Life History of the Scleractinian Coral *Seriatopora hystrix*: a Population Genetic Approach

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften

der Fakultät für Biologie

der Ludwig-Maximilians-Universität München

vorgelegt von

Elke Maier

München, 02. März 2010

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"The naturalist will feel this astonishment more deeply
after having examined the soft and almost gelatinous bodies
of these apparently insignificant creatures,
and when he knows that the solid reef increases only on the outer edge,
which day and night is lashed by the breakers
of an ocean never at rest."

Charles Darwin (1842). The Structure and Distribution of Coral Reefs.

CONTENTS

SUMMARY

Scleractinian corals show an extraordinary diversity of life-history strategies with regard to growth, reproduction and dispersal. Abundant intraspecific variation makes direct functional analyses of these key life-history traits possible. This is not only interesting from an evolutionary point of view but also a central issue for reef conservation.

A good candidate for such analyses is the widely distributed coral *Seriatopora hystrix*. It is a hermaphroditic species with internal larval development. In addition to outcrossing, *S. hystrix* is capable of selfing. Moreover, various modes of asexual reproduction have been discussed. Given this large repertoire of possible strategies, the aim of this thesis was to investigate which options are realised in natural *S. hystrix* populations from two different geographical regions, the Red Sea and the Great Barrier Reef (GBR).

In order to obtain a set of markers suitable for detailed population genetic studies in *S. hystrix*, I set out to develop microsatellite loci. The major problem with developing species-specific markers in scleractinian corals is the presence of endosymbiotic algae (zooxanthellae). Due to these algae, coral tissue contains DNA from two different organisms. Consequently, a method is required that assures the coral origin of any markers that have been identified. I developed a method to overcome this problem, based on manual removal of algal cells from minute amounts of coral tissue in combination with a whole genome preamplification of the extracted DNA. This way, a coral genomic library free of contamination with algal DNA was constructed. A screen with a fluorescently labelled $(CA)_{15}$ probe yielded a set of species-specific microsatellite markers. Additional tests confirmed the coral origin of these markers.

As a first approach to test whether larval dispersal in *S. hystrix* is spatially restricted, I focused on ten *S. hystrix* stands within the Red Sea. Mantel tests revealed isolation by distance effects on scales ≤ 20 km, indicating restricted dispersal. The data did not provide evidence against strictly sexual reproduction. At all sites, considerable heterozygote deficits occurred. However, as null alleles were likely to contribute to this pattern, the role of coral population processes and life history could not be assessed. Thus, more detailed analyses were required to investigate which mechanisms caused these large local heterozygote deficits that are frequently observed in *S. hystrix*.

To investigate which processes shape *S. hystrix* genetic population structure, all adult as well as juvenile colonies from two contiguous stands within the Red Sea were sampled for a fine-scale analysis on scales from decimetres to metres. The sexual mode of reproduction,

the likely occurrence of selfing and the level of immigration were in agreement with previous studies on this species. Contrary to other findings, both stands were in Hardy-Weinberg equilibrium. Also, no evidence for spatially restricted larval dispersal within the sampled areas was found.

Analyses on *S. hystrix* from the GBR revealed a very different picture. Whereas the three sites sampled at Lizard Island (northern GBR) were genetically almost homogeneous, five of the six sites at Heron Island (southern GBR) were highly substructured, consistent with colonisation from various genetically diverse source populations. The study revealed that Wahlund effects, small-scale dispersal of sperm and/or larvae and inbreeding, including selfing, contributed to the observed genetic population structure. The importance of different mechanisms differed largely among sites. The observed variation could reflect differences in life history and/or varying environmental conditions, which opens intriguing questions for future research.

To unravel within-population genetic processes, various approaches based on individual multilocus genotypes such as Bayesian clustering analyses, parentage assignment and identification of clonal replicates were used. Individual-based analyses allow for high resolution but could possibly be affected by undetected intracolonial genetic variation. Within a single colony, different genotypes may be present due to the accumulation of somatic mutations (= mosaicism) or fusion of genetically different larvae or colonies (= chimerism). Regarding the potential impact of intracolonial genetic variation on population genetic studies in *S. hystrix*, I set out to investigate the proportion of heterogeneous *S. hystrix* colonies in natural populations. Moreover, I aimed to distinguish between the two underlying mechanisms, mosaicism versus chimerism. I found a considerable proportion of colonies (~17%) that included more than a single genotype. Considering the low sampling effort per colony, the true value of intracolonial genetic variation is expected to be even higher. Whereas both, mosaicism and chimerism contributed, mosaicism was the major source of intracolonial genetic variation.

1. INTRODUCTION

"Modular growth, the rarity of senescence, curious reproductive schedules, the ability of a genet to function in bits, the absence of segregated germ plasm, and the effects that branching structure have on the capture of resources – all set modular organisms apart from those with unitary structure. The same set of properties brings most of the plant kingdom and a neglected part of the animal kingdom into a common category for generalizations about life-cycle biology and evolution. Moreover, it is in the nature of modular organisms that they provide exquisite experimental tools for the field and laboratory study of fundamental biological problems. It is odd that so much of the study of ecology and evolution has been based on the behavior of unitary organisms."

Harper (1985). Population Biology and Evolution of Clonal Organisms.

1.1 Background

1.1.1 Unitar versus modular organisms

The life history of an organism can be defined as the schedule of events that occurs between birth and death (Hall & Hughes 1996). Traditionally, life-history theory has mainly focused on unitary organisms which have a closed developmental programme, beginning with a single-celled zygote and ending with an adult individual that has a largely determinate body size and a maximum life span (Jackson 1985; Stearns 1992). Most unitary organisms reproduce strictly sexually. Thus, each individual in a population is genetically unique. Genotypes cannot be preserved intact across generations and usually survive only few reproductive cycles. Population dynamics are determined by the individuals' birth, growth, migration and death (Karlson 2002).

Modular organisms, which include most plants and representatives of 19 animal phyla such as sponges, bryozoans and cnidarians (Begon *et al.* 1998), are fundamentally different: Modular growth proceeds via the repeated, vegetative formation of identical modules, such as leaves in plants, zooids in bryozoans or polyps in cnidarians. All modules are ultimately derived from the same zygote (Jackson 1977, 1985). Modularity involves an open-ended developmental programme and allows for great flexibility of growth form and organisation (Wood 1999). Growth is often indeterminate and continues until severe injury or death (e.g. Jackson 1985; Sebens 1987; Hughes 1989). Usually, single modules show distinct age structure, however, in some long-lived taxa like trees and corals, the whole organism may experience little senescence (Hughes & Jackson 1980). Size increase cannot only be achieved by growth but also by fusion, while size decrease may occur due to fission, fragmentation or partial mortality (Wood 1999). Modules usually remain interconnected during growth but are generally capable to exist independent of the parental organism (Jackson 1985), which leads to the widespread occurrence of asexual reproduction. Unless somatic mutations occur, asexual offspring is genetically identical to the parent. This implies that genotypes can be transmitted intact to future generations. As a consequence of asexual reproduction, a single genetic individual (genet) may consist of multiple different parts (ramets) that are distributed across time and space and can experience different selective regimes. This can spread a genet's risk of localised mortality, e.g. due to predation, disease or environmental disturbance, and provide protection against extinction.

In addition to asexual reproduction, another characteristic of various modular organisms such as plants or sessile marine invertebrates is the ability to self-fertilise. Due to the

co-occurrence of different reproductive modes, population dynamics of modular organisms can be highly complex, both spatially and temporally, and involve processes that act on the genet level, the ramet level as well as on all individuals within a population, irrespective of their genetic identity (Karlson 2002). Due to their great variability of life-history traits, modular organisms are excellent study objects to test predictions of evolutionary theory.

1.1.2 Reproduction and dispersal

A central evolutionary issue is the significance of different reproductive strategies. Above all, explaining the widespread occurrence of sexual reproduction in the face of the two-fold advantage of asexual lineages (Maynard Smith 1978) is still the queen of problems in evolutionary biology (Bell 1982). Hypotheses for the maintenance of sexual reproduction can generally be classified into two groups, i.e. those emphasising that sexual reproduction impedes the accumulation of deleterious mutations (Kondrashov 1988) and those stressing the advantage of sex in heterogeneous environments (Bell 1982). For organisms that can reproduce both sexually and asexually, the first hypothesis can largely be ruled out, as a low percentage of recombination suffices to prevent mutation accumulation (Hurst & Peck 1996). This suggests that the production of recombinant offspring in these organisms may be promoted by environmental factors.

According to the classical view, sexually produced, genetically diverse offspring is advantageous in heterogeneous, unstable environments and should thus be widely dispersed (West *et al.* 1999). In contrast, a locally successful genotype can corroborate its fitness advantage via asexual propagation because advantageous gene combinations can be transmitted intact to the next generation. The role of sexual and asexual offspring in sessile organisms that multiply vegetatively in continuous habitats (such as strawberries and corals) is summarised in Williams' (1975) strawberry-coral model: The model predicts that these organisms produce sexual offspring to colonise new, unpredictable sites whereas they use asexual propagation to expand locally and exploit limited resources.

Another central evolutionary issue is the significance of selfing (reviewed in Jarne & Charlesworth 1993; Goodwillie *et al.* 2005; Jarne & Auld 2006). Selfing has been associated with negative fitness effects (i.e. inbreeding depression, Charlesworth & Charlesworth 1987). On the other hand, it may help to preserve favourable gene combinations and facilitate local adaptation (Shields 1982) while it allows for purging of deleterious recessive alleles (Lande *et al.* 1994). Both asexual reproduction and selfing can assure fertilisation in the absence of

mates and may therefore present important strategies, especially in sessile organisms (reviewed in Jarne & Charlesworth 1993).

Reproduction is closely linked to dispersal, i.e. the movement of organisms from their location at birth to other locations where they reproduce (Futuyma 1998). Dispersal leads to gene flow, i.e. the movement and integration of genes from one population into another (Ross 2001). Gene flow determines the amount and distribution of genetic variation on which selection can act and thus affects local adaptation and speciation (Barton 2001). Even though dispersal is perilous and mortality rates of dispersers are often high, it is an important strategy to reduce competition among relatives (Hamilton & May 1977), avoid negative effects of inbreeding (Shields 1982; Perrin & Goudet 2001), escape from deteriorating local conditions (Denno & Roderick 1992) and colonise new habitats (van Valen 1971). As dispersal influences the dynamics and persistence of populations, the abundance and distribution of species as well as the structure of communities (e.g. Dieckmann *et al.* 1999; Hanski 2001; Mouquet *et al.* 2001), it has profound evolutionary and ecological consequences. In addition, it is an important issue for conservation (Ronce *et al.* 2001).

1.2 Life history of scleractinian corals

1.2.1 Overview

Scleractinian corals (Phylum Cnidaria, Class Anthozoa, Order Scleractinia) are a group of modular marine organisms with an extraordinary diversity of life-history strategies among closely related or even within the same species (Carlon 1999). Thus, they are excellent model organisms from an evolutionary perspective. Many species do not only reproduce sexually but may also propagate asexually in various ways, e.g. via broken fragments, detached polyps or asexually produced larvae (reviewed in Harrison & Wallace 1990). Sexual and asexual reproduction is not mutually exclusive but may co-occur even within the same colony (Richmond 1997). Both sexual and asexual offspring can cover a range of dispersal distances. In terms of sexual reproduction, various coral species are capable of selfing (Carlon 1999). As corals live in spatially and temporally highly dynamic environments (Connell 1973), this raises the question about the adaptive value of different strategies.

Coral life history is not only interesting from an evolutionary point of view but also a central issue for reef conservation. Scleractinian corals are the major framework builders of coral reefs, the most diverse marine ecosystems with enormous ecological, economic and

social value (Done *et al.* 1996). As architectural species, they are the key to the persistence of thousands of other organisms (Knowlton 2001). At present, reefs face a large-scale crisis, mostly due to a combination of global stressors, especially climate change, coupled with regional and local detrimental factors including pollution, overfishing and habitat destruction associated with coastal development (e.g. Hughes *et al.* 2003; Bellwood *et al.* 2004; Hoegh-Guldberg *et al*. 2007). The prime global stressor is the rising CO_2-level, causing both ocean warming and acidification. The main results are coral bleaching, reduced calcification rates as well as reduced settlement abilities of larvae (e.g. Hoegh-Guldberg *et al*. 2007; De'ath *et al.* 2009; Munday *et al.* 2009). Of the world's 285.000 km^2 of coral reefs, about 20% are already destroyed and half of the remaining reefs are under imminent threat (Pennisi 2007). One third of all coral species face elevated extinction risk (Carpenter *et al.* 2008). This alarming situation calls for effective management strategies to avert further damage. The prerequisite to develop successful conservational plans is a sound knowledge of how reef systems will respond to environmental impact (Hughes & Tanner 2000).

Life history of reef-building corals is a critical factor to determine the resilience of reefs after local disturbance. As reproduction, dispersal and recruitment, i.e. the process by which propagules become part of the reef community, impact population dynamics as well as reef connectivity, they determine whether and at what time scales depleted reefs are replenished after local extinction (Richmond 1997). In addition, as reproductive mode and dispersal distance affect the distribution of genetic variation on which natural selection can act (Barton & Whitlock 1997), they largely influence to what extent reefs can adapt to local conditions (Hellberg 2006*a*; van Oppen & Gates 2006). Dispersal is critical for the design of marine reserves and should be considered in any decision about size and spacing of protected areas to ensure that they are self-sustaining and preferably send propagules to depleted areas nearby (Palumbi 2003). Not surprisingly, studies on coral reproductive biology and the associated process of dispersal have become a central topic in marine biology (e.g. Hellberg 2006*a*, 2007; van Oppen & Gates 2006; Baums 2008).

1.2.2 Growth

Coral life cycle includes a short-lived, often planktonic larval phase and a long-lived, sessile adult stage (Wood 1999). Of the approximately 1400 extant species of scleractinian corals, about 60% are colonial (reviewed in Baird *et al*. 2009). The term 'colony' refers to an organism that consists of modules that are also morphological individuals (Wood 1999). The modules of a coral colony are termed polyps (Figure 1). Each polyp is equipped with a whole set

of organs, i.e. mouth, gastric cavity, gonads etc. Colony growth proceeds via budding of polyps, which means that a polyp divides to form new individuals, thus creating replicates of itself. Polyps usually remain interconnected but may also separate from the underlying colony and persist as discrete units (Jackson 1985). All polyps and colonies derived from a single zygote present the genetic individual, or genet. A single genet may either remain intact throughout its life or propagate clonally, e.g. via separate polyps or daughter colonies (Hughes *et al.* 1992).

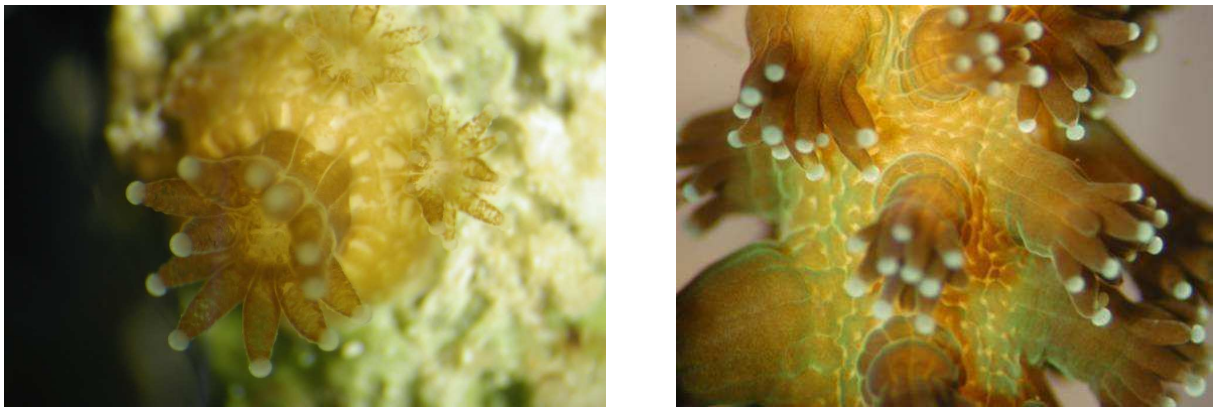

Figure 1: Juvenile coral (*Pocillopora damicornis*) showing three polyps (left) and branch detail of an adult *P. damicornis* colony (right). The polyps of a colony are interconnected by living tissue, the so-called coenosarc. It spreads along the surface of the calcareous skeleton. *Photographs: Christian Laforsch*

Due to fission and fusion, age and size are decoupled in coral whereas growth and longevity of any one colony are in principle unlimited. Size correlates with many fitness attributes such as survival, competitive ability and fecundity. For example, predation on small colonies mostly results in complete death, while large colonies may only suffer partial mortality (Hughes & Jackson 1980). Larger size can also increase survival in the face of competitors such as macroalgae (Hughes 1989). Finally, as fecundity in corals is proportional to the number of polyps that participate in reproduction, it is skewed towards large colonies (Babcock 1984; Hughes *et al.* 1992; Hall & Hughes 1996), which may thus dominate a population's gene pool (Wood 1999).

An important consequence of modular growth is that a single coral colony does not necessarily present a homogeneous genetic entity. Rather, it may include various genotypes, either due to somatic mutations, referred to as mosaicism, or fusion of genetically different newly settled larvae or colonies, called chimerism (Figure 2). Especially with regard to the

long life-time of some corals, a considerable amount of somatic mutations may accumulate within a single colony over time (Wood 1999). As corals lack a separate germ line, somatic mutations may be represented in the gametes and thus spread within the population in the course of sexual reproduction. Alternatively, they may be passed to clonal offspring (Orive 2001). Intracolonial genetic variation may considerably affect the amount and distribution of genetic variation within a population, with important ecological and evolutionary consequences.

Figure 2: Chimeric entity of the coral *Favia fragum*. These fused primary polyps resulted from two larvae settling in close proximity. *Photograph: Dirk Petersen*

1.2.3 Reproduction

Scleractinian corals display a diverse range of reproductive strategies, with various sexual and asexual modes of propagation. Sexual reproduction results in a motile larval stage, so-called planula larva (Figure 3). Planulae are ciliated, lipid-rich and equipped with chemosensors for detecting suitable substrate for settlement and metamorphosis (Harrison & Wallace 1990; Richmond 1997). The criteria for appropriate settlement sites include substratum type, water motion, salinity, adequate sunlight and limited sediment deposition. In addition, specific algal species or biological films of diatoms and bacteria may be required for successful settlement. Metamorphosis involves the secretion of a basal plate along with the first skeletal

cup as well as the formation of tentacles surrounding the mouth, resulting in a so-called primary polyp (Figure 3). Through continued growth, budding and calcification, this first polyp develops into a new coral colony (Richmond 1997).

Figure 3: Planula (left) and 1-month old primary polyp (right) of *Favia fragum*. *Photographs: Dirk Petersen*

Planulae are produced via two different developmental pathways (reviewed in Harrison & Wallace 1990; Richmond 1997; Baird *et al*. 2009): In broadcast spawners, which comprise ~85% of all coral species, eggs and sperm are shed into the water column where fertilisation and development take place (Figure 4). In brooders which include the remaining 15% of species, eggs are fertilised internally and embryos develop within the parental polyp for several weeks before being released. Whereas broadcasters often spawn annually at predictable times (Harrison & Wallace 1990), many brooders release their larvae monthly throughout the year or during certain reproductive periods (Richmond 1997). Control of reproductive timing is highly complex and may involve a wide range of environmental signals, including sea surface temperature and lunar phases (reviewed in Tanner 1996; Harrison & Wallace 1990; Baird *et al*. 2009).

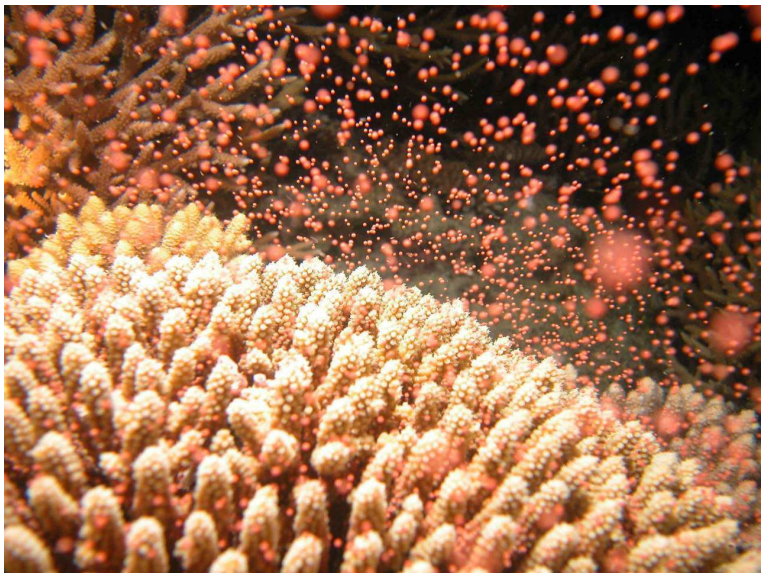

Figure 4: Spawning colony of the genus *Acropora*. In this hermaphroditic coral, the gametes are released as bouyant bundles of eggs and sperm packed together. HERON ISLAND, GREAT BARRIER REEF
Photograph: Ralph Tollrian

In terms of sexual expression, corals are either hermaphroditic or gonochoric (reviewed in Harrison & Wallace 1990; Richmond 1997; Baird *et al*. 2009). More than two thirds of species express both sexes at the same time (simultaneous hermaphrodites). In almost all cases, eggs and sperm are produced within a single polyp. The widespread occurrence of simultaneous hermaphroditism opens the possibility for selfing, either by syngamy of gametes originating from the same polyp or from different polyps of the same colony (Carlon 1999). The potential for selfing differs largely among corals but may be higher in brooders compared to broadcast spawners. *In vitro* trials in broadcasting species showed that eggs and sperm from the same colony either did not produce any viable embryos or did so at very low rates (reviewed in Carlon 1999; but see Stoddart *et al.* 1988). Few data are available on selfing in brooding corals, with selfing rates ranging from almost exclusive outcrossing to considerable levels of selfing (Brazeau *et al.* 1998; Ayre & Miller 2006; Sherman 2008). In brooders, outcrossing relies on sperm transfer between colonies, which means that limited sperm motility, sperm dilution and restricted lifespan of sperm may restrict successful outcrossed fertilisation to colonies in close proximity (Levitan & Petersen 1995; Coffroth & Lasker 1998). Selfing may therefore present an important reproductive strategy in brooding corals.

In addition to sexual reproduction, various modes of asexual propagation have been described. In branching corals like *Acropora* (Tunnicliffe 1981; Baums *et al.* 2006), asexual reproduction via broken fragments is common. Fragments are detached from the parental colony due to physical factors such as currents and storms or biological impact such as predators and bioeroders (Highsmith 1982). If a fragment falls on solid bottom, it can attach to the surface and continue growth (Richmond 1997). A study on *Montastrea annularis* showed that fragmentation may also occur in massive corals (Foster *et al.* 2007). Asexual reproduction may also take place via individual polyps: In a process called polyp bail-out, single polyps detach from the underlying skeleton, disperse and reattach to an appropriate surface (Sammarco 1982). Similarly, certain species form balls of coral tissue that separate from the coral calices to later differentiate into polyps, secrete a calcareous skeleton and form new colonies (Rosen & Taylor 1969; Highsmith 1982). Finally, coral larvae may arise from unfertilised eggs. This parthenogenetic production of larvae is best known from the widespread species *Pocillopora damicornis* (Stoddart 1983; Ayre & Miller 2004; Sherman *et al.* 2006; Yeoh & Dai 2010).

In summary, with their extraordinary diversity of reproductive strategies, scleractinian corals do not fit into traditional life-history schemes. Variation of reproductive mode and sexual expression may occur among closely related species, within single species or even within the same colony. For example, in *Pocillopora verrucosa* and *P. damicornis*, broadcasting and brooding colonies have been observed at different geographical locations. (Glynn *et al.* 1991; Ward 1992). In *Goniastrea aspera*, both strategies may co-occur within the same colony (Sakai 1997). In some corals, individual colonies or even individual polyps are hermaphroditic, while others are gonochoric (Harrison & Wallace 1990). Finally, while the rate of sexual versus asexual reproduction may vary greatly for a given species in different parts of its geographic range, both reproductive modes may also co-occur within a single colony (Richmond 1997). The occurrence of alternative strategies within the same species is of special interest, as it may provide insight into their adaptive significance in an ecological context. Corals are valuable study objects to test and extend evolutionary theory.

1.2.4 Dispersal

Dispersal in corals is mediated via gametes, sexual larvae and/or asexual propagules. Exceptionally, dispersal may also occur through rafting of small colonies attached to floating objects such as pumice or coconuts (Jokiel 1984, 1989, 1990). Dispersal distances are determined by various physical and biological factors (reviewed in Hohenlohe 2004). Physical

factors include hydrological conditions of the habitat (Dawson 2001), ocean current patterns (Palumbi 1994) as well as environmental conditions such as salinity or temperature that exceed larval tolerances and thus limit dispersal (Luppi *et al.* 2003). Also expanses of open ocean may present barriers to dispersal (Ayre & Hughes 2004). Biological factors that affect dispersal include a species' mode of reproduction and the properties of its offspring.

Larvae of brooders are generally larger than larvae of broadcast spawners and contain symbiotic algae (zooxanthellae) upon release from the parental polyp. They are competent to settle and metamorphose immediately and tend to stay close to their parent. In contrast, larvae of broadcast spawners generally lack zooxanthellae and require a developmental period of at least four to seven days in the water column to gain settlement competency (Babcock & Heyward 1986; Harrison & Wallace 1990; but see Miller & Mundy 2003). During this time they may be transported away from their natal reef. This suggests that broadcast spawners show larger minimum dispersal distances compared to brooders. Considering the upper limit of the dispersal phase, larvae of broadcasters must settle within three to four weeks, otherwise energy reserves will be too low for a successful metamorphosis (Richmond & Hunter 1990; but see Nishikawa *et al.* 2003; Graham *et al*. 2008). On the other hand, brooded larvae may show extended competency periods of more than 100 days, as zooxanthellae provide a source of energy (Richmond 1987; Harii *et al*. 2002). Thus, they may cover large distances.

Taken together, these findings indicate that larval biology does not provide a reliable clue to predict mean dispersal distances. Also comparative population genetic studies on various species of brooders and broadcast spawners did not reveal a clear picture: Some indicated a correlation between reproductive mode and larval dispersal distance (Nishikawa *et al*. 2003; Underwood *et al*. 2009) while others did not support such a relationship (Ayre & Hughes 2000; Miller & Ayre 2008). Especially in brooders, larval dispersal distances may vary considerably, with settlement from only decimetres to tens of kilometres away from the parental colony.

In principle, this vast range of dispersal distances may not only be covered by sexual but also asexual offspring. While fragmentation is a local process, as size and weight of broken fragments restrict dispersal (Lirman 2000), asexual larvae may show the same range of dispersal distances as their sexual counterparts (Stoddart 1983). Likewise, other small and light-weight forms of asexual offspring, like detached polyps, may be dispersed away from the parental colony (Sammarco 1982). This is in contrast to other organisms like various plants where asexual reproduction, e.g. via stolons or rhizomes, is restricted to local scales (Begon *et al.* 1998).

1.3 Population genetic studies on scleractinian corals

Studying life-history traits such as reproduction and dispersal in natural populations presents a major challenge, especially in marine populations where possibilities of direct observation are limited (but see Olson 1985; Carlon & Olson 1993; Planes *et al.* 2002; Jones *et al.* 2005). Usually, sexual and asexual offspring is visually indistinguishable, family relationships cannot be inferred by mere observation, and following tiny propagules in the vast ocean is an insoluble task (Hellberg *et al.* 2002). However, as reproduction and movement of propagules affect the distribution of neutral genetic variation, population genetic studies offer an indirect approach to gain insight into these processes.

During the last decades, such studies have challenged the traditional view that marine populations are highly interconnected across thousands of kilometres (reviewed in Hedgecock 1986; Benzie 1999). Also in corals, they revealed that the spatial scale of connectivity is generally smaller than previously assumed, and evidence for panmixia was only found across relatively small distances of several tens of kilometres or less (reviewed in van Oppen & Gates 2006). It was even shown that restricted dispersal may affect population genetic structure on scales below tens of metres (Underwood *et al.* 2007; Miller & Ayre 2008). For studies on such small spatial scales, genetic markers with high resolution are required which permit analyses on the individual level.

In recent years, a plethora of new population genetic tools have become available to study population level processes on ecological time scales (summarised in Excoffier & Heckel 2006), and new markers and analytical approaches keep being developed at an ever increasing pace. In corals, however, population genetic analyses were lagging behind many other marine taxa for a long time, mostly due to the lack of high-resolution genetic markers in the past (Ridgway 2002). The mitochondrial genome evolves at an extremely slow rate in anthozoans (e.g. Shearer *et al.* 2002; Hellberg 2006*b*; but see Chen *et al*. 2008) and thus does not provide suitable genetic markers for analyses on the individual level. In studies on coral speciation and hybridisation, nuclear introns have been analysed (e.g. Hatta *et al.* 1999; van Oppen *et al.* 2001; Vollmer & Palumbi 2002) but again the level of resolution is not suitable for fine-scale analyses. The same is true for internal transcribed spacer (ITS) markers that have also been employed in corals (e.g. Odorico & Miller 1997; Diekmann *et al.* 2001; Rodriguez-Lanetty & Hoegh-Guldberg 2002). Another major problem with the ITS region is the high level of intra-individual variation (Vollmer & Palumbi 2004; Lam *et al.* 2006). With multilocus techniques like AFLPs, the major obstacle is that the source of the observed bands is ambiguous (Barki *et al*. 2000; Brazeau *et al*. 2005).

Until recently, the majority of population genetic studies on corals were based on allozyme markers (reviewed in van Oppen & Gates 2006). Allozymes combine various advantages for studies on corals (reviewed in Ridgway 2005) but are notoriously slow in reaching genetic equilibrium such that present population genetic processes may be confounded by signatures of past events (Grosberg & Cunningham 2001). Moreover, they also show limited allelic diversity (Selkoe & Toonen 2006).

Today, the most widely used molecular markers to study fine-scale processes on ecological time scales are microsatellites (e.g. Ellegren 2004; Selkoe & Toonen 2006). Microsatellites are species-specific, co-dominant markers with Mendelian inheritance. They consist of tandem repeats of one to six nucleotides and are frequent in the nuclear genome of most taxa (Selkoe & Toonen 2006). Due to their specific mutational process, mainly slippage during DNA replication, many microsatellite loci show high mutation rates between 10^{-2} and 10^{-6} mutations per locus per generation (Schlötterer 2000). This results in high allelic diversity such that combining several microsatellite loci provides a 'unique genotype identification tag' for studies on the individual level (Queller *et al.* 1993; reviewed in Selkoe & Toonen 2006). For microsatellite amplification only a few DNA copies are sufficient, which has proved invaluable in many applications such as conservation studies or analyses of ancient DNA (Hutchinson *et al.* 2003). Especially in combination with sophisticated analytical tools, based on maximum likelihood, Bayesian probability theory and Markov chain Monte Carlo simulation (summarised in Excoffier & Heckel 2006), microsatellites promise to reveal detailed insight into recent population genetic processes.

In scleractinian corals, microsatellites were not available until a few years ago. By the end of the 1990's, only a single microsatellite marker had been published (Lopez *et al.* 1999). This was because the development of these markers had been hampered by the presence of zooxanthellae which occur in high numbers of 0.5 to 5 million algal cells per cm^2 coral tissue (Smith *et al.* 2005). For the development of species-specific markers, these algae present a major obstacle, as DNA extractions contain both coral host and symbiont DNA (Shearer *et al.* 2005). In recent years, various approaches have been applied to overcome this problem (e.g. Magalon *et al.* 2004; Baums *et al.* 2005*a*, 2009; Underwood *et al.* 2006; van Oppen *et al.* 2007) and to date, microsatellites have been successfully used in a number of population genetic studies on corals (Magalon *et al.* 2005; Baums *et al.* 2005*b*, 2006; Foster *et al.* 2007; Ridgway *et al.* 2008; Souter & Grahn 2008; Souter *et al*. 2009; van Oppen *et al.* 2008; Noreen *et al*. 2009; Nakajima *et al*. 2009; Puill-Stephan *et al*. 2009; Underwood 2009; Underwood *et al.* 2007, 2009; Yeoh & Dai 2010; Souter 2010). These studies revealed impor-

tant insight into population genetic processes in this highly diverse group and provide valuable clues for the establishment of efficient management strategies.

To gain a comprehensive picture of coral life history, knowledge about the factors that shape genetic population structure within a single species at different locations across a range of spatial scales is essential. So far, however, detailed analyses for any given species are scarce. Most studies focused on scales between tens of metres to hundreds of kilometres to evaluate connectivity patterns while very little is known about the processes that shape genetic structure on the smallest scales within local stands (but see Miller & Ayre 2008; Yeoh & Dai 2010).

1.4 The study species: *Seriatopora hystrix* (Dana 1846)

The present thesis focuses on the widespread scleractinian coral *Seriatopora hystrix* (Figure 5). This pocilloporid coral is commonly found in Indo-Pacific reefs (Veron 2000). It has been a focus of recent microsatellite-based research (Underwood *et al.* 2007, 2009; van Oppen *et al.* 2008; Noreen *et al*. 2009), thus data are available that can be combined and extended in order to obtain a comprehensive picture of life history.

S. hystrix is a simultaneous hermaphrodite that reproduces sexually via brooded larvae (Ayre & Resing 1986; Sherman 2008). Production of broods may involve a mixed mating strategy with considerable levels of selfing (Sherman 2008). Larval release in *S. hystrix* shows latitudinal variation, with seasonal reproductive phases in the Red Sea and on the GBR and year-round release of planulae in Palau (reviewed in Harrison & Wallace 1990). The onset of larval production was observed at a minimum colony diameter of 8 cm and a volume of 15 cm^3, corresponding typically to a colony age of one to two years (Stimson 1978). In the laboratory, most larvae settle within 24 h after release (Atoda 1951), which implies restricted dispersal.

Field studies supported this prediction: Two allozyme surveys on the GBR found very high levels of genetic differentiation across limited spatial scales, with F_{ST} values of 0.3 (Ayre & Dufty 1994) and 0.28 (Ayre & Hughes 2000) for populations within a single reef. On even smaller scales, microsatellite-based autocorrelation analyses in *S. hystrix* from Western Australia showed a tendency of larvae to settle within tens of metres of their parental colony (Underwood *et al*. 2007). Nevertheless, as the well-provisioned, brooded larvae may survive for a long time in the water column, at least a small proportion is expected to travel far

(Richmond 1987). Assignment tests that have successfully identified migrants confirmed this assumption (Underwood *et al.* 2007; van Oppen *et al.* 2008; Noreen *et al*. 2009). Laboratory observations imply that *S. hystrix* may even use differently sized planulae for short- and long distance dispersal, with larger, long-lived larvae adapted to long-distance dispersal (Isomura & Nishihira 2001).

Figure 5: Adult (left) and juvenile (right) colony of *Seriatopora hystrix*. The colour of colonies varies from pink to cream, blue or green (Veron 2000). LIZARD ISLAND, GREAT BARRIER REEF
Photographs: Christoph Haacke, Christian Laforsch

Like other corals, *S. hystrix* may not only reproduce sexually but also asexually. Given the colonies' delicately branched morphology, reproduction via fragmentation appears likely (Ayre & Dufty 1994; Ayre & Hughes 2000; see also Figure 6). Under laboratory conditions, polyp bail-out was observed as a response to exposure, excess or insufficient illumination. The mechanism was also reported from the field (Sammarco 1982). It was discussed that 'pregnant' polyps may bail out and release planulae upon resettling, thus enhancing the species' distribution (Sorokin 1990). In addition, it was suggested that polyp bail-out may contribute to the dominance of *S. hystrix* in some shallow reef habitats (Sammarco 1982).

While *S. hystrix* has the potential to reproduce asexually, previous population genetic surveys imply that local stands are largely maintained by sexual recruits (Ayre & Dufty 1994; Ayre & Hughes 2000; Underwood *et al.* 2007; Sherman 2008; van Oppen *et al.* 2008; Noreen *et al*. 2009). The resolution of these studies, however, was restricted due to the use of allozyme markers and/or coarse sampling design such that some clonal replicates may have been missed. Moreover, recent microsatellite data were interpreted as evidence for long-

distance dispersal of asexual propagules (van Oppen *et al.* 2008). Considering these points and the likely geographical variation in reproductive strategies, a definitive assessment of asexual reproduction in *S. hystrix* has yet to be reached.

Figure 6: Broken fragment of *S. hystrix*. Such fragments could possibly form new colonies. HERON ISLAND, GREAT BARRIER REEF *Photograph: Christian Laforsch*

In summary, *S. hystrix* is endowed with a variety of reproductive modes, including outcrossing, selfing and different modes of asexual propagation as well as the capability for short- and long-distance dispersal. This range of options offers a broad scope of strategies to cope with varying environmental conditions. *S. hystrix* shows an opportunistic ('weedy') life history that is characterised by rapid growth, high reproductive output and a relatively short life-span (Stimson 1978). The species is often among the first to recolonise disturbed areas (Underwood *et al.* 2007). Studying patterns of reproduction and dispersal that are associated with this specific life history can shed light on the adaptive value of alternative strategies. Moreover, such studies are important for conservation. Like other brooding corals with 'weedy' life histories, *S. hystrix* is particularly susceptible to light stress and elevated sea temperatures (Marshall & Baird 2000) and may thus be highly threatened by climate change. Knowledge of reproduction and dispersal patterns is essential to establish efficient management strategies for *S. hystrix* and potentially also other coral species with similar life-history attributes.

1.5 Thesis outline

This thesis focuses on *S. hystrix* in order to obtain a comprehensive picture of life-history strategies that are realised at the studied sites. I employed a population genetic approach using species-specific microsatellite markers and focused on *S. hystrix* populations in the Red Sea and on the GBR. Specifically, I aimed to 1) infer reproductive mode, 2) study dispersal patterns at different locations across a range of spatial scales and 3) investigate within-colony heterogeneity due to somatic mutations and/or allogeneic fusions.

The results are presented within five papers. Three of them (Paper 1, 2 & 3) have already been published, Paper 4 has been submitted and Paper 5 has been prepared for submission. Paper reprints or manuscripts, respectively, are included as attachments. Chapter 2 summarises each of the five papers. Chapter 3 brings together their results and discusses the major findings. Chapter 4 outlines potential areas for future research.

Paper 1: Here I present a method to develop species-specific microsatellite markers from minute amounts of symbiont-free tissue. The approach is based on manual removal of algal cells in combination with a whole-genome preamplification of the extracted DNA to obtain sufficient DNA for library construction. This way, a number of species-specific markers could be obtained.

Paper 2: This study focuses on ten *S. hystrix* stands in the Red Sea in order to investigate whether larval dispersal is spatially limited and correlated with distance. Analyses showed isolation by distance effects on a small geographic scale of ≤ 20 km, indicating limited dispersal of larvae. The data did not reveal any evidence against purely sexual reproduction.

Paper 3: To gain insight into the population genetic processes that work on the smallest possible scales from decimetres to metres, I studied two *S. hystrix* stands from the Red Sea in greater detail. The sexual mode of reproduction, the likely occurrence of selfing and the level of immigration were consistent with previous studies on this species. Contrary to other findings, genotypes in both stands were in Hardy-Weinberg proportions. Moreover, no evidence for spatially restricted larval dispersal was found within the sampled areas.

Paper 4: This paper addresses intracolonial genetic variation in *S. hystrix* and investigates the underlying mechanisms, mosaicism versus chimerism. It shows that natural *S. hystrix* populations include a considerable proportion of genetically heterogeneous colonies. Whereas both mosaicism and chimerism contribute, the findings imply that mosaicism is the major cause of within-colony heterogeneity.

Paper 5: In this study I focused on nine *S. hystrix* stands from two locations on the GBR, Lizard and Heron Island. Specifically, I aimed to infer the population genetic processes that are responsible for the large and significant heterozygote deficits that are common in *S. hystrix* and many other sessile marine invertebrates. The data revealed that Wahlund effects, small-scale dispersal of sperm and/or larvae as well as inbreeding, including selfing, all contribute, with varying importance at different sites. Consistent with all other studies on this species, asexual reproduction plays at most a marginal role.

2. PAPER SUMMARIES

2.1 Development of species-specific markers in an organism with endosymbionts: microsatellites in the scleractinian coral *Seriatopora hystrix*

Maier E, Tollrian R, Nürnberger B, *Molecular Ecology Notes*, 1, 157-159, 2001.

In order to obtain a set of markers suitable for population genetic analyses in *S. hystrix*, I developed species-specific microsatellites. The isolation of such markers in corals is hampered by the presence of algal endosymbionts (zooxanthellae) because DNA extracts from coral tissue contain a mixture of DNA from two different organisms. Thus, a method is required that assures the coral origin of any microsatellite loci that have been identified.

I constructed a genomic library free of algal DNA via a whole genome preamplification from minute amounts of symbiont-free tissue. Pure coral tissue was prepared by carefully removing all algal cells under a binocular microscope (40x magnification) and the absence of symbionts was confirmed at 400x magnification. The resulting fragments were cloned into the plasmid cloning vector pUC18, and *Escherichia coli* were transformed with the recombinant plasmids. Twenty-nine microsatellites were isolated from a library screen with a fluorescently labelled $(CA)_{15}$ probe.

To verify that the markers were species-specific, they were tested on DNA from a close relative, *Pocillopora damicornis*, as well as *S. hystrix* from the same aquarium as *P. damicornis* (i.e. shared pool of zooxanthellae). Amplification with DNA from *P. damicornis* did not yield any product whereas *S. hystrix* amplified successfully. Tests with primers Sh3.32 and Sh4.28 failed to amplify DNA from a pure zooxanthellae culture. These findings corroborated a species-specific coral origin of the primers.

2.2 Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea

Maier E, Tollrian R, Rinkevich B, Nürnberger B, *Marine Biology*, 147, 1109-1120, 2005.

In this study I focused on ten *S. hystrix* stands from the Red Sea, separated between 0.150 km and 610 km in order to evaluate whether larval dispersal is spatially limited such that genetic differentiation correlates with distance. Furthermore, I analysed the patterns of genotypic diversity for a possible signature of asexual reproduction.

I collected samples of *S. hystrix* at one site near Eilat, Israel (*Sa*: Satil), seven sites in the area around Dahab, Sinai Peninsula (*Be*: Bells; *Bh*: Blue Hole; *Eg*: Eel Garden; *Hr*: House Reef; *Is*: Islands; *La*: Lagoon and *Tp*: Three Pools) as well as two sites near Hamata, southern Egypt (*Za*: Zabargad; *Ma*: Malahi). From each stand, all colonies with diameters of about 20 cm within a contiguous area were sampled until the target sample size of about 20 was reached (two exceptions: N = 11 and 32). Allelic variation was analysed at three microsatellite loci.

The study revealed moderate genetic differentiation among sites, based on both F_{ST} and R_{ST} (0.089 vs. 0.136, respectively) as well as considerable heterozygote deficits. As null alleles presumably contributed, however, the impact of coral population processes on deviations from Hardy-Weinberg equilibrium could not be determined. Mantel tests showed isolation by distance effects on a small geographic scale (≤ 20 km), which indicates that dispersal of larvae is spatially restricted. The data did not reveal any evidence against purely sexual reproduction among the studied populations. While the present findings indicate that spatially restricted dispersal contributes to the population genetic structure of the *S. hystrix* study sites in the Dahab area, they did not allow estimating immigration rates, actual dispersal distances or mating patterns. Also it remained unclear which factors (e.g. null alleles, Wahlund effects, restricted dispersal of gametes and/or larvae or mating pattern) caused the observed heterozygote deficits. Thus, I set out to investigate population genetic structure on the smallest possible scale within stands to clarify these issues (Papers 3 & 5).

2.3 Fine-scale analysis of genetic structure in the brooding coral *Seriatopora hystrix* from the Red Sea

Maier E, Tollrian R, Nürnberger B, *Coral Reefs*, 28, 751-756, 2009.

To investigate small-scale genetic processes that shape *S. hystrix* populations in the Dahab area (Red Sea), I analysed two stands (~100 colonies each), House Reef 2 (*HR2*) and Golden Blocks (*GB*) on the smallest scale from decimetres to metres. Specifically, I aimed to 1) determine whether these sites are internally structured, and 2) examine the role of four processes that could cause such structure, i.e. immigration, clonal reproduction, selfing and small-scale larval dispersal.

At both sites, all colonies within a contiguous area were sampled. The position of each colony was mapped via *x*- and *y*-coordinates. Genotyping was performed using five microsatellite loci.

Both stands were most likely maintained by sexual recruits, which agrees with previous studies on this species. Assignment tests identified 13 (*HR2*) and 16 (*GB*) colonies as immigrants. The minimum estimate of *Nm* was seven (6.5%) for *HR2* and ten (9.1%) for *GB*. In comparison to a previous study on *S. hystrix* from the remote Scotts Reef in northern Western Australia (Underwood *et al.* 2007), this estimate is rather high and implies a relatively greater influx of immigrants into *HR2* and *GB*. This could be due to more numerous and larger source populations along the Dahab coast. Moreover, the comparatively simpler shape of the Dahab shoreline may facilitate larval transport. A simulation of selfing points towards the presence of selfed offspring at *GB*. The small F_{IS} estimates, however, suggest that selfing rates are low ($S < 10\%$). Much higher rates of self-fertilisation were found in *S. hystrix* from different sites on the GBR (Sherman 2008; Paper 5 in this thesis). In contrast to my previous study on *S. hystrix* from the Red Sea (Paper 2) as well as other studies on this species, both stands were in Hardy-Weinberg proportions. Also, no evidence for spatially restricted larval dispersal within the sampled areas was found. It is currently an open question whether differences between this and other studies on *S. hystrix* reflect variation in life history or abiotic forces.

2.4 Intracolonial genetic variation in the scleractinian coral *Seriatopora hystrix*

Maier E, Buckenmaier A, Tollrian R, Nürnberger B, submitted to *Molecular Ecology*.

Here I investigated intracolonial genetic variation at microsatellite loci in *S. hystrix*. Two different mechanisms could be responsible for the presence of distinct genotypes within a single colony, i.e. mosaicism, which is caused by somatic mutation, or chimerism, which originates from allogeneic fusion. The aims of this study were to 1) assess the level of within-colony heterogeneity in natural *S. hystrix* populations, 2) distinguish between the two underlying mechanisms, mosaicism versus chimerism and 3) test the hypothesis that the proportion of heterogeneous colonies should increase with colony size due to the accumulation of somatic mutations during growth.

From each of 155 *S. hystrix* colonies, both adults and juveniles, two or three samples were collected. The colonies were sampled at five sites on the GBR (see also Paper 5), i.e. Palfrey 1 (*PAL1*) and Horseshoe Reef (*HS*), both situated at Lizard Island (northern GBR) as well as Staghorn Bank (*SB*), Canyons (*CA*) and Harry's Bommie (*HB*), all located at Heron Island (southern GBR). To standardise (and presumably maximise) the probability of detecting different genotypes within a colony, two-fold samples were taken from opposing sites of the upper half of the colony, while three-fold samples were taken from opposing sites and approximately evenly spaced angles, i.e. 120° steps. Allelic variation was analysed at five or six loci.

Twenty-seven (17%) genetically heterogeneous colonies were found. Due to the low sampling effort per colony, however, the true level of heterogeneity is expected to be underestimated. The finding that a larger proportion of heterogeneous colonies occurred among three-fold sampled compared to two-fold sampled colonies supported this assumption. For most colonies, it was not possible to distinguish between mosaicism and chimerism due to limited resolution of marker loci and/or genetic homogeneity of the sampling site. Yet, statistical analyses indicated that both mosaicism and chimerism occurred. Specifically, two colonies were clearly identified as chimeras whereas three colonies were classified as mosaics. In most heterogeneous colonies, intracolonial variation was found only at a single allele. Corroborated by statistical tests, this implies that somatic mutation presented the major source of genetic heterogeneity. In contrast to the expectation that the proportion of heterogeneous colonies should increase with size, a higher level of heterogeneity was found among medium-sized compared to small and large colonies. This finding is presently unexplained and certainly needs further investigation. The study shows that intracolonial genetic variation is

an important aspect of coral population genetics that has largely been neglected to date. It should be relevant for any population genetic study in corals that employs individual-based analytical approaches.

2.5 Genetic population structure in the scleractinian coral *Seriatopora hystrix* on the Great Barrier Reef: patterns of reproduction and dispersal

Maier E, Tollrian R, Nürnberger B, to be submitted to *Marine Ecology Progress Series.*

In this study I analysed population genetic structure of *S. hystrix* from two geographical regions on the GBR (Lizard and Heron Island, max. distance: ~1185 km) in order to 1) evaluate migration patterns on different spatial scales, 2) infer reproductive mode and 3) identify causes of heterozygote deficits.

In total, 698 *S. hystrix* colonies from nine different sites were genotyped at five or six microsatellite loci. Three study sites, i.e. Palfrey 1 (*PAL1*), Horseshoe Reef (*HS*) and Palfrey 2 (*PAL2*) were located at Lizard Island (northern GBR). Six sites, Tenements II (*T*), Staghorn Bank (*SB*), Canyons (*CA*), Harry's Bommie (*HB*), South Reef (*SR*) and North-West-Wistari (*NWW*) were situated at Heron Island (southern GBR). From five sites (*PAL1*, *HS*, *SB*, *CA* and *HB*) all adult as well as juvenile colonies within contiguous stands were sampled (N = 106 to 125 per site). From four stands (*PAL2*, *SR*, *NWW* and *T*), only adult colonies were sampled (N = 30 to 34). At *SB* and *CA*, the position of each colony within the sampled area was recorded via *x*- and *y*-coordinates for detailed spatial analyses.

Levels of genetic differentiation among sites within regions were low at Lizard Island (F_{ST} = 0.006, max. distance: 1.6 km) and moderate at Heron Island (F_{ST} = 0.097, max. distance: 10.6 km). At Heron Island, cluster analyses revealed pronounced substructure within most stands (except *NWW*), pointing towards colonisation from several genetically differentiated sites. In contrast, Lizard Island sites appeared homogeneous, consistent with a single, well-mixed gene pool. All stands were largely maintained by sexual recruits. The significant heterozygote deficits that occurred at most sites (except *PAL2*) were attributed to Wahlund effects, restricted dispersal of gametes and/or larvae and inbreeding, including selfing. Specifically, at site *SB* at Heron Island, autocorrelation analyses revealed evidence for restricted dispersal on scales as small as 0-2 metres. Parentage analyses provided clear evidence for selfing at four of five sites where data on adults as well as juveniles were available. Considering the most likely parents, these analyses inferred that between 14% (*PAL1*) and 40%

(*CA*) of the juvenile colonies within each stand resulted from selfing. Simulations showed that these estimates differed significantly from the expectation of pure outcrossing at all but one site (*PAL1*). At all nine sites, the population substructure results from a combination of external factors and life-history attributes of *S. hystrix.*

3. DISCUSSION

In this thesis a population genetic approach was employed to study life history in the scleractinian coral *S. hystrix*, a modular organism with many options regarding growth, reproduction and dispersal. The main goal was to investigate which of the various life-history options are actually realised in nature.

I found that a single colony does not necessarily present a genetic entity but may include various genotypes. While mosaicism and chimerism both occur, most cases of within-colony heterogeneity were attributed to mosaicism. Various asexual modes of propagation are known from *S. hystrix*, yet local recruits are mostly sexual. This implies that *S. hystrix* does not follow Williams' (1975) strawberry-coral model which predicts that local stands are maintained asexually while sexual offspring is produced to colonise new sites. The present analyses revealed that selfing is a crucial part of this coral's life history, but that its prevalence varies geographically. Dispersal across larger distances is important for colonising new sites but recruitment on surprisingly small spatial scales also occurs. Within certain stands, localised dispersal on scales from decimetres to metres was inferred. This leads to small-scale genetic structure and contributes to the large heterozygote deficits that are found locally in *S. hystrix*. Within-population genetic structure shows large variation, ranging from genetically uniform to highly substructured sites. The underlying processes that are responsible for the observed pattern of neutral genetic variation are Wahlund effects in conjunction with small-distance dispersal and inbreeding, including selfing.

The present findings raise important general questions: To what extent does the population genetic pattern reflect life history versus the particular local environment, e.g. currents, habitat structure or local extinction events? How could the environment shape *S. hystrix* life history? Addressing these questions presents a challenge for future studies.

3.1. Microsatellite analyses in *Seriatopora hystrix*

As a starting point, I set out to develop species-specific microsatellite markers (Paper 1) which I intended to use together with advanced statistical methods such as Bayesian clustering to perform high-resolution analyses of population genetic processes. To overcome the problem associated with endosymbionts which had previously hampered the development of

coral microsatellites, I employed a whole genome preamplification of DNA extracted from minute amounts of symbiont-free tissue. To my knowledge, apart from a single microsatellite that was published for the *Montastrea annularis* complex (Lopez *et al.* 1999), this yielded the first set of microsatellite markers available for population genetic studies on scleractinian corals. The markers were developed from an aquarium specimen with unknown origin and tested on *S. hystrix* from the Red Sea. Unfortunately, most of the markers described in Paper 1 were associated with problems such as unreliable banding pattern, amplification failure or null alleles, thus I had to develop additional loci (Sh2.15 & Sh4.24, Paper 2). With *S. hystrix* from the GBR, most loci showed only weak amplification or did not amplify at all. One locus (Sh4.24) worked with samples from Heron Island but amplified poorly with samples from Lizard Island. Consequently, for genotyping samples from the GBR, I used five microsatellite markers that had been subsequently developed by Underwood *et al.* (2006) from Australian samples.

The finding that *S. hystrix* from two geographical regions, Red Sea and GBR, showed very different results with the same markers indicates that the coral is genetically sufficiently differentiated across its distribution range to prevent universal amplification with the same primers. Probably, the coalescence time between lineages from the Red Sea and the Indian Ocean is long enough such that many mutations could accumulate. Not only the geographical distance (~13.000 km) but also the shallow sill of Bab el Mandab between the Red Sea and the Indian Ocean act as barriers to gene flow and promote genetic isolation (Sidall *et al.* 2002).

A similar problem with microsatellite amplification is known from ostracods where samples from different sites around the Mediterranean failed to amplify with the same primers (D.K. Lamatsch, personal communication). In this case, sequencing revealed large variability of the microsatellite flanking regions. Analyses of mitochondrial DNA pointed towards a cryptic species complex in these ostracods. Also in corals, it was shown that the usefulness of microsatellite loci may vary spatially. Miller & Ayre (2008) found that a locus which worked with *Platygyra daedalea* from East Africa (Souter & Grahn 2008) proved unreliable with *P. daedalea* from the GBR. Recent studies on pocilloporid corals of the genus *Seriatopora* (Flot *et al*. 2008) and *Pocillopora* (Souter 2010) reported that putative species boundaries inferred from molecular data show little agreement with the current morphological species delimitation. It is currently an open question whether cryptic species are possibly involved in the present study.

3.2 Intracolonial genetic variation

The present findings indicate that a considerable number of *S. hystrix* colonies include more than a single genotype (Paper 4). Specifically, from a total of 155 colonies analysed at two or three positions using five or six loci, 27 colonies (17%) were genetically heterogeneous. The low sampling effort and the limited number of loci, however, suggest that the true level of intracolonial genetic heterogeneity should be much higher. This assumption was supported by the finding that a larger proportion of heterogeneous colonies were found among three-fold compared to two-fold sampled colonies (32% versus 14.6%). Moreover, in a study on *S. hystrix* from the Red Sea where ten colonies were sampled at 14 and 15 positions, respectively, and analysed at four microsatellite loci, 80% of colonies showed within-colony heterogeneity (Striewski 2009). In contrast, in the broadcast spawning coral *Acropora millepora* from the GBR where 124 adult colonies were sampled at eight positions and genotyped at eight to twelve microsatellite loci, only ~5.6% of colonies were heterogeneous (Puill-Stephan *et al*. 2009).

While both mosaicism and chimerism contribute, the present study identified mosaicism as the major source of intracolonial genetic variation. Data on mosaicism in corals are only just becoming available (Puill-Stephan *et al*. 2009). Also in other colonial marine invertebrates, very few studies documented somatic mutations (reviewed in Gill *et al.* 1995). Chimerism is known from various laboratory studies on corals (e.g. Hidaka 1985; Hidaka *et al.* 1997; Frank *et al.* 1997; Amar *et al.* 2008), but has rarely been reported from natural populations (Nozawa & Loya 2005; Puill-Stephan *et al*. 2009).

For the majority of heterogeneous colonies it was not possible to distinguish between mosaicism and chimerism due to limited resolution of marker loci and/or genetic homogeneity of the sampling site. Chimeras could only be unambiguously identified if fusion involved partners that were genetically clearly distinct. In contrast, fusion between genetically similar partners, possibly due to gregarious settlement of related larvae (see below), could not be distinguished from mosaicism. Nevertheless, two out of 27 heterogeneous colonies were clearly identified as chimeras whereas three colonies were classified as mosaics.

The fusion of distinct genotypes into a single colony has been related to both costs and benefits: Chimeras may suffer from cell parasitism, negative competitive interactions and developmental instability, caused by the presence of different cell lineages within a single colony (e.g. Rinkevich & Weissman 1987; Pineda-Krch & Lehtilä 2004; Amar *et al.* 2008). On the other hand, chimeras could benefit from increased genetic variability, earlier onset of

reproduction, enhanced competitiveness, higher survivorship rates and the assurance of a nearby genetically distinct mate (reviewed in Amar *et al.* 2008).

Whether positive or negative effects prevail possibly depends on the genetic relatedness of fusion partners: If cell lineages within a chimera are genetically similar, i.e. if fusion partners are closely related, internal conflicts are likely to be reduced. Indeed, gregarious settlement of kin larvae and the formation of multi-partner chimeras have been reported from laboratory studies on *P. damicornis* (Raymundo & Maypab 2004) and *S. pistillata* (Amar *et al.* 2008). These entities have been associated with benefits in the early phase of ontogeny, i.e. the ability to occupy large areas of substrate due to rapid size increase (Amar *et al.* 2008) as well as high survival rates (Raymundo & Maypab 2004). Benefits of fusion between related individuals have even been discussed as a selective force in the evolution of short-distance dispersal in sessile marine invertebrates (Jackson 1986; see also Chapter 3.3.3). In addition, the production of broods may involve high levels of selfing (Sherman 2008; Paper 5 in this thesis). Both factors should largely increase relatedness within stands, thus gregarious settlement of kin could facilitate the formation of stable chimeras.

Most studies of mosaicism have focused on plants, both at neutral (Cloutier *et al.* 2003; O'Connell & Ritland 2004) and selected loci (Whitham & Slobodchikoff 1981; Gill *et al.* 1995). It has been suggested that somatic mutations may compensate for the lack of recombination in primarily clonal species, providing genetic variability for selection to act upon (Mogie 1985). Genetic heterogeneity within a single plant can be adaptive to cope with fast-evolving pests, especially insect herbivores, and allow for 'fine tuning' to local selective regimes (Whitham & Slobodchikoff 1981). Such mechanisms may also apply to colonial animals like corals, thus further studies that focus on selected loci could be highly rewarding. Note, however, that levels of mosaicism in coding regions of the genome should be much lower than those seen at microsatellite loci.

The present results are important for any population genetic study on corals that relies on the identification of individual multilocus genotypes. This is particularly true for analyses based on the colonies' genotypic identity such as the identification of clonal replicates or parentage analyses. For example, assuming that all colonies within a given area are represented by a single sample in the dataset, some genotypes are missed if there are colonies that include more than a single genotype. As a result, certain clone mates or potential parents remain undetected. Depending on the specific question to be answered, this can cause misinterpretations.

Regarding the population genetic analyses conducted in this thesis, the impact of intracolonial genetic variation is expected to be low: First, all inferences on reproductive

mode (i.e. sexual versus asexual reproduction, outcrossing versus selfing) were based on the population level rather than on individual observations. Second, parentage analyses were conducted allowing for high genotyping error rates (Paper 3 & 5). Thus, the present inferences on *S. hystrix* mating patterns are expected to be robust in the light of within-colony heterogeneity.

3.3 Causes of heterozygote deficits

A striking finding was the large variation of within-population genetic structure among sites, ranging from stands that were consistent with panmixia to highly substructured stands showing pronounced heterozygote deficits. Whereas both *HR2* and *GB* within the Red Sea conformed Hardy-Weinberg expectations (Paper 3), moderate heterozygote deficits were found at *PAL1*, *HS* and *PAL2* at Lizard Island as well as *NWW* at Heron Island on the GBR (Paper 5). Large heterozygote deficits occurred at the other Heron Island sites, i.e. *T*, *SB*, *CA*, *HB* and *SR* (Paper 5). Also the Red Sea sites analysed in Paper 2 showed considerable heterozygote deficits. In this case, however, they do not necessarily reflect population processes but may result from null alleles.

Large and consistent local heterozygote deficits have also been reported in other studies on *S. hystrix* (Ayre & Dufty 1994; Ayre & Hughes 2000; Sherman *et al.* 2006; Underwood *et al.* 2007, 2009; van Oppen *et al.* 2008; Noreen *et al*. 2009) and a variety of sessile marine invertebrates such as sponges (Duran *et al.* 2004; Calderón *et al.* 2007), bivalves (Bierne *et al.* 1998), ascidians (Ben-Shlomo *et al.* 2001), sea anemones (Russo *et al.* 1994), gorgonians (Brazeau & Harvell 1994; Costantini *et al.* 2007) and scleractinian corals (e.g. Ridgway *et al.* 2001; Gilmour 2002; Whitaker 2004). A number of mechanisms have been proposed to explain these heterozygote deficits, e.g. null alleles, pooling of samples from genetically different subpopulations (Wahlund effects) as well as inbreeding, including selfing. Few studies, however, have directly investigated the underlying mechanisms (but see Costantini *et al.* 2007). Therefore, the aim of this thesis was to unravel the population genetic processes responsible for the observed heterozygote deficits. The present analyses revealed that reproductive mode, i.e. selfing, Wahlund effects, and most likely also small-scale dispersal of sperm and/or larvae contributed. The importance of different mechanisms varied between sites. In the following, these mechanisms are discussed in greater detail.

3.3.1 Reproduction

Asexual reproduction played, at most, a marginal role within any of the stands investigated here and thus does not provide an explanation for deviations from Hardy-Weinberg conditions (Paper 2, 3 & 5). Likewise, also other population genetic studies on *S. hystrix* found that local stands are largely maintained by sexual recruits (Ayre & Dufty 1994; Ayre & Hughes 2000; Underwood *et al.* 2007, 2009; Sherman 2008; van Oppen *et al.* 2008; Noreen *et al*. 2009). These studies, however, provided only limited resolution due to the use of allozyme markers, sampling on a coarse grid and/or deliberately avoiding clonal replicates. The sexual origin of larvae was also directly shown by comparing genotypes of maternal colonies and broods (Ayre & Resing 1986; Sherman 2008).

The finding that an organism like *S. hystrix* that is capable of both sexual and asexual reproduction relies on sexual offspring for local propagation challenges traditional life-history theory. Asexual reproduction has been related to benefits such as the maintenance of locally adapted gene complexes, propagation in the absence of mates and the possibility to reproduce year-round rather than being restricted to certain reproductive seasons (e.g. Williams 1975; Maynard Smith 1978; Bell 1982; Otto & Lenormand 2002). Specifically in corals, clonal propagation via fragmentation has been regarded beneficial because fragments are larger compared to planulae, which may result in higher survivorship after recruitment. Moreover, fragments can colonise areas that are not suited for larval recruitment such as soft-bottom habitats (reviewed in Lirman 2000).

Despite these benefits, *S. hystrix* reproduces largely sexually, which implies that sex involves immediate benefits that outweigh its costs (Williams 1975). Sexually produced, genetically diverse offspring could be favoured in spatially heterogeneous habitats (Bell 1982), in the face of strong intra- or interspecific competition (Hebert 1978) or fast evolving predators or pathogens (Glesener & Tilman 1978; Hamilton 1980). In addition, because *S. hystrix* is a relatively short-lived coral that faces a highly dynamic environment (Connell 1973), population turnovers are likely to be frequent. As a result, one of the proposed benefits of asexual reproduction, i.e. the preservation of locally adapted genotypes, may not play an important role. Moreover, selfing provides an alternative solution to the problem of mate limitation that colonisers may face.

While the marker variability was too low for exact parentage analyses, the data did provide insight into mating patterns within sites. Specifically, B. Nürnberger developed a statistical method to obtain estimates of selfing rates across the whole population (Paper 5). The results suggest that selfing contributes to the observed heterozygote deficits. In particular, high selfing rates at sites *SB*, *CA* and *HB* (Heron Island) can in principle explain the 're-

sidual' F_{IS} within the genetic clusters that were inferred via Bayesian clustering analyses with TESS (François *et al.* 2006; Chen *et al.* 2007). At Lizard Island, site *HS* yielded a high selfing estimate whereas the neighbouring site *PAL1* did not deviate significantly from pure outcrossing (Paper 5). Likewise, selfing rates at both *HR2* and *GB* within the Red Sea were low (Paper 3).

For comparison, a direct analysis of six *S. hystrix* colonies and their offspring from a single reef flat site at One Tree Island (GBR) revealed almost equal levels of outcrossing and selfing, with large variation among individual colonies (Sherman 2008). Few estimates on selfing rates are available from other brooding corals: In *Acropora palifera* from two sites on the GBR, broods were generated almost exclusively via outcrossing (Ayre & Miller 2006). In contrast, considerable selfing rates (34 and 49%, respectively) were inferred for two Caribbean brooders, *Porites astreoides* and *Favia fragum*, based on randomly amplified polymorphic DNA (RAPD; Brazeau *et al.* 1998). Note, however, that the authors interpreted the latter estimates with caution, as confidence limits could not be calculated. Taken together, the above examples indicate that brooders show considerable variation in selfing rates, both at the intra- and interspecific level.

With considerable levels of selfing, *S. hystrix* follows a life-history strategy that is common in the plant kingdom but rare among animals. Traditionally, selfing has mostly been related to severe negative fitness effects (i.e. inbreeding depression), basically due to unmasking of deleterious recessive alleles (Charlesworth & Charlesworth 1987). On the other hand, selfing presents an efficient way to preserve favourable gene combinations while avoiding the accumulation of detrimental mutations (Shields 1982). In this respect, selfing may be beneficial compared to asexual reproduction which can cause mutational meltdown (Muller 1964; Lynch *et al.* 1993). Empirical evidence from marine invertebrates has revealed both positive and negative selfing effects (Sabbadin 1971; Grosberg & Quinn 1986; Grosberg 1987; Knowlton & Jackson 1993).

Whether costs or benefits prevail may depend on a population's inbreeding history: In case of persistent inbreeding, negative effects are likely to be reduced because deleterious recessives are constantly purged (e.g. Shields 1982; Lande *et al.* 1994; Hedrick & Kalinowski 2000). Possibly, this applies to *S. hystrix* which shows some extremely short-distance dispersal (Paper 5, see also Chapter 3.3.3) such that closely related colonies are frequently engaged in consanguineous mating.

As mentioned above, the preservation of favourable gene combinations may not play an important role if population turnovers are frequent. Rather, selfing could be beneficial because it enables reproduction in the absence of conspecifics. *S. hystrix* is an opportunistic

species and often among the first to colonise new habitats (Underwood *et al*. 2007). Selfing probably is an important strategy that contributes to the high colonisation success of this species.

3.3.2 Wahlund effects

At most Heron Island sites, i.e. *T*, *SB*, *CA*, *HB* and *SR*, Bayesian clustering analyses with TESS (François *et al.* 2006; Chen *et al.* 2007) revealed pronounced genetic substructure (Paper 5). This is consistent with colonisation from various genetically distinct source populations, indicating that Wahlund effects considerably contribute to the observed heterozygote deficits. Interestingly, even sites separated by less than 1.5 km such as *CA* and *HB* were colonised by recruits from genetically distinct sites. In contrast *PAL1*, *HS* and *PAL2* at Lizard Island (GBR, Paper 5) as well as at *HR2* and *GB* near Dahab (Red Sea, Paper 3) were genetically homogeneous, pointing towards immigration from a well-mixed gene pool and unlimited dispersal distances within the sampled areas. Here, Wahlund effects are expected to play little role. Rather, the heterozygote deficits that were found at all three Lizard Island sites could be the result of internal processes such as selfing.

In sessile marine invertebrates, sites may be colonised by recruits from genetically different source populations because larval sources vary over time. Especially in species like *S. hystrix* where broods are released over several months (Harrison & Wallace 1990), varying oceanographic conditions during the time of larval release are likely to alter dispersal routes (Underwood *et al.* 2007). Moreover, larval sources may change beause some populations are decimated or go extinct while others are founded during the colonisation of new habitats. Frequent population turnovers are expected to produce temporally varying source and sink dynamics.

The present findings raise important issues for reef conservation: Are there any sites that are prone to local extinction and have to be considered as sink populations, dependent on colonists from other reefs? Conversely, do some stands act as source populations, providing colonists for distant sites? Over what distances does colonisation occur? Further studies that include oceanographic data on circulation patterns (see Chapter 4.3) are required for a deeper insight into the source and sink-dynamics of reef-building corals.

3.3.3 Dispersal

Autocorrelation analyses showed that population genetic structure in *S. hystrix* may be shaped by spatially restricted dispersal on scales of decimetres to metres (Paper 5). Small-scale dispersal is likely to add to the observed heterozygote deficits: First, it leads to the formation of genetically differentiated groups and thus promotes Wahlund effects on the smallest spatial scales. Second, it favours mating between close relatives, which increases inbreeding. Note, however, that highly restricted dispersal was observed at only one site, i.e. *SB* at Heron Island (Paper 5) out of four from which the relevant data were collected (Paper 3 & 5). In particular, there was no evidence for spatially restricted dispersal at both *HR2* and *GB* in the Red Sea (Paper 3).

For comparison, previous population genetic data on *S. hystrix* from northern Western Australia showed that a significant proportion of larvae recruit within 100 m of the parental colony (Underwood *et al.* 2007). Significant positive autocorrelations on scales of decimetres to metres that point towards highly restricted dispersal have been reported from various sessile marine organisms with free-swimming larvae, e.g. sponges (Calderón *et al.* 2007), bryozoans (Pemberton *et al.* 2007), black corals (Miller 1998) and scleractinian corals (Miller & Ayre 2008). Thus, in striking contrast to the traditional view that pelagic larvae are dispersed across vast distances in the ocean (reviewed in Hedgecock 1986; Benzie 1999), these studies show that recruitment may occur on surprisingly small spatial scales.

Dispersal distances are crucial both from an evolutionary perspective and from an ecological point of view. In an evolutionary context, small-scale dispersal leads to the formation of genetically differentiated clades, either by local selection and/or genetic drift. Moreover, it favours inbreeding, which is an efficient way to preserve favourable gene combinations (Shields 1982). Finally, as mentioned above (Chapter 3.2), short-distance dispersal possibly promotes allogeneic fusion (Paper 4). From an ecological viewpoint, short dispersal distances minimise the loss of larvae due to predation during the planktonic phase. This should play an important role especially in brooders like *S. hystrix* which produce a relatively small number of well-developed larvae (Harrison & Wallace 1990). On the other hand, short-distance dispersal enhances competition among relatives (Williams 1975). Moreover, it increases the risk of extinction after local disturbance. In *S. hystrix*, however, local recruitment is complemented by long-distance dispersal, which suggests that the species has the potential to revover from disturbances (van Oppen *et al.* 2008). In an applied context, dispersal distances are critical for the design of marine reserve networks (e.g. Palumbi 2003; Jones *et al*. 2009). Protected areas have to be sufficiently large to be self-sustaining and close enough to promote dispersal among them. Moreover, it is desirable that they also send re-

cruits to other reefs outside the protected area. Efficient management also requires that scales of larval dispersal are considered in relation to scales of disturbance (Sale *et al.* 2005; Underwood *et al.* 2009). Thus, extending our current understanding of dispersal patterns to a range of species with different life histories may be essential to protect reefs in the face of increasing threat.

3.4 Potential caveats and limitations

A number of analyses in this study were restricted by the limited number of loci that was available. More loci should probably have enabled to pinpoint mosaicism versus chimerism for a larger number of heterogeneous colonies (Paper 4). Likewise, a larger set of loci would have increased the power to detect immigrants (Paper 3 & 5). Finally, additional loci would have been essential for exact parentage analyses (Paper 5).

While a larger number of markers would have allowed for additional insight, however, none of the conclusions presented here should be unduly affected: With regard to mating patterns, the available allelic variation was sufficient to render almost all multilocus genotypes (MLG) within a given site unique. While more loci may have revealed additional differences in the observed sets of identical MLG, the conclusion that *S. hystrix* reproduces mostly sexually would still remain. Other inferences on within-population genetic structure such as inbreeding or isolation by distance are also expected to be robust because the processes in question equilibrate very rapidly. Once equilibrium is attained, the stochastic variation among loci is expected to be small such that the use of additional markers should not appreciably alter the conclusions. Regarding Bayesian clustering analyses, a larger number of loci would probably have revealed a finer resolution of within-population genetic structure. While this should have identified additional immigrants, the general findings, i.e. genetic homogeneity versus heterogeneity at different sites, are expected to remain unaltered.

4. RESEARCH OUTLOOK

Due to their extraordinary diversity of life-history strategies, corals provide excellent study objects to test and extend theoretical predictions. The present thesis revealed insight into the repertoire of strategies that is realised in the widely distributed, brooding species *S. hystrix*. One of the greatest challenges for future research on *S. hystrix* and other corals is to go beyond mere descriptive analyses in order to address which biotic or abiotic factors are responsible for the observed variation in life history such as short- versus long-distance dispersal or local variation of selfing rates. This would be the prerequisite to understand the adaptive value of different strategies. Population genetic approaches cannot yield all the answers to these questions and should be complemented by experimental analyses. A promising approach would be to combine field experiments and controlled experiments in the laboratory to test the importance of various biotic and abiotic factors.

4.1 Intracolonial genetic variation

The role of mosaicism and chimerism in natural coral populations is largely unknown to date and presents an exciting field for future research. Although the present study was only based on few samples per colony as well as a limited number of marker loci, it revealed a surprisingly high rate of intracolonial genetic variation in *S. hystrix*.

A sampling approach with larger sample sizes and additional marker loci is expected to provide a better estimate of the frequency of genetically heterogeneous colonies and should allow to reliably distinguish between mosaicism and chimerism in many cases. This would help to assess the impact of both mechanisms on population genetic studies on corals. Moreover, it would enable to address the evolutionary implications. For example, studies on natural chimeras could determine the level of relatedness among fusion partners and observe the performance of chimeras in the field. Following Amar *et al.* (2008), laboratory experiments could investigate aggregated settlement of related and non-related *S. hystrix* larvae. This may reveal under which conditions stable chimeras are formed and whether chimerism possibly presents a selective force in the evolution of short-distance dispersal.

In scleractinian corals, intracolonial genetic variation can involve both, the coral host and the associated zooxanthellae. Several studies have demonstrated that a single colony

can harbour multiple types of zooxanthellae (e.g. Rowan & Knowlton 1995; Rowan *et al.* 1997; Ulstrup & van Oppen 2003). Different types of zooxanthellae may vary with regard to their physiological properties such as thermal tolerance, photosynthetic response to irradiance or metabolism of photosynthetically fixed carbon (e.g. Savage *et al.* 2002; Berkelmans & van Oppen 2006; Loram *et al.* 2007). This allows for adaptive responses to varying selective regimes within a single colony. Small-scale adaptation may occur not only due to variability of zooxanthellae but also of the coral host itself, albeit to my knowledge, the latter has not been addressed in any coral to date. In this thesis, intracolonial genetic variation was studied at neutral loci, characterised by high mutation rates. This does not parallel variation at selected loci, yet the latter would be decisive for adaptation. Future studies that focus on selected loci may reveal intriguing insight into the importance of intracolonial genetic variation in corals.

4.2 Reproduction

This thesis confirmed that local *S. hystrix* stands are largely if not entirely maintained by sexual recruits. Moreover, it showed that the production of broods may involve high levels of selfing, yet that selfing rates vary considerably among locations.

More detailed insight into the reproductive mode could be gained by directly collecting larvae from their maternal colony. Direct comparisons of maternal and offspring genotypes together with analyses of local genotypic diversity could be performed to investigate whether *S. hystrix* possibly produces asexual larvae that are dispersed beyond the parental habitat. If broods involved asexual larvae but local stands did not show evidence for asexual recruitment, this could point towards long-distance dispersal of these larvae [see Ayre & Miller (2004) and Sherman *et al.* (2006) for examples from *P. damicornis*]. Long-distance dispersal of asexual propagules has also been suggested for *S. hystrix* (van Oppen *et al.* 2008). Comparing maternal and offspring genotypes also allows to exactly calculate selfing rates in individual colonies. So far, such estimates are only available for six *S. hystrix* colonies from a single reef flat site on the GBR (Sherman 2008).

In brooders, larvae of individual colonies can be collected by taking gravid colonies to the laboratory and holding them in separate tanks until planulae are released (e.g. Isomura & Nishihira 2001; Sherman 2008). Alternatively, they can be obtained *in situ* using nylon mesh traps that are placed over gravid colonies before sunset and removed the following morning

(Figure 7). For a detailed description of these planula traps, see Amar *et al.* (2007). I planned to collect *S. hystrix* larvae from individual colonies at Lizard and Heron Island *in situ*. Despite that field work was conducted during the season of *S. hystrix* larval release (e.g. Sherman 2008), due to unknown reasons only a small number of larvae (~20 in total) could be collected. With this low number of larvae, sound analyses could not be performed. Determining the reproductive status of single colonies based on stereoscopic examinations of gonadal development (changes in size, colour and volume; Shlesinger & Loya 1985) may help identifying gravid colonies and narrowing down the appropriate time for larval collection in future studies.

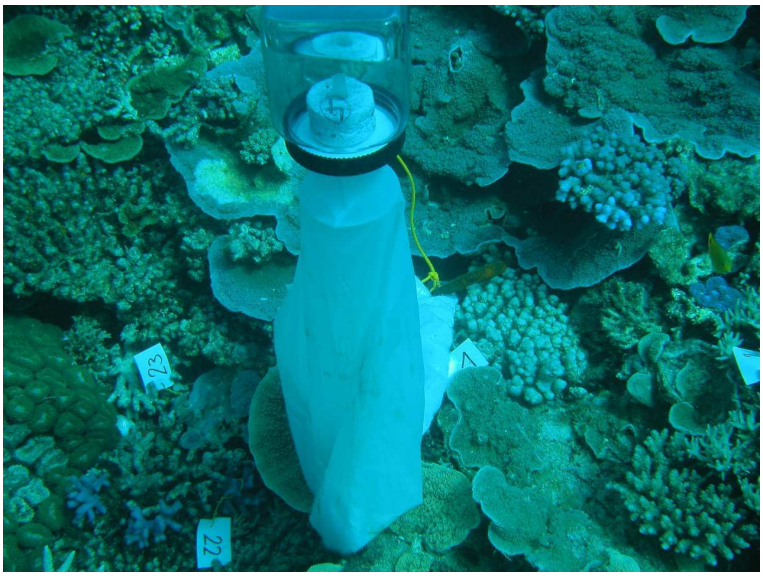

Figure 7: Nylon mesh trap for the collection of *Seriatopora hystrix* planulae *in situ*.
HERON ISLAND, GREAT BARRIER REEF *Photograph: Christoph Haacke*

Analyses of broods from a large number of colonies from different sites could be used to investigate whether variation of selfing rates between individual colonies (Sherman 2008) and between locations (Paper 5) reflect fixed differences in life history or an adaptive response to environmental conditions. If selfing was adaptive, subsequent studies should focus on the factors that influence the rate of outcrossing versus selfing. Various studies on marine invertebrates have employed *in situ* experiments on fertilisation rates, e.g. in tunicates (Grosberg 1987, 1991; Yund 1995), bryozoans (McCartney 1997), hydroids (Yund 1990) and gorgonian corals (Brazeau & Lasker 1992; Coffroth & Lasker 1998; Lasker *et al*. 2008).

These studies revealed that fertilisation success depends on the distance between colonies, local water flow regimes as well as male reproductive output (but see Lasker *et al*. 2008). They indicate that selfing possibly predominates in stands with low colony density or under unfavourable hydrological conditions. In *S. hystrix*, experimental manipulation of adult densities as well as the placement of colonies in relation to current directions would be a promising approach to investigate the biological and environmental factors that promote selfing.

An important step towards a better understanding of reproductive strategies in *S. hystrix* would be to test the fitness of different offspring (sexual versus asexual, outcrossed versus selfed) under different environmental conditions. This could be done via common garden experiments as well as reciprocal transplanting. Due to their modular life habit, corals are cut out for such analyses, as multiple genetically identical fragments can be obtained from a single colony (e.g. Potts 1984). They can be exposed to different treatments to test the fitness of different genotypes in the same environment, and *vice versa*, the same genotype in different environments.

4.3 Dispersal

Population genetics offers a powerful approach to infer connectivity patterns of marine larvae, yet it can only detect dispersal events that lead to successful recruitment while the actual number of migrants remains unknown. Moreover, the interpretation of population genetic data for connectivity estimates is hampered by the large number of unsampled source populations that are likely to be present.

One step towards improving the interpretation of data could be coupling population genetics with oceanographic models (Galindo *et al.* 2006). Based on environmental parameters, such models can simulate particle movements in ocean currents, which allows for quantitative estimates of larval transport. They can be used to test whether relatively simple dispersal scenarios such as isolation by distance adequately reflect dispersal in nature. They can also help inferring the robustness of gene flow estimates as well as determining to what extent oceanographic conditions influence dispersal distances.

To date, both population genetics and oceanographic modelling have largely remained disconnected, albeit a combined approach is highly promising: For example, in *Acropora palmata*, oceanographic simulations have successfully been applied to predict genetic structure and test these predictions against empirical genetic data collected throughout the

Caribbean (Galindo *et al.* 2006). Likewise, in marine mussels, physical circulation models were coupled with genetic data to assess the geographic scale of larval dispersal (Gilg & Hilbish 2003). These examples show the advantage of integrating approaches from different disciplines. As stated by Botsford *et al*. (2009), greater communication between empiricists and population modellers will add to a better understanding of the scales and patterns of connectivity in the ocean.

APPENDIX

Utility of Bayesian methods in population genetic studies on *S. hystrix*

To evaluate population genetic structure in *S. hystrix*, I employed traditional analyses such as Wright's (1965) *F*-statistics (Paper 2, 3 & 5) and statistical methods based on Bayesian probability theory (Paper 3, 4 & 5). In recent years, Bayesian approaches (e.g. Corander *et al.* 2003; François *et al.* 2006; Guillot *et al.* 2005; Piry *et al.* 2004; Pritchard *et al.* 2000) have gained considerable popularity in population genetics. Bayesian clustering methods attempt to identify cryptic population structure by assigning individuals to genetically divergent clusters, based on their individual multilocus genotypes (Corander & Marttinen 2006). This is done by minimising Hardy-Weinberg as well as linkage disequilibrium within clusters. Various methods can also incorporate prior information such as the spatial location of individuals (reviewed in Manel *et al.* 2005).

Bayesian clustering methods generate a grouping of individuals without any need to define population units in advance. This is particularly useful in species with continuous distributions where an *a priori* definition of population units may be arbitrary or even erroneous (Balloux & Lugon-Moulin 2002; Mank & Avise 2004; Pearse & Crandall 2004). Determining the number of genetic clusters (*K)* in the dataset, however, is a major challenge (e.g. Durand *et al.* 2009). Furthermore, there is dissent about the applicability of certain methods (e.g. François *et al.* 2008; Durand *et al.* 2009; Guillot 2009). Finally, features of the dataset such as the level of differentiation among groups or isolation by distance may influence the performance of Bayesian inference (Latch *et al.* 2006; Pritchard *et al.* 2007). Various authors stated that the use of different methods and the critical examination of results may be the best way to avoid misinterpretations and to obtain results of biological significance (e.g. Pearse & Crandall 2004; Excoffier & Heckel 2006; Latch *et al.* 2006).

Following this advice, various Bayesian clustering approaches were tested and the results were examined carefully. Specifically, I applied STRUCTURE version 2.2 (Pritchard *et al.* 2000), BAPS version 4.1 (Corander *et al*. 2003; Corander & Marttinen 2006), GENELAND version 1.0.5 (Guillot *et al.* 2005), TESS version 1.1 (François *et al.* 2006; Chen *et al.* 2007) as well as GeneClass2 (Piry *et al.* 2004) which implements the partial Bayesian criterion of Rannala & Mountain (1997). Especially the widely used software STRUCTURE is poorly suited for inferring *K* (S. Baird, personal communication). Indeed, when applying

STRUCTURE to the *S. hystrix* dataset from the GBR (Paper 5), it was not possible to reliably estimate the number of clusters because the corresponding values of posterior probabilities continued to increase with *K*. This could be because the dataset did not conform precisely to the STRUCTURE model, e.g. due to inbreeding (Pritchard *et al.* 2007). In an attempt to overcome this problem and obtain reliable estimates of *K,* I applied an *ad hoc* statistic Δ*K*, based on the rate of change in the log probability of data between successive *K* values (Evanno *et al.* 2005). With this method, the highest likelihood was obtained for $K = 2$. As the method does not provide a way to validate $K = 1$, this result could not be verified.

With BAPS analyses performed in the group mode, the highest number of clusters is defined by the maximum *K* that is predetermined by the user. As a result, BAPS could not be used to evaluate within-site population genetic structure due to limited resolution. In the individual mode, very large numbers of clusters were obtained, with considerable variation between runs. This tendency of BAPS to overestimate the number of underlying populations was also described by Latch *et al.* (2006).

The clustering algorithm implemented in GENELAND yields reliable estimates of *K* (S. Baird, personal communication). GENELAND performs well when the underlying population structure can adequately be described by polygons (Guillot *et al.* 2005). In the present analyses, a major problem with GENELAND was that the system frequently crashed when performing analyses in the 'non-spatial mode'. In the 'spatial mode', the program performed clustering on the level of sampling sites but did not enable to unravel genetic structure within stands.

Given these constraints, STRUCTURE, BAPS and GENELAND analyses were dropped. Instead, the final results were based on TESS (Paper 4 & 5) and GeneClass2 (Paper 3). According to François *et al.* (2006), TESS performs better than GENELAND when the underlying spatial population genetic structure is too complex to be described by simple polygons (but see Guillot *et al*. 2009). When applied to the *S. hystrix* dataset from the GBR, TESS revealed ten clusters, five of which were very clearly defined, and uncovered population genetic structure down to the smallest spatial scales within stands (Paper 5) or within single colonies (Paper 4). Three observations illustrate that the TESS clusters reflected relevant biological units instead of artefacts of the clustering algorithm: First, as shown for Heron Island, differentiation among the main clusters was much higher than among sites while mean heterozygote deficits within clusters were greatly reduced (see also Mank & Avise 2004). Second, a considerably lower number of significant inter-locus associations was found within clusters than within sites. Third, parentage analyses suggested that cluster member-

ship was 'inherited' because in most cases, the most likely parents of a given juvenile were assigned to the same cluster as the juvenile itself (Paper 5).

For the two *S. hystrix* stands from the Red Sea analysed in Paper 3, *HR2* and *GB*, TESS did not infer any hidden population substructure, presumably due to low levels of genetic differentiation. This was consistent to the low F_{IS} values calculated for these sites. To identify individual immigrants, the population exclusion method as implemented in GeneClass2 (Piry *et al.* 2004) was applied. With this method, the number of individuals classified as immigrants largely depends on the threshold of exclusion set by the user. To compensate for the limited power of the dataset to identify immigrants (i.e. unknown source populations, limited number of loci), a low stringency criterion of $\alpha = 0.05$ (95% exclusion probability) was applied. From the resulting estimate of *Nm*, the expected Type I error ($N * \alpha$) was subtracted to obtain a reliable minimum, as suggested by Paetkau *et al.* (2004).

Finally, with all Bayesian analyses, I abstained from assigning immigrant individuals to presumptive source populations because this would require sampling all possible source populations, which is clearly beyond the scope of this study. Taking these precautions, Bayesian clustering methods provide a powerful way to uncover hidden population genetic structure in corals that would remain undetected with other approaches.

REFERENCES

Amar KO, Chadwick NE, Rinkevich B (2007) Coral planulae as dispersion vehicles: biological properties of larvae released early and late in the season. *Marine Ecology Progress Series*, **350**, 71-78.

Amar KO, Chadwick NE, Rinkevich B (2008) Coral kin aggregations exhibit mixed allogeneic reactions and enhanced fitness during early ontogeny. *BMC Evolutionary Biology*, **8**, 126.

Atoda K (1951) The larva and postlarval development of the reef-building corals. V. *Seriatopora hystrix* Dana. *Science Reports of the Tohoku University, 4th series (Biology)*, **19**, 33-39.

Ayre DJ, Dufty S (1994) Evidence for restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia`s Great Barrier Reef. *Evolution*, **48**, 1183-1201.

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. *Evolution*, **54**, 1590-1605.

Ayre DJ, Hughes TP (2004) Climate change, genotypic diversity and gene flow in reef-building corals. *Ecology Letters*, **7**, 273-278.

Ayre DJ, Miller KJ (2004) Where do clonal larvae go? Adult genotypic diversity conflicts with reproductive effort in the brooding coral *Pocillopora damicornis*. *Marine Ecology Progress Series*, **277**, 95-105.

Ayre DJ, Miller KJ (2006) Random mating in the brooding coral *Acropora palifera*. *Marine Ecology Progress Series*, **307**, 155-160.

Ayre DJ, Resing JM (1986) Sexual and asexual production of planulae in reef corals. *Marine Biology*, **90**, 187-190.

Babcock RC (1984) Reproduction and distribution of two species of *Goniastrea* (Scleractinia) from the Great Barrier Reef province. *Coral Reefs*, **2**, 187-204.

Babcock RC, Heyward AJ (1986) Larval development of certain gamete-spawning scleractinian corals. *Coral Reefs*, **5**, 111-116.

Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. *Annual Review of Ecology, Evolution and Systematics*, **40**, 551-571.

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. *Molecular Ecology*, **11**, 155-165.

Barki Y, Douek J, Graur D, Gateño D, Rinkevich B (2000) Polymorphism in soft coral larvae revealed by amplified fragment-lenght polymorphism (AFLP) markers. *Marine Biology*, **136**, 37-41.

Barton NH (2001) The evolutionary consequences of gene flow and local adaptation: future approaches. In: *Dispersal* (eds. Clobert J, Danchin E, Dhondt AA, Nichols JD), pp. 329-340. Oxford University Press, Oxford.

Barton NH, Whitlock MC (1997) The evolution of metapopulations. In: *Metapopulation Biology: Ecology, Genetics, and Evolution* (eds. Hanski IA, Gilpin ME), pp. 183-210. Academic Press, San Diego, CA.

Baums IB (2008) A restoration genetics guide for coral reef conservation. *Molecular Ecology*, **17**, 2796-2811.

Baums IB, Hughes CR, Hellberg ME (2005*a*) Mendelian microsatellite loci for the Caribbean coral *Acropora palmata*. *Marine Ecology Progress Series*, **288**, 115-127.

Baums IB, Miller MW, Hellberg ME (2005*b*) Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. *Molecular Ecology*, **14**, 1377-1390.

Baums IB, Miller MW, Hellberg ME (2006) Geographic variation in clonal structure in a reef-building Caribbean coral, *Acropora palmata*. *Ecological Monographs*, **76**, 503-519.

Baums IB, Devlin-Durante MK, Brown L, Pinzón JH (2009) Nine novel, polymorphic microsatellite markers for the study of threatened Caribbean acroporid corals. *Molecular Ecology Resources,* **9**, 1155-1158.

Begon ME, Harper JL, Townsend CR (1998) *Ökologie*. Spektrum Akademischer Verlag, Heidelberg, Berlin.

Bell G (1982) *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. University of California Press, Berkeley.

Bellwood DR, Hughes TP, Folke C, Nyström M (2004) Confronting the coral reef crisis. *Nature*, **429**, 827-833.

Ben-Shlomo R, Douek J, Rinkevich B (2001) Heterozygote deficiency and chimerism in remote populations of a colonial ascidian from New Zealand. *Marine Ecology Progress Series*, **209**, 109-117.

Benzie JAH (1999) Genetic structure of coral reef organisms: ghosts of dispersal past. *American Zoologist*, **39**, 131-145.

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **273**, 2305-2312.

Bierne N, Launey S, Naciri-Graven Y, Bonhomme F (1998) Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. *Genetics*, **148**, 1893-1906.

Botsford LW, White JW, Coffroth M-A, Paris CB, Planes S, Shearer TL, Thorrold SR, Jones GP (2009) Connectivity and resilience of coral reef metapopulations in marine protected areas: matching empirical efforts to predictive needs. *Coral Reefs*, **28**, 327-337.

Brazeau DA, Harvell CD (1994) Genetic structure of local populations and divergence between growth form in a clonal invertebrate, the Caribbean octocoral *Briareum asbestinum*. *Marine Biology*, **119**, 53-60.

Brazeau DA, Lasker HR (1992) Reproductive success in the Caribbean octocoral *Briareum asbestinum*. *Marine Biology*, **114**, 157-163.

Brazeau DA, Gleason DF, Morgan ME (1998) Self-fertilization in brooding hermaphroditic Caribbean corals: evidence from molecular markers. *Journal of Experimental Marine Biology and Ecology*, **231**, 225-238.

Brazeau DA, Sammarco PW, Gleason DF (2005) A multi-locus genetic assignment technique to assess sources of *Agaricia agaricites* larvae on coral reefs. *Marine Biology*, **147**, 1141-1148.

Calderón I, Ortega N, Duran S, Becerro M, Pascual M, Turon X (2007) Finding the relevant scale: clonality and genetic structure in a marine invertebrate (*Crambe crambe*, Porifera). *Molecular Ecology*, **16**, 1799-1810.

Carlon DB (1999) The evolution of mating systems in tropical reef corals. *Trends in Ecology and Evolution*, **14**, 491-495.

Carlon DB, Olson RR (1993) Larval dispersal distance as an explanation for adult spatial patterns in two Caribbean reef corals. *Journal of Experimental Marine Biology and Ecology*, **173**, 247-263.

Carpenter KE, Abrar M, Aeby G, Aronson RB, Banks S, Bruckner A, Chiriboga A, Cortés J, Delbeek JC, DeVantier L, Edgar GJ, Edwards AJ, Fenner D, Guzmán HM, Hoeksema BW, Hodgson G, Johan O, Licuanan WY, Livingstone SR, Lovell ER, Moore JA, Obura DO, Ochavillo D, Polidoro BA, Precht WF, Quibilan MC, Reboton C, Richards ZT, Rogers AD, Sanciangco J, Sheppard A, Sheppard C, Smith J, Stuart S, Turak E, Veron JEN, Wallace C, Weil E, Wood E (2008) One third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science*, **321**, 560-563.

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, **18**, 237-268.

Chen C, Durand E, Forbes F, François O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes*, **7**, 747-756.

Chen C, Dai C-F, Plathong S, Chiou C-Y, Allen C, Chen CA (2008) The complete mitochondrial genomes of needle corals, *Seriatopora* spp. (Scleractinia: Pocilloporidae): An idiosyncratic *atp8*, duplicated *trnW* gene, and hypervariable regions used to determine species phylogenies and recently diverged populations. *Molecular Phylogenetics and Evolution*, **46**, 19-33.

Cloutier D, Rioux D, Beaulieu J, Schoen DJ (2003) Somatic stability of microsatellite loci in Eastern white pine, *Pinus strobus* L. *Heredity*, **90**, 247-252.

Coffroth MA, Lasker HR (1998) Larval paternity and male reproductive success of a broadcast-spawning gorgonian, *Plexaura kuna*. *Marine Biology*, **131**, 329-337.

Connell JH (1973) Population ecology of reef-building corals. In: *Biology and Geology of Coral Reefs*, 2 (eds. Jones O, Endean R), pp. 205-245. Academic Press, New York, USA.

Corander J, Marttinen P (2006) Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology*, **15**, 2833-2843.

Corander J, Waldmann P, Sillanpaa MJ (2003) Bayesian analysis of genetic differentiation between populations. *Genetics*, **163**, 367-374.

Costantini F, Fauvelot C, Abbiati M (2007) Fine-scale genetic structuring in *Corallium rubrum*: evidence of inbreeding and limited effective larval dispersal. *Marine Ecology Progress Series*, **340**, 109-119.

Dana JD (1846) *Zoophytes*. United States Exploring Exped 1838-1842, **7**, 740 pp.

Darwin C (1842) *The Structure and Distribution of Coral Reefs*. Smith, Elder & Company, London.

Dawson MN (2001) Phylogeography in coastal marine animals: a solution from California? *Journal of Biogeography*, **28**, 723-736.

De'ath G, Lough JM, Fabricius KE (2009) Declining coral calcification on the Great Barrier Reef. *Science*, **323**, 116-119.

Denno RF, Roderick GK (1992) Density-related dispersal in planthoppers: effects of interspecific crowding. *Ecology*, **73**, 1323-1334.

Dieckmann U, O'Hara B, Weisser W (1999) The evolutionary ecology of dispersal. *Trends in Ecology and Evolution*, **14**, 88-90.

Diekmann OE, Bak RPM, Stam WT, Olsen JL (2001) Molecular genetic evidence for probable reticulate speciation in the coral genus *Madracis* from a Caribbean fringing reef slope. *Marine Biology*, **139**, 221-233.

Done TJ, Ogden JC, Wiebe WJ, Rosen BR (1996) Biodiversity and ecosystem function of coral reefs. In: *Functional Roles of Biodiversity: A Global Perspective* (eds. Mooney HA, Cushman JH, Medina E, Sala OE, Schulze ED), pp. 393-429. John Wiley and Sons, Chichester.

Duran S, Pascual M, Estoup A, Turon X (2004) Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. *Molecular Ecology*, **13**, 511-522.

Durand E, Chen C, François O (2009) Comment on 'On the inference of spatial structure from population genetics data'. *Bioinformatics*, **25**, 1802-1804.

Ellegren H (2004) Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics*, **5**, 435-445.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611-2620.

Excoffier L, Heckel G (2006) Computer programs for population genetics data analysis: a survival guide. *Nature Reviews Genetics*, **7**, 745-758.

Flot JF, Licuanan WY, Nakano Y, Payri C, Cruaud C, Tiller S (2008) Mitochondrial sequences of *Seriatopora* corals show little agreement with morphology and reveal the duplication of a tRNA gene near the control region. *Coral Reefs*, **27**, 789-794.

Foster NL, Baums IB, Mumby PJ (2007) Sexual vs. asexual reproduction in an ecosystem engineer: the massive coral *Montastrea annularis*. *Journal of Animal Ecology*, **76**, 384-391.

François O, Ancelet S, Guillot G (2006) Bayesian clustering using hidden Markov random fields in spatial population genetics. *Genetics*, **174**, 805-816.

François O, Blum MGB, Jakobsson M, Rosenberg NA (2008) Demographic history of European populations of *Arabidopsis thaliana*. *PLoS Genetics*, **4**(5), e1000075.

Frank U, Oren U, Loya Y, Rinkevich B (1997) Alloimmune maturation in the coral *Stylophora pistillata* is achieved through three distinctive stages, 4 months post-metamorphosis. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **264**, 99-104.

Futuyma DJ (1998) *Evolutionary Biology*. Sinauer Associates, Sunderland, Massachusetts.

Galindo HM, Olson DB, Palumbi SR (2006) Seascape genetics: a coupled oceanographic-genetic model predicts population structure of Caribbean corals. *Current Biology*, **16**, 1622-1626.

Gilg MR, Hilbish TJ (2003) The geography of marine larval dispersal: coupling genetics with fine-scale physical oceanography. *Ecology*, **84**, 2989-2998.

Gill DE, Chao L, Perkins SL, Wolf JB (1995) Genetic mosaicism in plants and clonal animals. *Annual Review of Ecology and Systematics*, **26**, 423-444.

Gilmour JP (2002) Substantial asexual recruitment of mushroom corals contributes little to population genetics of adults in conditions of chronic sedimentation. *Marine Ecology Progress Series*, **25**, 81-91.

Glesener RR, Tilman D (1978) Sexuality and the components of environmental uncertainty: clues from geographic parthenogenesis in terrestrial animals. *The American Naturalist*, **112**, 659-673.

Glynn PW, Gassman NJ, Eakin CM, Cortes J, Smith DB, Guzman HH (1991) Reef coral reproduction in the eastern Pacific: Costa Rica, Panama, and Galapagos Islands (Ecuador). I. Pocilloporidae. *Marine Biology*, **109**, 355-368.

Goodwillie C, Kalisz S, Eckert CG (2005) The evolutionary enigma of mixed-mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology and Systematics*, **36**, 47-79.

Graham EM, Baird AH, Connolly SR (2008) Survival dynamics of scleractinian coral larvae and implications for dispersal. *Coral Reefs*, **27**, 529-539.

Grosberg RK (1987) Limited dispersal and proximity-dependent mating success in the colonial ascidian *Botryllus schlosseri*. *Evolution*, **41**, 372-384.

Grosberg RK (1991) Sperm-mediated gene flow and the genetic structure of a population of the colonial sea squirt *Botryllus schlosseri*. *Evolution*, **45**, 130-142.

Grosberg RK, Cunningham CW (2001) Genetic structure in the sea: from populations to communities. In: *Marine Community Ecology* (eds. Bertness MD, Gaines SD, Hay ME), pp. 61-83. Sinauer Associates, Sunderland, Massachusetts.

Grosberg RK, Quinn JF (1986) The genetic control and consequences of kin recognition by the larvae of a colonial marine invertebrate. *Nature*, **322**, 456-459.

Guillot G (2009) On the inference of spatial structure from population genetics data using the Tess program. *Bioinformatics*, **25**, 1796-1801.

Guillot G, Estoup A, Mortier F, Cosson JF (2005) A spatial statistical model for landscape genetics. *Genetics*, **170**, 1261-1280.

Guillot G, Leblois R, Coulon A, Frantz AC (2009) Statistical methods in spatial genetics. *Molecular Ecology*, **18**, 4734-4756.

Hall VR, Hughes TP (1996) Reproductive strategies of modular organisms: comparative studies of reef-building corals. *Ecology*, **77**, 950-963.

Hamilton WD (1980) Sex versus non-sex versus parasite. *Oikos*, **35**, 282-290.

Hamilton WD, May RM (1977) Dispersal in stable habitats. *Nature*, **269**, 578-581.

Hanski A (2001) Population dynamic consequences of dispersal in local populations and in metapopulations. In: *Dispersal* (eds. Clobert J, Danchin E, Dhondt AA, Nichols JD), pp. 283-298. Oxford University Press, Oxford.

Harii S, Kayanne H, Takigawa H, Hayashibara T, Yamamoto M (2002) Larval survivorship, competency periods and settlement of two brooding corals, *Heliopora coerulea* and *Pocillopora damicornis*. *Marine Biology*, **141**, 39-46.

Harper JL (1985) Modules, branches, and the capture of resources. In: *Population Biology and Evolution of Clonal Organisms* (eds. Jackson JBC, Buss LW, Cook RE), pp. 1-33. Yale University Press, New Haven, Connecticut.

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: *Ecosystems of the world 25: Coral Reefs* (ed. Dubinsky Z), pp. 133-207. Elsevier, New York.

Hatta M, Fukami H, Wang W, Omori M, Shimoike K, Hayashibara T, Ina Y, Sugiyama T (1999) Reproductive and genetic evidence for a reticulate evolutionary history of mass-spawning corals. *Molecular Biology and Evolution*, **16**, 1607-1613.

Hebert PN (1978) Adaptive significance of cyclomorphosis in *Daphnia*: more possibilities. *Freshwater Biology*, **8**, 313-320.

Hedgecock D (1986) Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? *Bulletin of Marine Science*, **39**, 550-564.

Hedrick PW, Kalinowski ST (2000) Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics*, **31**, 139-162.

Hellberg ME (2006*a*) Genetic approaches to understanding marine metapopulation dynamics, In: *Marine Metapopulations* (eds. Kritzer JP, Sale PF), pp. 431-455. Academic Press, Amsterdam, The Netherlands.

Hellberg ME (2006*b*) No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *BMC Evolutionary Biology*, **6**, 24.

Hellberg ME (2007) Footprints on water: the genetic wake of dispersal among reefs. *Coral Reefs*, **26**, 463-473.

Hellberg ME, Burton RS, Neigel JE, Palumbi SR (2002) Genetic assessment of connectivity among marine populations. *Bulletin of Marine Science*, **70** (Suppl.), 273-290.

Hidaka M (1985) Tissue compatibility between colonies and between newly settled larvae of *Pocillopora damicornis*. *Coral Reefs*, **4**, 111-116.

Hidaka M, Yurugi K, Sunagawa S, Kinzie RA (1997) Contact reactions between young colonies of the coral *Pocillopora damicornis*. *Coral Reefs*, **16**, 13-20.

Highsmith RC (1982) Reproduction by fragmentation in corals. *Marine Ecology Progress Series*, **7**, 207-226.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change. *Science*, **318**, 1737-1742.

Hohenlohe PA (2004) Limits to gene flow in marine animals with planktonic larvae: models of *Littorina* species around point conception, California. *Biological Journal of the Linnean Society*, **83**, 169-187.

Hughes RN (1989) *A Functional Biology of Clonal Animals*. Chapman and Hall, New York, USA.

Hughes TP, Jackson JBC (1980) Do corals lie about their age? Some demographic consequences of partial mortality, fission and fusion. *Science*, **209**, 713-715.

Hughes TP, Tanner JW (2000) Recruitment failure, life histories, and long-term decline of Caribbean corals. *Ecology*, **81**, 2250-2263.

Hughes TP, Ayre D, Connell JH (1992) The evolutionary ecology of corals. *Trends in Ecology and Evolution*, **7**, 292-295.

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JBC, Kleypas J, Lough JM, Marshall P, Nyström M, Palumbi SR, Pandolfi JM, Rosen B, Roughgarden J (2003) Climate change, human impacts, and the resilience of coral reefs. *Science*, **301**, 929-933.

Hurst LD, Peck JR (1996) Recent advances in understanding of the evolution and maintenance of sex. *Trends in Ecology and Evolution,* **11**, 46-52.

Hutchinson WF, Van Oosterhout C, Rogers S, Carvalho GR (2003) Temporal analysis of archieved samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **270**, 2125-2132.

Isomura N, Nishihira M (2001) Size variation of planulae and its effect on the lifetime of planulae in three pocilloporid corals. *Coral Reefs*, **20**, 309-315.

Jackson JBC (1977) Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. *The American Naturalist*, **111**, 743-747.

Jackson JBC (1985) Distribution and ecology of clonal and aclonal benthic invertebrates. In: *Population Biology and Evolution of Clonal Organisms* (eds. Jackson JBC, Buss LW, Cook RE), pp. 297-355. Yale University Press, New Haven, Connecticut.

Jackson JBC (1986) Modes of dispersal of clonal benthic invertebrates: consequences for species' distributions and genetic structure of local populations. *Bulletin of Marine Science*, **39**, 588-605.

Jarne P, Auld JR (2006) Animals mix it up too: the distribution of self-fertilisation among hermaphroditic animals. *Evolution*, **60**, 1816-1824.

Jarne P, Charlesworth D (1993) The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annual Review of Ecology and Systematics*, **24**, 441-466.

Jokiel PL (1984) Long distance dispersal of reef corals by rafting. *Coral Reefs*, **3**, 113-116.

Jokiel PL (1989) Rafting of corals and other organisms at Kwajalein Atoll. *Marine Biology*, **101**, 483-493.

Jokiel PL (1990) Long-distance dispersal by rafting: reemergence of an old hypothesis. *Endeavour*, **14**, 66-73.

Jones GP, Planes S, Thorrold SR (2005) Coral reef fish larvae settle close to home. *Current Biology*, **15**, 1314-1318.

Jones GP, Almany GR, Russ GR, Sale PF, Steneck RS, van Oppen MJH, Willis BL (2009) Larval retention and connectivity among populations of corals and reef fishes: history, advances and challenges. *Coral Reefs*, **28**, 307-325.

Karlson RH (2002) Population processes in modular benthic invertebrates. In: *Reproductive Biology of Invertebrates* (ed. Hughes R), Vol. XI, pp. 159-173. Oxford & IBH. Publishing Co. Pvt. Ltd., New Delhi, India.

Knowlton N (2001) The future of coral reefs. *Proceedings of the National Academy of Sciences of the USA*, **98**, 5419-5425.

Knowlton N, Jackson JBC (1993) Inbreeding and outbreeding in marine invertebrates. In: *The Natural History of Inbreeding and Outbreeding* (ed. Thornhill N), pp. 200-249. University of Chicago Press, Chicago.

Kondrashov AS (1988) Deleterious mutations and the evolution of sexual reproduction. *Nature*, **336**, 435-440.

Lam KKY, Loo AHB, Todd PA, Chew FT, Chou LM (2006) Existence of intra-colonial paralogues of the ribosomal internal transcribed spacer (ITS) impedes studies of intra-colonial genetic variation in the scleractinian coral *Diploastrea heliopora* (Lamark 1816). *The Raffles Bulletin of Zoology*, **54**, 485-489.

Lande R, Schemske DW, Schultze ST (1994) High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. *Evolution*, **48**, 965-978.

Lasker HR, Gutiérrez-Rodríguez C, Bala K, Hannes A, Bilewitch JP (2008) Male reproductive success during spawning events of the octocoral *Pseudopterogorgia elisabethae*. *Marine Ecology Progress Series*, **367**, 153-161.

Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, **7**, 295-302.

Levitan DR, Petersen C (1995) Sperm limitation in the sea. *Trends in Ecology and Evolution*, **10**, 228-231.

Lirman D (2000) Fragmentation in the branching coral *Acropora palmata* (Lamarck): growth, survivorship, and reproduction of colonies and fragments. *Journal of Experimental Marine Biology and Ecology*, **251**, 41-57.

Lopez JV, Kersanach R, Rehner SA, Knowlton N (1999) Molecular determination of species boundaries in corals: Genetic analysis of the *Montastrea annularis* complex using Amplified Fragment Length Polymorphisms and a microsatellite marker. *Biological Bulletin*, **196**, 80-93.

Loram JE, Trapido-Rosenthal HG, Douglas AE (2007) Functional significance of genetically different symbiotic algae *Symbiodinium* in a coral reef symbiosis. *Molecular Ecology*, **16**, 4849-4857.

Luppi TA, Spivak ED, Bas CC (2003) The effects of temperature and salinity on larval development of *Armases rubripes* Rathbun 1897 (Brachyura, Grapsoidea, Sesarmidae) and the southern limit of its geographical distribution. *Estuarine Coastal and Shelf Science*, **58**, 575-585.

Lynch M, Bürger R, Butcher D, Gabriel W (1993) The mutational meltdown in an asexual population. *Journal of Heredity*, **84**, 339-344.

Magalon H, Samadi S, Richard M, Adjeroud M, Veuille M (2004) Development of coral and zooxanthellae-specific microsatellites in three species of *Pocillopora* (Cnidaria, Scleractinia) from French Polynesia. *Molecular Ecology Notes*, **4**, 206-208.

Magalon H, Adjeroud M, Veuille M (2005) Patterns of genetic variation do not correlate with geographical distance in the reef-building coral *Pocillopora meandrina* in the South Pacific. *Molecular Ecology*, **14**, 1861-1868.

Manel S, Gagiotti OE, Waples RS (2005) Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology and Evolution*, **20**, 136-142.

Mank JE, Avise JC (2004) Individual organisms as units of analysis: Bayesian-clustering alternatives in population genetics. *Genetics Research*, **84**, 135-143.

Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. *Coral Reefs*, **19**, 155-163.

Maynard Smith J (1978) *The Evolution of Sex*. Cambridge University Press, Cambridge.

McCartney MA (1997) Sex allocation and male fitness gain in a colonial, hermaphroditic marine invertebrate. *Evolution*, **51**, 127-140.

Miller KJ (1998) Short-distance dispersal of black coral larvae: inference from spatial analysis of colony genotypes. *Marine Ecology Progress Series*, **163**, 225-233.

Miller KJ, Ayre DJ (2008) Population structure is not a simple function of reproductive mode and larval type: insights from tropical corals. *Journal of Animal Ecology*, **77**, 713-724.

Miller KJ, Mundy C (2003) Rapid settlement in broadcast spawning corals: implications for larval dispersal. *Coral Reefs*, **22**, 99-106.

Mogie M (1985) Morphological, developmental and electrophoretic variation within and between obligately apomictic *Taraxacum* species. *Biological Journal of the Linnean Society*, **24**, 207-216.

Mouquet N, Mulder GSSE, Jansen VAA, Loreau M (2001) The properties of competitive communities with coupled local and regional dynamics. In: *Dispersal* (eds. Clobert J, Danchin E, Dhondt AA, Nichols JD), pp. 311-325. Oxford University Press, Oxford.

Muller HJ (1964) The relation of recombination to mutational advance. *Mutation Research*, **1**, 2-9.

Munday PL, Leis JM, Lough JM, Paris CB, Kingsford MJ, Berumen ML, Lambrechts J (2009) Climate change and coral reef connectivity. *Coral Reefs*, **28**, 379-395.

Nakajima Y, Nishikawa A, Isomura N, Iguchi A, Sakai K (2009) Genetic connectivity in the broadcast-spawning coral *Acropora digitifera* analyzed by microsatellite markers on the Sekisei Reef, southwestern Japan. *Zoological Science*, **26**, 209-215.

Nishikawa A, Katoh M, Sakai K (2003) Larval settlement rates and gene flow of broadcast-spawning (*Acropora tenuis*) and planula-brooding (*Stylophora pistillata*) corals. *Marine Ecology Progress Series*, **256**, 87-97.

Noreen AME, Harrison PL, van Oppen MJH (2009) Genetic diversity and connectivity in a brooding reef coral at the limit of its distribution. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **276**, 3927-3935.

Nozawa Y, Loya Y (2005) Genetic relationship and maturity state of the allorecognition system affect contact reactions in juvenile *Seriatopora* corals. *Marine Ecology Progress Series*, **286**, 115-123.

O'Connell LM and Ritland K (2004) Somatic mutations at microsatellite loci in western redcedar (*Thuja plicata*: Cupressaceae). *Journal of Heredity*, **95**, 172-176.

Odorico DM, Miller DJ (1997) Variation in the ribosomal internal transcribed spacers and 5.8S rDNA among five species of *Acropora* (Cnidaria; Scleractinia): patterns of variation consistent with reticulate evolution. *Molecular Biology and Evolution*, **14**, 465-473.

Olson RR (1985) The consequences of short-distance larval dispersal in a sessile marine invertebrate. *Ecology*, **66**, 30-39.

Orive ME (2001) Somatic mutations in organisms with complex life histories. *Theoretical Population Biology*, **59**, 235-249.

Otto SP, Lenormand T (2002) Resolving the paradox of sex and recombination. *Nature Reviews Genetics*, **3**, 252-261.

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology*, **13**, 55-65.

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Reviews of Ecology and Systematics*, **25**, 547-572.

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications*, **13**, S146-S158.

Pearse DE, Crandall KA (2004) Beyond F_{ST}: Analysis of population genetic data for conservation. *Conservation Genetics*, **5**, 585-602.

Pemberton AJ, Hansson LJ, Craig SF, Hughes RN, Bishop JDD (2007) Microscale genetic differentiation in a sessile marine invertebrate with cloned larvae: investigating the role of polyembryony. *Marine Biology*, **153**, 71-82.

Pennisi E (2007) Spawning for a better life. *Science*, **318**, 1712-1717.

Perrin N, Goudet J (2001) Inbreeding, kinship and the evolution of natal dispersal. In: *Dispersal* (eds. Clobert J, Danchin E, Dhondt AA, Nichols JD), pp. 127-146. Oxford University Press, Oxford.

Pineda-Krch M, Lehtilä K (2004) Costs and benefits of genetic heterogeneity within organisms. *Journal of Evolutionary Biology*, **17**, 1167-1177.

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A (2004) GeneClass2: a software for genetic assignment and first generation migrant detection. *Journal of Heredity*, **95**, 536-539.

Planes S, Lecaillon G, Lenfant P, Meekan M (2002) Genetic and demographic variation in new recruits of *Naso unicornis*. *Journal of Fish Biology*, **61**, 1033-1049.

Potts DC (1984) Natural selection in experimental populations of reefbuilding corals (Scleractinia). *Evolution*, **38**, 1059-1078.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945-959.

Pritchard JK, Wen X, Falush D (2007) Documentation for structure software: version 2.2. http://www.pritch.bsd.uchicago.edu/software/structure22/readme.pdf

Puill-Stephan E, Willis BL, van Herwerden L, van Oppen MJH (2009) Chimerism in wild adult populations of the broadcast spawning coral *Acropora millepora* on the Great Barrier Reef. *PLoS ONE*, **4**(11), e7751.

Queller DC, Strassmann JE, Hughes CR (1993) Microsatellites and kinship. *Trends in Ecology and Evolution*, **8**, 285-288.

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the USA*, **94**, 9197-9201.

Raymundo A, Maypab AP (2004) Getting bigger faster: mediation of size-specific mortality via fusion in juvenile coral transplants. *Ecological Applications*, **14**, 281-295.

Richmond RH (1987) Energetics, competency, and long-distance dispersal of planula larvae of the coral *Pocillopora damicornis*. *Marine Biology*, **93**, 527-533.

Richmond RH (1997) Reproduction and recruitment in corals: critical links in the persistence of reefs. In: *Life and Death of Coral Reefs* (ed. Birkeland C), pp. 175-197. Chapman & Hall, New York.

Richmond RH, Hunter CL (1990) Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. *Marine Ecology Progress Series*, **60**, 185-203.

Ridgway T (2002) Testing the applicability of molecular genetic markers to population analyses of scleractinian corals. *Symbiosis*, **33**, 243-261.

Ridgway T (2005) Allozyme electrophoresis still represents a powerful technique in the management of coral reefs. *Biodiversity and Conservation*, **14**, 135-149.

Ridgway T, Hoegh-Guldberg O, Ayre DJ (2001) Panmixia in *Pocillopora verrucosa* from South Africa. *Marine Biology*, **139**, 175-181.

Ridgway T, Riginos C, Davis J, Hoegh-Guldberg O (2008) Genetic connectivity patterns of *Pocillopora verrucosa* in southern African Marine Protected Areas. *Marine Ecology Progress Series*, **354**, 161-168.

Rinkevich B, Weissman IL (1987) Chimeras in colonial invertebrates: a synergistic symbiosis or somatic- and germ-cell parasitism? *Symbiosis*, **4**, 117-134.

Rodriguez-Lanetty M, Hoegh-Guldberg O (2002) The phylogeography and connectivity of the latitudinally widespread scleractinian coral *Plesiastrea versipora* in the Western Pacific. *Molecular Ecology*, **11**, 1177-1189.

Ronce O, Olivieri I, Clobert J, Danchin E (2001) Perspectives on the study of dispersal evolution. In: *Dispersal* (eds. Clobert J, Danchin E, Dhondt AA, Nichols JD), pp. 341-357. Oxford University Press, Oxford.

Rosen BR, Taylor JD (1969) Reef coral from Aldabra: new mode of reproduction. *Science*, **166**, 119-121.

Ross KG (2001) How to measure dispersal: the genetic approach. The example of fire ants. In: *Dispersal* (eds. Clobert J, Danchin E, Dhondt AA, Nichols JD), pp. 29-42. Oxford University Press, Oxford.

Rowan R, Knowlton N (1995) Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proceedings of the National Academy of Sciences of the USA*, **92**, 2850-2853.

Rowan R, Knowlton N, Baker AC, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, **388**, 265.

Russo CAM, Solé-Cava AM, Thorpe JP (1994) Population structure and genetic variation in two tropical sea anemones (Cnidaria, Actinidae) with different reproductive strategies. *Marine Biology*, **119**, 267-276.

Sabbadin A (1971) Self-and cross-fertilisation in the compound ascidian, *Botryllus schlosseri*. *Developmental Biology*, **24**, 379-391.

Sakai K (1997) Gametogenesis, spawning, and planula brooding by the reef coral *Goniastrea aspera* (Scleractinia) in Okinawa, Japan. *Marine Ecology Progress Series*, **151**, 67-72.

Sale PF, Cowen RK, Danilowicz BS, Jones GP, Kritzer JP, Lindeman KC, Planes S, Polunin NVC, Russ GR, Sadovy YJ, Steneck RS (2005) Critical science gaps impede use of no-take fishery reserves. *Trends in Ecology and Evolution*, **20**, 74-80.

Sammarco PW (1982) Polyp bail-out: an escape response to environmental stress and a new means of reproduction in corals. *Marine Ecology Progress Series*, **10**, 57-65.

Savage AM, Trapido-Rosenthal H, Douglas AE (2002) On the functional significance of molecular variation in *Symbiodinium*, the symbiotic algae of Cnidaria: photosynthetic response to irradiance. *Marine Ecology Progress Series*, **244**, 27-37.

Schlötterer C (2000) Evolutionary dynamics of microsatellite DNA. *Chromosoma*, **109**, 365-371.

Sebens KP (1987) The ecology of indeterminate growth in animals. *Annual Review of Ecology and Systematics*, **18**, 371-407.

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters*, **9**, 615-629.

Shearer TL, van Oppen MJH, Romano SL, Worheide G (2002) Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology*, **11**, 2475-2487.

Shearer TL, Gutiérrez-Rodríguez C, Coffroth MA (2005) Generating molecular markers from zooxanthellate cnidarians. *Coral Reefs*, **24**, 57-66.

Sherman CDH (2008) Mating system variation in the hermaphroditic brooding coral, *Seriatopora hystrix*. *Heredity*, **100**, 296-303.

Sherman CDH, Ayre DJ, Miller KJ (2006) Asexual reproduction does not produce clonal populations of the brooding coral *Pocillopora damicornis* on the Great Barrier Reef, Australia. *Coral Reefs*, **25**, 7-18.

Shields WM (1982) *Philopatry, Inbreeding, and the Evolution of Sex*. State University of New York Press, Albany.

Shlesinger Y, Loya Y (1985) Coral community reproductive patterns: Red Sea versus the Great Barrier Reef. *Science*, **228**, 1333-1335.

Siddall M, Smeed DA, Matthiesen S, Rohling EJ (2002) Modelling the seasonal cycle of the exchange flow in Bab el Mandab (Red Sea). *Deep Sea Research Part I*, **49**, 1551-1569.

Smith DJ, Suggett DJ, Baker NR (2005) Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals? *Global Change Biology*, **11**, 1-11.

Sorokin YI (1990) *Coral Reef Ecology*. Springer, Berlin.

Souter P (2010) Hidden genetic diversity in a key model species of coral. *Marine Biology*, doi: 10.1007/s00227-009-1370-3.

Souter P, Grahn M (2008) Spatial genetic patterns in lagoonal, reef-slope and island populations of the coral *Platygyra daedalea* in Kenya and Tanzania. *Coral Reefs*, **27**, 433-439.

Souter P, Henriksson O, Olsson N, Grahn M (2009) Patterns of genetic structuring in the coral *Pocillopora damicornis* on reefs in East Africa. *BMC Ecology*, **9**, 19.

Stearns SC (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.

Stimson JS (1978) Mode and timing of reproduction in some common hermatypic corals of Hawaii and Enewetak. *Marine Biology*, **48**, 173-184.

Stoddart JA (1983) Asexual production of planulae in the coral *Pocillopora damicornis*. *Marine Biology*, **76**, 279-284.

Stoddart JA, Babcock RC, Heyward AJ (1988) Self-fertilisation and maternal enzymes in the planulae of the coral *Goniastrea favulus*. *Marine Biology*, **99**, 489-494.

Striewski S (2009) *Examination on the Intracolonial Genetic Variation in the Scleractinian Coral Seriatopora hystrix.* Masterthesis. Ruhr-Universität Bochum, Germany.

Tanner JE (1996) Seasonality and lunar periodicity in the reproduction of Pocilloporid corals. *Coral Reefs*, **15**, 59-66.

Tunnicliffe V (1981) Breakage and propagation of the stony coral *Acropora cervicornis*. *Proceedings of the National Academy of Sciences of the USA*, **78**, 2427-2431.

Ulstrup KE, van Oppen MJH (2003) Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef. *Molecular Ecology*, **12**, 3477-3484.

Underwood JN (2009) Genetic diversity and divergence among coastal and offshore reefs in a hard coral depend on geographic discontinuity and oceanic currents. *Evolutionary Applications*, **2**, 222-233.

Underwood JN, Souter PB, Ballment ER, Lutz AH, van Oppen MJH (2006) Development of 10 polymorphic microsatellite markers from herbicide bleached tissues of the brooding pocilloporid coral *Seriatopora hystrix*. *Molecular Ecology Notes*, **6**, 176-178.

Underwood JN, Smith LD, van Oppen MJH Gilmour JP (2007) Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. *Molecular Ecology*, **16**, 771-784.

Underwood JN, Smith LD, van Oppen MJH, Gilmour JP (2009) Ecologically relevant dispersal of corals on isolated reefs: implications for managing resilience. *Ecological Applications*, **19**, 18-29.

van Oppen MJH, Gates RD (2006) Conservation genetics and the resilience of reef-building corals. *Molecular Ecology*, **15**, 3863-3883.

van Oppen MJH, McDonald BJ, Willis B, Miller DJ (2001) The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or morphological convergence? *Molecular Biology and Evolution*, **18**, 1315-1329.

van Oppen MJH, Underwood JN, Murihead AN (2007) Ten microsatellite loci for the reef-building coral *Acropora millepora* (Cnidaria, Scleractinia) from the Great Barrier Reef, Australia. *Molecular Ecology*, **7**, 436-438.

van Oppen MJH, Lutz A, De'ath G, Peplow L, Kininmonth S (2008) Genetic traces of recent long-distance dispersal in a predominantly self-recruiting coral. *PLoS ONE*, **3**(10), e3401.

van Valen L (1971) Group selection and the evolution of dispersal. *Evolution*, **25**, 591-598.

Veron JEN (2000) *Corals of the World*, vol. 2, Australian Institute of Marine Science, Townsville.

Vollmer SV, Palumbi SR (2002) Hybridisation and the evolution of reef coral diversity. *Science*, **296**, 2023-2025.

Vollmer SV, Palumbi SR (2004) Testing the utility of internally transcribed spacer sequences in coral phylogenetics. *Molecular Ecology*, **13**, 2763-2772.

Ward S (1992) Evidence for broadcast spawning as well as brooding in the scleractinian coral *Pocillopora damicornis*. *Marine Biology*, **112**, 641-646.

West SA, Lively CM, Read AF (1999) A pluralist approach to sex and recombination. *Journal of Evolutionary Biology*, **12**, 1003-1012.

Whitaker K (2004) Non-random mating and population genetic subdivision of two broadcasting corals at Ningaloo Reef, Western Australia. *Marine Biology*, **144**, 593-603.

Whitham TG, Slobodchikoff CN (1981) Evolution by individuals, plant-herbivore interactions, and mosaics of genetic variability: the adaptive significance of somatic mutations in plants. *Oecologia*, **49**, 287-292.

Williams GC (1975) *Sex and Evolution*. Princeton University Press, Princeton, New Jersey.

Wood R (1999) *Reef Evolution*. Oxford University Press, Oxford.

Wright S (1965) The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution*, **19**, 395-420.

Yeoh S-R, Dai C-F (2010) The production of sexual and asexual larvae within single broods of the scleractinian coral, *Pocillopora damicornis*. *Marine Biology*, **157**, 351-359.

Yund PO (1990) An in situ measurement of sperm dispersal in a colonial marine hydroid. *Journal of Experimental Zoology*, **253**, 102-106.

Yund, PO (1995) Gene flow via the dispersal of fertilizing sperm in a colonial ascidian (*Botryllus schlosseri*): the effect of male density. *Marine Biology*, **122**, 649-654.

PAPER 1

Development of species-specific markers in an organism with endosymbionts: microsatellites in the scleractinian coral *Seriatopora hystrix*

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PRIMER NOTE

Development of species-specific markers in an organism with endosymbionts: microsatellites in the scleractinian coral *Seriatopora hystrix*

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Abstract

We report on the development of microsatellites in *Seriatopora hystrix*, a coral with algal endosymbionts. In order to obtain a genomic library free of algal DNA, we conducted a whole genome preamplification from minute amounts of symbiont-free tissue. The resulting fragments were cloned into pUC18, and *Escherichia coli* were transformed with the recombinant plasmids. Twenty-nine microsatellites were isolated from a library screen with a fluorescently labelled $(CA)_{15}$ probe. Five of these yielded reliable polymorphic markers.

Keywords: clonal structure, Cnidaria, corals, genome amplification, microsatellites, *Seriatopora hystrix*

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Reef-building corals (Scleractinia) show an extraordinary diversity of reproductive strategies. Various combinations of sexual and asexual modes of propagation as well as variation in dispersal capabilities within each mode have been documented (Harrison & Wallace 1990). Abundant intraspecific variation allows for direct functional analyses of these key life history traits. A good candidate for such analyses is the widely distributed species *Seriatopora hystrix* (Ayre & Hughes 2000). In order to obtain a set of highly variable single-locus markers for a detailed investigation of its reproductive strategies, we have set out to develop microsatellite loci in *S. hystrix*.

The isolation of molecular markers in corals is hampered by the presence of algal endosymbionts (zooxanthellae). However, if minute amounts of symbiont-free tissue can be obtained, then DNA fragments suitable for the construction of a genomic library can be generated via a whole genome amplification. In a so-called degenerate oligonucleotide primed–polymerase chain reaction (DOP–PCR), we used DOPs (Telenius *et al.* 1992), which include an *Xho*I restriction site that facilitates subsequent cloning of fragments.

Under a binocular microscope (40× magnification), we carefully removed all algal cells from a small piece of fresh *S. hystrix* tissue (100–200 μm^2). The absence of symbionts was confirmed at 400× magnification. DNA was extracted from the tissue sample by incubation in 20 µL DNAzol™ and 1 µL Polyacrylcarrier (Molecular Research Center, Inc.) for 10 min at room temperature and precipitated with 50 µL 100% ethanol. After washes in 95% and 70% ethanol, the DNA was air-dried and resuspended in 10 µL H_2O. The genome amplification reaction was set up in a 50-µL volume as follows: 2.0 µL template, 100 pmol of DOPs, 5 µL dNTP (2 mmol/nucleotide), 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 9.0, and 2.5 U *Taq* polymerase (Pharmacia rTaq). The following thermal cycling protocol was used (Hybaid Touchdown™ thermocycler): one cycle at 94 °C for 10 min, eight cycles of 93 °C for 60 s, 30 °C for 90 s and 72 °C for 3 min, then 28 cycles of 93 °C for 60 s, 58 °C for 60 s and 72 °C for 3 min. The polymerase was added after the initial denaturing step. A control reaction without template DNA was run in parallel.

On an agarose gel, the genome amplification generated a thick smear of DNA fragments mainly from 200 to several thousand base pairs (bp), whereas the control reaction showed only a very faint background shadow. *S. hystrix* fragments longer than 300 bp were purified (Qiaquick Gel Extraction Kit, Qiagen), digested to completion with *Xho*I (Gibco), and ligated overnight at 22 °C into *Sal*I-digested, dephosphorylated pUC18 (Appligene). The recombinant

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Table 1 Description of five microsatellite loci developed in *Seriatopora hystrix*

Locus (size of cloned insert, bp)	Primer sequences* (5′–3′)	GenBank accession no.	Repeat motif	T_a† (°C)	No. of genotyped colonies	No. of alleles	Allele size range	H_O	H_E
Sh3.13 (446)	CGTTTATTCCAATCATGAAGC CAGGCAGGCAACTTAACCTC	AF320765	$(CA)_4CGTA(CA)_8$	55	69	3	196–204	0.11	0.25
Sh3.32 (539)	CCAAAACCCTGCATTTTGAG* CCCCCTGTAAAAGTGTACCC	AF320766	$(CA)_{20}$	55 (52)	81	9	226–274	0.10	0.56
Sh3.39 (458)	AGTGAAAACACAGCACGTCG* ATTGTTCACACAATGGCGTG	AF320767	$(CA)_{12}$	59 (52)	98	4	217–229	0.42	0.58
Sh4.3 (647)	GGCTAGGATTGCACCAAGTG* CTGCCCCAAACTAAAAGCTG	AF320768	$(CA)_{12}$	60 (54)	31	3	188–200	0.22	0.50
Sh4.28 (140)	TGTGGTCTACAGTATATCTTTTGTG* CAAATTTGAATCTACAGTGGGG	AF320769	$(CA)_{11}$	55 (52)	94	6	102–116	0.47	0.55

*The oligonucleotides marked with *were Cy5-labelled for analysis on an automated sequencer.
†Annealing temperatures in parentheses apply to amplifications with Cy5-labelled primers. Optimal $MgCl_2$ concentrations were 1.5 mmol for loci Sh3.32, Sh3.39 and Sh4.3 and 2.5 mmol for loci Sh3.13 and Sh4.28.

plasmids were used to transform competent *Escherichia coli* (TOP10 One Shot™, Invitrogen), and the culture was grown overnight on selective agar plates.

Colonies were lifted onto nylon membranes, where DNA was immobilized by baking for 2 h at 80 °C. We screened the library with a fluorescently labelled $(CA)_{15}$ probe (ECL 3′-oligolabelling and detection system, Amersham). Hybridization was conducted overnight in a rotary oven at 52 °C. In all subsequent steps of the screen, we followed the manufacturer's instructions, except for the second stringency wash, which we performed in 6× SSC and 0.1% SDS. Colonies that gave a signal on the autoradiograph were streaked out in replicate onto fresh agar plates and subjected to a secondary screen. Plasmid DNA was extracted from overnight cultures of all positives of this second screen (42 clones). Thirty-seven clones were sequenced (ALFexpress™ AutoRead™ Sequencing Kit) and fragments were separated on an automated sequencer (ALFexpress, Pharmacia). We found 29 microsatellites (> 3 repeats). Primers were designed with PRIMER vs. 0.5 (Lincoln *et al*. 1991; Table 1) for nine loci with long repeats [> $(CA)_{10}$] and sufficient flanking sequence.

After some PCR optimization, all nine loci amplified reliably. PCR reactions were carried out in a total volume of 30 µL with 1 µL template (DNA extracted as above), 10 pmol of each primer, 3 µL dNTP (2 mmol/nucleotide), 50 mM KCl, 1.5–2.5 mM $MgCl_2$ (cf. Table 1), 10 mM Tris-HCl pH 9.0 and 0.5 U *Taq* polymerase. The cycling protocol was as follows: one cycle at 95 °C for 3 min followed by 35 cycles at 94 °C for 15 s, 52–60 °C for 30 s (cf. Table 1) and 72 °C for 30 s. In four loci, allelic variation was analysed by using fluorescently labelled primers and separating fragments on an ALFexpress sequencer. For the fifth locus (Sh3.13), electrophoresis was carried out through 10% polyacrylamide gels, and bands were visualized with silver staining. PCR reactions with DNA of *Pocillopora damicornis*, a close relative, did not yield any product. As amplifications of *S. hystrix* from the same aquarium (i.e. a shared pool of zooxanthellae) were successful, these results are in accordance with a species-specific coral origin of our primers. Also, tests with primers Sh3.32 and Sh4.28 failed to amplify DNA from a pure zooxanthellae culture. We genotyped samples of *S. hystrix* colonies from five different sites near Dahab (Gulf of Aquaba, Red Sea, Egypt) located within a 14-km stretch of coastline. In the field, roughly 1 cm long, fresh coral fragments were quickly blow-dried and then stored at ambient temperature until further processing.

Between three and nine alleles were detected per locus. All loci show some heterozygote deficit. At this point, we cannot rule out that null alleles are contributing to this pattern. Likely additional explanations are deviations from the Wright–Fisher model in the form of population subdivision and clonal replication through asexual reproduction. Larger sample sizes are needed to rigorously test these hypotheses.

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References

Ayre DJ & Hughes TP (2000) Genotypic diversity and gene flow in

brooding and spawning corals along the Great Barrier Reef, Australia. *Evolution*, **54**, 1590–1605.

Harrison PL & Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: *Ecosystems of the World*, **Vol. 25**. Coral Reefs (ed. Dubinsky Z), pp. 133–207. Elsevier, Amsterdam.

Lincoln SE, Daly MJ, Lander ES (1991) *Primer, Version 0.5 Manual*. The Whitehead Institute for Biomedical Research, Cambridge, MA.

Telenius H, Carter NP, Bebb CE, Nordenskjöld M, Ponder BAJ, Tunnacliffe A (1992) Degenerate oligonucleotide-primed PCR: General amplification of target DNA by a single degenerate primer. *Genomics*, **13**, 718–725.

PAPER 2

Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea

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RESEARCH ARTICLE

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Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea

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Abstract Pelagic dispersal of larvae in sessile marine invertebrates could in principle lead to a homogeneous gene pool over vast distances, yet there is increasing evidence of surprisingly high levels of genetic differentiation on small spatial scale. To evaluate whether larval dispersal is spatially limited and correlated with distance, we conducted a study on the widely distributed, viviparous reef coral *Seriatopora hystrix* from the Red Sea where we investigated ten populations separated between ~0.150 km and ~610 km. We addressed these questions with newly developed, highly variable microsatellite markers. We detected moderate genetic differentiation among populations based on both F_{ST} and R_{ST} (0.089 vs. 0.136, respectively) as well as considerable heterozygote deficits. Mantel tests revealed isolation by distance effects on a small geographic scale (≤ 20 km), indicating limited dispersal of larvae. Our data did not reveal any evidence against strictly sexual reproduction among the studied populations.

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Introduction

Pelagic dispersal of larvae in sessile marine invertebrates could in principle lead to large-scale panmixia and to a homogeneous spatial distribution of genetic variation. This would limit the formation of differentiated clades by either genetic drift or local selection, and most adaptive changes would occur in response to spatially averaged selection pressures. Yet, in contrast to this prediction, numerous studies have demonstrated moderate to high levels of genetic differentiation at genetic marker loci over relatively small spatial scales. Examples of F_{ST} estimates (Wright 1969) greater than 0.1 on relatively limited spatial scales come from sponges (Duran et al. 2004), ascidians (Ayre et al. 1997a; Ben-Shlomo et al. 2001; Paz et al. 2003), gorgonians (Gutiérrez-Rodríguez and Lasker 2004) and scleractinian corals (Stoddart 1984b; Ayre and Dufty 1994; Ayre and Hughes 2000; Nishikawa et al. 2003). Under the assumption of selective neutrality of the genetic markers, these results imply that genetic drift plays an important role in structuring the gene pool of these populations. This could be because gene flow is relatively rare and/or because population processes such as population turnover or highly skewed reproductive success generate large stochastic fluctuations in local allele frequencies. Disentangling the processes that lead to the observed balance between genetic drift and gene flow is clearly of interest, because it contributes towards a better understanding of the life history strategies of sessile marine invertebrates. Here, we focus on the contribution of spatially limited dispersal to neutral genetic differentiation among populations of the scleractinian coral *Seriatopora hystrix* in the Red Sea. Previously reported estimates of F_{ST} for *S. hystrix* from the Great Barrier Reef are among the highest found in any coral to date (Ayre and Dufty 1994; Ayre and Hughes 2000).

Dispersal distances of planula larvae depend on various parameters such as the pre-competency period, the survival rate of competent larvae, the availability of

suitable substrate and current patterns (Harrison and Wallace 1990; Petersen and Tollrian 2001; Hohenlohe 2004). On the basis of the specific properties of coral larvae, several predictions about dispersal distances have been formulated. For example, brooded larvae typically attain settlement competency earlier than larvae of broadcast spawners (Harrison and Wallace 1990) and could therefore settle relatively closer to their mother colonies. However, as brooded larvae possess symbiotic algae from which they may gain energy resources, they are able to survive long-distance dispersal (Richmond 1987). The above-mentioned predictions would argue for a larger variance in dispersal distances of brooders compared to broadcast spawners, but not necessarily for a difference in the mean. In fact, neither of them is strongly supported by the available evidence. Recent laboratory-based studies have shown that the timing of settlement in brooders versus broadcast spawners is not necessarily all that different (Miller and Mundy 2003) and that at least some broadcast spawners may have longer competency periods than brooders (Nishikawa et al. 2003). Thus, our current knowledge about the biology of coral larvae offers little clues about dispersal distances in nature. Moreover, while a study on *Acropora tenuis* and *Stylophora pistillata* supported the prediction that neutral genetic differentiation among populations is greater in brooders compared to broadcast spawners (Nishikawa et al. 2003), a large comparative study of five brooding and four spawning coral species did not find an effect of larval biology on the levels of neutral genetic differentiation (Ayre and Hughes 2000).

Population genetic analyses offer valuable insight into patterns of sexual and asexual reproduction and larval dispersal, as these traits shape the genetic structure of populations. However, the estimation of gene flow based on the amount of spatial genetic differentiation (viz. F_{ST}) is complicated by the fact that a given pattern of neutral genetic variation could be caused by various combinations of migration between and genetic drift within demes (Whitlock and McCauley 1999). Considering the diverse reproductive strategies of corals and their often erratic local population trajectories (Hughes et al. 1992), it seems likely that highly skewed reproductive success and/or frequent population turnover cause large stochastic fluctuations in local allele frequencies and thus inflate F_{ST} (Whitlock and McCauley 1999). As an alternative, spatially restricted gene flow can be inferred from allele frequency data based on Wright's (1943) isolation by distance model. If the sampling scale is larger than the average dispersal distance, then pairwise estimates of F_{ST} from populations that are separated by some distance x should increase with x (Slatkin 1993). Previous studies have applied this approach to a variety of marine invertebrates including sponges (Duran et al. 2004), bivalves (Launey et al. 2002), cephalopods (Perez-Losada et al. 2002), sea stars (Skold et al. 2003; Perrin et al. 2004), soft corals (Bastidas et al. 2002) as well as scleractinian corals, i.e. the solitary species *Balanophyllia elegans* (Hellberg 1994, 1995, 1996) and *Paracyathus stearnsii* (Hellberg 1996) as well as the deep-sea coral *Lophelia pertusa* (Le Goff-Vitry et al. 2004).

Seriatopora hystrix is a hermaphroditic coral species commonly found in Indo-Pacific reefs from the Red Sea to the Western Pacific (summarised in Veron 2000). It reproduces sexually via brooded larvae (Ayre and Resing 1986). In the laboratory most larvae have been observed to settle within 24 h after release (Atoda 1951), which suggests that dispersal may be spatially limited. This behaviour may contribute to the very high levels of genetic differentiation: two allozyme studies on the Great Barrier Reef revealed F_{ST} values of 0.3 (Ayre and Dufty 1994) and 0.28 (Ayre and Hughes 2000) for populations within a single reef.

Several asexual modes of reproduction have also been suggested for *S. hystrix*: based on the colonies' delicately branched morphology, reproduction via fragmentation appears likely (Ayre and Dufty 1994; Ayre and Hughes 2000). Moreover, in a laboratory study, single polyps were observed to detach from the underlying skeleton and resettle once they came in contact with an appropriate surface (polyp bail-out; Sammarco 1982). However, allozyme-based population surveys have so far not found any conclusive evidence for a contribution of asexual reproduction to recruitment on the Great Barrier Reef (Ayre and Dufty 1994; Ayre and Hughes 2000). In the present study of populations from the Red Sea, we analysed the patterns of genotypic diversity for a possible signature of asexual reproduction, because life history strategies in corals show enormous intraspecific, geographic variation (e.g. Stoddart 1984a, b; Ayre et al. 1997b) and because we would need to exclude obvious clonal replicates from any analysis of isolation by distance.

The power of the present analyses is aided by the use of highly variable microsatellite loci (Maier et al. 2001). The chances to detect isolation by distance have been shown to increase with the level of allelic variation per locus (Hellberg 1994). The same is true for the analysis of multilocus genotypic diversity in view of detecting asexual reproduction (cf. Discussion). We investigated the genetic structure of ten *S. hystrix* populations within the Red Sea along a distance of ~610 km. We found evidence for spatially restricted dispersal on a scale of ≤ 20 km. There was no evidence that asexual reproduction played an important role in structuring local populations. Given the likely occurrence of null alleles in our data set, we developed a statistical method to test that our inferences about spatial population structure are robust to possible null allele effects.

Materials and methods

Collection and storage of samples

Tissue samples of *S. hystrix* were collected between 1999 and 2002 from ten sites within the Red Sea region, separated between ~0.150 km and ~610 km. The

northernmost population was situated near the city of Eilat, Israel (29°33′40″N, 34°57′06″E) at the dive site Satil (*Sa*). The two southern populations Zabargad (*Za*) and Malahi (*Ma*) were located near Hamata, Egypt (25°05′N, 34°54′E). The remaining seven sites Bells (*Be*), Blue Hole (*Bh*), Eel Garden (*Eg*), House Reef (*Hr*), Islands (*Is*), Lagoon (*La*) and Three Pools (*Tp*) were chosen in the Dahab region, Egypt (28°29′00″N, 34°32′00″E; Fig. 1). Depths of sampling sites ranged from 3 m to 24 m. We sampled all colonies with a diameter of about 20 cm in contiguous stands of *S. hystrix* until the target sample size of about 20 was reached (two exceptions: $n = 11$ and 32). Depending on colony density, the sampled areas ranged from 30 m^2 to 150 m^2. Size-selective sampling was done in order to reduce the confounding effects of allele frequency variance among age cohorts (Waples 1987). An exception was the population *Sa* where colonies were sampled irrespective of size. From each colony, a single branch tip of approximately 1 cm length was pruned carefully underwater and, upon return to the shore, immediately blow-dried for the preservation of DNA. Samples were initially stored at ambient temperature and then frozen at −60°C prior to DNA extraction.

Laboratory analysis

DNA was extracted with DNAzol™ and Polyacrylcarrier (Molecular Research Center, Inc.) as described by Maier et al. (2001). PCR reactions were set up in a total volume of 30 µl with 1 µl template DNA, 10 pmol of each primer (one per pair labelled with *Cy5*), 3 µl dNTPs (2 mmol per nucleotide), 50 mM KCl, 1.5–2.5 mM $MgCl_2$ (cf. Table 1), 10 mM Tris-HCl (pH 9.0 at RT), 0.5 U Taq polymerase (rTaq, Amersham Biosciences) and overlaid with mineral oil. Amplifications were carried out on a Hybaid Touchdown thermocycler. The following cycling protocol was used: an initial denaturation step for 3 min at 95°C was followed by 35 cycles at 94°C for 15 s, 52–54°C for 30 s (cf. Table 1) and 72°C for 30 s. Allelic variation was analysed on an ALFexpress automated sequencer (Amersham Biosciences).

To date, seven microsatellite markers have been developed for *S. hystrix* (this study; Maier et al. 2001). But only four (Sh2.15, Sh3.32, Sh3.39 and Sh4.24) were analysed in the present study, as the other three produced unreliable banding patterns. From our final data set, we also had to exclude locus Sh3.39 because unambiguous scoring of alleles proved impossible, probably due to another microsatellite locus that co-amplified with our primers. In locus Sh3.32, the two most common alleles that differed in lengths by 2 bp were pooled because of confounding stutter bands. Moreover, in 19 isolated sample/locus combinations from seven populations, a clear distinction between two neighbouring alleles was not feasible, also because of stutter bands. In these instances, we adopted a conservative approach of assigning the rarer allele in the particular population to the colony in question, as this tends to bias F_{ST} downward and thus favours the null hypothesis of no spatial structure among populations. In no case did this assignment affect the number of different multilocus genotypes observed within the population.

Statistical analysis

When inferring levels of genetic population subdivision with microsatellite markers, the specifics of their mutation process have to be taken into account: microsatellites mutate to different allele lengths mostly by the addition or loss of single repeat units (e.g. Crozier et al. 1999; Kayser et al. 2000; for exceptions see Di Rienzo et al. 1994; Anderson et al. 2000). The stepwise mutation model (viz. R_{ST}, Slatkin 1995) therefore infers the relatedness between alleles from their similarity in length and derives from this a measure of population divergence. In contrast, the infinite allele model posits that every mutation introduces a novel allele and determines population subdivision solely based on probabilities of identity by descent (viz. F_{ST}, Wright 1969). However, any allelic size homoplasy violates the infinite allele assumption. Considering this concern and the observed preponderance of small mutational steps, it appears that R_{ST} better estimates spatial population structure at mi-

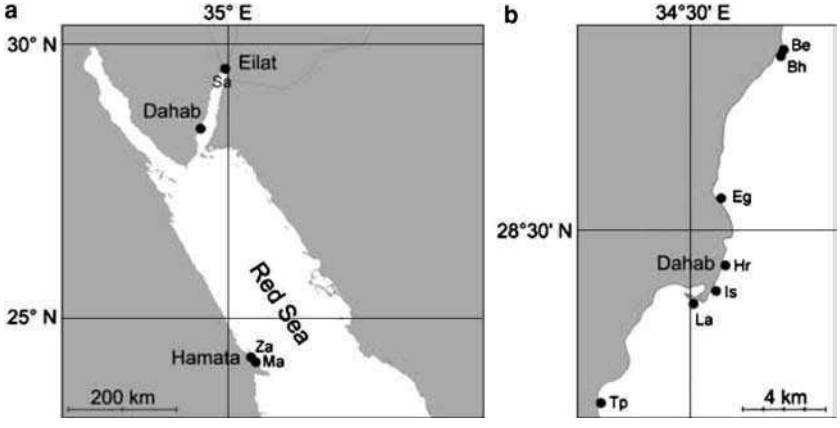

Fig. 1 Map of sampling sites. (**a**) Northern Red Sea including the site near Eilat (*Sa*: Satil) and the two sites near Hamata (*Za*: Zabargad; *Ma*: Malahi). (**b**) Location of sites in the area around Dahab (*Be*: Bells; *Bh*: Blue Hole; *Eg*: Eel Garden; *Hr*: House Reef; *Is*: Islands; *La*: Lagoon; *Tp*: Three Pools)

Table 1 Description of three microsatellite loci used in this study on *Seriatopora hystrix*

Locus	Primer sequences [a] (5′ → 3′)	GenBank accession number	Repeat motif	T_a [b] (°C)	No. of alleles	Allele size range
Sh2.15	CGTGCCACTGTGATTTCTTC* AACAAAAACGTCTCCATTACCC	AY604005	$(CA)_{18}$	57 (52)	15	263–315
Sh3.32	CCAAAACCCTGCATTTTGAG* CCCCCTGTAAAAGTGTACCC	AF320766	$(CA)_{20}$	55 (52)	15	219–252
Sh4.24	TCCTCCAGATGAATTTGAACG* TTCAGGGAAGATTTGCCG	AY604006	$(CA)_{37}$	59 (54)	26	178–292

[a]The oligonucleotides marked with * were Cy5-labelled for analysis on an automated sequencer
[b]Annealing temperatures in parentheses apply to amplifications with Cy5-labelled primers. Optimal $MgCl_2$ concentrations were 1.5 mmol for locus Sh2.15 as well as Sh3.32 and 2.5 mmol for locus Sh4.24

crosatellite loci. To check the robustness of our results, we follow the common approach (Gaggiotti et al. 1999; Balloux and Goudet 2002; Reichow and Smith 2001) of using both estimators, F_{ST} as well as R_{ST}, to evaluate genetic differentiation between populations.

Allele frequencies as well as Weir and Cockerham's (1984) estimators of Wright's (1965) *F*-statistics, f ($=F_{IS}$, the inbreeding coefficient) and θ ($=F_{ST}$, the fixation index), were calculated using *FSTAT* Version 2.9.3 (Goudet 2001). R_{ST} was calculated with R_{ST} calc., Version 2.2 (Goodman 1997), with 5,000 iterations performed for the bootstrap and permutation tests. To assess genetic variation, we calculated expected heterozygosity (H_e) for each locality and compared it with the observed heterozygosity (H_o). H_e was calculated as $1-\Sigma p_i^2$ where p_i is the frequency of the ith multilocus genotype in the population. H_o was calculated using IBD (Bohonak 2002).

Because our results suggested that null alleles were present in the data set (see below), we checked that they did not unduly affect the inference of spatial population structure. Using a maximum-likelihood approach, we computed an alternate, hypothetical data set in which a number of apparent homozygotes were converted into heterozygotes with the observed and a null allele. The alternate data set is a compilation of the resulting maximum-likelihood genotype sets per locus and population based on the assumption of random mating. Details of this method are given in the Appendix. F_{ST} estimates from the alternate and the observed data set were compared in order to check the robustness of our results for the possible occurrence of null alleles.

As a preliminary test to check whether each locus behaved independently, we used *FSTAT* Version 2.9.3 (Goudet 2001) to estimate pairwise genotypic disequilibria based on 60 permutations. Inter-locus associations may result from either physical linkage between loci, which would rule out their use as independent markers, or from population genetic processes such as the mixing of populations and/or asexual reproduction.

To assess the contribution of asexual reproduction to the genotypic composition within populations, we calculated the observed number of distinct genotypes (N_g) over the sample size (N). Observed (G_o) and expected (G_e) genotypic diversity was estimated according to Stoddart and Taylor (1988). To assess the probability of detecting identical multilocus genotypes by chance within a sexually reproducing population, a simulation approach was used as described in Duran et al. (2004). The program generates simulated populations by randomly selecting alleles using the observed frequencies, but maintaining the observed heterozygosity at each locus. The major advantage of this approach is that it does not rely on the assumption of random mating, as this condition is often violated, especially in sessile marine invertebrates with low dispersal capabilities.

To test for a correlation between geographic and genetic distances, we performed a Mantel test (Mantel 1967) using IBD (Bohonak 2002), which compares the matrices of pairwise genetic and geographic distances. We estimated geographic distances as the shortest distance connecting populations by sea. The matrices contained the unscaled (=linear) geographic distance and $F_{ST}/(1-F_{ST})$ as a measure of genetic distance, respectively. In a one-dimensional habitat, one expects a linear relationship between these two variables (Rousset 1997). A second, analogous set of tests was carried out using $R_{ST}/(1-R_{ST})$. Each Mantel test was based on 30,000 matrix randomisations. Moreover, slopes and intercepts of the relationships were calculated via reduced major axis (RMA) regression. We considered two spatial scales: the small-scale analysis involved only populations around Dahab (maximum pairwise distance: ~20 km), whereas the analysis on large scale included all populations (maximum pairwise distance: ~610 km).

Results

Allelic variation

A total of 207 *S. hystrix* colonies were sampled and genotyped. All three microsatellite loci amplified reliably and were highly polymorphic, revealing 15–26 alleles per locus (mean: 18.66). The longest and shortest alleles per locus differed by 32 bp (Sh3.32), 52 bp (Sh2.15) and 114 bp (Sh4.24), and the frequencies of the most common alleles were 0.212 (Sh4.24), 0.389 (Sh2.15) and

0.519 (Sh3.32), respectively. Averages of expected heterozygosity within sites for the three loci were 0.611 (Sh3.32), 0.747 (Sh2.15) and 0.849 (Sh4.24). The minima and maxima overall were 0.320 (Sh3.32 in population *La*) and 0.933 (Sh4.24 in population *Is*, Table 2), respectively.

Allele frequencies varied markedly among populations, even over short distances (Fig. 2). For example, at locus Sh4.24, allele *232* was the most frequent in population *Eg* (frequency = 0.325). Only 3.5 km to the south, at population *Hr*, its frequency was only 0.053, and it was absent in the next population to the south (*Is*, 4.8 km distance from *Hr*). Several rare alleles were found only in a single population each (alleles *220*, *250* and *252* of locus Sh2.15 as well as alleles *202* and *210* of locus Sh4.24, see Fig. 2). Thus, our data revealed high allelic diversity within and between the ten studied populations.

Genotypic structure within populations

Heterozygote deficits were observed at all three loci. Mean values of observed heterozygosity (H_o) per locus were 0.354 (Sh3.32), 0.581 (Sh2.15), and 0.747 (Sh4.24), which resulted in average F_{IS} estimates of 0.44, 0.21 and 0.11, respectively (Table 2). These relatively high estimates and in particular, the large variance among loci suggested that our samples might include null alleles. This motivates the use of a hypothetical, alternate data set with null alleles being included to check the robustness of our F_{ST}-estimates (see Material and methods, Appendix and below). We evaluate the null allele hypothesis in the light of alternative explanations in the discussion.

Sexual versus asexual reproduction

Preliminary analyses revealed no deviation from linkage equilibrium for any pair of loci ($P < 0.05$, based on 60 permutations). Hence, we considered all loci as statistically independent. In all ten study populations, the expected genotypic diversity (G_e) was greater than 90% of the sample size (Table 3). Thus, our results provide high statistical power for tests of sexual versus asexual reproduction (see Discussion). In eight populations, all colonies were genotypically distinct ($G_o = N$). Somewhat lower genotypic diversity was found in the populations *Eg* and *La*, with the lowest G_o: G_e ratio of 0.85 overall in the latter population ($P < 0.13$, where P corresponds to the proportion of the null distribution with genotypic diversity smaller or equal to G_o). The proportion of simulated populations (based on 100,000 replicates, keeping sample size, allele frequencies and heterozygosity as observed) with at least two colonies with identical multilocus genotypes ranged from 0.09 (location *Tp*) to 0.71 (location *Bh*), with 0.29 for *Eg* and 0.70 for *La*. These values revealed a high probability that the small proportion of identical multilocus genotypes within two of our populations was generated by chance under strictly sexual reproduction. As we did not find any evidence for asexual reproduction, no further adjustment of the data set (viz. removal of clonal replicates) was needed. Non-amplifying alleles do not change these conclusions, since they would tend to increase G_o and we already detected the maximum possible number within most of our populations.

Genetic differentiation among populations

Averaged over all three loci and all populations, our results revealed moderate and highly significant genetic differentiation ($F_{ST} = 0.089$; $R_{ST} = 0.136$, $P < 0.001$ for each locus and overall, Table 4). Considering all population comparisons, the minimum and maximum pairwise estimates for F_{ST} were < 0.001 for the comparison *Hr-Is* (~1.3 km distance) and 0.306 for *La-Bh* (~11 km distance), respectively. Those for R_{ST} were −0.017 for

Table 2 Expected (H_e) and observed (H_o) heterozygosity and F_{IS} estimates for the three loci among different collections

Site	Sh2.15			Sh3.32			Sh4.24		
	H_e	H_o	F_{IS}	H_e	H_o	F_{IS}	H_e	H_o	F_{IS}
Sa	0.778	0.678	0.13	0.718	0.387	0.46[a]	0.892	0.846	0.05
Be	0.721	0.650	0.10	0.729	0.333	0.54[a]	0.796	0.632	0.23
Bh	0.755	0.556	0.26	0.682	0.400	0.41	0.594	0.529	0.11
Eg	0.814	0.550	0.33	0.580	0.316	0.46[a]	0.843	0.950	−0.13
Hr	0.839	0.750	0.11	0.642	0.353	0.45[a]	0.928	0.842	0.09
Is	0.780	0.700	0.10	0.369	0.294	0.20	0.933	0.889	0.05
La	0.322	0.200	0.38	0.320	0.250	0.22	0.908	0.833	0.08
Tp	0.906	0.600	0.34	0.372	0.300	0.19	0.889	0.700	0.21
Za	0.720	0.571	0.21	0.879	0.647	0.26[a]	0.807	0.600	0.26
Ma	0.829	0.550	0.34	0.822	0.263	0.68[a]	0.904	0.647	0.29
Mean	0.747	0.581	0.21 ± 0.04	0.611	0.354	0.44 ± 0.05	0.849	0.747	0.11 ± 0.04

F_{IS} was calculated using *FSTAT*, Version 2.9.3 (Goudet 2001). Mean F_{IS} estimates over populations were estimated according to Weir and Cockerham (1984). Significant values have been determined after sequential Bonferroni corrections. For abbreviations of sampling sites, see Fig. 1

[a] Indicate adjusted α-level (5%) ≤ 0.0017

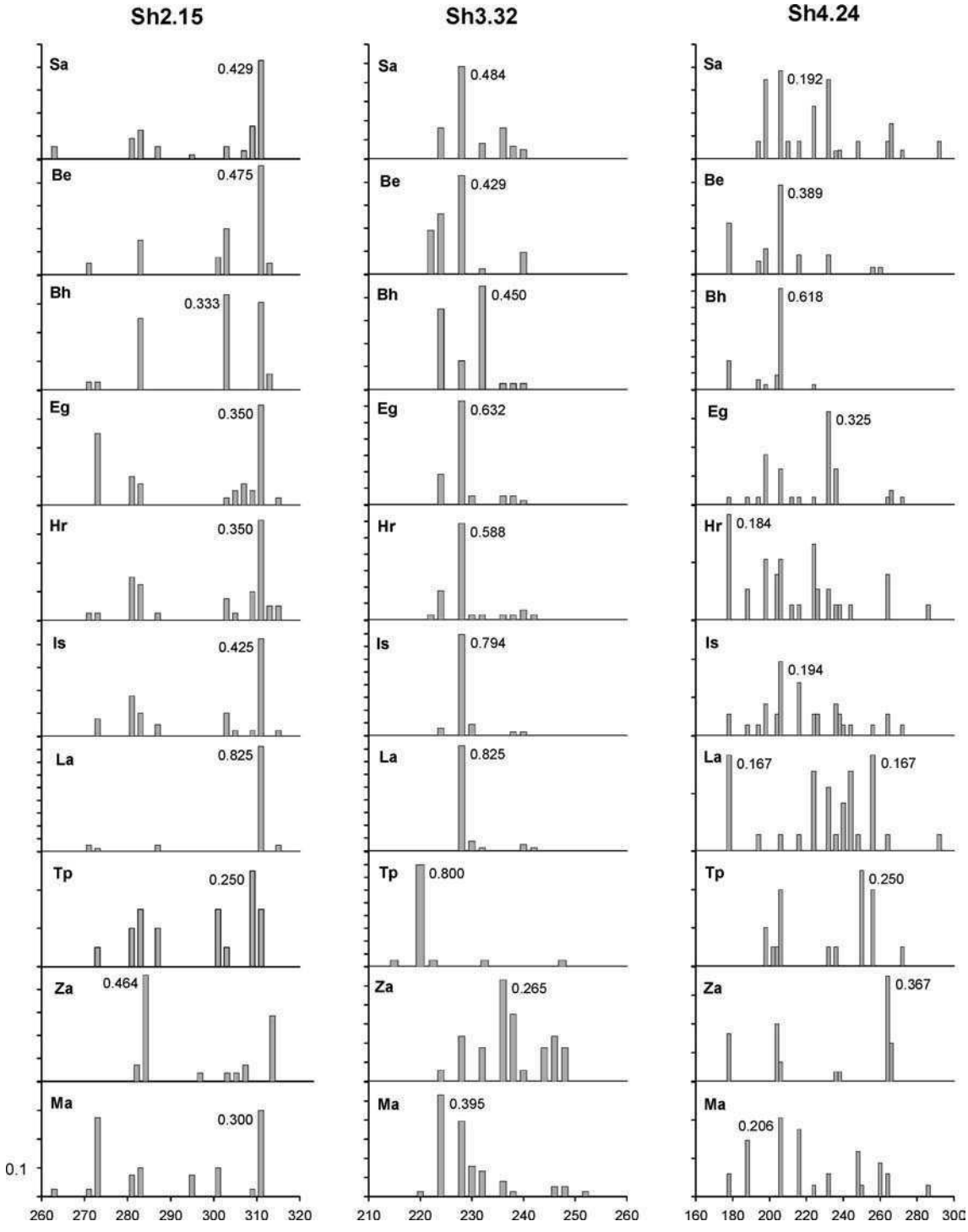

Fig. 2 Allele frequencies at the three loci across all populations (for details see Fig. 1 legend). Alleles were classified according to their sizes (bp). The scales on the *y* axes vary among graphs. The frequency of the most common allele is given for each population

the comparison *Eg-Is* (~4.8 km distance) and 0.453 for *Be-Za* (~475 km distance) (Table 5). There was little change in the overall level of differentiation when we allowed for null alleles: F_{ST} in the alternate data set was 0.097 as opposed to 0.089 (observed). Note that we could not compute R_{ST} for the alternate data set because the length of the inferred null allele is unspecified.

Isolation by distance

We considered the relationship between genetic and geographic distance on two spatial scales: (a) populations around Dahab (max. distance ~20 km) and (b) all populations (max. distance ~610 km). To assess differ-

Table 3 Multilocus genotypic diversity per population. Listed are the sample sizes (N), the ratio of the observed number of distinct genotypes (N_g) over the sample size as well as the observed (G_o) and expected (G_e) genotypic diversity estimates

Site	N	N_g/N	G_o	G_e	p	*sim*
Sa	32	1.00	32.0	31.8	1.00	0.45
Be	21	1.00	21.0	20.6	1.00	0.42
Bh	20	1.00	20.0	18.5	1.00	0.71
Eg	20	0.95	18.2	19.7	0.14	0.29
Hr	20	1.00	20.0	19.9	1.00	0.21
Is	20	1.00	20.0	19.9	1.00	0.15
La	20	0.85	15.4	18.1	0.13	0.70
Tp	11	1.00	11.0	10.9	1.00	0.09
Za	22	1.00	21.0	20.7	1.00	0.40
Ma	21	1.00	22.0	21.9	1.00	0.23

The proportion of the null distribution with genotypic diversity smaller than or equal to G_o is given by p. The proportion of simulated populations with at least one clone of size 2 is given by *sim*. Abbreviations of sampling sites are as in Fig. 1

Table 4 Estimates of F_{ST} (here: Weir & Cockerham's θ) and R_{ST} (ρ, normalised across loci according to Goodman, 1997)

Locus	F_{ST} (s.e.)	R_{ST}
Sh2.15	0.072 (0.035)	0.063
Sh3.32	0.125 (0.055)	0.255
Sh4.24	0.074 (0.024)	0.118
Total	0.089	0.136

Standard errors (s.e.) for θ are based on jackknifing over populations

entiation based on allelic identity, we computed Mantel correlations between $F_{ST}/(1-F_{ST})$ and geographic distance, given the nearly linear arrangement of habitats (Rousset 1997). The correlation was not significant ($r=0.4452$, $P=0.069$). Thus this analysis, based on F_{ST}, does not provide evidence for a distance effect. On the large spatial scale, there is no indication for a distance relationship whatsoever ($r=0.0647$, $P=0.4496$). Null alleles had no effect on these results as correlation coefficients of the observed versus the alternate data set were very similar and the patterns of significance were identical. For the analogous quantity, $R_{ST}/(1-R_{ST})$, Mantel tests gave a highly significant correlation between genetic and geographic distance on the small spatial scale ($r=0.6314$, $P=0.018$, Fig. 3). There was also a significant, albeit weaker correlation on the large spatial scale ($r=0.490$, $P=0.0408$). The opposing results for R_{ST} versus F_{ST} for the entire data set are illustrated in Fig. 4.

Discussion

This study on the brooding coral species *S. hystrix* from the Red Sea revealed marked genetic differentiation at microsatellite loci on a small spatial scale ($\leq$ 20 km). Thus, our findings are in general agreement with earlier allozyme studies on *S. hystrix* from the Great Barrier Reef, which showed high levels of genetic differentiation within and between reefs (Ayre and Dufty 1994; Ayre and Hughes 2000). Since a given estimate of neutral genetic variation can be translated into measures of gene flow only for populations that conform to Wright's (1969) island model (Whitlock and McCauley 1999), we abstain from a direct translation of F_{ST} estimates into the number of migrants (Nm). Instead, we used the observed increase in pairwise genetic distances with geographic distance to infer spatially limited dispersal in *S. hystrix* on a small scale of $\leq$ 20 km. Moreover, while several potential mechanisms of asexual reproduction have been discussed for *S. hystrix*, our data are in agreement with strictly sexual reproduction.

Population differentiation

Our inference of spatially limited dispersal is based on the significant regression of pairwise $R_{ST}/(1-R_{ST})$ on geographic distance. Since the mutation process of microsatellites is better approximated by the stepwise as opposed to the infinite allele model (Slatkin 1995), we consider this to be the more appropriate analysis relative to the analogous regression using $F_{ST}/(1-F_{ST})$. The latter is based only on the identity of alleles and ignores information based on similarity of allele lengths. The F_{ST}-based correlation was not significant ($P=0.069$). Moreover, spatially limited dispersal should lead to ever

Table 5 Pairwise estimates of F_{ST} (above diagonal) and R_{ST} (below diagonal) between populations of *S. hystrix*

Site	*Sa*	*Be*	*Bh*	*Eg*	*Hr*	*Is*	*La*	*Tp*	*Za*	*Ma*
Sa		0.034	0.125	0.016	0.004	0.030	0.097	0.049	0.096	0.034
Be	0.150		0.070	0.063	0.024	0.054	0.130	0.090	0.125	0.053
Bh	**0.150**	0.030		0.185	0.135	0.191	0.306	0.208	0.155	0.121
Eg	0.011	0.123	0.131		0.017	0.028	0.109	0.048	0.146	0.034
Hr	0.020	0.029	0.026	0.010		<0.001	0.080	0.029	0.092	0.027
Is	0.009	0.084	0.098	−0.017	−0.014		0.063	0.027	0.155	0.041
La	0.038	**0.191**	**0.259**	0.108	0.085	0.092		0.152	0.252	0.134
Tp	−0.012	0.206	**0.223**	−0.006	0.055	0.016	0.057		0.163	0.057
Za	**0.222**	**0.453**	**0.404**	**0.292**	**0.306**	**0.324**	**0.329**	0.233		0.085
Ma	0.043	**0.212**	0.156	0.039	0.076	0.066	**0.175**	0.035	0.115	

Abbreviations of sampling sites as in Fig.1. Significant R_{ST} values are given in bold. To maintain an experiment-wise error level of 0.05, the critical α-level was 0.001 (Dunn-Šidák method). No significance estimates were provided by IBD (Bohonak 2002) for F_{ST}

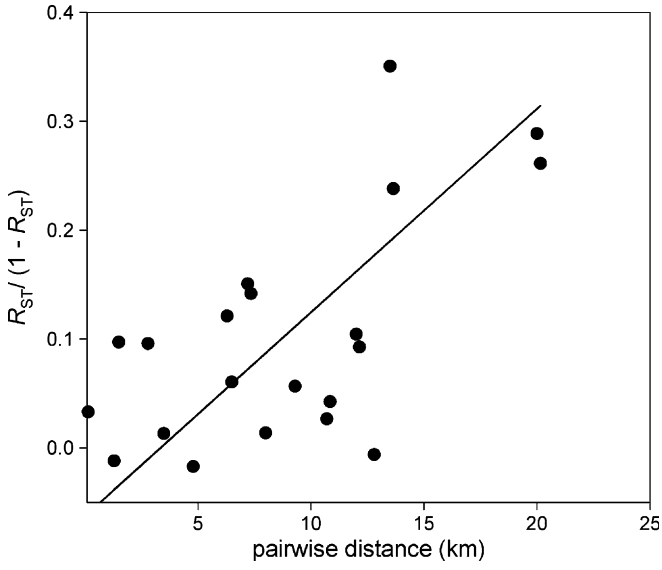

Fig. 3 Isolation by distance within the Dahab area. Plotted is $R_{ST}/(1-R_{ST})$ as a function of geographic distance. The *line* reflects the reduced major axis regression ($P=0.0181$)

greater levels of differentiation with increasing geographic distances. Indeed, the highest estimates of R_{ST} were found for pairs of populations that were 500 km apart, but overall the regression slope was lower for the entire set of sites than for the small-scale analysis around Dahab, and the correlation was lower as well ($r=0.4901$ vs. $r=0.6314$). Given that our data set included only a small number of very distant sites, this difference may simply be a sampling artefact. It could also be due to constraints on allele size at our microsatellite loci, which would cause pairwise R_{ST} values to asymptote at greater geographic distances. Furthermore, historical large-scale population processes may impede the attainment of equilibrium between genetic drift and migration across the Red Sea (Hellberg 1995).

The difference between Mantel test results based on R_{ST} versus F_{ST} might be explained by the different behaviour of these two estimators under the stepwise mutation model. When the difference in average coalescence times of pairs of genes sampled from the same or from different populations is large, new mutations will accumulate and affect pairwise differences in allelic state without causing much divergence in allele frequencies (Slatkin 1995). Given that we observed relatively strong differentiation (max. pairwise $F_{ST}=0.3$), this effect should play a role in this data set and could explain the observed results. This motivates our choice of R_{ST} in the Mantel tests. We acknowledge, however, that a large sampling variance is often associated with R_{ST} when the number of loci is small (Balloux and Lugon-Moulin 2002). Our findings provide evidence of surprisingly small-scale patterns of larval dispersal in *S. hystrix*. They are consistent with laboratory observations which imply that the majority of larvae in this species settle shortly after release (Atoda 1951). Isolation by distance effects on small and large scales (50 and 3,000 km, respectively) were found in the coral *Balanophyllia elegans* (Hellberg 1995). In this species larvae are typically not pelagic but disperse by crawling over the substrate and usually settle very close to the mother colonies. No isolation by distance was detected in the deep-sea coral *Lophelia pertusa* based on samples from around the rim of the North Sea (LeGoff-Vitry et al. 2004). As pointed out, this does not necessarily imply panmixia but could result from a lack of equilibrium between genetic drift and migration. It also does not preclude distance-dependent genetic differentiation on smaller spatial scales.

In this study, the absolute level of differentiation among populations was lower compared to previous investigations of *S. hystrix*: for the Dahab sites only, F_{ST} was 0.1, whereas within-reef estimates on the Great Barrier Reef ranged from 0.28 to 0.3 (Ayre and Dufty 1994; Ayre and Hughes 2000). This could be because the

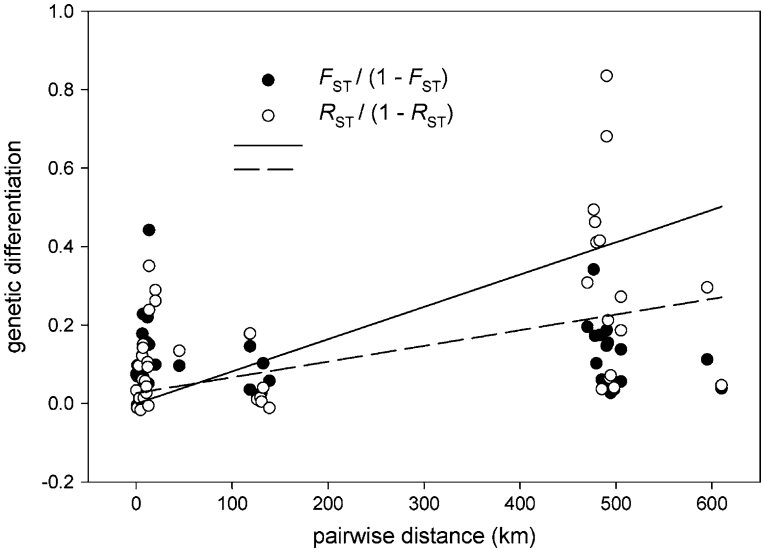

Fig. 4 Relationship between pairwise differentiation and geographic distance between all populations. *Solid circles* represent pairwise population comparisons calculated as $F_{ST}/(1-F_{ST})$, whereas *open circles* are used for pairwise population comparisons calculated as $R_{ST}/(1-R_{ST})$. The corresponding reduced major axis regressions are shown as a *broken line* for $F_{ST}/(1-F_{ST})$ ($P=0.4496$) and a *solid line* for $R_{ST}/(1-R_{ST})$ ($P=0.0408$), respectively

Red Sea populations are truly less differentiated due to weaker genetic drift effects and/or more frequent gene flow. Alternatively, the difference might stem from the use of differently variable markers (microsatellites versus allozymes). Following the definition of F_{ST} in terms of probabilities of identity of alleles sampled either from the same (f_0) or from different populations (f_1), $F_{ST}=(f_0-f_1)/(1-f_1)$, F_{ST} is sensitive to the absolute within-population variation. This effect is most clearly seen in the extreme case when no alleles are shared between populations ($f_1=0$). In this case, F_{ST} equals f_0 (Rousset 1997). Thus, for the same set of populations, highly variable microsatellites are expected to yield lower F_{ST} estimates than less variable allozymes (Hedrick 1999). The possible occurrence of homoplasy at microsatellite alleles may further enhance this effect (Viard et al. 1998). The currently available data sets are therefore not suited for a comparison of *S. hystrix* population structures in the Red Sea versus on the Great Barrier Reef. While we could not use as many loci as we intended, high levels of polymorphism of our three microsatellites should serve to reduce the coefficient of variation around our parameter estimates (Kalinowski 2005).

Genotypic structure within populations

We observed considerable heterozygote deficits at all three marker loci. Similar deviations from Hardy-Weinberg expectations have been reported from a variety of marine sessile invertebrates such as sea anemones (Russo et al. 1994), gorgonians (Brazeau and Harvell 1994) and scleractinian corals (Ayre et al. 1997b; Miller 1997, 1998; Yu et al. 1999; Dai et al. 2000; Ridgway et al. 2001; Gilmour 2002), including *S. hystrix* populations from the Great Barrier Reef (Ayre and Dufty 1994; Ayre and Hughes 2000). These findings point towards non-random mating within sites that would result if, for example, dispersal distances of sperm were short relative to the size of the sampling area. Several random-mating subpopulations would then be contained in a given site, and any variance in allele frequency among them would produce a deficit of heterozygotes in the pooled sample (Wahlund 1928). Direct evidence that fertilisation in marine brooders occurs over short distances comes from colonial ascidians and bryozoans (Yund and McCartney 1994). Immigrant recruits from other such demes would further add to the observed pattern.

In microsatellites, null alleles are a common cause for apparent, large heterozygote deficits (e.g. Callen et al. 1993; Pemberton et al. 1995). Since average F_{IS} estimates varied strongly between loci (min: 0.11, max: 0.44), it seems likely that non-amplifying alleles affected our results, especially at locus Sh3.32. The observed levels of heterozygote deficit could thus reflect the combined effect of population processes and null alleles, so that the strength of the former cannot be quantified. Null alleles might also lead to an erroneous assessment of population differentiation, especially if their frequencies vary among populations. However, the close agreement between overall F_{ST} estimates based on the observed and the alternate data sets (0.089 and 0.097, respectively) shows that non-amplifying alleles, if present, introduced little bias in this measure of differentiation. We note that biases due to the presence of more than one null allele remain, but these effects should become progressively weaker as more alleles of this type are involved.

In most of the studied populations, all colonies were genotypically distinct. Simulations suggested that the small proportion of identical multilocus genotypes in two of our study populations may have resulted by chance under strictly sexual reproduction. We have therefore no evidence for clonal reproduction in *S. hystrix* in our samples, despite good statistical resolution afforded by our microsatellite loci. The power to detect a small proportion of clonal replicates in a sample of otherwise sexual origin depends strongly on the allelic diversity (B. Nürnberger, unpublished simulations). In the case of three loci, an effective number of alleles (n_e) per locus of about 3.5 (corresponding to $H_e=0.72$) is required to insure that a clonal contribution of 10% to a sample of 20 colonies is statistically detectable. This power of detection drops off steeply as allelic diversity declines below this level. Our data set corresponds roughly to the example case just given. Recall, though, that sampling was carried out on a coarse grid, as colonies were sampled according to their size. This involves the possibility that some clonal replicates established close to the mother colony might have been missed.

A highly clonal population structure has been described from a variety of marine invertebrates, including sea anemones (e.g. Ayre 1984; Hoffman 1986), soft corals (e.g. McFadden 1997) and scleractinian corals (e.g. Stoddart 1984a; Adjeroud and Tsuchiya 1999). Indeed, a large proportion of *S. hystrix* populations at the Great Barrier Reef (37 and 44%) showed significantly reduced genotypic diversity (G_o) (Ayre and Dufty 1994; Ayre and Hughes 2000, respectively). But in combination with the observed deficit of heterozygotes, non-random mating within sites presents an alternative explanation as this would also lead to an apparent overrepresentation of certain genotypes and thus reduce G_o (see Duran et al. 2004 for a recently published test that allows for non-random mating). Thus, conclusive evidence for asexual reproduction is lacking at the Great Barrier Reef. In the present study, the observed genotypic diversity was high even though observed heterozygote deficits were strong. Thus, clonal reproduction does not play a detectable role in our studied populations.

In this study, we have shown that spatially restricted larval dispersal contributes to the population structure of *S. hystrix* in the area around Dahab in the Red Sea. Judging by the high heterozygote deficits, it appears likely that also movement of gametes within populations is spatially limited. Any such localised movement opens

up the possibility for adaptation to locally acting selection pressures. The scatter of pairwise F_{ST} estimates around the regression plots (cf. Figs. 3, 4) also shows that genetic drift strongly affects local allele frequencies. The levels of gene flow and genetic drift together set the stage for adaptation and determine the minimal selection strength to which an adaptive response could occur. In order to evaluate these possibilities for any coral, we still need to know more about the actual migration rates (i.e. *m* rather than *Nm*) and the patterns of mating within sites. Intriguing insights into the detailed workings of coral populations could be obtained from the identification of migrants and the assessment of parentage of recruits based on multilocus microsatellite profiles.

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Appendix

For a given population and locus with *n* apparent homozygotes, we computed the likelihood of all possible genotype sets in which 1, 2,... *n* homozygotes had been replaced by heterozygotes involving a null allele. Non-amplifying colony/locus combinations were interpreted as null homozygotes provided that the other two loci of the same colony had amplified, thus controlling for poor DNA quality. The likelihood computations were based on allele frequency estimates from the modified genotype sets under the assumption of random mating. The alternate data set was assembled from the maximum-likelihood genotype sets per population and locus. The estimated maximal local frequencies of null alleles that were inferred by our method were 0.21 (Sh4.24), 0.25 (Sh2.15) and 0.37 (Sh3.32). The minimal local estimates were zero for all three loci. Whenever our method detected the presence of a null allele in a given locus/site combination (ML frequency > 0), its frequency correlated very strongly ($r > 0.75$) with estimates from two published methods (Chakraborty et al. 1992; Brookfield 1996). These approaches compute null allele frequencies solely based on observed heterozygote deficits, either with (Brookfield 1996) or without (Chakraborty et al. 1992) including non-amplifying samples as data (= null homozygotes). It is interesting to note, though, that there were cases in which our ML frequency of the null allele was zero, while the F_{IS}-based methods inferred appreciable non-zero frequencies. In all of these cases, the likelihood curve was rather flat so that no particular estimate was strongly supported. It appears that the consideration of the specific genotypic constellations by our method (rather than just F_{IS}) tended to favour the hypothesis of no null allele in these cases. In keeping with these observations, F_{IS} estimates based on the alternate data set were not necessarily zero but they were non-significant throughout.

References

Adjeroud M, Tsuchiya M (1999) Genetic variation and clonal structure in the scleractinian coral *Pocillopora damicornis* in the Ryukyu Archipelago, southern Japan. Mar Biol 134:753–760

Anderson TJC, Su XZ, Roddam A, Day KP (2000) Complex mutations in a high proportion of microsatellite loci from the protozoan parasite *Plasmodium falciparum*. Mol Ecol 9:1599–1608

Atoda K (1951) The larva and postlarval development of the reef-building corals. V. *Seriatopora hystrix* Dana. The Science Reports of the Tohoku University, Series 4:33–39

Ayre DJ (1984) The effects of sexual and asexual reproduction on geographic variation in the sea anemone *Actinia tenebrosa*. Oecologia 69:222–229

Ayre DJ, Dufty S (1994) Evidence for restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia's Great Barrier Reef. Evolution 48:1183–1201

Ayre DJ, Resing JM (1986) Sexual and asexual production of planulae in reef corals. Mar Biol 90:187–190

Ayre DJ, Davis AR, Billingham M, Llorens T, Styan C (1997a) Genetic evidence for contrasting patterns of dispersal in solitary and colonial ascidians. Mar Biol (Berl) 130:51–61

Ayre DJ, Hughes, TP, Standish, RJ (1997b) Genetic differentiation, reproductive mode, and gene flow in the brooding coral *Pocillopora damicornis* along the Great Barrier Reef, Australia. Mar Ecol Prog Ser 159:175–187

Ayre DJ, Hughes, TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54:1590–1605

Balloux F, Goudet J (2002) Statistical properties of population differentiation estimators under stepwise mutation in a finite island model. Mol Ecol 11:771–783

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. Mol Ecol 11:155–165

Bastidas C, Benzie JAH, Fabricius KE (2002) Genetic differentiation among populations of the brooding soft coral *Clavularia koellikeri* on the Great Barrier Reef. Coral Reefs 21:233–241

Ben-Shlomo R, Douek J, Rinkevich B (2001) Heterozygote deficiency and chimerism in remote populations of a colonial ascidian from New Zealand. Mar Ecol Prog Ser 209:109–117

Bohonak AJ (2002) IBD (Isolation by distance): a program for population genetic analyses of isolation by distance. Available from http://www.bio.sdsu.edu/pub/andy/IBD.html

Brazeau DA, Harvell CD (1994) Genetic structure of local populations and divergence between growth form in a clonal invertebrate, the Caribbean octocoral *Briareum asbestinum*. Mar Biol 119:53–60

Brookfield JFY (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. Mol Ecol 5:453–455

Callen DF, Thompson, AD, Shen Y, Phillips HA, Richards RI, Mulley JC, Sutherland GR (1993) Incidence and origin of "null" alleles in the $(AC)_n$ microsatellite markers. Am J Hum Gen 52:922–927

Chakraborty R, de Andrade M, Daiger SP, Budowle B (1992) Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. Ann Hum Gen 56:45–57

Crozier RH, Kaufmann B, Carew ME, Crozier YC (1999) Mutability of microsatellites developed for the ant *Camponotus consobrinus*. Mol Ecol 8:271–276

Dai CF, Fan TY, Yu JK (2000) Reproductive isolation and genetic differentiation of a scleractinian coral, *Mycedium elephantosus*. Mar Ecol Prog Ser 201:179–187

Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple-sequence repeat loci in human populations. Proc Natl Acad Sci 91:3166–3170

Duran S, Pascual M, Estoup A, Turon X (2004) Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. Mol Ecol 13:511–522

Gaggiotti OE, Lange O, Rassmann K, Gliddons C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. Mol Ecol 8:1513–1520

Gilmour JP (2002) Substantial asexual recruitment of mushroom corals contributes little to population genetics of adults in conditions of chronic sedimentation. Mar Ecol Prog Ser 25:81–91

Goodman SJ (1997) R_{ST} calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and a determining of their significance. Mol Ecol 6:881–885

Goudet J (2001) *FSTAT*, a program to estimate and test gene diversities and fixation indices Version 2.9.3. Available from http://www.unil.ch/izea/softwares/fstat.html [updated from Goudet (1995)]

Gutiérrez-Rodríguez C, Lasker HR (2004) Microsatellite variation reveals high levels of genetic variability and population structure in the gorgonian coral *Pseudopterogorgia elisabethae* across the Bahamas. Mol Ecol 13:2211–2221

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystems of the world 25: Coral reefs. Elsevier, New York, pp 133–207

Hedrick PW (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution 53:313–318

Hellberg ME (1994) Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. Evolution 48:1829–1854

Hellberg ME (1995) Stepping stone gene flow in the solitary coral *Balanophyllia elegans*: equilibrium and non-equilibrium at different spatial scales. Mar Biol 123:573–581

Hellberg ME (1996) Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution 50:1167–1175

Hoffman RJ (1986) Variation in contributions of asexual reproduction to the genetic structure of populations of the sea anemone *Metridium senile*. Evolution 40:357–365

Hohenlohe PA (2004) Limits to gene flow in marine animals with planktonic larvae: models of *Littorina* species around point conception, California. Biol J Linn Soc 82:169–187

Hughes TP, Ayre D, Connell JH (1992) The evolutionary ecology of corals. Trends Ecol Evol 7:292–295

Kalinowski ST (2005) Do polymorphic markers need large sample sizes to estimate genetic distances? Heredity 94:33–36

Kayser M, Roewer L, Hedman M et al. (2000) Characteristics and frequency of germline mutations at microsatellite loci from the human Y chromosome, as revealed by direct observation in father/son pairs. Am J Hum Gen 66:1580–1588

Launey S, Ledu C, Boudry P, Bonhomme F, Naciri-Graven Y (2002) Geographic structure in the European flat oyster (*Ostrea edulis* L.) as revealed by microsatellite polymorphism. J Hered 93:331–338

Le Goff-Vitry MC, Pybus OG, Rogers AD (2004) Genetic structure of the deep-sea coral *Lophelia pertusa* in the northeast Atlantic revealed by microsatellites and internal transcribed spacers. Mol Ecol 13:537–549

Maier E, Tollrian R, Nürnberger B (2001) Development of species-specific markers in an organism with endosymbionts: microsatellites in the scleractinian coral *Seriatopora hystrix*. Mol Ecol Notes 1:157–159

Mantel N (1967) The detection of desease clustering and a generalized regression approach. Cancer Res 27:209–220

McFadden CS (1997) Contributions of sexual and asexual reproduction to population structure in the clonal soft coral, *Alcyonium rudyi*. Evolution 51:112–126

Miller KJ (1997) Genetic structure of black coral populations in New Zealand's fjords. Mar Ecol Prog Ser 161:123–132

Miller KJ (1998) Short-distance dispersal of black coral larvae: inference from spatial analysis of colony genotypes. Mar Ecol Prog Ser 163:225–233

Miller K, Mundy C (2003) Rapid settlement in broadcast spawning corals: implications for larval dispersal. Coral Reefs 22:99–106

Nishikawa A, Katoh M, Sakai K (2003) Larval settlement rates and gene flow of broadcast-spawning (*Acropora tenuis*) and planula-brooding (*Stylophora pistillata*) corals. Mar Ecol Prog Ser 256:87–97

Paz G, Douek J, Mo C, Goren M, Rinkevich B (2003) Genetic structure of *Botryllus schlosseri* (Tunicata) populations from the Mediterranean coast of Israel. Mar Ecol Prog Ser 250:153–162

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. Mol Ecol 4:249–252

Perez-Losada M, Guerra A, Carvalho GR, Sanjuan A, Shaw PW (2002) Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) around the Iberian Peninsula indicated by microsatellite DNA variation. Heredity 89:417–424

Perrin C, Wing SR, Roy MS (2004) Effects of hydrographic barriers on population genetic structure of the sea star *Coscinasterias muricata* (Echinodermata, Asteroidea) in the New Zealand fjords. Mol Ecol 13:2183–2195

Petersen D, Tollrian R (2001) Methods to enhance sexual recruitment for restoration of damaged reefs. Bull Mar Sci 69:989–1000

Reichow D, Smith MJ (2001) Microsatellites reveal high levels of gene flow among populations of the California squid *Loligo opalescens*. Mol Ecol 10:1101–1109

Richmond RH (1987) Energetics, competency, and long-distance dispersal of planula larvae of the coral *Pocillopora damicornis*. Mar Biol 93:527–533

Ridgway T, Hoegh-Guldberg O, Ayre DJ (2001) Panmixia in *Pocillopora verrucosa* from South Africa. Mar Biol 139:175–181

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219–1228

Russo CAM, Solé-Cava AM, Thorpe JP (1994) Population structure and genetic variation in two tropical sea anemones (Cnidaria, Actinidae) with different reproductive strategies. Mar Biol 119:267–276

Sammarco PW (1982) Polyp bail-out: an escape response to environmental stress and a new means of reproduction in corals. Mar Ecol Prog Ser 10:57–65

Skold M, Wing SR, Mladenov PV (2003) Genetic subdivision of a sea star with high dispersal capability in relation to physical barriers in a fjordic seascape. Mar Ecol Prog Ser 250:163–174

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462

Stoddart JA (1984a) Genetical structure within populations of the coral *Pocillopora damicornis*. Mar Biol 81:19–30

Stoddart JA (1984b) Genetic differentiation amongst populations of the coral *Pocillopora damicornis* off Southwestern Australia. Coral Reefs 3:149–156

Stoddart JA, Taylor JF (1988) Genotypic diversity: estimation and prediction in samples. Genetics 118:705–711

Veron JEN (2000) Corals of the World, vol 2, Australian Institute of Marine Science, Townsville

Viard F, Franck P, Dubois MP, Estoup A, Jarne P (1998) Variation of microsatellite size homoplasy across electromorphs, loci, and populations in three invertebrate species. J Mol Evol 47:42–51

Wahlund S (1928) Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas 11:65–106

Waples RS (1987) A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41:385–400

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{st} \neq 1/(4\ Nm+1)$. Heredity 82:117–125

Wright S (1943) Isolation by distance. Genetics 28:114–138

Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395–420

Wright S (1969) The evolution and genetics of populations, vol 2. The theory of gene frequencies. University of Chicago Press, Chicago, IL

Yu JK, Wang HY, Lee SC, Dai CF (1999) Genetic structure of a scleractinian coral, *Mycedium elephantosus*, in Taiwan. Mar Biol 133:21–28

Yund PO, McCartney MA (1994) Male reproductive success in sessile invertebrates: competition for fertilizations. Ecology 75:2151–2167

PAPER 3

Fine-scale analysis of genetic structure in the brooding coral *Seriatopora hystrix* from the Red Sea

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NOTE

Fine-scale analysis of genetic structure in the brooding coral *Seriatopora hystrix* from the Red Sea

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Abstract The dispersal of gametes and larvae plays a key role in the population dynamics of sessile marine invertebrates. Species with internal fertilisation are often associated with very localised larval dispersal, which may cause small-scale patterns of neutral genetic variation. This study on the brooding coral *Seriatopora hystrix* from the Red Sea focused on the smallest possible scale: Two *S. hystrix* stands (~100 colonies each) near Dahab were completely sampled, mapped and analysed at five microsatellite markers. The sexual mode of reproduction, the likely occurrence of selfing and the level of immigration were in agreement with previous studies on this species. Contrary to previous findings, both stands were in Hardy–Weinberg proportions. Also, no evidence for spatially restricted larval dispersal within the sampled areas was found. Differences between this and previous studies on *S. hystrix* could reflect variation in life history or varying environmental conditions, which opens intriguing questions for future research.

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Introduction

In sessile marine invertebrates, scales of larval dispersal play a fundamental role in determining population dynamics. Small-scale dispersal increases the chance of inbreeding and promotes genetic divergence among populations due to selection and/or genetic drift. Species with internal fertilisation have often been associated with the occurrence of localised larval dispersal and pronounced neutral genetic variation on surprisingly small spatial scales. Few population genetic studies, however, focused on scales of decimetres to metres (e.g., Calderón et al. 2007).

Seriatopora hystrix is a hermaphroditic, brooding coral distributed from the Red Sea to the Western Pacific (Veron 2000). It reproduces largely sexually but can pursue a mixed mating strategy of outcrossing and selfing (Sherman 2008). Large heterozygote deficits within local stands of colonies (Ayre and Dufty 1994; Ayre and Hughes 2000; Underwood et al. 2007; Sherman 2008) and isolation by distance effects on limited spatial scales of ≤20 km (Maier et al. 2005) or even below tens of metres (Underwood et al. 2007) imply very localised larval dispersal. Nevertheless, at least some of the well-provisioned larvae are expected to travel far, and assignment tests have successfully identified immigrants (Underwood et al. 2007). Taken together, these findings suggest that the population genetic structure of *S. hystrix* is shaped by processes that act on a broad range of spatial scales. Identifying these processes should shed light on a coral life history and its variability across a vast distribution range. Previous studies on *S. hystrix* focused on scales from tens of metres to hundreds of kilometres.

The present study was intended as a first step to understand fine-scale patterns of genetic structure on scales from decimetres to metres.

Two *S. hystrix* stands from the Red Sea (~100 colonies each) were analysed using five microsatellites in order to (1) determine whether these sites are internally structured, and (2) examine the role of four processes that could cause such structure, i.e., immigration, clonal reproduction, selfing and small-scale larval dispersal.

Materials and methods

Sample collection

Seriatopora hystrix was sampled in 2002 from two locations in the Dahab region, Egypt, separated by ~8.0 km (Fig. 1). House Reef 2 (*HR2*, 28°29′24.4″ N, 34°30′58.1″ E) and Golden Blocks (*GB*, 28°26′18.9″ N, 34°27′46.6″ E) were situated on the reef slope at 21 and 13 m depth, respectively. The sampled areas were ~600 m^2 (*HR2*) and ~200 m^2 (*GB*) in size. From all colonies, branch tips of approximately 1 cm length were collected (*HR2*: $n = 107$; *GB*: $n = 110$) and the position of each colony was mapped via *x*- and *y*-coordinates. DNA was preserved as described in Maier et al. (2005).

Genotyping

DNA was extracted following Maier et al. (2001). Genotypes were scored at five species-specific microsatellite loci: Sh2-002, Sh3-004 and Sh4-001 (Underwood et al. 2006, 2007) as well as Sh2.15 and Sh4.24 (Maier et al. 2005). Allelic variation was analysed on a MegaBACE 1000, scored with MegaBACE Genetic Profiler v2.2 (GE Healthcare) and checked manually.

Statistical analysis

Allele frequencies, observed (H_o) and expected (H_e) heterozygosities as well as F_{IS} (*f*, Weir and Cockerham 1984) were calculated in *FSTAT* version 2.9.3 (Goudet 2001) and GENEPOP web version 3.4 (Raymond and Rousset 1995). Tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibria were performed in *FSTAT*, based on 1,000 and 600 permutations, respectively. *FSTAT* was also used to calculate F_{ST} (θ, Weir and Cockerham 1984) and R_{ST} (ρ, Goodman 1997). Significance of F_{ST} was estimated using Fisher's exact test in GENEPOP (1,000 dememorizations, 100 batches, 1,000 iterations per batch). The software FREENA (Chapuis and Estoup 2007) was used to check the robustness of results in the light of potential null alleles.

In order to identify immigrants, the population exclusion method implemented in GeneClass2 (Piry et al. 2004) was applied. For each colony, the likelihood that it originated within its sampling site was calculated using the partial Bayesian criterion of Rannala and Mountain (1997) and compared to the distribution of likelihoods of 10^4 simulated genotypes, generated using a Monte Carlo algorithm (Paetkau et al. 2004). In order to compensate for the limited power of this data set to identify migrants (unknown source populations, small number of loci), the low stringency criterion of $\alpha = 0.05$ (95% exclusion probability) was applied. From the resulting estimate of *Nm*, the expected Type I error ($N^*\alpha$) was subtracted to obtain a reliable minimum, as suggested by Paetkau et al. (2004).

As a descriptive statistic, the ratio of the observed number of distinct genotypes (N_g) over the sample size (N) was computed. In order to test for asexual reproduction, the probability of finding the observed number of identical multilocus genotypes (MLG) under the null hypothesis of purely sexual, biparental reproduction was assessed using a simulation approach described in Calderón et al. (2007),

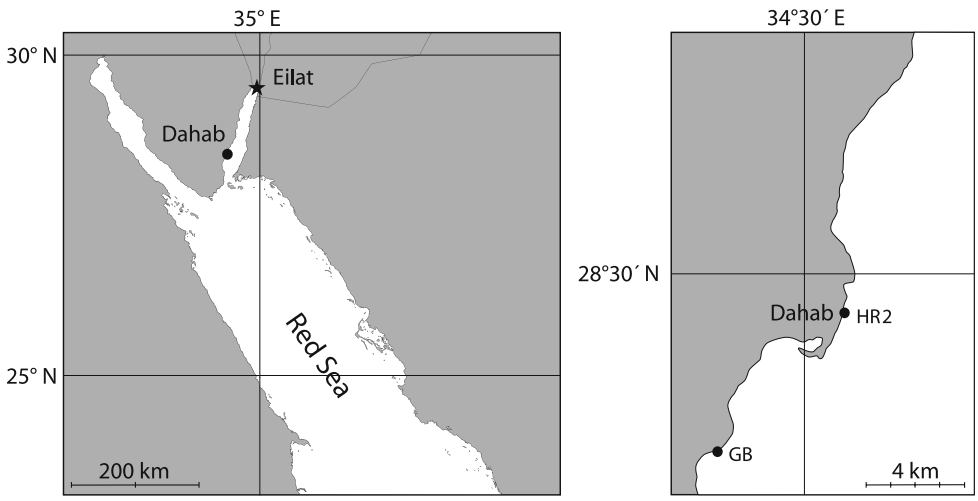

Fig. 1 Location of *Seriatopora hystrix* sampling sites, House Reef 2 (*HR2*) and Golden Blocks (*GB*)

based on 100,000 replicates, keeping sample size, allele frequencies and heterozygosities as observed.

In order to test whether colonies were more closely related than expected under panmixia, pairwise relatedness (R, Queller and Goodnight 1989) was calculated in IDENTIX (Belkhir et al. 2002). The mean and variance of R were compared to the expected distribution under the null hypothesis of panmixia, based on 1,000 permutations of single-locus genotypes. Autocorrelation analyses in SPAGEDI version 1.2 (Hardy and Vekemans 2002) were used to test whether R decreased with increasing distance. In order to avoid bias from potential clonal replicates, identical MLG were removed from analyses of relatedness (randomly keeping one per group).

Results and discussion

A total of 217 *S. hystrix* colonies were analysed (Table 1). Tests for linkage disequilibria were consistent with independent segregation of marker loci. The null-corrected data set generated almost identical differentiation estimates (F_{ST}: 0.0346 vs. 0.0349) and supported all of the conclusions of this study. The mean deviations from HWE per site were small ($F_{IS} = 0.034$ and 0.041, Table 1) and non-significant. This is in contrast to all previous studies on *S. hystrix* which reported large heterozygote deficits based on allozymes (Ayre and Dufty 1994; Ayre and Hughes 2000; Sherman 2008) and microsatellites (Maier et al. 2005; Underwood et al. 2007). These were attributed to factors such as restricted dispersal of sperm and larvae, inbreeding (including selfing) and Wahlund effects. In contrast, the present findings were consistent with free mixing of gametes and larvae within the sampled areas. In the only other study on *S. hystrix* from the Red Sea (Maier et al. 2005), null alleles presumably contributed to the observed large heterozygote deficits. Thus, it is presently unknown whether (approximate) Hardy–Weinberg proportions are common in this region.

Assignment tests in GeneClass2 identified 13 (*HR2*) and 16 (*GB*) colonies as immigrants. After subtracting the expected Type I error (cf. Materials and Methods), the minimum estimate of *Nm* was seven (6.5%) for *HR2* and 10 (9.1%) for *GB*. The true values may well be twice as large as the inferred minimum (Paetkau et al. 2004). Note that between one and five *S. hystrix* colonies per site were identified as immigrants (6% overall, uncorrected, $P \leq 0.05$) at the Scotts Reef in northern Western Australia (Underwood et al. 2007). Especially given the reduced power of immigrant detection in this study (five loci vs. eight used by Underwood et al. 2007), the comparison implies a relatively greater influx of immigrants into *HR2* and *GB*. This may be due to more numerous and larger source populations along the Dahab coast. The small Scott Reef is in a remote location 270 km from the mainland, and most of its *S. hystrix* populations had not yet recovered from a catastrophic bleaching event six years prior to genetic sampling. Moreover, the comparatively simpler shape of the Dahab shoreline may facilitate the transport of larvae through coastal waters (Johnson and Black 2006).

While the source populations of the immigrants remain unknown, data from a previous study help to determine plausible minimum dispersal distances. For seven sites in the Dahab region (max. distance between sites: 20 km),

Table 1 Genetic variation at five microsatellite loci in *Seriatopora hystrix*

	Locus					Overall
	Sh2-002	Sh3-004	Sh4-001	Sh2.15	Sh4.24	
HR2 ($n = 107$)						
N_A	5	4	9	14	31	
N_{PA}	1	0	3	3	11	
H_o	0.514	0.514	0.570	0.757	0.858	
H_e	0.507	0.497	0.605	0.805	0.911	
F_{IS}	−0.015	−0.033	0.058	0.060	0.058	0.034
GB ($n = 110$)						
N_A	5	5	9	16	31	
N_{PA}	1	1	3	5	11	
H_o	0.509	0.361	0.536	0.806	0.798	
H_e	0.446	0.359	0.555	0.834	0.945	
F_{IS}	−0.142	−0.007	0.033	0.034	0.155*	0.041
Total						
N_A	6	5	12	19	42	
Repeat motiv[a]	$(CA)_{18}$	$(AAC)_{12}$	$(CAAT)_{10}$	$(CA)_{18}$	$(CA)_{37}$	
Allele size (bp)	128–155	154–166	130–160	215–331	178–298	

n Sample sizes, N_A number of alleles, N_{PA} number of private alleles, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} inbreeding coefficient (Weir and Cockerham 1984)

Significant values were determined after sequential Bonferroni corrections

* Indicates significance at a nominal α-level of 0.05, $P \leq 0.0042$

[a] Number of repeats are given for the cloned allele

Table 2 Estimates of F_{ST} (θ. Weir and Cockerham 1984) and R_{ST} (ρ, normalised across loci according to Goodman 1997)

Locus	F_{ST}	R_{ST}
Sh2-002	0.015	−0.003
Sh3-004	0.108	0.311
Sh4-001	0.012	0.031
Sh2.15	0.027	−0.004
Sh4.24	0.029	0.167
Total	0.035* (0.012)	0.109

Standard error (s.e.) for θ is based on jackknifing over loci

* $P < 0.0001$ according to Fisher's exact test in GENEPOP. No test for significance was carried out on R_{ST}

Maier et al. (2005) found a mean F_{ST} of 0.094. Eight out of 21 pairwise comparisons yielded F_{ST} estimates greater than 0.1 (min. pairwise distance within this subset: 6.3 km). At that level of differentiation, the power of assignment tests to detect immigrants approaches one for the set of markers used here (Paetkau et al. 2004). Immigrants that are detectable by the present approach may therefore have originated within the Dahab region. However, it is unlikely that any exchange of migrants between *HR2* and *GB* would have been picked up: differentiation between both sites was low if highly significant ($F_{ST} = 0.035$, $P < 0.0001$). The corresponding R_{ST} value was 0.109 (Table 2). While the inferred immigrants appear to be randomly located within site *HR2*, those at site *GB* lie mostly on a line that runs diagonally through the study site (Fig. 2). This curious pattern is presently unexplained. It does not match any features of the substrate such as a ridge or a trough.

At *HR2*, no repetition of MLG occurred ($N_g/N = 1$). At *GB*, five groups of colonies with identical MLG were found, each group consisting of two colonies. No identical MLG were shared between both sites. Thus, genotypic structure at *HR2* was consistent with purely sexual, biparental reproduction. Under that assumption, the observed repetition of MLG at *GB* was highly improbable ($P < 10^{-5}$), but can be explained by selfing (see below). Members of a given putative clone did not occupy closely neighbouring locations but were scattered across the study site (Fig. 2b). In agreement with all previous studies on *S. hystrix* (Ayre and Resing 1986; Ayre and Dufty 1994; Ayre and Hughes 2000; Maier et al. 2005; Underwood et al. 2007; Sherman 2008), the analysis shows that reproduction was largely if not entirely sexual.

Neither mean nor variance of pairwise relatedness (R) was significantly higher than expected under panmixia at *HR2* (Table 3). At *GB*, the mean of R was consistent with panmixia whereas the variance of R was significantly increased. The latter could be due to immigrants that are only distantly related to local colonies. However, removal of presumptive immigrants did not change the results

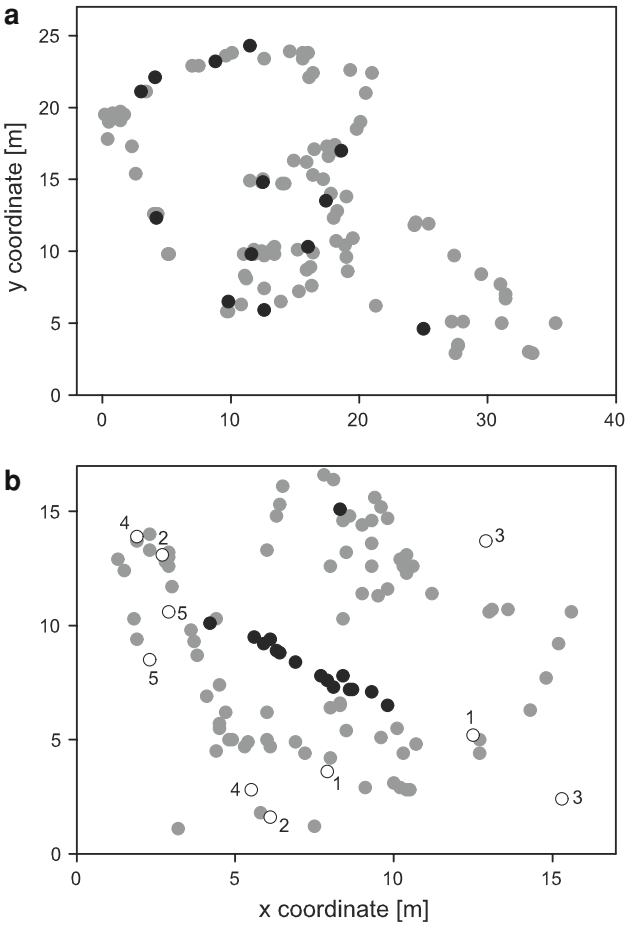

Fig. 2 Location of colonies within the sampled areas, **a** *HR2* and **b** *GB*. Inferred immigrants are marked with black symbols whereas putative clonal replicates (*open symbols*) are denoted with identical numbers. Note that each set of immigrants contains more colonies than the estimated minimum number referred to in the text

Table 3 Mean (R_{mean}) and variance (R_{var}) of pairwise relatedness (R, Queller and Goodnight 1989)

	All colonies		Without immigrants	
	R_{mean}	R_{var}	R_{mean}	R_{var}
Site				
HR2	−0.006	0.071	−0.007	0.075
GB	−0.013	0.071*	−0.019	0.077*

* Indicates significance at a nominal α-level of 0.05, $P \leq 0.013$

(Table 3). In fact, the distribution was skewed to the right, i.e., there was an excess of high relatedness values. This suggests selfing as an alternative explanation for the increased variance. A simulation of selfing among observed *GB* genotypes showed that the largest observed R values ($R > 0.5$) may represent pairs of selfed offspring and their parent. All of these observations also hold for *HR2*, albeit with no greater than expected variance in

R. However, the small F_{IS} estimates suggest selfing rates (*S*) under 0.1 (assuming equilibrium conditions with each individual producing a proportion *S* of selfed offspring, Pollak 1987). In contrast, Sherman (2008) inferred on average almost equal levels of outcrossing and selfing at One Tree Island (GBR) based on the direct comparison of adult and offspring genotypes.

Autocorrelation analyses did not reveal any association between pairwise relatedness and spatial distance. At each site, none of the correlation coefficients was significant, while sample sizes were large (min. $N = 225$ pairs in the seven smallest distance classes). An existing spatial autocorrelation may be obscured by (a) thinning processes after recruitment that weaken allelic aggregations, (b) overlapping dispersal shadows of different maternal colonies that reduce the relatedness among adjacent colonies (reviewed in Asuka et al. 2005) or (c) the random settlement of immigrants within a site. The latter point can be ruled out, as the same results were obtained after removal of presumptive immigrants. As it stands, the data are consistent with unrestricted larval dispersal (cf. Underwood et al. 2007) and random mating within sites, as shown by this analysis and the prevailing Hardy–Weinberg proportions. Thus, in *S. hystrix*, the smallest documented scale for spatially restricted dispersal in the Dahab area is 20 km (Maier et al. 2005).

Approximate panmixia as reported here is the exception rather than the rule in *S. hystrix* populations and, more generally, in marine invertebrates. The examination of four underlying processes revealed both differences and similarities in comparison with previous studies on *S. hystrix*. While there was no detectable tendency of larvae to settle near their parents, such a tendency may still exist yet be obscured by external forces such as water currents. These may also generate well-mixed samples of immigrants from various sites that appear to arrive in a steady stream without causing admixture effects. In keeping with previous studies, asexual reproduction played at most a marginal role in either population. Finally, the data hint at a mixed mating strategy of selfing and outcrossing but with a much lower selfing rate than was found elsewhere (Sherman 2008).

Among the potential causes for the divergent genetic structures of *S. hystrix* populations, there are a few on which we can comment here. Approximate Hardy–Weinberg proportions are not restricted to the Red Sea but have also been reported for several populations on the Great Barrier Reef (Ayre and Dufty 1994; van Oppen et al. 2008). The present study contradicts the notion that panmixia in *S. hystrix* is only found in lagoon habitats (Ayre and Dufty 1994; van Oppen et al. 2008). Instead, *HR2* and *GB* are situated at the reef slope. Both sites may have been populated for some time so that any admixture effects in the colonising cohort would have been eroded by subsequent random mating. If population turnover is generally common in *S. hystrix*, then 'mature' populations will simply not be sampled very often. Life history differences among populations, e.g., with regard to selfing rates, would not be unexpected. Yet, the spatial scale of such variation is presently unknown. Apportioning the observed population differences to variation in either life history or abiotic forces presents a challenge for future studies.

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References

Asuka Y, Tomaru N, Munehara Y, Tani N, Tsumura Y, Yamamoto S (2005) Half-sib family structure of *Fagus crenata* saplings in an old-growth beech-dwarf bamboo forest. Mol Ecol 14:2565–2575

Ayre DJ, Dufty S (1994) Evidence for restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia's Great Barrier Reef. Evolution 48:1183–1201

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54:1590–1605

Ayre DJ, Resing JM (1986) Sexual and asexual production of planulae in reef corals. Mar Biol 90:187–190

Belkhir K, Castric V, Bonhomme F (2002) IDENTIX, a software to test for relatedness in a population using permutation methods. Mol Ecol Notes 2:611–614

Calderón I, Ortega N, Duran S, Becerro M, Pascual M, Turon X (2007) Finding the relevant scale: clonality and genetic structure in a marine invertebrate (*Crambe crambe*, Porifera). Mol Ecol 16:1799–1810

Chapuis M-P, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24: 621–631

Goodman SJ (1997) R_{ST} calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining of their significance. Mol Ecol 6: 881–885

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2), http://www2.unil.ch/popgen/softwares/fstat.htm

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618–620

Johnson MS, Black R (2006) Islands increase genetic subdivision and disrupt patterns of connectivity of intertidal snails in a complex archipelago. Evolution 60:2498–2506

Maier E, Tollrian R, Nürnberger B (2001) Development of species-specific markers in an organism with endosymbionts: microsatellites in the scleractinian coral *Seriatopora hystrix*. Mol Ecol Notes 1:157–159

Maier E, Tollrian R, Rinkevich B, Nürnberger B (2005) Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea. Mar Biol 147:1109–1120

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a

simulation-based exploration of accuracy and power. Mol Ecol 13:55–65

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A (2004) GeneClass2: a software for genetic assignment and first-generation migrant detection. J Hered 95:536–539

Pollak E (1987) On the theory of partially inbreeding finite populations. I Partial selfing. Genetics 117:353–360

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci USA 94:9197–9201

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Sherman CDH (2008) Mating system variation in the hermaphroditic brooding coral, *Seriatopora hystrix*. Heredity 100:296–303

Underwood JN, Souter PB, Ballment ER, Lutz AH, van Oppen MJH (2006) Development of 10 polymorphic microsatellite markers from herbicide-bleached tissues of the brooding pocilloporid coral *Seriatopora hystrix*. Mol Ecol Notes 6:176–178

Underwood JN, Smith LD, van Oppen MJH, Gilmour JP (2007) Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. Mol Ecol 16: 771–784

van Oppen MJH, Lutz A, De'ath G, Peplow L, Kininmonth S (2008) Genetic traces of recent long-distance dispersal in a predominantly self-recruiting coral. PLoS ONE 3(10):e3401. doi: 10.1371/journal.pone.0003401

Veron JEN (2000) Corals of the world, vol 2. Australian Institute of Marine Science, Townsville

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370

PAPER 4

Intracolonial genetic variation in the scleractinian coral *Seriatopora hystrix*

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Intracolonial genetic variation in the scleractinian coral *Seriatopora hystrix*

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Running title: Intracolonial genetic variation in a coral

Abstract

In recent years, increasing numbers of studies revealed intraorganismal genetic variation, primarily in modular organisms like plants or colonial marine invertebrates. Two underlying mechanisms are distinguished: Mosaicism is caused by somatic mutation whereas chimerism originates from allogeneic fusion. While the evolutionary impact of mosaicism and chimerism is still being debated, it is clear that the presence of various genotypes within a single organism needs to be taken into consideration for any population genetic study based on the identification of individual multilocus genotypes. We investigated the occurrence of intracolonial genetic variation at microsatellite loci in five natural populations of the scleractinian coral *Seriatopora hystrix* on the Great Barrier Reef. This coral is a widely distributed, brooding species which is at present a target of intensive population genetic research on reproduction and dispersal patterns. From each of 155 *S. hystrix* colonies, either two or three samples were genotyped at five or six loci. Twenty-seven (~17%) genetically heterogeneous colonies were found. Statistical analyses indicated the occurrence of both mosaicism and chimerism. In most cases, intracolonial variation was found only at a single allele. Our analyses suggest that somatic mutations present the major source of genetic heterogeneity within a single colony. As we detected a surprisingly high rate of intracolonial genetic variation in *S. hystrix*, our findings should be relevant for any population genetic study in corals that employs individual-based analytical approaches.

Introduction

Traditionally, intraorganismal genetic variation has been regarded merely as an exception (Santelices 2004), yet increasing numbers of studies challenge this view (reviewed in Pineda-Krch & Lehtilä 2004). Two underlying mechanisms are distinguished: Mosaicism refers to genetic variation caused by intrinsic genetic change, basically somatic mutations. It may be widespread especially in large and long-lived organisms such as plants and corals where somatic mutations can accumulate over time (Gill *et al*. 1995). Chimerism is caused by extrinsic variation due to fusion of genetically distinct (allogeneic) entities. It has mainly been described in modular organisms characterised by low levels of terminal differentiation, plastic development and long life spans (reviewed in Pineda-Krch & Lehtilä 2004). Examples come from plants and various sessile marine invertebrates such as sponges (Maldonado 1998), bryozoans (Hughes *et al*. 2004), hydroids (Shenk & Buss 1991; Hart & Grosberg 1999), ascidians (Sommerfeldt & Bishop 1999; Ben-Shlomo *et al*. 2001; Sommerfeldt *et al*. 2003) and corals (Frank *et al*. 1997; Barki *et al*. 2002; Nozawa & Loya 2005; Amar *et al*. 2008; Puill-Stephan *et al*. 2009). While mosaicism and chimerism are likely to be more common than previously believed, few studies have focused on intraorganismal genetic variation in nature (Sommerfeld & Bishop 1999; Sommerfeldt *et al*. 2003; Puill-Stephan *et al*. 2009).

Scleractinian corals are a group of sessile marine invertebrates where intraorganismal genetic variation should be frequent. Especially with regard to the longevity of some corals, somatic mutations can accumulate during growth and lead to a significant degree of genetic variation within a single colony (Hughes *et al*. 1992). As corals lack a discrete germ line, somatic mutations may be represented in the gametes and spread during sexual reproduction (Buss 1987). Alternatively, they can be passed to independent clonal offspring. As a result, somatic mutations may not only increase variation within a colony but also add to ge-

netic variation at the population level (Orive 2001). Despite its importance, very little is known about mosaicism in natural coral populations.

Corals often settle and grow in close proximity, which promotes tissue contact, fusion and the formation of chimeras (Rinkevich & Loya 1983; Amar *et al*. 2008). Chimeras have been reported from various laboratory studies, either due to fusion of genetically different fragments (e.g. Resing & Ayre 1985; Willis & Ayre 1985; Frank *et al*. 1997) or newly settled juveniles (e.g. Hidaka 1985; Hidaka *et al*. 1997; Amar *et al*. 2008). Evidence for chimerism in natural coral populations was found in a recent study on *Acropora millepora* (Puill-Stephan *et al*. 2009). Possibly, chimeras may benefit from increased genetic variability, rapid size increase and facilitation of mating. On the other hand, they may suffer from cell parasitism, competitive interactions and developmental instability (e.g. Buss 1982; Rinkevich & Weissman 1987; Pineda-Krch & Lehtilä 2004; Amar *et al*. 2008). Several studies suggested that the chimeric state may be unstable over longer periods of time due to internal conflicts (e.g. Rinkevich & Loya 1983; Barki *et al*. 2002; Nozawa & Loya 2005; Amar *et al*. 2008), yet chimerism presents a potential source of intracolonial genetic variation in corals.

Population genetic studies in corals are at present a focus of marine research, not least because coral reefs are worldwide under severe threat (Hughes *et al*. 2003; Bellwood *et al*. 2004). Consequently, knowledge about reef connectivity has become a central issue for conservation (van Oppen & Gates 2006). Especially microsatellite markers (e.g. Magalon *et al*. 2004; Baums *et al*. 2005; van Oppen *et al*. 2007) together with sophisticated statistical methods, based on maximum likelihood, Bayesian probability theory and Markov chain Monte Carlo simulation (summarised in Excoffier & Heckel 2006) promise to reveal detailed insight into population genetic processes in this highly diverse group. Many of these methods rely on the identification of individual genotypes and usually, researchers treat single colonies as genetic entities, which will be misleading if intracolonial genetic variation is common. Therefore, mosaicism and/or chimerism should be considered as a source of error in popula-

tion genetic studies. This is especially true for microsatellites where mutation rates between 10^{-6} and 10^{-2} per locus and generation were inferred (Schlötterer 2000).

The present study focuses on intracolonial genetic variation at microsatellite loci in the scleractinian coral *Seriatopora hystrix* and formed part of a larger investigation on reproduction and dispersal in this common brooding species. *S. hystrix* (Fam. Pocilloporidae) is widely distributed in Indo-Pacific reefs, shows high growth rates and forms delicately branched colonies (Veron 2000). In recent years, a number of species-specific microsatellites were developed (Maier *et al*. 2001, 2005; Underwood *et al*. 2006) and successfully applied to study larval dispersal patterns on different spatial scales (Maier *et al*. 2005, 2009; Underwood *et al*. 2007, 2009; van Oppen *et al.* 2008; Noreen *et al*. 2009). Given that these and current surveys include individual-based analyses, the specific aim of our study was to 1) assess the level of within-colony heterogeneity in natural populations of *S. hystrix* with respect to population genetic studies, 2) distinguish between the two underlying mechanisms, mosaicism versus chimerism and 3) test the hypothesis that the proportion of heterogeneous colonies should increase with colony size due to the accumulation of somatic mutations. Based on a number of statistical approaches, the study provides evidence for both, mosaicism and chimerism. It shows that intracolonial genetic variation is an important aspect of coral population genetics that has been largely neglected to date.

Materials and Methods

Sampling and preservation of DNA

Samples of *Seriatopora hystrix* were collected in November and December 2002 from five different sampling sites on the Great Barrier Reef (GBR). Palfrey Island (*PAL1*) and Horseshoe Reef (*HS*) were situated at Lizard Island (14°40´S, 145°28´E, northern GBR), Staghorn

Bank (*SB*), Canyons (*CA*) and Harry's Bommie (*HB*) were located at Heron Island (23°27´S, 151°55´E, southern GBR). Depths of sampling sites ranged from 3 m to 17 m. One hundred fifty-five colonies were subjected to multiple sampling (two or three samples/colony), with each of 130 colonies sampled at two positions and each of 25 colonies sampled at three positions. Additional colonies at each site were sampled once. The total numbers of sampled colonies were 106 (*PAL1*), 120 (*HS*), 125 (*SB*), 108 (*CA*) and 106 (*HB*). In a companion study, we used these genotype data to infer the mechanisms that govern the observed population substructure (Maier *et al*., ms).

To standardise (and presumably maximise) the probability of detecting different genotypes within a colony, two-fold samples were taken from opposing sites of the upper half of the colony, while three-fold samples were taken from opposing sites and approximately evenly spaced angles, i.e. 120° steps. Sampling included colonies of different sizes, with maximum diameters between 5 and 60 cm. The diameter of each colony was measured to the nearest 5 cm interval. Samples consisted of branch tips, approximately 1 cm in length, which were preserved as previously described (Maier *et al*. 2005). DNA was extracted and stored according to Maier *et al*. (2001).

Molecular analyses

All samples were genotyped at five or six microsatellite loci. Sh2-002, Sh2-006, Sh3-004, Sh3-007 and Sh4-001 were developed by Underwood *et al*. (2006) and previously used in *S. hystrix* from Australia (Underwood *et al*. 2007; van Oppen *et al*. 2008; Noreen *et al*. 2009). Locus Sh4.24 was originally employed in *S. hystrix* from the Red Sea (Maier *et al*. 2005). With GBR samples, Sh4.24 did not reliably amplify, thus primers were redesigned. The sequences of the new forward and reverse primers were 5'-CCTAACAAAAGGACTGATTGGC-3' (Sh4.24A3) and 5'-TTGAACATCTGGTTTGAAATG-3' (Sh4.24B3). We obtained reliable genotypes for the Heron Island sites, *HB*, *CA* and *SB*. For the Lizard Island sites, *HS* and *PAL1*, even the new

primers yielded weak or unscorable PCR products, thus locus Sh4.24 was dropped from the analysis of these sites. Consequently, the data sets for *HB*, *CA* and *SB* comprised six loci while the data for *HS* and *PAL1* were based on five loci. Details on the amplification procedure were published elsewhere (Maier *et al*. 2005; Underwood *et al*. 2006). Allelic variation was analysed on a MegaBACE 1000 automated sequencer (GE Healthcare) relative to an internal size standard (ET 400-R). Alleles were scored according to their length in base pairs using the software MegaBACE GENETIC PROFILER 2.2 (Amersham Biosciences) and checked manually. Samples which indicated the presence of within-colony variation were reamplified and rescored from the same DNA extraction to exclude PCR artefacts. Missing alleles due to PCR failure or unscorable banding patterns were marked as zero. Statistical analyses were performed in a conservative way to minimise errors due to missing data.

Statistical analyses

Mosaicism

Intracolonial genetic variation due to somatic mutation should be discernible if genetic variation within the same colony is significantly smaller than variation between colonies at the same sampling site. Based on this assumption, we applied an approach modified from Hart & Grosberg (1999) to identify specific colonies that were heterogeneous due to somatic mutations. It is based on a similarity index (*SI*) defined as the number of shared alleles for a pair of multilocus genotypes. First, we created a reference population for each sampling site, including all colonies. From multiply sampled colonies, we randomly chose one sample for the reference population. To account for allelic dropouts, incomplete or missing single-locus genotypes in each reference population were randomly replaced according to genotypic frequencies at this locus. This 'fill-in' procedure is preferable to interpolation, as the latter does not account for differences in allelic variation among loci. It also preserves the genotypic structure for that locus. A locus was omitted from the analysis at a given sampling site if its proportion of missing genotypes was larger than 30%. This procedure is conservative, as it

tends to lower genotypic differences among colonies. Second, within each heterogeneous colony, we computed similarity indices $SI_{C\text{-}C}$ for all possible pairs of distinct samples (i.e. one $SI_{C\text{-}C}$ for two-fold sampled colonies and at most three $SI_{C\text{-}C}$ for three-fold sampled colonies). Third, for each sample from a heterogeneous pair, we computed the similarity indices $SI_{C\text{-}R}$ between this sample and every colony of the reference population and from these the mean similarity $SI_{C\text{-}R,mean}$ and its standard deviation. If a within-colony pairwise comparison involved missing data at a given locus, this locus was omitted from calculations of both $SI_{C\text{-}C}$ and $SI_{C\text{-}R}$. For each sample of a heterogeneous pair we compared $SI_{C\text{-}C}$ to $SI_{C\text{-}R,mean}$ using an adjusted two-sample *t*-test for single observations (Sokal & Rohlf 1994). To assess significance of similarity, we chose the test with the larger *P*-value for a conservative estimate (i.e. we chose the sample of a pair that was most similar to the reference population). The significance level was set to 0.05. Finally, significance levels were adjusted using the sequential Bonferroni approach [Rice 1989, but see Moran (2003) for a critical view on Bonferroni corrections]. For all putative mosaics, we tabulated single-step mutations and mutations of larger sizes.

To assess the overall probability of somatic mutation we focused on single-allele differences between multilocus genotypes within heterogeneous colonies compared to allelic differences between colonies at the same site. Since mutations are rare events, they should generate few allelic differences within colonies when only five or six loci are used, whereas a greater number of allelic differences is expected between two randomly chosen colonies which resulted from sexual reproduction.

We computed the similarity indices $SI_{R\text{-}R}$ for all possible pairs of the reference population and counted the number of pairs that differed at a single allele. The frequency of this number divided by the total number of pairs (omitting identical pairs) gives the probability *p* that two randomly chosen, genetically distinct samples differ at a single allele. Approximating the hypergeometric by the binomial distribution, we expect in *n* randomly chosen pairs *np* pairs with a single-allele difference, given that the pairs differ at all. We analysed the data in two different ways. Missing data in samples from heterogeneous colonies were either in-

serted in such a way as to minimise the number of single-allele differences or, alternatively, the locus in question was dropped. At each site, we compared the observed frequency of single-allele differences within heterogeneous colonies to its expected frequency calculated from *p* using a binomial test and sequential Bonferroni correction. The *P*-value is the probability to get at least the observed number of single-allele variations in *n* chosen pairs.

Chimerism

We applied Bayesian clustering analyses using the program TESS (François *et al*. 2006; Chen *et al*. 2007) as a conservative approach to identify chimeras. Samples from the same colony that were assigned to different genetic clusters were taken as evidence for chimerism. Three of the five sites, *SB*, *CA* and *HB*, showed pronounced genetic substructure (see below), which provided high statistical power to detect at least a subset of colonies that resulted from allogeneic fusion. TESS seeks population structure from individual multilocus genotypes and groups individuals without assuming predefined populations. We based our inference of chimerism only on samples that were assigned to a specific cluster with a high membership coefficient $q \geq 0.9$. The input dataset included multilocus genotypes of all colonies sampled at one position as well as distinct multilocus genotypes from each heterogeneous colony. The 'Generate Spatial Coordinates' option in TESS was used to generate individual random spatial coordinates for each colony, based on sampling site coordinates. TESS was run using the MCMC algorithm (burn-in 10000 sweeps, running period 50000 sweeps) under the default options (no admixture, no F-model, interaction parameter of HMRF = 0.6). To infer the number of genetic groups, or clusters (*K*), we started with $K = 2$ and increased *K* until the estimated number of clusters stabilised. Once the number of clusters was inferred, 200 runs were performed with the predetermined *K*. After choosing 20% of runs with the highest likelihoods, the software *CLUMPP* (Jakobsson & Rosenberg 2007) was employed to calculate averages over these runs using the LargeKGreedy algorithm, with GREEDY_OPTION = 2 and 1000 random input orders of the runs.

Level of heterogeneity

If a mutation arises somewhere at a branch, it may be transmitted to descendant cells of this branch during growth. Assuming selective neutrality, the mutant branch is expected to grow like any other branch and its newly formed tips will occupy a constant part of the growing sphere with respect to the total sphere. Taking a single sample from a heterogeneous colony with primal genotype A, any different genotype B (mutant or chimeric partner) will be obtained with probability *s*. Thus, *s* is the proportion of the colony sphere that is occupied by any genotype different from genotype A.

Heterogeneity within a colony will be detected from two samples with probability $r = 2s$. Correspondingly, $r = 3s$ for three-fold sampled heterogeneous colonies. Factor 2 (3) arises because samples are taken from two (three) opposite positions each of which may be genotype B.

Heterogeneity depending on colony size

To test whether the proportion of heterogeneous colonies increased with colony size, *S. hystrix* colonies were grouped into three size classes, based on maximum colony diameter ($d \leq 15$ cm, $15 < d \leq 30$ cm, $d > 30$ cm). Sampling effort per colony was standardised by randomly excluding one sample from all three-fold sampled heterogeneous colonies. To test for differences between size classes, a G-test (Sokal & Rohlf 1994) was applied.

Results

The level of genetic variation of the six loci is best illustrated with data from the total sample of 565 *S. hystrix* colonies from the five study sites. Between five (Sh3-007) and 32 (Sh4.24) different alleles were found per locus (Sh2-006 and Sh3-004: 10 alleles, Sh4-001: 13 alleles,

Sh2-002: 18 alleles) and expected heterozygosities per locus across the five sites were 0.74 (Sh2-002), 0.62 (Sh2-006), 0.66 (Sh3-004), 0.38 (Sh3-007), 0.52 (Sh4-001) and 0.87 (Sh4.24; Maier *et al*., ms). Among the 155 multiply sampled colonies, intracolonial variation was detected in 27 colonies, yielding a total of 184 different multilocus genotypes. In two out of eight three-fold sampled heterogeneous colonies, three different genotypes were detected. Most heterogeneous colonies (N = 18) varied at a single locus and mostly at a single allele (N = 16). In nine heterogeneous colonies, variation occurred at several loci.

Mosaicism

The distribution of similarity indices in the reference populations ($SI_{R\text{-}R}$) for the five *S. hystrix* sampling sites is given in Figure 1. At *PAL1* and *HS* the distribution of $SI_{R\text{-}R}$ was unimodal, consistent with low levels of genetic substructure. At *SB* and *CA* the distribution of $SI_{R\text{-}R}$ was bimodal, indicating pronounced substructure. At *HB*, the distribution of $SI_{R\text{-}R}$ was unimodal, too, but with a considerably smaller mode compared to *CA* and *SB*. This suggests the presence of several distinct genetic groups. These observations agree with the population substructure revealed by the TESS analysis (see below) and with the observed heterozygote deficits ($F_{IS} \approx 0.18$ for *PAL1* and *HS*, $F_{IS} > 0.3$ for *SB*, *CA* and *HB*, Maier *et al*., ms). Similarity indices ($SI_{C\text{-}C}$) varied between 0.42 and 0.92 and $SI_{C\text{-}R,mean}$ ranged from 0.27 to 0.76 (Table 1). In three colonies, *SB-55, CA-23* and *HB-70*, $SI_{C\text{-}C}$ were significantly larger compared to the mean of $SI_{C\text{-}R,mean}$ after sequential Bonferroni correction, indicating somatic mutation. In all three cases, within-colony variation involved only one allele at a single locus. For all other genetically heterogeneous colonies (N = 24), $SI_{C\text{-}C}$ did not differ significantly from $SI_{C\text{-}R,mean}$. Note, however, that without Bonferroni correction, nine out of 27 colonies (33%) would be attributed to somatic mutation.

The probability p of randomly choosing two samples from the reference population that differ at a single allele ranged from 0.003 to 0.011 (Table 2). For sites *PAL1*, *HB* and *SB*, the number of multiply sampled colonies with a single-allele difference was significantly lar-

ger than in randomly chosen pairs from the reference population, indicating somatic mutations. This was true if the numbers of single-allele differences were conservatively estimated for incomplete genotypes (Table 3*a*) and also when loci with missing data were omitted (Table 3*b*). *HS* and *CA* gave significant results in the latter case.

In the three colonies that were classified as mosaics, the mutant allele differed from its progenitor in length by one (*HB-70*) or two (*SB-55, CA-23*) repeat unit(s).

Chimerism

Clustering analyses with TESS indicated the presence of ten clusters (data not shown). Sites *PAL1* and *HS* at Lizard Island were each genetically homogeneous, with most samples (98%) not clearly assigned to any cluster. Our analysis therefore concentrated on the remaining three sites, *SB*, *CA* and *HB* at Heron Island, which were highly substructured such that samples from the same site clearly fell into different genetic clusters ($q \geq 0.9$). At the within-colony level, samples were mostly assigned to the same cluster with similar or even identical values of q (Table 4). This was true for all colonies considered as genetic mosaics, i.e. *SB-55*, *CA-23* and *HB-70*. In two colonies from site *HB*, *HB-103* and *HB-104*, samples from the same colony fell into different genetic clusters. *HB-103* showed variation at three out of five informative loci, with both alleles differing at two loci. *HB-104* showed variation at four out of five informative loci, with both alleles varying at three loci. Such high levels of within-colony heterogeneity are unlikely to arise from mutation alone but rather indicate the presence of genetically different fusion partners. Thus, we considered these colonies as chimeras. Both, *HB-103* and *HB-104* were morphologically indistinguishable from genetically homogeneous colonies. By mere observation in the field, however, it was not possible to decide whether the genetically distinct entities were connected via fusion of skeletons and overlying soft tissues or simply grew in close contact without being fused.

Level of genetic heterogeneity

Among two-fold sampled colonies, the proportion of heterogeneous colonies (r) was 19/130 = 0.146. This figure is affected both by the true proportion of heterogeneous colonies at our study sites and by the probability of detection, i.e. by the proportion of the colony sphere that is made up of the 'secondary' genotype. If we assume for the moment that *all* colonies were heterogeneous, then following our argument above (cf. Materials and Methods) on average 7.3% of sphere-tips belonged to any genotype different from the genotype *A* ($s = r/2 = 0.073$). This corresponds to a centred cone with an apex angle equal to 62.8°. For three-fold sampled colonies, $r = 8/25 = 0.32$ and thus $s = r/3 = 0.107$. This difference suggests that more than 7.3% of colony branch tips were genotype B. However, as only few colonies were sampled at three positions, this result could not be statistically substantiated. These considerations imply that taking a single sample per colony will miss the primal genotype in ~7-10% of cases. We return to the assumptions underlying this analysis in the discussion.

Heterogeneity depending on colony size

The proportion of genetically heterogeneous *S. hystrix* colonies was not evenly distributed across all size classes. Rather, a significantly larger proportion of heterogeneous colonies was detected among medium-sized colonies ($15 < d \leq 30$ cm) compared to smaller ($d \leq 15$ cm) and larger ($d > 30$ cm) ones (Table 5: G (adj) = 8.79, $df = 2$, $P = 0.01$).

Discussion

The present study on intracolonial genetic variation in the scleractinian coral *S. hystrix* revealed a considerable proportion of genetically heterogeneous colonies. From a total of 155

colonies analysed at two or three positions using five or six microsatellite loci, 27 colonies (~17%) were genetically heterogeneous. We showed that both mosaicism and chimerism occur. While for most colonies it was not possible to distinguish between mosaicism and chimerism, the data suggest that mosaicism prevails. Clearly, both mechanisms may co-occur within the same colony. In their study on the broadcast spawning coral *Acropora millepora* from two sampling sites on the GBR, Puill-Stephan *et al*. (2009) sampled eight branches from 124 adult *A. millepora* colonies (15 to 40 cm in diameter) and genoyped them at eight to twelve microsatellite loci. They identified only ~5.6% of sampled colonies as genetically heterogeneous.

Mosaicism versus chimerism

For genetically heterogeneous colonies, we aimed to distinguish between mosaicism and chimerism. The comparison of similarity indices, $SI_{C\text{-}C}$ and $SI_{C\text{-}R,mean}$, attributed three colonies to mosaicism, *SB-55, CA-23* and *HB-70*. This method strongly depends on genetic structure within sites: High levels of heterogeneity, possibly due to the presence of distinct family groups or immigrants from genetically distinct source populations, may lead to low $SI_{C\text{-}R,mean}$ estimates und thus unduly inflate the power of the tests. We therefore repeated the analysis for colony *HB-70* by computing $SI_{C\text{-}R,mean}$ only with colonies that fell into the same genetic cluster as *HB-70* itself. We obtained a unimodal distribution of $SI_{C\text{-}R}$. $SI_{C\text{-}C}$ was still significant ($P = 2.36 \times 10^{-4}$), which confirmed *HB-70* as a mosaic. For *SB-55* and *CA-23* we could not repeat the analysis due to insufficient sample size.

The comparison of similarity indices is very sensitive to the standard deviation of the distribution of $SI_{C\text{-}R}$ and has limited power due to numerous Bonferroni corrections. In contrast, the comparison of single-allele variation in repeated samples from a single colony versus in randomly chosen colony pairs provides a more general test for the prevalence of somatic mutations. At all sites, it was highly unlikely to observe even one pair of samples with a single-allele variation. Thus, if two samples from a colony differed at a single allele, they

were most likely the result of somatic mutation. Based on the less stringent calculation from Table 3*b*, 16 out of 27 heterogeneous colonies differed at a single allele. This further supports our conclusion that somatic mutation is a common source of intracolonial genetic variation in *S. hystrix*.

The TESS analyses identified two presumptive chimeras, i.e. *HB-103* and *HB-104*. In both cases, samples from the same colony clearly fell into different clusters. Pronounced genetic substructure at site *HB* facilitated this analysis, which consequently is not applicable to genetically homogeneous sites such as *PAL1* and *HS*. And, of course, any chimeras consisting of genetically similar (e.g. related) partners will have been missed even at site *HB*.

Alternatively, we may identify chimeras based on large numbers of allele differences. For example, if we regard a difference of at least 5 (4) alleles of 6 (5) informative loci as evidence for chimerism, colonies *HS-36* and *Sb-21* also qualify as chimeras. *Sb-21* even revealed evidence for both mosaicism and chimerism. In this three-fold sampled colony, one pairwise comparison of samples showed a single-allele difference, pointing towards mosaicism, while another pairwise comparison revealed differences at six alleles, indicating chimerism.

Mutational mechanism

The three colonies that were identified as mosaics, based on similarity indices, revealed one single-step (*HB-70*) and two two-step mutations (*SB-55, CA-23*). If all colonies with single-allele differences were considered as mosaics, seven single-step, two two-step and seven larger mutations occurred. The mutation process of microsatellites is a central issue for population genetic studies, as information on the mutational mechanism helps to choose the most appropriate method for data analysis (e.g. Slatkin 1995; Balloux *et al*. 2000; Balloux & Lugon-Moulin 2002).

Level of genetic heterogeneity

As outlined above, we could not infer the true proportion of heterogeneous colonies from the observed proportion, given our limited sampling effort. However, considering the high mutation rates of microsatellites (Schlötterer 2000), we conjecture that intracolonial genetic variation can be considered the norm rather than the exception, at least at these marker loci. We expect that additional samples per colony would have revealed a much higher proportion of heterogeneous colonies. In a study on *S. hystrix* where 14 or 15 samples were taken from each of ten colonies and analysed at four microsatellite loci, 80% of colonies were heterogeneous (Striewski 2009). Thus, our assumption that in fact all colonies were heterogeneous should not be too far from the truth. It suggests that scoring five to six loci from a single sample per colony will fail to uncover the primal genotype in 7.3% (10.7%) of cases. We may take the mean of both values, i.e. 9%, as an overall estimate. Note, however, that this is only an approximate value because calculations were performed across all size-classes. Given that the level of heterogeneity varied with size (see below), this could have biased the analysis.

Heterogeneity depending on colony size

Genetically heterogeneous colonies occurred across all size classes but were most frequent among medium-sized colonies ($15 < d \leq 30$ cm). If somatic mutation was the main source of within-colony variation, we would expect the highest proportion of heterogeneous colonies in the largest size class, since mutations should accumulate with increasing number of cell divisions during growth (Gill *et al*. 1995). Chimerism might in principle explain the lower prevalence of heterogeneity in the largest size class if intracolonial, allogeneic competition caused the break-up of fused entities in the long term (see below). However, as our data did not allow estimating the frequency of chimeras, we cannot readily explain the observed size effect. Possibly, sampling artefacts accounted for this pattern. For example, colony sizes as well as relatedness among colonies may have varied simultaneously among sites. Given that fusions

are expected to be more frequent among related colonies (e.g. Grosberg & Quinn 1986), this could have contributed to the finding that the proportion of heterogeneous colonies varied among size classes. Finally, our assumption that growth and thus branching rates are constant throughout all parts of a colony may not be correct. More rapidly growing sections of a colony could have been overrepresented in our samples, especially in large colonies.

Previous studies on chimerism in corals

Whereas data on mosaicism are only just becoming available, chimerism is known from previous studies on corals, including *S. hystrix*. Atoda (1951) reported that *S. hystrix* larvae settling in close proximity fused to form an aggregated colony. In this study, however, it was not specified whether planulae were released from the same or from different colonies. Allogeneic fusion was shown in grafting experiments in *S. hystrix* (Nozawa & Loya 2005). They suggest that the onset of fusion reactions is restricted to an early life stage and that most allografts do not form stable chimeras over longer periods of time. On the other hand, natural *Seriatopora* chimeras were reported, i.e. between *S. hystrix* and its congener *S. caliendrum* (Nozawa & Loya 2005). This suggests that chimerism also does play a role in adult *S. hystrix* and calls for further studies *in situ*. In their study on *A. millepora*, Puill-Stephan *et al*. (2009) interpreted a greater proportion of heterogeneous colonies, i.e. six of seven colonies, as chimeras. Their inference relied on the assumption of the stepwise mutation model (Ohta & Kimura 1973). The authors counted single allele differences between samples from the same colony as evidence for chimerism if the alleles differed in length by more than one repeat unit. In two colonies, multiple allelic differences across several loci provided strong evidence for chimerism.

Implications for population genetic studies

The present study on *S. hystrix* highlights the importance of considering intracolonial genetic variation as a potential source of error in population genetic studies in corals. This is espe-

cially true with regard to the increasing use of individual-based approaches such as assignment tests or parentage analyses in studies on reef connectivity. Mosaicism and chimerism can affect such studies in various ways. In principle, effects of within-colony heterogeneity should add to those caused by genotyping errors (Bonin *et al*. 2004; Hoffman & Amos 2005). While analyses based on average allele frequencies (i.e. Wright's *F*-statistics, Wright 1965) are relatively robust, individual-based approaches are particularly affected, especially if large numbers of loci are used. For example, as shown for genotyping errors, an error rate of 5% per locus in a three-locus data set results in 95% accuracy in estimates of allele frequencies but causes that only 85% of individuals are genotyped correctly at all three loci (Selkoe & Toonen 2006). As individual genotypes are usually compared with others, the problem gets even more severe. If we take our estimate of 9% as an example, then only about 82% of pairwise comparisons would reveal two genotypes that one might consider 'representative' for the colony from which they were collected. In studies on reproductive mode, somatic mutations may obscure asexual reproduction, as they can cause that colonies derived from the same progenitor differ genetically (Lowe *et al*. 2004). Parentage analyses are particularly prone to error, mostly due to the exclusion of 'true' parents if somatic mutations caused a mismatch between parent and offspring (Hoffman & Amos 2005; Slavov *et al*. 2005). Undetected chimeras may hide 'true' parents, thus causing flaws in analyses that assume exhaustive sampling of potential parents. In the light of the present results (i.e. within-colony variation mostly involved single alleles), special attention should be paid to single mismatches in order to avoid misinterpretations. Choosing appropriate algorithms for data analysis which account for genotyping errors may help to confine error rates.

The present study on *S. hystrix* revealed a substantial level of intracolonial genetic variation. Whereas both mosaicism and chimerism contributed, somatic mutations presented the major source of heterogeneity. The preliminary observation that a higher proportion of heterogeneous colonies occurred among medium-sized compared to small and large colonies calls for further investigation. A sampling approach with larger numbers of samples per

colony should provide for better resolution. The ongoing development of microsatellite markers for various coral species will open up new possibilities to study intracolonial genetic variation as an important aspect of coral population genetics.

References

Amar KO, Chadwick NE, Rinkevich B (2008) Coral kin aggregations exhibit mixed allogeneic reactions and enhanced fitness during early ontogeny. *BMC Evolutionary Biology*, **8**, 126.

Atoda K (1951) The larva and postlarval development of the reef-building corals. V. *Seriatopora hystrix* Dana. *Science Reports of the Tohoku University, 4th series (Biology),* **19**, 33-39.

Balloux F, Brünner H, Lugon-Moulin N, Hausser J, Goudet J (2000) Microsatellites can be misleading: an empirical and simulation study. *Evolution*, **54**, 1414-1422.

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. *Molecular Ecology*, **11**, 155-165.

Barki Y, Gateño D, Graur D, Rinkevich B (2002) Soft-coral natural chimerism: a window in ontogeny allows the creation of entities comprised of incongruous parts. *Marine Ecology Progress Series*, **231**, 91-99.

Baums IB, Hughes CR, Hellberg ME (2005) Mendelian microsatellite loci for the Caribbean coral *Acropora palmata*. *Marine Ecology Progress Series*, **288**, 115-127.

Bellwood DR, Hughes TP, Folke C, Nyström M (2004) Confronting the coral reef crisis. *Nature*, **429**, 827-833.

Ben-Shlomo R, Douek J, Rinkevich B (2001) Heterozygote deficiency and chimerism in remote populations of a colonial ascidian from New Zealand. *Marine Ecology Progress Series*, **209**, 109-117.

Bonin A, Bellemain E, Eidesen PB, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetics studies. *Molecular Ecology*, **13**, 3261-3273.

Buss LW (1982) Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proceedings of the National Academy of Sciences of the USA,* **79**, 5337-5341.

Buss LW (1987) *The evolution of individuality*. Princeton University Press, Princeton, NJ.

Chen C, Durand E, Forbes F, François O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes*, **7**, 747-756.

Excoffier L, Heckel G (2006) Computer programs for population genetics data analysis: a survival guide. *Nature Reviews Genetics*, **7**, 745-758.

François O, Ancelet S, Guillot G (2006) Bayesian clustering using hidden Markov random fields in spatial population genetics. *Genetics*, **174**, 805-816.

Frank U, Oren U, Loya Y, Rinkevich B (1997) Alloimmune maturation in the coral *Stylophora pistillata* is achieved through three distinctive stages, 4 months post-metamorphosis. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **264**, 99-104.

Gill DE, Chao L, Perkins SL, Wolf JB (1995) Genetic mosaicism in plants and clonal animals. *Annual Review of Ecology and Systematics*, **26**, 423-444.

Grosberg RK, Quinn JF (1986) The genetic control and consequences of kin recognition by the larvae of a colonial marine invertebrate. *Nature*, **322**, 456-459.

Hart MW, Grosberg RK (1999) Kin interactions in a colonial hydrozoan (*Hydractinia symbiolongicarpus*): population structure on a mobile landscape. *Evolution*, **53**, 793-805.

Hidaka M (1985) Tissue compatibility between colonies and between newly settled larvae of *Pocillopora damicornis*. *Coral Reefs*, **4**, 111-116.

Hidaka M, Yurugi K, Sunagawa S, Kinzie RA (1997) Contact reactions between young colonies of the coral *Pocillopora damicornis*. *Coral Reefs*, **16**, 13-20.

Hoffman JI, Amos W (2005) Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Molecular Ecology*, **14**, 599-612.

Hughes RN, Manríquez PH, Morley S, Craig SF, Bishop JDD (2004) Kin or self-recognition? Colonial fusibility of the bryozoan *Celleporella hyalina. Evolution and Development*, **6**, 431-437.

Hughes TP, Ayre D, Connell JH (1992) The evolutionary ecology of corals. *Trends in Ecology and Evolution*, **7**, 292-295.

Hughes TP, Baird AH, Bellwood DR *et al*. (2003) Climate change, human impacts, and the resilience of coral reefs. *Science*, **301**, 929-933.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801-1806.

Lowe A, Harris S, Ashton P (2004) *Ecological genetics: design, analysis, and application*. Blackwell Publishing, Oxford.

Magalon H, Samadi S, Richard M, Adjeroud M, Veuille M (2004) Development of coral and zooxanthella-specific microsatellites in three species of *Pocillopora* (Cnidaria, Scleractinia) from French Polynesia. *Molecular Ecology Notes*, **4**, 206-208.

Maier E, Tollrian R, Nürnberger B (2001) Development of species-specific markers in an organism with endosymbionts: microsatellites in the scleractinian coral *Seriatopora hystrix*. *Molecular Ecology Notes*, **1**, 157-159.

Maier E, Tollrian R, Rinkevich B, Nürnberger B (2005) Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea. *Marine Biology*, **147**, 1109-1120.

Maier E, Tollrian R, Nürnberger B (2009) Fine-scale analysis of genetic structure in the brooding coral *Seriatopora hystrix* from the Red Sea. *Coral Reefs*, **28**, 751-756.

Maldonado M (1998) Do chimeric sponges have improved chances of survival? *Marine Ecology Progress Series*, **164**, 301-306.

Moran MD (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos*, **100**, 403-405.

Noreen AME, Harrison PL, van Oppen MJH (2009) Genetic diversity and connectivity in a brooding reef coral at the limit of its distribution. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **276**, 3927-3935.

Nozawa Y, Loya Y (2005) Genetic relationship and maturity state of the allorecognition system affect contact reactions in juvenile *Seriatopora* corals. *Marine Ecology Progress Series*, **286**, 115-123.

Ohta T, Kimura M (1973) A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetics Research*, **22**, 201-204.

Orive ME (2001) Somatic mutations in organisms with complex life histories. *Theoretical Population Biology*, **59**, 235-249.

Pineda-Krch M, Lehtilä K (2004) Costs and benefits of genetic heterogeneity within organisms. *Journal of Evolutionary Biology*, **17**, 1167-1177.

Puill-Stephan E, Willis BL, van Herwerden L, van Oppen MJH (2009) Chimerism in wild adult populations of the broadcast spawning coral *Acropora millepora* on the Great Barrier Reef. *PLoS ONE*, **4** (11), e7751.

Resing JM, Ayre DJ (1985) The usefulness of the tissue grafting bioessay as an indicator of clonal identity in scleractinian corals (Great Barrier Reef – Australia). *Proceedings of the 5th International Coral Reef Congress*, **6**, 75-81.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223-225.

Rinkevich B, Loya Y (1983) Intraspecific competitive networks in the Red Sea coral *Stylophora pistillata*. *Coral Reefs*, **1**, 161-172.

Rinkevich B, Weissman IL (1987) Chimeras in colonial invertebrates: a synergistic symbiosis or somatic- and germ-cell parasitism? *Symbiosis*, **4**, 117-134.

Santelices B (2004) Mosaicism and chimerism as components of intraorganismal genetic heterogeneity. *Journal of Evolutionary Biology*, **17**, 1187-1188.

Schlötterer C (2000) Evolutionary dynamics of microsatellite DNA. *Chromosoma*, **109**, 365-371.

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters*, **9**, 615-629.

Shenk MA, Buss LW (1991) Ontogenetic changes in fusibility in the colonial hydroid *Hydractinia symbiolongicarpus*. *Journal of Experimental Zoology*, **257**, 80-86.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457-462.

Slavov GT, Howe GT, Gyaourova AV, Birkes DS, Adams WT (2005) Estimating pollen flow using SSR markers and paternity exclusion: accounting for mistyping. *Molecular Ecology*, **14**, 3109-3121.

Sokal RR, Rohlf FJ (1994) *Biometry*. 3rd edn, Freeman, San Francisco.

Sommerfeldt AD, Bishop JDD (1999) Random amplified polymorphic DNA (RAPD) analysis reveals extensive natural chimerism in a marine protochordate. *Molecular Ecology*, **8**, 885-890.

Sommerfeldt AD, Bishop JDD, Wood CA (2003) Chimerism following fusion in a clonal ascidian (Urchordata). *Biological Journal of the Linnean Society*, **79**, 183-192.

Striewski S (2009) Examination on the intracolonial genetic variation in the scleractinian coral *Seriatopora hystrix*. Masterthesis. Ruhr-Universität Bochum, Germany.

Underwood JN, Souter PB, Ballment ER, Lutz AH, van Oppen MJH (2006). Development of 10 polymorphic microsatellite markers from herbicide-bleached tissues of the brooding pocilloporid coral *Seriatopora hystrix*. *Molecular Ecology Notes*, **6**, 176-178.

Underwood JN, Smith LD, van Oppen MJH, Gilmour JP (2007) Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. *Molecular Ecology*, **16**, 771-784.

Underwood JN, Smith LD, van Oppen MJH, Gilmour JP (2009) Ecologically relevant dispersal of corals on isolated reefs: implications for managing resilience. *Ecological Applications*, **19**, 18-29.

van Oppen MJH, Gates RD (2006) Conservation genetics and the resilience of reef-building corals. *Molecular Ecology*, **15**, 3863-3883.

van Oppen MJH, Underwood JN, Muirhead AN. Peplow L (2007) Ten microsatellite loci for the reef-building coral *Acropora millepora* (Cnidaria, Scleractinia) from the Great Barrier Reef, Australia. *Molecular Ecology Notes*, **7**, 436-438.

van Oppen MJH, Lutz A, De'ath G, Peplow L, Kininmonth S (2008) Genetic traces of recent long-distance dispersal in a predominantly self-recruiting coral. *PLoS ONE*, **3**(10), e3401.

Veron JEN (2000) *Corals of the World*. Australian Institute of Marine Science, Townsville.

Willis BL, Ayre DJ (1985) Asexual reproduction and genetic determination of growth form in the coral *Pavona cactus*: biochemical genetic and immunogenic evidence. *Oecologia (Berlin)*, **65**, 516-525.

Wright S (1965) The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution*, **19**, 395-420.

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Figure Legends

Figure 1 Distribution of similarity indices in the reference populations ($SI_{R\text{-}R}$) for the five *S. hystrix* sampling sites. The distributions differed among the five reference populations but covered the full range of possible values at each site. Note that at *HS* and *PAL1* only five loci were analysed, thus $SI_{R\text{-}R}$ ranges from 0 to 10 and single-allele variation corresponds to $SI_{R\text{-}R}$ = 9. At *HB*, *CA* and *SB*, $SI_{R\text{-}R}$ ranges from 0 to 12, thus single-allele variation corresponds to $SI_{R\text{-}R}$ = 11.

Tables

Table 1 Comparison of similarity indices, $SI_{C\text{-}C}$ and $SI_{C\text{-}R,mean}$ in 27 genetically heterogeneous *Seriatopora hystrix* colonies. Given are the colony identification (*Colony-ID*), the maximum colony diameter (*Size*), the number of samples analysed per colony (#), the pairwise within-colony similarity index ($SI_{C\text{-}C}$) together with the number of loci that were included in its calculation (in parentheses), the mean between-colony similarity index ($SI_{C\text{-}R,mean}$), calculated between the heterogeneous colony and all other colonies from the same site, as well as the probability of $SI_{C\text{-}C}$ under the null hypothesis (*P*). * significant at $\alpha = 0.05$ after sequential Bonferroni correction. For a conservative estimate, the highest *P*-value was considered for each colony.

Colony-ID	***Size [cm]***	**#**	$SI_{C\text{-}C}$ **(# loci)**	$SI_{C\text{-}R,mean}$	***P***
PAL1-16	40	2	0.83 (3)	0.41	0.024
PAL1-51	15	2	0.90 (5)	0.42	0.003
PAL1-61	40	2	0.88 (4)	0.61	0.109
PAL1-79	30	2	0.90 (5)	0.49	0.013
PAL1-91	40	2	0.70 (5)	0.57	0.301
HS-36	30	2	0.60 (5)	0.56	0.532
HS-111	30	2	0.88 (4)	0.45	0.042
SB-21	25	3	0.50 (6)	0.63	0.803
SB-31	30	3	0.90 (5)	0.52	0.094
SB-45	20	3	0.92 (6)	0.49	0.035
SB-52	50	3	0.83 (5)	0.62	0.303
*SB-55**	30	3	0.92 (6)	0.30	3.46×10^{-7}
SB-69	20	3	0.80 (5)	0.58	0.171
SB-124	30	2	0.92 (5)	0.65	0.219
CA-2	30	2	0.90 (5)	0.62	0.129
*CA-23**	20	2	0.90 (5)	0.36	4.44×10^{-5}
CA-31	20	2	0.90 (5)	0.58	0.078
CA-66	7	2	0.88 (4)	0.59	0.140
CA-82	30	2	0.83 (3)	0.76	0.516
CA-87	20	2	0.67 (3)	0.50	0.365
HB-66	25	2	0.50 (4)	0.40	0.454
*HB-70**	40	2	0.92 (6)	0.27	8.70×10^{-4}
HB-74	20	2	0.75 (4)	0.34	0.096
HB-86	25	3	0.92 (6)	0.37	0.012
HB-102	20	2	0.80 (5)	0.43	0.053
HB-103	45	2	0.50 (5)	0.43	0.461
HB-104	20	3	0.42 (6)	0.40	0.523

Table 2 Counts of single-allele variations among all pairwise comparisons at a sampling site. The last row gives the probability *p* that two random colonies differ at a single allele, provided that they differ at all.

Sampling site	***PAL1***	***HS***	***SB***	***CA***	***HB***
# Single-allele variations	40	44	87	15	20
# Pairwise comparisons	5564	7133	7702	5765	5545
p	0.007	0.006	0.011	0.003	0.004

Table 3 Assessment of single-allele variation within heterogeneous *S. hystrix* colonies.

a. Conservative assessment, shifting as many heterogeneous colonies with missing data as possible away from single-allele variation. ***b.*** Assessment of single-allele variation, ignoring loci with unknown allelic identity.

'# Single-allele variations' gives the number of heterogeneous colonies with variation at a single allele and, in parentheses, the total number of heterogeneous colonies at each site. *P* is the probability to get at least the observed number of single-allele variations. If no single-allele variation is detected, the probability of getting zero successes is approximately 1. $\alpha_{Bonferroni}$ is the significance threshold after sequential Bonferroni correction, respectively.

	Sampling site	***PAL1***	***HS***	***SB***	***CA***	***HB***
a.	# Single-allele var. (total)	2 (5)	0 (2)	2 (7)	0 (6)	2 (7)
	P	0.0005	1.0	0.0026	1.0	0.0003
	$\alpha_{Bonferroni}$	0.0127	0.0500	0.0170	0.0253	0.0102
b.	# Single-allele var. (total)	4 (5)	1 (2)	4 (7)	5 (6)	2 (7)
	P	1.3×10^{-8}	0.0123	5.5×10^{-7}	7.1×10^{-13}	0.0003
	$\alpha_{Bonferroni}$	0.0127	0.0500	0.0170	0.0102	0.0253

Table 4 Cluster membership coefficient *q* for 27 genetically heterogeneous *S. hystrix* colonies according to TESS, *S* = Sample-ID. The cluster membership data follows the format 'cluster number: q / ...'. For colonies sampled at three positions, only genetically distinct samples are listed. Cluster membership coefficients ≤ 0.05 were omitted. Presumed chimeras are denoted with *.

Colony-ID	***S***	***Cluster membership***
PAL1-16	A	3: 0.29 / 6: 0.43 / 9: 0.27
	B	3: 0.31 / 6: 0.40 / 9: 0.27
PAL1-51	A	3: 0.40 / 6: 0.29 / 9: 0.31
	B	3: 0.40 / 6: 0.29 / 9: 0.31
PAL1-61	A	3: 0.33 / 6: 0.39 / 9: 0.28
	B	3: 0.33 / 6: 0.36 / 9: 0.31
PAL1-79	A	3: 0.30 / 6: 0.31 / 9: 0.39
	B	3: 0.31 / 6: 0.32 / 9: 0.37
PAL1-91	A	3: 0.33 / 6: 0.41 / 9: 0.25
	B	3: 0.38 / 6: 0.38 / 9: 0.24
HS-36	A	3: 0.40 / 6: 0.32 / 9: 0.28
	B	3: 0.29 / 6: 0.27 / 9: 0.43
HS-111	A	3: 0.35 / 6: 0.23 / 9: 0.42
	B	3: 0.37 / 6: 0.24 / 9: 0.39
SB-21	A	1: 0.85 / 5: 0.14
	B	1: 0.70 / 4: 0.14 / 5: 0.15
	C	1: 0.93
SB-31	A	1: 0.99
	B	1: 0.72 / 5: 0.26
SB-45	A	4: 0.13 / 5: 0.87
	B	4: 0.13 / 5: 0.87
SB-52	A	1: 0.24 / 5: 0.71
	B	1: 0.09 / 5: 0.88
SB-55	A	8: 1.00
	B	8: 1.00
SB-69	A	1: 1.00
	B	1: 0.99
	C	1: 0.92 / 10: 0.08

Colony-ID	***S***	***Cluster membership***
SB-124	A	1: 0.11 / 5: 0.86
	B	1: 0.11 / 5: 0.86
CA-2	A	1: 0.30 / 5: 0.63
	B	1: 0.69 / 5: 0.29
CA-23	A	1: 1.00
	B	1: 0.99
CA-31	A	1: 0.14 / 4: 0.11 / 5: 0.74
	B	1: 0.48 / 4: 0.04 / 5: 0.48
CA-66	A	1: 0.09 / 4: 0.06 / 5: 0.85
	B	1: 0.05 / 5: 0.92
CA-82	A	1: 0.38 / 5: 0.52
	B	1: 0.70 / 5: 0.23 / 10:
CA-87	A	1: 0.59 / 4: 0.10 / 5: 0.31
	B	1: 0.40 / 4: 0.11 / 5: 0.49
HB-66	A	1: 0.56 / 5: 0.38
	B	9: 0.98
HB-70	A	2: 1.00
	B	2: 1.00
HB-74	A	2: 1.00
	B	2: 1.00
HB-86	A	1: 0.95
	B	1: 0.92 / 10: 0.08
HB-102	A	1: 0.99
	B	1: 0.54 / 4: 0.06 / 5: 0.40
*HB-103**	A	1: 0.99
	B	8: 1.00
*HB-104**	A	8: 1.00
	B	1: 0.93 / 5: 0.06

Table 5 Number of *S. hystrix* colonies showing intracolonial genetic variation within different size classes. For each size class, the total number of colonies (*total*) and the corresponding number of heterogeneous colonies (*het.*) that were genotyped at two (*N-2*) or three positions (*N-3*) are given, together with their summarised values (*N*). Size was measured as the maximum colony diameter. For details, see text.

	N-2		***N-3***		***N***	
Size in cm	***total***	***het.***	***total***	***het.***	***total***	***het.***
x ≤ 15	33	2	5	0	38	2
15 < x ≤ 30	51	12	11	7	62	19
x > 30	46	5	9	1	55	6
All sizes	130	19	25	8*	155	27

* Six of the three-fold sampled colonies revealed two different genotypes whereas two of them revealed three different genotypes.

Figures

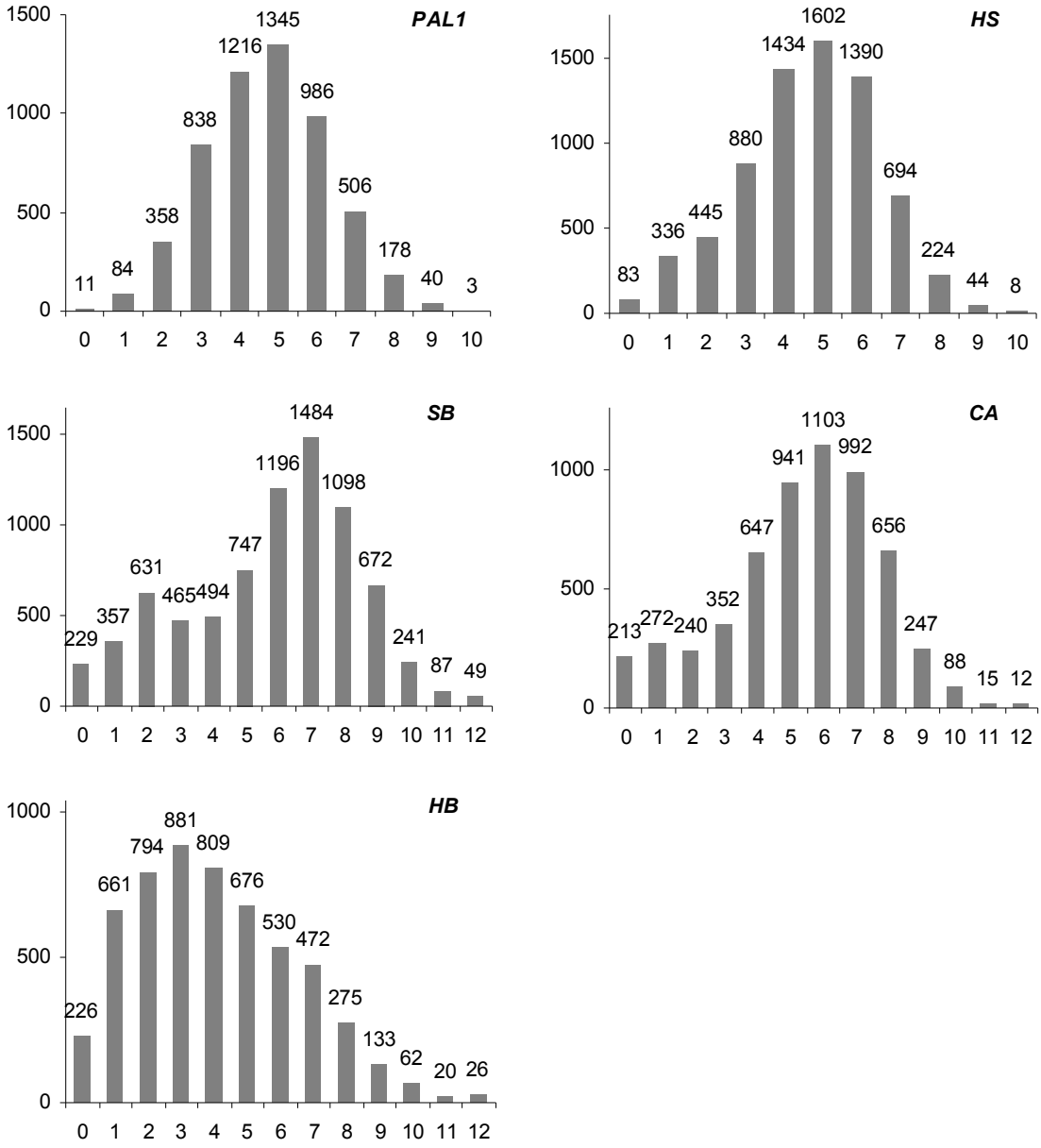

Figure 1

PAPER 5

Genetic population structure of the scleractinian coral *Seriatopora hystrix* on the Great Barrier Reef: patterns of reproduction and dispersal

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Genetic population structure in the scleractinian coral *Seriatopora hystrix* on the Great Barrier Reef: patterns of reproduction and dispersal

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Running title: Population genetics of *Seriatopora hystrix*

Abstract

Reproduction and larval dispersal are fundamental in population dynamics and evolution of sessile marine invertebrates, as they affect the rate and pattern of adaptation and determine the dynamics of local extinction and recolonisation. This is critical for the persistence of populations and a central issue for the design of marine reserves. In this study on the brooding coral *Seriatopora hystrix* from the Great Barrier Reef we used microsatellites to investigate population genetic structure of nine *S. hystrix* stands from two geographical regions (Lizard and Heron Island, max. distance: ~1185 km). Specifically, we aimed to 1) evaluate migration patterns on different spatial scales, 2) infer reproductive mode and 3) identify causes of heterozygote deficits. Levels of genetic differentiation among sites within regions were low at Lizard Island (F_{ST} = 0.006, max. distance: 1.6 km) and moderate at Heron Island (F_{ST} = 0.093, max. distance: 10.6 km). At Heron Island, cluster analyses revealed pronounced substructure within most sites, pointing towards colonisation from several genetically differentiated sites. In contrast, Lizard Island sites appeared homogeneous, consistent with a single, well-mixed gene pool. All stands were largely maintained by sexual recruits. Using various analyses based on individual multilocus genotypes, we attributed the significant heterozygote deficits that occurred at most sites to a combination of Wahlund effects, restricted dispersal of gametes and/or larvae and inbreeding, including selfing. We observed large variation of population genetic structure between sites. Apportioning this variation to differences in either life history or abiotic forces presents a challenge for future studies.

Introduction

Scleractinian corals are sessile marine organisms with complex life histories that include a wide range of both reproductive modes and offspring dispersal capabilities (Harrison & Wallace 1990). Since considerable life-history variation can be found in closely related species or even within a single species, corals offer the opportunity to test the adaptive value of different strategies and are thus valuable study objects from an evolutionary point of view. From an ecological perspective, scleractinian corals are the major framework builders of coral reefs which are worldwide under increasing threat from climate change and other human-induced disturbances (e.g. Hughes et al. 2003). For the development of efficient management strategies, it is crucial to understand coral population dynamics and connectivity patterns (Palumbi 2003). Not surprisingly, scleractinian corals have become a major target of marine studies (reviewed in van Oppen & Gates 2006, Baums 2008).

Various coral species are capable of both sexual and asexual reproduction (Harrison & Wallace 1990) or follow a mixed mating strategy of outcrossing and selfing (Carlon 1999). Especially in species with internal fertilisation and development (so-called brooders), with larvae that are capable of settlement immediately after release, dispersal distances are often short (Harrison & Wallace 1990). As outcrossed fertilisation is usually restricted to colonies in close proximity, this promotes mating between close relatives (e.g. Levitan & Petersen 1995, but see Ayre & Miller 2006). Together with selfing, short-distance dispersal of gametes and/or larvae increases genetic relatedness within local stands and thus favours inbreeding (Jackson 1986). A combination of inbreeding and Wahlund effects (Wahlund 1928) due to admixture of genetically distinct larval cohorts may cause local heterozygote deficits that are common in corals (e.g. Ayre & Hughes 2000, Whitaker 2004, Magalon et al. 2005). Disentangling the causes of heterozygote deficits would add to our understanding of small-scale processes that govern coral populations. To date, however, most population genetic studies

on scleractinian corals focused on scales between tens of metres and hundreds of kilometres while data on small-scale processes from decimetres to metres are scarce (but see Miller & Ayre 2008, Yeoh & Dai 2010).

In this study, we focused on the scleractinian coral *Seriatopora hystrix* (Fam. Pocilloporidae). *S. hystrix* is a hermaphroditic species commonly found in the Indo-Pacific. It is widespread in most major habitat types of Australia's Great Barrier Reef (GBR, Veron 2000). *S. hystrix* reproduces via sexually produced, brooded larvae (Ayre & Resing 1986, Sherman 2008). In the laboratory, most larvae settle shortly after release (Atoda 1951), which should promote small-scale dispersal. This was confirmed by two population genetic studies that found isolation by distance on spatial scales ≤20 km (Maier et al. 2005) or even below tens of metres (Underwood et al. 2007). Yet, occasional dispersal between reefs also occurs (Underwood et al. 2007, 2009, van Oppen et al. 2008, Noreen et al. 2009). Short- and long-distance dispersal may even involve different types of larvae (Isomura & Nishihira 2001). Progeny array analyses indicated that broods can include both outcrossed and selfed offspring (Sherman 2008). Various modes of asexual reproduction have also been discussed: Given the colonies' delicately branched morphology, reproduction via fragmentation seems likely (Ayre & Dufty 1994, Ayre & Hughes 2000). In the laboratory, single polyps where observed to detach from the skeleton and resettle on appropriate substratum (polyp bail-out, Sammarco 1982). Finally, like the related species *Pocillopora damicornis*, *S. hystrix* could possibly produce asexual larvae (van Oppen et al. 2008). While most analyses indicate that asexual reproduction does not play a role for local propagation (Ayre & Dufty 1994, Ayre & Hughes 2000, Maier et al. 2005, 2009, Underwood et al. 2007, Sherman 2008, Noreen et al. 2009), a recent study suggests that asexual propagules might be involved in long-distance dispersal (van Oppen et al. 2008). Taken together, these findings imply that *S. hystrix* populations are shaped by processes that act on a wide range of spatial scales, from decimetres/metres to hundreds of kilometres. While previous studies focused on scales ≥tens of metres, population genetic structure on the smallest scales (i.e. decimetres to metres) has

not been analysed. Likewise, causes for the local heterozygote deficits that are common in *S. hystrix* (e.g. Ayre & Dufty 1994, Maier et al. 2005, Underwood et al. 2007) have not specifically been addressed.

We used five to six microsatellite markers to analyse the population genetic structure of nine *Seriatopora hystrix* stands on the GBR, separated between ~0.3 km and 1185 km. We proceed from summary statistics (*F*-statistics, pairwise linkage disequilibria *D*) to more in-depth analyses based on individual multilocus genotypes to uncover the underlying biological processes. With a focus on small spatial scales, we concentrate on modes of reproduction (sexual, asexual, selfing) and patterns of larval transport within and between sites. Our statistical analyses include Bayesian clustering as well as a novel method to infer population-wide selfing rates (using CERVUS, Kalinowski et al. 2007). Details for the latter are provided in the Appendix.

Materials and Methods

Collection and storage of samples

A total of 698 *Seriatopora hystrix* colonies were sampled between November and December 2002 from nine sites on the GBR (Fig. 1). Three sites, Palfrey 1 (*PAL1*), Horseshoe Reef (*HS*) and Palfrey 2 (*PAL2*) were located at Lizard Island, northern GBR (14°40´S, 145°28´E). Six sites, Tenements II (*T*), Staghorn Bank (*SB*), Canyons (*CA*), Harry's Bommie (*HB*), South Reef (*SR*) and North-West-Wistari (*NWW*) were situated at Heron Island, southern GBR (23°27´S, 151°55´E). Depths of sampling sites ranged from 3 m to 17 m. At *PAL1*, *HS*, *SB*, *CA* and *HB*, tissue samples from all adult as well as juvenile colonies within contiguous stands were collected (*N* = 106 to 125 per site). At *PAL2*, *SR*, *NWW* and *T*, only adult colonies were sampled (*N* = 30 to 34). According to Stimson (1978), colonies <8 cm were classi-

fied as juveniles whereas those ≥8 cm were regarded as adults. Depending on *S. hystrix* density, the size of the sampled areas ranged from ~50 to 600 m^2. At *SB* and *CA*, the position of each colony was recorded via *x*- and *y*-coordinates for spatial analyses of genetic population structure. From each colony, a single branch tip of ~1 cm length was collected. From a total of 155 colonies, additional samples (one or two/colony) were sampled for an analysis of intracolonial genetic variation. The results of this study will be presented in a separate report. DNA was preserved according to Maier et al. (2005).

Molecular analyses

DNA was extracted as described in Maier et al. (2001). All samples were genotyped at five or six microsatellite loci. Loci Sh2-002, Sh2-006, Sh3-004, Sh3-007 and Sh4-001 were developed by Underwood et al. (2006) and previously used in *Seriatopora hystrix* from Australia (Underwood et al. 2007, van Oppen et al. 2008, Noreen et al. 2009). Locus Sh4.24 was originally employed in *S. hystrix* from the Red Sea (Maier et al. 2005). With GBR samples, Sh4.24 did not reliably amplify, thus primers were redesigned. The sequences of the new forward and reverse primers were 5'-CCTAACAAAAGGACTGATTGGC-3' (Sh4.24A3) and 5'-TTGAACATCTGGTTTGAAATG-3' (Sh4.24B3). Even these primers worked well only for samples from Heron Island but yielded weak or unscorable PCR products for samples from Lizard Island. Consequently, locus Sh4.24 was excluded from Lizard Island samples such that colonies from sites *HB*, *CA* and *SB* were genotyped at six loci while colonies from *HS* and *PAL1* were genotyped at five loci. For detailed amplification protocols see Maier et al. (2005) and Underwood et al. (2006). Allelic variation was analysed on a MegaBACE 1000 automated sequencer (GE Healthcare) relative to an internal size standard (ET 400-R). Alleles were scored according to their length in base pairs using the software MegaBACE GENETIC PROFILER 2.2 (Amersham Biosciences) and checked manually.

Statistical analyses

From colonies that were sampled at two or three positions, one sample was randomly chosen for the present analyses. Allele frequencies as well as observed (H_o) and expected (H_e) heterozygosities were calculated in *FSTAT* version 2.9.3 (Goudet 2001) and GENEPOP version 3.4 (Raymond & Rousset 1995). To estimate microsatellite diversity standardised for variation in sample size, allelic richness (A) according to El Mousadik & Petit (1996) was calculated in *FSTAT*. Tests for Hardy-Weinberg equilibrium (HWE) and linkage disequilibria were performed in *FSTAT*, based on 1000 and 2700 permutations, respectively.

We expressed the prevalence of repeated multilocus genotypes per site as the number of distinct genotypes (N_g) divided by the sample size (N). To assess the probability of observing a given number of identical multilocus genotypes (MLG) by chance under biparental sexual reproduction, we carried out simulations as described in Calderón et al. (2007), because this method does not assume random mating.

Weir and Cockerham's (1984) estimators of Wright's (1965) F-statistics, f (= F_{IS}, the inbreeding coefficient), θ (= F_{ST}, the fixation index) as well as R_{ST}, a microsatellite-based differentiation estimate (Rousset 1996) were calculated in *FSTAT*. For consistency, locus Sh4.24 was omitted from all comparisons between regions. Hedrick's (2005) standardised genetic differentiation parameter, F'_{ST}, was calculated using RECODEDATA version 0.1 (Meirmans 2006) and *FSTAT*. Estimates of F'_{ST} are insensitive to levels of allelic variation and thus allow for a comparison of genetic markers with different mutation rates (e.g. microsatellites versus allozymes, Balloux & Lugon-Moulin 2002, Hedrick 2005).

To test for a correlation between geographic and genetic distances, we performed a Mantel test (Mantel 1967) for the six Heron Island sites (max. distance: 10.6 km). Matrices contained $F_{ST}/(1-F_{ST})$ as a measure of genetic distance and log-transformed geographic distances, as proposed by Rousset (1997) for two-dimensional habitats. A second test was carried out for the analogous quantity $R_{ST}/(1-R_{ST})$. Geographic distances were estimated as the shortest distance connecting sampling sites by sea. Tests were performed in GENEPOP,

with 100000 matrix randomisations. To exclude the influence of potential clonemates, only one representative of each distinct MLG (see below) was used in the analyses.

As large heterozygote deficits were observed at most sites, we set out to identify the underlying causes: First, we focused on the potential impact of null alleles (e.g. Pemberton et al. 1995). The software FREENA (Chapuis & Estoup 2007) was used to estimate null allele frequencies following Dempster et al. (1977). Missing genotypes were coded as null alleles if a given sample failed to amplify at one or two loci while amplification was successful for all other loci. If more than two loci did not amplify, allelic dropout was attributed to technical factors (e.g. DNA quality). FREENA was used to infer maximum null allele frequencies per site as well as to compute an alternate dataset corrected for nulls to check the robustness of our results where applicable. We note, though, that this is a simplified approach, as it assumes that a) null alleles are the only source of heterozygote deficits whereas population processes are likely to contribute as well and b) a single null allele is common to all sites whereas possibly, multiple allelic states are involved.

Bayesian clustering analyses were used to group colonies based on their individual multilocus genotypes. We used the software TESS version 1.1 (François et al. 2006, Chen et al. 2007), with the 'Generate Spatial Coordinates' option to generate random spatial coordinates for each colony, based on sampling site coordinates. We ran the program under default options (no admixture, no F-model), using the MCMC algorithm (burn in = 10000 sweeps, running period = 50000 sweeps). We first estimated the number of clusters (*K*) and performed 250 runs with this predetermined *K* value (interaction parameter of HMRF = 0.6, parameter of allele frequency model = 1.0, burn-in = 10000 sweeps, running period = 50000 sweeps). We selected the 20% highest likelihood runs and used the software *CLUMPP* (Jakobsson & Rosenberg 2007) to compute averages over these runs, using the LargeKGreedy algorithm with GREEDY_OPTION = 2 and 1000 random input orders of the runs. To visualise estimated membership coefficients for each of the 698 colonies, we used the program *DISTRUCT* (Rosenberg 2004).

To test for restricted dispersal within sites, we performed autocorrelation analyses in SPAGEDI version 1.2 (Hardy & Vekemans 2002) to evaluate whether pairwise relatedness (*R*, Queller & Goodnight 1989) decreased with increasing distance between colonies. Analyses were performed for *SB* and *CA* where all colonies had been mapped. To avoid bias from potential clonal replicates, identical MLG were removed from analyses of relatedness (randomly keeping one per group).

To test whether selfing contributed to the observed heterozygote deficits, we carried out parentage analyses with CERVUS version 3.0 (Kalinowski et al. 2007) and tested with simulations whether the inferred selfing rates differed significantly from zero. This approach provided estimated selfing rates for *PAL1*, *HS*, *SB*, *CA* and *HB* where juveniles as well as adults had been sampled. Details on this analysis are provided in the Appendix.

Results

The total number of alleles per locus ranged from six for Sh3-007 to 34 for Sh4.24 (mean: 15.66, Table 1). Allelic richness (El Mousadik & Petit 1996) across loci varied between 4.393 (*PAL1*) and 5.008 (*HS*) at Lizard Island and between 5.937 (*NWW*) and 7.929 (*HB*) at Heron Island (standardised to a minimum sample size of 23). Private alleles occurred at six out of nine sites (Table 1). Expected heterozygosities varied between 0.351 (Sh3-007) and 0.854 (Sh4.24).

Within-population structure

Observed heterozygosities, averaged across sites, ranged from 0.249 (Sh3-007) to 0.609 (Sh4.24, Table 1). Individual F_{IS} values ranged from -0.167 (Sh3-004 at *NWW*) to 0.787 (Sh2-006 at *CA*). Averages across loci and per site tended to be lower at Lizard Island

(range: 0.138 to 0.178) compared to Heron Island (0.306 to 0.369, except *NWW*: 0.140). They were all significant with one exception (*PAL2* at Lizard Island).

Tests for pairwise linkage disequilibria (*D*) showed 32 significant inter-locus associations out of 120 pairwise comparisons (adjusted 5% α-level = 0.00037). These occurred at six out of nine sites (Table 1). Note that all significant *D* estimates were observed at Heron Island sites with high heterozygote deficits (F_{IS} > 0.3, see above). Across all sites, *D* values were significant for all pairwise inter-locus associations except Sh4-001/Sh2-006 and Sh2-006/Sh3-007.

As expected, the null-corrected data for each site were in Hardy-Weinberg equilibrium (max. F_{IS} per site: 0.024 at *SR*). However, 29 pairwise disequilibria at four Heron Island sites remained significant: *T* (2/15), *SB* (10/15), *CA* (6/15), *HB* (11/15). Removal of identical MLG (see below) resulted in very similar F_{IS} values but a considerably lower number of significant pairwise inter-locus associations [16 in total, *SB* (7/15), *HB* (7/15), *CA* (1/15), *T* (1/15)].

Population differentiation

There was strong differentiation between the two regions (F_{ST} = 0.305, max. distance: 1185 km), moderate differentiation among sites at Heron Island (F_{ST} = 0.093, max. distance: 10.6 km) and very little differentiation among Lizard Island sites (F_{ST} = 0.006, max. distance: 1.6 km). In most cases, somewhat higher differentiation estimates were obtained for R_{ST} (Tables 3a, 4). Among Heron Island sites, no significant correlation between geographic and genetic distance was detected (F_{ST}: r = 0.6370, P = 0.1341; R_{ST}: r = 0.3727, P = 0.3072).

F_{ST} estimates based on the null-corrected data set were typically about 10% lower, but the pattern of strong, moderate and low differentiation for the three comparisons above remained unchanged. Thus, null alleles had at most a small effect on our differentiation estimates.

Asexual reproduction

At sites *SR* and *NWW* all colonies were genotypically distinct (N_g/N = 1.00, Table 2). At all other sites, groups of identical MLG occurred. In most cases, a given MLG was shared by two colonies, but larger groups of identical MLG were also found at *HB*, *SB* and *T* (max. group size: six). When individuals with a single missing genotype were included, even one group of eight identical MLG was present (*HB*). Identical MLG were also shared between sites (data not shown). The simulations of purely sexual, biparental reproduction showed that groups of size two were likely to arise by chance at each site. The occurrence of larger groups, however, was much less probable (Table 2). The observed groups of size six at *T, SB* and *HB* argue against purely sexual, biparental reproduction when stringent significance criteria are applied (cf. Table 2).

Admixture

Bayesian clustering analysis with TESS indicated the presence of ten clusters (Fig. 2, Table 5). We considered genotypes that were assigned to a particular cluster with membership coefficients $q \geq 0.8$ as clearly associated with that cluster and referred to all other genotypes as unassigned. Figure 2 gives a graphical representation of proportionate cluster membership for all individuals. This analysis revealed striking differences between the Lizard and Heron Island sites. TESS inferred the existence of four clusters for *PAL1, HS* and *PAL2* at Lizard Island. On average the three stands were assigned to each of three of these clusters with equal proportions. Overall, however, none of the colonies were clearly associated with a particular cluster (exception: five colonies at *HS*, see below). In other words, TESS found at best a subtle level of within-population substructure, and the clustering pattern was largely shared among all three sites. The latter observation agrees with the very low F_{ST} estimates among them.

For the Heron Island sites, another six clusters were proposed by TESS and five of these contained a core group of individuals that were clearly associated with them. Per site,

more than half of the colonies clearly belonged to a specific cluster (except *T*: 29%) and a minimum of four well defined clusters were present (except *NWW*: two clusters). Overall, the composition of samples varied markedly in terms of cluster membership (Fig. 2, Table 5). While the adjacent sites *SB* and *CA* showed a similar genetic structure dominated by cluster 2, *HB* was clearly structured by clusters 1, 6 and 8. Site *SR* included large proportions of clusters 3 and 6. Cluster 6 was also important at *T*. The geographically most distant site *NWW* was genetically most distinct from the others and homogeneous, with over 90% of colonies assigned to cluster 1 (Table 5). Analyses based on the dataset corrected for nulls gave very similar results (data not shown).

The TESS analysis identified an important component of population structure, as evidenced by the following observations: At Heron Island, differentiation among the five main clusters was much stronger (F_{ST} = 0.441, based on assigned colonies only) than among sites (F_{ST} = 0.093). Mean heterozygote deficit, on the other hand, was much reduced within the clusters (F_{IS} = 0.086, compared to 0.302 within sites), yet still significant ($p < 0.001$, Table 3b). A considerably lower number of significant pairwise inter-locus associations was found within clusters (2/42) than within sites (32/90). Furthermore, parentage analysis with CERVUS suggested that cluster membership was 'inherited'. For the juvenile colonies that had been assigned to a specific cluster (*N* = 73), the most likely parents belonged to the same cluster in 75% of all cases. At least one most likely parent had been assigned to the same cluster as its putative offspring in 91% of all cases.

First-generation immigrants

The identification of first generation immigrants is best accomplished in an otherwise panmictic population. Clearly, this ideal is not fulfilled here. In fact, the overall population substructure, in particular at the Heron Island sites, can be explained by colonisation from several genetically distinct sources in the not too distant past (see Discussion). Two observations suggest recent immigration: At the highly homogeneous Lizard Island sites (*PAL1*, *HS* and

PAL2), five out of 258 colonies were assigned to a separate genetic cluster (cluster 4) with $q \geq 0.99$. No other colony was assigned to that cluster at any site. At *NWW* (Heron Island), all colonies with $q \geq 0.8$ belonged to cluster 1, except for two colonies that were assigned to cluster 6. This cluster was well represented at other Heron Island sites.

Spatially restricted dispersal within sites

If sperm and larvae typically dispersed over very small distances (on the scale of a few decimetres), this would generate spatial aggregation of closely related individuals and cause inbreeding within sites. The spatial distribution of colonies at sites *SB* and *CA* is shown in Figure 3. At *SB*, there was indeed a significant trend of decreasing pairwise relatedness (*R*) with distance between colonies (Fig. 4). In particular, a significant positive autocorrelation amongst colonies occurred in the 0-2 m distance class. Significant negative autocorrelations were found between colonies that were 6 to 10 m apart. At *CA*, the autocorrelations were non-significant.

The autocorrelation effects at *SB* could be due to spatial aggregations of genetic clusters and thus possibly due to inhomogeneous settlement of larvae from different sources during the colonisation stage. If, however, pairwise relatedness declined with distance for pairs of colonies that belonged to the *same* cluster*,* this would directly implicate spatially restricted dispersal within sites. Closer inspection of the *SB* data suggests that both components were present: a) pairs of colonies assigned to the same cluster were more frequent over small as opposed to large distances and b) there was a clear trend that pairwise relatedness within clusters declined with distance (Fig. 5). At *CA*, there was no indication of any spatial aggregation of clusters, but pairwise relatedness within clusters did drop off with distance (data not shown). Here, more uniformly distributed genetic clusters may have overshadowed an existing pattern of spatially limited dispersal.

Inbreeding and selfing

The parentage analysis provided clear evidence for selfing at four of five sites. Considering the most likely parent pairs, CERVUS inferred that between 14% (*PAL1*) and 40% (*CA*) of the juvenile colonies at each site resulted from selfing (Table 6). Simulations (cf. Appendix) showed that these estimates differed significantly from the expectation of pure outcrossing in all but one site (*PAL1*). Furthermore, these conclusions were fully supported by simulations with the null-corrected data sets (Table 6).

Discussion

In the present study of nine *Seriatopora hystrix* stands on the Great Barrier Reef we observed marked within-population genetic structure in terms of heterozygote deficits and pairwise linkage disequilibria. These deviations from equilibrial, random mating conditions were particularly large at Heron Island sites. In contrast, sites at Lizard Island were much more homogeneous and only showed small deviations from Hardy-Weinberg-equilibrium. Pronounced local heterozygote deficits were also detected in most previous studies on *S. hystrix*, i.e. on the GBR (Ayre & Dufty 1994, Ayre & Hughes 2000, Sherman 2008, van Oppen et al. 2008), northern Western Australia (Underwood et al. 2007, Underwood et al. 2009) and the Red Sea (Maier et al. 2005). They are, in fact, common also in other sessile marine invertebrates (e.g. Johnson & Black 1984, Calderón et al. 2007). We investigated four population processes that could be responsible for them. As we will argue, Wahlund effects, inbreeding due to restricted dispersal of sperm and larvae, and selfing all contributed to the observed patterns, albeit with large variation between sites. Asexual reproduction played at most a marginal role at any site. Our observations also shed light on dispersal and colonisation processes of *S. hystrix* at different locations.

Population history and connectivity

The within-population genetic structure at the three Lizard Island sites is remarkably similar as seen by a comparison of allele frequencies (F_{ST} = 0.006), average heterozygote deficits and multilocus associations (viz. clustering patterns). This suggests that their gene pools are shaped by similar processes. Ongoing and frequent gene flow among them appears to be the most likely explanation. As a possible alternative, consider colonisation from the same source or from genetically similar source populations without subsequent gene flow. This would involve stochastic effects in the make-up of propagules that settle, in the present case, up to 1.6 km apart, including on either side of a small island (cf. Johnson & Black 2006). The initial level of differentiation would be augmented by subsequent genetic drift within sites. And if one allows, very plausibly, for a more diverse set of source populations, even greater differentiation would be expected. Given the extremely low F_{ST} estimates, any such colonisation scenario without ongoing gene flow appears unlikely. Possibly, the hydrological conditions around Lizard Island, situated in the Great Barrier Reef Lagoon, promote the retention of larvae and favour a well-mixed gene pool (Frith et al. 1986). Finally, the observed weak population substructure may be due to an internal process such as selfing that operates similarly within each site.

The spatial extent of the gene pool that comprises the three sampled Lizard Island sites remains unknown. It certainly does not include all *Seriatopora hystrix* stands around Lizard Island, because van Oppen et al. (2008) sampled three different sites in the same area and found much larger pairwise F_{ST} estimates (0.06 to 0.31). The five presumptive immigrants at *HS* could therefore in principle have come from sites nearby.

The six sites around Heron Island, all situated at the reef slope, presented a very different picture. Strong heterozygote deficits, abundant pairwise linkage disequilibria and clear clustering patterns indicated strong population substructure, and the sites were clearly differentiated (average F_{ST} = 0.093). All observations are consistent with recent colonisation from a limited number of genetically distinct source populations. The varying representation of

clusters among sites (cf. Table 5) and the lack of a spatial autocorrelation of allele frequencies may be due to the stochasticity associated with the colonisation process. Currents that vary within a season and across years (Johnson & Black 2006) and the prolonged release of larvae by *Seriatopora hystrix* over several months per year (Harrison & Wallace 1990) may contribute importantly to this stochasticity. The hypothesis of recent colonisation agrees with known aspects of *S. hystrix* life history: it is an opportunistic species and often among the first settlers in recently disturbed areas (Underwood et al. 2007).

Genetic variability among settling larvae has been documented in other marine invertebrates, e.g. bivalves (David et al. 1997), limpets (Johnson & Black 1984) or polychaetes (Virgilio et al. 2006). In contrast, immigrants identified in two *Seriatopora hystrix* stands from the Red Sea were most likely a well-mixed sample from surrounding sites, because they did not cause any detectable population substructure (F_{IS} < 0.042, ns, Maier et al. 2009).

As in the case of Heron Island, the sources of immigrants may be fairly close, as judged by the high levels of differentiation: The maximum pairwise F_{ST} estimate at Heron Island was 0.25. The average was almost identical to estimates of among-site differentiation in *Seriatopora hystrix* from Western Australia (F_{ST}: 0.095, max. distance: 60 km, Underwood et al. 2007) and from the Red Sea (F_{ST} = 0.094, max. distance: 20 km, Maier et al. 2005). Our results are also comparable to previous allozyme studies of *S. hystrix* on similar spatial scales on the GBR (F_{ST} = 0.3 and 0.28, Ayre & Dufty 1994, Ayre & Hughes 2000), when we compute Hedrick's (2005) standardised genetic differentiation parameter (mean F'_{ST} = 0.22). The recurrence of such differentiation on relatively small spatial scales further supports the hypothesis that local extinctions and recolonisations are a key feature of *S. hystrix* population dynamics.

While Wahlund effects can explain a large part of the observed population substructure at Heron Island, other factors must contribute as well. There were still significant heterozygote deficits within TESS clusters (mean F_{IS} = 0.086) as there were at the Lizard Island sites (F_{IS} = 0.138 to 0.178). Moreover, while F_{IS} is expected to decline to zero in the first off-

spring generation under random mating, heterozygote deficits were still present amongst juveniles (data not shown). We therefore consider in the following three life-history features of *Seriatopora hystrix* that may also contribute.

Asexual reproduction

Sets of identical multilocus genotypes were found in all but two sites, but only in three instances, these sets differed significantly from the expectation of biparental sexual reproduction. This agrees with previous findings that *Seriatopora hystrix* reproduces largely sexually (e.g. Maier et al. 2005, 2009, Underwood et al. 2007, 2009, Noreen et al. 2009). Some identical multilocus genotypes may have been generated by asexual larvae (van Oppen et al. 2008), polyp bail-out (Sammarco 1982) or fragmentation (Ayre & Dufty 1994, Ayre & Hughes 2000). Alternatively, even the significant cases can be explained by selfing (see below). If in fact *S. hystrix* is a 'weedy' species with high rates of population turnover, then one of the proposed advantages of asexual reproduction, i.e. the preservation of locally adapted genotypes, may be irrelevant. And selfing provides an alternative solution to the problem of mate limitation that colonisers may face.

In a companion study, we discovered a large number of genetically heterogeneous colonies across all nine study sites. Specifically, 27 out of 155 colonies that were sampled at two or three positions included more than one genotype (Maier et al., ms). We therefore expect some genotypes to be missed even in the intensively sampled sites. Consequently, the size of some groups of identical MLGs may have been underestimated and additional small groups may have been missed entirely. However, our tests are valid for a given sampling effort and, hence, the conclusion that asexual reproduction is, at best, rare remains true.

Selfing

Our approach to estimate selfing rates at the population level is based on the most likely parent pair for a given juvenile as determined by CERVUS (Kalinowski et al. 2007). As we show in the Appendix, this inference holds information about the kinds of matings that take place (i.e. selfing versus outcrossing), even though the resolution of the dataset is insufficient to determine actual parent individuals with confidence. Our approach illustrates that added information about population processes can be gained by using genealogical data in the widest sense (Pemberton 2008).

Specifically, we compared per site the observed juvenile age category with simulated offspring cohorts based on purely outcrossed, random mating in terms of the proportion of colonies that CERVUS deems to be selfed. Current methods to estimate selfing rates either depend on the screening of progeny arrays (Ritland 2002) or they employ a population model of partially selfing individuals which yields a characteristic level of heterozygote deficit under equilibrium conditions (Pollak 1987). The latter approach attributes the observed heterozygote deficit solely to selfing and therefore leads at all but one site (*HS* at Lizard Island) to considerably higher selfing estimates than the CERVUS analysis (Table 6). As it stands, our analyses demonstrate substantial levels of selfing at 4/5 sites (approx. 30 to 40% of offspring). Note that the non-significant site had both the smallest juvenile cohort and the smallest number of markers such that the power to detect selfing was low.

Given that sperm dispersal is likely to be highly localised, one might argue that the assumption of random mating in the simulations is not appropriate: in the real dataset, small-scale clustering of related individuals as observed at *SB* (see below) might inflate the inferred selfing rates relative to our null hypothesis. However, selfing imposes stringent criteria on the feasible genotypes of parent and offspring (cf. Appendix). In our simulations, fewer than 5% of outcrossed offspring were wrongly classified as selfed. And even in the smallest distance class at both mapped sites (*CA* and *SB*), fewer than 0.5% of adult colony pairs had $R = 1$ and would thus produce only offspring that appear to be selfed.

While intracolonial genetic variation would need to be considered in true *parentage* analyses, it does not invalidate our conclusions regarding selfing: firstly, we performed CERVUS analyses allowing for high error rates of 0.025, and, secondly, we derived only population level estimates of *mating patterns*. Since selfing retards the breakdown of heterozygote deficits generated by Wahlund effects, it is an important contributor to the observed population structure.

Considerable selfing rates were also found in *Seriatopora hystrix* from One Tree Island on the GBR, with large variation between individual colonies (Sherman 2008). Moreover, selfing was reported from two brooding corals *Favia fragum* and *Porites astreoides* (Brazeau et al. 1998) as well as the broadcast spawner *Goniastrea favulus* (Stoddart et al. 1988). In brooding species, outcrossing relies on sperm transfer between colonies, which means that limited sperm motility, sperm dilution and restricted lifespan of sperm should restrict successful outcrossed fertilisation to colonies in close proximity (e.g. Levitan & Petersen 1995, but see Ayre & Miller 2006). Thus, it seems likely that selfing presents an important reproductive strategy in *S. hystrix* that contributes to the species' high colonisation success.

Small-scale dispersal

Especially in brooders, restricted dispersal of larvae should cause heterozygote deficits (Harrison & Wallace 1990). We found a significant positive autocorrelation within the smallest distance class of 0-2 m at *SB*, indicating spatial clustering of related colonies. Colony pairs separated between 6 and 10 m were significantly less related than expected under the null hypothesis. At *CA*, no significant autocorrelation was found. We propose that two factors contribute to these effects at *SB*: First, the proportion of colony pairs assigned to the same cluster decreased with distance. This indicates spatial clumping at the level of clusters, possibly due to non-homogeneous colonisation and/or small-scale dispersal of selfed offspring. Second, the level of relatedness within clusters decreased with distance, which clearly points

towards small-scale larval dispersal. The latter effect was also found at *CA*, albeit without spatial clumping of genetic clusters. Possibly, *SB* and *CA* show similar effective larval dispersal distances, but at *CA* positive autocorrelation effects are overshadowed by a more homogeneous cluster distribution. A significant autocorrelation within stands was also detected in *Seriatopora hystrix* from Western Australia on scales from 0 to 20 m (Underwood et al. 2007). But note that no such effects were found within two *S. hystrix* stands from the Red Sea (Maier et al. 2009).

The contribution of small-scale dispersal to the observed heterozygote deficits may depend on how long a population has existed because it takes time to build up local kin groups. At highly structured sites such as *SB* and *CA*, the overwhelming majority (80 to 90%) of outcrossed matings within the smallest distance class involved colonies that were not assigned to the same cluster. Thus even highly localised biparental reproduction should mainly erode heterozygote deficits due to Wahlund effects in the short run. In the longer term, however, the formation of kin groups due to small-scale larval dispersal may cause a renewed build-up of heterozygote deficits. This suggests that selfing currently represents the more important mechanism that maintains the signature of recent colonisation for longer than expected under biparental random mating.

Conclusions

Our analyses suggest that the population substructure at all nine study sites results from a combination of external factors and life-history attributes of *Seriatopora hystrix*. The similarity among the three Lizard Island sites points towards ongoing gene flow that might be facilitated by the lagoon setting and that may have eroded admixture effects due to colonisation in the not too distant past. Whether the observed weak clustering pattern is a remnant of such events or results from selfing remains at present an open question. Selfing is expected to contribute to the significant heterozygote deficit at site *HS*, whilst its occurrence at site *PAL1* is uncertain. Null alleles cannot fully account for the observed population structure, because

the null-corrected dataset produced nearly identical clustering patterns. The pronounced population substructure of the Heron Island sites stems most likely from a combination of recent colonisation from genetically distinct source populations and selfing. Both factors are required, because on the one hand, F_{IS} estimates were still significant within the TESS clusters and on the other hand, the estimated selfing rates were too low to explain all of the observed heterozygote deficit (cf. Table 6). As before, null alleles do not provide an alternative explanation, because they failed to account for the clustering patterns and about 50% of linkage disequilibria. Small-scale dispersal of larvae, as demonstrated at *SB*, also generates heterozygote deficit, as it builds up local kin groups with every new cohort following colonisation. Overall, immigration is important at all nine sites, but appears to be more stochastic around Heron Island. It is currently an open question whether these *S. hystrix* populations differ appreciably in any life-history features. The observed differences in population structure might be entirely due to varying population histories and/or current patterns that channel the arrival of immigrants.

Intriguing insights could be gained from high-resolution parentage analyses that provide accurate estimates of selfing rates and sperm dispersal distances. Our findings raise important questions for reef conservation: Are there any sites that act consistently as either source or sink populations? Over what distances does recolonisation occur after local extinction? Answering these questions presents a challenge for future studies that is crucial for reef conservation (Palumbi 2003, van Oppen & Gates 2006). Coupling genetic analyses with oceanographic models (e.g. Galindo et al. 2006) could greatly improve our knowledge about reef connectivity.

References

Atoda K (1951) The larva and postlarval development of the reef-building corals. V. *Seriatopora hystrix* Dana. Science Reports of the Tohoku University, 4th series (Biology) 19: 33-39

Ayre DJ, Dufty S (1994) Evidence for restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia`s Great Barrier Reef. Evolution 48:1183-1201

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54:1590-1605

Ayre DJ, Miller K (2006) Random mating in the brooding coral *Acropora palifera*. Mar Ecol Prog Ser 307:155-160

Ayre DJ, Resing JM (1986) Sexual and asexual production of planulae in reef corals. Mar Biol 90:187-190

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. Mol Ecol 11:155-165

Baums IB (2008) A restoration genetics guide for coral reef conservation. Mol Ecol 17:2796-2811

Brazeau DA, Gleason DF, Morgan ME (1998) Self-fertilization in brooding hermaphroditic Caribbean corals: evidence from molecular markers. J Exp Mar Biol Ecol 231:225-238

Calderón I, Ortega N, Duran S, Becerro M, Pascual M, Turon X (2007) Finding the relevant scale: clonality and genetic structure in a marine invertebrate (*Crambe crambe*, Porifera). Mol Ecol 16:1799-1810

Carlon DB (1999) The evolution of mating systems in tropical reef corals. Trends Ecol Evol 14:491-495

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621-631

Chen C, Durand E, Forbes F, François O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. Mol Ecol Notes 7:747-756

David P, Perdieu M-A, Pernot A-F, Jarne P (1997) Fine-grained spatial and temporal population genetic structure in the marine bivalve *Spisula ovalis*. Evolution 51:1318-1322

Dempster AP, Laird NM, Rubin DB (1977) Maximum likelihood from incomplete data via the EM algorithm. J Royal Stat Soc 39:1-38

El Mousadik A, Petit RJ (1996) High levels of genetic differentiation for allelic richness among populations of the argan tree [Argania spinosa (L.) Skeels] endemic to Morocco. Theor Appl Genetics 92:832-839

François O, Ancelet S, Guillot G (2006) Bayesian clustering using hidden Markov random fields in spatial population genetics. Genetics 174:805-816

Frith, CA, Leis JM, Goldman B (1986) Currents in the Lizard Island region of the Great Barrier Reef Lagoon and their relevance to potential movements of larvae. Coral Reefs 5: 81-92

Galindo HM, Olson DB, Palumbi SR (2006) Seascape genetics: a coupled oceanographic-genetic model predicts population structure of Caribbean corals. Curr Biol 16:1622-1626

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2), Available at: http://www2.unil.ch/popgen/softwares/fstat.htm

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618-620

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystems of the World: Coral Reefs, pp. 133-207. Elsevier Publishers, Amsterdam

Hedrick PW (2005) A standardized genetic differentiation measure. Evolution 59:1633-1638

Hughes TP, Baird AH, Bellwood DR et al. (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301:929-933

Isomura N, Nishihira M (2001) Size variation of planulae and its effect on the lifetime of planulae in three pocilloporid corals. Coral Reefs 20:309-315

Jackson JBC (1986) Modes of dispersal of clonal benthic invertebrates: consequences for species' distributions and genetic structure of local populations. Bull Mar Sci 39: 588-605

Jakobsson M, Rosenberg NA (2007) *CLUMPP*: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801-1806

Johnson MS, Black R (1984) The Wahlund effect and the geographical scale of variation in the intertidal limpet *Siphonaria* sp. Mar Biol 79:295-302

Johnson MS, Black R (2006) Islands increase genetic subdivision and disrupt patterns of connectivity of intertidal snails in a complex archipelago. Evolution 60:2498-2506

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099-1106

Levitan DR, Petersen C (1995) Sperm limitation in the sea. Trends Ecol Evol 10:228-231

Magalon H, Adjeroud M, Veuille M (2005) Patterns of genetic variation do not correlate with geographical distance in the reef-building coral *Pocillopora meandrina* in the South Pacific. Mol Ecol 14:1861-1868

Maier E, Tollrian R, Nürnberger B (2001) Development of species-specific markers in an organism with endosymbionts: microsatellites in the scleractinian coral *Seriatopora hystrix*. Mol Ecol Notes 1:157-159

Maier E, Tollrian R, Rinkevich B, Nürnberger B (2005) Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea. Mar Biol 147:1109-1120

Maier E, Tollrian R, Nürnberger B (2009) Fine-scale analysis of genetic structure in the brooding coral *Seriatopora hystrix* from the Red Sea. Coral Reefs 28:751-756

Mantel N (1967) The detection of desease clustering and a generalized regression approach. Cancer Res 27:209-220

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639-655

Meirmans PG (2006) Using the AMOVA framework to estimate a standardized genetic differentiation measure. Evolution 60:2399-2402

Miller KJ, Ayre DJ (2008) Population structure is not a simple function of reproductive mode and larval type: insights from tropical corals. J Anim Ecol 77:713-724

Noreen AME, Harrison PL, van Oppen MJH (2009) Genetic diversity and connectivity in a brooding reef coral at the limit of its distribution. Proc R Soc B 276:3927-3935

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecol Appl 13 (Suppl.):S146-S158

Pemberton JM (2008) Wild pedigrees: the way forward. Proc R Soc B 275:613-621

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. Mol Ecol 4:249-252

Pollak E (1987) On the theory of partially inbreeding finite populations. I. Partial selfing. Genetics 117:353-360

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258-275

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248-249. Available at: http://genepop.curtin.edu.au/

Ritland K (2002) Extensions of models for the estimation of mating systems using *n* independent loci. Heredity 88:221-228

Rosenberg NA (2004) *Distruct*: a program for the graphical display of population structure. Mol Ecol Notes 4:137-138

Rousset F (1996) Equilibrium values of measures of population subdivision for stepwise mutation processes. Genetics 142:1357-1362

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145:1219-1228

Sammarco PW (1982) Polyp bail-out: an escape response to environmental stress and a new means of reproduction in corals. Mar Ecol Prog Ser 10:57-65

Sherman CDH (2008) Mating system variation in the hermaphroditic brooding coral, *Seriatopora hystrix*. Heredity 100:296-303

Stimson JS (1978) Mode and timing of reproduction in some common hermatypic corals of Hawaii and Enewetak. Mar Biol 48:173-184

Stoddart JA, Babcock RC, Heyward AJ (1988) Self-fertilisation and maternal enzymes in the planulae of the coral *Goniastrea favulus*. Mar Biol 99:489-494

Underwood JN, Smith LD, van Oppen MJH, Gilmour JP (2007) Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. Mol Ecol 16:771-784

Underwood JN, Smith LD, van Oppen MJH, Gilmour JP (2009) Ecologically relevant dispersal of corals on isolated reefs: implications for managing resilience. Ecol Appl 19:18-29

Underwood JN, Souter PB, Ballment ER, Lutz AH, van Oppen MJH (2006) Development of ten polymorphic microsatellite markers from herbicide bleached tissues of the brooding pocilloporid coral *Seriatopora hystrix*. Mol Ecol Notes 6:176-178

van Oppen MJH, Gates RD (2006) Conservation genetics and the resilience of reef-building corals. Mol Ecol 15:3863-3883

van Oppen MJH, Lutz A, De'ath G, Peplow L, Kininmonth S (2008) Genetic traces of recent long-distance dispersal in a predominantly self-recruiting coral. PLoS ONE 3(10):e3401

Veron JEN (2000) Corals of the World. Australian Institute of Marine Science, Townsville

Virgilio M, Backeljau T, Abbiati M (2006) Mitochondrial DNA and allozyme patterns of *Hediste diversicolor* (Polychaeta: Nereididae): the importance of small scale genetic structuring. Mar Ecol Prog Ser 326:157-165

Wahlund S (1928) Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas 11:65-106

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358-1370

Whitaker K (2004) Non-random mating and population genetic subdivision of two broadcasting corals at Ningaloo Reef, Western Australia. Mar Biol 144:593-603

Wright S (1965) The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution 19:395-420

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Appendix

Estimating selfing rates with CERVUS

Ever since its first release over ten years ago (Marshall et al. 1998), the computer program CERVUS (Kalinowski et al. 2007) has been the most widely used package to determine parentage in natural populations based on multilocus genotypes. For a given offspring, it determines the most likely mother-father-offspring trio from a list of potential parents. The mother's genotype may be either known or unknown. Statistical confidence for assignment is based on the difference Δ in LOD scores of the most likely and the second most likely trio. Simulations are used to determine thresholds for Δ that correspond to a desired level of confidence (e.g. 80% or 95% confidence of correct assignment).

The resolution of our *Seriatopora hystrix* dataset in terms of the number of loci and their allelic variation is insufficient for confident parentage assignment for all but a very small number of juvenile colonies. Nevertheless, the CERVUS analysis does provide information on the kinds of matings (selfed versus outcrossed) that have occurred. To demonstrate this, we simulated mating among the observed adult genotypes within each of the five sites that were exhaustively sampled. Note that this maintained the actual genotypic structure in each adult cohort. In each replicate run, a proportion *s* of the offspring was generated by selfing whereas each of the remaining offspring was derived from two randomly chosen adult genotypes. CERVUS was then used to assign parentage to the simulated offspring. Per site, the simulated number of offspring was equal to the observed number of juveniles. All adult genotypes of a site were considered as candidate maternal or paternal parents, with selfing allowed. The inferred selfing rate was determined as the proportion of simulated offspring for which the most likely parent pair featured the same colony twice.

In the CERVUS analysis, we used the improved likelihood functions of Kalinowski et al. (2007). The genotyping error rate was set to 0.025. We chose this relatively high value,

because in addition to the typical sources of error such as scoring mistakes and null alleles, we needed to accommodate intracolonial genetic variation (Maier et al., submitted). Most cases of within-colony heterogeneity involve variation at one locus only and are consistent with somatic mutations rather than colony fusion. All identified distinct genotypes within colonies were included in the list of potential parents. For groups of colonies with identical multilocus genotypes, only one representative was retained in this list.

Figure A1 shows the results for simulated selfing rates ranging between 0 and 1 for the two sites that gave the best (*HB,* six loci, Fig. A1a) and the worst (*PAL1,* five loci, Fig. A1b) correspondence between simulated and inferred selfing rates. The curves for the other three sites are qualitatively the same and most closely resemble the plot shown here with the same number of loci. The CERVUS inference is more accurate for low selfing rates, whereas it underestimates *s* when selfing is the predominant mode of reproduction. This is true especially for the two sites in which five rather than six loci were scored. Note that the success of parentage assignment shows a trend in the opposite direction (top panel in each plot of Fig. A1): the proportion of offspring for which the most likely parents are the true ones reaches maxima of 85% and 55% for pure selfing at sites *HB* and *PAL1*, respectively.

These results show that outbred offspring are only rarely mistaken as selfed in our CERVUS analyses even though the success of parentage assignment for them is low. This presumably reflects the stringent requirements that selfing places on the feasible genotypes of parent and offspring: an offspring genotype cannot have any alleles that are not present in the parent and, consequently, an offspring genotype cannot have more heterozygous loci than its parent. Especially when allelic variation is high and/or many loci have been typed, it is unlikely that an outbred individual fits in this manner to any other genotype in the population. These considerations also imply that even with the limited level of resolution provided by our dataset, we can test for the occurrence of selfing against the null hypothesis of pure outcrossing with good statistical power.

On the other hand, selfed offspring are more often wrongly considered as outbred by CERVUS, presumably because the particular subset of alleles present in their genotype can alternatively be assembled from a variety of genotype pairs. Our results for sites with five versus six loci suggest that this error becomes smaller as the number of loci increases. In fact, it may disappear as more loci are typed, because greater genetic resolution will increase the chances that the true parent(s) attain(s) the top LOD score. In our simulations, correct inferences of selfing are mostly based on a maximum LOD score for the correct parent, which explains why the success of parentage assignment increases with the selfing rate.

Simulations with null alleles

Null alleles influence these analyses because they feign the effects of selfing by increasing the number of observed homozygotes. We studied their possible effect for the case of pure outcrossing. As before, the hypothetical dataset with inserted null alleles (generated with the software FREENA, Chapuis & Estoup 2007, see above) was used to generate simulated offspring. Null alleles were inherited in a Mendelian fashion, but offspring genotypes were coded as follows: (a,null) → (a,a) and (null,null) → missing data. The CERVUS analysis was then carried out with the simulated offspring genotypes and with the original adult genotypes. In other words, the null alleles remained invisible in the CERVUS analysis, as they would in any real dataset. The results are shown in Table 6. As expected, null alleles generate higher inferred selfing rates with larger error bars for purely outcrossed offspring. Thus, they reduce the power to demonstrate selfing. As we argue above, this test is overly conservative in the context of our study, because FREENA attributes the entire observed heterozygote deficit to null alleles, whereas we have shown that other effects also contribute.

Figure Legends

Fig. 1 Map of the northeastern coast of Australia showing the location of *Seriatopora hystrix* sampling sites. *PAL1*: Palfrey 1; *HS*: Horseshoe Reef; *PAL2*: Palfrey 2; *T*: Tenements II; *SB*: Staghorn Bank; *CA*: Canyons; *HB*: Harry's Bommie; *SR*: South Reef; *NWW*: North-West-Wistari. Key to inserts: Lizard Island: dark shading: continental islands, light shading: fringing reef; Heron Island: dark shading: cay, light shading: platform reef

Fig. 2 Results of the Bayesian clustering analyses in TESS. Each of the 698 *Seriatopora hystrix* colonies is represented by a thin vertical line that is divided into *K* coloured segments, visualising the colony's membership coefficient in *K* clusters. In total, ten clusters are distinguishable, represented by distinct colours.

Fig. 3 Spatial distribution of *Seriatopora hystrix* colonies at *SB* and *CA*. The size of the colonies reflects colony diameter (in five size classes). Different colours denote cluster membership: cluster 1: white, 2: blue, 3: red, 6: green, 7: turquoise, 8: pink, admixed ($q < 0.8$): grey. For clarity, Fig 3a shows only a detail of *SB* including 75% of colonies at this site. Maximum coordinates: x (0.40,12.25); y (1.45,10.30).

Fig. 4 Spatial autocorrelation analyses of pairwise relatedness at sites *SB* and *CA*.
r = autocorrelation coefficient. Observed values are given in black. Error bars correspond to one standard deviation of the null hypothesis (white symbols). Significant values: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

Fig. 5 Components of the distance effect at site *SB*. *R* = pairwise relatedness. Top panel: Proportion of all colony pairs in a given distance class that have been assigned to the same

TESS cluster. Bottom panel: R_{mean} for all colony pairs in a given distance class that have been assigned to the same TESS cluster.

Appendix

Fig. A1 Selfing rates as inferred by CERVUS for simulated offspring for two sites, **a.** *HB* and **b.** *PAL1*. See text for details. For each simulated selfing rate, 50 replicate offspring cohorts were generated. The number of offspring in each cohort was equal to the observed number of juvenile colonies within the respective site. The bottom panels show the mean inferred selfing rates ±1 standard deviation. The top panels show the proportions of inferred most likely parents that were the true parents over all replicates.

Tables

Table 1 Summary of genetic variation at six *Seriatopora hystrix* microsatellite loci, including sample sizes (N), the number of alleles per locus (N_A), allelic richness (A_R, El Mousadik & Petit 1996), based on a minimum sample size of 23 individuals, expected (H_e) and observed (H_o) heterozygosity, F_{IS} (Weir & Cockerham 1984) as well as null allele frequency (p_0) according to Dempster et al. (1977). Also given is the number of private alleles at each site (N_{PA}) as well as the number of significant inter-locus associations in relation to the total number of pairwise comparisons for each site (D). Locus Sh4.24 was not analysed for Lizard Island samples (*na*).

*Indicates significance at a nominal α-level of 0.05, $P \leq 0.00093$

		Lizard Island			*Heron Island*						
Locus		***PAL1***	***HS***	***PAL2***	***T***	***SB***	***CA***	***HB***	***SR***	***NWW***	***Overall***
	N	106	120	32	30	125	108	106	34	37	698
Sh2-002	N_A	6	14	6	9	12	10	12	10	8	18
	A_R	5.870	8.094	5.754	8.492	8.383	7.664	9.935	9.116	7.580	7.876
	H_e	0.725	0.638	0.667	0.751	0.728	0.757	0.874	0.792	0.790	0.747
	H_o	0.604	0.508	0.467	0.828	0.576	0.743	0.667	0.500	0.486	0.598
	F_{IS}	0.167	0.203*	0.300	-0.103	0.209*	0.019	0.238*	0.369*	0.385*	0.199
	p_0	0.070	0.085	0.114	0.040	0.110	0.075	0.137	0.147	0.176	0.106
Sh2-006	N_A	7	6	5	5	6	7	6	6	3	11
	A_R	4.722	4.470	5.547	7.578	6.337	5.938	8.403	8.208	4.676	6.209
	H_e	0.670	0.662	0.646	0.439	0.618	0.672	0.480	0.640	0.312	0.571
	H_o	0.530	0.496	0.531	0.185	0.200	0.143	0.277	0.419	0.306	0.343
	F_{IS}	0.209	0.251*	0.177	0.578*	0.676*	0.787*	0.423*	0.345	0.020	0.385
	p_0	0.083	0.111	0.092	0.267	0.283	0.493	0.265	0.230	0.043	0.207
Sh3-004	N_A	8	7	6	8	8	8	9	9	5	11
	A_R	4.240	4.669	3.000	3.980	4.854	5.935	6.031	5.843	5.552	4.900
	H_e	0.494	0.569	0.607	0.727	0.713	0.698	0.846	0.724	0.630	0.668
	H_o	0.392	0.541	0.469	0.517	0.577	0.476	0.610	0.545	0.735	0.540
	F_{IS}	0.207	0.049	0.228	0.289	0.190*	0.318*	0.279*	0.247	-0.167	0.182
	p_0	0.091	0.078	0.055	0.174	0.112	0.141	0.160	0.086	0.000	0.100

Sh3-007	N_A	4	3	4	3	4	5	3	3	5	6
	A_R	4.414	4.652	4.976	4.704	4.762	5.661	4.880	5.615	2.956	4.736
	H_e	0.522	0.561	0.531	0.217	0.161	0.223	0.433	0.351	0.160	0.351
	H_o	0.413	0.383	0.500	0.167	0.065	0.106	0.230	0.235	0.139	0.249
	F_{IS}	0.208	0.316	0.059	0.233	0.599	0.525	0.468	0.330	0.134	0.319
	p_0	0.124	0.137	0.005	0.070	0.155	0.160	0.181	0.111	0.000	0.105
Sh4-001	N_A	6	8	3	4	7	10	8	6	6	14
	A_R	2.717	3.155	3.438	2.987	3.337	3.639	2.998	2.991	4.023	3.254
	H_e	0.657	0.680	0.616	0.459	0.314	0.303	0.665	0.515	0.724	0.548
	H_o	0.588	0.637	0.677	0.296	0.137	0.137	0.353	0.344	0.583	0.417
	F_{IS}	0.105	0.063	-0.099	0.355	0.564*	0.548*	0.470*	0.332	0.194	0.281
	p_0	0.072	0.048	0.000	0.139	0.152	0.148	0.172	0.083	0.080	0.099
Sh4.24	N_A	*na*	*na*	*na*	12	24	22	21	11	11	34
	A_R	*na*	*na*	*na*	12.000	12.559	13.458	15.328	10.639	10.832	12.469
	H_e	*na*	*na*	*na*	0.825	0.838	0.873	0.913	0.850	0.822	0.854
	H_o	*na*	*na*	*na*	0.304	0.786	0.727	0.727	0.400	0.708	0.609
	F_{IS}	*na*	*na*	*na*	0.631*	0.062	0.166*	0.204*	0.529*	0.139	0.289
	p_0	*na*	*na*	*na*	0.320	0.067	0.088	0.127	0.271	0.077	0.158
Multilocus	A_R	4.393	5.008	4.543	6.624	6.705	7.049	7.929	7.069	5.937	
	N_{PA}	0	5	1	0	4	3	2	0	2	
	Mean H_e	0.614	0.622	0.613	0.570	0.562	0.588	0.702	0.645	0.573	
	Mean H_o	0.505	0.513	0.529	0.383	0.390	0.389	0.477	0.407	0.493	
	F_{IS}	0.178*	0.175*	0.138	0.328*	0.306*	0.339*	0.320*	0.369*	0.140*	
	D	0/10	0/10	0/10	4/15	11/15	4/15	12/15	1/15	0/15	32/120

Table 2 Genotypic diversity in *Seriatopora hystrix*. Listed are the sample sizes (*N*) as well as the ratio of the observed number of distinct multilocus genotypes (N_g) over the sample size (*N*). *Obs(i)* denote observed numbers of groups with *i* identical multilocus genotypes. Given that there are missing data, identity was judged based on complete multilocus genotypes only or, in parentheses, on comparisons with at least *n-1* informative loci (*n* = total number of loci for that sampling site). In the latter case, the minimum value of N_g was given. *Sim(i)* denote the proportion of simulated samples with at least the observed number of groups of size i (complete genotypes only). Significant *P*-values (at an 'experiment-wide' α of 0.05) are printed in bold.

	Lizard Island			*Heron Island*					
	PAL1	***HS***	***PAL2***	***T***	***SB***	***CA***	***HB***	***SR***	***NWW***
N	87 (104)	104 (117)	29 (32)	19 (27)	105 (117)	55 (93)	68 (89)	26 (32)	23 (33)
N_g/N	0.99 (0.94)	0.94 (0.93)	0.97 (0.97)	0.68 (0.74)	0.71 (0.72)	0.87 (0.80)	0.85 (0.85)	1.00 (0.97)	1.00 (1.00)
Obs(2)	1 (6)	6 (8)	1 (1)	1 (2)	8 (9)	7 (12)	5 (6)	- (1)	-
Sim(2)	0.958	0.976	0.472	0.174	0.649	0.500	0.019	0.113	0.079
Obs(3)	-	-	-	-	4 (5)	1 (2)	-	-	-
Sim(3)	0.289	0.409	0.053	0.011	0.067	0.040	≤ 0.0001	0.006	0.001
Obs(4)	-	-	-	-	3	-	-	-	-
Sim(4)	0.055	0.107	0.008	≤ 0.001	0.007	0.004	≤ 0.0001	≤ 0.001	≤ 0.0001
Obs(6)	-	-	-	**1**	**1**	- (1)	**1**	-	-
Sim(6)	0.002	0.008	≤ 0.001	**≤ 0.0001**	**≤ 0.001**	≤ 0.0001	**≤ 0.0001**	≤ 0.0001	≤ 0.0001
Obs(8)	-	-	-	-	-	-	- (1)	-	-
Sim(8)	≤ 0.001	≤ 0.001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001

Table 3 Genetic subdivision in *Seriatopora hystrix* based on five microsatellite loci (excluding locus Sh4.24). Given are Weir and Cockerham's (1984) f (= F_{IS}, the inbreeding coefficient), θ (= F_{ST}, the fixation index), Hedrick's (2005) standardised differentiation measure F'_{ST} and Rousset's (1996) R_{ST}. Linkage disequilibria (D) are shown as the number of significant associations relative to all possible pairwise comparisons. *S. hystrix* samples were grouped according to **a.** all sampling sites *(PAL1)* (*HS) (PAL2) (T*) *(SB) (CA) (HB) (SR) (NWW)*; three Lizard Island sites (*PAL1*) (*HS*) (*PAL2*); six Heron Island sites (*SB*) (*CA*) (*HB*) (*SR*) (*NWW*); two regions, Lizard/Heron I. (*PAL1*, *HS*, *PAL2*) (*SB*, *CA*, *HB*, *SR*, *NWW*) and **b.** five TESS clusters (all clusters including a minimum of 28 colonies with membership coefficients $q \geq 0.8$. Standard errors (s.e.) are based on jackknifing over loci. For consistency, all estimates involving Lizard Island sites are based on five loci.

Grouping	F_{IS} **(s.e.)**	F_{ST} **(s.e.)**	F'_{ST}	R_{ST}	***D***
a.					
Nine sampling sites	0.271 (0.050)	0.236 (0.048)	0.559	0.291	32/120
Lizard Island sites	0.172 (0.034)	0.006 (0.005)	0.016	0.004	0/30
Heron Island sites	0.302 (0.069)	0.093 (0.010)	0.238	0.144	32/90
Lizard/ Heron I.	0.308 (0.049)	0.305 (0.078)	0.786	0.371	not applicable
b.					
TESS clusters	0.086 (0.027)	0.441 (0.088)	0.754	0.751	2/42

Table 4 Pairwise estimates of F_{ST} (below diagonal) and R_{ST} (above diagonal) between *S. hystrix* sampling sites.

	Lizard Island			***Heron Island***					
Site	***PAL1***	***HS***	***PAL2***	***T***	***SB***	***CA***	***HB***	***SR***	***NWW***
PAL1		0.006	-0.003	0.404	0.565	0.589	0.170	0.424	0.209
HS	0.009		0.006	0.328	0.503	0.524	0.139	0.349	0.185
PAL2	0.005	0.000		0.424	0.602	0.634	0.181	0.448	0.253
T	0.343	0.320	0.362		0.040	0.061	0.063	-0.026	0.188
SB	0.380	0.357	0.387	0.094		-0.004	0.216	0.033	0.370
CA	0.376	0.354	0.379	0.107	0.005		0.241	0.057	0.417
HB	0.273	0.258	0.282	0.042	0.101	0.113		0.075	0.023
SR	0.317	0.299	0.324	0.008	0.065	0.067	0.048		0.194
NWW	0.286	0.283	0.318	0.174	0.230	0.251	0.095	0.167	

Table 5 TESS cluster membership of nine *Seriatopora hystrix* sites, based on two different criteria. **a.** The upper value in each row represents the mean proportion (averaged across 250 TESS runs) of each stand that was assigned to a specific cluster. Values smaller than 0.05 were omitted. Cluster 4 never included more than 5% of a stand and was thus left out. **b.** The lower value shows the proportion of colonies that were on average assigned to a specific cluster with $q \geq 0.8$. If this proportion was larger than 0.15, the cell was highlighted in grey.

	Lizard Island			***Heron Island***					
Cluster	***PAL1***	***HS***	***PAL2***	***T***	***SB***	***CA***	***HB***	***SR***	***NWW***
1				0.06 0.03	0.06 0.06		0.18 0.18	0.08 0.06	0.91 0.91
2					0.35 0.30	0.39 0.32	0.05 0.02		
3				0.11 0.03	0.13 0.11	0.21 0.10		0.34 0.32	
5	0.31 0	0.33 0	0.32 0						
6				0.17 0.17	0.07 0.07	0.07 0.07	0.33 0.33	0.21 0.18	0.06 0.06
7				0.21 0	0.29 0.06	0.15 0.01	0.14 0.04	0.16 0	
8				0.41 0.07	0.08 0.02	0.13 0.06	0.24 0.17	0.16 0.03	
9	0.33 0	0.31 0	0.35 0						
10	0.35 0	0.32 0	0.32 0						

Table 6 Summary of the selfing analysis in *Seriatopora hystrix* using CERVUS (Kalinowski et al. 2007). For each of the exhaustively sampled sites, the proportion of juveniles is listed that was classified by CERVUS as selfed. Also shown are the results of two sets of simulations. **a.** The same number of juveniles as observed was generated under the assumption of biparental random mating (cf. Appendix for details). 50 replicates were run for each site. For these, the mean and maximum inferred selfing rates are listed. The minimum was zero in all cases. **b.** As for *a.*, except that the null-corrected dataset was used to generate the offspring genotypes (cf. Appendix). Minima ranged between 0.02 and 0.05. The last column lists the selfing rate *s* estimated from F_{IS} (Pollak 1987): $s = 2 / [(1 / F_{IS}) + 1]$

			Proportion of selfed offspring as inferred by CERVUS					
			Observed data	**a. Simulations of pure outcrossing**		**b. Simulations with null alleles**		**Estimate from F_{IS}**
Site	**#Juveniles**	**#Adults**		**Mean**	**Max**	**Mean**	**Max**	
PAL1	22	89	0.14	0.086	0.21	0.132	0.27	0.302
HS	48	71	0.29	0.074	0.14	0.128	0.22	0.297
SB	26	77	0.35	0.043	0.18	0.105	0.29	0.469
CA	48	54	0.40	0.037	0.10	0.130	0.28	0.506
HB	39	61	0.26	0.020	0.12	0.117	0.23	0.485

Figures

Fig. 1

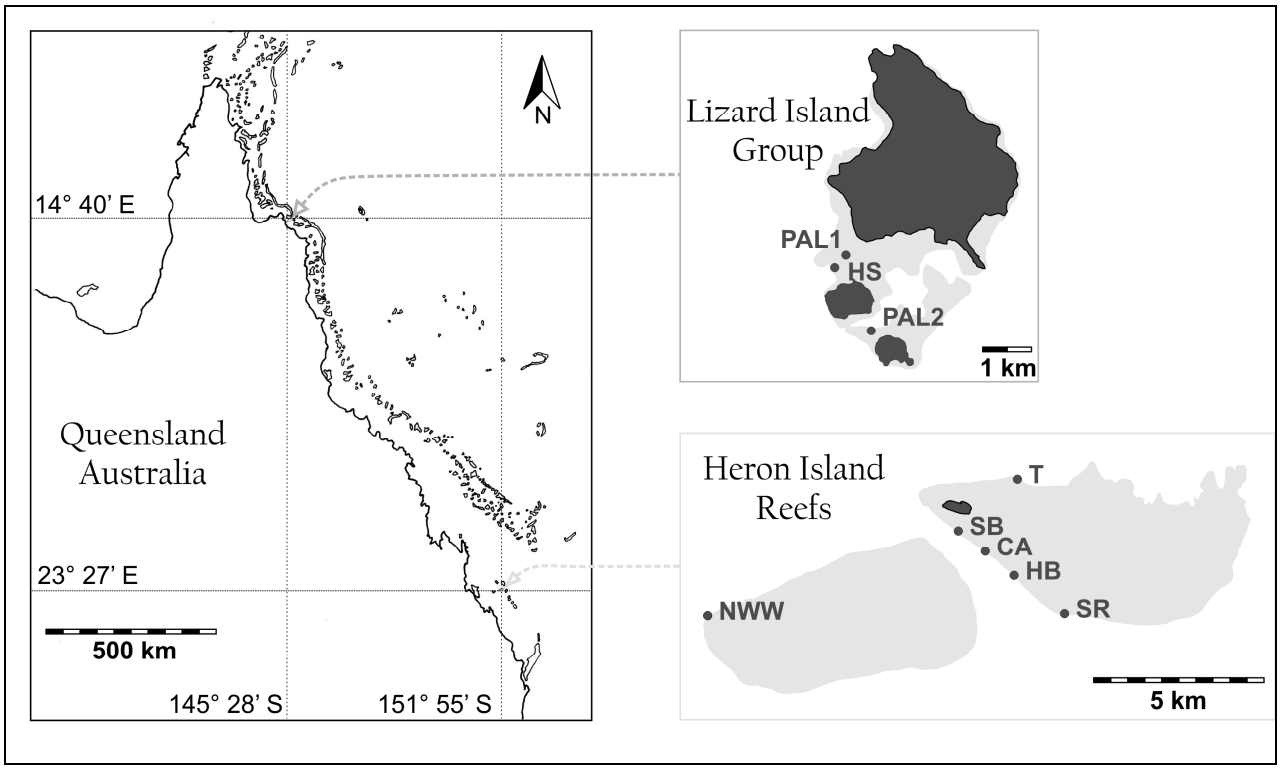


Fig. 2

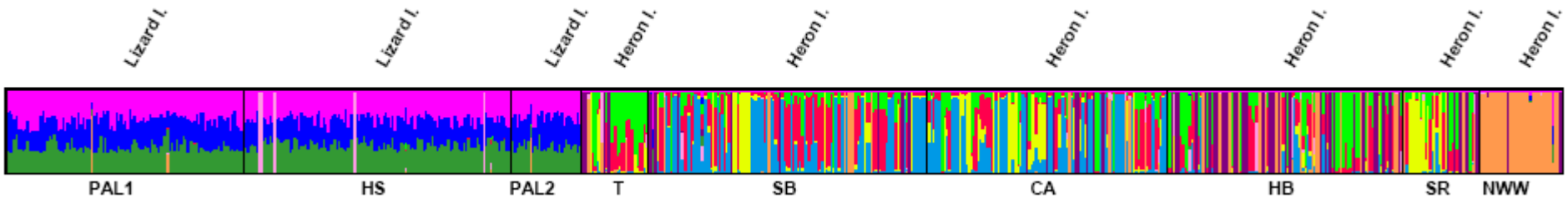

Lizard I.
Lizard I.
Lizard I.
Heron I.
Heron I.
Heron I.
Heron I.
Heron I.
Heron I.
PAL1
HS
PAL2
T
SB
CA
HB
SR
NWW

Fig. 3

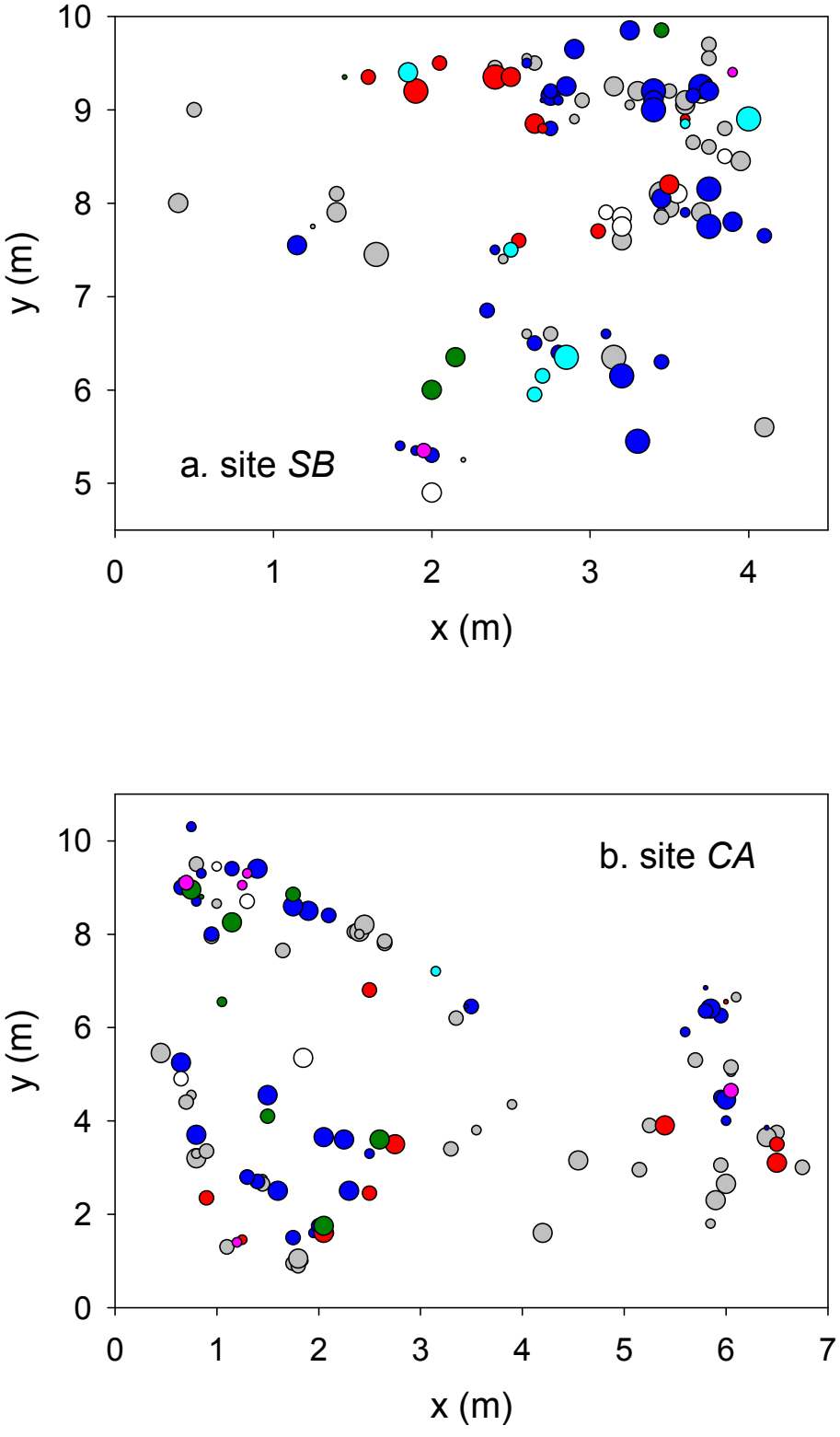


Fig. 4

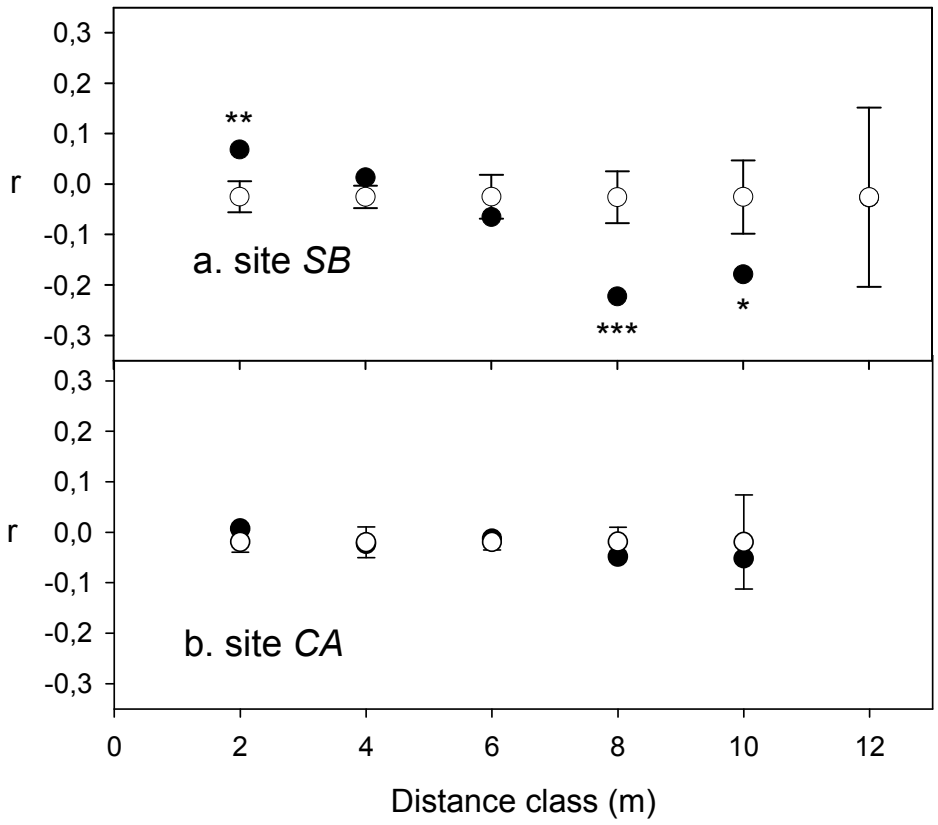
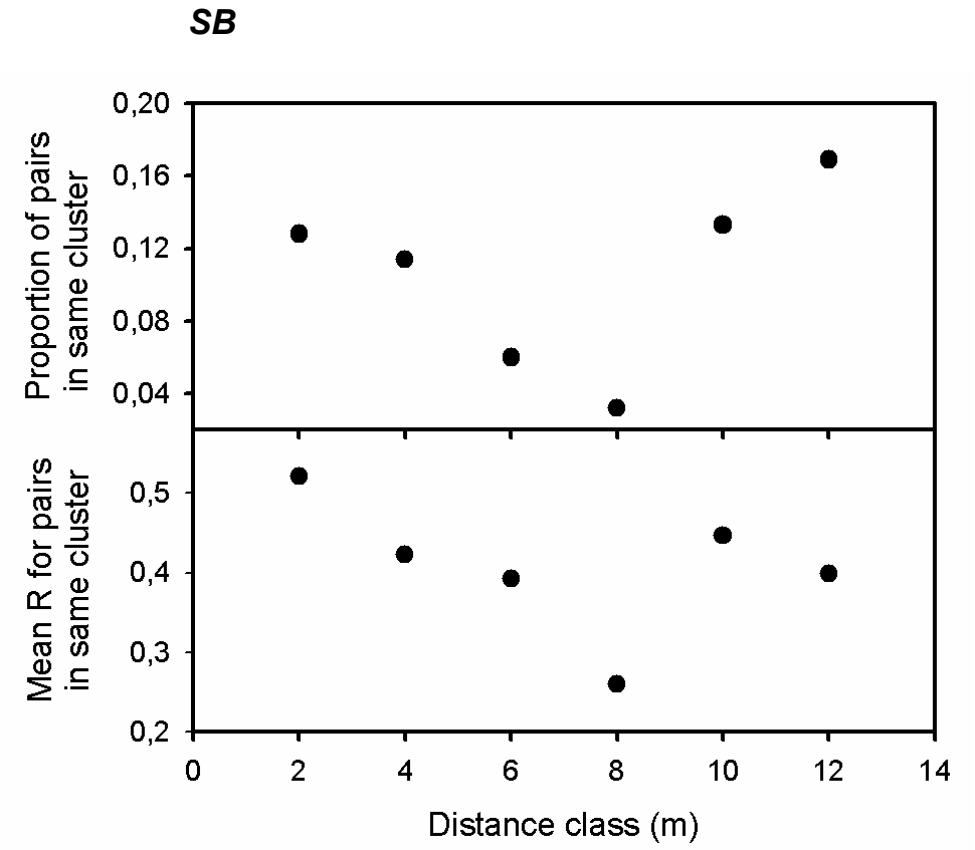
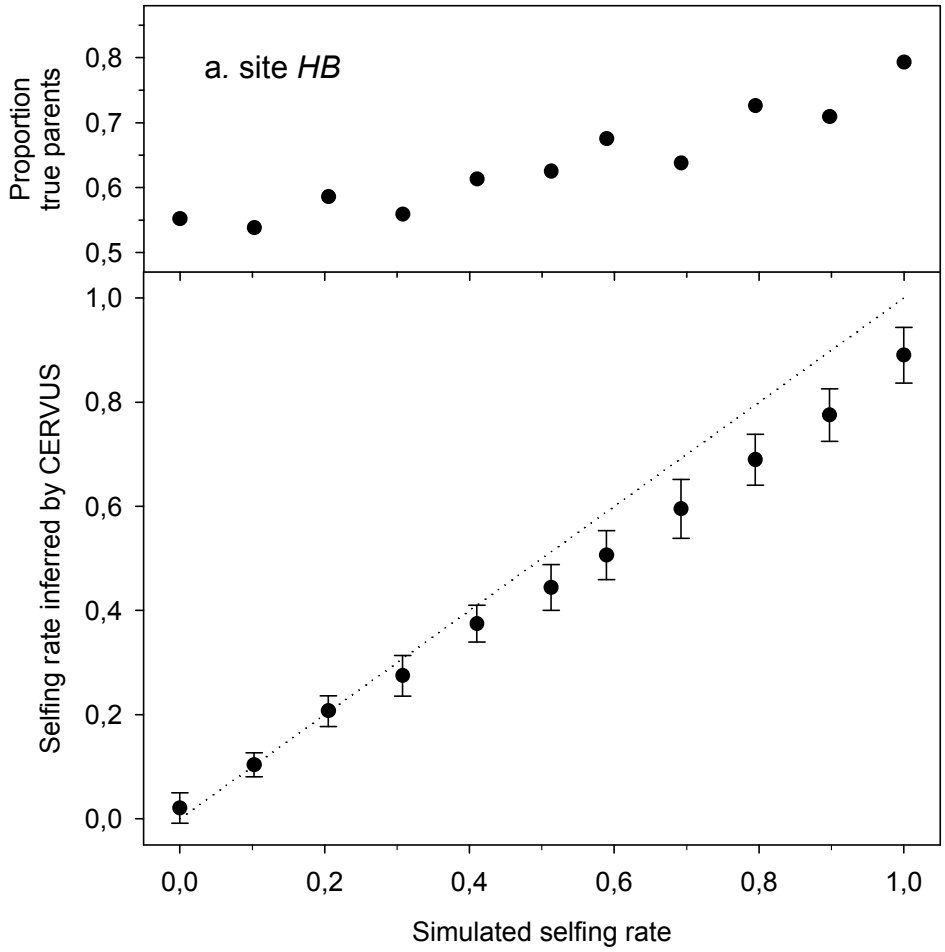

0,3
0,2
0,1
0,0
-0,1
-0,2
-0,3
r
**
a. site SB
***
*
b. site CA
0
2
4
6
8
10
12
Distance class (m)

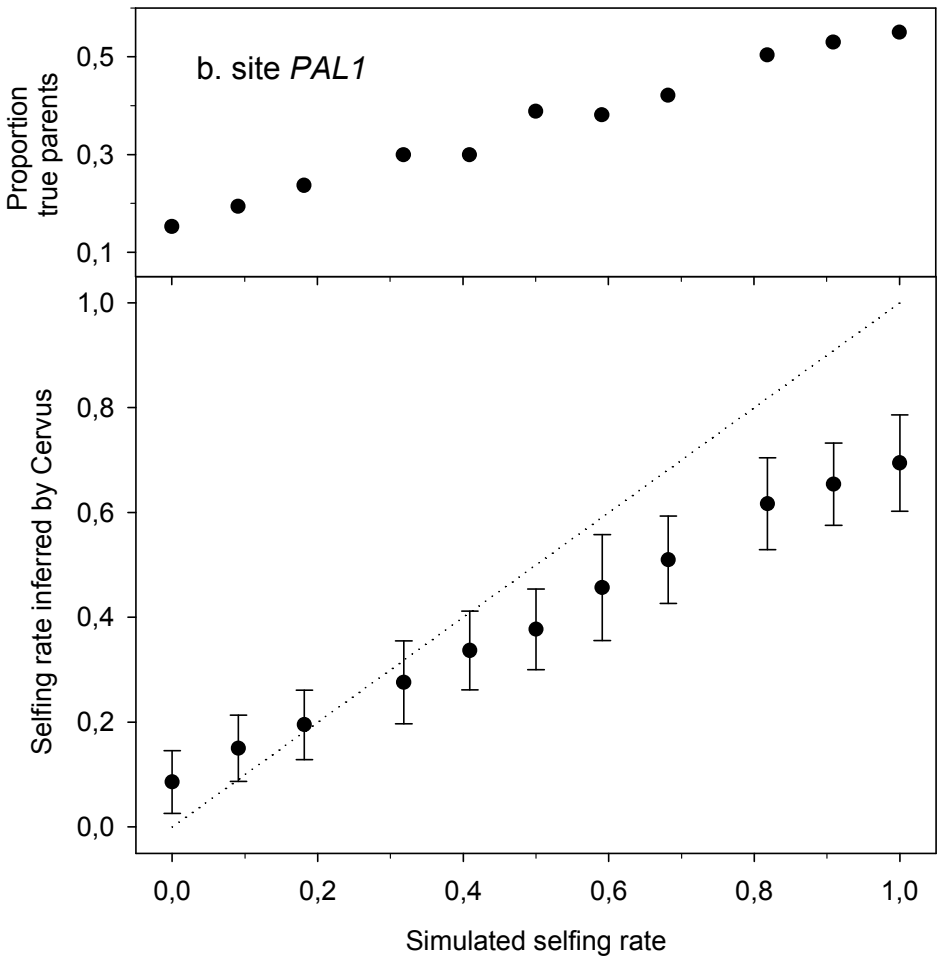
Fig. 5


SB
Proportion of pairs in same cluster
0,20
0,16
0,12
0,08
0,04
Mean R for pairs in same cluster
0,5
0,4
0,3
0,2
0
2
4
6
8
10
12
14
Distance class (m)

Appendix

Fig. A1




b. site *PAL1*
Proportion true parents
0,1
0,3
0,5
Selfing rate inferred by Cervus
0,0
0,2
0,4
0,6
0,8
1,0
Simulated selfing rate

ACKNOWLEDGEMENTS

PERSONAL NOTES

DECLARATION

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This thesis is dedicated to my family, with many thanks for their support.

PERSONAL NOTES

Curriculum Vitae

Name	Elke Maier
Date and place of birth	9th July 1974 in Munich, Germany
Nationality	German

Working and Teaching Experience

08/2009 - present	Work as a freelancer with the public relations department of the Max Planck Society Headquarters
06/2009 - 07/2009	Internship with the public relations department of the Max Planck Society Headquarters
02/2009 - 05/2009	Internship with the publisher "oekom Verlag", followed by one month work as a freelancer
2001 - 2008	Research assistant at the Institute of Ecology, Division of Evolutionary Ecology (Ludwig-Maximilians-Universität Munich)
2006 - 2007	Teaching mandate in the seminar "Coral Reef Ecology" and in the practical course "Biology and Ecology of Corals"
2000 - 2004	Teaching assistant in the practical field courses "Ecology of the Mediterranean" in Sardinia, Italy and "Coral Reef Ecology" in Dahab, Egypt, including leading of dive groups, each course two weeks at a time

Education

1994 - 2000	Study of biology at the Ludwig-Maximilians-Universität Munich; major: zoology, minors: ecology, anthropology & human genetics, palaeontology; final grade: 1.0
1981 - 1994	Primary school and grammar school in Munich, graduation: Abitur; grade: 2.0

Grants

07/2008	DFG travel grant for visiting the "11th International Coral Reef Symposium", Fort Lauderdale, USA
09/2005 - 08/2006	Grant within the HWP Program (Ludwig-Maximilians-Universität Munich)
07/2001 - 06/2003	Minerva Fellowship (Max Planck Society)

Presentations

07/2008	"11th International Coral Reef Symposium", Fort Lauderdale, USA: "Population genetic structure of the scleractinian coral *Seriatopora hystrix* as revealed by microsatellites: patterns of reproduction and dispersal" (poster presentation)
12/2006	Invited talk: "Reproductive strategies in the scleractinian coral *Seriatopora hystrix*: a population genetic approach". Ruhr-University Bochum, Germany
11/2006	VW-workshop "Advances in molecular evolutionary ecology" Evangelische Akademie Tutzing, Germany: "Reproduction and dispersal in the scleractinian coral *Seriatopora hystrix*" (poster presentation)

08/2005	"10th Congress of the European Society for Evolutionary Biology" in Cracow, Poland: "Patterns of reproduction and dispersal in the scleractinian coral *Seriatopora hystrix*: a population genetic approach" (poster presentation)
03/2001	"7th Meeting of PhD Students in Evolutionary Biology" in Bernried, Germany: "Microsatellites in *Seriatopora hystrix*: a new method to investigate the clonal structure of coral populations" (poster presentation)

Publications & Manuscripts

Maier E, Tollrian R, Nürnberger B (2001) Development of species-specific markers in an organism with endosymbionts: microsatellites in the scleractinian coral *Seriatopora hystrix*. *Molecular Ecology Notes*, 1, 157-159.

Maier E, Tollrian R, Rinkevich B, Nürnberger B (2005) Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea. *Marine Biology*, 147, 1109-1120.

Maier E, Tollrian R, Nürnberger B (2009) Fine-scale analysis of genetic structure in the brooding coral *Seriatopora hystrix* from the Red Sea. *Coral Reefs*, 28, 751-756.

Maier E, Buckenmaier A, Tollrian R, Nürnberger B. Intracolonial genetic variation in the scleractinian coral *Seriatopora hystrix*. Submitted to *Molecular Ecology*.

Maier E, Tollrian R, Nürnberger B. Genetic population structure of the scleractinian coral *Seriatopora hystrix* on the Great Barrier Reef: patterns of reproduction and dispersal. To be submitted to *Marine Ecology Progress Series*.

Reviewer

Invited reviewer for *Coral Reefs*

DECLARATION

Erklärung

Diese Promotion wurde im Sinne §12 der Promotionsordnung von PD Dr. Beate Nürnberger betreut. Ich erkläre hiermit, dass die Dissertation keiner anderen Prüfungskommission vorgelegt worden ist und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

Ehrenwörtliche Versicherung

Ich versichere hiermit, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt wurde.

München, den 02. März 2010

Elke Maier

Beiträge der Koautoren

PD Dr. Beate Nürnberger[1,2,3,4,5] betreute die Dissertation, insbesondere die Laborarbeiten, und half bei der Auswertung der Daten (Paper 1 bis 5). Sie entwickelte Computerprogramme zur Erstellung von Nullallel-korrigierten Datensätzen (Paper 3) sowie zur Abschätzung von Selbstbefruchtungsraten (Paper 4 & 5). Sie trug zur Interpretation und Diskussion der Ergebnisse bei und half beim Verfassen der Manuskripte (Paper 1 bis 5).

Prof. Dr. Ralph Tollrian[1,2,3,4,5] half, die Probensammlung zu konzipieren, betreute die Freilandarbeiten in Ägypten (Paper 2 & 3) und Australien (Paper 4 & 5) und beteiligte sich an Kartierung und Beprobung. Er trug zur Diskussion der Ergebnisse bei und kommentierte die Manuskripte (Paper 1 bis 5).

Prof. Dr. Baruch Rinkevich[2] stellte die notwendige Infrastruktur für die Probensammlung in Eilat (Israel) bereit und half als Kooperations- und Ansprechpartner vor Ort. Er beteiligte sich an der Diskussion der Ergebnisse und kommentierte das Manuskript (Paper 2).

Andreas Buckenmaier[4] entwickelte Computerprogramme zur Analyse genetisch heterogener Korallenkolonien, um somatische Mutationen von Fusionen verschiedener Individuen zu unterscheiden. Er trug zur Interpretation der Daten bei und beteiligte sich am Verfassen des Manuskripts (Paper 4).

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[1]Paper 1: Development of species-specific markers in an organism with endosymbionts: microsatellites in the scleractinian coral *Seriatopora hystrix* (Maier E, Tollrian R, Nürnberger B, *Molecular Ecology Notes*, 1, 157-159, 2001.)

[2]Paper 2: Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea (Maier E, Tollrian R, Rinkevich B, Nürnberger B, *Marine Biology*, 147, 1109-1120, 2005.)

[3]Paper 3: Fine-scale analysis of genetic structure in the brooding coral *Seriatopora hystrix* from the Red Sea (Maier E, Tollrian R, Nürnberger B, *Coral Reefs*, 28, 751-756, 2009.)

[4]Paper 4: Intracolonial genetic variation in the scleractinian coral *Seriatopora hystrix* (Maier E, Buckenmaier A, Tollrian R, Nürnberger B, submitted to *Molecular Ecology*.)

[5]Paper 5: Genetic population structure in the scleractinian coral *Seriatopora hystrix* on the Great Barrier Reef: patterns of reproduction and dispersal (Maier E, Tollrian R, Nürnberger B, to be submitted to *Marine Ecology Progress Series*.)