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T. Ridgway · O. Hoegh-Guldberg · D.J. Ayre

Panmixia in *Pocillopora verrucosa* from South Africa

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Abstract The genetic structure of six local collections of *Pocillopora verrrucosa* from six coral reefs in KwaZulu-Natal, South Africa, was examined using allozyme electrophoresis. The six separate reefs lie within two different reef complexes. Twenty-two enzymes were screened on five buffer systems, but only five polymorphic loci (*Gpi-1, Gdh-1, Lgg-2, Lpp-1, Est-1*) could be consistently resolved. No significant differences in allelic frequencies were detected among the six sites. All local collections were genotypically diverse, with evidence of only very limited clonal replication at each site. Indeed, the ratio of observed to expected genotypic diversity (mean $Go:Ge = 0.64 \pm 0.05$ SD), the ratio of observed number of genotypes to the number of individuals (mean $Ng:N = 0.65 \pm 0.04$ SE), and deviations from the Hardy–Weinberg equilibrium indicate that sexual reproduction plays a major role in the maintenance of the populations. No genetic differentiation was found either within ($FSR = 0.026 \pm 0.003$ SE) or between ($FRT = 0.000 \pm 0.001$ SE) reef complexes. The homogeneity of the gene frequencies across the six reefs strongly supports the assumption that the KwaZulu-Natal reef complexes are highly connected by gene flow ($Nem = 44$). The reefs in the southern and central reef complexes along the northern Maputaland coastline can therefore be considered part of a single population.

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T. Ridgway (✉) · O. Hoegh-Guldberg
Centre for Marine Studies, University of Queensland,
St. Lucia, Qld. 4072, Australia

E-mail: tyrone@uq.edu.au

D.J. Ayre
Australian Flora and Fauna Research Centre,
Department of Biological Sciences,
University of Wollongong, Wollongong, NSW 2522,
Australia

Introduction

The coral-reef communities that line the Maputaland coastline in northern KwaZulu-Natal (27°50′S, South Africa) are at the south-western extremity of the Indo-Pacific reef fauna. These coral reefs are also among the most southerly of any in the world. They are small reefs (<20 km^2 in total area) based on a fossilized substratum of submerged dune and beachrock systems (Ramsay and Mason 1990) and therefore lack most of the geomorphological traits typical of coral reefs growing at lower latitudes. Nevertheless, these reefs are diverse (90 species of coral) with a strong component of endemic genera and species of coral (Riegl 1993). Furthermore, the reefs are among South Africa's major tourist attractions (over 120,000 dives logged per year) and provide an important source of revenue for conservation. However, as with other reefs in the Western Indian Ocean (WIO) region, very few scientific studies have been conducted on the South African reefs. This is despite the fact that local conservation and management authorities are highly concerned about the degradation of the coral communities under the increasing local and global stresses. A crucial issue is the need to understand the extent to which these reefs, and others to the north, are interconnected by larval dispersal. Current management plans assume that reefs in this region are interconnected, yet there is a lack of information as to the extent to which these reefs are self-seeding or dependent upon recruitment from external sources. This study addresses this issue for reef systems that range over most of the coral coast of north-eastern South Africa.

The need to understand patterns of larval dispersal is especially acute for the high-latitude southern African reefs, because they are currently managed on the basis of the untested assumption that it is sufficient to exclude human activities on the most northern and southern of the three reef complexes. The northern reefs are expected to provide sufficient widely dispersed larvae to maintain the diversity of the central and southern complexes

within the St. Lucia and Maputaland Marine Reserves (Fig. 1). At present, no human activities are permitted in the northern and southern reef complexes, with all the diving and fishing effort being concentrated on the central (Sodwana) complex. In addition, there is increasing user-group pressure to open up these areas to recreational and subsistence harvesting, which would place further pressure on the health of these reefs.

Attempts to manage or conserve coral populations world-wide are hampered by the complexity of coral life histories. Even within a species, corals may utilize multiple modes of sexual and of asexual reproduction (Ward 1992) and display highly variable longevity of individual colonies and clones. Asexual reproduction may occur through various forms of fission and fragmentation (reviewed by Highsmith 1982) and may include the production of planula larvae (Stoddart 1983; Ayre and Resing 1986). Sexual reproduction may occur through broadcast spawning or internal fertilization and production of brooded planulae (Harrison and Wallace 1990), and both may occur within a single species (Stoddart and Black 1985; Ward 1992 – both studies in the eastern Indian Ocean at high latitudes, therefore sharing some of the characteristics of the present study). There is considerable debate about the ecological roles of larvae generated by each of these processes. However, genetic evidence from corals of Australia's Great Barrier Reef (GBR) implies that populations of some brooding and broadcasting corals are at least partially maintained by localized settlement of larvae and yet produce sufficient widely dispersed larvae to maintain high levels of gene flow along the GBR (Ayre and Hughes 2000). Nevertheless, brooding and broadcast-spawning species appear unable to maintain high levels of gene flow to the isolated coral reefs of Lord Howe Island (some 2,000 km south-east; Ayre and Hughes, in preparation).

The current study uses genetic (allozyme) data to infer the predominant modes of reproduction and strength of larval connections within the central and southern reef complexes of northern KwaZulu-Natal for the coral *Pocillopora verrucosa* Ellis and Solander 1780. *Pocillopora verrucosa* was chosen because it is locally abundant in each of the three reef complexes. It is a broadcast-spawning coral (Kruger and Schleyer 1998) with a branching morphology, and this life history seems likely to favour widespread larval dispersal (see Ayre and Hughes 2000).

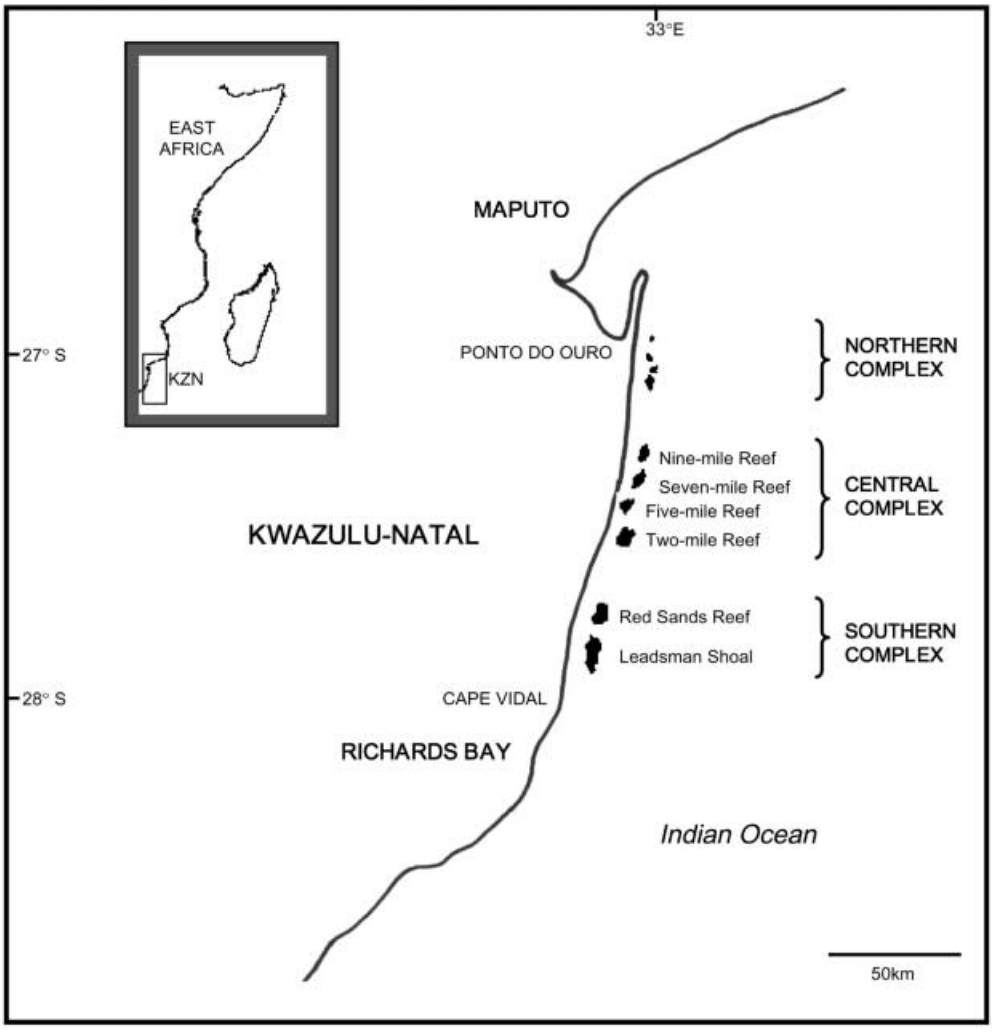

Fig. 1 Location of the six sampling sites of *Pocillopora verrucosa* along the coast of northern KwaZulu-Natal, South Africa

Materials and methods

Sample collection

Samples of *Pocillopora verrucosa* were collected from six sites within the central and southern reef complexes of the Maputaland coastline in northern KwaZulu-Natal, South Africa. All the reefs lie parallel to the coastline and are no more than two nautical miles from the mainland high-water mark. The sampled reefs were Nine-mile reef (27°25′S), Seven-mile reef (27°27′S), Five-mile reef (27°29′S) and Two-mile reef (27°31′S) from the central reef complex, and Red Sands reef (27°45′S) and Leadsman Shoal (27°51′S) from the southern reef complex (Fig. 1). All reefs were < 2 km^2 and their small size made it difficult to collect from replicate sites (local populations). To minimize genetic variation due to habitat type, all samples were taken from a depth of 12–15 m on reef slopes. At each site, tissue samples were collected randomly by removing small fragments (approximately 3 cm) from the branch tips of up to 48 attached adult colonies, from reef areas averaging 650 m^2. We avoided collecting multiple fragments from adjacent colonies, by sampling colonies that were separated by at least 1 m. The high wave action at the six sites makes it unlikely that detached fragments would remain close to the parent colony. Samples were placed in individual bags under water and transferred to liquid nitrogen until final storage at –70°C in the laboratory.

Electrophoresis

Allozyme electrophoresis was used to determine the five-locus genotype of the 267 colonies collected, in order to compare the genetic structure of the six reefs. Electrophoretic methods were modified from Harris and Hopkinson (1976) and Richardson et al. (1986). Assays were conducted using horizontal starch gels (11% w/v). Coral fragments were ground in a solution of 0.04% B mercaptoethanol and 0.014-*M* Tris-HCl. Electrophoretic buffers and assay conditions followed Selander et al. (1971). Twenty-two enzymes were screened initially on five buffer systems using up to eight samples from each of the six sample sites. Of these 22 enzymes, 5 produced no detectable activity, 10 were monomorphic and 7 were polymorphic. Of the 7 polymorphic enzymes, only 5 yielded activity that could be consistently resolved. The enzymes and buffer systems used were as follows: leucyl-glycyl-glycyl peptidase (LGG, E.C. 3.4.11) and leucyl-proline peptidase (LPP, E.C. 3.4.11) on buffer number 2; glutamate dehydrogenase (GDH, E.C. 1.4.1.2) and esterase (EST, E.C. 3.1.1.) on buffer number 6; and glucose-phosphate isomerase (GPI, E.C. 5.3.1.9) on buffer number 9. Between two and six alleles were detected at each locus (Table 1). Two standards of known mobility were run on all gels in order to standardize the scoring of the alleles. As with *Pocillopora damicornis*, *P. verrucosa* is the host for symbiotic zooxanthellae. However, Stoddart (1983), in comparing bleached with non-bleached colonies of *P. damicornis*, detected no contribution of the zooxanthellae to the activity of the loci he assayed (see also Willis and Ayre 1985). Since we used similar loci, we assume that the zooxanthellae were not influencing the zymograms that we were scoring.

Analyses

General population genetic statistics were calculated using TFPGA (Miller 1997). The magnitude and direction of departures from the Hardy–Weinberg equilibrium were assessed to infer the effects of dispersal and reproductive mode on the genotypic composition of the populations. These departures were expressed as Wright's (1978) fixation index (*f*), where negative and positive values represent excess or deficit of heterozygotes, respectively. Chi-square (χ^2) tests, using a Bonferroni correction, were used to test for

Table 1 Allelic frequencies, allelic diversity and observed heterozygosity of six populations of *Pocillopora verrucosa*. *N* Number of individuals (*$P < 0.05$)

	Nine-mile	Seven-mile	Five-mile	Two-mile	Red Sands	Leadsman
Gpi-1						
(*N*)	40	43	48	48	34	43
A	0.025	0.109	0.188	0.021	0.073	0.024
B	0.013	0.239	0.125	0.021	0.059	0.081
C	0.625	0.391	0.479	0.638	0.662	0.698
D	0.025	0.054	0.021	0.192	–	0.081
E	0.275	0.163	0.156	0.128	0.206	0.116
F	0.037	0.044	0.031	0.000	0.000	0.000
Gdh-1						
(*N*)	46	48	48	48	34	43
A	1.000	0.750	0.937	0.979	0.824	0.861
B	0.000	0.250	0.063	0.021	0.176	0.139
Lgg-2						
(*N*)	46	48	48	48	34	43
A	0.065	0.167	0.291	0.187	0.147	0.233
B	0.576	0.510	0.615	0.490	0.529	0.593
C	0.196	0.146	0.021	0.115	0.074	0.081
D	0.163	0.177	0.073	0.208	0.250	0.093
Lpp-1						
(*N*)	46	48	48	48	34	43
A	0.935	0.900	0.969	0.875	0.897	0.977
B	0.065	0.100	0.031	0.125	0.103	0.023
Est-1						
(*N*)	46	48	48	48	34	43
A	0.956	0.948	0.906	0.844	0.971	0.953
B	0.044	0.052	0.094	0.156	0.029	0.047
Mean no. alleles/locus	2.8 (0.7 SE)	3.2 (0.8 SE)	3.2 (0.8 SE)	3.0 (0.6 SE)	2.8 (0.5 SE)	3.0 (0.6 SE)
Observed heterozygosity	0.126 (0.05 SE)*	0.144 (0.05 SE)*	0.108 (0.05 SE)*	0.185 (0.07 SE)*	0.153 (0.07 SE)*	0.153 (0.07 SE)*

*$P < 0.05$

significant departures from expected numbers of homozygotes and heterozygotes.

Two approaches were used to infer the relative frequencies of sexually and asexually derived adults within the collected populations. Firstly, for all six sites, we determined the multi-locus genotype of each colony (*N*), and then counted the number of unique genotypes detected (*N*g). The ratio *N*g:*N* provides the simplest index of the effects of asexual reproduction on genotypic diversity. However, because only a small portion of the genome can be sampled electrophoretically, genotypically identical colonies may still be non-clonemates (Ayre et al 1997), and the ratio *N*g:*N* therefore provides the maximum estimate of the contribution of asexual reproduction to local recruitment. It is assumed that *P. verrucosa* will recruit either by locally generated asexual propagules, or by locally produced or widely dispersed sexual propagules. Thus, we calculated the observed genotypic diversity (*G*o) at each site and compared it with values expected under conditions of random sexual reproduction (*G*e). Departures of the ratio *G*o:*G*e from unity provide an index of the combined effects of departures from single-locus Hardy–Weinberg equilibrium and linkage disequilibria. Such departures are a predictable consequence of asexual reproduction, but may result from other factors, such as population subdivision. A genetically variable population with high levels of sexually derived recruits will display a high ratio (close to unity) of *G*o:*G*e. The statistical significance of the differences between *G*o and *G*e was assessed using an unpaired *t*-test (Stoddart and Taylor 1988).

We used Wright's *F* statistic, calculated as Weir and Cockerham's (1984) estimator θ, to hierarchically partition genetic variation within and among reefs. We therefore estimated the standardized genetic variation among sites within reef complexes (*F*SR) and among reef complexes (*F*RT). These statistics were calculated as Weir and Cockerham's (1984) θ using the program TFPGA (Miller 1997) which executes numerical jack-knifing to provide estimates of variance for each locus, and to provide estimates of variances across loci. Values of θ range from zero (no variation due to panmixia) to a theoretical maximum of one (population fixed for alternative alleles). However, in some cases where θ is close to zero, negative values may be obtained (Weir 1990). Values of θ were judged to be statistically significant when zero lay outside the 95% confidence interval of the mean.

Results

Genetic diversity

Samples of *Pocillopora verrucosa* from all six sites were genetically diverse, with no apparent pattern of variation in allele frequencies or allelic diversity (Table 1). Two loci (*Gpi-1* and *Lgg-2*) were highly variable, and were represented by six and four alleles, respectively. The other three loci (*Gdh-1*, *Lpp-1*, *Est-1*) were much less variable and the frequency of the most common allele at each site was always greater than 75%. The mean number of alleles per locus ranged from 2.8 to 3.2 and was similar for all six local collections. Similarly, observed heterozygosity ranged from 0.108 to only 0.185.

Genotypic structure of local populations

Genotypic frequencies for all five loci differed significantly from expectations for Hardy–Weinberg equilibria at the six sites (χ^2, $P < 0.05$) (Table 2), but were not consistent with the predicted effects of asexual reproduction. Both *Gdh-1* (except monomorphic at Nine-mile reef) and *Lgg-2* deviated significantly at all sites, whereas *Est-1* only differed significantly at two sites. Asexual reproduction should produce both deficiencies and excesses of heterozygotes (e.g. Ayre and Willis 1988). However, only five cases of excess were found, and of the 22 significant deviations from the Hardy–Weinberg equilibrium (Table 2), 19 were produced by deficits.

Examination of multi-locus genotype frequencies also revealed little evidence of localized asexual reproduction (Table 3). At every site the majority of colonies displayed unique multi-locus genotypes (i.e. *N*g:*N* ranged from 0.55 to 0.81) and no genotype was represented by more than nine colonies. The *G*o:*G*e ratio was significantly different from expectations for sexual reproduction in only two of the six sites (*t*-test, $P < 0.05$), with values ranging from 0.52 to 0.79 (mean 0.64 ± 0.05 SD). The lowest *G*o:*G*e ratios were 0.52 and 0.53, and the 43 and 48 colonies sampled displayed 35 and 27 distinct five-locus genotypes, respectively. Of these, 40 and 41 respectively, were represented by only one colony. However, this seems likely to reflect the ubiquitous occurrence of large heterozygous deficits at all loci. Such heterozygous deficits are unlikely to reflect asexual reproduction because, as mentioned before, this would be expected to generate similar numbers of heterozygous excesses and deficits.

Geographic variation

As inferred from inspections of the allelic frequency data, our estimation of hierarchical *F* statistics revealed no significant variation within or among reef complexes for any of the five loci examined. That is, *F*SR and *F*RT values were not significantly different from zero ($P > 0.05$). *F*SR ranged from 0.019 to 0.039, and values of *F*RT were always negative (mean -0.006 ± 0.001 SE),

Table 2 Wright's fixation index (*f*) for six populations of *Pocillopora verrucosa*, for each of five enzyme-encoding loci. Significant departures from Hardy–Weinberg equilibria are noted (after Bonferroni correction) as * $P < 0.05$, ** $P < 0.01$; (–) locus monomorphic for population

Population	*Gpi-1*	*Gdh-1*	*Lgg-2*	*Lpp-1*	*Est-1*
Nine-mile	+0.650**	(–)	+0.528**	+0.287	–0.045
Seven-mile	+0.709**	+0.889**	+0.557**	+0.630**	–0.005
Five-mile	+0.610**	+1.000**	+0.726**	+0.656**	+0.387**
Two-mile	+0.447**	+1.000**	+0.408**	+0.619**	+0.447**
Red Sands	+0.194	+1.000**	+0.673**	+0.522*	–0.030
Leadsman	+0.330*	+1.000**	+0.478**	–0.024	–0.049

Table 3 Comparison of observed and expected multi-locus genotypic diversity for six collections of *Pocillopora verrucosa*. *N*: number of individual colonies; *N*g number of unique multi-locus genotypes; *G*o observed multi-locus genotypic diversity; *G*e expected multi-locus genotypic diversity. (unpaired *t* test, * $P < 0.05$)

Population	*N*	*N*g	*N*g:*N*	*G*o	*G*e	*G*o (SD)	*G*o:*G*e
Nine-mile	40	22	0.55	9.41	16.74	3.77	0.56
Seven-mile	43	35	0.81	20.76	39.87	4.69	0.52*
Five-mile	48	27	0.56	13.55	25.48	5.12	0.53*
Two-mile	48	29	0.60	17.99	29.12	5.36	0.62
Red Sands	34	24	0.71	16.99	21.28	4.56	0.79
Leadsman	43	28	0.65	15.03	18.88	4.74	0.79
Mean	42.7	27.5	0.65	15.62	25.23	–	0.64
(±SE)	±2.15	±1.84	±0.04	±1.60	±3.45		±0.05

indicating that there was no detectable variation among the reef complexes (Table 4).

Discussion

Our hierarchical analysis of the levels of genetic variation of populations of *Pocillopora verrucosa* within and among reef complexes in northern KwaZulu-Natal, South Africa, implies that the central and southern reef complexes support a single, genetically homogenous population. This is the first such study of reefs in the WIO. Our data suggest that the reefs seem likely to be maintained by high levels of localized sexual recruitment (*G*o:*G*e; *N*g:*N*; deviations from Hardy–Weinberg), together with sufficiently high levels of long-distance larval dispersal to prevent the development of any significant geographic differentiation. Despite the small size of the reefs in question (which made it impossible to assess within-reef differentiation), these results are similar to the population structure that Ayre et al. (1997) report for *Pocillopora damicornis* on Australia's GBR. Furthermore, Ayre and Hughes (2000) showed similar results for four broadcast-spawning species of *Acropora* on the GBR. In this case, populations of the *Acropora* spp. showed some within-reef differentiation, but no variation among reefs, even over larger distances. There is a clear contrast, however, with the brooding-population structures of *Stylophora pistillata* and *Seriatopora hystrix* which display much more genetic subdivision (Ayre and Hughes 2000), and the clonal population structures of *Pavona cactus* (Ayre and Willis 1988) and *Pocillopora damicornis* from high latitudes in Western Australia (Stoddart 1984).

Table 4 *Pocillopora verrucosa*. Summary results from *F* statistic estimates of *F*SR (variation among sites within reef complexes) and *F*RT (total variation among reef complexes) at five loci. NS Not significant, $P > 0.05$

Locus	*F*SR	*F*RT
Gpi-1	0.019NS	–0.009NS
Gdh-1	0.020NS	–0.005NS
Lgg-2	0.039NS	–0.003NS
Lpp-1	0.028NS	–0.006NS
Est-1	0.027NS	–0.007NS
Average	0.0254	–0.0057
	(0.014 SE)	(0.004 SE)

Localized recruitment

Our data show that localized asexual recruitment had little influence on the maintenance of the studied populations of *P. verrucosa*; rather, they were maintained by recruitment of sexually generated larvae. All local populations were genotypically diverse and, on average, unique five-locus genotypes could be assigned to 65% of all colonies (i.e. mean $Ng:N = 0.65$). Some colonies shared identical five-locus genotypes. This is, however, an expected consequence of sexual reproduction when only a small number of loci are used with limited underlying genetic variability. In any case, no potentially clonal genotype was represented by more than nine colonies at any one site. Indeed, on average, we detected 64% of the levels of five-locus genotypic diversity expected for randomly mating populations. The ratio of observed to expected genotypic diversity was similar to values reported for Great Barrier Reef (GBR) populations of *P. damicornis* (Benzie et al. 1995; Ayre et al. 1997) and *Seriatopora hystrix* (Ayre and Dufty 1994). These values were also much higher than those reported for similarly sampled but highly clonal populations of the corals *P. damicornis* in Western Australia (Stoddart 1984) and *Pavona cactus* (Ayre and Willis 1988) on the GBR. A similar contrast holds for the highly clonal sea anemones *Actinia tenebrosa* (Ayre et al. 1991) and *Anthothoe albocincta* (Billingham and Ayre 1997), and the fissiparous sea star *Coscinasterias calamaria* (Johnson and Threlfall 1987).

Some depression of the *G*o:*G*e ratio is expected for sexually reproducing populations that do not mate in a completely random manner (Stoddart and Taylor 1988). In this and in other studies of sexually reproducing corals (Ayre and Dufty 1994; Ayre et al. 1997; Ayre and Hughes 2000), single-locus genotype frequencies were characterized by large and significant heterozygous deficits. Heterozygote deficits are a common feature of many marine populations, and many explanations have been put forward to account for this effect (see Ayre et al. 1997). The consistently large deficits that we detected

seem most likely to be a consequence of inbreeding mediated either by self-fertilization or restricted dispersal of gametes or larvae within *P. verrucosa* populations (Wahlund effect).

The heterozygous deficits reported here are so large that one possible source of heterozygous deficiencies should not be discounted. Currently, the extent of scientific and taxonomic knowledge of the corals of the WIO region is poor, and hence it is possible that *P. verrucosa* collected in the current study is actually a multi-species complex. Sampling the genotype frequencies of one or more cryptic species could lead to substantial Wahlund effects, which would result from the incorrect pooling of two separate gene pools. Several sets of cryptic coral species have been detected using similar genetic approaches (Ayre et al. 1991; Stobbart and Benzie 1994; Lopez et al. 1999) and our data appear to justify a careful comparison of morphometric and genetic data. However, differences between samples could not be aligned with categories of morphometric data using established methods (data not shown). Nevertheless, the simplest explanation of our findings is that *P. verrucosa,* like all nine coral species examined by Ayre and Hughes (2000), shows large heterozygous deficits produced by limited dispersal of larvae or genets.

Geographic variation and gene flow

P. verrucosa shows no variation in allele frequencies in the central and southern reef complexes in South Africa. For each of the five apparently unlinked loci, there was no detectable variation among samples collected from reefs separated by up to 70 km (mean $F\mathrm{RT} = 0.000$). This low level of variation is similar to that reported for other broadcast-spawning corals (Ayre and Hughes 2000), as well as for other broadcasting taxa (see Bohonak 1999 for review). Much greater spatial variation has been reported for brooding corals (Hellberg 1994; Ayre and Hughes 2000), with the exception of *P. damicornis*, which has been suggested as displaying both brooding and broadcast spawning in some populations (Stoddart and Black 1985; Ward 1992).

Treating the central and southern reef complexes as separate sub-populations, and using Wright's (1969) Island Model [$Nem = (1/F\mathrm{RT} - 1)/4$, where $N\mathrm{e}$ = effective population size and m = proportion of migrants per generation], it was estimated that the effective number of migrants moving between the two reef complexes is 44 per generation. This level of gene flow between the reef complexes is rather large in an evolutionary context, reflecting the low levels of differentiation between distant reefs. However, Wright's (1969) Island Model assumes that gene flow is bi-directional and at a stable equilibrium – neither of which is realistic. The most important large-scale oceanographic feature of the area under investigation is the Agulhas Current (Ramsay 1994), which forms off the northern KwaZulu-Natal/Mozambique coast. As a consequence of a narrow continental shelf in the study area, the Agulhas Current flows close inshore in a southerly direction, reaching a maximum speed of approximately 1.5 m/s (Ramsay 1994). Furthermore, larval settlement rates vary among coral species (Ayre and Hughes 2000) and our estimate of gene flow is similar to that for species with relatively long larval durations (*Acanthaster planci*, $Nem = 27$–50; *Tridacna* spp., $Nem = 20$ to ∞; see Ayre et al. 1997). Thus, given the direction and speed of the prevailing current, coupled with the closeness of the reefs in question, it is not surprising that the populations of *P. verrucosa* in this study are functioning as a single population.

Based on the present data, there is no doubt that the genotypic composition of the southern and central South African reef complexes reflects episodes of strong genetic connectivity and maintenance by high levels of self-seeding. However, it would be premature to make any substantial management suggestions based on the data presented above, for several reasons. First, as emphasized by recent reviews (e.g. Bohonak 1999; Whitlock and McCaughley 1999) indirect estimates of gene flow cannot be equated with current levels of migration or even gene flow; i.e. our estimate of gene flow is based on several restrictive assumptions, including the requirement that current levels of genetic differentiation reflect an equilibrium between gene flow and drift, and that gene flow is equally probable among all local populations (Wright 1969). In fact current levels of differentiation may reflect historically higher levels of gene flow or recent colonization events, and may be driven by higher levels of gene flow from the still unsampled northern reef complex. Secondly, the small size and the difficulty in accessing many of these reefs made it impossible to collect replicate populations at each of the sites, and it is possible that there are restricted patterns of dispersal within individual reefs. Ideally, future studies will provide more refined estimates of the scale of dispersal and frequency of dispersal through inclusion of populations from the northern reef complex, utilization of more sensitive DNA markers and determination of the source of new recruits.

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