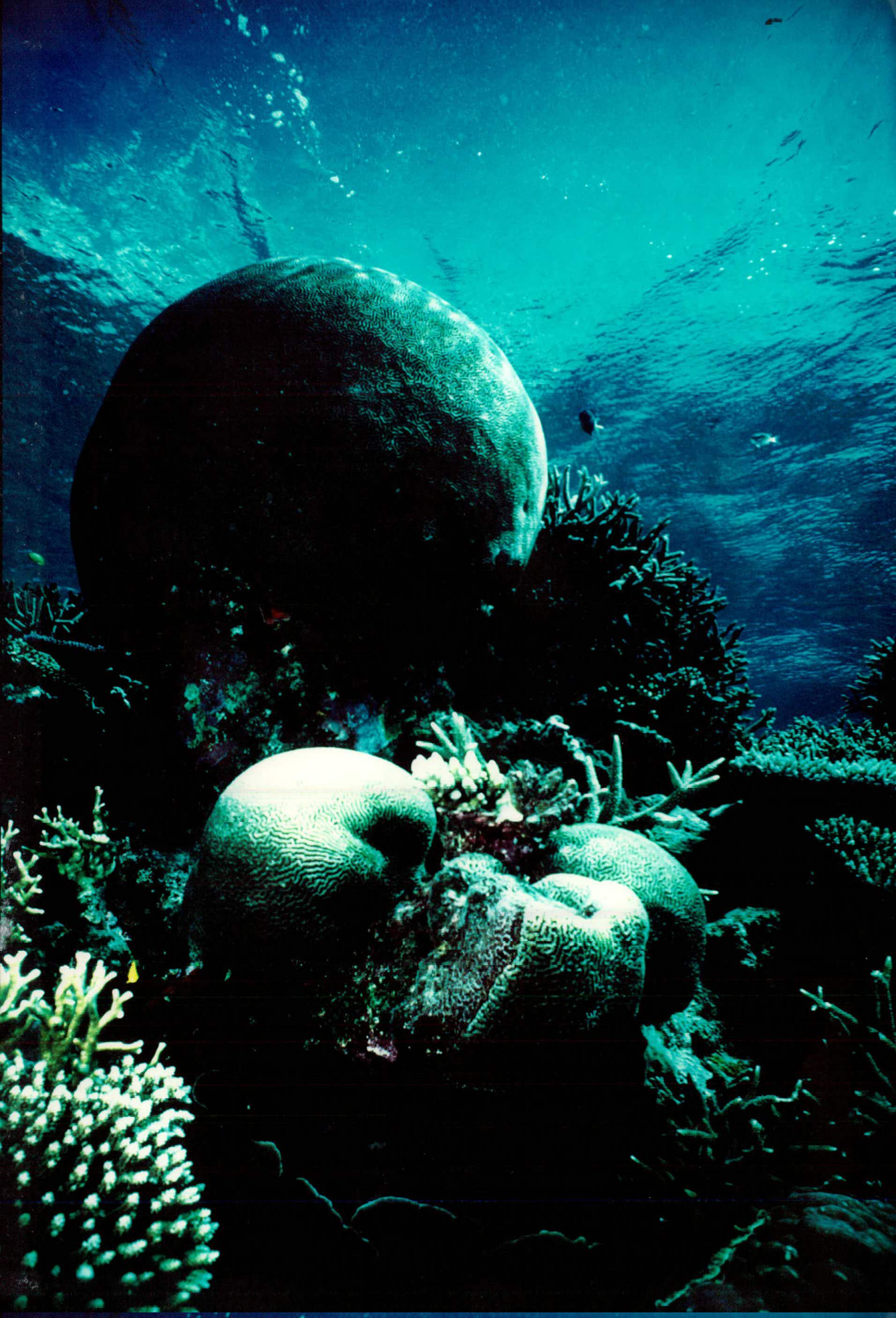

THE *PLATYGYRA* SPECIES COMPLEX: IMPLICATIONS FOR CORAL TAXONOMY AND EVOLUTION

Thesis submitted by
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for the degree of Doctor of Philosophy in
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O sweet spontaneous
earth how often
has the naughty thumb
of science prodded
thy
beauty
thou answereth
them only with
spring

e.e. cummings

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MARCH 1994

ABSTRACT

The majority of species boundaries are based on morphology, under the assumption that morphological discontinuities equate with reproductive incompatibility. In scleractinian corals, morphological boundaries between some species are indistinct, particularly where skeletal characters overlap to form a morphologic continuum between species. It has recently been discovered that at least some morphological species of coral are capable of interbreeding *in vitro* and it may be that events such as hybridisation could explain the high degrees of morphological variation recorded in many coral species and genera. In order to better understand the nature of species relationships and evolutionary processes within the Scleractinia, a detailed analysis of species boundaries within a single, representative genus - *Platygyra* - was carried out.

A multivariate morphometric analysis based on nine skeletal characters indicated seven morphologically distinct species (including two undescribed species) were present within the genus *Platygyra* on the Great Barrier Reef. However, no diagnostic species characters were found. The range of skeletal variation within a morphological species was also examined and was found to be high, with discrete morphotypes occurring within the species *Platygyra daedalea*. Intra-specific morphological variation in corals is often attributed to environmental influences whereby colonies of the same species will display different morphologies in different environments. A transect survey of all species and morphs of *Platygyra* on Davies Reef, central Great Barrier Reef, indicated that all morphs and species occurred sympatrically in six different reef habitats. Similarly, a broad scale survey of *Platygyra* colonies on reefs around Australia showed all species and morphs were widespread and common across their range. The sympatric distribution of *Platygyra* species suggest morphological variation in these corals is not a result of environmental influences at either a between-reef or within-reef scale.

Fertilisation trials between morphological species of *Platygyra* showed all seven species are capable of hybridisation *in vitro*. Hybridisation rates were equivalent to rates of within-species fertilisation (50-60%) and hybrid corals developed normally, surviving at least 3½ years. Fertilisation data suggest that morphological species of *Platygyra* are not reproductively isolated. This is further supported by the absence of temporal barriers to hybridisation between species and preliminary evidence which suggests there is no

species-specific sperm attraction acting to reproductively isolate species.

Studies using allozyme electrophoresis at nine polymorphic loci confirmed the findings from fertilisation trials and suggested hybridisation occurs naturally between species of *Platygyra* on the reef. There were no fixed gene differences between morphological species and values of Nei's D between species were low (< 0.2), which is within the range usually associated with intra-specific variation. Deviations from Hardy-Weinberg equilibria show there is some degree of non-random mating within species of *Platygyra*, however this is most likely to be associated with aspects of their reproductive biology rather than species-specific differences. Histocompatibility tests between morphological species of *Platygyra* suggested there was no relationship between morphology and interaction outcome, nor was there any association between genetic difference between colonies and interactive response.

By combining results from morphometric, ecological, reproductive and genetic studies of *Platygyra*, I conclude that hybridisation occurs naturally between the seven morphological species of *Platygyra*. Natural hybridisation will be an important consideration when defining species boundaries and evolutionary processes in corals. By drawing analogies between corals and plants, I propose that processes such as reticulate evolution and introgression, as have been described in plants, will also be taking place in the Scleractinia. In light of these findings, traditional species concepts which assume reproductive isolation between species will not be appropriate to define coral species. In the future it will be important to realise that coral taxonomy, which defines morphologically distinct species, may not necessarily reflect breeding relationships or have any bearing on evolutionary processes.

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

MARCH 1994

CHAPTER 1

GENERAL INTRODUCTION

1.1 Species boundaries in scleractinian corals

To date, most taxonomy in the scleractinia has been based on morphology, and on skeletal characters in particular (ie. Vaughan & Wells, 1943; Chevalier, 1975; Veron *et al.*, 1977). However, corals exhibit high degrees of morphological variation and this variability has confounded taxonomy. Morphological variants can form a continuum between species and the transition from intraspecific variation to interspecific difference is often unclear (Brakel, 1977; Wijsman-Best, 1977, Wallace, 1978). Traditional taxonomic techniques rely strongly on diagnostic morphological characters for the construction of taxonomic keys. Skeletal characters in scleractinian corals are highly variable and the presence or absence of such characters may be insufficient criteria with which to group species. Presence of a feature may differ from coral to coral within a species, even from polyp to polyp within a colony, and is often highly inconsistent between species (Veron *et al.*, 1977; Veron, 1981; Lang, 1984).

As a consequence of high degrees of morphological variation, scleractinian species have been continually merged, split, re-synonymised and discarded due largely to a lack of understanding of the variability of the organisms themselves. Veron and his co-workers, in their monograph series (Veron and Pichon, 1976; Veron et al., 1977; Veron & Pichon, 1980; Veron & Pichon, 1982; Veron & Wallace, 1984), were able to allay much of the confusion associated with coral taxonomy by taking this natural variability into account when defining species boundaries. However, while it is now widely accepted that a single coral species may display a wide variety of morphotypes, species boundaries in some groups remain unclear. Faviid corals in particular are a highly variable taxonomic group in which morphological characters of different species intergrade (Veron *et al.*, 1977; Wijsman-Best, 1974). The designation of species within such variable groups

becomes largely subjective with individual interpretations differing markedly (eg Chevalier, 1975; Wijsman-Best, 1976). Alternative methods of classifying coral species have been variously employed, including histocompatibility, biochemical techniques and internal structures, although no single technique has proven universally applicable (Lang, 1984).

1.2 The origin of morphological variation in corals

Morphological variation in coral species is often associated with environmental heterogeneity. Veron and Pichon (1976) introduced the term "ecomorph" to describe intraspecific environmental variants, the concept of which is similar in theory to the clines (Huxley, 1938) and ecotypes (Turesson, 1922) used in the botanical literature. Thus, a coral species may display different morphologies in different environments and each of these environmental variants is an ecomorph. Pocilloporids are a typical example of this phenomenon. Colonies of *Pocillopora damicornis* can vary from stunted with thick branches in shallow exposed regions to colonies with thin elongate branches in turbid or protected environments (Veron and Pichon, 1976). Similar correlations between morphology and environment have been described in many other coral species (eg. Barnes, 1973; Wijsman-Best, 1974; Graus and Macintyre, 1982) and transplant experiments have been used to demonstrate the effects of environment on skeletal characters of corals (eg. Foster, 1979a; Oliver *et al.*, 1983; Willis, 1985).

Morphological variation may be genetically controlled in some coral species (eg. *Pavona cactus*; Willis, 1985). It has recently been hypothesised that morphological variation may reflect the presence of sibling-species which are adapted to different environments, rather than intra-specific variation or ecomorphs (Knowlton *et al.*, 1992; Knowlton, 1993; Knowlton and Jackson, 1994). Historical events (on a geological time-scale) are also likely to be an important influence in structuring morphological variation in corals. Potts (1983, 1984) suggested corals in the Indo-Pacific had not undergone complete speciation during the Pleistocene

due to repeated sea-level fluctuations which were occurring at a faster rate than speciation processes. As a result, coral species boundaries and hence morphology, were not stable. Veron (in press) suggested morphological variation in corals was due, in part, to processes of reticulate evolution. In a reticulate system, evolution involves a network of diverging and converging species via differing processes of speciation and hybridisation. However, evolutionary disequilibrium and reticulate evolution are not mutually exclusive and, if they occur within the Scleractinia, it is possible that both processes may be operating simultaneously. The evidence for either is meagre in corals.

Natural hybridisation will be an important process in reticulate evolution, particularly in the merging of species. Hybridisation may also result from the process of divergent evolution where speciation processes are incomplete and species have not attained complete reproductive isolation ie. semispecies (Grant, 1981). While an early study suggested species of coral were reproductively isolated (Hodgson, 1988), recent studies have demonstrated that some species of coral are capable of hybridising *in vitro* (Willis *et al.*, 1992). Reproductive trials *in vitro* may not, of course, be representative of reproductive relationships on the reef. Genetic surveys will be important to determine whether gene flow occurs between species within a reef system. Ayre *et al.* (1991) confirmed the morphological species *Acropora cuneata* and *A. palifera* were genetically distinct and were reproductively isolated. However, within the genus *Porites*, morphological groupings do not equate with genetic groupings and the presence of cryptic species of *Porites* has been suggested (Potts and Garthwaite, 1986; Potts *et al.*, 1992). Similarly, within the genus *Acropora*, genetic groupings do not correspond with morphological species groupings (McMillan *et al.*, 1991). These latter studies suggest relationships between many morphological species of coral will not be straight-forward and discrepancies between traditional and molecular taxonomies may reflect incomplete reproductive isolation between species.

If hybridisation does occur on the reef, resultant hybrid corals are likely to be

intermediate in morphology between parental species. The presence of intermediate hybrid corals may explain the high degrees of morphological variation within species. Similarly, large amounts of hybridisation and backcrossing with parental populations may account for perceived morphological continuums within genera where morphological characters overlap and species intergrade.

The high morphological variation within coral species coupled with the potential for hybridisation between species suggests species relationships may be more complex within the Scleractinia than previously thought. The previous assumption that morphological species boundaries will equate with reproductive isolation and subsequently evolutionary units may not necessarily be justified and traditional species theory (the Biological Species Concept) may not be the most appropriate for scleractinian corals.

1.3 Parallels between corals and plants

The inapplicability of widely accepted species and evolutionary concepts to plants has been recognised for the last 20-30 years, consequently, traditional assumptions of reproductive isolation between species (Mayr, 1942) and of hierarchical evolution and speciation (Darwin, 1859) have been questioned by botanists (Grant, 1981). Many parallels exist between plants and corals in terms of their life history strategies (eg. Hughes, 1984; Hughes and Connell, 1987) reproductive modes and patterns (Harrison and Wallace, 1990) and their ability to alter morphology in response to the environment (eg. Brakel, 1977; Foster, 1979a). Hence, in many respects, corals are more plant-like than animal-like and it may be that evolutionary processes described in plants will be relevant when explaining species relationships in corals.

Morphological species of plants are capable of interbreeding and hybridisation may be common between some plant species (eg. Burger, 1975; Levin, 1979; Potts

and Reid, 1985; Mosseler, 1989). The absence of reproductive isolation in many plant groups has led botanists to discard the Biological Species Concept (BSC) in favour of the more flexible Evolutionary Species Concept (ESC) which incorporates processes of hybridisation and reticulate evolution. Processes such as reticulate evolution, natural hybridisation and introgression have adequately accounted for patterns of morphological variation and genetic relationships between plants (Arnold *et al.*, 1990; Whittemore and Schaal, 1991; Cruzan *et al.*, 1993). Similar processes may also occur within the Scleractinia. Therefore, it will be important to study species relationships in detail to fully understand the basis of morphological variability and genetic relationships in corals.

1.4 The Biological Species Concept in scleractinian corals

Crucial to the understanding of speciation and evolution within the Scleractinia is the species definition. The Biological Species Concept (BSC) is the most widely recognised species definition. It was originally conceptualised by Dobzhansky (1937) and later defined by Mayr (1942) where "a species is a group of actually or potentially interbreeding populations which is reproductively isolated from other such groups". However, the majority of species defined both within the Scleractinia and across all phyla are largely based on morphological differences, with the assumption that morphological dissimilarities are highly correlated with reproductive compatibility. Recently, it has become clear in many taxa that morphological species do not conform to the biological definition of species and hence the assumption that morphological change will always be congruent with speciation (considering speciation as a genetic/reproductive change) is clearly wrong (Larson, 1989). These inconsistencies include species which are capable of hybridising and producing fertile offspring eg. the white oaks - *Quercus* spp. (Burger, 1975), *Eucalyptus* spp. (Potts and Reid, 1988) and species which consist of more than one reproductively isolated group within a single morphological entity (sibling-species eg. Mayr, 1963).

The BSC has also been widely criticised for a number of other reasons. It is considered non-operational as reproductive isolation cannot be completely tested and subsequently, classifications will remain arbitrary (Dobzhansky, 1935; Sokal and Crovello, 1970). It is also inapplicable to asexual taxa (Cracraft, 1987). Theoretical downfalls of the BSC include being relational ie. expressed in terms of other species (Paterson, 1985) and that selection for reproductive isolation may be irrelevant in the process of speciation (Dobzhansky, 1935; Paterson, 1985; Templeton, 1989). The BSC is also considered inapplicable in situations where the biological species can be reduced to smaller units (eg. sub-species, semi-species) (Sokal and Crovello, 1970; Cracraft, 1989).

In contrast to the BSC, the Evolutionary Species Concept (ESC), which has be largely adopted by botanists (eg. Grant, 1981), attempts to deal with processes of speciation, rather than just patterns of speciation (Endler, 1989). Thus an evolutionary species is "a lineage (an ancestor-descendent sequence of populations) evolving separately from others and with its own evolutionary role and tendencies" (Simpson, 1961). It has advantages over the BSC as it can be applied to both sexual and asexual species and incorporates different breeding systems such as those of cryptic species, semispecies and agamospecies. Identity of an evolutionary species rests not only on reproductive isolation but may be based on phenotypic, behavioural, biochemical or ecological differences (Wiley, 1981).

By defining corals on morphology, it has been assumed that morphological differences equate with reproductive isolation. Willis (1990) found no evidence to the contrary and suggested the BSC may well hold in corals. However, she indicated further studies were needed to confirm this. In light of the many parallels in the biology and ecology of corals and plants, particularly the evidence which suggests that patterns of hybridisation and asexual reproduction described in plants may also be present in corals, it seems important to question the applicability of the BSC in scleractinian corals. If the BSC is not the most

appropriate for describing species groups within the Scleractinia, it may be that the ESC, as used by botanists, is more suitable.

1.5 The coral genus *Platygyra*

The genus *Platygyra* is widespread throughout the Indo-Pacific and displays considerable morphological variation, a trait typical of the family Faviidae, of which it is a member. Colonies are generally massive with long, meandroid valleys, although some colonies may become plate-like. Valleys range from monocentric (*P. pini*) to continuous (*P. daedalea*) with lengths of 1 to 10's of centimetres. Colonies of all species are common on the Great Barrier Reef (Veron *et al.*, 1977) and *Platygyra* colonies form an important reef-building group of corals.

The biology of *Platygyra* species has been comprehensively studied on the Great Barrier Reef and much is known of their gametogenic cycles (Babcock, 1986) timing of reproduction (Babcock *et al.*, 1986), larval development (Babcock and Heyward, 1986), population structures and life history (Babcock, 1985; Babcock, 1991). Colonies are sessile, long lived and slow growing, and preliminary studies suggest that some morphological species of *Platygyra* are capable of interbreeding (Babcock, pers comm; Willis *et al.*, 1992). In many respects of both their biology and ecology, species of *Platygyra* closely parallel plants from terrestrial ecosystems and hence they are ideal models with which to investigate species relationships in corals.

1.6 The taxonomic status of *Platygyra*

There are currently five species of Platygyra identified from the Great Barrier Reef and at least ten recorded world-wide (Veron *et al.*, 1977; Veron, 1992). The previous taxonomy of these corals has been conflicting. The current genus *Platygyra* was originally part of the *Madrepora* (Ellis and Solander, 1786) and

later *Meandrina* (Ellis and Solander, 1786) then *Astroria* (Edwards and Haime, 1849) and *Coeloria* (Edwards and Haime, 1848). The genus *Platygyra* was originally described by Ehrenberg (1834), although it initially consisted of corals now included in the genus *Leptoria* (Edwards and Haime, 1848). Below is a brief summary of the species which have been described previously within the genus *Platygyra* (or its historical equivalents) and their status relevant to those species currently recorded from the Great Barrier Reef (as grouped by Veron *et al.*, 1977; Veron, 1992). A recent synonymy of *Platygyra* species is provided by Veron *et al.* (1977).

Current species from the Great Barrier Reef

Platygyra daedalea (Ellis and Solander, 1786)

Platygyra sinensis (Edwards and Haime, 1849)

Platygyra lamellina (Ehrenberg, 1834) - was synonymised with *P. daedalea* (Stephenson and Wells, 1955) reinstated by Wijsman-Best (1972) then re-synonymised by Chevalier (1975). Veron *et al.* (1977) considered the two species on the Great Barrier Reef to be distinct.

Platygyra pini Chevalier, 1975

Platygyra ryukyuensis Yabe and Sugiyama, 1935 - was synonymised with *P. sinensis* (Chevalier, 1975; Veron *et al.*, 1977) although reinstated from the Great Barrier Reef by Veron (1992)

Species from regions other than the Great Barrier Reef:

Platygyra exigua Nemenzo, 1959; recorded only from the Philippines (Nemenzo, 1959; 1981)

Platygyra verweyi Wijsman-Best, 1976; originally described from New Caledonia and Java sea. Also recorded from Western Australia, Japan and the Philippines (Veron 1992)

Platygyra contorta Veron, 1990; Recorded from Japan as far south as Papua New Guinea (Veron, 1992)

Platygyra crosslandi (Matthai, 1928); currently recorded only from the Red Sea (Vine, 1986) although was synonymised with *P. daedalea* by Chevalier (1975)

Platygyra yaeyamensis (Eguchi and Shirai, 1977); only found in Japan (Veron, 1992)

Previously identified species worldwide:

Platygyra delicatula (Ortmann, 1888); Chevalier, (1968)

Platygyra stricta (Edwards & Haime, 1857); Chevalier, (1968)

- both now synonymised with ***P. sinensis*** (see Veron *et al.*, 1977)

Coeloria bottai Edwards and Haime, 1849

Coeloria laticollis Edwards and Haime, 1849

Coeloria forskaliana Edwards and Haime, 1849

Coeloria leptoticha Klunzinger, 1879

Coeloria arabica Klunzinger, 1879

- all five synonymised with ***P. lamellina*** (see Veron *et al.*, 1977)

Platygyra astreiformis (Edwards and Haime, 1849); Ma, (1959)

Platygyra rustica (Dana, 1846); Stephenson and Wells (1955); Ma (1959)

Platygyra esperi (Edwards and Haime, 1849); Ma, (1959)

- all now synonymised with ***P. daedalea*** (see Veron *et al.*, 1977)

Platygyra gracilis (Edwards and Haime, 1857) Matthai, (1928)

Platygyra phrygia (Ellis and Solander); Matthai, (1928)

- both now synonymised with *Leptoria phrygia* (see Veron *et al.*, 1977)

Platygyra zelli Veron , Pichon and Wijsman-Best, 1977
- now *Australogyra zelli* (see Veron, 1986)

Coeloria gigantea Yabe and Sugiyama, 1935
- now *Oulophyllia crispa* (see Veron *et al.*, 1977)

Other species

The species listed below have previously been described but have no reference in current literature. However, Veron *et al.* (1977) suggested current synonymies may extend to other fossil and nominal species which is likely to include these species.

Platygyra pachychila / Leptoria pachychila (Ehrenberg)
Platygyra euaensis (Hoffmeister, 1925)
Platygyra mareensis Chevalier, 1968 (described only from fossil specimens)
Platygyra equisepta (Gregory)
Coeloria klunzingeri Matthai, 1928 (described from the Red Sea)
Platygyra planulata (Edwards and Haime, 1849) - perhaps synonymised with *Goniastrea pectinata* as *G. planulata* (see Wijsman-Best, 1972)

Fossil Records

The earliest evidence of the genus *Platygyra* in the fossil record is from the Eocene (Wells, 1964) although the species remains unidentified. Chevalier (1968) recorded fossil *Platygyra* from the Pleistocene at Mares Island (New Caledonia) including the species *P. daedalea*, *P. lamellina*, *P. stricta*, *P. equisepta*, *P. delicatula*, *P. planulata*, *P. cf. euaensis*. Similarly, Veron and Kelley (1988) found Pliocene fossil specimens of *P. sinensis* and *P. pini* in Papua New Guinea. A single species, *Platygyra mareensis* (Chevalier, 1968) has only been described from fossil specimens.

1.7 Aims

The aim of this thesis is to fully explore inter-relationships between morphological species of corals in the genus *Platygyra* from both a taxonomic and evolutionary perspective. Using the framework of Veron (1981) I intend to take the working taxonomic unit, ie. the morphological species, and explore inter- and intra-specific morphological variation using multivariate morphometrics. In addition, I use genetic techniques (allozyme electrophoresis) and reproductive trials to explore the basis of morphological differentiation, and the nature of relationships between species, in particular to 1) determine if morphological variants represent ecomorphs (sensu Veron and Pichon, 1976) pseudo sibling-species (taxa that are distinct when alive or considered in their entirety; *sensu* Knowlton, 1993) or hybrids (Willis *et al.*, 1992); 2) examine levels of speciation within the genus and explore the significance of concepts such as reticulate evolution (Veron, in press) and evolutionary disequilibrium (Potts, 1983; 1984) in *Platygyra* and 3) test the applicability of current species theory to morphological species of corals.

CHAPTER 2

MORPHOLOGICAL SPECIES WITHIN THE GENUS *PLATYGYRA*: A QUANTITATIVE ANALYSIS

2.1 Introduction

2.1.1. Quantitative taxonomy.

Species based on morphology are often considered subjective (Mayr, 1964) particularly in Scleractinians which display high degrees of morphological variation (eg. Faviids; Wijsman-Best, 1974; Veron *et al.*, 1977). Numeric methods such as cluster analysis, principle components analysis and canonical variate analysis, have been introduced in taxonomy in order to quantify differences between classes or groups and to reduce the subjectivity associated with species identification. Such techniques have been broadly used for a variety of organisms to address questions relating to growth forms (eg. Shea, 1985; Ray, 1990; Balla and Walter, 1991), geographic variation (eg. Blondel *et al.*, 1984) and taxonomic differences (eg. Lindberg, 1990; Weber & Galleguillos, 1991). Marcus (1990) provides a comprehensive summary of many of the traditional methods used in morphometrics including their applications and assumptions. More recently, methods for analysing shapes of organisms rather than size of particular characters have been introduced eg. Fourier shape analysis, eigenshape analysis (Rohlf, 1986) and the analysis of landmark data (Bookstein, 1990).

2.1.2. Quantitative taxonomy in corals

Numerical taxonomic methods have been applied to some groups of scleractinian corals in an attempt to remove subjectivity associated with the assignment of corals to genera and species (eg Potts *et al.*, 1992; Weil, 1992). Powers (1970) and Powers & Rohlf (1972) used cluster analysis to group corals from Hawaii and the Caribbean, and their numeric classifications closely paralleled those of the original classification

of Vaughan & Wells (1943). Similarly, quantitative analysis of *Acropora* skeletons (Wallace, 1974) also corresponded with traditional species groupings. The close correspondence between numerical species groups and traditional species groups in these two studies may not be surprising since both numerical and traditional taxonomic methods use morphological characteristics as grouping criteria. In contrast, numerical examination of morphological variation within the genus *Porites* using cluster analysis and principal components analysis (Brakel, 1976; 1977) showed variation to be continuous within the genus, rather than discontinuous with distinct species groups being formed. However, depending on the level of difference used as a species cut-off, species groups which overlapped to a large extent with original taxonomies could be isolated from the resultant clusters. Thus, numeric methods and traditional methods in taxonomy are not mutually exclusive and even quantitative studies will still contain certain levels of subjectivity likely to be driven by predetermined ideas of what constitutes a species.

Canonical discriminant analysis (or canonical variate analysis) [CDA] has been used as a classification method to determine differences between species of corals, particularly in comparisons of extinct and extant coral species (Foster, 1984; Budd, 1988), but also to examine variation within species across environmental gradients (Foster, 1979a; 1980), variation in species across geological time scales (Budd, 1989) and corallite variation within and between colonies of various species (Foster, 1977; 1983; 1985). Cairns (1989) used CDA as a method for confirming *a priori* species groups in fossil corals, an approach which has also been used in extant corals to quantitatively confirm taxonomic groupings in Acroporiids (Wallace *et al.*, 1991) and to aid in the delineation of sibling species of *Montastrea annularis* (Knowlton *et al.*, 1992).

Shape analysis has also been applied to questions of coral taxonomy. Pandolfi and Burke (1989) combined discriminant function analysis with fourier shape analysis to address taxonomic problems in the fossil coral genus *Pleurodictyum*. While such an approach may be useful, they caution against using shape analysis alone to distinguish fossil species as growth form may evolve in response to the environment

and may be parallel in both closely and distantly related species.

2.1.3. Morphological species in Platygyra

Current species definitions within the scleractinian genus *Platygyra* are qualitative, based only on skeletal descriptions which encompass a broad range of variation. Skeletal variation is high within *Platygyra* (Wijsman-Best, 1974; Chevalier, 1975; Veron *et al.*, 1977) and there is much apparent overlap in skeletal characteristics across species (see Stephenson & Wells, 1955). While 'typical' specimens of a species group are easily assigned, it is not always possible to allocate any one skeleton to a particular species. Similarly, it is often an arbitrary decision to determine at what point two groups are different enough to warrant specific status. A further problem confounding delineation of species within *Platygyra* is the potential of a species to alter its morphology in response to the environment. Habitat may have an important influence on the morphology of *Platygyra* colonies and Wijsman-Best (1974) suggested there was some correlation between habitat and morphology in *P. daedalea*. Similarly, Veron *et al.* (1977, page 98) state in reference to *Platygyra*... "specific differences may become obscure in large collections from a variety of biotopes, where the skeletal characters of one species overlap with those of another from a different biotope".

This aim of this chapter is to apply numerical taxonomic methods, in particular canonical variate analysis, to examine morphological variation in the genus *Platygyra*. In particular, quantitative results will be used to better define morphological species boundaries in the genus and to understand the range and nature of intra-specific variation. Spatial distribution patterns of morphological species of *Platygyra* will be documented and morphological variation examined relative to environmental gradients in Chapter 3.

2.2. Methods

2.2.1. Morphological groups based on field appearance

Following an extensive survey across a number of reefs on the Great Barrier Reef, eleven morphological groups of *Platygyra* were defined for this study, based on field appearances. Five of these groups corresponded with the existing species *Platygyra pini*, *P. sinensis*, *P. lamellina* and *P. daedalea* (as described by Veron *et al.*, 1977) and *P. ryukyuensis* (as described by Nishihira, 1988). Representative colonies of each of these species are shown in Plates 2.1 - 2.5. These groups will, for simplicity, be referred to as species. However, use of the word "species" throughout this thesis (unless otherwise stated) is not intended to imply their reproductive or genetic relationships, merely that they represent discrete morphological entities.

Seven different morphological variants of *P. daedalea* (hereafter referred to as morphs) were also distinguished by field appearance and grouped as follows:

1. Classic (PDC) - Has a massive, hemispherical growth form and long, meandroid valleys. Theca width is constant within the colony, corallite walls are steep sided forming U-shaped valleys in cross-section and septa are moderately exert. Colonies can be uniform in colour or with contrasting mouths and theca (ie. green mouths with brown theca). See Plate 2.5

2. Short-valley (PDSV) - Very similar to PDC with a massive, hemispherical growth form, however valleys are short to monocentric. Theca width is constant within the colony, corallite walls are steep sided forming U-shaped valleys in cross-section and septa are moderately exert. Colonies can be uniform in colour or with contrasting mouths and theca. See Plate 2.6.

3. Encrusting (PDSE) - Colonies are flattened and encrusting, often plate-like. Valleys generally run parallel to each other, perpendicular to the line of colony growth. Corallite walls are steep, the theca thin and septa highly exert. Colony colour is variable, either uniform or with contrasting mouths and theca. See Plate 2.7.

4. Fat (PDF) - Colonies are similar in appearance to the classic morph. However, the theca is wider and septa more rounded (but not as much as *P. lamellina*). Colonies can be uniform in colour or with contrasting mouths and theca. See Plate 2.8

5. Fat/intermediate (PDFI) - These colonies have thecae intermediate in width between the classic and fat morphs, have moderately exert septa and a massive growth form. Colouration is either uniform or with contrasting mouths and theca.

6. Hillocky (PH) - Colonies have a massive, hillocky growth form. Valleys are long and meandroid and are V-shaped. Theca thickness is variable within a colony, ranging from wide with rounded septa on convex surfaces to thin with highly exert septa on concave surfaces. Colonies are uniform in colour, either yellowish-brown or greyish-green. See Plate 2.9.

7. Miniature (PB) - A massive colony often with a hillocky growth form and neat regular appearance. Valleys are long and meandroid with polyps smaller in size than the other morphs. Septa are regularly spaced and meet above the theca forming a ridge. PB superficially resembles *Leptoria phrygia* in the field however PB septa are less rounded than those of *L. phrygia* and polyp mouths are often iridescent blue or green with valley walls being brown. Some colonies are uniform brown in colour. See Plate 2.10.

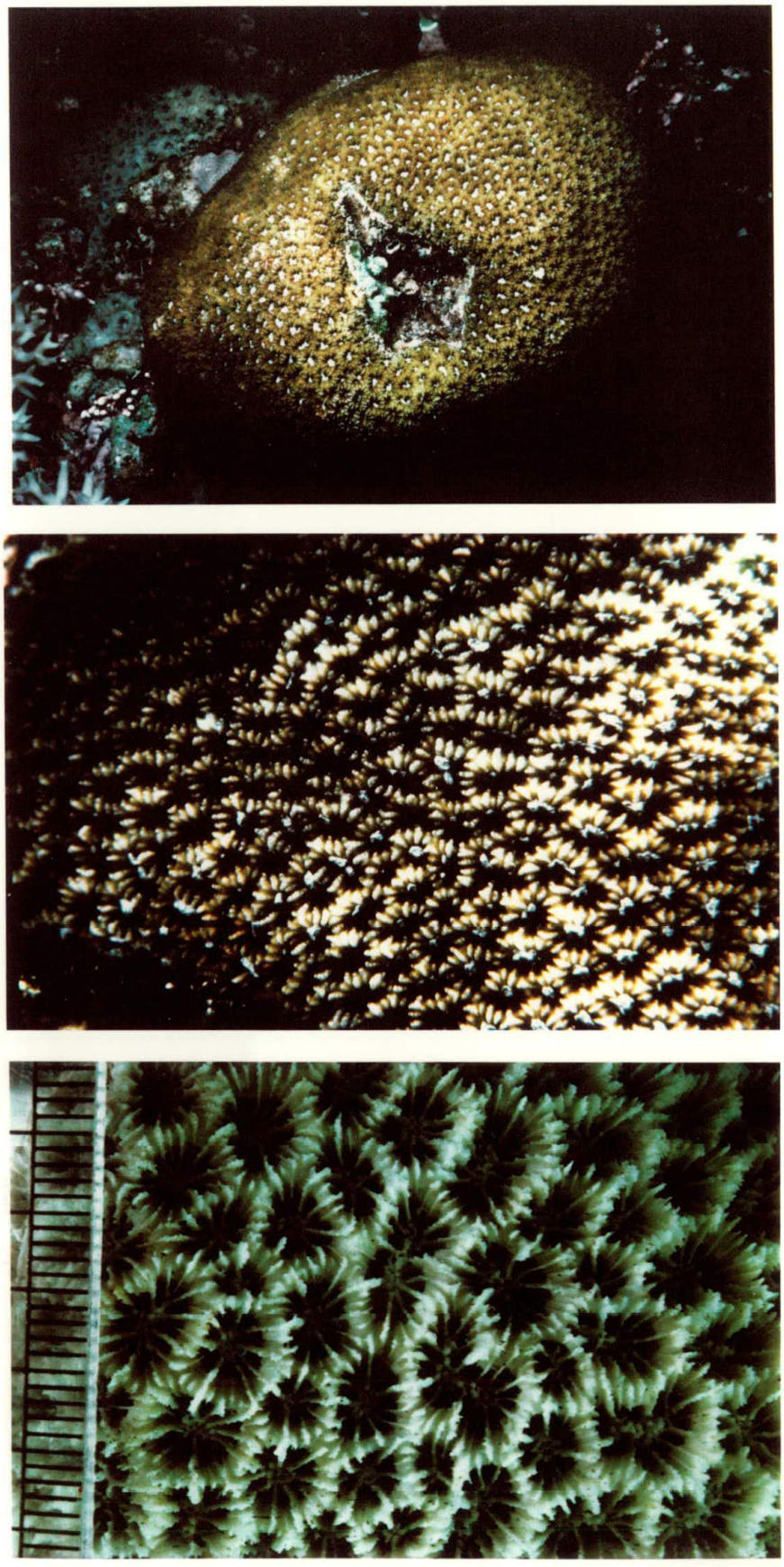
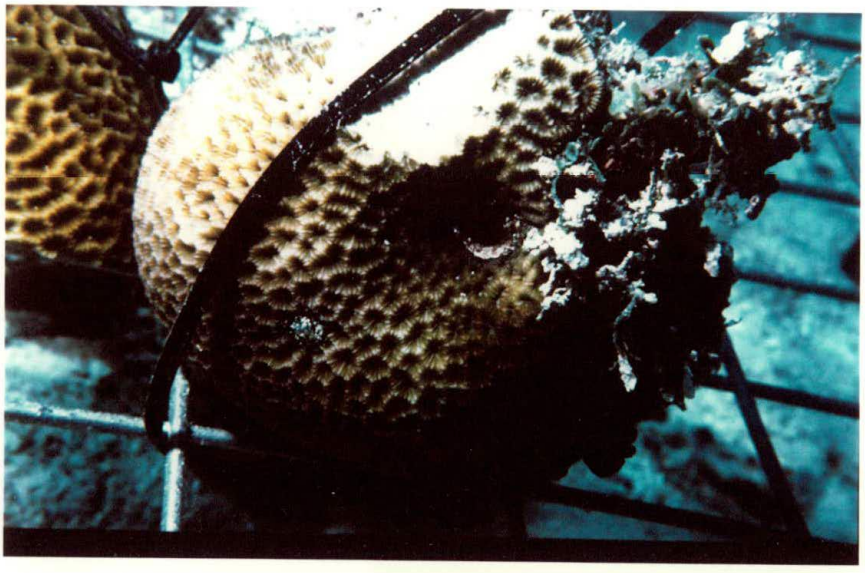


Plate 2.1. Photographs of live colonies and skeletal detail of *Platygyra pini*



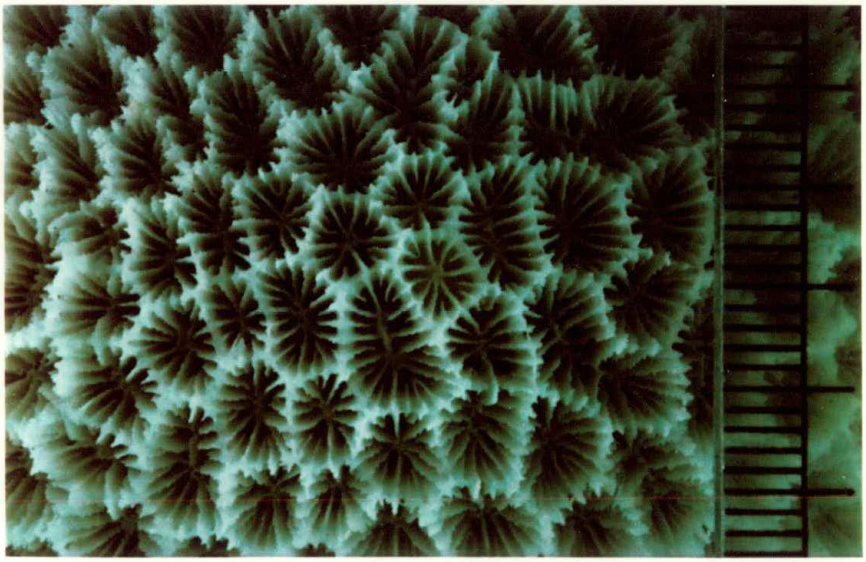


Plate 2.2. Photographs of a live colony and skeletal detail of *Platygyra ryukyuensis*.

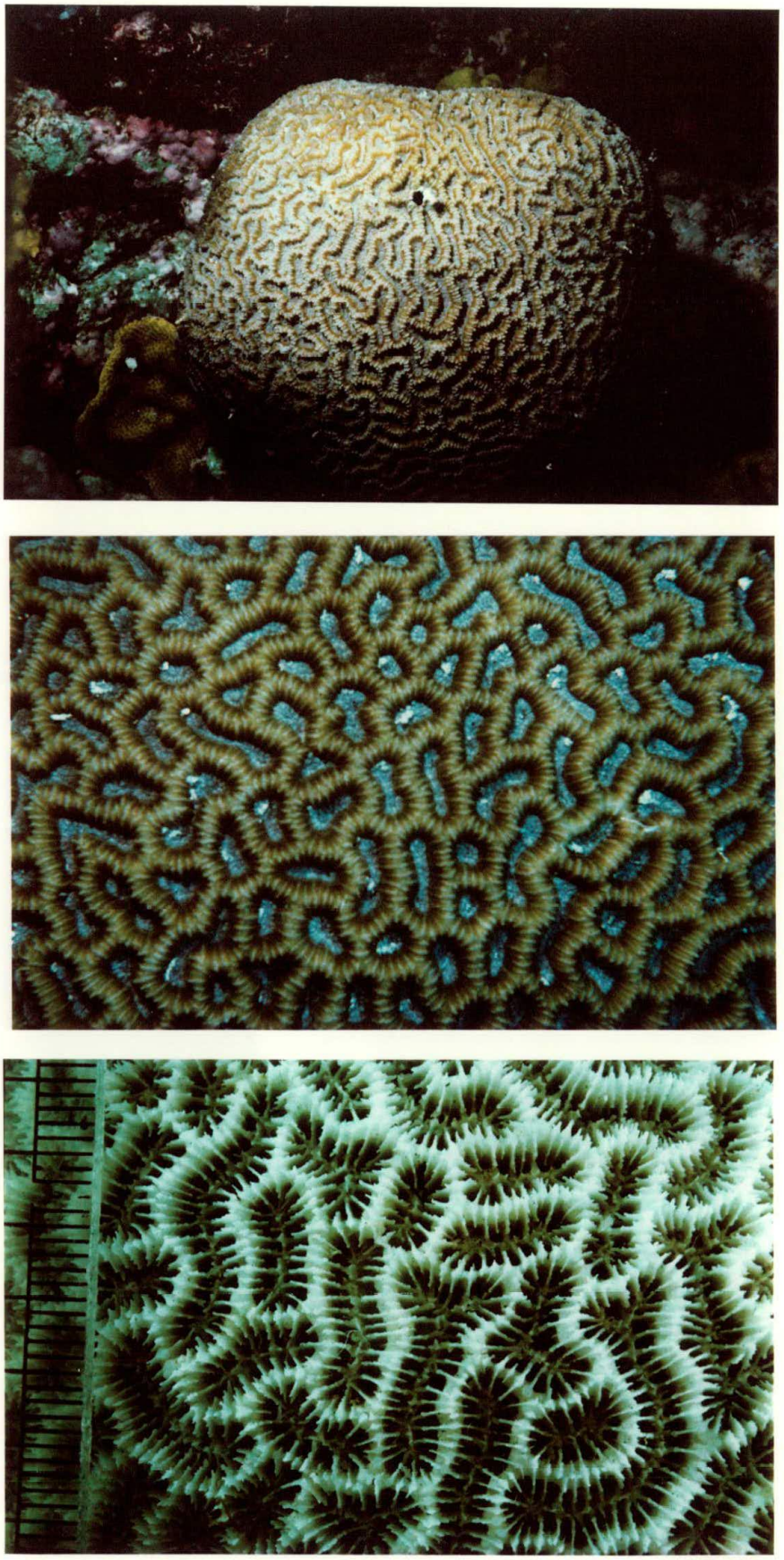


Plate 2.3. Photographs of live colonies and skeletal detail of *Platygyra sinensis*

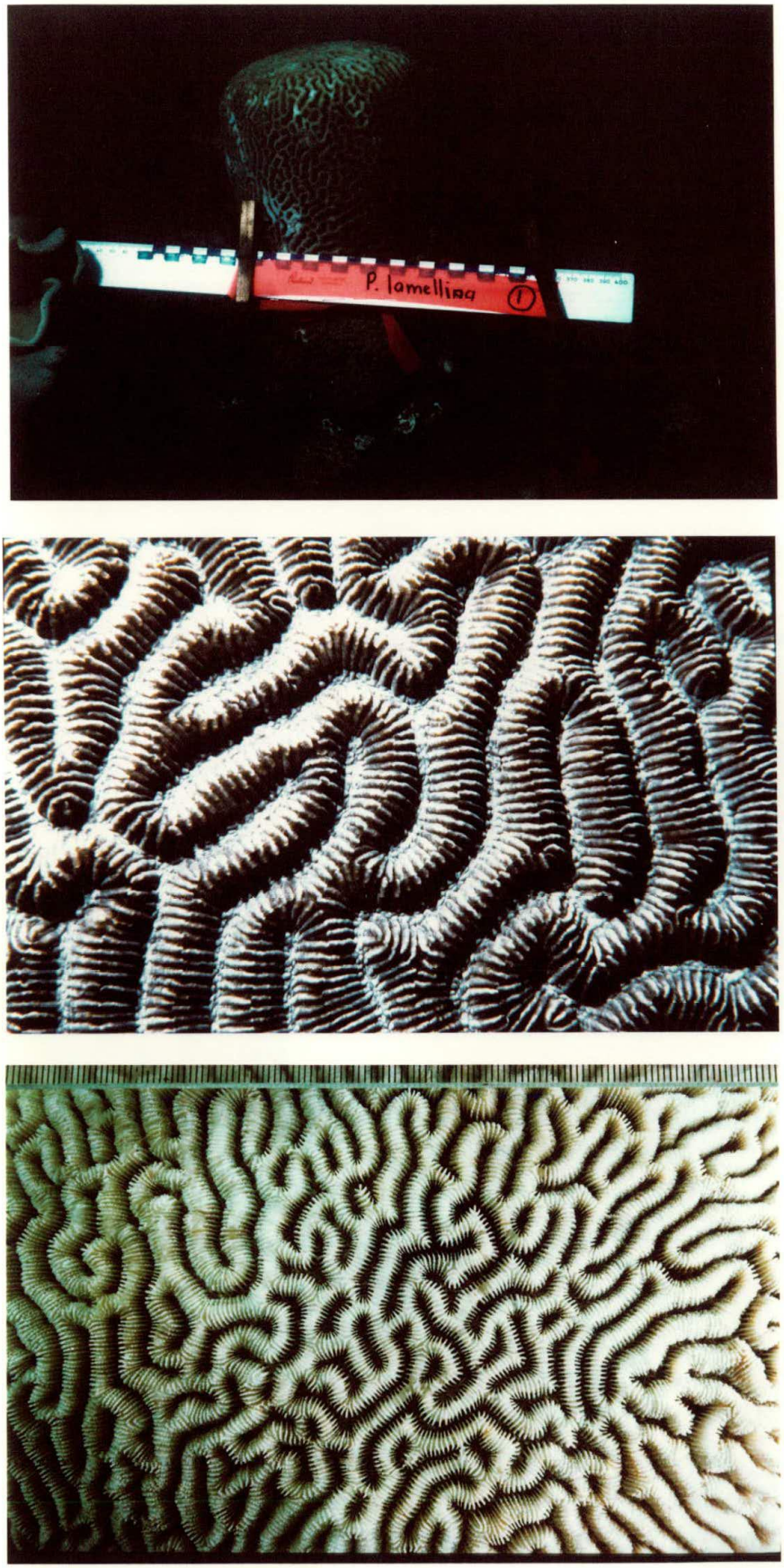


Plate 2.4. Photographs of live colonies and skeletal detail of *Platygyra lamellina*

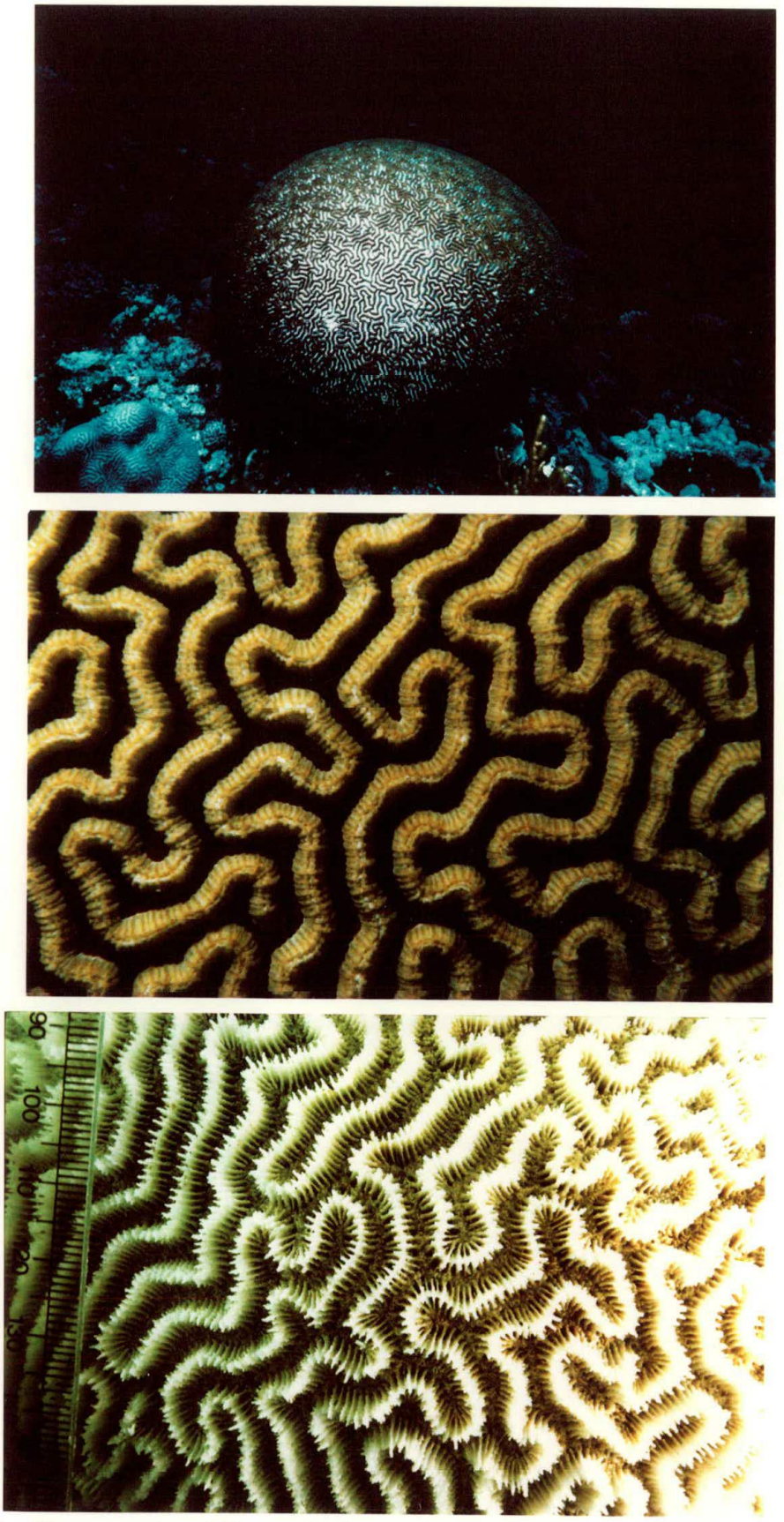


Plate 2.5. Photographs of live colonies and skeletal detail of *Platygyra daedalea* 'classic' morph (PDC).

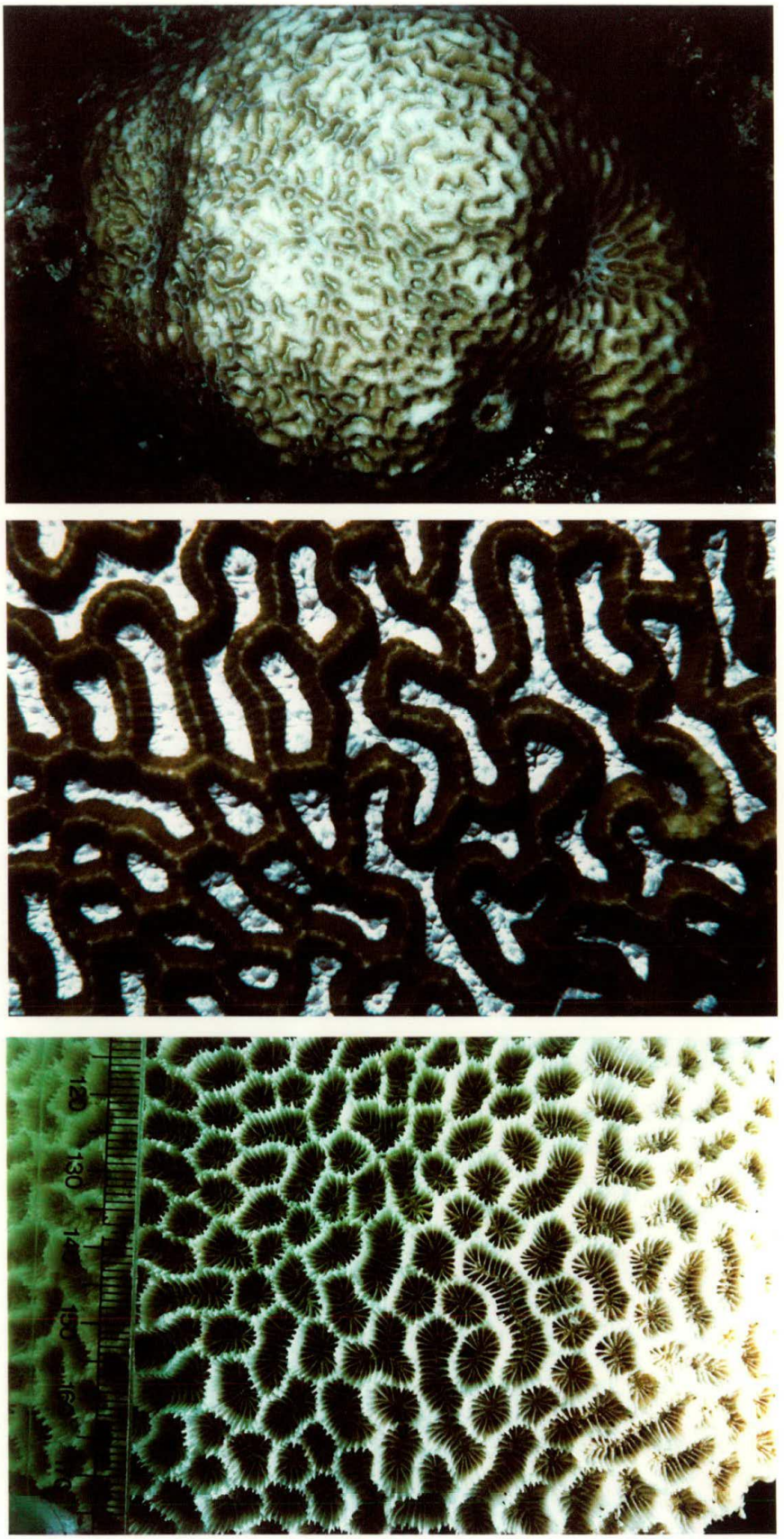


Plate 2.6. Photographs of live colonies and skeletal detail of *Platygyra daedalea* 'short-valley' morph (PDSV).

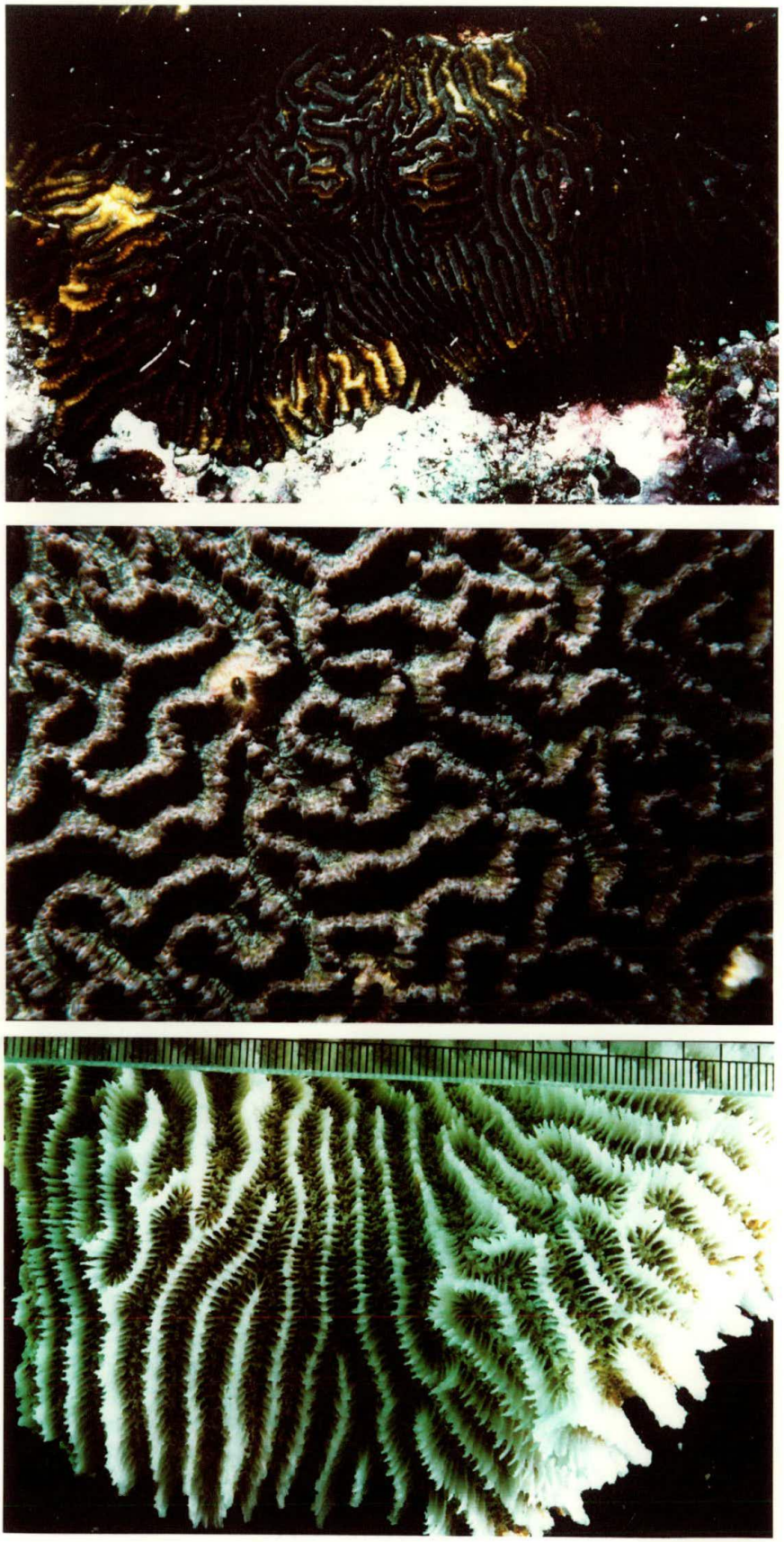


Plate 2.7. Photographs of live colonies and skeletal detail of *Platygyra daedalea* 'encrusting' morph (PDSE).

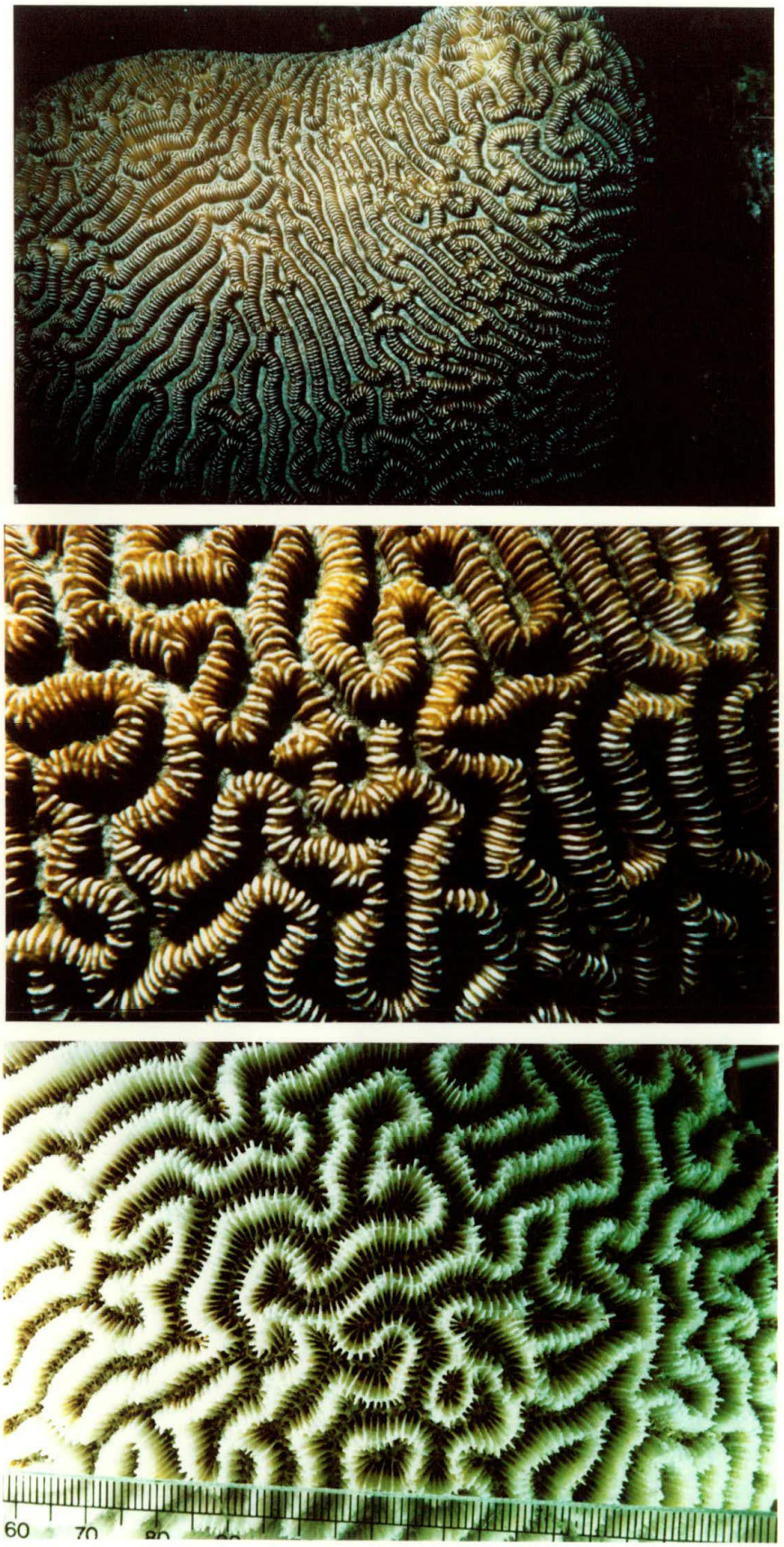


Plate 2.8. Photographs of live colonies and skeletal detail of *Platygyra daedalea* 'fat' morph (PDF).

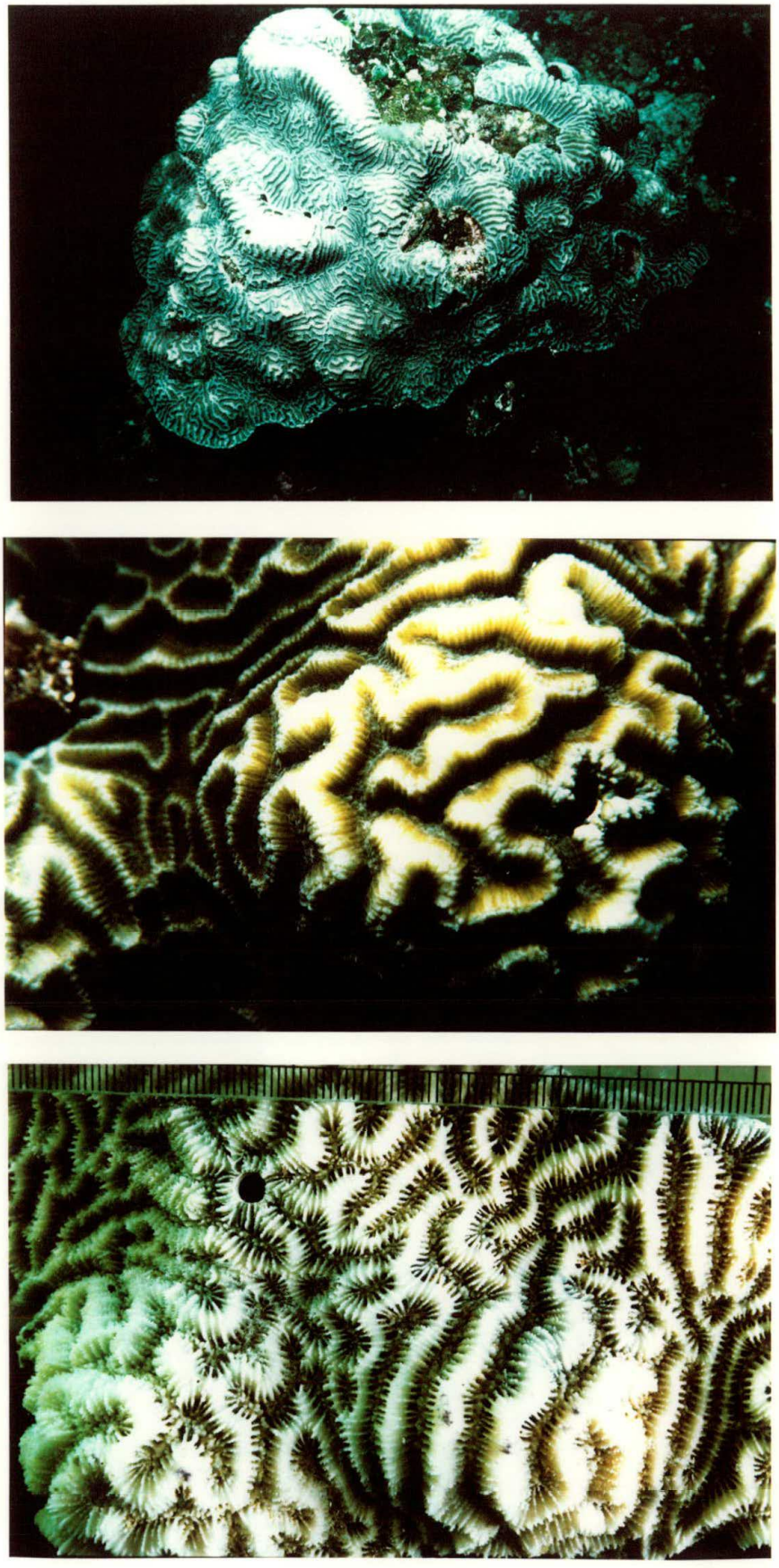


Plate 2.9. Photographs of live colonies and skeletal detail of PH.

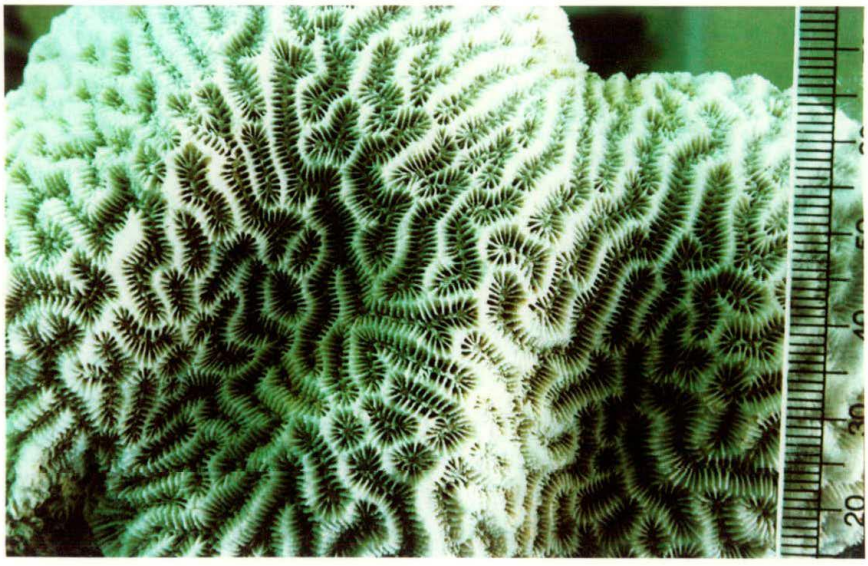


Plate 2.10. Photographs of a live colony and skeletal detail of PB.

2.2.2. Colony collection

Corals for morphometric analysis were collected from Davies Reef in the central Great Barrier Reef, 80km off the coast of Townsville. *Platygyra lamellina* was extremely rare on Davies reef (only one colony was found during this study) hence colonies from Orpheus Island were used for morphometric analyses. Colonies were photographed and measured *in situ* (the largest diameter and the diameter perpendicular to it, to the nearest 5mm). Their morphology, colour and location on the reef were recorded and a tissue sample was collected for genetic analysis (see chapter 5). Due to the large size of most *Platygyra* colonies, only a small sample (approximately 20 - 30 cm in diameter) of each colony was removed for skeletal analysis.

2.2.3. Preparation of skeletons

Skeletons were cleaned by placing colonies in fresh water for two to three days prior to removing the tissue with a high pressure hose. Skeletons were dried in the sun for a further two to three days before being used for morphometric analysis.

2.2.4. Quantitative morphometrics

2.2.4.1. Measurement apparatus

Skeletal measurements were taken using V-TRACE 2.2 (E-Soft 1990) a program written for Commodore-Amiga computers. Microscope images of the skeleton were displayed on the computer monitor via a video camera. Measurements were taken on the screen using a mouse to position the cursor. The microscope set-up allowed for measurements over an area approximately 3cm x 3cm. For those colonies with valleys longer than 3cm (eg *P.daedalea* and *P.lamellina*) valley lengths and areas were determined using consecutive frames.

To minimise parallax error in the video measurements the 'orgasmatron' was created. It consisted of a large ball-and-socket-like apparatus (made of wood) which allowed for multi-dimensional rotation of the skeleton until the region of the skeleton being measured was horizontal under the microscope.

2.2.4.2. Skeletal characters measured

The characters measured using the computer system described above were as follows (see also Figure 2.1):

Valley Length (VL) - the length of a valley (or polyp for monocentric colonies) measured from the top centre of the theca at each end of the valley.

Valley width (VW) - the width measured perpendicular to valley length, from the middle of the theca on either side of the valley.

Valley area (VA) - the area, in plane projection, of the region encompassed by a line drawn around the margins of each valley along the central ridge of the theca.

Septal cycles (SC) - the pattern of septal arrangement; either primary, secondary or tertiary.

Number of septa per cm (SCM) - total number of septa (of all cycles) occurring along a 1cm length of theca.

Columella length (CL) - measured along the entire length of the valley from where the columella meets the primary septa.

Columella width (CW) - columella width, measured parallel with valley width, from the edges of the columella (as above).

Septa thickness (ST) - the width of an individual primary septa measured parallel with the theca edge.

Theca thickness (TT) - width of the theca, measured approximately half way up its height.

A further group of characters were measured by hand using vernier callipers or counted directly from the skeleton (see Figure 2.1):

Valley depth (VD) - depth of the valley measured vertically from the columella to the top of the septa.

Septa exertness (SE) - exertness of the septa measured vertically from the top of the theca to the top of the septa.

Number of polyps per valley (PPV) - where possible the number of polyps per valley was counted based on the number of distinct centres apparent on the columella. Where centres were indistinct, the number of polyps was estimated by regression methods (see section 2.2.4.3.).

Valleys for measurements were selected haphazardly. Recently budded or budding polyps and valleys were avoided. Similarly, to minimise the effect of atypical skeletal patterns often apparent at the growing edge of a colony (Barnes, 1973) only calices from the central region of each colony were measured.

2.2.4.3. Calculations

From the measurements described above, the following values were calculated.

1. Polyp area (PA). The approximate area of an individual polyp in a valley, calculated as: Polyp Area = Valley Area ÷ Number of polyps per valley.

2. Number of polyps per valley (PPV). For those valleys where an exact count of polyps was impossible due to indistinct centres, the number of polyps per valley was estimated using a regression equation. A regression was carried out with columella length (independent variable) and the number of polyps per valley (dependent variable) on valleys with known numbers of polyps. A strong positive relationship was found between columella length and polyps per valley for all species, with r^2 between 0.74 and 0.99 (Table 2.1). The regression equation (see Table 2.1) was used to estimate the number of polyps in valleys where a direct count could not be made.

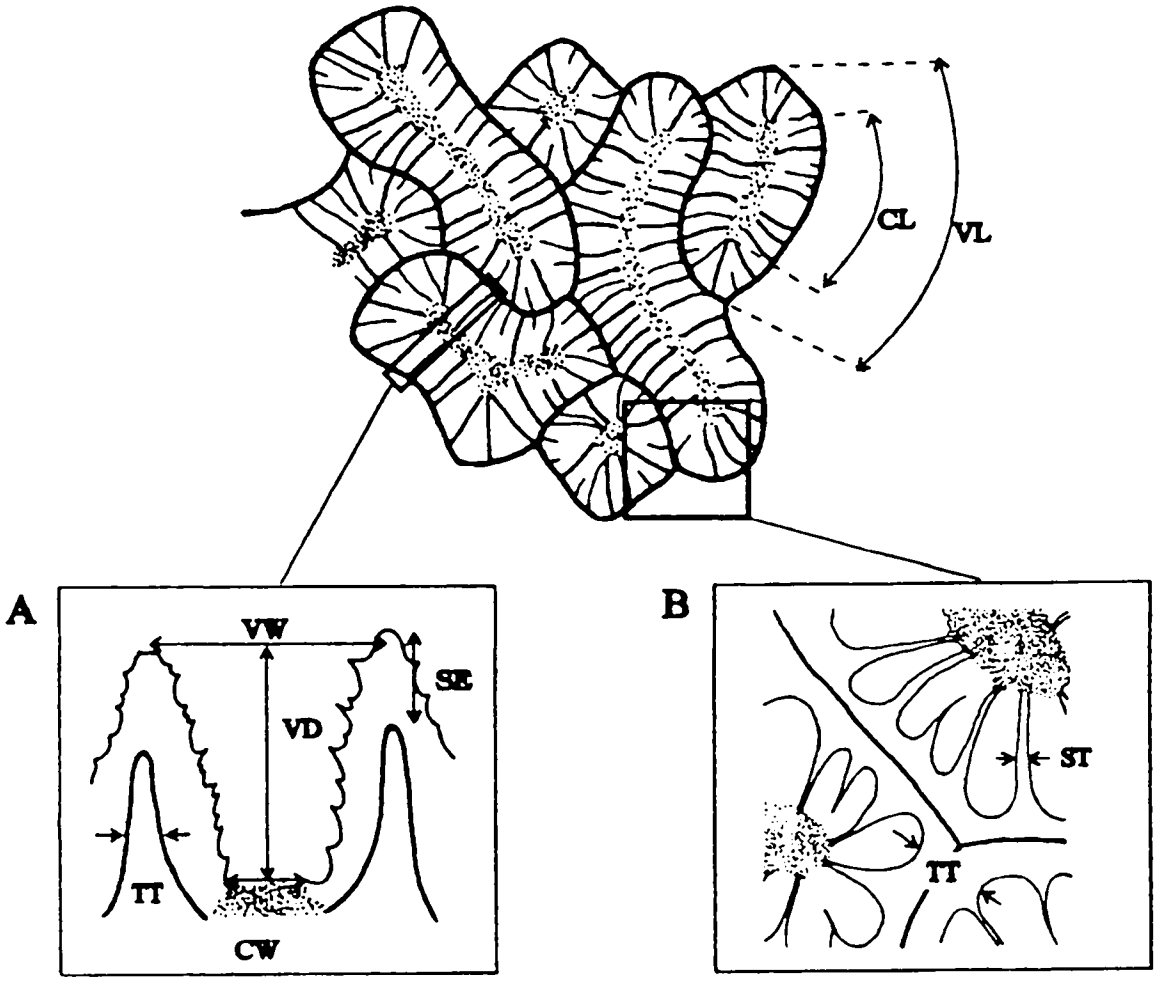


Figure 2.1. Diagrammatic representation of skeletal measurements taken on colonies of *Platygyra*. VL, valley length, VW - valley width, CL, columella length, CW - columella width, ST - septa thickness, TT - theca thickness, VD - valley depth, SE - exertness of septa.

Species	no. valleys	r^2		Intercept	Slope
PB	11	0.99	***	0.49	0.20
PDC	79	0.94	***	1.01	0.15
PDF	8	0.74	**	3.38	0.09
PDFI	21	0.92	***	2.18	0.11
PH	12	0.79	***	0.91	0.13
PDSE	29	0.93	***	1.05	0.15
PDSV	55	0.89	***	0.69	0.14
PL	14	0.99	***	0.85	0.17

Table 2.1. Relationship between columella length (independent variable) and number of polyps per valley (dependant variable). Significance level of linear regression; *** $P < 0.001$, ** $P < 0.01$. The number of polyps per valley (PPV) was estimated for valleys where direct counts could not be made using the regression equation: PPV = intercept + CL x slope

2.2.4.4. Determination of sample sizes for morphometric analysis

A preliminary study was carried out in order to determine the appropriate number of replicate skeletal measurements needed to adequately describe variation within and between colonies and species groups. In particular, it was necessary to determine the number of measurements to be taken per colony and the number of colonies of each species to be sampled. Two species were used for preliminary investigations, *Platygyra pini* and *P. sinensis* as both are common and widely distributed on the reef. Three colonies of each of the two species were examined. On each of the colonies three 9 cm^2 quadrats were haphazardly placed and measurements taken for all valleys within the boundaries of the quadrat (see section 2.2.4.2). Multiple measurements were taken, where appropriate, for each of eight different characters within a valley; VL (valley length), VW (valley width), VA (valley area), CL (columella length), CW (columella width), SCM (number of septa per cm), ST (septa thickness) and TT (theca thickness).

Sample sizes were determined based on calculations of precision and from estimates of variance within and between measured colonies.

1. Calculation of precision

Precision was calculated for each character for each colony, pooling quadrats. Precision levels <0.1 were obtained for each character measured from each of the six colonies based on a sample size between 25 and 125 depending on the character and the colony. The formula for precision,

$$Precision\ (P) = \frac{S}{\bar{x}\sqrt{n}}$$

where S = standard error
n = number of replicate measurements
x = mean value for each character

was rearranged as...

$$n = \left(\frac{S}{P\bar{x}}\right)^2$$

to calculate the sample size required to obtain reasonable precision. Values for P of 0.2, 0.1 and 0.05 were substituted into this equation and the resultant n value calculated for each character (Table 2.2). From these calculations, precision of at least 0.20 can be achieved for all characters (except VA) if ten measurements are taken on each colony.

	Number of replicates							
Precision	VL	VA	VW	CL	CW	SCM	ST	TT
0.05	54	186	8	139	18	22	19	20
0.1	13	46	2	35	5	5	4	5
0.2	3	12	1	9	1	1	1	1

Table 2.2. Calculated values for the number of replicate measurements of each of eight skeletal characters per colony to achieve desired precision.

2. Examination of variance within and between colonies

A single factor model II ANOVA was used to examine variation within and between colonies for each species. Variance components were calculated for each of the terms in the ANOVA model to give a relative measure of the contribution of each term to the overall variance. The majority of variation for a given morph (up to 99%) occurs within a colony (between measurements) rather than between colonies (Table 2.3).

		σ^2 (%)							
Species	source	VL	VW	VA	SCM	CL	CW	ST	TT
P. pini	between colony	12.9	2.1	1.8	22.0	10.3	0.9	18.5	26.4
	within colony	87.1	97.9	97.2	78.0	89.7	99.1	81.5	73.6
P. sinensis	between colony	1.3	53.8	6.5	47.7	4.5	43.3	17.2	5.7
	within colony	98.7	46.2	93.5	52.3	95.5	56.7	82.8	94.3

Table 2.3. Relative contribution of variance components for each character expressed as a percentage of total variance

Variance of the sample mean was calculated for each skeletal character as :

$$V\bar{x} = \frac{\sigma^2 C}{c} + \frac{\sigma^2}{cn}$$

where δ^2C = variance component for colony
c = number of colonies measured
δ^2 = residual variance component
n = number of replicate measurements per colony

By substituting a range of different values for *c* and *n* it is possible to determine the smallest sample size that will maintain or decrease the sample variance. Results of this substitution indicate that for both species and most characters, a sample size of 6 colonies and 10 replicate measurements per colony will optimise the sampling procedure (Table 2.4). Based on the assumption that the two species tested (*P. pini* and *P. sinensis*) were representative of the genus as a whole, I decided to sample at least 6 colonies of each morphological group and take 10 measurements (ie. measure characters from 10 separate valleys or calices) of each skeletal character. This should provide adequate replication and realistically represent variation within the populations sampled.

Species		VL	VW	VA	SCM	CL	CW	ST	TT
P. pini	*c*	5	6	6	5	5	6	4	4
	n	10	20	20	10	10	20	10	10
P. sinensis	*c*	6	4	6	4	6	6	5	7
	n	20	10	10	10	10	10	10	10

Table 2.4. Minimum values of *c* (number of colonies) and *n* (number of replicates per colony) which minimise variance of each of the measured characters.

2.2.5. Analysis of morphometric data.

Of the 14 characters measured and calculated, only nine were used for the final analyses. VL, VA, CL and PPV were all highly correlated with each other. As the inclusion of all four highly correlated variables would not provide information additional to the results obtained using only one, only the character VL was used for the morphometric analyses. The character SC (septal cycles) was found to be highly variable and inconsistent between valleys in all species and morphs and therefore was excluded. The nine characters used for the final analyses were VL, VW, SCM, CW, ST, TT, VD, SE and PA.

Canonical discriminant analysis (CDA) was used to describe the relationship between the eleven pre-defined groups of *Platygyra*. CDA determines functions which

minimise differences within groups and maximise differences between group means (Wiley 1981, Clifford and Stephenson 1975). It is also a useful technique for testing the appropriate assignment of individuals to a population. Canonical discriminant analysis has a number of underlying assumptions, including random sampling and normal distributions. The final nine morphometric characters were tested for univariate normality and heteroscedasticity using the SAS procedure GLM. Six of the nine variables displayed normal distributions and were homoscedastic (VW, SCM, ST, TT, VD and SE). The variable PA was non-normal and homoscedastic and the remaining two variables (VL and CW) were non-normal and heteroscedastic. Linearity of variables is also an assumption of multivariate analyses, however a transformation to produce linearity (ie log transform) is likely to remove normality (Marcus 1990). Tabachnick & Fidell (1989, p79) emphasise normality is more important than linearity in multivariate analyses and state "it is more likely that the assumption of multivariate normality is met if all the variables are normally distributed" (page 79). Both raw and log-transformed data sets were analysed initially and both produced identical results. Since canonical discriminant analysis is considered moderately robust to deviations from normality (Wiley, 1981) and as the majority of the variables in this study were already normally distributed, no transformations were used on the data prior to analysis and the results presented are based only on raw data.

2.2.5.1 Inter-specific variation

A total of 98 colonies, including representatives from the five field-defined species and the seven morphs of *P. daedalea*, were analysed by multivariate analysis of variance (MANOVA) to assess whether groups classified by field appearance also displayed skeletal differences detectable by morphometric methods. Analyses were based on the mean of 10 replicate measurements of each of the nine skeletal characters (see 2.2.4) for each of six colonies of each species (except *P. ryukyuensis* where only three colonies were sampled). Canonical discriminant analysis (SAS, procedure CANDISC) was then used to examine the differences between the *a priori* groups of *Platygyra*.

2.2.5.2 Intra-specific variation

A separate analysis of the morphs of *Platygyra daedalea* was undertaken to better describe variation within the species complex which may have been concealed in the inter-specific analysis. Seven different morphological variants within the species *P. daedalea* were identified *a priori* based on field appearances and gross colony form (see 2.2.1). Morphometric measurements (mean values of ten replicate measurements of each of nine characters for six colonies of each morph, except PB where only five colonies were used) were analysed using MANOVA and CDA.

2.3. Results.

2.3.1. Inter-specific variation

The application of numerical taxonomic methods to the genus *Platygyra* suggests the existence of seven, rather than five, morphologically distinct species. These correspond with the existing species *P. daedalea*, *P. lamellina*, *P. pini*, *P. ryukyuensis* and *P. sinensis*, and include two undescribed species - PB and PH - which were initially considered variants within the species *P. daedalea*.

There is no single character suitable for isolating the seven different species since the ranges of variation in all characters overlap between groups (Table 2.5). However, mean values indicate some features may be useful for characterising a few of the morphological species. Theca thickness is generally larger in *P. lamellina* colonies (3.06 mm vs. 1-2 mm in other species; Table 2.5) and *P. ryukyuensis* has considerably more septa per cm on average than the other species (15 vs. 9-11; Table 2.5). Similarly, the division of the genus into two groups - large and small polyped species - is evident based on mean values of skeletal characters such as polyp area, valley length and valley width (Table 2.5). In addition, MANOVA based on the nine skeletal characters measured was significant (Pillai's Trace=3.79, F=5.55, df=100, $P<0.001$) indicating morphological differences do exist between the seven species groups when all characters are considered simultaneously.

	P.daedalea									
	PDC	PDF	PDSE	PDSV	*P. lamellina*	*P. pini*	*P. ryukyuensis*	*P. sinensis*	PB	PH
Valley length	96.2 (17.0 - 627.8)	117.6 (41.9 - 345.2)	74.3 (26.1 - 292.4)	20.3 (8.7 - 52.1)	76.3 (32.3 - 198.5)	6.8 (4.4 - 12.1)	5.9 (4.2 - 9.1)	15.7 (6.2 - 40.2)	63.1 (16.5 - 247.9)	127.2 (27.9 - 526.8)
Valley width	7.2 (5.4 - 10.1)	7.4 (6.0 - 11.3)	7.0 (5.5 - 8.9)	7.5 (5.9 - 9.7)	7.4 (5.8 - 9.8)	5.2 (3.3 - 7.6)	4.7 (3.7 - 5.5)	5.9 (4.3 - 7.5)	5.2 (4.4 - 6.7)	7.1 (3.9 - 11.0)
No. of septa/cm	9.1 (6 - 13)	9.2 (7 - 12)	9.0 (7 - 11)	9.4 (7 - 13)	10.6 (8 - 13)	11.4 (8 - 16)	15.0 (12 - 18)	10.7 (7 - 15)	11.6 (9 - 14)	9.4 (7 - 12)
Columella width	1.13 (0.72 - 2.50)	1.14 (0.68 - 2.05)	1.21 (0.84 - 2.58)	1.34 (0.71 - 2.03)	0.80 (0.60 - 1.15)	1.15 (0.59 - 2.10)	1.06 (0.76 - 1.50)	1.16 (0.76 - 1.88)	0.82 (0.50 - 1.30)	1.12 (0.62 - 1.87)
Septa thickness	0.25 (0.12 - 0.43)	0.28 (0.19 - 0.43)	0.27 (0.11 - 0.45)	0.20 (0.10 - 0.35)	0.21 (0.14 - 0.30)	0.20 (0.08 - 0.33)	0.19 (0.13 - 0.26)	0.19 (0.07 - 0.36)	0.17 (0.09 - 0.27)	0.24 (0.14 - 0.40)
Theca thickness	1.70 (0.66 - 3.55)	1.88 (0.73 - 3.23)	1.81 (1.13 - 2.90)	1.48 (0.66 - 2.90)	3.06 (2.11 - 4.44)	1.41 (0.61 - 2.52)	0.84 (0.48 - 1.29)	1.03 (0.52 - 1.71)	1.41 (0.56 - 2.78)	2.36 (0.88 - 4.54)
Valley depth	5.0 (3.1 - 7.4)	4.4 (2.0 - 5.7)	4.8 (3.5 - 7.3)	3.7 (2.2 - 6.0)	5.0 (4.2 - 6.5)	2.6 (1.1 - 4.5)	3.2 (2.3 - 4.0)	2.6 (1.2 - 4.4)	1.7 (0.8 - 2.5)	3.3 (1.8 - 4.6)
Exertness of septa	2.2 (0.1 - 3.7)	1.8 (0.4 - 2.8)	2.8 (1.3 - 5.5)	1.1 (0 - 3.3)	1.8 (1.3 - 2.4)	1.3 (0 - 2.9)	1.0 (0.1 - 1.9)	1.2 (0.5 - 2.1)	0.4 (0 - 1.3)	1.3 (0.1 - 2.0)
Polyp area	49.3 (28.0 - 95.9)	61.6 (39.2 - 91.3)	44.9 (22.0 - 65.6)	53.9 (65.6 - 91.4)	43.5 (31.8 - 58.5)	25.4 (13.4 - 52.4)	20.3 (12.1 - 28.1)	34.1 (13.5 - 73.6)	26.9 (19.0 - 35.3)	52.7 (37.5 - 86.3)
n	300	60	60	60	60	240	30	60	50	60

Table 2.5. Mean values of nine skeletal characters measured from seven morphological species of *Platygyra*, including four morphological variants of *P.daedalea* (NB. the morph PDC includes colonies originally grouped as PDFI as they were not separated in analyses). Ranges for each character are in parentheses and n indicates the total number of measurements taken. All measurements are in mm except Polyp area which is mm^2.

Canonical discriminant analysis shows that 86% of the variation between groups is explained by the first three canonical variables (Table 2.6), and a plot of the first two canonical variables clearly identifies differences between four of the species groups - *P. lamellina*, *P. sinensis*, *P. daedalea* and the group PH (initially considered here to be a morphological variant of *P. daedalea*) (Figure 2.2) . Plots of other canonical variates were also examined but revealed no further groupings. Overall size is represented by the first canonical variable which is strongly influenced by all characters except columella width (Table 2.6, Figure 2.2). The character theca thickness is important in the separation of *P. lamellina* and the group PH (Figure 2.2). *Platygyra lamellina* has a mean theca thickness of 3.1 mm, compared to 2.4 mm for PH and between 0.8 and 2 mm for the other groups (Table 2.5). Polyp size follows a distinct gradient along the x-axis from those with the smallest polyps (*P. pini*, *P. ryukyuensis*, *P. sinensis* and PB) through to those with larger polyps (*P. daedalea*, *P. lamellina* and PH) (Figure 2.2). Separation of the small-polyped group of species is unclear based on this analysis, probably due to the influence of the large-polyped group of species (Figure 2.2). A second CDA was performed on only the small-polyped groups which clearly defines differences between the species *P. pini*, *P. ryukyuensis*, *P. sinensis* and the group PB (initially considered a morph of *P. daedalea*) (Figure 2.3). The group PB was separated largely on valley length, *P. sinensis* on polyp area and valley width and *P. ryukyuensis* on number of septa per cm (Table 2.7, Figure 2.3).

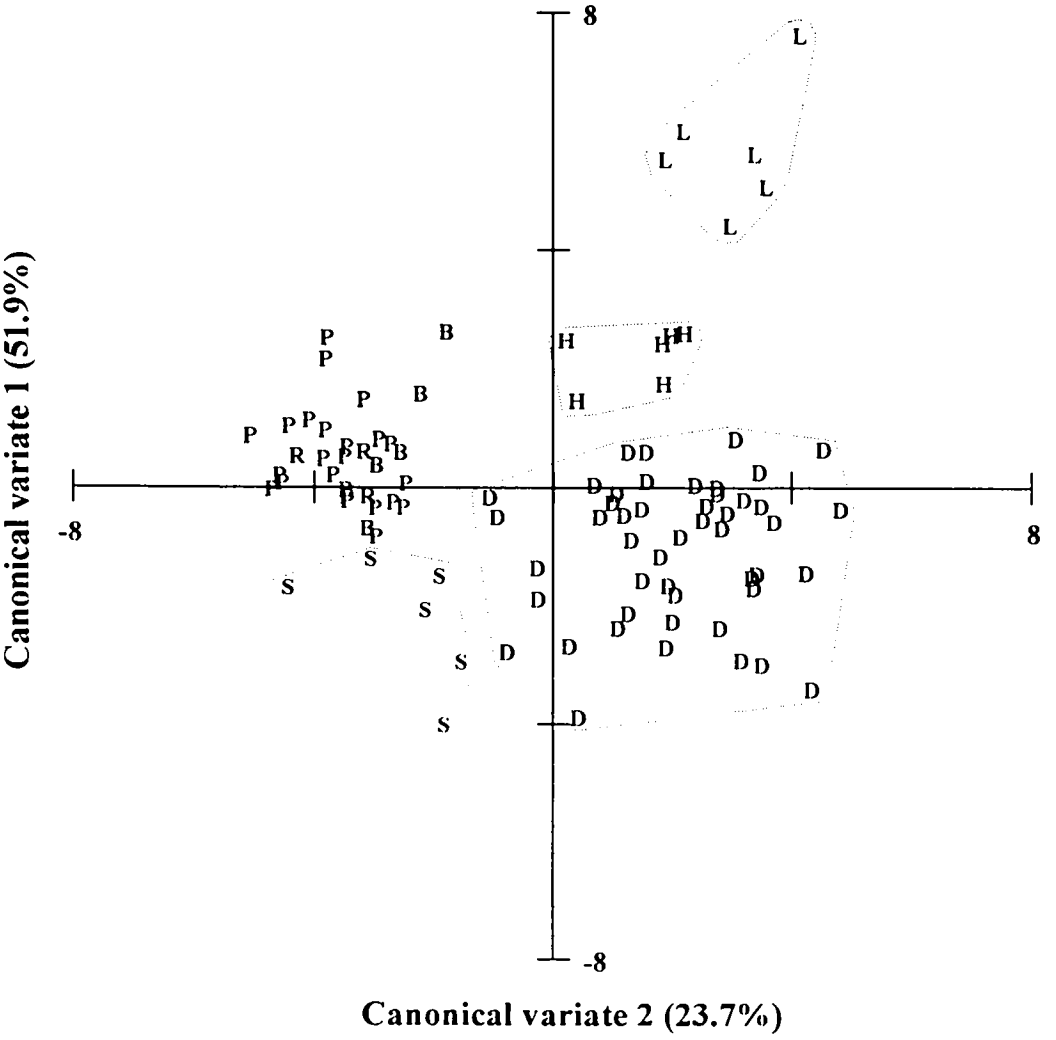
All specimens were assigned correctly to *a priori* species groups (ie the plots of individuals indicate no colonies were misclassified). However, while PB and PH were originally included as morphs of *P.daedalea* based on field observations, the results of morphometric analyses indicate they are as morphologically distinct from *P. daedalea* as are the other four species (Figure 2.2). Consequently, these two morphs were considered different morphological species in subsequent chapters.

Character	Canonical variable Can 1	Can 2	Can 3
Valley length	0.732	0.041	-0.183
Valley width	0.868	-0.092	-0.188
No. septa per cm	-0.721	0.305	0.434
Columella width	-0.054	-0.461	-0.197
Septa thickness	0.492	-0.149	-0.050
Theca thickness	0.572	0.687	-0.241
Valley depth	0.842	-0.092	0.346
Septa exertness	0.554	-0.166	0.256
Polyp area	0.819	-0.201	-0.331
Eigenvalue	8.2941	3.7355	1.7329
% variance	51.87	23.36	10.84
cum. % variance	51.879	75.23	86.06

Table 2.6 Correlation co-efficients between canonical variables and original variables for discriminant analysis of seven morphological species groups based on nine skeletal characters.

	Canonical variable Can 1	Can 2	Can 3
Valley length	0.814	0.378	0.294
Valley width	0.246	0.111	-0.646
No. septa per cm	0.022	-0.628	0.581
Columella width	-0.514	-0.163	-0.420
Septa thickness	-0.368	-0.004	-0.040
Theca thickness	-0.243	0.699	0.212
Valley depth	-0.354	-0.432	-0.108
Septa exertness	-0.449	-0.066	-0.249
Polyp area	0.141	0.106	-0.443
Eigenvalue	10.6929	3.0767	2.5509
% variance	65.52	18.85	15.63
cum. % variance	65.52	84.37	100.00

Table 2.7. Correlation co-efficients between canonical variables and skeletal characters for discriminant analysis of small - polyped morphological species groups.



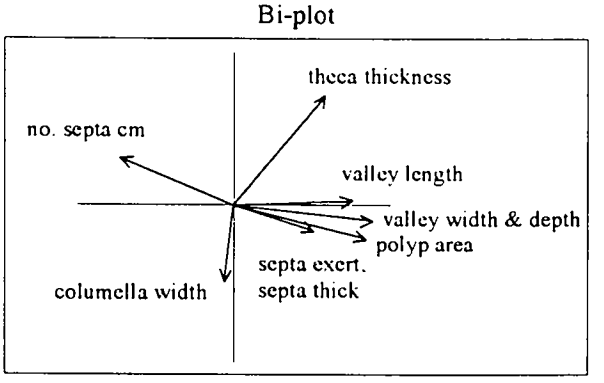


Figure 2.2. Results from discriminant analysis of all *Platygyra* colonies; plot of first two canonical variates. D - *P. daedalea*, S - *P. sinensis*, L - *P. lamellina*, R - *P.ryukyuensis*, P - *P. pini*, H - PH, B - PB. Bi-plot shows relative contributions of each skeletal character.

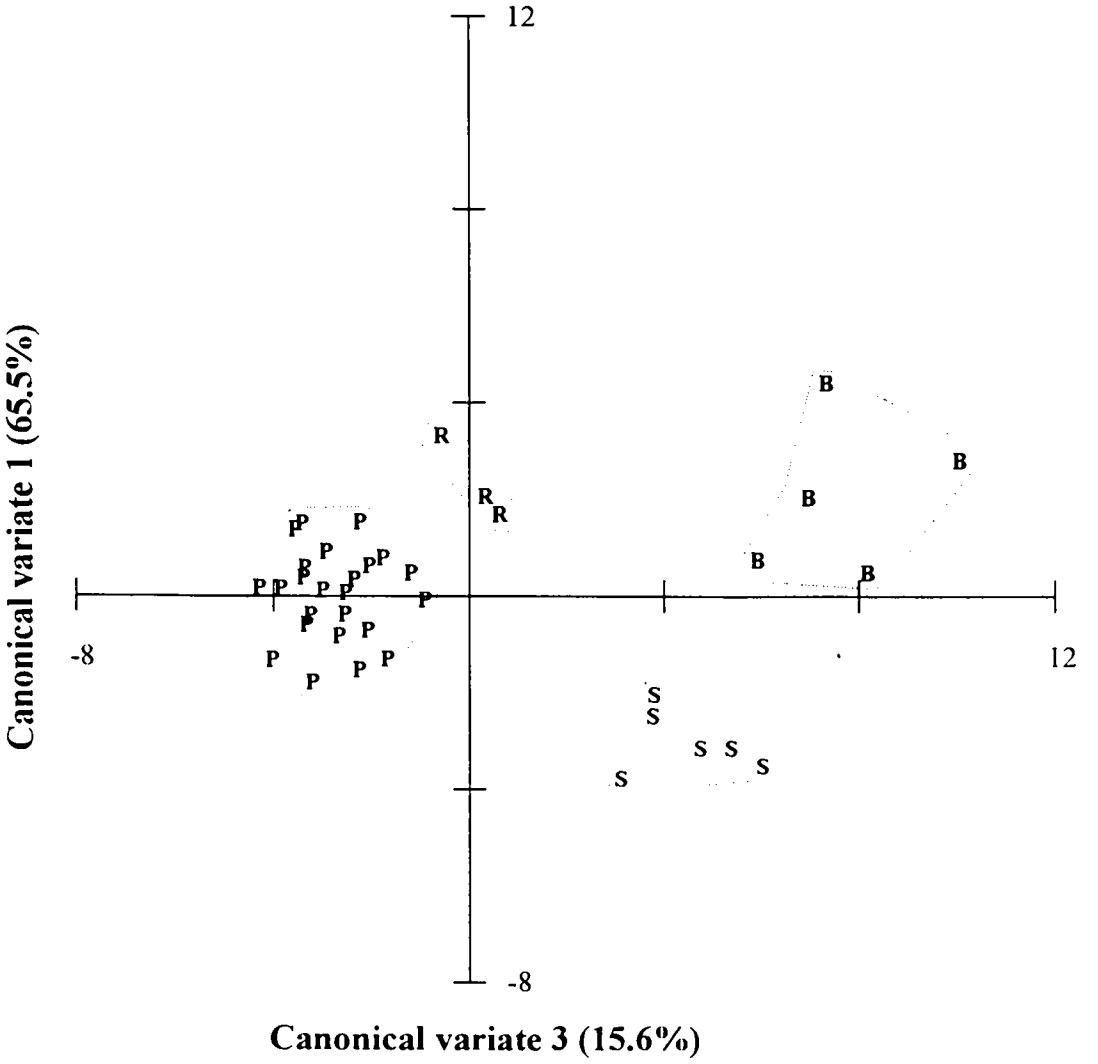


Figure 2.3. Results from discriminant analysis of small-polyped *Platygyra* species; plot of first and third canonical variates. B - PB, S - *P. sinensis*, P - *P. pini*, R - *P. ryukyuensis*. Bi-plot shows relative contributions of each skeletal character

2.3.2. *Intra-specific variation in Platygyra daedalea*

While *Platygyra daedalea* was initially divided into seven different morphological variants based on field appearances, the analyses described above suggest the groups PB and PH differ considerably from the other *P. daedalea* morphs in their skeletal characteristics. To examine variation within *P. daedalea*, a CDA was performed only on those colonies which clustered together in the initial analyses as group 'D' (morphs PDC, PDF, PDFI, PDSV, PDSE; Figure 2.2).

The first three canonical variates explain 96% of the variation within the species *P. daedalea* (Table 2.8). Plots of canonical variates (Figure 2.4) show clusters corresponding with the morphs PDC, PDSE, PDF and PDSV although there is some overlap between groups. Morph PDFI does not form a separate cluster in either of these plots nor was there evidence suggesting its morphological distinctness in plots of other canonical variables. Hence, the morph PDFI was combined with PDC (which it most closely resembles in the field) for the remainder of the analyses. The absence of discrete, non-overlapping groups in this analysis confirms that the four morphs represent variants of a single species, rather than separate species (as is the case for *P. pini*, *P. ryukyuensis*, *P. sinensis* and PB in Figure 2.3).

The morph PDF is characterised by large polyp area and wide theca thickness (Table 2.8, Figure 2.4a) and the group PDSV is strongly influenced by theca thickness, columella width and to a lesser extent septa per cm (Table 2.8, Figure 2.4a). Valley length is not a factor for discriminating PDSV, although mean values are considerably lower than for the other morphs (Table 2.1). The morph PDSE is characterised by exertness of the septa and septa thickness (Table 2.8, Figure 2.4b). These results indicate that colonies representing the range of variation within a single species, *P.daedalea*, form part of a morphologic continuum and that the different morphs occur predictably at regions along this continuum.

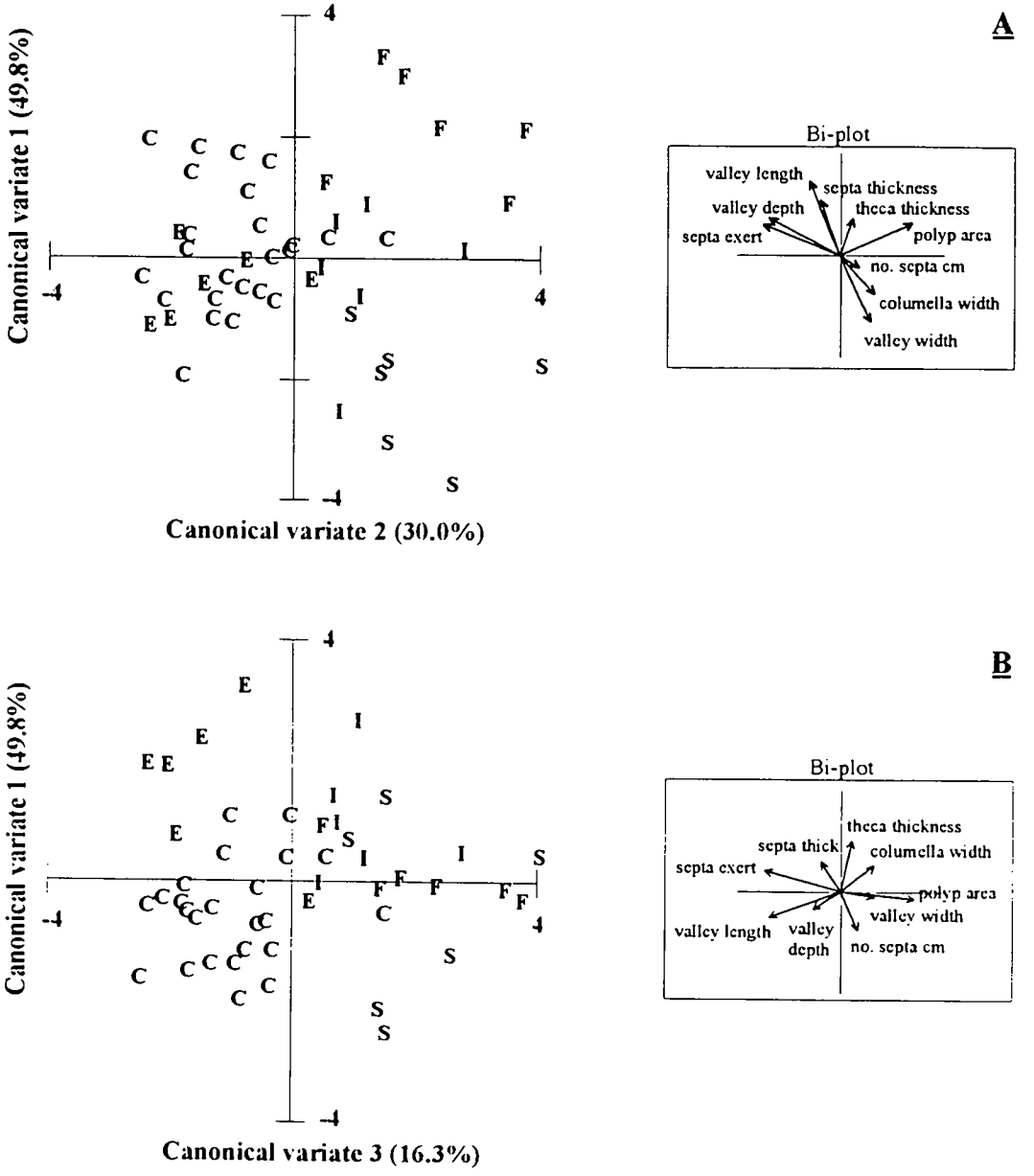


Figure 2.4. Plot of canonical variables from CDA on morphs of *Platygyra daedalea*. **A** - plot of canonical variates 1 and 2; **B** - plot of canonical variates 2 and 3. Letters correspond to morphological groupings: C - morph PDC; E - morph PDSE; F - morph PDF; I - morph PDFI; S - morph PDSV. Bi-plots show relative contributions of each skeletal character

	Canonical variable		
	Can 1	Can 2	Can 3
Valley length	-0.264	0.698	-0.137
Valley width	0.281	-0.620	-0.042
No. septa per cm	0.153	-0.115	-0.360
Columella width	0.300	-0.370	0.254
Septa thickness	-0.183	0.498	0.275
Theca thickness	0.103	0.315	0.479
Valley depth	-0.656	0.334	-0.273
Septa exertness	-0.709	0.283	0.202
Polyp area	0.651	0.300	-0.083
Eigenvalue	2.228	1.341	0.731
% variance	49.75	29.95	16.33
cum. % variance	49.75	79.70	96.03

Table 2.8. Correlation co-efficients between canonical variables and skeletal characters for discriminant analysis of morphological groups within *P.daedalea*.

2.4 Discussion.

2.4.1 Morphological differences between species

Where skeletal characters appear to form a continuum between species, numerical methods will be extremely useful in the delineation of species groups. No single diagnostic skeletal character exists between *Platygyra* species and skeletal characters are highly variable with overlapping ranges (Table 2.5). However, multivariate analysis successfully isolated seven discrete morphological groups (Figures 2.2, 2.3). Five of these morphological groups correspond well with the species *P. daedalea*, *P. lamellina*, *P. pini*, *P. ryukyuensis* and *P. sinensis* which are currently recognised in the taxonomic literature. Two groups - PB and PH - do not appear to have any parallels in earlier literature and may represent new morphological species. The specific status of *P. lamellina* has been questioned in the past and it has often been synonymised with *P. daedalea* (Stephenson and Wells, 1955; Chevalier, 1975). This

study has shown that the two species are morphologically distinct and that their separate status should be maintained. Similarly, *P. ryukyuensis* is sometimes synonymised with *P. sinensis* (Chevalier, 1975; Veron *et al.*, 1977). However the two species have been shown to be morphologically different in this study (Figure 2.3).

It is not surprising that the morphological groups from the analyses closely resemble previous taxonomies as many of the same skeletal characters are used in both classifications. However, the use of multivariate methods has advantages over traditional methods including unbiased assignment of unknown skeletons to morphological groups and the removal of much of the 'user-associated' variation in classifications. However, there are some limitations to numerical methods. Some types of data may be unsuitable (eg. binary data; Wallace, 1974) and the use of some characters may result in artificial groups being formed (Fedorowski, 1984). While it may never be possible to know which characters will cause artificial groupings, the characters used in this study appear to be appropriate as morphometric groups do approximate original taxonomies and there were no misclassifications of skeletons to *a priori* groups.

Previous quantitative morphometric work on *Platygyra* species is sparse. Veron *et al.* (1977) compared valley length, valley width and wall width between the four species *P. daedalea*, *P. sinensis*, *P. lamellina* and *P. pini* but did not use these data as species delimiters. My study recorded similar values for valley width and wall width (theca thickness) to those reported for the four species by Veron *et al.* (1977). Valley length in *P. pini* and *P. sinensis* are also comparable between studies. However, Veron *et al.* (1977, page 99) reports valley lengths ranging between 10 and 90 mm for *P. daedalea* and *P. lamellina* with means lying between 20 and 30 mm. This is quite different to the results of this study, in which mean valley lengths are in excess of 60mm and range as high as 250mm (Table 2.5). Due to the sampling method used in this study, where only portions of colonies were collected for morphometric analysis, valley length for meandroid species may have been underestimated. Only complete valleys were measured and any valleys which

continued beyond the sampled piece of skeleton were not included. It is likely therefore that in reality, valley lengths of both *P. daedalea* and *P. lamellina* are longer than the lengths presented here. Lengths described by Veron *et al.* (1977) are lower than the values I have described, however a direct comparison between the two data sets is not possible without further details of methods used in their study.

Skeletal characters in *P. daedalea* may display geographic variation. In a study of Faviids from New Caledonia, Wijsman-Best (1974) reported values between 1 and 7 for the number of corallites per meander in *P. daedalea.* I found mean values of polyps per valley for *P. daedalea* on Davies Reef ranging from 2 (PDSV) through to 14 (PDC), with as many as 50 in some valleys (Table 2.5). As explained above, these figures are likely to be an underestimate. In the same study, Wijsman-Best documents values of the number of septa per cm in *P. daedalea* between 11 and 17. This study shows mean numbers of septa per cm of approximately 9, ranging between 7 and 11 (Table 2.5). Sample sizes did differ between the two studies and this may account for the difference in the ranges. Alternatively, corals measured by Wijsman-Best (1974) may only include some of the morphs measured in this study.

Chevalier (1975) documented numerous skeletal features on type specimens of *Platygyra* and summarises variation in measurements for the three species *P. daedalea*, *P. sinensis* and *P. pini* from New Caledonia. However, Chevalier (1975) synonymises *P. lamellina* with *P. daedalea* and *P. ryukyuensis* with *P. sinensis.* Due to differences in taxonomy, ranges of variations for *P. daedalea* and *P. sinensis* are likely to differ from values reported here. However, a comparison of *P. pini* can still be made. Many of the measurements of Chevalier (1975) coincide with measurements taken in this study and mean values are very similar to those reported here (Table 2.9). Thus geographic variation in *P. pini* appears to be minimal between the Great Barrier Reef and New Caledonia.

	Chevalier (1975)	This study
valley width	5.0	5.2
columella width	1.2	1.15
septa exertness	1.4	1.3
septa thickness	0.25	0.2
theca thickness	1.4	1.41
valley depth	2.7	2.6

Table 2.9. Comparison of mean values of measured skeletal characters from this study with documented values from New Caledonia. Records from Chevalier (1975) are based on the mean of maximum and minimum values presented for each character. All measurements are in millimetres.

2.4.2 Variation within morphological species.

Platygyra daedalea can be divided into four different morphs (PDC, PDSV, PDSE, PDF) which differ in both colony form (see methods) and in skeletal characters (Figure 2.4). Variants within the species *P. daedalea* form a discrete species group in the discriminant analysis when compared with other species (Figure 2.2) and, although the four morphological variants occur predictably within the group, they do form a continuum (Figure 2.4). The analysis did not reveal any differences between the morphs PDC and PDFI, which will therefore be combined as a single morph PDC for this thesis.

Morphological variation within species of *Platygyra* is often attributed to environmental influences. Wijsman-Best (1974) showed that skeletal characters in *P. daedalea* from New Caledonia were highly variable. She hypothesised that this variation was attributable to environmental influences, in particular, the influence of depth and associated changes in light and food availability. Similarly, Veron *et al.* (1977) divide *P. daedalea* into three different 'ecomorphs' which correspond with different reef habitats. The growth form Veron *et al.* (1977) refer to from areas of "relatively poor illumination" appears to correspond morphologically to the morph PDSE. However, my other three morphs (PDC, PDF and PDSV) appear to be variations within their morphs from "exposed biotopes" and "microatolls". The four

different morphs I have described are distinguishable on the basis of skeletal characteristics and colony growth form. However, the basis of this variation - either as a response to environmental influences or genetic in origin - is unknown. The relationship between habitat and morphology in colonies of *Platygyra* will be addressed in Chapter 3.

2.4.3 Conclusions: morphological 'species' of Platygyra

By definition, a morphological species is any group of individuals which look different to any other group. Thus *Platygyra* can be divided into seven different morphological "species". These species correspond with the current taxa *P. pini* (PP), *P. sinensis* (PS), *P. lamellina* (PL), *P. ryukyuensis* (PR) and *P. daedalea* (PD) and include two new classifications, *Platygyra 'H'* (PH) and *Platygyra 'B'* (PB). The analysis also distinguishes four variants within the species *P. daedalea* (PDC, PDSV, PDF, PDSE) which display morphological differences but nevertheless, merge into one another to form a single species continuum.

CHAPTER 3

DISTRIBUTION PATTERNS OF PLATYGYRA SPECIES: THE EFFECT OF ENVIRONMENT ON COLONY MORPHOLOGY

3.1 Introduction

3.1.1. Phenotypic plasticity

Many species display a wide range of phenotypes in response to habitat differences. Such environmental influences on morphology have been documented in a variety of taxa, particularly those with a sessile mode of life such as plants and corals. The ability to adapt morphology according to habitat is likely to be highly advantageous particularly in sessile organisms with passive dispersal (Bradshaw, 1965; Harper, 1967). Similarly, phenotypically plastic species may be able to inhabit a wider range of environments than species with more stable phenotypes (eg. Yonge, 1935; Briggs and Walters, 1984). The suggestion that phenotypic responses to environmental influences may be more diverse in heterogeneous environments (eg. Schlichting and Levin, 1984) is particularly relevant in coral reef systems which are highly heterogeneous. Thus the response of corals to environmental influences will be important when considering the range of variation that can be exhibited by a single species and ultimately when considering species boundaries.

3.1.2. Environment-related variation in coral morphology

Much of the intra-specific morphological variation documented in scleractinian corals has been attributed to the environment, whereby colonies of a single species will display different morphologies in various habitats. Such variation has been reported to correlate with environmental parameters including depth (Barnes, 1973), light attenuation (Jaubert, 1977; Graus and Macintyre, 1982) and wave action (Veron and Pichon, 1976). Morphological variation in corals has also been considered in terms of genetic variation (eg. Willis, 1985; Willis and Ayre, 1985) which will be discussed

in further chapters.

Some corals, such as *Pocillopora damicornis* and *Seriatopora hystrix* display distinct colony morphotypes corresponding to habitat (Veron and Pichon, 1976). Similarly, *Turbinaria mesenterina* colonies are convoluted in shallow water but become plate like in deep water (Willis, 1985). *Acropora formosa* displays differing growth and branching patterns with depth (Oliver *et al.*, 1983) and *Favia stelligera* colonies at Enewetok Atoll change from rounded in shallow water to columnar in deeper water (Haggerty *et al.*, 1980). Brakel (1976) related differences in colony morphology in *Porites* spp. from the Caribbean to micro-habitat variation, although he found no relationship between environment and variation in corallite characters. Veron *et al.* (1977) described differing colony types for *Platygyra daedalea* from exposed and protected habitats and under differing light regimes. Environmental variation has also been documented in fossil scleractinians where colony morphology can be correlated with substratum type (Hofling, 1989).

Growth rates of scleractinian corals have also been reported to vary with depth, eg. *Favia pallida*, *Goniastrea retiformis* and *Porites lutea* (Highsmith, 1979) and *Montastrea annularis* (Baker and Weber, 1975). These changes may be associated with changes in calcification rates in lowered light regimes which may be mediated through the zooxanthellae (Goreau, 1959). In fact, Dustan (1979) suggested changes in colony morphology in *Montastrea annularis* may be the result of discrete zooxanthellae populations responding to differing light regimes ultimately effecting calcification rates and colony form of their host corals. Similarly, Jaubert (1977) found growth forms in *Synaraea convexa* varied predictably with depth, and that growth was correlated with light intensity and photosynthetic rates of symbiotic zooxanthellae. Differences in growth patterns in corals have also been associated with environmental influences such as nutrient availability and water temperature (eg. Highsmith, 1979; Wellington and Glynn, 1983; Dodge and Lang, 1983).

Calicular skeletal characters may also vary in response to environmental parameters. Wijsman-Best (1974) found an inverse relationship between depth and polyp skeletal

characteristics (eg. number of septa per cm and number of corallites per valley) in Faviid corals including *P. daedalea*. Foster (1977, 1980) correlated skeletal variation in *Montastraea annularis* with light intensity and food supply, while *Siderastrea siderea* corallite structures varied with sedimentation rates in different environments. Transplanted colonies of these two species were shown to alter corallite morphological characters in response to different habitats (Foster, 1979a). Foster (1979b) also suggested corallite variation in the fossil coral *Solenastrea fairbanksi* varied in response to environmental pressures.

In the previous chapter, I identified seven different morphological "species" within the genus *Platygyra* (plus 4 morphs within *P. daedalea*) based on measured skeletal characters. It is important to understand the effect of environment on the morphology of these species to determine the taxonomic status of each of these morphological groups. Relative abundances of ecomorphs (*sensu* Veron and Pichon, 1976) of a single coral species should vary predictably across environmental gradients of light, depth and wave action. Hence, if these morphological groups of *Platygyra* represent ecomorphs of a single species, it should be reflected in their distribution patterns ie. different morphs should occur in different environments. The aim of this chapter, is to examine the spatial distribution of the morphological species and morphs of *Platygyra* over both large and small geographic scales. At the same time the degree of variation within morphological species will be examined relative to environmental variables in order to understand the role of habitat on morphology of *Platygyra* species.

3.2 Methods

3.2.1 Large scale (between reef) distributions of Platygyra

To determine how widely the different morphological species of *Platygyra* were distributed and also to examine variation within each of the species at a number of different locations, I qualitatively examined *Platygyra* populations from seven different reefs along the Great Barrier Reef. These reefs ranged from Lizard Island

in the north (14°38' S, 145°24' E) to Heron Island in the south (23°26' S, 159°59' E) (Figure 3.1). Two other reef systems were also explored, Lord Howe Island in New South Wales, and Scott Reef in the Timor Sea off Western Australia. (Figure 3.1). The majority of these observations were carried out opportunistically, and while not exhaustive, these surveys allow at least some insight to the broad scale distributions (in terms of presence or absence) of the different morphological groups.

3.2.2 Small scale (within reef) distributions of Platygyra

A detailed quantitative study of distribution patterns of each of the morphological groups of *Platygyra* was carried out at Davies Reef, central Great Barrier Reef (Figure 3.1) to examine variation in abundances of the species and morphs across different environments. It is well known that coral reefs are exposed to gradients of wind and wave energy, light, turbidity and sedimentation. These gradients have produced geomorphological reef zones (eg. Flood, 1984) which display different suites of characters related to environmental effects (eg. Done, 1982; Sheppard, 1982). If *Platygyra* colonies respond morphologically to these environmental differences, variation in their morphology should be apparent parallel with these ecological reef zones.

3.2.2.1 Relative abundances of morphs in different reef zones

The abundance of the seven different morphological species of *Platygyra* (and the four morphs of *P. daedalea*) was examined across six different reef zones on Davies Reef (see Figure 3.2).

1. The front reef slope at 10 m depth (F10)
2. The front reef slope at 5 m depth (F5)
3. The front reef flat (a region approx 20 m behind the reef crest) (FRF)
4. The lagoon (approx 3 m deep) (LAG)
5. the back reef flat (BRF)
6. the back reef slope at approx 5 m depth. (BRS)

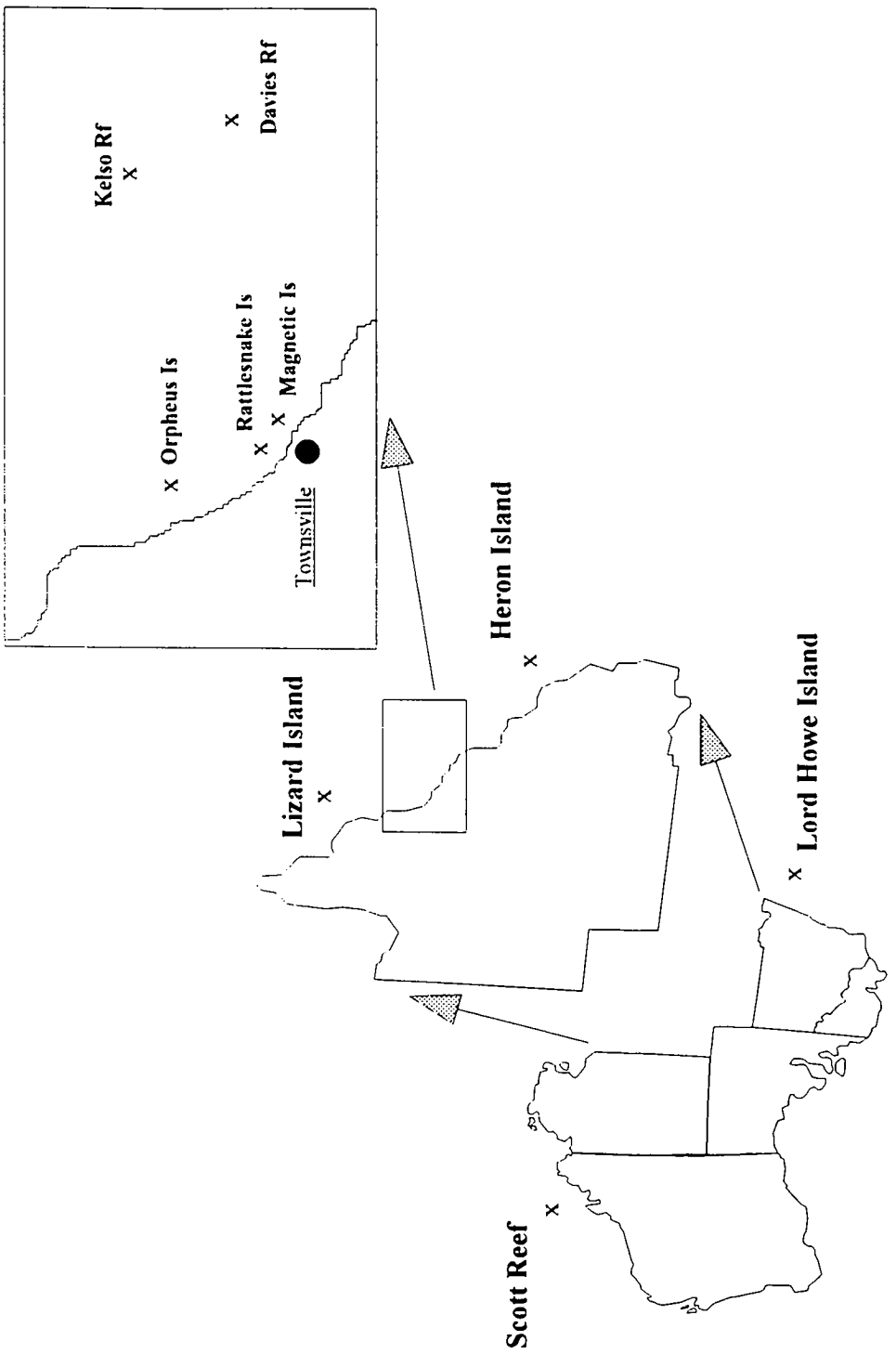


Figure 3.1. Map of Australia showing approximate locations of reefs surveyed for colonies of *Platygyra*

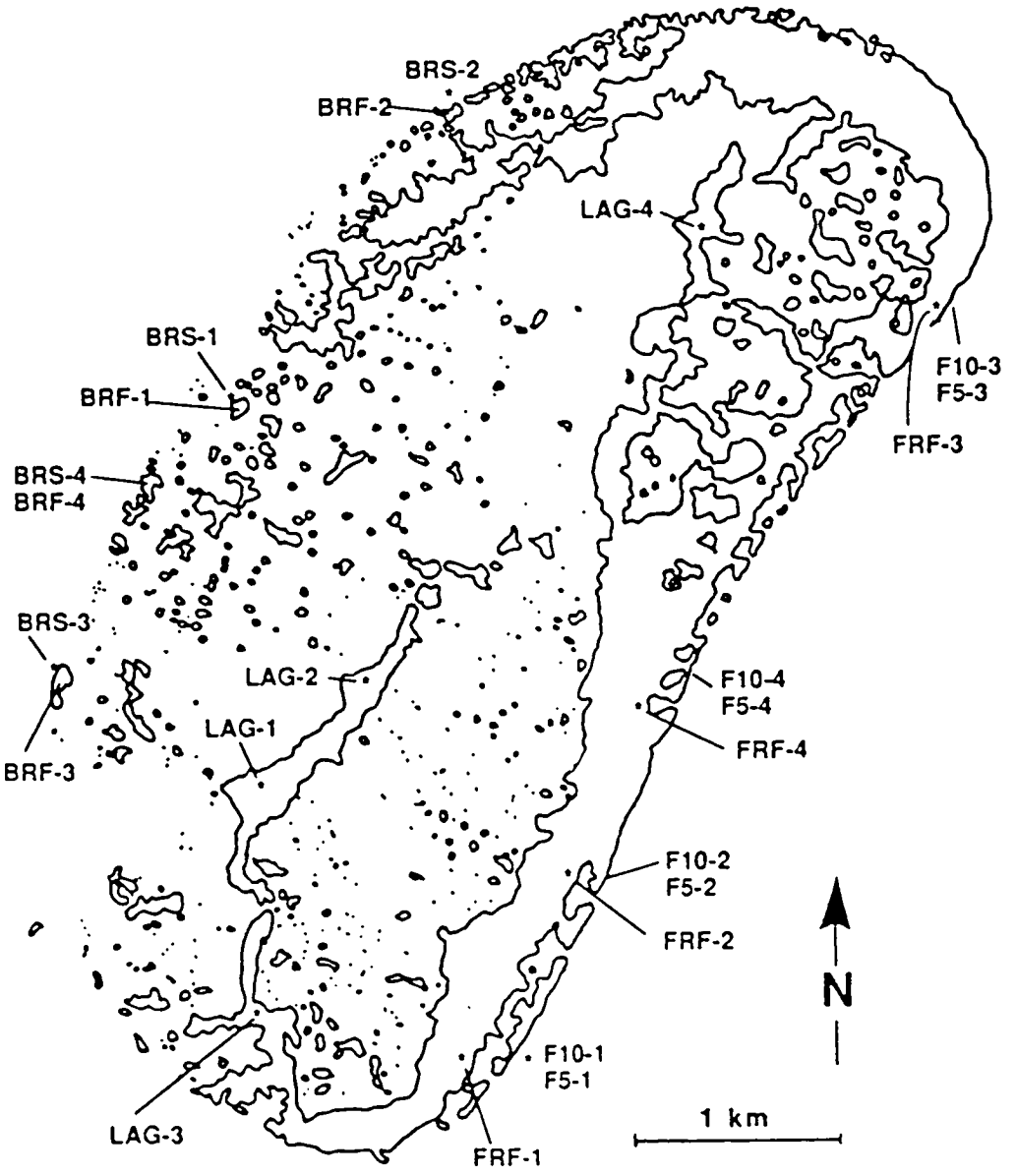


Figure 3.2. Location of transect sites in six different reef zones at Davies Reef. F10 - front reef slope at 10 m depth; F5 - front reef slope at 5 m depth; FRF - front reef flat; LAG - lagoon; BRF - back reef flat; BRS - Back reef slope.

Within each zone four different sites were chosen (approximately equidistant along the reef length, Figure 3.2) and within each site eight 20 x 1 metre replicate belt transects were surveyed, recording the size and morphological type of all *Platygyra* colonies. The occurrence of rare morphs not found in the transects but which were seen adjacent to the transects was also noted. The size of each colony was recorded (to the nearest 5 mm) as the largest diameter and the diameter perpendicular, and the approximate surface area calculated based on the formula for area of an ellipse:

$$Surface\ area = \pi \frac{ab}{4}$$

where a and b are diameter 1 and diameter 2 respectively.

The transect data were analysed using a one way nested analyses of variance (ANOVA) to test for differences in the abundance of each species between reef zones. The main effect was zone (six treatments; BRF, BRS, LAG, FRF, F5, F10) with site nested within zone (four sites per zone) and eight replicate transects within each site. The size range of colonies was compared for each species and morph on Davies Reef to determine if any relationship existed between size and morphology (ie. ontogenetic change).

3.2.2.2 Morphological variation across habitats

To further assess the influence of environment on skeletal characters within a morphological species, measured skeletal characters were compared across four of the reef zones described above (F10, F5, LAG, BRS). Colonies of two species - *P. daedalea* and *P. pini* - were used for this comparison. Both species are common, widely distributed and represent either end of the morphological gradient within the genus. *Platygyra pini* has relatively small, monocentric polyps and small colony size. In contrast, *P. daedalea* has long, meandroid valleys, relatively large polyps and forms large colonies. Nine characters (valley length, valley width, valley depth,

columella width, number of septa per cm, septa thickness, exertness of septa, theca thickness, polyp area - as described in Chapter 2) from each of six colonies of *P. daedalea* (morph PDC) and six colonies of *P. pini* from each zone were measured. Skeletal characters of conspecific colonies from the four zones were compared using multivariate analysis of variance (MANOVA) to determine if measured skeletal characters varied significantly between different environmental regions.

3.3 Results

3.3.1 Large scale distribution of Platygyra

The genus *Platygyra* is widespread and occurs commonly on most of the reefs surveyed in this study (Table 3.1). In particular, the morphological species *P. sinensis*, *P. pini*, *P. daedalea* (morph PDC) and *P. daedalea* (morph PDSE) are widely distributed and were common on the majority of reefs visited. *Platygyra lamellina*, while extremely common in the Palm Island group (including Orpheus Island) and at Scott Reef (Western Australia), was extremely rare at both Magnetic Island and Davies Reef and was not found elsewhere (Table 3.1). Similarly the species PH has a more restricted distribution and was only found on reefs in the northern section of the Great Barrier Reef (from Davies Reef northwards). *Platygyra ryukyuensis* was numerically more abundant on inshore reefs than on offshore reefs although the limited ranges of the species *P. ryukyuensis* and PB may reflect the limitations of the survey conducted. Both groups are restricted to very shallow water (see below) whereas most observations were made on SCUBA in deeper water. Similarly observations were only made opportunistically, not systematically, and the absence of any species in Table 3.1 does not necessarily confirm the absence of that species on a reef, only that it was not found in this study.

Morphs of *P. daedalea* appear to be differentially distributed across wide geographic regions. The morph PDF was not found on the two inshore reefs (Magnetic and Rattlesnake) however it was found on offshore reefs in the same locality (Kelso and Davies Reef). Similarly only one morph (PDSE) was present on Lord Howe Island.

	Species/morph									
Reef	PDC	PDF	PDSE	PDSV	PB	PH	PL	PP	PR	PS
Lizard Is. (o)	✓	✓	✓	✓		✓		✓		✓
Orpheus Is. (i/o)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Kelso Reef (o)	✓	✓	✓			✓		✓		✓
Rattlesnake Is. (i)	✓		✓			✓				✓
Magnetic Is. (i)	✓		✓	✓		✓	✓	✓	✓	✓
Davies Reef (o)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Heron Is. (o)	✓	✓	✓	✓	✓			✓		✓
Lord Howe Is. (o)			✓							
Scott Reef (o) *	✓			✓			✓	✓	✓	✓

Table 3.1. Presence of *Platygyra* species and morphs on various reefs around Australia. '✓' denotes presence. Reefs are listed according to increasing latitude from 14° south to 35° south (* except Scott Reef which is in Western Australia). (o) - denotes offshore reefs, (i) denotes inshore reefs. Orpheus Island (i/o) is located inshore but has reef characteristics more similar to offshore reefs. See chapter 2 for reference to species codes.

3.3.2 Small scale distributions of Platygyra at Davies Reef

The majority of *Platygyra* species occur sympatrically within each of the reef zones on Davies Reef (Table 3.2). However, *P. lamellina* was not found in the transect survey and occurs only rarely on Davies Reef. Similarly, *P. ryukyuensis* and PB were confined to reef flat habitats (Table 3.2).

The relative abundances of species and morphs differs between the six reef zones (Figure 3.3). ANOVAs to test for differences in abundances (number of colonies) of each species across the six reef zones produced varying results (Table 3.3). There was some variation in colony abundance between sites within zones. In particular, abundances of *P. daedalea* (morphs PDC and PDF) and PB were significantly different between sites although the other species (*P. daedalea* morphs PDSE and PDSV, *P. pini*, *P. sinensis* and PH) were more uniformly distributed between sites (Table 3.3).

There was no significant difference in abundance of *P. sinensis*, *P. daedalea* (morph PDF), PB or PH across the six zones. In contrast, *P. daedalea* (morphs PDC, PDSE and PDSV) and *P. pini* were significantly more abundant in some zones (Table 3.3, Figure 3.3). PDC was significantly more abundant on shallow slopes (F5) than on the front reef flat (FRF). Similarly PDSE was more common on the reef slope (both F5 and F10) than in other parts of the reef. PDSV had fewer colonies in the lagoon (LAG) and at F10 than on the front reef flat. *Platygyra pini* was most common at F10 but was uncommon in the lagoon and on reef flats.

	Species/morph									
Reef zone	PDC	PDF	PDSE	PDSV	PB	PH	PL	PP	PR	PS
F10	✓	✓	✓	✓	-	✓	-	✓	-	✓
F5	✓	✓	✓	✓	-	✓	-	✓	-	✓
FRF	✓	✓	-	✓	✓	✓	-	-	✓	✓
LAG	✓	✓	✓	✓	-	✓	-	✓	-	✓
BRF	✓	✓	✓	✓	✓	✓	-	✓	-	✓
BRS	✓	✓	✓	✓	-	✓	-	✓	-	✓

Table 3.2. Presence/absence of *Platygyra* species (including morphs of *P. daedalea*) in different reef zones on Davies Reef. '✓' denotes presence, '-' denotes absence. F10 - front reef slope at 10m depth; F5 - front reef slope at 5 m depth; FRF - front reef flat; LAG - lagoon; BRF - back reef flat; BRS - back reef slope.

The size range of colonies from the five morphological species (and the four morphs of *P. daedalea*) were largely overlapping, Small colonies (< 50 cm^2) were found in all species and morphs and mean colony size (across the entire reef) was similar in all species (< 200 cm^2) (Figure 3.4). The maximum colony size of *P. daedalea* (morph PDC) and *P. sinensis* were similar, approximately 1800 cm^2, which is almost five times larger than maximum colony sizes of the other species.

Species/morph	Source of variation	df	MS	F	
P. daedalea					
- PDC	zone	5	34.20	4.15	**
	site(zone)	18	8.24	3.75	***
- PDF	zone	5	2.67	1.91	ns
	site (zone)	18	1.39	1.74	*
- PDSE	zone	5	73.27	19.03	***
	site (zone)	18	3.85	1.05	ns
- PDSV	zone	5	2.08	3.11	*
	site (zone)	18	0.67	1.09	ns
P. pini	zone	5	76.93	12.33	***
	site (zone)	18	6.24	1.60	ns
P. sinensis	zone	5	3.16	1.93	ns
	site (zone)	18	1.64	1.15	ns
PB	zone	5	6.85	1.66	ns
	site (zone)	18	4.14	3.68	***
PH	zone	5	0.26	1.48	ns
	site (zone)	18	0.17	0.78	ns

Table 3.3. Results from ANOVA's comparing species abundance (number of colonies) across six reef zones. Species that occurred in only one zone are not included. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, ns - not significant.

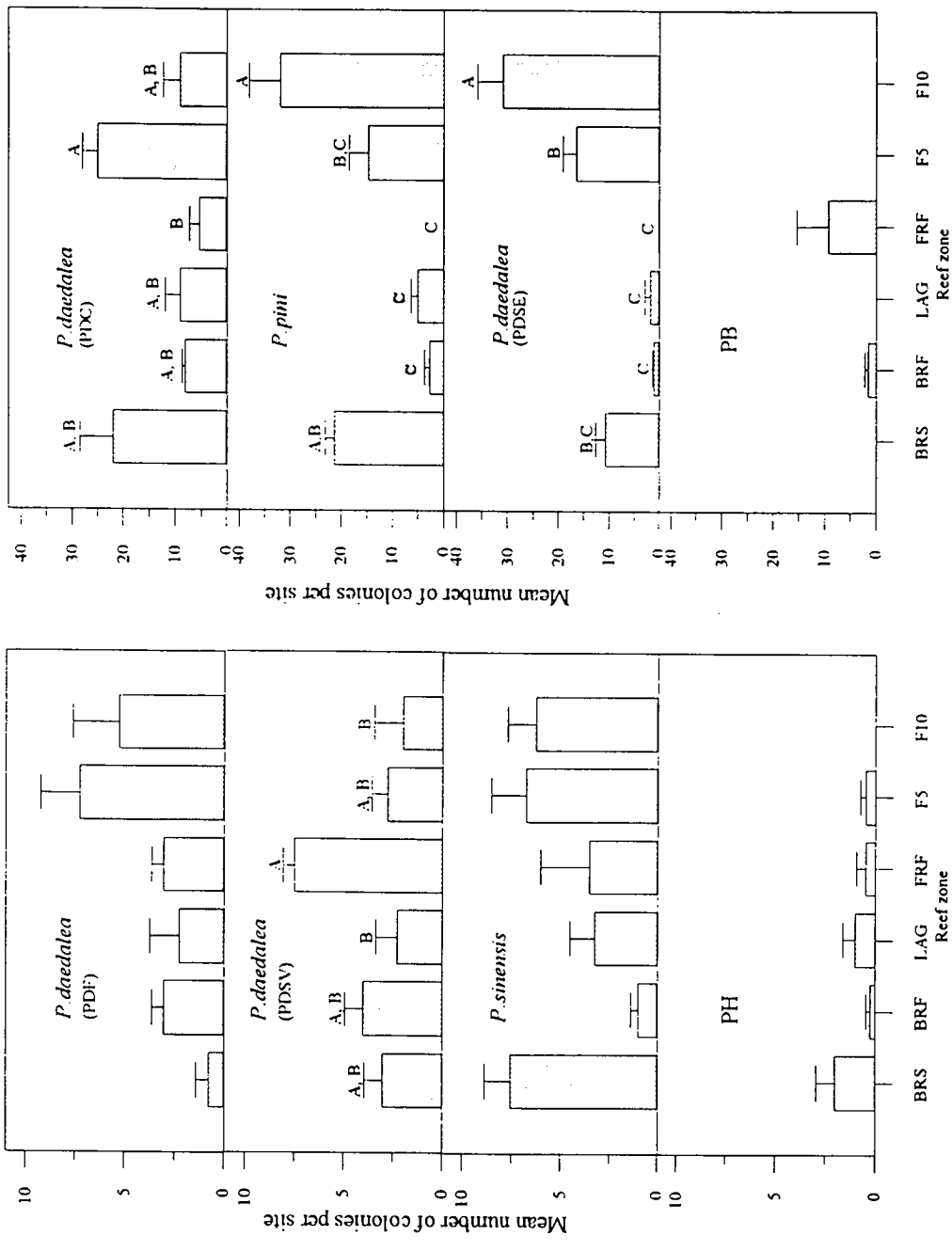


Figure 3.3. Distributions of *Platygyra* species and morphs across six different reef zones at Davies Reef. Sites consist of 8 replicate 20 x 1 metre belt transects. Mean values are based on four sites in each zone. Error bars are standard errors. NB. *P. ryukyuensis* was not found on the transect survey, however colonies were seen on the front reef flat (FRF) adjacent to the transect sites. Similarly, PH was also seen adjacent to the transects at F10 (front reef slope, 10m depth). Letters (A,B,C) represent Tukey's groupings for those species/morphs with significant distributions (see Table 3.3).

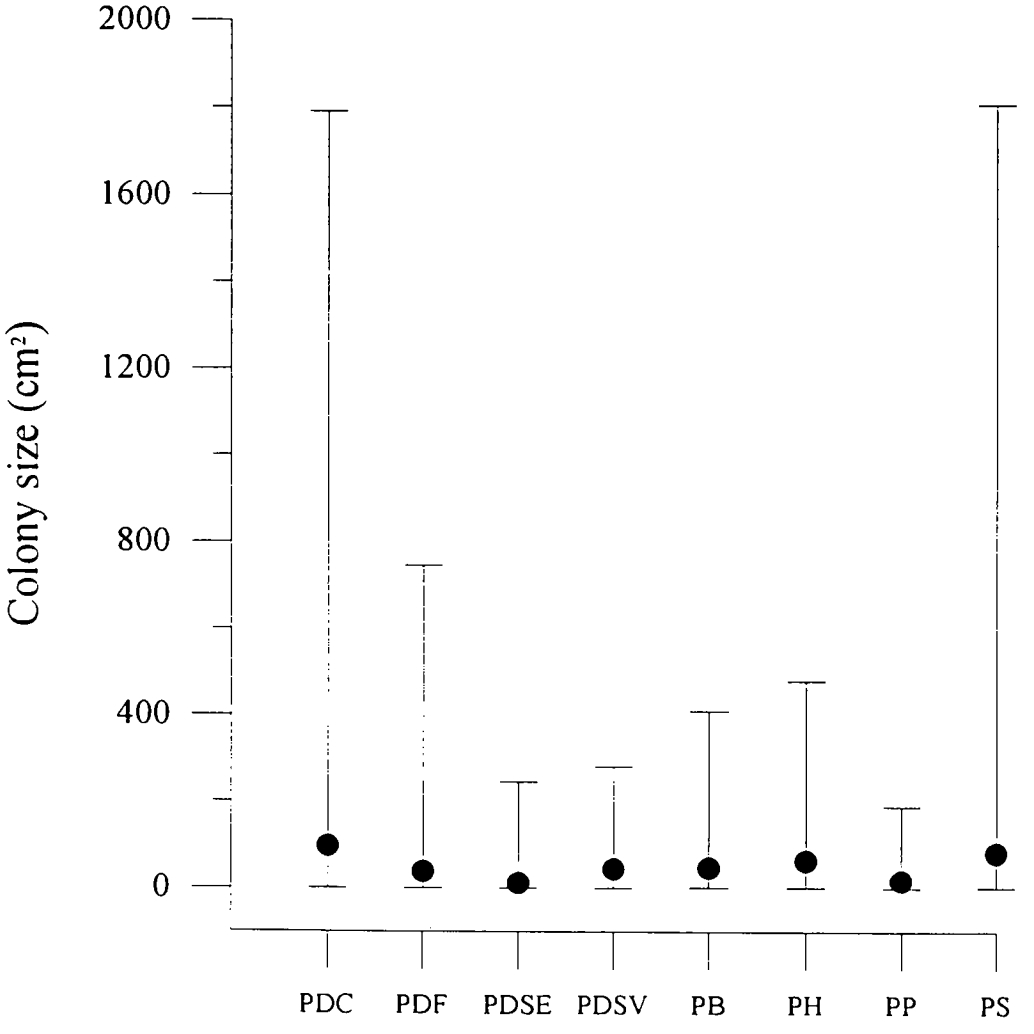


Figure 3.4. Size range of colonies (measured as approximate surface area) of each species of *Platygyra* on Davies Reef (all sites combined). • represents mean colony size for each species. Total number of colonies of each species measured are; PDC - 313, PDF - 86; PDSE - 244; PDSV - 86, PB - 43; PH - 17; PP - 303; PS - 113.

3.3.2.2 *Intra-specific morphological variation across habitats*

Measured skeletal characteristics in *P. daedalea* (morph PDC) and *P. pini* did not differ significantly between reef zones (Table 3.4, MANOVA; *P. daedalea*: Pillai's trace=1.39, F=1.34, df=27, P<0.19; *P. pini*: Pillai's trace=0.96, F=0.73, df=27, P<0.79). A non-significant result may reflect the inadequacy of the test to detect a difference, which in this case may be a result of insufficient replication. However, the initial pilot study indicated 10 replicate measurements of characters on six colonies was more than adequate to represent the population variance (see chapter 2) and variances on most characters are very low (Table 3.4). Therefore, non-significant results are likely to be real. Hence, these results indicate habitat does not affect any of the nine skeletal characteristics measured in these two species.

	P. daedalea				*P. pini*			
	F10	F5	LAG	BRS	F10	F5	LAG	BRS
Valley length	125.5 (110.9)	116.6 (72.4)	74.5 (47.3)	83.2 (55.4)	6.7 (1.4)	6.6 (1.3)	7.0 (1.1)	7.0 (1.2)
Valley width	6.9 (0.8)	6.7 (0.8)	7.2 (0.9)	7.7 (1.0)	5.1 (0.7)	4.9 (0.7)	5.3 (0.7)	5.3 (0.9)
No. septa/cm	9.1 (1.0)	9.6 (1.2)	8.9 (1.1)	8.8 (1.0)	11.7 (1.6)	11.2 (1.3)	11.2 (1.4)	11.4 (1.4)
Columella width	1.09 (0.25)	1.06 (0.31)	1.11 (0.19)	1.28 (0.21)	1.19 (0.26)	1.06 (0.23)	1.11 (0.20)	1.23 (0.21)
Septa thickness	0.27 (0.06)	0.24 (0.04)	0.23 (0.05)	0.27 (0.07)	0.21 (0.04)	0.19 (0.03)	0.22 (0.04)	0.20 (0.05)
Theca thickness	1.59 (0.39)	1.51 (0.38)	1.87 (0.46)	1.71 (0.50)	1.39 (0.26)	1.30 (0.26)	1.49 (0.27)	1.47 (0.40)
Valley depth	5.1 (0.8)	5.1 (0.8)	4.6 (0.9)	5.6 (0.8)	2.3 (0.7)	2.7 (0.7)	2.7 (0.8)	2.8 (0.7)
Exertness of septa	2.4 (0.5)	2.2 (0.5)	2.0 (0.7)	2.4 (0.6)	1.0 (0.7)	1.3 (0.5)	1.3 (0.7)	1.5 (0.6)
Polyp area	48.4 (6.8)	45.9 (4.6)	49.2 (10.1)	54.3 (11.7)	24.0 (6.5)	22.7 (5.3)	26.8 (6.5)	27.9 (8.1)

Table 3.4. Mean values for nine skeletal characters of *P. daedalea* and *P. pini* from four different reef zones on Davies Reef (n=60 for each zone; six colonies of each species with 10 replicate measurements of each character). Standard deviations are in parentheses. All measurements are in mm (except polyp area which is mm^2).

3.4 Discussion

3.4.1 Large scale patterns of distribution

Platygyra is a widespread genus which is common across its range (Veron *et al*, 1977). This is reflected in the distributions described here, although some species appear to be patchy in their distribution (eg. *P. lamellina*, Table 3.1). *Platygyra* has been recorded from Eastern Australia as far south as Lord Howe Island, including Moreton Bay and the Solitary Islands (Veron *et al.*, 1977; Peter Harrison, pers comm). Colonies have also been found in Western Australia, Japan, Papua New Guinea, Vanuatu, Philippines, Singapore, Malaysia, Hong Kong, Vietnam, Indonesia and Thailand (Veron, 1992) in New Caledonia (Chevalier, 1975) and the Red Sea (Vine, 1986). *Platygyra* has been recorded as early as the Eocene, and fossil records exist from Enewetok (Wells 1964), Papua New Guinea (Veron and Kelly, 1988) and Mares Island (Chevalier, 1968) suggesting the distribution of *Platygyra* in the past was also widespread.

Biogeographically, the relative abundances of the species and morphs of *Platygyra* are interesting. While multiple species of *Platygyra* were recorded from most of the reefs I surveyed, only one species, *P. daedalea*, was found on Lord Howe Island and it did not display the range of variation found on the Great Barrier Reef (Table 3.1). This may be associated with limited larval input and founder events in an isolated area at the latitudinal extremes of the species range. Alternatively, it could be due to differential physiological tolerances of the morphs to extremes in temperature variation. Scott Reef (Western Australia) is also isolated, however *Platygyra* populations are diverse and do not appear to differ in gross morphology from populations on the Great Barrier Reef. In contrast to Lord Howe Island, Scott Reef is in a tropical region and has a much less variable climate. In addition, the magnitude and frequency of larval supply to Scott Reef is unknown and may be higher than to Lord Howe Island. Thus, higher larval input coupled with more favourable climatic conditions may account for the differences in diversity of *Platygyra* species on the two isolated reefs.

The relative abundances of some species also differed between reefs. *Platygyra lamellina* was common at Orpheus Island and Scott Reef but was rare elsewhere. Similarly, *P. ryukyuensis* was abundant on inshore reef flats but was less common on offshore reefs. Reasons for the apparent differences in the abundance of these two species across a wide geographic scale can only be speculated upon and may be associated with differential larval distribution, survival and physiological tolerances. More detailed studies of the distributions of *Platygyra* species and morphs (including genetic comparisons) may reveal interesting patterns of large-scale connectivity or separation between geographic regions.

3.4.2 Within-reef distribution patterns of Platygyra

The transect surveys indicate that the majority of *Platygyra* species identified in Chapter 2 occur sympatrically in different zones across the reef (Table 3.2). This distribution pattern suggests that the morphological differences between these species do not occur in response to environmental variables. However, although the seven morphological species are sympatric, there are differences in their relative abundances in the different reef zones (Figure 3.3, Table 3.3). These differences may be accounted for by factors such as larval availability, habitat selection by larvae or differential post-settlement mortality. Low level outbreaks of crown-of-thorns starfish have been occurring on Davies Reef over the past six to eight years which may have affected the distributions of *Platygyra*. However, *Platygyra* is considered a low preference food source (Peter Moran, pers comm). Therefore, while the starfish are likely to have impacted *Platygyra* populations to some extent, the effects on the overall distributions of these species are likely to be relatively low.

The four different morphs of *P. daedalea* were also differentially distributed across Davies Reef (Figure 3.3, Table 3.3)). PDF was uniformly distributed across zones, however PDC was significantly more abundant in F5 than on the FRF. This may reflect the abundances of all corals in these two zones, since coral cover is much higher on the upper reef slope than on the reef flat at Davies Reef (pers obs). PDSE was more abundant in deeper water than in shallow water while PDSV showed the

opposite pattern. Barnes (1973) suggested some coral species may become platelike with increasing depth, perhaps in relation to light availability (see also Dustan 1975, Graus and Macintyre, 1982) and this has been demonstrated experimentally in some species (eg. *Montastrea annularis* Dustan, 1979; *Turbinaria. mesenterina* Willis, 1985). While the morph PDSE, which has a plate-like or encrusting morphology, is most abundant below 5 metres (Figure 3.3) it can be found in all reef zones, in depths ranging from 1 m to 10 m and beyond. Similarly, short-valleyed morphs are also widely distributed across zones (Figure 3.3). Graus and Macintyre (1982) suggested that the presence of plate-like *Montastrea annularis* colonies in shallow water was due to patchy areas of low light levels. In terms of *Platygyra* distributions, such an explanation would seem unlikely as the converse situation of rounded colonies in deep water (eg. PDC, PDSV, PDF) is probably not due to patches of increased light. Based on these results, difference between environments cannot be the primary factor responsible for the observed variation in colony morphology in *P. daedalea* (see also Miller, in press).

3.4.3. Skeletal variation across habitats

Intra-specific morphological variation in corals is often correlated with environmental influences (eg. Foster, 1980; Haggerty *et al.*, 1980; Oliver *et al.*, 1983; Willis, 1985). Within *Platygyra* species, however, there appears to be no significant skeletal variation in either calicular characters or colony morphology that is related to habitat. Skeletal characters of colonies of both *P. daedalea* (morph PDC) and *P. pini* from four different reef zones were very similar (Table 3.5). Other species of *Platygyra* were also sympatric (Table 3.2, Figure 3.3) and there were no obvious changes in their morphology between reef zones. The absence of any association between morphology and habitat in this study differs from the findings of Wijsman-Best (1974) who found decreases in the number of corallites per meander and the number of septa per cm with increasing depth in *P. daedalea.* As discussed in Chapter 2, geographic variation or differences in sampling regime may account for the discrepancies between my results and those of Wijsman-Best. Observations by Veron *et al.* (1977) also suggested the morphology of *P. daedalea* varied predictably

between reef habitats which has not been demonstrated here. However, the morphs of *P. daedalea* described in this study do have differential distributions across reef habitats and these differences could be perceived as environment-related variation at a qualitative level. As discussed above, the quantitative survey of distributions of *P. daedalea* morphs has shown that all four morphs are sympatric in different reef zones. Therefore environment cannot be the primary influence on morphology in this species. Similar studies on some other coral species have also found little or no relationship between environment and variation in skeletal characters or coral growth (eg Brakel, 1976; 1977; Isdale, 1977). Thus, while phenotypic plasticity is an environmental response in many species of corals, it is clear that factors other than environment control morphology in some coral species, including *P. daedalea.*

An alternative method to determine environmental effects on morphology is to conduct transplant experiments (eg. Foster, 1979a; Oliver *et al.*, 1983; Willis, 1985). Similar experiments were not feasible with colonies of *Platygyra* due to their extremely slow growth rates. Babcock (1991) recorded mean growth rates of adult *P.sinensis* colonies at Orpheus Is and Magnetic Is. between 6 and 7 mm per year. At these rates, it would be well in excess of three years before any changes in the morphology of transplanted colonies could be observed.

3.4.4. Micro-environmental effects on morphology

The results presented here relate only to environmental effects on a large scale (across reef) and do not address the possibility of micro-environmental effects on morphology. Small-scale (ie. within zone) influences may be important, particularly in highly heterogeneous environments such as coral reefs. Observations of living colonies *in situ* suggest micro-environment has little effect on overall colony morphology. For example, a single *P. daedalea* colony can reach several metres in diameter (the largest recorded at Davies reef in this study had a diameter of 1.64 metres). A colony this large will be subject to a number of different micro-environments. However, in cases I have observed, skeletal characters remain uniform and do not vary with factors such as light, depth or wave action in terms of position

on the colony. Prevailing environmental conditions at the time of settlement could potentially affect the growth form of a colony, however this effect may be masked due to sampling a population comprising a number of different cohorts. Finally, it is unlikely that colony morphology changes with size as both small and large colonies of all species and morphs were seen on the reef (Figure 3.4).

3.4.5. Conclusions: the effects of environment on Platygyra morphology

The results from this chapter confirm the existence of seven discreet morphological species of *Platygyra*. These species occur sympatrically in different reef zones and are widely distributed geographically. The absence of any association between morphology and habitat in *Platygyra* suggests that the environment is not the dominant influence on morphology in these species. However, some of these species are significantly more common in certain habitats suggesting that environment may have some effect on their distribution.

CHAPTER 4

REPRODUCTIVE RELATIONSHIPS BETWEEN MORPHOLOGICAL SPECIES OF *PLATYGYRA*

4.1. Introduction.

4.1.1. Reproduction, hybridisation and isolating mechanisms in scleractinian corals

The majority of corals on the Great Barrier Reef reproduce annually during a mass spawning event (Harrison *et al.*, 1984; Willis *et al.*, 1985; Babcock *et al.*, 1986). Many aspects of the mass spawning phenomenon have been investigated for a variety of coral species, including gametogenic cycles (eg. Kojis and Quinn, 1981a; 1982; Babcock, 1984; Harriott, 1983), timing cues (eg. Harrison *et al.*, 1984; Willis *et al.*, 1985), fertilisation ecology (Oliver and Babcock, 1992), larval development (eg. Kojis and Quinn, 1981b; Babcock and Heyward, 1986), larval dispersal (Oliver and Willis, 1987; Willis and Oliver, 1990) and subsequent recruitment (eg. Wallace and Bull, 1981; Wallace, 1985; Babcock, 1988) (see also reviews by Fadlallah, 1983; Harrison and Wallace, 1992).

More than 133 different species of coral have been recorded spawning during the annual mass-spawning (Willis *et al.*, 1985). Such multi-species spawning events, whereby gametes of many species become mixed within the water column, infer the presence of some mechanism to prevent hybridisation and hence maintain species boundaries (Willis, 1990). In *Montipora*, hybridisation is considered to be maladaptive and is thought to result in gamete wastage due to arrested development in hybrid embryos (Hodgson, 1988). More recently however, fertilisation experiments have indicated hybridisation is possible *in vitro* between many coral species including *Platygyra* spp. (Willis *et al.*, 1992) although how experimental fertilisation relates to field fertilisation is unknown.

Isolating mechanisms preventing hybridisation in coral species are likely to be varied. Sperm chemotaxis is used by a variety of marine invertebrates to attract con-specific sperm (eg Miller, 1979; 1985) and chemical sperm attractants have been implicated as a mechanism to reduce hybridisation in the genus *Montipora* (Coll *et al.*, in press). However, little is known of sperm chemotaxis even in *Montipora* (Coll *et al.*, in press; Richard Miller, pers comm), and its role in preventing hybridisation during mass-spawning can only be speculated. Species of *Platygyra* are hermaphroditic broadcast spawners that spawn once a year during the mass spawning event, generally 4-5 nights following the first full moon in October or November on the Great Barrier Reef (Harrison *et al.* 1984, Babcock *et al.* 1986). All five species (*P. daedalea*, *P. sinensis*, *P. pini*, *P. lamellina* and *P. ryukyuensis*) spawn on the same night, although there is some suggestion of temporal differences in spawning times between species. Babcock *et al.* (1986) recorded the spawning time of *P. lamellina* (2 hrs and 40 minutes following sunset), *P. pini* (1 hr 15 minutes and 1 hr 40 minutes following sunset) and *P. sinensis* (between 3 - 4 hours after sunset). These observations only involved a few colonies and it is not known how cohesive species spawning times are between colonies. Gametes may be viable for at least 5 hours in the water column (Oliver and Babcock, 1992). Thus it is unlikely that the observed time differences act as a substantial barrier to hybridisation. In Japan, *Platygyra* spp have been recorded spawning 2-3 nights (*P. pini*) and 7-8 nights (*P. ryukyuensis*) following full moon (Heyward *et al.*, 1987). While differences of a number of days between species are likely to be effective isolating mechanisms, more recent observations show there is overlap in spawning days between some species of *Platygyra* in Japan (Hayashibara *et al.*, 1993). In the Red Sea, records exist only for *P. lamellina*, which spawns 3-5 nights after the full moon (Shlesinger and Loya, 1991).

4.1.2. Reproduction and embryogenesis in Platygyra species

Spawning *Platygyra* colonies release gamete bundles containing both eggs and sperm which break apart on the water's surface releasing the gametes for

fertilisation (Babcock *et al.*, 1986). Eggs cannot, however, be fertilised until 20 - 30 minutes after spawning (Babcock and Heyward, 1986; Shlesinger & Loya, 1991) and this may be a mechanism to prevent or minimise self-fertilisation which has been recorded in some species of corals (Heyward and Babcock, 1986; Stoddart *et al.*, 1988). Sperm clots have been observed on the surface of *Platygyra* eggs (Babcock and Heyward, 1986; personal observation) although the site of fertilisation is unknown for these species. Development of *Platygyra* embryos has been well documented. Initial cleavage takes place approximately 2-3 hrs after fertilisation and follows a radial pattern eventually becoming pseudospiral (Babcock and Heyward, 1986; Shlesinger & Loya, 1991). Multi-cellular blastulae develop within 6-8 hrs after which pharynx invagination occurs, the gut cavity is formed (24 - 36 hrs) and embryos develop into ciliated larvae within 48hrs (Babcock and Heyward, 1986; Shlesinger & Loya, 1991). Ciliated larvae are competent to settle after 3-4 days and the majority of larvae will settle within a week (Babcock, 1985; Babcock and Heyward, 1986).

4.1.3. Species boundaries and reproductive isolation in Platygyra

Morphologically and ecologically, there are seven species of *Platygyra* on the Great Barrier Reef. However, to fully understand species boundaries within the genus *Platygyra*, it is important to determine if morphological species are reproductively isolated. Similarly, if species of *Platygyra* are reproductively isolated, some mechanism must exist to prevent hybridisation during a mass spawning.

Due to the high predictability of spawning in *Platygyra* species, it is possible to explore reproductive relationships between these corals by conducting experimental fertilisation trials and larval rearing in the laboratory. Results from such trials will demonstrate if there is potential for hybridisation and self-fertilisation among the morphological species of *Platygyra* and also test the ability of any hybrids to develop as embryos and subsequently settle and grow. Observations of egg-sperm interactions will provide evidence of any species-

specific sperm attraction in eggs of *Platygyra* which may act as a potential isolating mechanism in these corals. At the same time, field observations of spawning times will be used to determine if any temporal barriers to hybridisation exist between the morphological species. Genetic comparisons of colonies used in the fertilisation trials will determine if there is any genetic structuring related to breeding groups within the genus, independent of morphology. Thus the aim of this chapter is to use a number of different fertilisation trials and experimental methods to determine if barriers to gene flow exist between the ten different morphological groups of *Platygyra* or, alternatively, if morphological groups are capable of interbreeding.

4.2 Methods

4.2.1 Study sites and colony collection

Corals on inshore reefs around Townsville (eg Magnetic Island) generally spawn one month earlier than offshore reefs, associated with an earlier increase in water temperature (Babcock *et al.*, 1986). It is therefore possible to exploit two spawning periods in any one year, the inshore spawning and the offshore spawning. Fertilisation experiments were done over a 3½ year study period during seven consecutive spawnings; at Magnetic Island (1990, 1991, 1992, 1993), Davies Reef (1990, 1991) and Orpheus Island (1992).

Coral colonies that are ripe and hence likely to spawn at the predicted time are readily identified by the presence of pigmented eggs within the polyps. These can easily be seen by chipping off a small portion of the coral colony and examining polyps underwater. Using this technique over the three to four days prior to the predicted mass spawning event, ripe colonies of *Platygyra* were identified and usually relocated underwater to a central collection point. Colonies of *Leptoria phrygia* (LP) and *Goniastrea aspera* were also collected for use in fertilisation experiments. On the afternoon of the predicted spawning, all colonies were transferred individually into separate buckets on the beach (or back deck of boat

depending on the reef) and kept under natural light to simulate normal conditions. Colonies generally commenced spawning from dusk onwards.

4.2.2. Fertilisation Trials

4.2.2.1. Experimental methods

Once a colony had spawned, gamete bundles were sucked from the surface of the water using a wide-mouthed glass pipette and collected in a beaker in order to isolate eggs and sperm from colonies of different morphotypes. Gamete bundles were gently agitated to facilitate their breaking up, imitating the normal effects of surface wave action on the bundles. Once all bundles had broken up, sperm and eggs were separated using a 105µm sieve. Sperm were stored in a concentrated form at room temperature (approximately 27°) until required for fertilisation. Eggs were taken through a series of between 8 and 10 washes of 'sperm-free water' (water collected ½ a kilometre off the edge of the reef in the afternoon prior to the commencement of spawning) to ensure the absence of any trace sperm. Eggs were stored in glass jars and agitated with a gentle flow from an aquarium aerator until needed for experiments.

Once separated, eggs and sperm from different colonies were combined experimentally in 20 ml glass vials to examine fertilisation success between colonies of different morphologies. Concentrations of the stored sperm were calculated using an haemocytometer and, just prior to fertilisation, diluted to a concentration of approximately 2.5×10^6 sperm per ml which is optimal for fertilisation (Oliver and Babcock, 1992). Approximately 100 eggs were added to 20 ml of diluted sperm in a glass scintillation vial which had been conditioned in sea water for 24 hrs prior to the experiment. Each cross between two colonies was replicated three times to allow for any effects of vial contamination that might affect fertilisation success. Experiments were designed in a grid system (see Figure 4.1) in which all colonies were crossed reciprocally with all other colonies (both eggs A x sperm B and eggs B x sperm A etc.). All colonies were

also tested for ability to self-fertilise (eggs A x sperm A, eggs B x sperm B etc.). Replicate controls were done by placing eggs from each colony in 20 ml of sperm-free water to indicate whether there had been any sperm contamination of washed eggs. Glass vials containing eggs and sperm were kept at ambient sea temperature with gentle agitation to allow fertilisation to take place. This was achieved by placing vials in a mesh bag buoyed with floats (designed to keep the bag at the surface of the water) and attaching the bag to a mooring on the reef.

Fertilisation in all replicate vials was recorded after 2-3 hours (first count) and again after 6-8 hours (second count) by censusing the first 100 eggs in any one vial. Vials were returned to the reef between counts. Fertilisation of the egg could easily be seen as the initiation of cleavage, apparent 2-3 hrs after fertilisation (Babcock and Heyward, 1986). After approximately 7 hrs eggs underwent multiple divisions and developed into blastulae. Repeated observations determined if fertilised eggs were developing normally. Similarly, any increase in the number of fertilised eggs with increased egg-sperm contact time was monitored. The 'health' of both eggs and embryos was recorded as either 'regular' or 'irregular'.

Eggs → / Sperm ↓	Colony A (species 1)	Colony B (species 1)	Colony C (species 2)
Colony A	self	WITHIN-SPECIES	**HYBRID**
Colony B	WITHIN-SPECIES	self	**HYBRID**
Colony C	**HYBRID**	**HYBRID**	self
No sperm	control	control	control

Figure 4.1. Experimental design of fertilisation trials to examine rates of within-species fertilisation and hybridisation between species. Each colony was crossed reciprocally with all other colonies and all crosses were replicated three times. Tests for self fertilisation involved combining eggs and sperm from the same colony. Eggs were placed in sperm-free water to control for contamination of washed eggs.

4.2.2.2. Analysis of fertilisation data

Fertilisation rates between any two colonies in count 1 were expressed as a percent, based on the number of fertilised eggs and the total number of fertilised and unfertilised eggs. Percent fertilisation from count 2 was calculated in two ways; a) based on the total number of fertilised and unfertilised eggs in the first count in order to look at changes in the percent fertilisation over time (percentages were based on the total from the first count as unfertilised eggs often lysed by the time the second count is carried out) and b) based only on the total number of fertilised eggs from the first count to determine what proportion of cleaved eggs have developed through to blastulae. This calculation will be referred to as count 3. Mean percent fertilisation of the three replicate vials in each cross (colony pairing) was used for analyses. Results from the seven spawnings at three reefs were combined at the level of morphological species for analysis.

Due to the logistic complications associated with fertilisation experiments, the resultant data sets are unbalanced. *Platygyra* colonies only spawn over one or two nights per year and there are physical (and mental!) limitations to the number of colonies that can be crossed in any one night. Not all collected colonies will spawn on the desired night, hence the actual crosses done tend to be somewhat opportunistic despite original systematic intentions. The number of times colonies of any two species were crossed over the entire study period varies considerably from one (PB x PDC) to thirty-one (PDC x PDC) (see Table 5.1). Although replication is unequal and will result in a reduction of power of statistical analyses, strong patterns will still result in significant differences. Because fertilisation rates were expressed as a percent, initial analyses were done on both raw data (ie percentages) and arcsine transformed data. Both produced identical results hence the final analyses and results presented are based only on untransformed data.

A two-way analysis of variance (ANOVA) based on the percent fertilisation data from count 1 was carried out to a) determine if hybrid fertilisations differed from within-species fertilisation rates and b) to determine if there were any differences in hybrid and within-species fertilisation rates between the different morphological species (based on maternal colony with all morphs of *P. daedalea* pooled). The main effects were fertilisation type (2 treatments; hybrid and within-species) and egg species (7 treatments; PB, PD, PH, PL, PP, PR, PS). The analysis was done using SAS proc GLM which handles unbalanced designs. The analysis was repeated using the data from count 2. A further two-way ANOVA was carried out on data from count 3 to compare the development of cleaved eggs through to blastulae in both hybrid and within-species fertilisations for each different species.

Fertilisation rates in counts 1 and 2 were compared to determine if hybridisation or within-species fertilisation increased with increased egg-sperm contact time. Similarly, the incidence of irregular and regular embryos was compared between hybrid and within-species crosses (for both count 1 and count 2) to determine if irregular embryos were more common in hybrid crosses.

Self-fertilisation rates were compared between species and between counts 1 and 2 for each species to determine if self-fertilisation increased with egg-sperm contact time. The difference between percent fertilisation rates between counts 1 and 2 (ie % fertilisation (count 2) - % fertilisation (count 1)) for each species was compared using a one way ANOVA. The main effect was egg species and there were 7 treatments; PD, PH, PL, PP, PR, PS, LP. Similarly, the difference between counts was assessed for significant departure from zero using the method of visual comparison of mean differences as described by Andrews *et al.* (1980). A difference of zero between fertilisation rates from count 1 to count 2 will indicate no change in self-fertilisation with time. Hence if the difference in fertilisation between counts is not significantly different to the hypothetical zero population, it can be concluded there is no difference in self-fertilisation between counts.

4.2.3. Effect of egg age on fertilisation results - a test of experimental methods

One of the potential artefacts of the experimental method described above (section 4.2.2.1) is the delay between spawning and fertilisation time. Oliver and Babcock (1992) showed fertilisation rates decreased dramatically in *P. sinensis* 4-5 hour after spawning. Due to the complex washing procedures and the nature of the experiments, it can be up to six hours after spawning before eggs and sperm are combined in fertilisation trials. This time delay may be seriously influencing observed fertilisation results. Sperm life can be prolonged by keeping it highly concentrated and this treatment does not adversely effect sperm mobility once diluted (Chia and Bickell, 1983; Oliver and Babcock, 1992). The effect of age on the egg, however, is unknown. It is possible that as an unfertilised egg increases in age, species specificity (in terms of fertilisation) may be lost.

To test if egg age was influencing the results of fertilisation trials conducted previously, an experiment was carried out to look at hybrid and normal fertilisation rates in eggs of varying ages. The aim was to cross eggs from a colony within half an hour of spawning and to repeat the same egg-sperm cross

every two hours, keeping sperm samples concentrated so only the egg was effectively ageing. Three colonies each of *P. daedalea* and *P. sinensis* from Magnetic Island were used. Once two colonies had spawned, gametes were washed as described above (section 4.2.2.1) and immediately crossed with each other. Remaining eggs and sperm were then stored as in normal experiments. As each successive colony spawned, gamete bundles were washed and immediately crossed with all other colonies that had already spawned. This was repeated until all six colonies had spawned, after which all crosses were repeated at two hourly intervals until eggs were 8 hours old. All crosses were reciprocal and each cross was replicated twice at each time interval. Controls with sperm-free water were done at all stages of the experiment. Fertilisation (in the first 100 eggs) was recorded after 2-3 hours and again after 6-7 hours. Regression analysis was used to detect general trends associated with the effect of egg age on fertilisation success for all cross types (within-species, hybrid, self and control). Percent fertilisation of hybrid crosses and normal crosses at various egg ages were compared for both *P. daedalea* and *P. sinensis* at both count 1 and count 2.

4.2.4. Temporal separation in spawning times

To determine if any real temporal separation existed in the spawning of morphological species of *Platygyra*, colonies were observed *in situ* during two spawning periods. In November 1992 at Pioneer Bay, Orpheus Island, up to six ripe colonies of various species and morphs of *Platygyra* were relocated (with a minimum of handling) to a central point in the bay. These colonies and nearby un-manipulated colonies were then monitored continually over a six hour period following sunset, recording the natural spawning times. In November 1993 at Geoffrey Bay, Magnetic Island, 45 tagged ripe *Platygyra* colonies (un-manipulated) were monitored for spawning activity. *In situ* spawning times were compared with spawning times of experimental colonies held in buckets in both instances to determine if spawning times of manipulated colonies reflected actual spawning times.

4.2.5. Observations of sperm-egg interactions.

Egg-sperm interactions were monitored in gametes from two colonies of *P. daedalea* and two colonies of *P. sinensis* for a preliminary investigation of species-specific sperm attraction (possibly chemical) as has been documented in other species of corals (Coll *et al.*, in press). These observations were carried out at Magnetic Island in 1993. Washed gametes of individual colonies were combined in a 20 ml vial and preparations (containing up to 12 eggs) of within-species, hybrid and self crosses were observed under a compound microscope for any signs of sperm activity or sperm aggregations at the egg surface. Sperm attraction is usually obvious as increased activity of sperm around one region of the eggs surface (which may be associated with the site of polar body release; Richard Miller, pers comm). Preparations were monitored for up to six hours to follow the development of eggs through sperm attraction, fertilisation and subsequent cleavage. Video records were taken at regular intervals.

4.2.6. Fertilisation success as a function of genotype

Tissue samples from all colonies used in the fertilisation trials in 1991 and 1992 were collected for genetic analysis (see chapter 5) to determine if there was any genetic structuring, independent of morphology which was associated with breeding groups within the genus. The genetic difference between mated colonies, based on the nine-loci genotype, was calculated as a percentage based on the number of different alleles (ie alleles not shared by the two colonies). The fertilisation success between any two colonies and the genetic difference between them was then compared graphically.

4.2.7. Larval rearing

In addition to the fertilisation experiments described above (4.2.2.1.) large numbers (>1000) of both hybrid and within-species larvae were reared and allowed to settle to monitor their subsequent development through planulae to

polyp. I also intended to compare the genotype of hybrid colonies to that of the two parent colonies. Larvae were raised using the methods described by Babcock and Heyward (1986). Excess eggs and sperm from fertilisation trials were combined as "bulk" crosses in two litre plastic jars with plankton mesh inserts in cut out lids. Jars were maintained from an anchor rope on the water surface for up to five days after which larvae were transferred to aquaria at the Australian Institute of Marine Science. Larvae were kept in closed aquaria which were aerated to provide surface agitation and water was changed every other day. Settlement substrata (either coral rubble or clay paving tiles) were provided. Once all larvae had settled, settlement plates were transferred to a flow-through aquarium system and the progress of colonies was monitored.

4.3 Results.

4.3.1. Fertilisation trials

4.3.1.1 Intra-generic crosses

The fertilisation trials between colonies of *Platygyra* show that hybridisation is possible between all the morphological species tested (Table 4.1). The nine distinct morphological species tested were, in general, highly intercompatible and mean fertilisation rates among different species were similar (usually between 50% and 70%, Table 4.1). However, fertilisation success between some species and morphs was depressed. PDF and PDC had very low fertilisation rates when crossed, with reciprocal mean fertilisation rates of only 0.4% and 2.6% (n=6 pairs, Table 4.1). Similarly, PDC eggs x PDSE sperm (n=4 pairs), PH eggs x PDC sperm (n=5 pairs) and PP sperm (n=3 pairs), PP eggs x PS sperm (n=2 pairs) and PB eggs x PDC sperm (n=1 pair) had fertilisation rates less than 20%. Fertilisation success was highly variable between and within species, with most differences occurring at the individual level eg. fertilisation success between any two PDC colonies ranged from 0% to 100% (Table 4.1). The low fertilisation rates between the species and morphs mentioned above are within the range

observed between colonies of the same species (Table 4.1) and may be influenced by individual differences in compatibility and small sample sizes rather than a reflection of overall species incompatibility. Larvae produced by crosses with low fertilisation rates were generally regular in appearance and appeared to develop normally.

♂ \ ♀	PDC	PDF	PDSE	PH	PL	PP	PR	PS	PB
PDC	67.9 (0-100) n= 31	0.4 (0-4.9) n=6	73.5 (38-94) n=4	18.9 (0-58.9) n=5	69.1 (41-89) n=11	65.3 (0-100) n=10	47.6 (0-84.1) n=4	63.8 (0-99.1) n=22	1.6 (0-6.3) n=1
PDF	2.6 (0-13.2) n=6	52.9 (26-74) n=2	-	-	-	-	-	-	-
PDSE	10.3 (0-53.3) n=4	-	48.4 (1.2-95) n=2	-	83.8 (78-90) n=2	-	32.0 (1.8-57) n=4	70.5 (41-87) n=4	-
PH	47.2 (0-94.2) n=5	-	-	50.7 (9 -95.8) n=2	-	23.4 (0-62.5) n=4	-	72.3 (0-100) n=2	-
PL	63.5 (1-100) n=11	-	86.5 (78-95) n=2	-	58.2 (41-84) n=6	-	63.6 (40-77) n=2	72.3 (41-91) n=2	-
PP	66.4 (6.5-99) n=10	-	-	17.3 (0-41.8) n=3	-	57.3 (1-96.7) n=4	-	76.5 (26-98) n=2	-
PR	63.9 (7-100) n=4	-	68.8 (32 -93) n=4	-	90.5 (90-92) n=2	-	57.7 (31-71) n=2	70.6 (27-92) n=4	-
PS	66.4 (0-98.8) n=24	-	62.0 (0-100) n=4	27.1 (13 - 44) n=2	45.4 (0-90.8) n=2	7.3 (0-27.7) n=2	65.9 (7-100) n=4	68.7 (0-100) n=14	-
PB	44.2 (31-57) n=1	-	-	-	-	-	-	-	-

Table 4.1. Mean fertilisation rates from fertilisation trials between morphological species and morphs of *Platygyra* based on all crosses tried over the 3½ year study period. n = number of crosses ie. colony pairs. All crosses between colony pairs were replicated three times and the range of % fertilisation over all replicates of all crosses is presented in parentheses. '-' denotes cross not yet tried. PDC - *P. daedalea* (classic morph), PDF - *P. daedalea* (fat morph), PDSE - *P. daedalea* (encrusting morph), PL - *P. lamellina*, PP - *P. pini*, PR - *P. ryukyuensis*, PS - *P. sinensis*.

Crosses between species were generally reciprocal ie eggs and sperm from two different species readily fertilised each other. However, this was not apparent in PP and PS crosses where PS eggs were readily fertilised by PP sperm (76.5% fertilisation,) while PP eggs were poorly fertilised by PS sperm (7.3% fertilisation) (Table 4.1). A similar pattern existed between PB and PDC (Table 4.1). Again sample sizes for these crosses are low (n=2 pairs, PP x PS; n=1 pair, PB x PDC) and they should be repeated using different colonies to determine if this pattern is consistent.

The overall rates of hybridisation (all species pooled) were not significantly different to the rates of within-species fertilisation (Table 4.2, Figure 4.2) suggesting hybridisation can occur at the same rate as cross-fertilisation in *Platygyra*. Overall fertilisation (hybrid and within-species crosses combined) compared between species was not significantly different in count 1 (Table 4.2). However, significant differences in mean fertilisation rates were observed between species in both counts 2 and 3 (Table 4.2). *Platygyra lamellina* had significantly more fertilisations than both *P. daedalea* and *P. ryukyuensis* in count 2. Similarly *P. sinensis* had higher survivorship of embryos than *P. daedalea* and *P. ryukyuensis* in count 3 and *Platygyra lamellina* had greater survivorship than *P. daedalea* in count 3.

Within-species fertilisation rates among the different species were not significantly different by ANOVA (Table 4.2; see also Figure 4.3). Similarly, rates of hybridisation did not differ significantly among species (Table 4.2, Figure 4.3). There was also no difference in patterns of hybridisation rates or within-species fertilisation rates between counts 1 and 2 (Figures 4.2, 4.3), hence fertilisation (either hybrid or within-species) did not increase significantly with egg-sperm contact time. Approximately 80% of all fertilised eggs in the first count had developed into blastulae by the second count and hybrid embryos survived at the same rate as non-hybrid embryos (Figure 4.3).

A comparison of regular and irregular fertilisations and embryos for both count 1 and count 2 showed the number of irregular fertilisations were similar in both hybrid and within-species crosses and between counts one and two (Figure 4.4). Irregular embryos made up approximately 10-15% of the 100 eggs counted (fertilised and unfertilised) in both count 1 and count 2, whereas regular fertilisations were, on average, 40-50% of the 100 counted. The occurrence of irregular larvae does not appear to be due to their hybrid origin and may simply be a consequence of the experimental method (see Oliver and Babcock,1992).

	Source of variation	df	Mean Square	F	Pr > F
count 1	fertilisation type	1	76.35	0.08	0.77 (ns)
	egg species	6	1475.81	1.64	0.13 (ns)
	fert-type x egg species	5	1578.38	1.75	0.12 (ns)
count 2	fertilisation type	1	1596.25	0.53	0.46 (ns)
	egg species	6	6610.83	2.20	0.04 *
	fert-type x egg species	5	1262.52	0.42	0.83 (ns)
count 3	fertilisation type	1	1012.43	1.03	0.31 (ns)
	egg species	6	4511.54	4.60	0.00 ***
	fert-type x egg species	5	567.12	0.59	0.71 (ns)

Table 4.2. Results from multiple two-way analyses of variance to compare fertilisation success in different species (both hybrid and within-species) for counts 1, 2 and 3. (ns) - not significant, * $P<0.05$, *** $P<0.001$.

The ability of a coral to self-fertilise appears to vary from individual to individual, but the mean rate of self-fertilisation was consistently low in the first count (Figure 4.5). While the mean self-fertilisation rate is higher in the second count than in the first count, differences between count 1 and count 2 did not vary significantly from zero for any of the species (Figure 4.6). This result demonstrates that there was no overall change (increase or decrease) in the rate of self fertilisations with time. The differences in fertilisation rates between the first and second counts were not significantly different between species (ANOVA; df = 6, MS=1730.73, F=0.82, Pr>F=0.5595) ie. all species had equivalent rates of selfing (see also Figure 4.6).

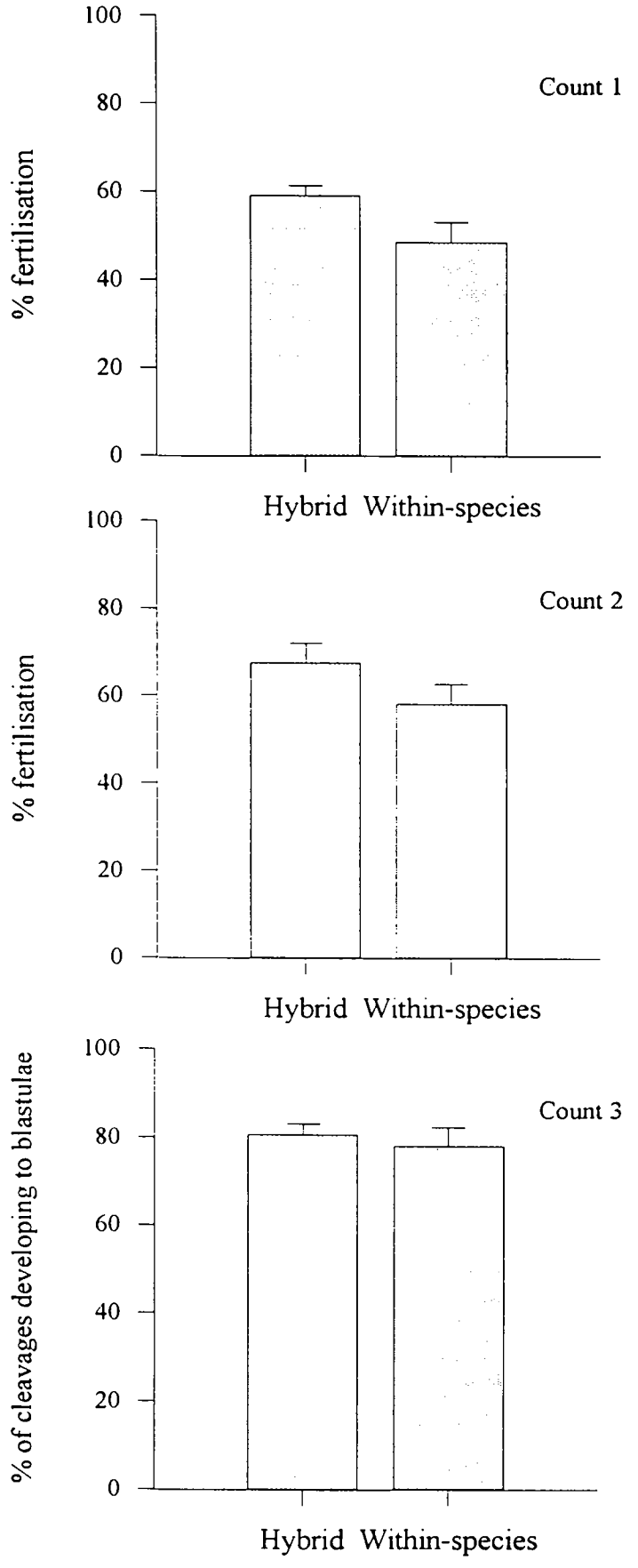


Figure 4.2. Mean rates of within-species fertilisation and hybridisation in fertilisation trials between morphological species of *Platygyra*. Bars denote standard errors.

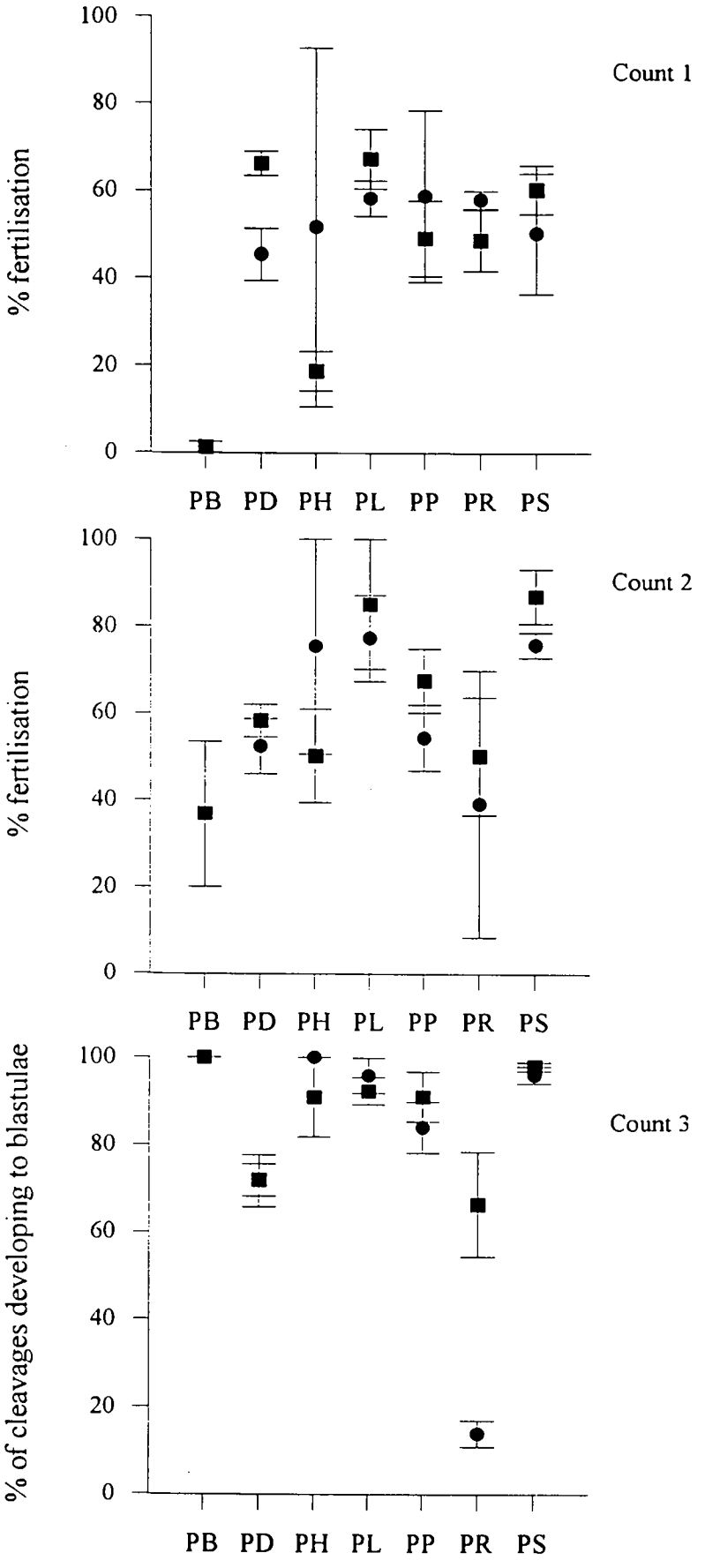


Figure 4.3. Mean rates of within-species fertilisations and hybridisation in seven morphological species of *Platygra*. Bars denote standard errors. ■ - hybrid crosses; ● - within-species crosses.

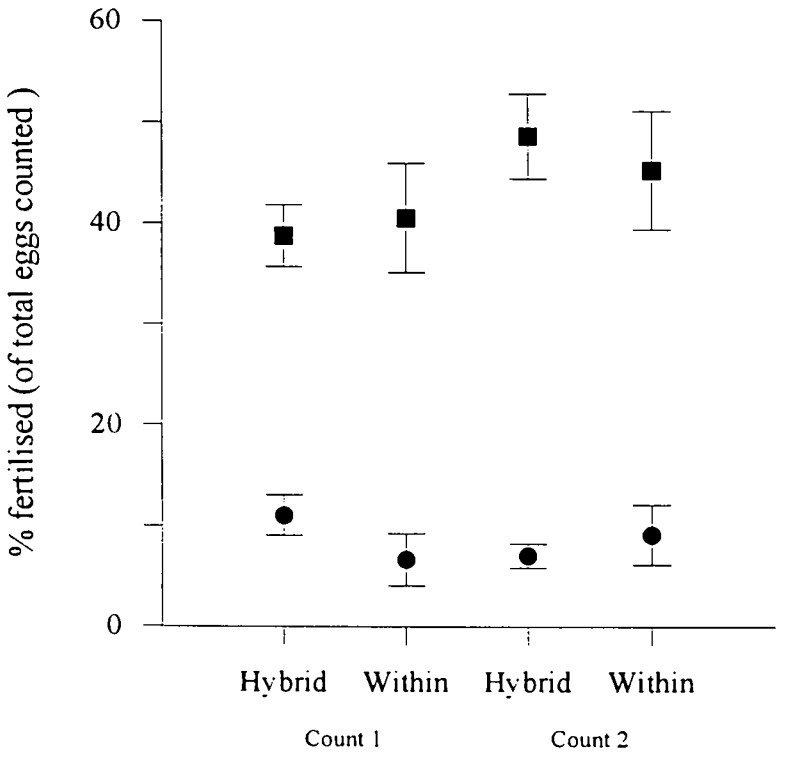


Figure 4.4. Occurrence of regular and irregular embryos in both hybrid and within-species fertilisation trials expressed as a percent of the first 100 eggs counted per vial (all species pooled). Bars represent 95% confidence limits. ■ - regular embryos; ● - irregular embryos.

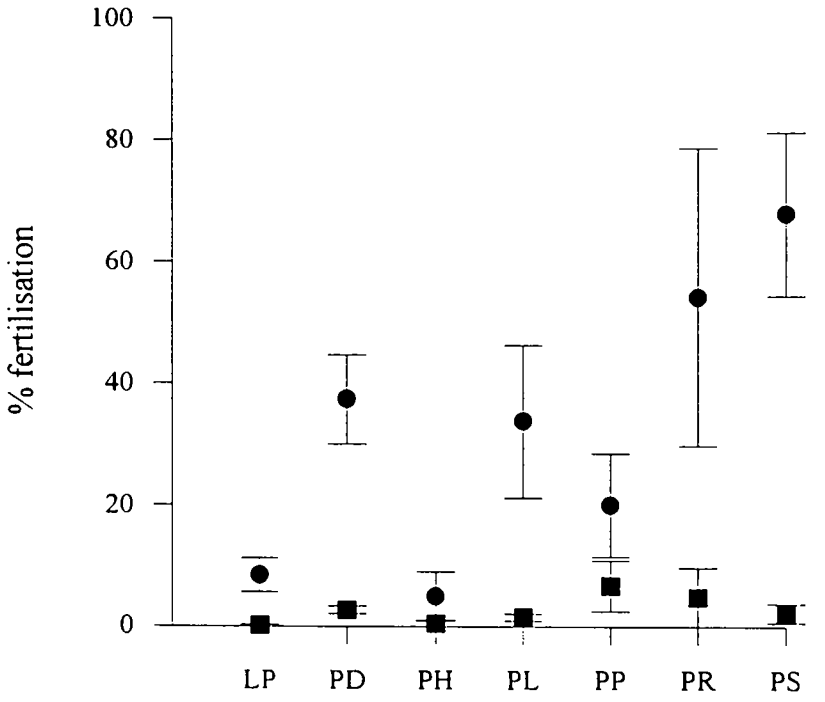


Figure 4.5. Mean self-fertilisation rates for six species of *Platygyra* and *Leptoria phrygia* at both count one and count two. Bars denote standard errors. ■ - count 1; ● - count 2

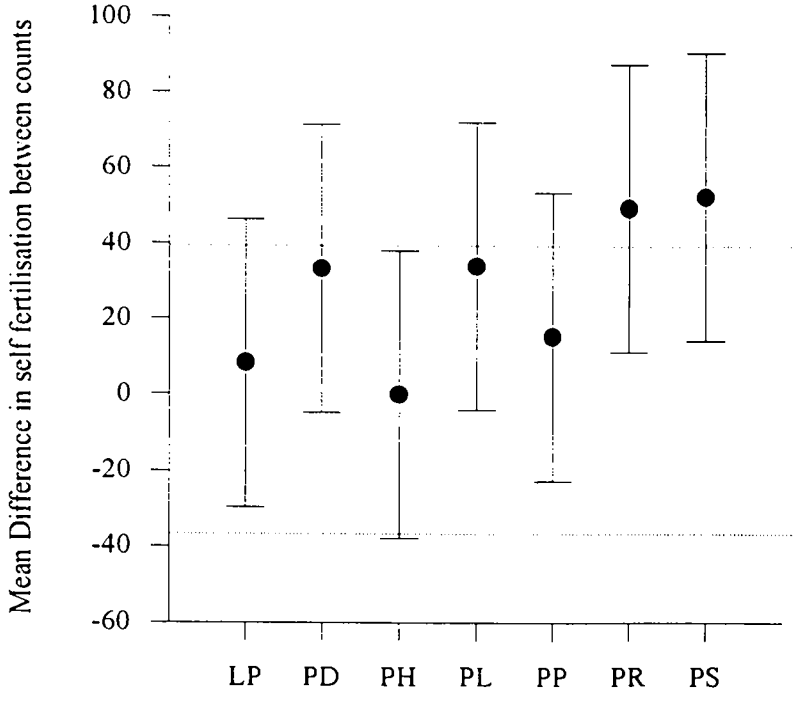


Figure 4.6. Mean values of the difference in percent fertilisation between the first and second counts of experimental vials for self crosses. Error bars are Tukey's Honestly Significant Intervals (HSI) and dotted represent the HSI for the 0 population.

4.3.1.2. Inter-generic crosses

Fertilisation trials indicated hybridisation was possible between *Platygyra* colonies and the genus *Leptoria*. Crosses with *Platygyra* as the maternal colony were unsuccessful and fertilisation rates were similar to control levels (Table 4.3). However, eggs from *L. phrygia* were capable of being fertilised by *Platygyra* sperm at levels approaching within-species rates of fertilisation (Table 4.3) although fertilisations were not apparent until the second count. "Bulk" crosses involving gametes from *L. phrygia* and *P. daedalea* produced thousands of hybrid larvae which appeared to develop normally (see section 4.3.5).

Hybridisation did not occur between *Goniastrea aspera* and *P. daedalea* or between *G. aspera* and *P. sinensis* . While there were low levels of fertilisation initially in some crosses between *G.aspera* and *Platygyra* colonies, these rates had dropped by the second count to control levels suggesting hybrid fertilisations did not develop through to blastulae (Table 4.3).

4.3.2. Effects of egg age on fertilisation

The ability of an egg to hybridise with sperm from a different species does not appear to be a function of egg age. There was no significant change in hybridisation rates with egg age in either *P. daedalea* or *P. sinensis*, nor was there any change in within-species fertilisation rates. Regression analysis of percent fertilisation with egg age was not significant for either species for any fertilisation type - hybridisation, within-species, self or controls (Table 4.4, Figure 4.7). Although r^2 was not significant (possibly due to high variation in fertilisation rates; see Figure 4.8) there was an overall decrease in fertilisation (both hybrid and normal) with egg age in *P. daedalea* while fertilisation generally increased with egg age in *P. sinensis* (Figure 4.7). The mean hybridisation rates of either *P. daedalea* or *P. sinensis* eggs were not significantly different for the different egg ages and variation was high (Figure 4.8). Mean fertilisation rates between counts were equivalent in both species (Figure 4.8). Hybridisation rates

were generally higher than within-species fertilisation rates in *P. daedalea* whereas hybridisation rates were lower than within-species fertilisations in *P. sinensis* (Figure 4.8). However, neither difference was significant.

		Mean percent fertilisation			
Cross (♀ x ♂)	n	count 1	control	count 2	control
PD x *L. phrygia*	6	0.08 (0-1)	0	1.6 (0-8)	0.1 (0-1)
PL x *L. phrygia*	6	1.4 (0-3)	1.1 (0-6)	2.7 (0-6)	1.3 (0-6)
L. phrygia x PD	6	0	0	5.7 (0-29)	0.2 (0-1)
L. phrygia x PL	6	0	0	25.2 (0-100)	0.2 (0-1)
L. phrygia x *L. phrygia*	2	38.5 (32-47)	0	36 (13-59)	0.2 (0-1)
PD x *G. aspera*	6	7.2 (0-37)	1.8 (0-6)	0.8 (0-9)	0.5 0-2)
PS x *G. aspera*	6	1.3 (0-5)	1.0 (0-5)	1.3 (0-4)	3.5 (0-7)
G. aspera x PD	6	4.8 (0-23)	2.7 (0-9)	5.7 (0-28)	4.4 (0-14)
G. aspera x PS	6	10.7 (0-45)	2.7 (0-9)	5.2 (0-34)	4.4 (0-14)
G. aspera x *G. aspera*	3	94 (86-98)	2.7 (0-9)	65 (4-100)	4.4 (0-14)

Table 4.3. Mean percent fertilisations in cross-generic fertilisation trials. n denotes the number of times each cross was tried using different colony pairs. All crosses were replicated three times and ranges (in brackets) are of all replicate vials in all crosses. Control counts are of eggs in sperm-free water.

	P. daedalea				*P. sinensis*			
	Normal	Hybrid	Self	Control	Normal	Hybrid	Self	Control
r^2	0.005	0.04	0.07	0.0004	0.05	0.1	0.1	0
	ns	ns	ns	ns	ns	ns	ns	ns

Table 4.4. Summary of r^2 values from regression analysis of fertilisation and egg age. r^2 is shown for each fertilisation type for the two species *P. daedalea* and *P. sinensis* . 'ns' denotes not significant ($P<0.05$)

4.3.3. Temporal separation in spawning times

There was some evidence of temporal separation of spawning times between certain species of *Platygyra* (Table 4.5), however, these differences are not clear-cut and overlap exists between the different species. *Platygyra daedalea*, *P. lamellina* and *P. pini* tend to spawn earlier in the evening (mean spawning times between 0 and 3 hours following sunset) while *P. sinensis*, *P. ryukyuensis* and PH generally spawn later (more then 3 hrs following sunset) (Table 4.5). However, while mean spawning times differ between certain species, the ranges of observed spawning times in the field show there is considerable overlap in spawning, and gametes of different morphological species may be released simultaneously. Times of observed spawnings of *Platygyra* colonies in experimental buckets generally fall within the range of field spawning observations (Table 4.5) hence the handling and relocation of colonies prior to spawning does not appear to greatly effect their synchrony.

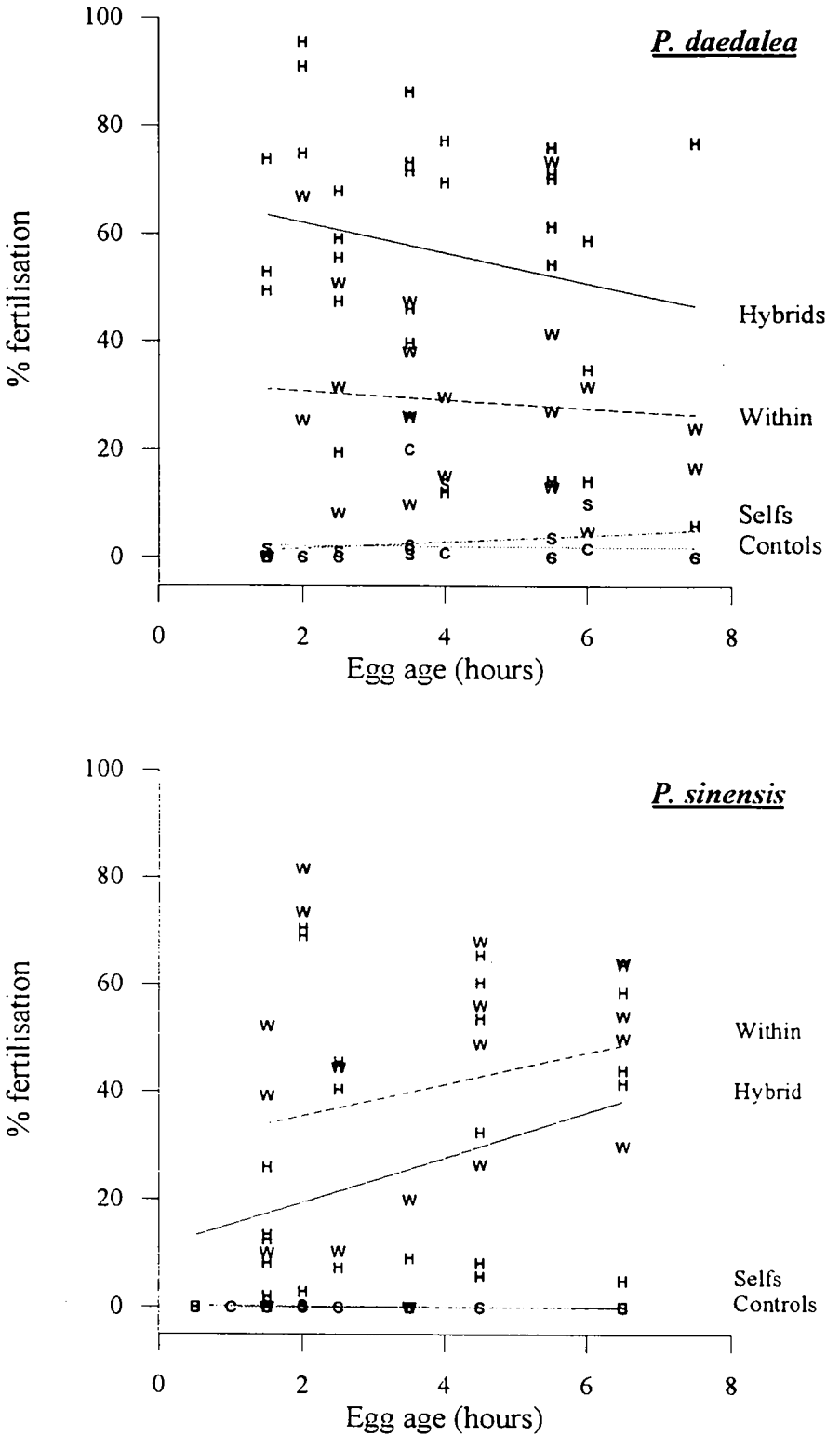


Figure 4.7. Regression analyses of fertilisation rates with egg age. Letters denote fertilisation type; H - hybrid, W - within species, S - self, C - controls.

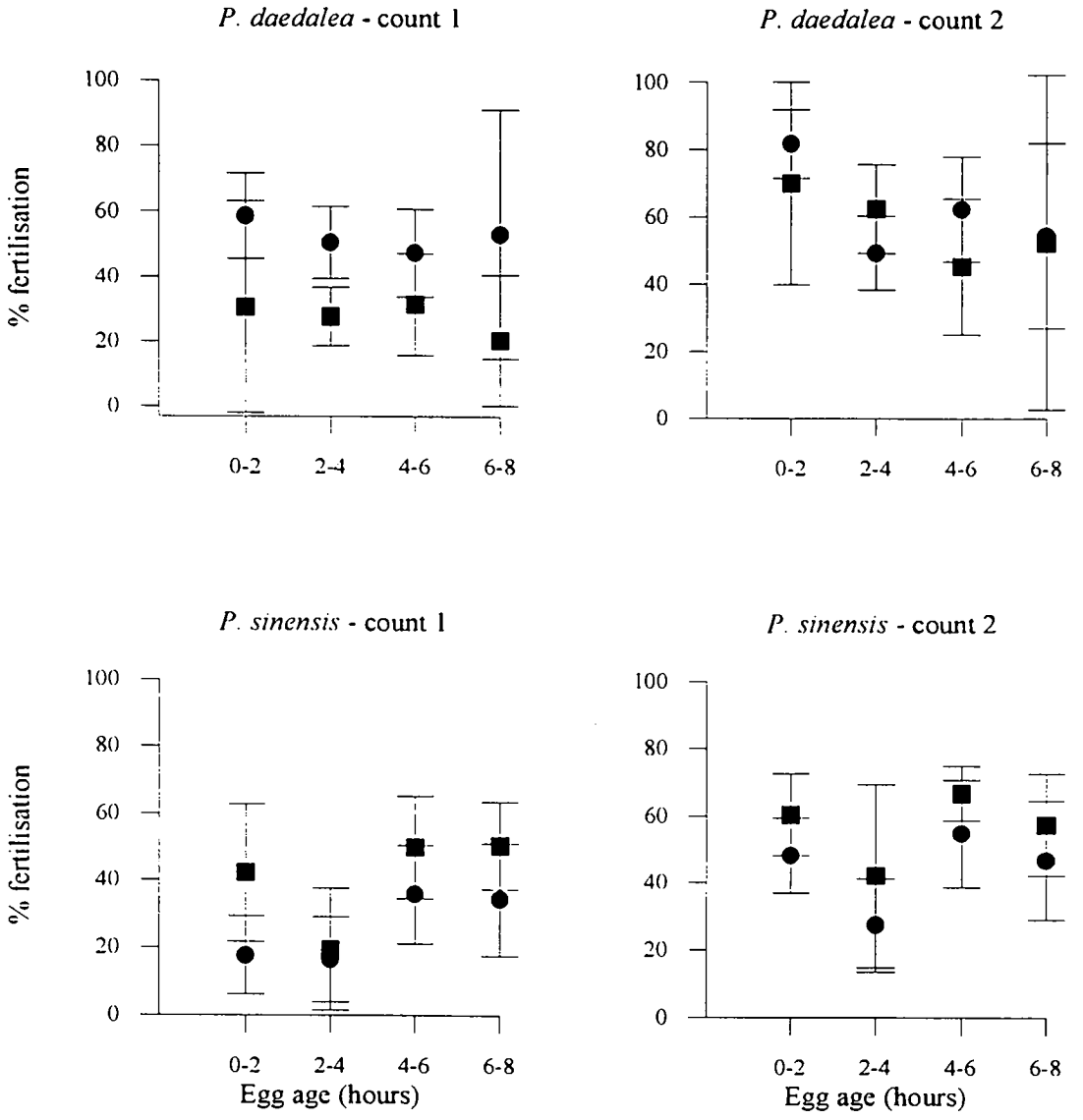


Figure 4.8. Mean fertilisation rates of hybrid and within-species crosses with increasing egg age. Bars represent 95% confidence limits. ■ - within-species crosses, ● - hybrid crosses.

	MAGNETIC ISLAND			ORPHEUS ISLAND			DAVIES REEF
Species	manipulated colonies	field observations	literature**	manipulated colonies	field observations	literature**	manipulated colonies
PDC	0:10, 0:11, 0:12, 0:15, 0:30,0:45, 1:50, 2:01, 2:13, 2:24, 2:25, 2:31 **(1:18)**	0:40,1:15, 1:18, 2:50, 2:55, 3:00, 3:05, 3:40, 3:45, 4:00* **(2:35)**	-	0:02 (2), 0:12, 0:32 **(0:12)**	1:17, 1:36 **(1:26)**	-	1:18 (3), 1:25 (3), 3:55 **(1:44)**
PDF	-	-	-	-	-	-	1:18 (2), 1:35 **(1:23)**
PDSE	1:06, 2:10, 2:13 **(1:50)**	1:45*, 3:05, 3:15*, **(2:41)**	-	-	-	-	1:25
PDSV	-	1:15*, 3:10, 3:30* **(2:38)**	-	-	-	-	-
PB	-	-	-	5:30	-	-	-
PH	-	3:40 (setting)	-	3:42, 3:47 **(3:44)**	-	-	5:55 (2), >5:30 (3), 8:30 **(6:08)**
PL	1:41	-	-	2:27, 2:42, 2:52, 3:02 (3) **(2:51)**	1:27, 1:37, 2:10, 2:22, 2:27 (many), 2:32, 2:43, 2:38, 2:55 **(2:19)**	2:40	-
PP	-	-	-	1:02, 3:17, 3:32 **(2:37)**	1:17, 1:27 **(1:22)**	1:15, 1:40 **(1:28)**	4:10 (2), 4:55 **(4:25)**
PR	1:16, 3:11 **(2:14)**	2:58, 3:40, 4:00* **(3:33)**	3:25, 3:45 *** **(3:35)**	-	3:32	3:10***	-
PS	2:56, 3:11, 3:39, 3:45, 3:55, 4:04, 4:34, **(3:43)**	1:33, 3:00 (2), 3:05, 3:10 (2), 3:23, 3:40 (2), 3:45 (4), 3:50,4:00*(2), 4:10 **(3:27)**		-	1:02, 1:32, 3:07, 3:32 **(2:18)**	-	1:25

Table 4.5. Comparison of spawning times of experimental colonies and field spawning times of *Platygyra* and times recorded in the literature (**, Babcock *et al.* 1986). Times are expressed as hours:minutes following sunset and mean spawning time is in **bold**. Number in brackets denote multiple colonies seen spawning at a given time, * denotes spawning time inferred from the disappearance of egg-sperm bundles from the polyp mouths. *** recorded as *P. sinensis* in Babcock *et al.* (1986) but probably *P. ryukyuensis* (Russ Babcock, pers comm).

4.3.4. Observations of egg-sperm interactions

There was little evidence of species specific sperm attraction in colonies of *Platygyra*. Assymetrical sperm aggregations (the increased activity and migration of sperm around one region of the egg's surface - used here to imply sperm attraction) were observed in crosses between *P. daedalea* colonies, *P. sinensis* colonies and in hybrid crosses between *P. daedalea* and *P. sinensis* (Table 4.6). Eggs of *P. daedalea* appeared to attract sperm of *P. sinensis* although *P. sinensis* eggs did not attract *P. daedalea* sperm. Results were, however, variable and this may be due to individual compatibility. No sperm attraction was evident in preparations containing eggs and sperm from the same colony except in one case with irregular eggs (Table 4.6). Results from these experiments suggest eggs and sperm can discriminate between self but not discriminate at the species level. These findings are, however, only preliminary and further work will be necessary to confirm these observations.

4.3.5. Fertilisation success as a function of genotype

There was no relationship between the genetic difference between parent colonies and fertilisation success in colonies of *Platygyra*. Theoretically, colonies which are genetically similar (same species) would have high fertilisation whereas colonies that are genetically different (ie different species), would have low fertilisation rates. Similarly, fertilisation between clone-mates and in self crosses should be low. *Platygyra* does not, however, appear to conform to this ideal. Colonies that were between 40% and 60% different genetically had fertilisation rates varying between 0 and 100% (Figure 4.9). Similarly, amounts of self fertilisation varied from colony to colony (up to 100% for some individuals) (Figure 4.9).

Cross (♀ x ♂)	Fertilisation type	Observations
PD2 x PD1	within-species	Prep # 1: sperm active, questionable attraction in one egg only. Prep # 2: distinct attraction to one region on the egg surface, eventually disappears Prep # 3: activity in one region of egg surface, near potential polar body. Egg eventually cleaved.
PS1 x PS2	within-species	Prep # 1: aggregation at one end of egg, sperm making distinct turns back to egg; apparent in a number of eggs
PD1 x PD1	self	Prep # 1: eggs irregular/deformed - distinct attraction to one egg
PD2 x PD2	self	Prep # 1: no attraction
PS1 x PD2	hybrid	Prep # 1: no attraction Prep # 2: no attraction
PD1 x PS2	hybrid	Prep # 1: weak attraction, sperm slow
PD2 x PS2	hybrid	Prep # 1: attraction in a number of eggs, continued for over 45 minutes. Prep # 2: attraction in a number of eggs. Some damaged eggs with no attraction.

Table 4.6. Summary of observations of egg-sperm interactions in various fertilisation types between colonies of *Platygyra*. (PD - *P. daedalea*, PS - *P. sinensis*).

4.3.6. Larval rearing

Rearing of larvae under aquarium conditions proved highly successful and both hybrid and normal larvae appeared to develop similarly. All larvae had developed into ciliate planulae within 2 days following spawning and were competent to begin settling 4-5 days after spawning. Most settlement was observed within a week following spawning although some larvae remained in the water column for up to six weeks before settling onto the substrata provided. Metamorphosed larvae gained zooxanthellae within 2 days after settlement and subsequently commenced skeletal construction. Post-settlement mortality rates were high (estimated in the vicinity of 80-90%), however, survival rates of hybrid larvae seemed to be higher than those of normal larvae (although this was not quantified). After five months,

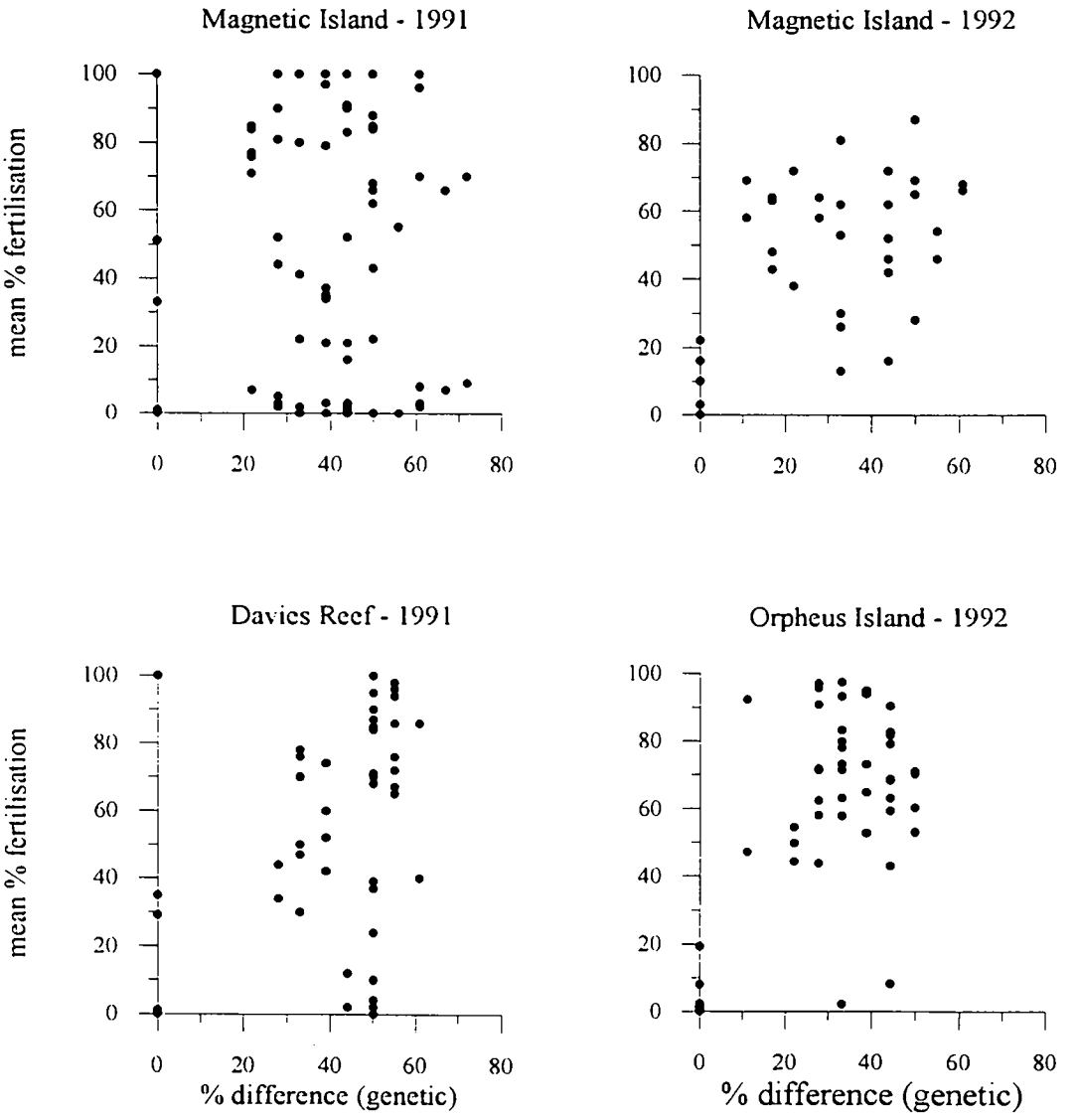


Figure 4.9. Comparison of percent fertilisation (based on the mean of three replicate vials) and genetic difference between colonies used in fertilisation trials in 1991 and 1992.

recruits ranged in size from 2-10 mm in diameter. Those on the upper surface of settlement plates grew much faster than those on the under surface, possibly due to much higher light levels. Unfortunately, all colonies in the aquarium were killed due to a system 'failure' in March, 1993. At this time, the oldest hybrid colonies had reached 3½ years of age (with diameters between 2 and 5 cm, see Plate 4.1) while the oldest colonies from within-species crosses were 1½ years old. Development of hybrid colonies to this point appeared to be normal, although their skeletal characteristics were not well enough developed to determine if they resembled any of the pre-defined morphological groups or were perhaps intermediate in morphology between the parent colonies (Plate 4.2).

Due to the unexpected mass mortality of hybrid colonies, tissue samples were not collected and hence hybrid and parent colonies have not been compared genetically. I plan to rear more larvae in the future so a genetic comparison can be done to look at inheritance in hybrid corals.

Larvae from *Platygyra* x *Leptoria* bulk crosses (see 4.3.1.2) were also reared successfully in the aquarium - both *Platygyra* ♀ x *Leptoria* ♂ and *Leptoria* ♀ x *Platygyra* ♂. These cross-generic hybrids settled, obtained zooxanthellae and began building skeletons (see Plate 4.2). Unfortunately these corals were lost at four months of age due to the aquarium system failure. Prior to this they appeared to be developing normally.

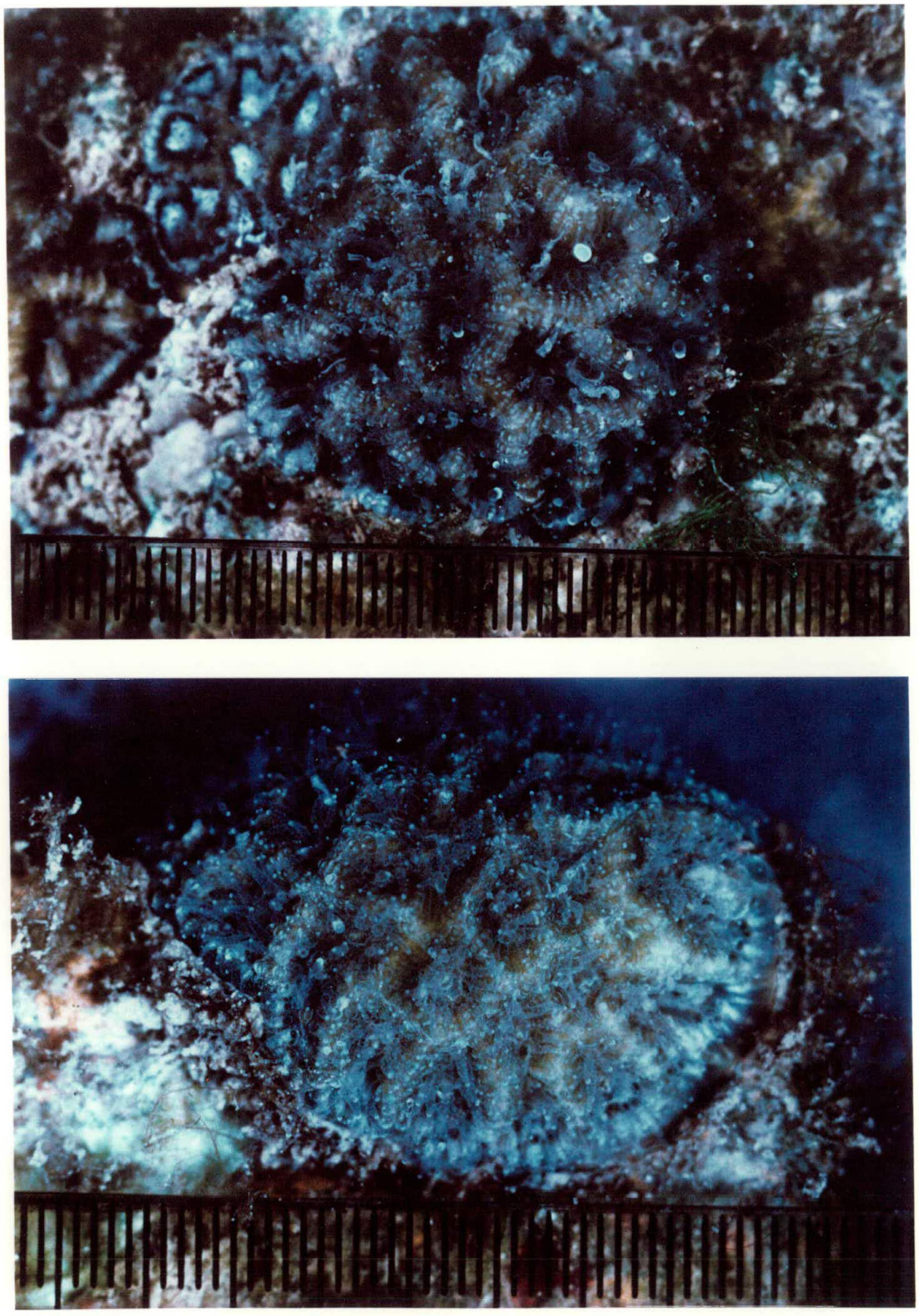


Plate 4.1. Photographs of hybrid *Platygyra* colonies at 3½ years of age. A - cross PD x PS; B - cross PS x PD.

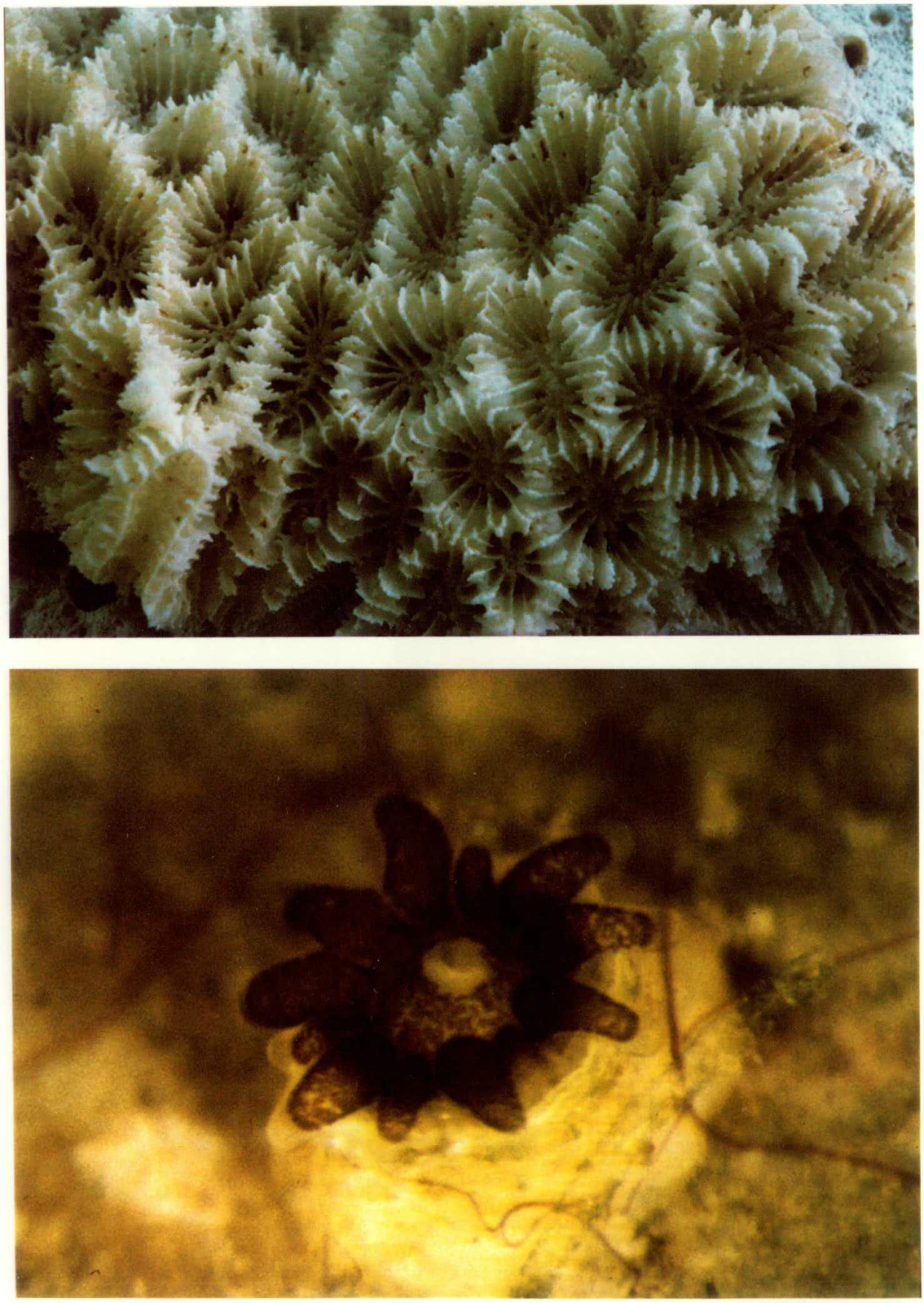


Plate 4.2. Photographs of hybrid colonies. A - skeleton of 3½ year old colony, cross PD x PS; B - 12 week old cross-generic hybrid, *Leptoria phrygia* x *P. daedalea*

4.4. Discussion.

4.4.1. Hybridisation in Platygyra

Results from the fertilisation trials show that hybridisation is possible *in vitro* between the different morphological species of *Platygyra*. There appears to be no relationship between morphology and ability to reproduce since fertilisation was recorded in all crosses. Hybridisation occurred between most *Platygyra* species at rates equivalent to normal fertilisations (Table 4.1, Figures 4.2, 4.3) and hybrids did not appear to have reduced survivorship (Figures 4.2, 4.3). There may be incomplete barriers to fertilisation between some of the morphs of *P. daedalea* (ie PDF and PDC, Table 4.1) and all crosses may not be reciprocal (ie *P. pini* x *P. sinensis*, Table 4.1) however more fertilisation trials between these morphs and species need to be done to conclusively test this. Trials between some species have not yet been tried due to the time constraints associated with coral spawning. However, it is highly likely, based on the results so far, that un-tried crosses would also result in some degree of fertilisation.

4.4.2. Effect of egg age on fertilisation success

High numbers of fertilised eggs were apparent in vials after 2 hrs (the minimum time after fertilisation in which cleavage becomes evident) and hybridisation rates did not increase with egg-sperm contact time. This suggests fertilisation occurred immediately between heterospecific gametes since eggs were already over 30 minutes old when eggs and sperm were combined. Thus sperm may have been actively attracted to the fertilisation site on the eggs, even in hybrid crosses, rather than fertilisation occurring as a result of chance encounters between eggs and sperm in a small volume. Similarly, ability to fertilise was not a factor of egg age. Equivalent fertilisation rates (both in hybrid and within-species crosses) were recorded in eggs from 0.5 - 8 hrs old (Figures 4.7, 4.8). This is in contrast to Oliver and Babcock (1992) who reported marked decreases in fertilisation (within-species) of *P. sinensis* eggs after 4-5 hrs. The difference between my results and

those of Oliver & Babcock may be related to sperm ageing, as Oliver & Babcock (1992) used simultaneously aging gametes in their experiments.

Hybridisation may occur in sea urchins as a result of changes to the egg surface permitting the binding of non-specific sperm (Longo, 1977). However, similar changes, such as the degeneration of the vitelline layer which may prevent non-specific sperm from penetrating the egg, do not appear to be occurring as a consequence of egg age in *Platygyra*. While the potential for some change in the egg surface due to handling procedures cannot be completely ruled out, I found no evidence of this. Furthermore, other species of coral eg. *Montipora digitata* and *M. tortuosa* do not hybridise under identical experimental treatments (Ben Stobart, personal communication). Thus it seems likely that observed hybrids are real and not an experimental artefact.

4.4.3. Possible mechanisms preventing hybridisation in Platygyra

If hybridisation between morphological species of *Platygyra* is not prevented at the level of egg-sperm interaction, as fertilisation trials suggest, other mechanisms of pre- or post-zygotic barriers may have evolved to prevent hybridisation and the potential loss of gametes (assuming hybridisation equates with gamete wastage and also assuming different morphological species are in fact different biological species).

4.4.3.1. Pre-zygotic barriers

Pre-zygotic barriers to hybridisation may be responsible for maintaining biological species boundaries. Heterospecific sperm is prevented from penetrating the egg in some free-spawning animals eg. sea urchins (Palumbi, 1992) and other species of coral (Ben Stobart, personal communication), although this is obviously not the case in *Platygyra* as fertilisation was recorded between morphological species. Sperm chemotaxis has been demonstrated in species of *Montipora* and this may be important for reducing hybridisation in this genus (Coll *et al.*, in press). There is

no evidence to suggest a similar mechanism in *Platygyra*. While preliminary studies showed limited evidence of sperm attraction to *Platygyra* eggs (Table 4.6) this attraction does not appear to be species specific and hence is unlikely to be an effective pre-zygotic isolating mechanism. Hybridisation in vials between *Platygyra* and *Leptoria* were much lower than incidences of hybridisation between morphological species of *Platygyra* (Table 4.3). This may be due to the absence of sperm attractants between these corals and fertilisations may only have occurred at random within the vial, dependant solely on the chances of eggs and sperm encountering each other (see also section 4.4.2). Observations of self crosses suggest there is some level of egg-sperm recognition in *Platygyra* colonies (Table 4.6) and this is supported by the low rates of self-fertilisation in reproductive trials (Figure 4.5). Thus, unlike *Goniastrea favulus* which readily self-fertilises (Heyward & Babcock, 1986; Stoddart *et al.*, 1988), colonies of *Platygyra* do not largely self-fertilise. The high variability in fertilisation rates also suggests some level of incompatibility between individuals, although this does not seem to be related to morphotype (Table 4.1) or 9-locus allozyme genotype (Figure 4.9).

Differences in spawning times have also been demonstrated to be effective pre-zygotic isolating mechanisms in animals with external fertilisation eg. sea urchins (Uehara *et al.*, 1990) and will likely isolate species of corals which spawn a number of days apart (eg. Harrison *et al.*, 1984; Willis *et al.*, 1985; Babcock *et al.*, 1986) While some temporal differences in spawning exist between species of *Platygyra*, many species have overlapping spawning periods (Table 4.5). Eggs are not capable of being fertilised for at least 30 minutes following spawning (Babcock & Heyward, 1986; Shlesinger & Loya, 1991) and may still be fertilised successfully up to 8 hrs following release (Figures 4.7, 4.8). For temporal barriers to be completely effective, separations in spawning times of at least 8 hrs would be necessary, far longer than the observed differences in the spawning times of the different species of *Platygyra*. Therefore, while there is some separation among species spawning times, this does not constitute a temporal barrier to hybridisation. There is clearly potential for gametes of all species to

mix on any given spawning night. If temporal barriers did act as isolating mechanisms between some morphological species of *Platygyra* (eg PDC and PH, Table 4.3), it could be expected that those morpho-species which spawn simultaneously (eg *P. daedalea* and *P. pini*, Table 4.5) may have developed alternative isolating mechanisms (such as fertilisation barriers). However, no evidence exists to suggest this has occurred (Table 4.1). The possibility of sperm chemotaxis acting as an isolating mechanism in synchronously spawning morphological species cannot be completely ruled out and warrants further investigation.

4.4.3.2. Post-zygotic barriers

The apparent normal development of hybrid *Platygyra* indicates no post-zygotic barriers to hybridisation. Arrested development was not observed in hybrid *Platygyra*, unlike hybrids of *Montipora* which did not develop beyond the 4-cell stage (Hodgson, 1988) and cross generic hybrids with *Goniastrea* which do not develop into blastulae (Table 4.3). Both hybrid and normal *Platygyra* larvae appeared to develop identically and the numbers of hybrid larvae developing into planulae were the same as for normal crosses (Figures 4.2, 4.3). In addition, hybrids were competent to settle and were capable of growing and surviving for at least 3½ years. Growth rates of hybrid *Platygyra* were similar to those reported for *P. sinensis* in the literature. Babcock (1991) reported a mean growth rate of 6.8 mm per year for colonies in the field, which would equate to a diameter of 2-3 cm after 3½ years. Hybrid colonies had reached between 2 and 5 cm in diameter in this time under aquarium conditions. These results suggest there is no hybrid disadvantage associated with ability to settle, nor with their subsequent growth and survivorship.

Sterility of hybrid *Platygyra* colonies may be the only isolating mechanism, if one exists, between these morphological species of corals. Costs associated with hybrid sterility may be high, particularly in terms of gamete wastage and loss of resources. However, whether hybrid *Platygyra* are sexually reproductive remains

unclear. Colonies of *Platygyra* reach sexual maturity between 5 and 8 years of age (Babcock, 1991). Hence, all hybrid colonies were immature at their untimely death. More hybrid colonies will be raised in the future to conclusively determine if hybrids are capable of reproducing.

Circumstantial evidence points, however, to the viability of hybrid corals. Known hybrids from a number of different animal and plant groups are generally intermediate in appearance between the two parents (eg. McDade, 1990). Assuming hybrid corals exist (as my results suggest) and that they were completely sterile, it would be highly likely that one would find significant numbers of colonies in one or more morphological species (say intermediate between two of the species described here) in which all colonies were infertile. This is not the case in *Platygyra* where all discernible species were capable of producing eggs and sperm and of being fertilised. However, hybrids may not always produce morphological intermediates. Processes such as introgression contribute to the maintenance of discrete morphological groups in hybridising species of plants (Grant, 1981). For introgression to occur, hybrid progeny must be reproductive. If such a system is operating within the genus *Platygyra*, this also implies the viability of hybrid corals. Alternatively, hybrid corals may only express the morphological traits of one parent (eg. Byrne & Anderson, in press; Strathmann, 1981) and if this is the case in *Platygyra*, sterile hybrids may not be easily detected within a population. However, in the 389 fertilisation trials carried out over 3½ years, no colony was ever found that did not fertilise with at least one other colony. This suggests either sterile hybrids which are morphologically identical to one parent are rare within the population or alternatively that hybrids are not sterile.

4.4.4. Conclusions: hybridisation between morphological species of Platygyra

Results from this chapter have shown that hybridisation is possible between morphological species of *Platygyra*. However, fertilisation trials in a 20 ml vial may not necessarily be representative of what actually happens on the reef.

Perhaps during mass spawning conspecific sperm may easily out compete heterospecific sperm and consequently species boundaries would be maintained. In order to determine if the hybridisation observed *in vitro* also occurs on the reef, it will be necessary to conduct genetic surveys to determine if gene flow occurs between the morphological species or if they exhibit fixed gene differences as would be expected if they were reproductively isolated. Genetic relationships between the morphological species of *Platygyra* will be investigated in the following chapter.

CHAPTER 5

GENETIC RELATIONSHIPS BETWEEN MORPHOLOGICAL SPECIES OF *PLATYGYRA*

5.1. Introduction

5.1.1. Corals and electrophoresis.

Allozyme electrophoresis was first applied to scleractinian corals by Lamberts (1979) and later by Ohlhorst (1984) who confirmed the usefulness of electrophoresis for detecting species differences in corals and also demonstrated the potential for detecting geographic variation within populations. Subsequently, electrophoresis has been applied to questions of taxonomy and genetic population structure in a number of groups of scleractinians.

The corals *Acropora palifera* and *Acropora cuneata*, which are difficult to distinguish morphologically and often considered the same taxonomically (eg. Potts, 1978), were shown to be genetically distinct species using electrophoresis (Ayre *et al.*, 1991). Similarly, electrophoresis has been used in conjunction with morphometrics to examine species boundaries in the genus *Porites* (Weil, 1992; Garthwaite *et al.*, 1994). Morphological variants of *Montastrea annularis* are genetically different (Knowlton *et al.*, 1992; Van Veghel and Bak, 1993) and have been referred to as sibling-species based on these and other differences (Knowlton *et al.*, 1992).

Electrophoresis has also been used to investigate genetic relationships in coral populations, particularly the nature of gene exchange both within and between coral species. Morphological variants of *Porites compressa* differ genetically (Hunter, 1985). Similarly, in *Pavona cactus*, morphological variation is genetically rather than environmentally determined and rates of asexual reproduction by

fragmentation were found to be high (Willis and Ayre, 1985; Ayre and Willis,1988). The production of asexually produced planulae in the coral *Pocillopora damicornis* has been documented from electrophoretic comparisons of parents and larvae (Stoddart, 1983). Similarly, the role of asexual propagation in population maintenance and population structure of *P. damicornis* has been determined by electrophoretic surveys of adult populations in both Hawaii and Western Australia (Stoddart, 1983; 1984; 1986). Electrophoresis has been used to imply restricted gene flow between reef populations of *Seriatopora hystrix* and, in contrast to *Pavona cactus*, localised recruitment was found to be predominantly sexually derived rather than from asexual fragments (Ayre and Dufty, in press).

5.1.2 Histo-incompatibility between coral species.

Histo-incompatibility in corals (ie. tissue graft rejection) has been used as a criteria for separating coral species (eg. *Scolymia cubensis*; Lang, 1971). However, there is no one-to-one relationship between genotype and rejection response and intra-specific tissue grafts may give varied results including aggressive responses (Hildemann *et al.*, 1975). Fusion has been demonstrated between genetically identical colonies (clones) and also between genetically distinct colonies in the corals *Pavona cactus* (Willis and Ayre, 1985), *Montipora dilatata* and *Montipora verrucosa* (Heyward & Stoddart, 1985). Similarly, self-recognition may not always be evident in tissue grafting assays (Resing & Ayre, 1985) and not all species will necessarily interact (eg. Lang, 1973; Logan, 1984). Despite the limitations of the method when used to imply genetic relationships within a species, it may still be a useful technique for differentiating corals at a species-level. Where tissue rejection occurs consistently this provides strong evidence of species level differences.

5.1.3 Genetic relationships between morphological species of coral

It has previously been assumed that differences between morphological species of

coral equate with reproductive isolation and consequently with genetic differentiation. Genetic studies of some coral genera suggest this is not always the case. McMillan *et al.* (1991) found genetic relationships between some species of *Acropora* were contrary to morphological taxonomic groupings. Similarly, discrepancies exist between morphological and genetic species groups within the genus *Porites* (Potts and Garthwaite, 1986). A more recent study by Garthwaite *et al.* (1994) reported fixed gene differences between morphologically distinct `typical' Poritiid species. However, they did suggest that genetic analysis of the `atypical' morphological intermediates may reveal gene flow occurs between some species. Results from the previous chapter suggest morphological species of *Platygyra* are capable of interbreeding, although to what extent this occurs on the reef is unknown. If hybridisation occurs commonly between morphological species of *Platygyra*, it is highly likely that there will be little correspondence between morphological and genetic groupings within the genus.

The aim of this chapter is to examine populations of the seven morphological species of *Platygyra*, and the out-group *Leptoria phrygia*, using protein electrophoresis, to determine if the morphological species exhibit fixed gene differences as would be expected if they were reproductively isolated. The absence of fixed gene differences between the seven morphological species would indicate that gene exchange occurs between the morphological species, confirming the results from Chapter 4 that morphological species of *Platygyra* hybridise on the reef. Histo-incompatibility tests will also be used to determine whether consistent tissue rejection occurs which might suggest species-level differences.

5.2. Methods

5.2.1. Study sites

A total of 146 *Platygyra* colonies were collected from Davies Reef for electrophoretic comparison plus two colonies of the out-group *Leptoria phrygia*.

An out-group from a higher taxonomic level (ie. genera) was used to provide a framework for comparison of genetic differentiation at the lower taxonomic level (ie. between species). Colonies of each of the morphological species were sampled from five zones across Davies Reef - F5, F10, FRF, LAG, BRS - to examine genetic variation between habitats (see Chapter 3 for descriptions of habitats). *Platygyra lamellina* was not found at Davies Reef. In order to assess genetic differences between *P. lamellina* and the six other morphological species, eight samples of *P. lamellina* were collected from Orpheus Island. In addition, small samples were collected from Orpheus Island, Magnetic Island and Heron Island to confirm identities between these and the species at Davies Reef.

5.2.2. Electrophoresis

Tissue samples were chiselled from the surface of live colonies (from a region approximately 3 cm square), frozen immediately in liquid nitrogen and later transferred to a -80° freezer for storage. The presence of symbiotic algae (zooxanthellae) in coral tissues has been found not to prevent reliable scoring of coral genotypes on electrophoretic gels (Resing and Ayre, 1985; Willis and Ayre, 1985) hence whole tissue samples were used in enzyme assays. Prior to electrophoresis on starch gels (w/v 12%), tissue samples were homogenised in grinding buffer containing 0.04% β-mercaptoethanol and bromophenol blue.

A minimum of three samples is usually recommended when testing for species differences (Richardson *et al.*, 1986) although often a single individual is enough to characterise a species (Avise, 1975). I screened a minimum of 5 individuals of each of the morphological species of *Platygyra* (including the four morphs of *P. daedalea*) from Davies Reef. However, only three colonies of *P. ryukyuensis* were screened as this species is rare on Davies Reef. The total numbers of samples screened for each species were *P. daedalea* - 70 colonies (41 of morph PDC, 5 of morph PDF, and 12 each of PDSE and PDSV); *P. pini* - 28 colonies; *P. ryukyuensis* - 3 colonies; *P. sinensis* - 20 colonies; PH - 14 colonies; PB - 11

colonies and *Leptoria phrygia* - 2 colonies.

Of the thirty six enzyme systems initially tested, only 12 showed activity and only seven were consistently scorable. Gels were stained for phosphoglucomutase (PGM: EC 2.7.5.1), glucose-phosphate isomerase (GPI: EC 5.3.1.9), mannose-phosphate isomerase (MPI: EC 5.3.1.8), creatine kinase (CK: EC 2.7.3.2), leucyl-glycylglycine peptidase, leucyl-tyrosine peptidase and leucyl-proline peptidase (LGG, LT, LP: EC 3.4.11/13) A summary of enzyme systems and running conditions is presented in Table 5.1. Nine loci were examined (*Ck*, *Lt-1*, *Lt-2*, *Lp*, *Lg-1*, *Lg-2*, *Gpi*, *Pgm* and *Mpi*) and all nine were polymorphic. Alleles were labelled alphabetically commencing with the allele with greatest mobility from the origin, and proportional mobilities were calculated based on the most common allele. Zymogram patterns at each locus are represented in figures 5.1, 5.2. and 5.3.

Enzyme	E.C. number	Locus	Quaternary structure	Buffer * (pH)	*Running conditions***	
					current	*hrs*
Phosphoglucomutase	2.7.5.1	*Pgm*	monomeric	TEC (7.9)	30-35 mA (200 V)	5
Glucose-phosphate isomerase	5.3.1.9	*Gpi*	dimeric	TEC (7.9)	"	"
Creatine kinase	2.7.3.2	*Ck*	monomeric	TC (8)	30-35 mA (100-120 V)	7
Leucyl-proline peptidase	3.4.11/13	*Lp*	monomeric	TC (8)	"	"
Leucyl-glycylglycine peptidase	"	*Lg-1* *Lg-2*	monomeric monomeric	TEB (8.4)	30-35 mA (200-400 V)	7
Leucyl-tyrosine peptidase	"	*Lt-1* *Lt-2*	monomeric monomeric	TEB (8.4)	"	"
Mannose-phosphate isomerase	5.3.1.8	*Mpi*	monomeric	TEB (8.4)	"	"

Table 5.1. Summary of electrophoretic conditions and enzymes assayed. *All buffers as per Selander *et al.* (1971) ** All gels were run at 4° C

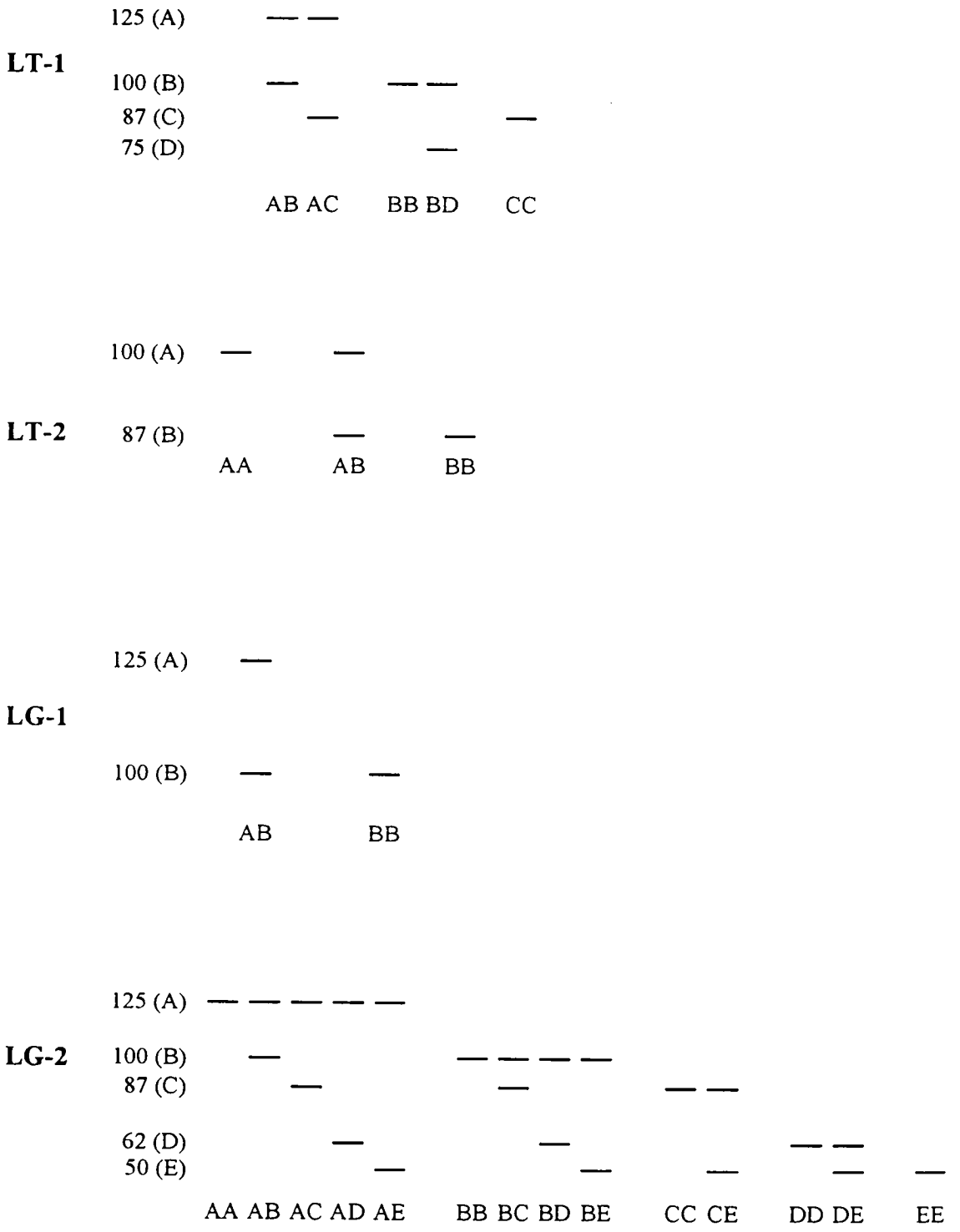


Figure 5.1. Zymogram patterns at the loci *Lt-1*, *Lt-2*, *Lg-1* and *Lg-2*

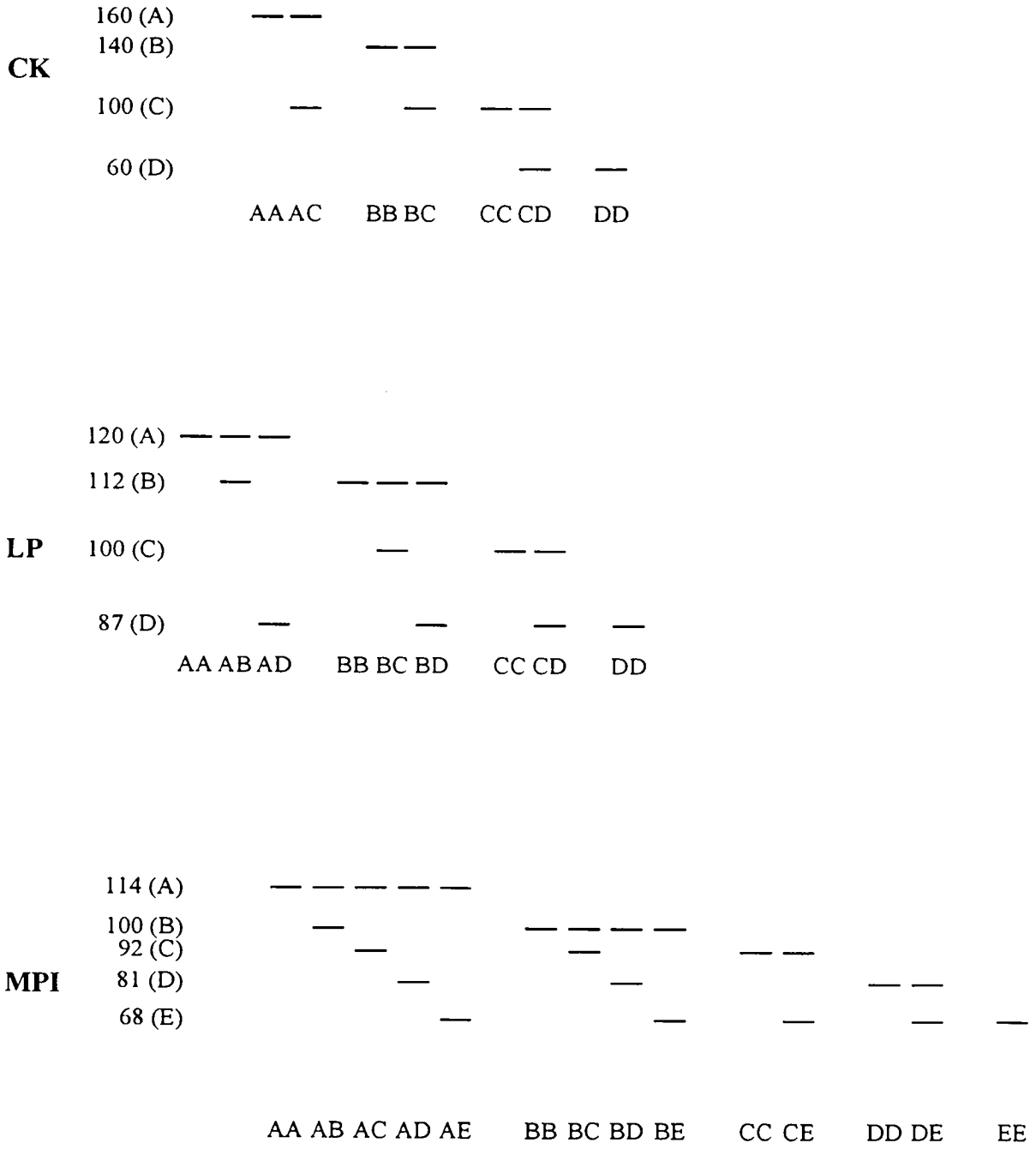


Figure 5.2. Zymogram patterns at the loci *Ck*, *Lp* and *Mpi*.

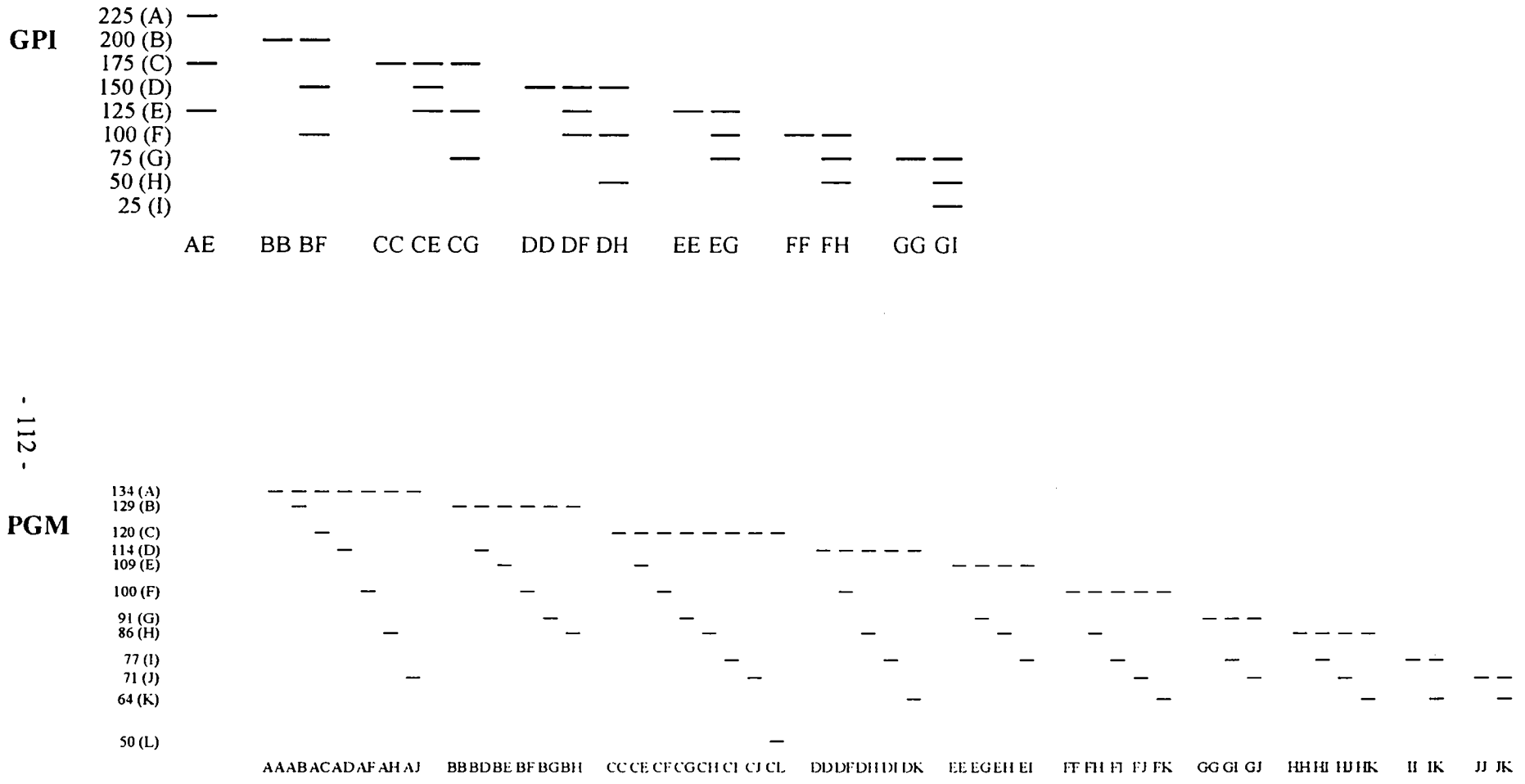


Figure 4.2.3. Zymogram patterns at the loci *Gpi* and *Pgm*

5.2.3. *Analysis of electrophoretic data*

Gene frequencies and basic population statistics were calculated using Biosys (Swofford and Selander, 1981). F-statistics (F_{is}: genetic variance within species and F_{st}: genetic variation between species) were calculated according to Weir and Cockerham (1984) to account for differences in size among populations. Significance levels of F-statistic values were tested using χ^2 calculations from equations given in Waples (1987). Exact tests (Elston and Forthofer, 1977) for conformation of gene frequencies to those expected under conditions of Hardy-Weinberg equilibrium were corrected for multiple simultaneous tests (Miller, 1966). Genetic distance between populations was calculated as Nei's D (unbiased genetic distance). Principle co-ordinates analyses based on nine-locus genotypes (performed using PATN; Belbin, 1987) were used to examine genetic groupings within the data set relative to morphotype and habitat.

5.2.4. *Histo-incompatibility experiments*

The interaction experiment used to examine species-level differences within the genus *Platygyra*, was based on the assumption that consistent tissue rejection will reflect species differences. Whole colonies or large fragments of colonies (>900 cm^2) were relocated to approximately 2 metres depth in the lagoon at Davies Reef (see Plate 5.1). Where possible, colonies were collected from the surrounding area in order to minimise stress of relocation. A platform was made of 6.5 cm reinforcing mesh and was raised 10 cm from the sandy bottom on lumps of coral rubble. Pairs of colonies were attached to the mesh using cable-ties so that the two colonies were separated by a gap of between 5 and 10 mm (see Plate 5.1). Adjacent pairs were separated by at least 30 cm so as not to confound interactive responses. Each morphological pairing was replicated twice using independent colonies and within-species pairings (two replicates) were also done. Replicate pairs were placed randomly on the grid to avoid localised positional effects confounding results.

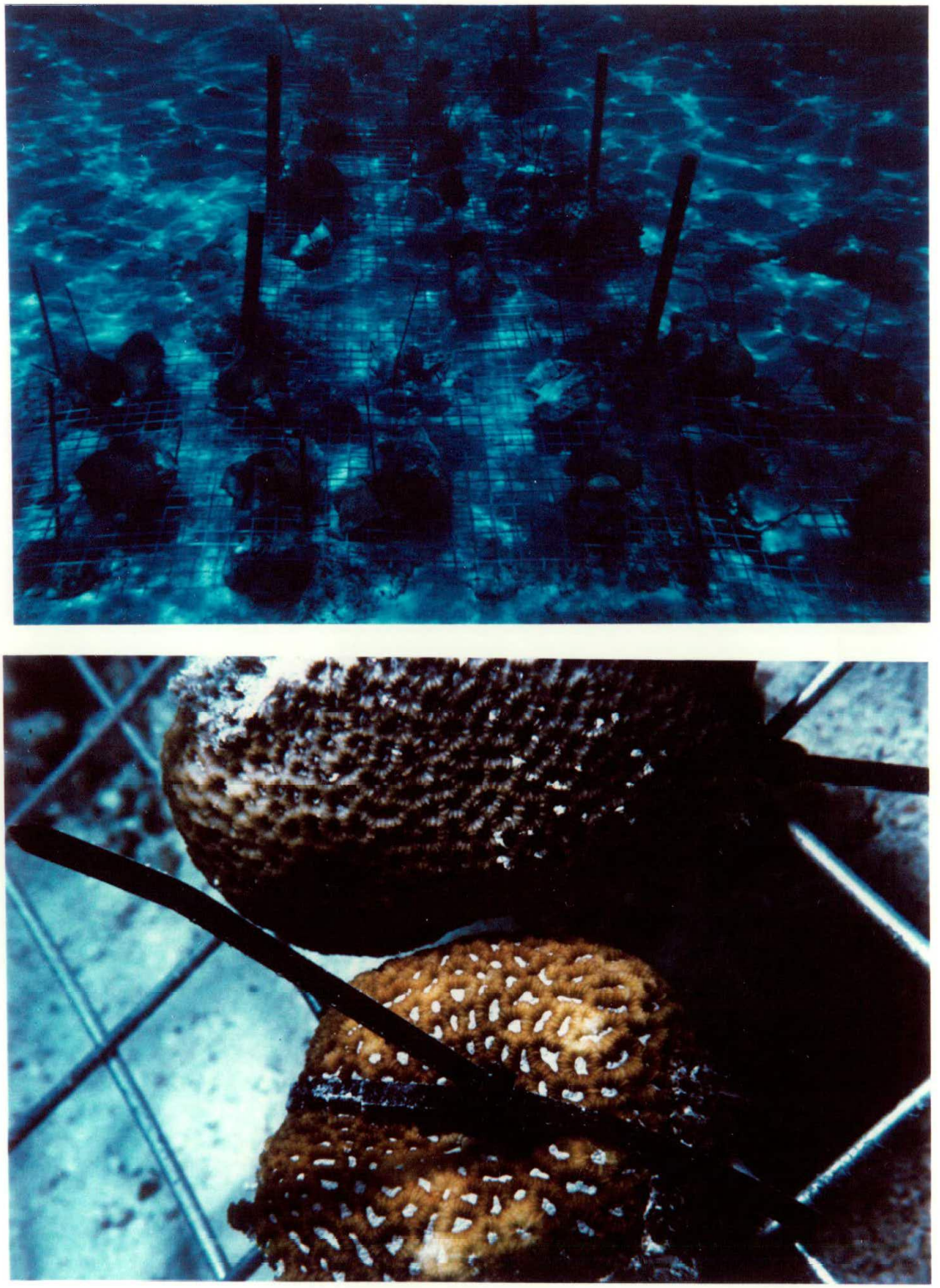


Plate 5.1. Photographs of interaction experiment performed in the lagoon at Davies Reef. A - Experimental platform with colonies attached. B - Close up of colony pair attached to platform.

Pairs were observed after 24 hours, 2 days, 3 days, 4 days, 3 months and 4 months and outcomes of the interactions were recorded. Due to logistical constraints, only a subset of all possible combinations of species were tested, a total of 27 colony pairs (see Table 5.7). Tissue samples of all colonies used in interaction experiments were collected for electrophoretic analysis (as described in 5.2.2) and the genetic difference between colony pairs were calculated as the % of different alleles (ie. alleles not shared). Genetic difference and interaction outcome were then compared to determine if there was any relationship between the two which might suggest that interaction outcome reflected genetic differences between colonies.

5.3 Results

5.3.1. Genetic relationships between morphological species.

No fixed gene differences were observed at any of the nine loci between any of the morphological species identified from Davies Reef, nor between the morphs of *P. daedalea* (Table 5.2). A comparison between *Platygyra* species and the outgroup *Leptoria phrygia* also showed no fixed gene differences between the two genera on Davies Reef (Table 5.2). There was some variation in gene frequencies among the morphological species at Davies Reef (Table 5.2) and values of F_{st} indicated significant genetic differences between morphological species at five of the nine loci (Table 5.3).

Values of Nei's genetic distance (D) between species at Davies Reef were very low, ranging between 0.020 and 0.157 (Table 5.4). However, Nei's D between *Leptoria phrygia* and the species of *Platygyra* was much higher (between 0.401 and 0.570, Table 5.4).

Locus / allele		SPECIES PDC	PDF	PDSE	PDSV	PH	PB	PP	PR	PS	LP
Pgm	A	-	-	0.042	0.042	-	-	-	0.333	0.150	-
	B	0.037	-	0.417	-	-	0.045	0.071	-	-	-
	C	0.134	0.100	0.125	0.083	0.071	0.045	0.161	-	-	-
	D	0.024	-	-	0.292	-	0.045	0.036	-	0.050	-
	E	-	-	-	-	0.107	-	0.036	-	0.050	-
	F	0.427	0.400	0.042	0.208	0.036	0.364	0.179	0.500	0.150	-
	G	0.049	-	0.042	-	0.321	-	0.054	-	0.025	-
	H	0.171	0.200	0.208	0.208	0.143	0.455	0.268	0.167	0.275	0.500
	I	0.049	-	0.083	0.083	0.250	0.045	0.143	-	0.075	-
	J	0.110	0.100	0.042	0.083	0.036	-	0.018	-	0.175	0.500
	K	-	0.200	-	-	0.036	-	-	-	0.050	-
	L	-	-	-	-	-	-	0.036	-	-	-
Mpi	A	0.220	0.300	0.167	0.083	-	0.227	0.054	-	0.050	0.500
	B	0.598	0.200	0.542	0.333	0.750	0.364	0.286	0.667	0.400	-
	C	0.061	0.100	0.083	0.042	0.107	0.091	0.107	-	0.100	-
	D	0.098	0.200	0.208	0.292	0.143	0.318	0.536	0.333	0.450	0.500
	E	0.024	0.200	-	0.250	-	-	0.018	-	-	-
Ck	A	0.037	-	0.083	0.042	-	-	-	-	-	-
	B	0.024	-	-	-	0.179	0.136	0.054	-	0.100	-
	C	0.939	1.000	0.917	0.875	0.821	0.864	0.946	1.000	0.875	1.000
	D	-	-	-	0.083	-	-	-	-	0.025	-
Lp	A	0.476	0.200	0.417	0.167	0.714	0.500	0.179	-	0.350	0.500
	B	0.049	0.100	0.333	0.625	0.179	0.318	0.339	0.333	0.400	-
	C	0.183	0.200	0.125	0.083	0.071	0.182	0.482	0.667	0.200	-
	D	0.293	0.500	0.125	0.125	0.036	-	-	-	0.050	0.500
Lt-1	A	0.061	0.100	0.083	-	0.286	0.045	-	-	0.025	0.500
	B	0.939	0.900	0.917	0.917	0.679	0.955	1.000	1.000	0.975	0.500
	C	-	-	-	-	0.036	-	-	-	-	-
	D	-	-	-	0.083	-	-	-	-	-	-
Lt-2	A	0.963	1.000	1.000	0.958	0.929	1.000	1.000	1.000	1.000	1.000
	B	0.037	-	-	0.042	0.071	-	-	-	-	-
Lg-1	A	-	0.100	-	-	0.036	0.273	-	-	-	1.000
	B	1.000	0.900	1.000	1.000	0.964	0.727	1.000	1.000	1.000	-
Lg-2	A	0.049	0.100	0.083	-	0.036	-	0.232	0.333	0.025	1.000
	B	0.439	0.400	0.750	0.292	0.500	0.364	0.554	0.333	0.350	-
	C	0.037	0.100	-	-	0.250	0.273	0.089	-	0.250	-
	D	0.085	0.200	0.042	0.083	0.179	0.045	0.036	0.333	0.125	-
	E	0.390	0.200	0.125	0.625	0.036	0.318	0.089	-	0.250	-
Gpi	A	0.012	-	0.125	-	-	-	-	-	-	-
	B	0.012	0.100	-	-	-	-	0.037	-	-	-
	C	0.146	0.100	0.042	0.042	0.107	0.136	0.037	0.333	0.125	-
	D	0.232	-	0.083	0.250	0.107	0.455	0.167	0.167	0.075	-
	E	0.354	0.700	0.500	0.375	0.250	0.318	0.296	0.333	0.125	-
	F	0.232	0.100	0.167	0.333	0.429	0.091	0.296	-	0.525	0.500
	G	-	-	-	-	0.071	-	0.074	-	0.150	-
	H	0.012	-	0.083	-	0.036	-	0.093	0.167	-	0.500
	I	-	-	-	-	-	-	-	-	-	-
n		41	5	12	12	14	11	28	3	20	2

Table 5.2. Frequency of alleles in morphological species of *Platygyra* from Davies Reef (including the outgroup *Leptoria phrygia*).

Locus	No. of alleles	F_{is}	χ^2	df		F_{st}	χ^2	df	
Pgm	12	0.127	25.9	66	ns	0.065	208.8	88	***
Mpi	5	0.767	343.5	10	***	0.064	74.8	32	***
Ck	4	0.436	83.3	6	***	-0.008	-7.0	24	ns
Lp	4	0.898	353.2	6	***	0.087	76.2	24	***
Lt-1	4	-0.089	3.5	6	ns	0.115	100.7	24	***
Lt-2	2	0.668	65.1	1	***	-0.023	-6.7	8	ns
Lg-1	2	0.624	56.8	1	***	0.116	33.9	8	***
Lg-2	5	0.790	364.5	10	***	0.035	40.9	32	ns
Gpi	9	0.698	569.0	36	***	0.025	58.4	64	ns
$\bar{x}$		0.547	183.5	11	***	0.053	64.9	34	**

Table 5.3. F-statistics on all loci for *Platygyra* species and morphs from Davies Reef. (ns - not significant; ** P<0.01; *** P<0.001)

	PB	PDC	PDF	PH	PDSE	PDSV	PP	PR	PS	LP
PB	-									
PDC	0.044	-								
PDF	0.057	0.020	-							
PH	0.106	0.076	0.143	-						
PDSE	0.070	0.048	0.048	0.067	-					
PDSV	0.062	0.069	0.065	0.157	0.083	-				
PP	0.061	0.082	0.063	0.117	0.048	0.072	-			
PR	0.079	0.068	0.053	0.150	0.081	0.101	0.024	-		
PS	0.050	0.066	0.083	0.068	0.069	0.044	0.032	0.067	-	
LP	0.414	0.519	0.401	0.537	0.542	0.602	0.483	0.570	0.476	-

Table 5.4. Values of Nei's D between morphological species of *Platygyra* and the out-group *Leptoria phrygia*.

Samples from Orpheus Island showed no fixed gene differences between *P. lamellina* and the other morphological species of *P. daedalea* (Appendix 1). Similarly, small samples from Magnetic, Orpheus and Heron Islands confirmed identities between these and the species at Davies Reef, and provided no evidence that populations were strongly differentiated between the three reefs (Appendix 1).

5.3.2. Genetic variation within morphological species

Genetic variability was high within all the species from Davies Reef with an average of 2 to 4 alleles per locus, and up to 100% of the nine loci were polymorphic (Table 5.5). Only two out of the 148 colonies sampled had identical genotypes - both *P. ryukyuensis* colonies - all remaining colonies had unique genotypes.

Values of F_{is} showed significant genetic variation ocurred within species at seven of the nine loci scored (Table 5.3). Mean heterozygosity values were lower than expected in all species, ranging between 0.130 and 0.333 (Table 5.5). Chi-squared tests for deviance from Hardy-Weinberg equilibrium showed the species *P. daedalea* (morphs PDC and PDSE), *P. pini*, *P. sinensis* and PH from Davies Reef deviated significantly from expected values (Table 5.6). These deviations were due to heterozygote deficiencies in all five cases. Those populations which conformed to Hardy-Weinberg equilibrium, *P. daedalea* (morphs PDF and PDSV), PB, *P. ryukyuensis* and *Leptoria phrygia* had small sample sizes ($n \leq 12$) and consequently little allelic variation. If larger numbers had been sampled, it is possible these species would also show departures from Hardy-Weinberg equilibrium.

Species/Morph		Mean sample size per locus	Mean no. of alleles per locus	% loci poly-morphic	Mean heterozygosity: direct- count	Mean heterozygosity: Hdywbg expected
Davies:	PB	11.0 (0.0)	3.1 (0.5)	88.9	0.162 (0.075)	0.471 (0.097)
	PDC	41.0 (0.0)	4.1 (0.8)	88.9	0.154 (0.070)	0.413 (0.109)
	PDF	5.0 (0.0)	3.2 (0.6)	77.8	0.244 (0.080)	0.464 (0.122)
	PDSE	12.0 (0.0)	3.6 (0.8)	77.8	0.130 (0.044)	0.403 (0.109)
	PDSV	12.0 (0.0)	3.4 (0.6)	88.9	0.167 (0.067)	0.436 (0.106)
	PH	14.0 (0.0)	3.9 (0.7)	100.0	0.286 (0.085)	0.459 (0.087)
	PP	27.9 (0.1)	3.9 (1.0)	66.7	0.176 (0.093)	0.405 (0.123)
	PR	3.0 (0.0)	2.0 (0.4)	55.6	0.296 (0.152)	0.385 (0.127)
	PS	20.0 (0.0)	3.8 (0.8)	77.8	0.161 (0.082)	0.434 (0.119)
	LP	2.0 (0.0)	1.6 (0.2)	55.6	0.333 (0.167)	0.370 (0.117)

Table 5.5. Genetic variability at nine loci in *Platygyra* species and morphs at Davies Reef (± SE). A locus was considered polymorphic if more than one allele was detected. Expected heterozygosities are unbiased estimates following Nei (1978)

5.3.3. Genetic groups within the genus Platygyra

Lack of conformance to Hardy-Weinberg equilibrium suggests some genetic structuring within species. To further examine the data set for the presence of genetic groups, a Principal Co-ordinates analysis was carried out on the nine-locus genotype of all colonies collected from Davies Reef. Results from this analysis (Figures 5.4, 5.5) further emphasise the absence of any structuring within the genus. There are no genetic groups associated with either morphology (Figure 5.4) or with habitat (Figure 5.5).

	P. daedalea									
	PDC	PDF	PDSE	PDSV	PB	PH	PP	PR	PS	LP
Pgm	1.000	1.003	0.030	0.277	0.543	0.585	1.017	0.400	1.040	1.000
Mpi	0.000*	0.625	0.004	0.077	0.001	0.135	0.000*	0.200	0.000*	0.333
Ck	0.002	-	0.043	0.130	1.000	0.326	1.000	-	0.236	-
Lp	0.000*	0.365	0.000*	0.006	0.011	0.000*	0.000*	0.200	0.000*	1.000
Lt-1	1.000	1.000	1.000	1.000	1.000	1.000	-	-	1.000	1.000
Lt-2	0.037	-	-	1.000	-	0.037	-	-	-	-
Lg-1	-	1.000	-	-	0.002	1.000	-	-	-	-
Lg-2	0.000*	0.048	0.088	0.006	0.001	0.128	0.000*	1.267	0.000*	-
Gpi	0.000*	0.333	0.281	0.007	0.001	0.010	0.002	1.267	0.000*	0.333
n	41	5	12	12	11	14	28	3	20	2

Table 5.6. Probabilities that the observed genotypic frquencies deviated from those expected under conditions of Hardy-Weinberg equilibrium derived from the exact test (Elston & Forthofer, 1977) in morphological species of *Platygyra* and the outgroup *Leptoria phyrigia*. (* denotes $P < 0.05$). (significance thresholds corrected for multiple simultaneous tests)

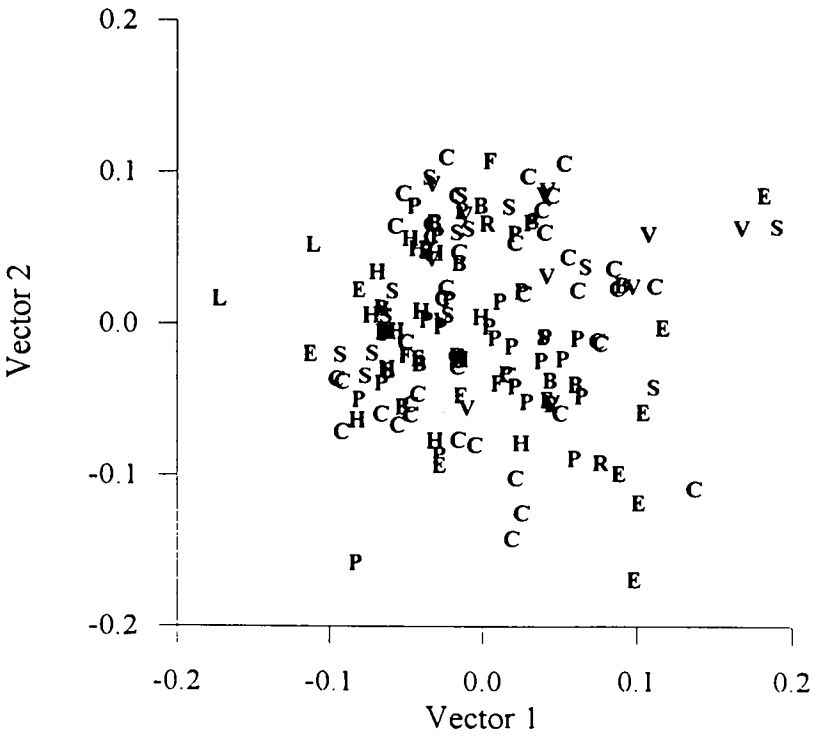


Figure 5.4. Plot of the first two vectors from principal co-ordinates analysis based on nine-locus genotype. Letters correspond to morphological species. H - PH, B - PB, S - *P. sinensis*, P - *P. pini*, R - *P. ryukyuensis*, L - *Leptoria phrygia*. Morphs of *P. daedalea* are C - PDC, E - PDSE, V- PDSV, F - PDF.

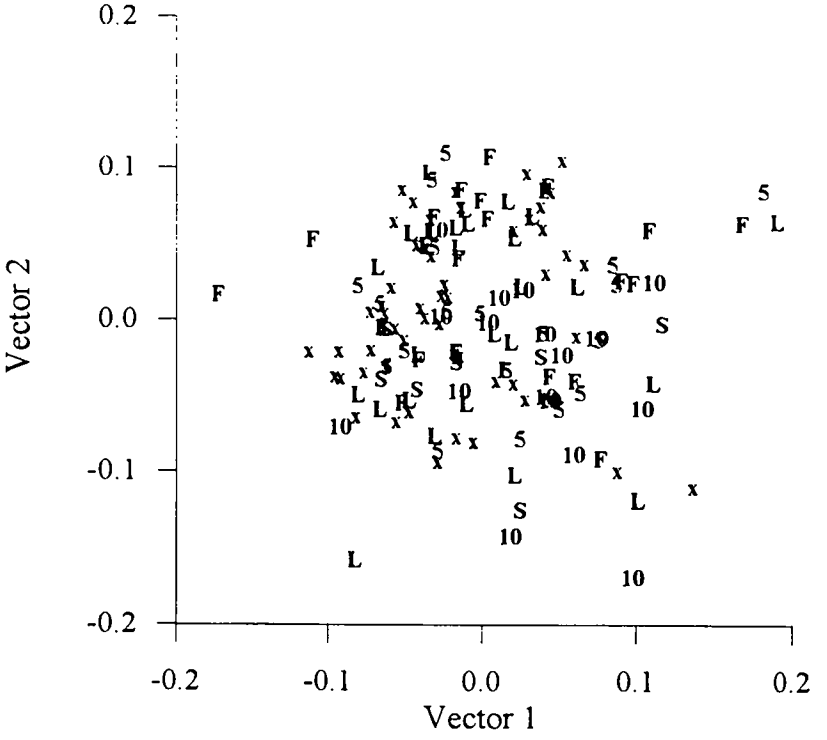


Figure 5.5. Plot of first two vectors from principal co-ordinates analysis based on nine-locus genotypes. Letters correspond to colony habitat ie. reef zone. 5 - front reef slope at 5 metres, 10 - front reef slope at 10 metres, F - front reef flat, L - lagoon, S - back reef slope. x signifies habitat of colony not recorded.

5.3.4. Histo-incompatibility between morphological species

No interactions between any of the colony pairs were evident after 48 hours, and no colonies showed signs of stress. After 72 hours, pair # 24 (*P. sinensis* x PB) showed signs of incompatibility, with the *P. sinensis* colony extending sweeper tentacles across to the PB colony. No other pairs interacted after 72 or 96 hours. After three months, many of the pairs exhibited signs of an interaction, which were consistent at the four month observation. Fifteen of the twenty-seven pairs were aggressive, resulting in tissue death after three months (Table 5.7). Outcomes of aggression were obvious as a band of dead tissue (up to 8 cm wide) on the subordinate colony. In only two instances did the replicate interactions produce the same outcome. *Platygyra sinensis* was dominant in both pairings with PB, and PB dominated *P. daedalea* (morph PDC) on both occasions (Table 5.7). No interaction was considered to have taken place if either colony had completely died as this could not conclusively be attributed to a competitive outcome. This occurred in five of the twenty-seven pairs (Table 5.7). Seven of the colony pairs showed no sign of incompatibility, and many of these showed signs of tissue indifference when colonies dislodged and came into close contact (Table 5.7). Only *P. daedalea* (morphs PDC and PDSE) were consistently compatible.

5.3.5 *Genetic comparison of interacting colonies*

There was no relationship between interaction outcome and percent genetic difference (Table 5.7). Colonies as much as 61% different genetically were found fusing (pair # 1, Table 5.7) whereas colonies which were genetically similar (17% different, pair # 22 and 24, Table 5.7) interacted. Mean genetic difference between interacting vs. non-interacting pairs was not significant using a t-test ($t = -1.9889$, $p < 0.05$).

Species	Pair #	Interaction outcome	Interaction response	% diff on genotype
PDC x PDC	1	PDC = PDC	contacting - no reaction	61
	7	*inconclusive*	one completely dead	22
PDC x PH	3	PDC > PH	6 cm dead band	39
	9	PDC <> PH	both interacting	22
PDC x PDSE	4	PDC = PDSE	no reaction	50
	10	PDC = PDSE	contacting - no reaction	44
PDC x PDSV	2	PDC = PDSV	contacting - no reaction	39
	8	PDC < PDSV	7 cm dead band	33
PDC x PP	6	PDC = PP	no reaction	33
	12	PDC > PP	8 cm dead band	50
PDC x PS	5	*inconclusive*	both dead	33
	11	PDC < PS	4.5 cm dead band	50
PDC x PB	13	PDC < PB	dead band	28
	16	PDC < PB	5 cm dead band	44
PH x PDSE	15	PH > PDSE	5.5 cm dead band	44
	18	PH < PDSE	4 cm dead band	39
PH x PB	19	PH < PB	6.5 cm dead band	33
	22	PH > PB	6 cm dead band	17
PS x PDSV	14	PS > PDSV	6.5 cm dead band	33
	17	*inconclusive*	both dying	39
PS x PP	20	*inconclusive*	PP completely dead	33
	23	PS > PP	7 cm dead band	50
PS x PB	21	PS > PB	4 cm dead band	33
	24	PS > PB	3 cm dead band	17
PP x PP	25	PP = PP	no reaction	39
	26	*inconclusive*	one completely dead	39
PR x PS	27	PR = PS	no reaction	50

Table 5.7. Summary of results of interaction experiment. Percent difference in genotype was calculated based on the number of different alleles at all nine loci examined. "=" denotes no interaction between pairs , ">" and "<" signs denote direction of aggressive interaction between pairs (eg. PDC > PP indicates PDC was dominant over PP) and "*inconclusive*" is due to the complete mortality of one or both interacting colonies, most likely due to external factors.

5.4 Discussion

5.4.1 Genetic relationships between morphological species of Platygyra

Results of the electrophoretic survey of morphological species of *Platygyra* revealed no fixed gene differences between species as would be expected if the morphological groups corresponded with reproductively isolated species. While results from the previous chapter demonstrated hybridisation was possible, genetic results suggest that gene exchange actually occurs between the morphological species of *Platygyra*. Significant F_{st} values are likely to be the result of the small sample sizes for many of the species, rather than real genetic differences between species.

The low values of Nei's D between the morphological species groups (Table 5.4), fell within the usual range for intra-specific variation in invertebrates (Ayala, 1982). This result further suggests that morphological species of *Platygyra* from Davies Reef constitute a set of reproductively connected populations. While Nei's D cannot be used for determining cut-off points for taxonomic boundaries and may not be directly comparable between taxonomic groups, it can give at least some idea as to the genetic similarity or dissimilarity between populations, and it is robust even if sample size is small (Nei, 1978; Gorman and Renzi, 1979). Based on the values of D from this study (Table 5.4), morphological groups of *Platygyra* are only as different as local populations of the same species.

Speciation may take place with little genetic change, and this has been demonstrated in a variety of taxa (eg. Avise *et al.*, 1974; Gartside *et al.*, 1977; Solé-Cava *et al.*, 1985). Thus the morphological species of *Platygyra* may have only recently arisen and as such, have not undergone considerable genetic divergence nor developed complete reproductive isolation. Alternatively, it may be that insufficient loci were examined to detect differences between these morphological groups, and further electrophoretic screening may reveal fixed differences.

The comparison between *Platygyra* species and the out-group *Leptoria phrygia* suggests the allozyme data is sensitive enough to detect generic differences. While there were no fixed gene differences found between *Platygyra* species and *L. phrygia*, values of Nei's D are considerably higher between the two genera (Table 5.4) and approach levels usually associated with closely related species and genera (Ayala, 1982). Fertilisation between the two genera is possible (see Chapter 4), however increased genetic distance between them suggests hybridisation may occur only rarely on the reef.

Due to concern that the protein electrophoretic results were inappropriate for addressing the question of genetic differentiation between morphological species of *Platygyra*, preliminary work was done using more sensitive genetic techniques to determine if genetic differences did exist between the morphological species of *Platygyra*. DNA sequences from the cytochrome oxidase 1 gene (mtDNA) and the D-1 domain of the 28s ribosomal gene have been obtained for some *Platygyra* colonies. Both of these genes are rapidly evolving regions and are likely to be sensitive to species-level differences. However, if morphological species of *Platygyra* have only recently speciated or comprise a complex of hybridising species, genetic differences may still not be detected. Unfortunately, due to time constraints, these methods could not be fully pursued. Sequences obtained to date are promising and it is likely that continuation of this approach will yield meaningful results.

The absence of diagnostic loci between species and the lack of genetic groups independent of morphology in the principle co-ordinates analysis (Figures 5.4, 5.5) suggests no subdivisions within the genus *Platygyra* corresponding with genetically differentiated groupings such as sub-, semi- or sibling species. Similar patterns of non-conformance of genetic groups with morphological groups have been documented in other corals. Stoddart (1986) found no clear relationship between phenotype and genotype in *Pocillopora damicornis* from Kanehoe Bay and Potts and Garthwaite (1986) found discontinuities between genetic and morphological species

groups in *Porites*. Similarly, McMillan *et al.* (1991) found that genetic subdivisions within the genus *Acropora* based on DNA sequences were contrary to taxonomic groupings based on morphology. The apparent disjunction between morphology and genotype in all of these genera suggests that patterns similar to those I have documented for *Platygyra* may occur throughout the Scleractinia. Recent studies have shown that hybridisation is possible between other coral species, including some species of *Acropora* (Willis *et al.*, 1992). It may be that hybridisation occurs commonly within a variety of coral genera on the reef including the genus *Platygyra*.

5.4.2. Genetic variation within morphological species of Platygyra

Genetic variation within morphological species from Davies Reef was high. Deviations from Hardy-Weinberg equilibria, caused by heterozygote deficiencies, were recorded in four of the seven morphological species of *Platygyra*, and it is highly probable that this pattern is widespread within the genus. Even though sample sizes were small, these results suggest there is some structuring within species associated with non-random mating. Similar patterns are commonly found in sedentary invertebrate populations which have external fertilisation and planktonic larvae eg. mussels (Tracey *et al.*, 1975), limpets (Johnson and Black, 1984), anemones (Ayre *et al.*, 1991) and other corals (Stoddart, 1984; Ayre and Willis, 1988; Van Veghel and Bak, 1993; Ayre & Dufty, in press). Several explanations have been proposed to explain heterozygote deficiencies in these populations; the Wahlund effect (the mixing of individuals from groups with different allelic frequencies), selection against heterozygotes, population inbreeding, asexual reproduction and the presence of null alleles.

Platygyra species do not produce asexual planulae (Babcock, 1986) and consequently heterozygote deficiencies within species are unlikely to result from asexual reproduction. However, reproductive patterns associated with mass spawning are likely to have important implications in terms of genetic population structure. Large numbers of unique genotypes were found within *Platygyra* populations on

Davies Reef and these are likely to reflect high gene flow and sexual reproduction within the genus. *Platygyra* are hermaphroditic broadcast spawners which reproduce annually (Babcock, 1986). Fertilisation is likely to take place over a relatively localised region (Oliver and Babcock, 1992), however, planktonic larvae are capable of surviving up to six weeks before settling (see Chapter 4). As a result, larvae are likely to be widely dispersed and will be transported between reefs (Willis and Oliver, 1990). The Wahlund effect has been related to heterozygote deficiencies in the coral *Seriatopora hystrix* which may experience occasional gene flow between otherwise philopatric populations (Ayre and Dufty, in press). Although the data were limited, there was no evidence of major genetic differentiation among populations of *Platygyra* species even on widely separated reefs (Appendix 1) and the Wahlund effect may therefore not be the most appropriate explanation of heterozygote deficiencies in *Platygyra* populations.

Other aspects of mass spawning are likely to promote non-random mating in coral populations. *Platygyra* species are long-lived and colonies can reach large sizes. It has been shown that a single large coral colony may contribute disproportionally to reproductive output of a population (Hall, 1992) and the subsequent small effective population sizes may contribute to heterozygote deficiencies. It is also likely that coral eggs will be fertilised by close neighbours (Oliver and Babcock, 1992) but structuring within populations associated with such non-random mating may be masked by larval dispersal (Grosberg, 1991). Results from Chapter 4 have shown that mating is non-random within the genus *Platygyra* associated with individual incompatibilities (ie. not all colonies will mate with all others) although the basis for this and its effect in terms of the population genetic structure is unknown. In addition, while the degree of temporal separation in spawning times between species is not enough to prevent hybridisation (see Chapter 4) it may result in some degree of non-random mating. It is not possible to test these hypotheses with my data set, however it is likely that all of these factors act simultaneously on the reef, and may contribute to the observed patterns of heterozygote deficiencies within populations of *Platygyra*.

Results from principal co-ordinates analysis show no genetic structuring within species associated with reef habitat (Figure 5.5). This is in contrast to some corals where genetic differences between populations in different habitats have been recorded. Stoddart (1988) found most of the variation within populations of *Acropora digitifera* occurred between sites on a single reef. Similarly, Benzie *et al.* (in press) found differences in gene frequencies between *Pocillopora damicornis* populations in reef front compared with lagoon habitats. Ayre & Willis (1988) found patchiness in genetic composition of *Pavona cactus* within reefs as did Stoddart (1984) in *P. damicornis* populations around Western Australia. Similar patterns of variation between localised populations have been described in other organisms eg. starfish (Hunt, 1993) and sea anemones (Ayre *et al.*, 1991). Habitat variation and small scale patchiness in coral populations often exists as a result of localised recruitment of asexual planulae or by fragmentation resulting in the local dominance of genotypes (Stoddart, 1984; 1988; Willis and Ayre, 1985; Ayre and Willis, 1988). However, asexual reproduction by fragmentation is unlikely to play an important role in the population structure of *Platygyra* species (Babcock, 1991) and this is reflected in the low frequency of clonal genotypes among all the individuals sampled. The apparent absence of genetic structure associated with habitat is likely to reflect wide dispersal and random settlement of these species relative to within-reef habitat differences. However larger sample sizes will be necessary to test this conclusively.

5.4.3 Histo-incompatibility between morphological species of Platygyra

The complexity of relationships both within and between morphological species of *Platygyra* is further highlighted by the results from the interaction experiments. Incompatibility responses between species appear to be multi- rather than uni-directional and are not always consistent regardless of morphology (Table 5.7). The consistent incompatibility between PB and both *P. daedalea* (morph PDC) and *P. sinensis* suggest there may be differentiation between these species. However, this is not reflected in genetic distances between the two species (Table

5.4) nor in their mating compatibility (see Chapter 4).

The high variability of interaction response at the species level may not be surprising in light of variation in reproductive compatibility between individuals (see Chapter 4). There was no relationship between interaction outcome and degree of allozyme genetic dissimilarity in *Platygyra*. This is similar to results in an analogous study of *Montastrea annularis* which also found no relationship between genotype and interaction (Van Veghel and Bak, 1993). Clearly there is no simple relationship between morphotype, genotype and aggressive response in *Platygyra* and this phenomenon is likely to be widespread across a variety of taxa (Grosberg, 1988; 1989). Stoddart (1988) reviewed various models of historecognition based on genetic identity (ie. exact genotype match or matching response at a single locus level). While interactive response did not appear to be related to genotype in *Platygyra*, it may be that none of the allozyme loci examined are linked to the region responsible for interactive response. However, it has been shown that interaction response is an unreliable genetic marker (Willis and Ayre, 1985; Resing and Ayre, 1985; Heyward and Stoddart, 1985) and it may be that the recognition system may be non-genetic, affected by factors such as age (Hidaka, 1985) or familiarisation (Hildemann *et al.*, 1977).

5.4.4. Conclusions

The morphological species of *Platygyra* are not genetically distinct, nor does there appear to be any structuring within the genus that might reflect the occurrence of reproductively isolated species. The genetic results are consistent with the results from fertilisation trials and suggest hybridisation occurs naturally between the seven morphological species of *Platygyra*. The importance of these findings to theories of evolution and speciation in the Scleractinia will be discussed in the following chapter.

CHAPTER 6

GENERAL DISCUSSION

6.1. Species boundaries in the genus *Platygyra*

Traditionally, species boundaries within the Scleractinia are morphological, based on skeletal characteristics. Within the genus *Platygyra*, five morphological species are currently described from the Great Barrier Reef (Veron, 1992). However, this study has revealed the existence of seven, rather than five, morphologically distinct species within the genus (Chapter 2). The range of variation within morphological species was high, as exemplified by *P. daedalea* which consists of four morphological variants that can be defined on gross colony morphology and skeletal characteristics. While skeletal characters are highly variable within species of *Platygyra*, I found little evidence of environmental influences on morphology in these species either within-reef or across large geographic scales (Chapter 3). Most of the seven morphological species show no clear habitat preference either within or between reefs. Some species are differentially distributed across habitats however these patterns do not appear to reflect the existence of ecomorphs or species boundaries.

Previous studies of corals have assumed that morphological species boundaries will equate with reproductive and genetic differences. However, fertilisation trials in this study have shown that morphological species of *Platygyra* are capable of hybridising and that hybrid fertilisation rates are equivalent to rates of within-species fertilisation (Chapter 4). Similarly, I have shown that hybrid *Platygyra* are capable of normal settlement and growth. Genetic results also suggest that morphological species share a common gene pool (Chapter 5) and this implies that hybridisation occurs naturally between morphological species of *Platygyra*. While there is evidence that mating is not random within the genus, non-random reproductive relationships are likely to be associated with aspects of coral biology such as reproductive timing, distribution patterns and individual incompatibilities,

rather than inter-specific barriers to fertilisation.

The results from this thesis demonstrate the complexity of reproductive and genetic relationships between morphological species of *Platygyra* and highlight the relevance of reassessing species relationships and evolutionary processes within the Scleractinia. Clearly, within the genus *Platygyra*, morphological species boundaries cannot imply breeding incompatibility or genetic isolation. Other coral species have also been shown to hybridise *in vitro* (eg. *Acropora* spp. and *Montipora* spp, Willis *et al.*, 1992) and there are other genera in which morphological and genetic species groups are inconsistent (eg. *Porites*, Potts and Garthwaite, 1986; Potts *et al.*, 1992; *Acropora*, McMillan *et al.*, 1991). It may be that hybridisation occurs commonly between many morphological species of coral and consequently, *Platygyra* will serve as a suitable model to explore the implications of these findings to both coral taxonomy and evolution. It will be important to determine if analogies exist in other organisms to explain the complex reproductive and genetic relationships I have described in *Platygyra*. In addition, comparisons with similar species complexes in other animal and plant groups might provide an explanation for the existence of hybridisation in *Platygyra* and establish the nature of circumstances leading to the maintenance of morphological species boundaries within the genus.

6.2 Hybridisation between morphological species: the syngameon

Hybridisation between morphologically, ecologically and biologically distinct species has been widely documented in all major groups of plants (eg Grant, 1981; Briggs & Walters, 1984). The term syngameon has been introduced in the plant literature to describe a group of interbreeding species (Grant, 1957) and this term may also be extremely useful for describing morphological species of coral which are capable of hybridising.

Until recently, the phenomenon of hybridisation was thought to be widespread in plants due to their relative simplicity compared with animals (Grant, 1981). Similarly, instances of hybridisation in animals were thought to be restricted to lower taxa, between species with relatively simple mating procedures and external fertilisation (Mayr, 1963). Species of *Platygyra* are plant-like in many respects of their biology (eg. life history strategies, phenotypic plasticity - see Chapter 1) and also have external fertilisation. Hybridisation may therefore be more common in *Platygyra* and other coral species because of these factors. However, more recent research indicates hybridisation in vertebrates may also be reasonably common (eg Templeton, 1989; Arnold, 1992; Smith, 1992, Grant and Grant, 1992) and it may form an important part of the biology of many different organisms, regardless of their relative simplicity or complexity (Arnold, 1992). Hence syngameons may be common in a variety of taxa.

Two of the most frequently cited examples of plant syngameons are those of the White Oaks (*Quercus*) and of Irises. Hybridisation between species of *Quercus* has been implied from the presence of morphological intermediates within populations (Palmer, 1948; Burger, 1975). Allozyme studies of *Quercus* species revealed high genetic identities between taxa supporting morphological evidence of hybridisation within the genus (Guttman and Weight, 1988). Recent DNA studies have also found that morphological species of oak share a common gene pool and that genetic groupings reflect geographic location rather than morphological species boundaries (Whittemore and Schaal, 1991). In Irises, the existence of plants intermediate in morphology between species has been used to imply hybridisation between species (Riley, 1938). However, morphological variation is high in Irises, and genetic studies have demonstrated that Irises form localised hybrid swarms (populations comprising an array of morphotypes including F1 hybrids, backcrosses and parental plants) (Grant, 1981; Arnold and Bennett, 1993). Other genetic studies of syngameons have shown evidence of hybrid speciation (eg. *Helianthus annus* ssp. *texanus*; Rieseberg *et al.*, 1990) and have provided conclusive evidence that hybridisation has occurred between species

of both plants and animals (Templeton, 1989; Meagher and Dowling, 1991; Arnold, 1992; Rieseberg and Wendel, 1993).

The results of this thesis suggest that like oaks and irises, species of *Platygyra* form a syngameon. The seven morphological species are capable of interbreeding, and genetic data suggest hybridisation occurs naturally between them. The frequency of natural hybridisation events can only be speculated upon, however the relative abundance of morphological intermediates suggest hybridisation rates may be low. If hybridisation occurred as frequently as within-species fertilisation (and hybrids were intermediate in morphology to parent species), in a community of two hybridising species where fertilisation is random, 50% of the population would be hybrids (ie. intermediate). This percentage increases with the number of potentially hybridising species. Within *Platygyra*, some species could be considered intermediates although it is unclear which species represent the end points of this process. For example, PH is intermediate in morphology between *P. lamellina* and *P. daedalea*, *P. sinensis* is intermediate between *P. daedalea* and *P. pini* and PB is intermediate between *P. daedalea* and *Leptoria phrygia*. At Davies Reef, species such as *P. daedalea*, *P. pini* and *L. phrygia* are very common whereas species with intermediate morphologies (PH, PB) are rare. This pattern suggests hybridisation only occurs at low levels between the morphological species although the assumption that hybrids will always be intermediate may not necessarily be correct (Byrne and Anderson, in press). Similarly, mating within *Platygyra* is not entirely random and this may also influence the abundance of morphological species and intermediates.

6.3 The maintenance of distinct morphology in the face of hybridisation.

If hybridisation does occur naturally in corals, even at relatively low levels, questions concerning the maintenance of discrete morphological species groups, as have been described in *Platygyra*, need to be addressed.

6.3.1 Introgression

If hybrid corals are viable (as evidence suggests, see Chapter 4), there is potential for introgression to occur. This possibility is further increased in *Platygyra* by mass spawning where gametes from many species and generations are mixed within the water column. Introgression, the repeated backcrossing of a natural hybrid to one or both parental populations, results in the infiltration of genes from one species to the second. Such processes have been demonstrated in numerous plant syngameons from both morphological and genetic data (eg. Grant, 1981; Arnold *et al.*, 1990; Rieseberg and Soltis, 1991; Whittemore and Schaal, 1991). One factor promoting introgression in populations is the relative rarity of hybrid progeny (Grant, 1981). If hybrids are rare, there will be an increased chance that hybrids will mate with parental types. As discussed above, hybrid *Platygyra* appear to be rare on the reef and consequently it is possible that introgression occurs in these corals.

Introgression often results in the merging of species however, in cases such as Oaks, the effects of introgression may be balanced by selection for groups of co-adapted alleles resulting in morphologically discrete taxa that are genetically similar (Whittemore and Schaal, 1991). Morphologically discrete hybridising populations may also be maintained due to ecological segregation (Grant, 1981). In such cases, environmental selection for morphology favours hybrids which resemble parental types and local differentiation in the presence of gene flow has been demonstrated in a variety of taxa (Endler, 1973). In *Platygyra*, discrete morphological species exist, even in the presence of hybridisation. However there is no relationship between morphology and habitat variation associated with zonation patterns on Davies Reef (Chapter 3). Micro-geographic selection for morphological traits may result in discrete morphology in *Platygyra* species, however, due to the high heterogeneity of reef environments, such patterns may be difficult to detect. It is therefore likely that selection will be operating in conjunction with introgression within *Platygyra*, resulting in morphologically

distinct species which are genetically similar.

There are numerous consequences of introgression which have been described in plants (reviewed by Rieseberg and Wendel, 1993) that are equally applicable to hybridising species of coral such as *Platygyra*. Introgression may lead to an increase in genetic diversity. Hence, measures of genetic diversity may be higher in introgressant populations. Similarly, morphological variation will be high in introgressed populations. Certainly in *Platygyra*, both morphological and genetic variation is high (Chapter 2, Chapter 5) and this may be a consequence of interbreeding species and populations. Genetic variability may be advantageous in heterogenous environments (Lewontin, 1957; Marshall and Jain, 1968) and this may promote the maintenance of processes such as introgression in populations. Other consequences of introgression include the origin and transfer of unique characters (not shared with parents) which may have adaptive potential (Rieseberg and Wendel, 1993). Introgression may also lead to the origin of new ecotypes/ecomorphs and similarly, may result in hybrid speciation (eg. Levin, 1967; Rieseberg *et al.*, 1990).

Introgression in plant species is generally associated with hybrid zones where distinct individuals or populations meet and mate (Barton and Hewitt, 1989; Harrison, 1990). Hybrid zones can take the form of disjunctions between sympatric species or of clines across ecological gradients and their size is often related to the dispersal capabilities of the organism (see Harrison, 1993 for a review of hybrid zones). Hybrid zones are particularly common in anthropogenically disturbed habitats where the opening up of a new habitat provides opportunities for hybrid progeny to colonise an area perhaps not as favourable to parental types and, once established, hybrid individuals can subsequently breed with parent populations (eg Grant, 1981; Pacheco *et al.*, 1991). Disturbances such as cyclones and crown-of-thorns outbreaks on reefs may be important in creating new habitats for hybrid corals to colonise, however their effects in terms of introgression can only be speculated.

Classic hybrid zone distributions were not found in this study of *Platygyra* species, and all morphotypes occur sympatrically across a number of different habitats and reefs (Chapter 3). Distinct hybrid zones are generally maintained by hybrid disadvantage which prevents the dispersal and colonisation of hybrids across wide regions (Harrison, 1993). However, there is no evidence to suggest *Platygyra* hybrids are disadvantageous (Chapter 4) . This factor, coupled with the potential for wide dispersal in *Platygyra*, may explain the absence of distinct hybrid zones on the reef. However, where habitats are patchy, mosaic hybrid zones may occur resulting in a complex distribution of hybrids and parental types (Harrison and Rand, 1989). Coral reefs are highly variable, patchy environments. Hence mosaic hybrid zones are more likely to occur on reefs than distinct, well defined hybrid zones. In addition, wide dispersal of *Platygyra* larvae will contribute to patchiness in hybrid and parental distributions. Certainly the possibility that introgression may be occurring between species of *Platygyra*, mediated in part by the existence of mosaic hybrid zones and habitat disturbance, needs to be investigated further.

6.4 Evolutionary consequences of hybridisation

The discovery that some species of corals hybridise will have important consequences for concepts of coral evolution and speciation. Adaptive advantages associated with hybridisation may have promoted its occurrence in coral populations. Hybrids may have higher fitness because they have phenotypes capable of dealing with environmental variation (Grant & Grant, 1992). Certainly hybrid *Platygyra* had higher survivorship in aquaria than did normal corals (Chapter 4) and this may also be the case in highly variable reef environments. Similarly, hybridisation will contribute to higher genetic variation in corals which may also confer fitness in heterogeneous environments (Lewontin, 1957; Lewontin and Birch, 1966; Marshall and Jain, 1968; Schlichting and Levin, 1984). The ability to hybridise may also be advantageous during a mass spawning because the ability of a coral egg to be fertilised by non-specific sperm may reduce gamete

wastage.

From an evolutionary perspective, hybridisation may be viewed in three different ways; firstly as a result of divergent evolution; secondly as the process of convergent evolution and finally as evolutionary stasis. The three alternatives and their implications in terms of speciation processes in corals are discussed below:

6.4.1 Divergent evolution

Hybridisation may be an indication of recent or current speciation in *Platygyra*. Thus the morphological species may be sympatric semispecies (Grant, 1981) which represent intermediate stages of divergence and reproductive isolation from a common ancestor. Examples of divergent evolution in some species can be found in the fossil record. Geary (1992) found intermediates between two gastropod species over a 1 million year time period before they disappeared from the fossil record. He hypothesised that hybrid intermediates existed during the speciation process and subsequently disappeared once complete speciation had taken place and the two species had achieved reproductive isolation. However, in corals species divergence may not be so straight-forward. Potts (1983, 1984) hypothesised that complete speciation had been inhibited in corals since the beginning of the Quaternary due to combined effects of heterogeneous environments (fluctuating sea-levels), long generation times and the subsequent inbreeding between generations. He proposed that evolutionary disturbance, in the form of fluctuating sea-levels (every 3,200 years on average), was occurring too frequently for long-lived corals to diverge sufficiently to reach reproductive isolation before having to colonise new habitats. Based on this hypothesis, incomplete reproductive isolation between species of *Platygyra* could represent incomplete speciation resulting from evolutionary disequilibrium. Similarly, the high degrees of genetic and morphological variation in *Platygyra* populations may reflect the high levels of variation which have been proposed to be associated with non-equilibrium, speciating populations with inbreeding generations (Potts and

Garthwaite, 1992).

6.4.2 Convergent evolution

In contrast to theories of divergent evolution, morphological species of *Platygyra* may actually be converging. Introgression between species may result in the convergence of morphological and ecological characters. Hence, two species will merge into one. Alternatively, hybrid speciation - the production of a novel third species as a result of hybridisation - may also occur. Both convergent speciation and hybrid speciation form part of the process of reticulate evolution which has been described in plants (Grant, 1981; Arnold, 1992). Recent genetic studies have confirmed that introgression and hybrid speciation has occurred in plants (eg. Rieseberg and Soltis, 1991; Arnold *et al.*, 1991) and it may be that similar processes have occurred in corals. The "hybrid complexes" (Grant, 1953) which are the product of reticulate evolution have a number of distinct characteristics including species with diverse, intergrading forms and ecological preferences, and widespread natural hybridisation (Grant, 1981). These are very similar to the patterns I have described in *Platygyra*. As has been emphasised, many parallels can be drawn between plants and corals and Palumbi (1992) suggested speciation in broadcasting marine animals (eg. corals) may be more similar to plants than terrestrial animals. In line with evolutionary theories in plants, Veron (in press) has proposed that reticulate evolution occurs within the Scleractinia. Thus, morphological species of coral may form a network comprising numerous reproductive and genetic links and barriers between species, and my data from *Platygyra* supports such a view.

6.4.3 Evolutionary stasis

The third possible explanation is that hybridisation between morphological species may simply be normal and may not necessarily represent active processes of evolution. Templeton (1989) describes a pertinent example of balsam poplars and

cottonwoods, two species which have been morphologically distinct for at least 12 million years (according to the fossil record) but which have hybridised throughout that time. Hybridisation is often attributed to species with relatively young evolutionary ages, although hybridisation has also been recorded in older species (Grant and Grant, 1992). Scleractinian corals of the family Faviidae arose during the Tertiary and fossil records of *Platygyra* have been found from as early as the Eocene (Wells, 1964). Little is known of the evolutionary age of many coral species (Potts, 1983) although the average age of Indo-Pacific species is probably young and has been estimated to be 20 million years (Veron and Kelley, 1988). Fossil records from Papua New Guinea suggest the morphology of some *Platygyra* species has not changed dramatically in that region since the Pliocene (Veron and Kelley, 1988). Morphological stability over a 2-5 million year period may indicate species stability, particularly in regions where speciation rates have been estimated to be high (Veron and Kelley, 1988). Therefore, hybridisation may not represent evolutionary divergence or convergence in *Platygyra* and the existence of morphologically discrete, hybridising species of corals may not be unusual.

6.4.4 Evolution in Platygyra

It is impossible, based on my data set, to conclusively determine which of the three evolutionary alternatives - divergence, convergence or stasis - is currently taking place within the genus *Platygyra*. However, in light of the many similarities between plants and corals, particularly evidenced by the results of this thesis, similar evolutionary processes (ie. reticulate evolution) are likely to be occurring within the two groups. Indeed in a reticulate system, divergence, convergence and stasis are likely to occur simultaneously across geographic scales and all three will most certainly occur over geological time scales. Fossil records of *Platygyra* are few, and more palaentological studies may provide further insights to evolutionary changes within the genus. Similarly, studies of conserved and rapidly evolving genetic markers (eg. mtDNA and rRNA, Moritz *et al.*, 1987)

will also be useful to estimate the time-scale associated with past speciation processes within the genus. It has been shown that cytoplasmic DNA is introgressed more readily than nuclear genes (Whittemore and Schaal, 1990; Rieseberg and Wendel, 1993). Hence genetic studies comparing the distributions of these differentially inherited genetic markers between species may provide insight into the frequency of hybridisation in *Platygyra* and role of introgression and selection in the maintenance of species boundaries.

6.5 Species concepts in scleractinian corals

The presence of complex reproductive and genetic inter-relationships between morphological species of *Platygyra* raises questions as to the applicability of current species concepts and the relevance of species theory. The Biological Species Concept (BSC) (Mayr, 1942) is the most widely used species paradigm. However, while the BSC defines species based on reproductive isolation, the majority of coral species currently identified are based on morphology. The assumptions of the BSC appear to hold for some morphological species of coral eg. Isoporan Acroporiids (Ayre *et al.*, 1991) and digitate species of *Montipora* (Ben Stobart, pers comm) however these species may be atypical (see 6.1). The BSC is clearly not applicable to morphological species of *Platygyra* which are not reproductively isolated. Related research suggests the Biological Species Concept may also be inappropriate when applied to other species of coral which have been shown to be capable of hybridising (Willis *et al.*, 1992).

The Biological Species Concept has been criticised for a number of reasons, including practical and theoretical limitations (reviewed in Chapter 1). Botanists have adopted the Evolutionary Species Concept (ESC) as a more appropriate descriptor of the plant species and in light of the many parallels between plants and corals, the ESC may also be more appropriate for describing coral species. However, the ESC is not without its own shortcomings, including conceptual problems associated with defining species based on its ancestors (Templeton,

1989) and the difficulties in defining evolutionary roles and distinguishing them between species (Sokal, 1973).

Numerous other alternative species concepts have been proposed, including the Recognition Concept (Paterson, 1985), the Phenetic Concept (Sokal and Crovello, 1970; Sokal, 1973), the Phylogenetic Concept (Cracraft, 1983; 1987; Mishler and Brandon, 1987) and the Cohesion Concept (Templeton, 1989). While these alternate concepts offer some improvements, similar criticisms to those of the BSC and the ESC can be made about all of them (eg. Sokal, 1973; Templton, 1989; Endler, 1989) and none of them appears to be universally applicable. Indeed, "no solution to the species problem has been generally accepted" (Cracraft 1989). There is, however, general acceptance within the literature that a species concept must be pluralistic, ie. that species should be defined on a number of different criteria which may not necessarily be the same for all species. Apart from proponents of the recognition concept and biological species concepts which hinge only on reproductive criteria, it is widely recognised that both species and processes of speciation are variable.

In corals, the species is the morphological unit, made up of groups of corals that have similar skeletal characters. However, the coral species as a morphological unit may not bear any relation to breeding groups nor behave as evolutionary units. Thus, morphological species of coral may interbreed, may have a common gene pool, or may not, and these relationships need to be taken into account when considering evolution and speciation within the Scleractinia.

6.5. Conclusions

This study has used a combination of approaches in coral taxonomy to better describe inter-relationships between morphological species groups. By combining the results from morphometric, ecological, reproductive and genetic studies of *Platygyra*, I have demonstrated the nature of reproductive and genetic

complexities associated with the genus, which in turn has lead to the realisation that species relationships in these corals are far from simple. Morphological variants within the genus *Platygyra* do not represent ecomorphs, nor is there any evidence to suggest the presence of sibling species within the genus. One of the most important outcomes of this research is the discovery that morphological species of coral hybridise and it is likely that the high degree of morphological variation within species occurs as a result of hybridisation. Evidence of hybridisation within the genus *Platygyra* provides strong evidence that reticulate evolution occurs within the Scleractinia and this will be an important consideration when interpreting evolutionary patterns and species relationships in corals. Similarly, the occurrence of hybridisation between morphological species of coral demonstrates the assumptions of the Biological Species Concept are not applicable to scleractinian corals. The results of this thesis will have important implications for future studies of evolution and speciation in corals, and it will be particularly relevant when determining what constitutes the minimal evolutionary unit in *Platygyra*. It will also be important to undertake furhter comprehensive studies to elucidate the extent and effect of processes such as hybridisation, introgression and subsequent selection in coral species.

APPENDIX 1

GENETIC RESULTS FROM SAMPLES COLLECTED FROM MAGNETIC, HERON AND ORPHEUS ISLANDS.

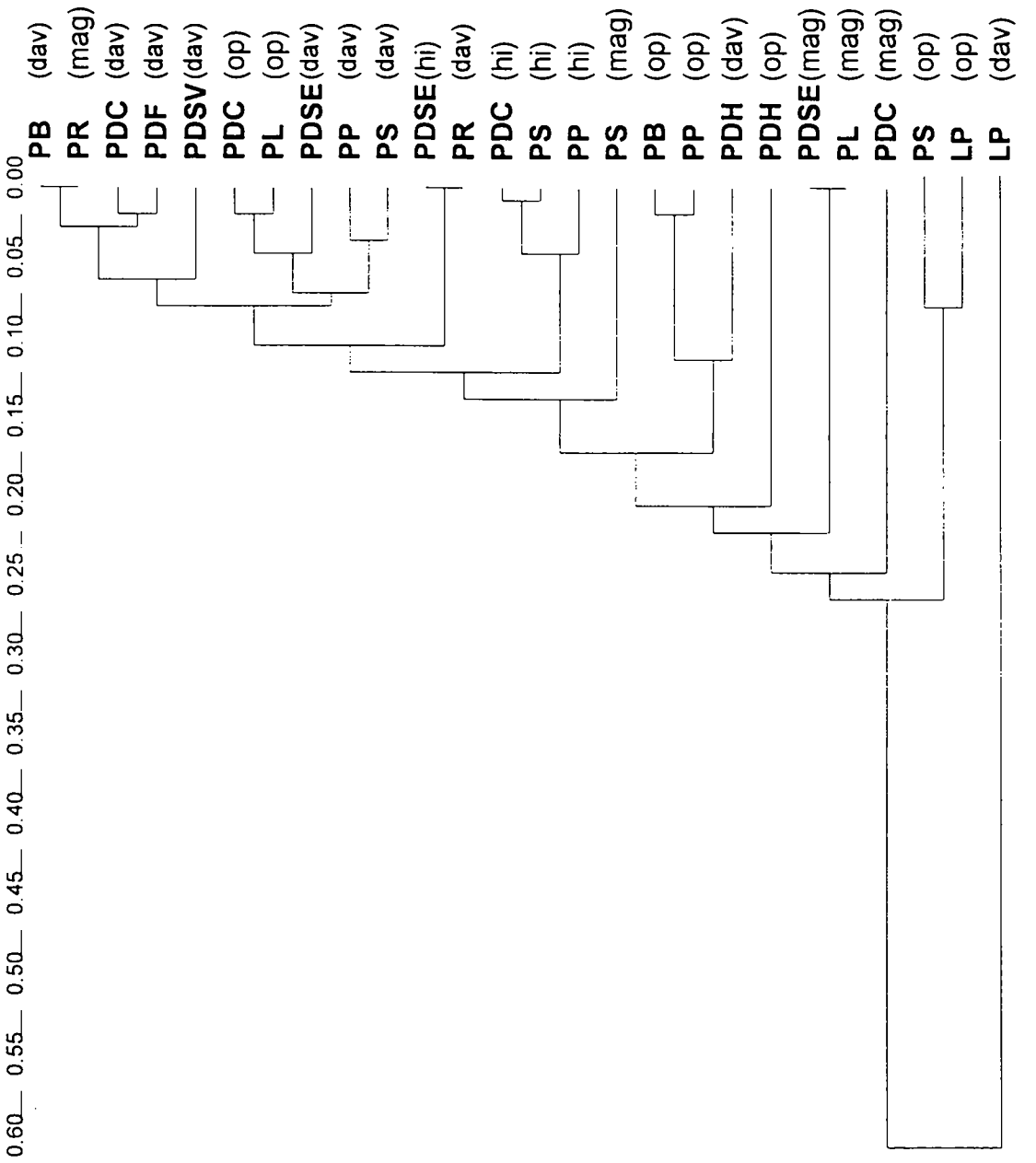


Figure A.1. Cluster analysis based on Nei's D for *Platygyra* species and morphs from four different reefs - Davies Reef, Magnetic Island, Heron Island and Orpheus Island.

Locus/allele		Species/Morph PDC	PDSE	PR	PS	PL
PGM	A	-	-	-	-	-
	B	0.167	-	-	0.100	-
	C	-	-	0.250	-	-
	D	-	-	-	0.100	-
	E	-	-	-	-	-
	F	-	-	0.250	0.400	0.500
	G	-	-	-	-	-
	H	0.167	-	0.250	0.100	-
	I	-	0.250	0.250	0.200	-
	J	0.500	0.250	-	0.100	0.500
	K	0.167	0.500	-	-	-
	L	-	-	-	-	-
MPI	A	0.667	-	0.500	-	-
	B	0.167	1.000	0.500	1.000	1.000
	C	-	-	-	-	-
	D	-	-	-	-	-
	E	0.167	-	-	-	-
CK	A	-	-	-	-	-
	B	-	-	-	-	-
	C	0.500	0.750	0.750	0.700	1.000
	D	0.500	0.250	0.250	0.300	-
LP	A	0.333	-	0.250	0.200	-
	B	0.667	1.000	0.500	0.200	1.000
	C	-	-	-	0.600	-
	D	-	-	0.250	-	-
LT-1	A	-	-	0.250	-	-
	B	1.000	1.000	0.750	1.000	1.000
	C	-	-	-	-	-
	D	-	-	-	-	-
LT-2	A	0.333	0.750	1.000	1.000	0.500
	B	0.667	0.250	-	-	0.500
LG-1	A	0.167	0.250	0.250	-	-
	B	0.833	0.750	0.750	1.000	1.000
LG-2	A	-	-	-	-	-
	B	0.500	0.750	0.250	1.000	1.000
	C	-	-	0.250	-	-
	D	-	-	-	-	-
	E	0.500	0.250	0.500	-	-
GPI	A	-	-	-	-	-
	B	-	-	-	-	-
	C	0.333	-	-	0.100	-
	D	-	0.250	0.500	0.700	-
	E	0.667	0.500	0.500	0.100	1.000
	F	-	0.250	-	0.100	-
	G	-	-	-	-	-
	H	-	-	-	-	-
	I	-	-	-	-	-
n		3	2	2	5	1

Table A.1. Frquency of alleles in *Platygyra* species and morphs from Magnetic Island.

Locus / allele		Species/Morph PDC	PDSE	PP	PS
Pgm	A	0.071	0.500	-	0.200
	B	0.071	-	0.250	0.300
	C	0.214	0.500	0.250	0.300
	D	-	-	-	-
	E	-	-	-	-
	F	0.286	-	0.250	0.100
	G	-	-	-	-
	H	0.357	-	0.250	0.100
	I	-	-	-	-
	J	-	-	-	-
	K	-	-	-	-
	L	-	-	-	-
Mpi	A	0.143	-	-	0.200
	B	0.571	1.000	0.250	0.700
	C	-	-	-	-
	D	0.143	-	0.750	0.100
	E	0.143	-	-	-
Ck	A	-	-	-	-
	B	-	-	-	-
	C	0.429	1.000	0.500	0.400
	D	0.571	-	0.500	0.600
Lp	A	0.429	-	0.500	0.700
	B	-	0.500	-	-
	C	0.571	0.500	0.500	0.200
	D	-	-	-	0.100
Lt-1	A	-	-	-	-
	B	1.000	1.000	1.000	1.000
	C	-	-	-	-
	D	-	-	-	-
Lt-2	A	0.857	1.000	1.000	0.800
	B	0.143	-	-	0.200
Lg-1	A	0.071	-	-	-
	B	0.929	1.000	1.000	1.000
Lg-2	A	0.071	0.250	0.250	0.200
	B	0.286	0.500	.500	0.500
	C	-	-	-	-
	D	0.214	0.250	-	-
	E	0.429	-	0.250	0.300
Gpi	A	-	0.250	-	-
	B	-	-	-	-
	C	-	0.500	-	0.200
	D	0.071	-	0.500	0.200
	E	0.143	0.250	0.500	0.200
	F	0.286	-	-	0.400
	G	0.143	-	-	-
	H	0.357	-	-	-
	I	-	-	-	-
n		7	2	2	5

Table A.2. Frequency of alleles in *Platygyra* species and morphs from Heron Island.

Locus / allele		Species/Moprh PDC	PB	PH	PP	PL	LP
Pgm	A	-	-	-	-	-	-
	B	0.143	-	-	-	0.125	0.625
	C	-	-	-	-	0.188	-
	D	0.071	-	-	-	0.063	-
	E	-	-	-	-	0.063	-
	F	0.214	0.500	0.500	0.250	0.250	0.250
	G	0.071	-	-	-	-	-
	H	0.214	0.500	0.500	0.500	0.250	0.125
	I	0.214	-	-	-	0.063	-
	J	-	-	-	-	-	-
	K	0.071	-	-	0.250	-	-
	L	-	-	-	-	-	-
Mpi	A	0.357	-	-	-	0.250	0.250
	B	0.571	1.000	-	0.750	0.250	-
	C	-	-	-	-	-	0.250
	D	-	-	0.500	-	0.250	0.500
	E	0.071	-	0.500	0.250	0.250	-
Ck	A	-	-	-	-	-	0.125
	B	0.143	-	-	-	0.063	-
	C	0.857	1.000	1.000	1.000	0.938	0.875
	D	-	-	-	-	-	-
Lp	A	0.286	1.000	1.000	0.500	-	-
	B	0.143	-	-	-	0.250	-
	C	0.571	-	-	0.500	0.625	1.000
	D	-	-	-	-	0.125	-
Lt-1	A	-	-	-	-	-	-
	B	1.000	1.000	0.500	1.000	1.000	1.000
	C	-	-	0.500	-	-	-
	D	-	-	-	-	-	-
Lt-2	A	1.000	1.000	1.000	1.000	0.813	1.000
	B	-	-	-	-	0.188	-
Lg-1	A	-	-	0.250	-	-	-
	B	1.000	1.000	0.750	1.000	1.000	1.000
Lg-2	A	-	-	-	-	-	0.250
	B	0.500	-	0.750	0.500	0.688	0.500
	C	-	-	-	-	-	-
	D	0.357	1.000	0.250	0.500	0.125	-
	E	0.143	-	-	-	0.188	0.250
Gpi	A	-	-	-	-	-	-
	B	-	-	-	-	-	-
	C	-	-	-	-	0.063	0.250
	D	-	-	0.500	-	0.188	-
	E	0.643	-	0.500	-	0.438	-
	F	0.286	1.000	-	1.000	0.188	-
	G	0.071	-	-	-	0.063	0.750
	H	-	-	-	-	-	-
	I	-	-	-	-	0.063	-
n		7	2	2	2	8	4

Table A.3. Frequency of alleles in *Platygyra* species from Orpheus Island (including the outgroup *Leptoria phyrigia*)

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