28 chromosomes, their were many instances where fewer chromosomes could be found. Complements containing as few as 13 chromosomes were occasionally encountered, although it was more typical to find 25 to 27. One obvious cause of this variation was experimental artifact. Examples were noted where individual chromosomes were widely dispersed during squashing, resulting in incomplete nuclear complements. In some preparations such disassociated chromosomes formed large aggregations of stained material. A further difficulty with squash preparations was related to overlapping chromosomes being obscured due to depth of field problems and related poor resolution.

The karyotypes of *Montipora dilatata*, *M. digitata*, *Lobophyllia hemprichii* and *Goniopora lobata* have been arranged according to chromosome length and centromere position (Figure 5.5 a,b,c,d). All of these species had a complement of elongate chromosomes ranging from 1 to 5 µm. The means and standard deviations of the relative lengths and centromeric indices of the fourteen chromosome pairs are

given in Table 5.1. The idiograms (Figure 5.6) are derived from these values. According to the nomenclature of Levan *et al.*, (1964) both species of *Montipora* have 14 metacentric pairs. *Lobophyllia hemprichii* has 2 submetacentric and 12 metacentric pairs, while *Goniopora lobata* has 5 submetacentric and 9 metacentric pairs. A potentially useful marker of the *G. lobata* karyotype is a subtelocentric secondary constriction present on the metacentric fifth pair.

The general similarity of the karyotypes for all species is obvious, yet differences do exist between comparable chromosome pairs e.g. the relative lengths of pair 2 in *M. digitata* and *G. lobata* (Figure 5.6). There are significant ($p<0.05$) differences in the position of the centromere in a few cases, e.g. chromosome 13 in *M. digitata* and *L. hemprichii*. The relative position of the centromere on chromosome 7 is also markedly different in the two *Montipora* species (Table 5.1 b,c). Nevertheless, for the majority of chromosomes the confidence limits for both relative length and centromeric index overlap for all four species.

Advanced techniques:

Cell monolayer preparations provided very few chromosome plates. Most of the embryonic cells were lost in processing. Those complements encountered were, however, at least equal in quality to the best of the squash preparations. In two cells from *Montipora digitata*, which contained the entire complement in the same plane, additional small particles of chromatin were observed (Figure 5.7). This material may represent an hitherto unseen microchromosome. The limited success of the techniques precluded further

elucidation of this material.

The presence of C-bands was demonstrated in *Acropora* species and *Favia pallida*. These species showed some banding in the chromosomes after a seven minute treatment in the barium hydroxide. Longer treatment times removed the chromatin altogether, while shorter times were not able to differentiate the bands. In general the C-bands appeared as a block of dark staining material extending a small distance from either side of the centromere (Figure 5.4). One micrograph of *Acropora valida* suggests that all the heterochromatin is to one side of the centromere on one chromosome (Figure 5.4). Similarly some preparations of *Favia pallida* appeared to have chromosomes with multiple bands. In general these C-band preparations were both rare and difficult to interpret, but did suggest that future banding studies would prove fruitful.

Figure 5.1 *Montipora dilatata*. Two chromosome complements are visible, surrounded by the maternally inherited zooxanthellae. The coral chromosomes (C) are reasonably well spread in the central cell. The complements of the zooxanthellae are tightly packed but in two cells (Z) the nuclei appear to be undergoing fission. Scale bar = 5 μm.

Figure 5.2 *Acropora formosa*. A micrograph showing part of the complement at metaphase. The chromosomes adopt the classic X shape prior to separation of the chromatids. Due to the contracted nature of the arms such plates were not very useful for distinguishing different chromosomes. Where separation of the chromosomes was good, nevertheless, they were used for estimates of chromosome number. Scale bar = 5 μm.

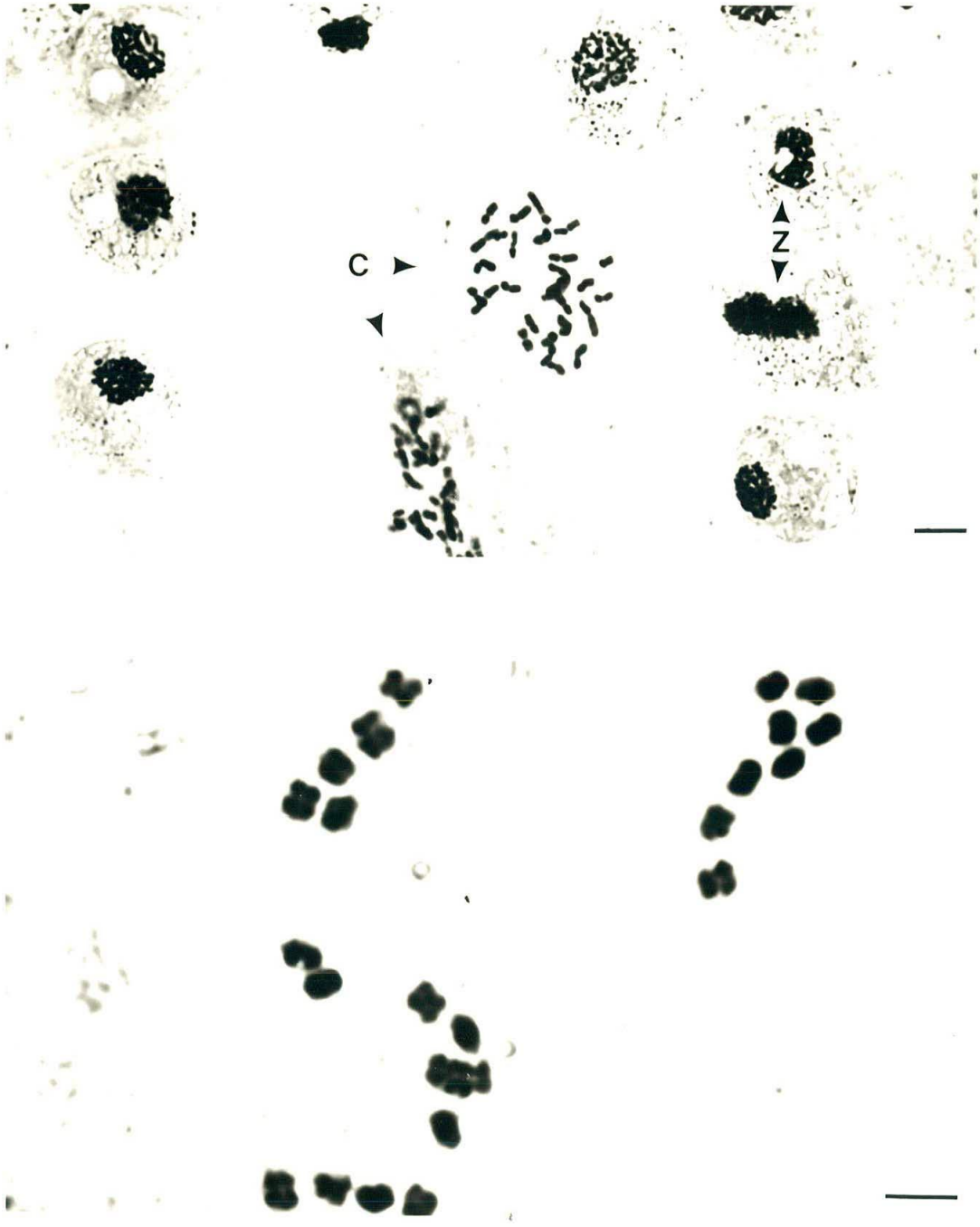

C
Z

Figure 5.3 *Acropora formosa*. A partial complement of chromosomes at late prophase. These elongate structures were typical of those used to compare the karyotypes of different species. Scale bar = 5 μm.

Figure 5.4 *Acropora valida*. Chromosomes exhibiting C-bands (B). The dark staining bands, which are due to the presence of heterochromatin, are usually located immediately either side of the centromere. One chromosome appears to have a large block of heterochromatin at one end (E). Scale bar = 5 μm.

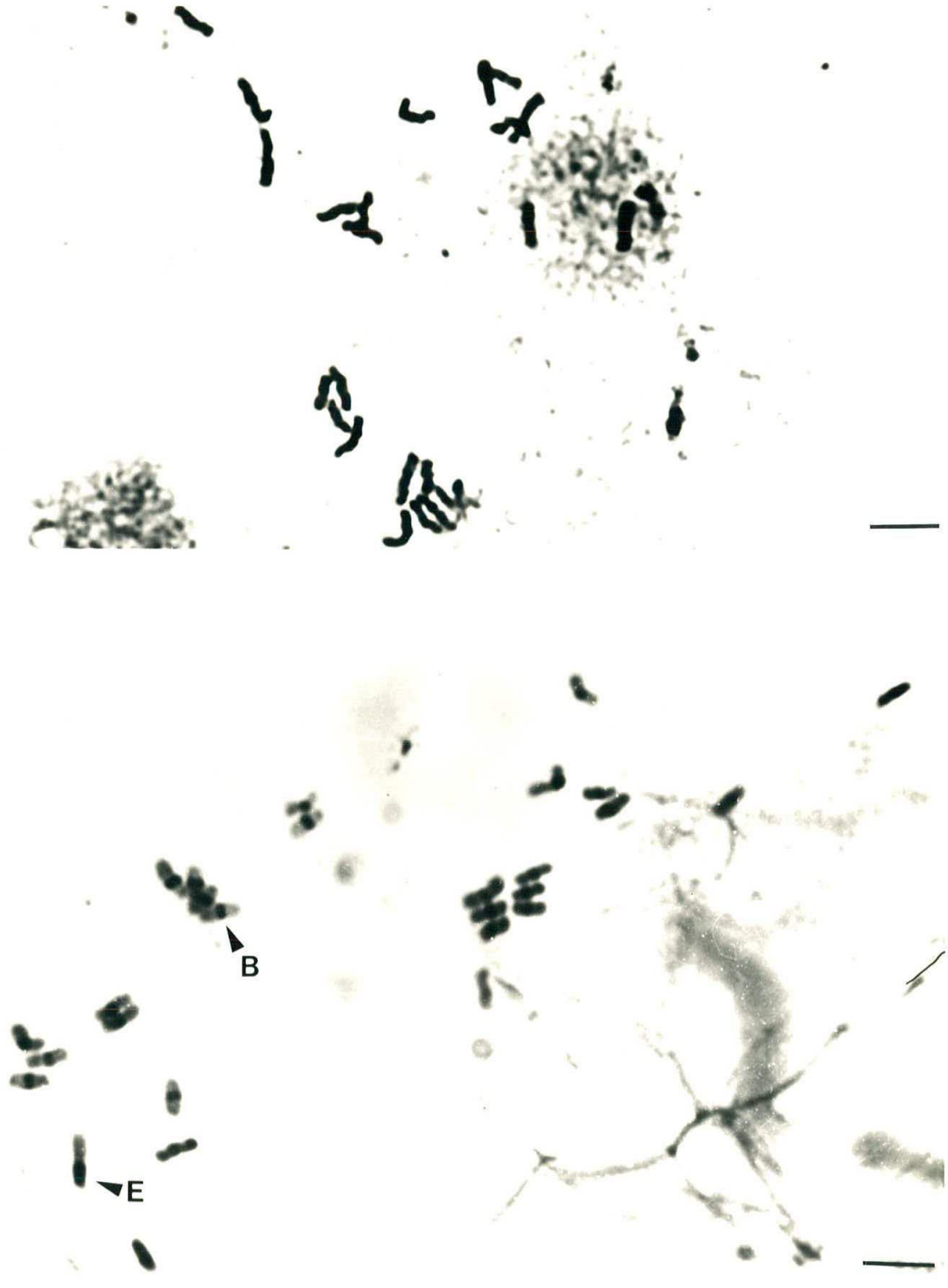
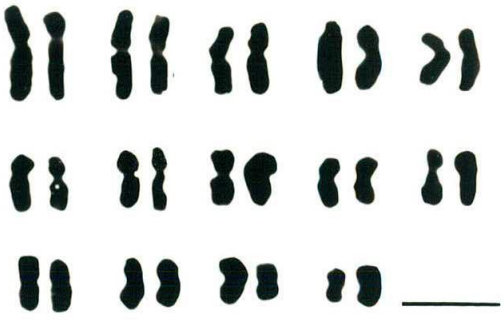
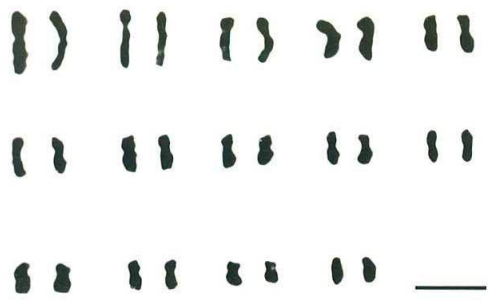

B
E

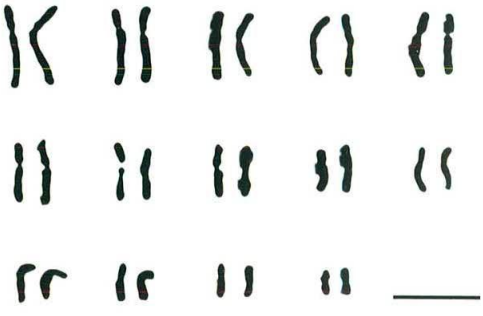
Figure 5.5 *Montipora dilatata*, *Montipora digitata*, *Goniopora lobata* and *Lobophyllia hemprichii*. Typical paired arrangements, based on chromosome length, of the karyotypes for each species. Scale bars equal 5μm.



Montipora dilatata

Montipora digitata





Goniopora lobata

Lobophyllia hemprichii

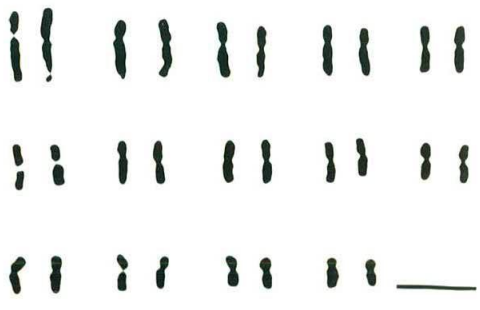


Figure 5.6 *Lobophyllia hemprichii, Montipora dilatata, Montipora digitata* and *Goniopora lobata*. Idiograms of one set of chromosomes based on the data from Table 5.1. Despite the general similarities in the overall complements, there are differences in internal position of the centromere. Note also the location of a secondary constriction on chromosome 5 in *G. lobata*.

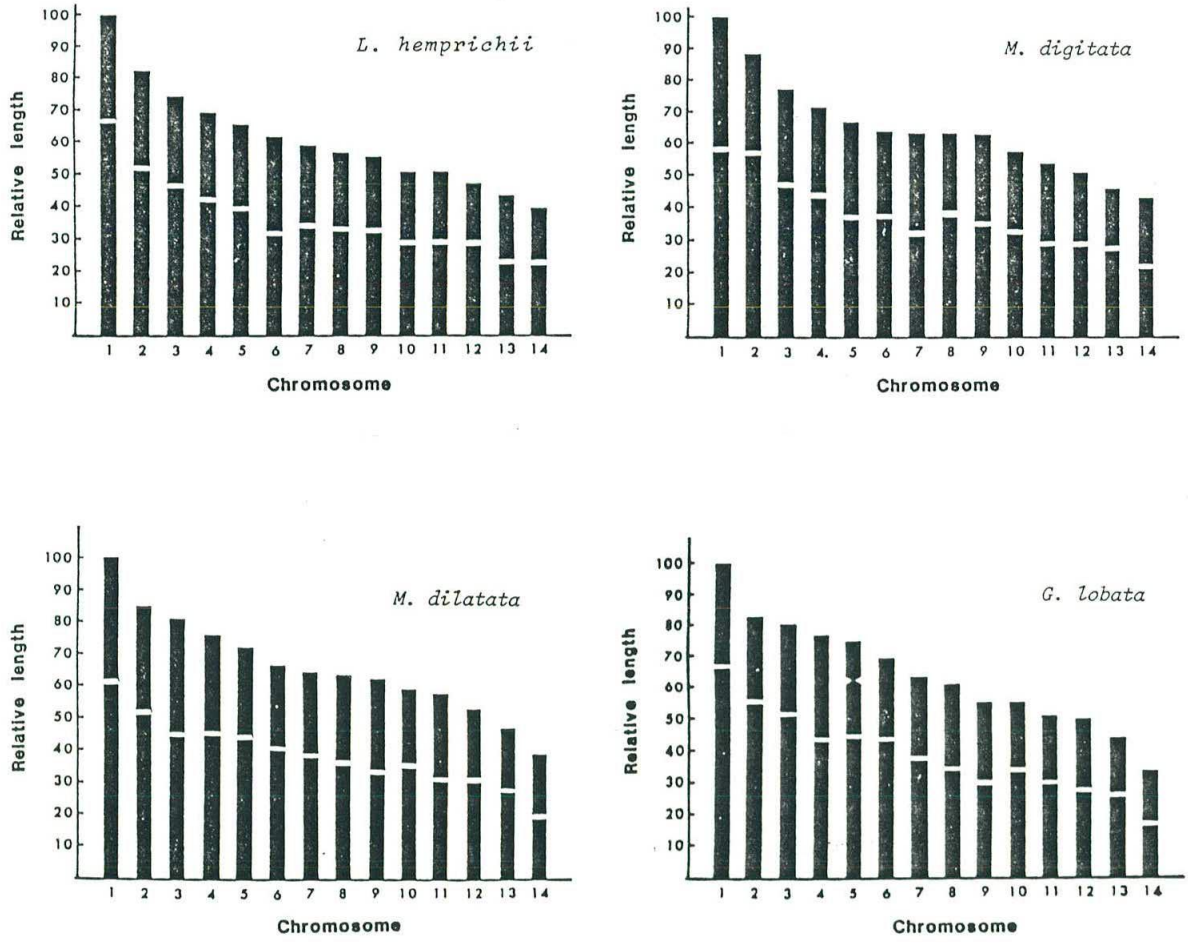


Figure 5.7 *Montipora digitata.* A complement as it was seen in the cell and the subsequent karyotypic arrangement by length. This micrograph was taken from a cell monolayer preparation. The chromosomes had been treated for C-banding but were not left in the barium hydroxide for long enough. The result is staining very similar to straightforward Giemsa staining. Additional very small structures (?) appear in this plate. This material may be hitherto unobserved microchromosomes. Scale bar = 5μm.

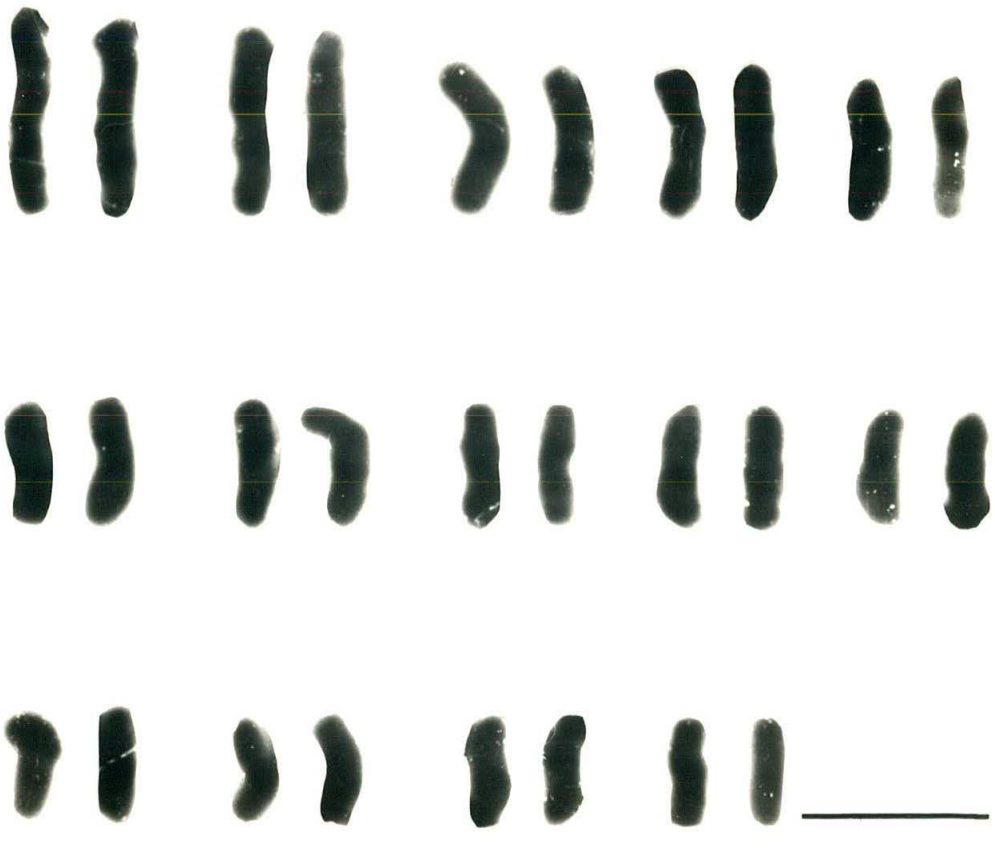


Table 5.1 *Lobophyllia hemprichii*, *Montipora dilatata*, *Montipora digitata* and *Goniopora lobata*. Relative lengths and centromeric indices of the fourteen chromosome pairs; means and standard deviations based on five plates.

(a) Lobophyllia hemprichii

Chromosome	Relative length	Centromeric index
1	100.0 ± 6.6	33.5 ± 3.5
2	82.6 ± 4.1	37.2 ± 4.3
3	74.6 ± 3.9	38.4 ± 5.5
4	69.4 ± 6.5	39.1 ± 3.4
5	65.6 ± 3.6	40.5 ± 3.5
6	60.8 ± 3.5	48.1 ± 2.5
7	58.1 ± 4.8	41.1 ± 3.6
8	56.6 ± 3.3	42.3 ± 2.7
9	54.7 ± 3.6	41.0 ± 4.7
10	50.6 ± 2.7	43.4 ± 1.2
11	50.6 ± 2.9	43.7 ± 1.2
12	47.0 ± 0.8	39.7 ± 5.3
13	43.4 ± 3.0	47.8 ± 3.7
14	39.2 ± 2.1	40.5 ± 4.7

(b) Montipora dilatata

Chromosome	Relative length	Centromeric index
1	100.0 ± 3.5	38.6 ± 3.6
2	84.7 ± 5.6	38.2 ± 6.6
3	80.9 ± 4.9	43.6 ± 1.5
4	75.9 ± 3.3	40.5 ± 2.3
5	72.2 ± 4.4	38.8 ± 1.6
6	66.1 ± 3.8	38.0 ± 1.2
7	64.6 ± 2.6	38.3 ± 1.9
8	63.1 ± 1.2	42.5 ± 1.6
9	62.1 ± 5.7	45.2 ± 0.6
10	59.3 ± 2.8	39.6 ± 2.0
11	57.6 ± 3.5	45.1 ± 1.2
12	52.8 ± 1.6	41.6 ± 2.6
13	47.3 ± 2.9	40.9 ± 2.1
14	39.4 ± 1.9	50.0 ± 0.5

(c) Montipora digitata

Chromosome	Relative length	Centromeric index
1	100.0 ± 1.9	42.1 ± 2.8
2	88.2 ± 3.4	36.0 ± 3.7
3	77.6 ± 10.1	39.7 ± 4.6
4	71.4 ± 4.9	38.7 ± 3.8
5	66.7 ± 8.1	44.8 ± 5.0
6	63.8 ± 10.1	42.4 ± 3.4
7	63.4 ± 10.3	49.5 ± 0.9
8	63.0 ± 7.8	40.5 ± 2.5
9	62.3 ± 1.9	42.8 ± 1.4
10	56.8 ± 9.9	43.5 ± 1.0
11	53.8 ± 8.5	46.1 ± 3.8
12	50.1 ± 6.4	42.2 ± 1.9
13	46.3 ± 6.1	39.6 ± 0.4
14	42.9 ± 8.3	48.9 ± 1.2

(d) Goniopora lobata

Chromosome	Relative length	Centromeric index
1	100.0 ± 4.8	32.5 ± 1.3
2	83.6 ± 1.5	33.2 ± 2.2
3	80.2 ± 5.1	36.1 ± 4.6
4	76.9 ± 3.6	43.4 ± 3.8
5	75.1 ± 4.6	40.3 ± 3.6
6	69.3 ± 3.1	37.3 ± 3.0
7	63.0 ± 2.0	40.9 ± 2.7
8	60.2 ± 4.7	42.9 ± 2.6
9	54.4 ± 3.4	45.0 ± 5.2
10	53.7 ± 5.5	37.3 ± 3.6
11	49.8 ± 2.2	41.7 ± 2.5
12	49.3 ± 3.7	45.6 ± 3.5
13	43.1 ± 4.7	41.8 ± 1.8
14	32.8 ± 3.5	50.0 ± 2.7

DISCUSSION.

These are the first quantitative data on coral chromosomes (Heyward, 1985a, b). The four karyotypes presented in detail (Table 5.1) and the additional species, together represent four taxonomic families of the Scleractinia. Conservatism of the karyotype was the major discovery. All species appeared to be diploid, with a typical chromosome number of 28 (n=14). In other recent work on cnidarian chromosomes, counts of 2n=30 for *Hydra* (Rahat *et al.*, 1985) and 2n=30 and 34 for two species of abyssal actinia (Van-Praet and Colombera, 1985) are of the same order of magnitude as for the corals in this study. In these and the present study, variations in chromosomal counts have been attributed to observational error and artifactual loss during tissue processing. The considerable uniformity, particularly with respect to the relative lengths of chromosomes within the complement, suggests that large quantitative changes in DNA have not been associated with speciation. Similarly, fusion or fission rearrangements were not detected. The additional chromatin seen in two complements of *Montipora digitata* and a complement of n=15 noted for one *Acropora formosa* cell may be the result of chromosome fission. Too few such plates, however, were obtained for karyometric analysis.

Intra-chromosomal changes, such as differing centromere positions, were the most significant differences found between species. The most likely chromosomal alterations which would change the position of the centromeres without otherwise altering the complement would be unequal reciprocal translocations or pericentric inversions (John, 1976). The

difficulty in exactly pairing all homologous chromosome pairs from the squash preparations does, nevertheless, introduce extra variability into the karyotypes. It was hoped, after the intra-chromosomal variations were found, that C-banding would allow precise homologue matching and shed light on the internal chromosome structures involved in the rearrangements. Unfortunately, large scale application of banding techniques to coral chromosomes proved elusive. There is no doubt, however, that such an analysis is feasible.

Wijsman and Wijsman-Best (1973) suggested that some coral species may have very large numbers of extremely small chromosomes. This hypothesis stemmed from preparations of *Cladocera cespitosa* in which enormous accumulations of stained dots were visible. In the light of the present study it is possible that such accumulations were composed of chromosomes which had become disassociated from their nuclei during tissue processing. On the other hand their approximations of 2n=28 for chromosome number in the dendrophyllid *Leptosammia pruvoti* and the caryophyllid *Caryophyllia smithi* var. *smithi* agree well with this study.

Twenty eight chromosomes between 1 and 5 μm long represent an encouraging karyotype for further study. As with chromosome studies of vertebrates, the role of chromosome changes in the evolution of the Scleractinia will become increasingly clear as advances are made in techniques for identifying homologous chromosome structures. Information on internal chromosome structure, using banding techniques, is required for the broadcast spawning species.

Other species may, in fact, display less stability in chromosome number than seen for these broadcast spawners.

Among the plants, Darlington (1963) has found a positive correlation between length of the reproductive cycle and stability of the chromosome numbers. Ford (1975, p4) points out that in short-lived plants the chromosome number is less stable and "is of importance below the taxonomic level of genus", while noting that stability in chromosome number "often transcends families in long-lived shrubs and trees". An analogous hypothesis could be proposed and tested for corals and interestingly,also among the Hydrozoa, where Makino (1951) has recorded considerable variation in chromosome numbers. Perhaps the greatest life history contrast among the Scleractinia occurs between species which brood larvae and those which broadcast gametes. Early maturing, 'weedy' brooders such as *Stylophora pistillata* (Loya, 1976) would be expected to have a higher probability of variation in chromosome number than many broadcast spawning species. Interestingly in Panama populations of the brooder *Pocillopora damicornis* , Richmond (pers. comm.) had difficulty in interpreting isoenzyme electrophoretic banding patterns using diploid models. The possibility that those populations are polyploids, which were established by the spread of asexual planulae (see Stoddart, 1983a) after arrival, needs further investigation. Analysis of brooding species will require techniques different to those employed in the present study. Tissue repair responses may elevate the mitotic index significantly to allow somatic tissue to be used successfully.

General Conclusions.

Genetic systems and hereditary structures.

This study brings to light differences in the genetic systems operating in populations of coral species which broadcast gametes. While the patterns of spawning and embryogenesis were similar, species differences were detected for karyotypes, mating systems and histocompatibility specificity.

On the basis of the self- and cross-fertilization studies (Chapter 3), it is tempting to suggest that many coral species utilize reproductive systems which combine means for fixing local adaptations (clonal proliferation and self-fertilization) and for generating large amounts of genetic variation (synchronous relase of gametes and external fertilization). *Montipora digitata* is an obligate outbreeder, yet is known to recruit via fragmentation, while *Goniastrea favulus*, although unlikely to clone extensively through fragmentation, can self-fertilize effectively. Wright (1940) has pointed out that such a strategy can be more effective than sexual or asexual systems alone. Harrigan 's (1972) observation that *Pocillopora damicornis* releases two types of planulae, some apparently dispersive while others settle rapidly, also fits this pattern in light of Stoddart's (1983) findings that locally recruited larvae are asexually produced. Among the broadcast spawning species, however, the probability of self-seeding in local populations seems much lower than for brooders. As a consequence, mechanisms such as self-fertilization, while enhancing reproductive fitness, are less likely to influence the genetic structure of local

populations than fragmentation.

Further study of mating systems will contribute greatly to our knowledge of the nature of genetic differences between species. The facultative self-fertilization of *G. favulus* and the obligate out-crossing of *M. digitata* are examples of recognition systems with different specificities. *M. digitata* eggs must have a mechanism for recognising and excluding sibling sperm, while recent electrophoretic evidence (Stoddart et al, unpublished data) suggests that self- and cross-fertilization in *G. favulus* are random. The study of adult recognition systems (Chapter 1) suggested that that specificity of histocompatibility responses, which varied with species, was a product of life history characteristics. It would be particularly interesting to test levels of self-fertilization within a group of corals which had the same mode of spawning, but different degrees of success with fragmentation eg. various *Acropora* species (see Wallace, 1985).

An additional aspect of genetic recognition, namely hybridization, needs particular attention now that controlled breeding trials are feasible. In some locations there is premating reproductive isolation due to temporal differences in spawning. Nevertheless, the failure to demonstrate successful hybridization between phenetically close, congeneric species which spawn synchronously (Harrison and Oliver, personal communication; author, unpublished data) implies either a fertility block or hybrid embryo inviability. One would expect considerable selective pressure for such an isolating mechanism in situations such as the

multi-specific coral spawning on the GBR.

It was hoped that the cytogenetic investigations would shed light on some patterns of evolution in the Scleractinia. The detected variability was small, but with refined banding techniques, cytogenetic studies have considerable promise. Among the plants, studies of speciation and evolution place emphasis on both hybridization and polyploidy (Jameson, 1977), neither of which appear to have major significance in corals which broadcast gametes. It would seem that while many analogies can be drawn between plant and coral demography, processes such as polyploidy and hybridization follow patterns more common to animals.

These observations come at a time when broadcasting of gametes as a mode a sexual reproduction has recently been recorded in coral communities around the globe. In Australia, using gametes from the mass spawning event on the GBR, DNA hybridization using coral sperm (McMillan and author, unpublished data), coral cell culture from embryonic tissue (Hutchinson and author, unpublished data) and electrophoretic analysis of inheritance in controlled breeding trials (Stoddart et al, unpublished data) have all proved successful. Clearly, these predictable spawning events, which provide access to gametes, will facilitate a wide range of future genetic studies.

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Appendix 1

Recipes for solutions used in C-band staining of chromosomes.

Sodium citrate solution

Sodium citrate - 22.0 g

Sodium chloride - 43.8 g

Make up to 2.5 l, adjust to pH 7.3 with conc. HCl.

Phosphate buffer

KH_2PO_4 - 2.75 g

Na_2HPO_4 - 2.00 g

pH - 6.8

Barium hydroxide

Use a 1 l glass-stoppered bottle. Fill 3/4 full with water, then pour in the barium hydroxide until it forms a layer 2 cm thick on the bottom. Top up bottle with water and put in a magnetic stirrer. Heat and stir on a hotplate until all the crystals dissolve. Let cool and sit overnight.

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