Marine Biology (1995) 122: 665–673

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Patterns of genetic subdivision in populations of a clonal cnidarian, *Zoanthus coppingeri*, from the Great Barrier Reef

Received: 24 January 1995 / Accepted: 27 January 1995

Abstract Samples of an intertidal zoanthid, *Zoanthus coppingeri*, Haddon and Shackelton, 1891, were collected from three localities in the Great Barrier Reef region during 1992–1993, and subjected to allozyme electrophoretic analysis at seven polymorphic loci. The reduced ratio of observed to expected genotypic diversity indicated that populations were partly clonal, but they were not dominated by a few clones as occurs in some other cnidarians. Regular disturbance by wave action is postulated to prevent the formation of large stands of particular clones by clearing space and mixing genotypes over small scales. The sexual origin of clonal genotypes was confirmed by conformance to Hardy–Weinberg predictions of genotype frequencies at all but one locus. Values of the standardised genetic variance among populations, F_{ST}, were highly significant between localities and between replicate sites within localities separated by only 50 m. Strong genetic structure has not previously been described in a Great Barrier Reef invertebrate species, and is considered to be the consequence of stochastic changes in gene frequencies as a result of low levels of gene flow. High clonal longevity and low recruitment rates may maintain genetic differences over long periods. Similar effects may be seen in other Great Barrier Reef invertebrate species with comparable reproductive patterns.

Introduction

The Zoanthidea (zoanthids) are an order of predominantly colonial cnidarians in the class Anthozoa, subclass Hexacorallia. Other orders in the Hexacorallia include the Actiniaria (anemones), Antipatharia (black corals), Ceriantharia (tube anemones), Corallimorpharia and Scleractinia (hard corals). Members of the largest family of Zoanthidea, the Zoanthidae, are ubiquitous on coral reefs and tropical rocky shores around the world. There are many described species (see Walsh and Bowers 1971 for a bibliography) and their taxonomy is highly confused (Muirhead and Ryland 1985; Ryland and Muirhead 1993). As a consequence zoanthids are notoriously difficult to identify, and have been largely ignored in ecological surveys.

Zoanthus coppingeri, Haddon and Shackleton, 1891, is a common member of intertidal and shallow sublittoral communities on rocky shores and coral reefs in the Great Barrier Reef (GBR) region. In partially sheltered, unstable habitats it may dominate communities, approaching 100% cover in some cases (W.J.B. personal observation). The success of *Zoanthus* spp. in similar habitats in the Caribbean has been attributed to extremely rapid recolonisation of newly available substratum following disturbance events (Karlson 1983). This is enabled by very rapid growth rates (Karlson 1988) and the ability of colonies to survive fragmentation during storms (Karlson 1983). Colony fission also occurs frequently under endogenous control (Muirhead and Ryland 1985; Karlson 1986), and the average size of physically discrete *Zoanthus* spp. colonies is very small, from < 2 to 5 polyps (Karlson 1986). Survival rates of these colonies are low, but continual growth and fragmentation mean that survivorship of clonal genotypes is high, provided mortality does not occur too soon after recruitment (Karlson 1991). Karlson (1988) has shown greater relative growth rate and delayed sexual reproduction in one species, *Z. sociatus*, compared with a second, *Z. solanderi*, which has lower rates of mortality. Rapid somatic growth appears adaptive in the face of high mortality. Partitioning of resources to ensure colony survival may reduce sexual reproductive output in *Z. sociatus* compared with

Communicated by J. Mauchline, Oban

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Z. solanderi, and in *Zoanthus* spp. as a whole compared to other groups with higher survival rates. Studies of sexual reproduction (Cooke 1976; Karlson 1981; Fadlallah et al. 1984) have all shown that *Zoanthus* spp. are broadcast spawners, probably with extended pelagic development and the potential to disperse over long distances. Rates of settlement and survival of sexual propagules of *Zoanthus* spp. are low, however (Karlson 1991); much less than the recruitment of asexually produced fragments (Karlson 1988).

Asexual reproduction has profound effects on population structure. In clonal cnidarians which utilise mixed sexual and asexual modes of reproduction, populations can be dominated by a small number of genotypes (Shick et al. 1979; Sebens 1982; Ayre 1983, 1984; Neigel and Avise 1983; Stoddart 1984; Shaw et al. 1987; Ayre et al. 1991) which are particularly well-adapted to local conditions, and which are able to multiply without recombination and subsequent loss of fitness. High rates of gene flow and recruitment in many strictly sexually reproducing marine invertebrates (Hedgecock 1986; Underwood and Fairweather 1989; Williams and Benzie 1993; Benzie 1994) reduce habitat-selection effects and stochastic contributors to genetic differentiation among populations (founder events and genetic drift). Low recruitment rates make many clonal species prone to such factors and, in combination with very long clonal lifetimes, maintain inter-population differences as a consequence of low genetic turnover (Hoffmann 1987).

Predictions have been made about population structure of intertidal *Zoanthus* spp. populations (Karlson 1991). Simulations show that clonal structure is highly dependent on rates of survivorship, recruitment and colony fission. The aim of the present study was to examine patterns of clonal structure within populations of *Z. coppingeri* from the GBR, subject to real, though undetermined, levels of recruitment, survivorship, asexual reproduction and disturbance.

Materials and Methods

Collections from intertidal *Zoanthus coppingeri* Haddon and Shackelton, 1891 populations were made at three localities: Cockle Bay on Magnetic Island, Low Island, Low Isles, and Kissing Point in Townsville, during 1992–1993 (Fig. 1). At each locality, samples were collected every 40 cm along four 10 m transects, 2.5 m apart and parallel to each other and the shoreline, forming a grid ("site") 7.5 m × 10 m, for a potential total of 100 samples per site. No sample was collected from points at which there was no colony within 10 cm. Two collections were made at each of the Cockle Bay and Low Isles localities. Replicate sites within localities were at the same tidal height and 50 m apart. The Kissing Point population is restricted to a small area, which provided only one site.

Polyps were carefully removed from the substratum using forceps, placed in individually numbered 4 ml polypropylene screw-cap vials, snap-frozen in liquid nitrogen or on dry ice within 2 h of collection, and stored long-term at − 80 °C.

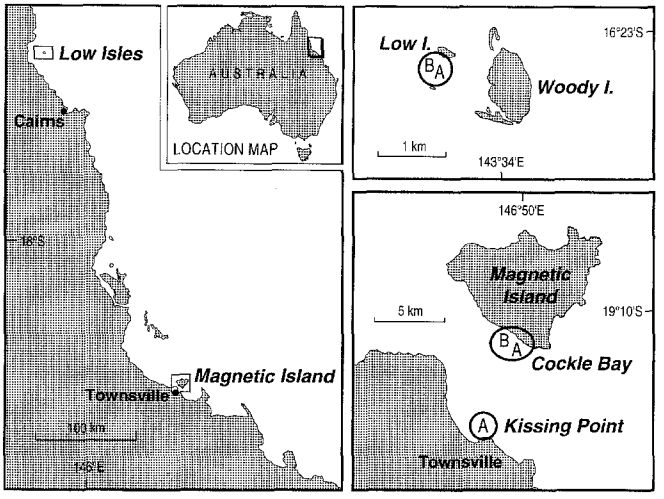

Fig. 1 *Zoanthus coppingeri*. Sampling locations in Great Barrier Reef Marine Park. There were two sampling sites (*A* and *B*) separated by 50 m at both Cockle Bay and Low Isles, and one site only at Kissing Point

Tissue extracts were prepared by homogenising one polyp from each sample in one drop of aqueous 0.04% β-mercaptoethanol (plus trace bromophenol blue). Sediment, mucous and zooxanthellae were removed by centrifugation (10 min, 15900 × *g*) and the clarified homogenates were refrozen and stored at − 80 °C until needed.

Allozyme electrophoresis was carried out using 12% horizontal starch gels (Sigma S-4501) and cellulose acetate gels (Cellogel™, Chemotron, Milan) following the protocols of Harris and Hopkinson (1976) and Richardson et al. (1986). Extracts were thawed and 12 µl of clarified supernatant were carefully removed and used to load cellulose acetate gels. The remainder was absorbed onto filter paper wicks (Whatman No. 3) and analysed on starch gels.

Following an initial survey of 40 enzymes on 9 buffer systems, regions of activity corresponding to 7 putative enzyme loci, coding for 6 different enzymes, were found to be polymorphic and consistently resolvable, and were used in the analysis. These enzymes are enolase (ENO, E.C. 4.2.1.11), malate dehydrogenase (MDH, E.C. 1.1.1.37), esterase-D (EST-D, E.C. 2.7.1.1), hexokinase (HK, E.C. 2.7.1.1), phosphoglucomutase (PGM, E.C. 5.4.2.2) and peptidase using leucylglycylglycine substrate (LGG, E.C. 3.4.11/13). ENO was assayed on cellulose acetate gels using a continuous phosphate buffer, pH7.0 (Phos7: Richardson et al. 1986), run at constant potential (200 V) for 2 h. MDH was assayed on starch gels using a continuous Tris-citrate buffer, pH7.0 (TC7: Shaklee and Keenan 1986) run under constant current (50 mA) for 6 h. EST-D, HK and PGM were assayed on starch gels using a continuous Tris-EDTA-citrate buffer, pH7.9 (TEC7.9: Goodall and Stoddart 1989) run under constant current (35 mA) for 6 h. LGG was assayed on starch gels using a discontinuous Tris-citrate/lithium borate buffer, pH8.4/8.15 (Li: Ballment et al. 1993) run at constant potential (400V) for 6 h. Gels were refrigerated during running (4 °C), and ice was applied to the top surface if they became warm to the touch.

Zymograms were visualised using stain recipes modified from Harris and Hopkinson (1976), using agar overlays. Full details of stain recipes and results of the initial survey of enzyme activity given elsewhere (Burnett 1995). Alleles were assigned mobilities relative to the most common allele in the zoanthid *Palythoa caesia* [to allow interspecific comparisons to be made elsewhere (W.J.B., Ph.D. thesis, University of Wales)]. Occasionally a sample of several polyps contained more than one genotype, presumably due to the small size and mixing of clonal fragments; therefore all enzymes were assayed for a particular sample on the same day using extract from the same polyp.

Statistical analyses

Each site was divided into four quarters ("subsites"), and statistics were calculated and assessed at subsite, site and locality level.

Values of observed genetic diversity (G_0) were calculated from $G_0 = 1/\sum_{i=1}^{k} g_i^2$ (Stoddart 1983), where g_i is the relative frequency of the ith genotype and k is the number of genotypes. Expected genetic diversity under conditions of strictly sexual reproduction was estimated from $G_E^* = (1/d + p/N)$, where $d = \sum g_i^2$ for genotypes where $(g_i N) > 1$, and $p = \sum g_i$ for genotypes where $(g_i N) < 1$, and N is sample size (Stoddart 1983). As no genotypes were predicted to occur once or more in any population G_E^* was equal to N in all populations.

Contrary to expectations under assumptions of strictly sexual reproduction, and in accordance with the colony fragmentation patterns observed in *Zoanthus* spp., many samples were found to share identical genotypes. The spatial distribution of samples with identical genotype within sites was tested for randomness by using χ^2 and Poisson expectations of the number of samples with identical genotypes per subsite.

Analysis of gene-frequency data was carried out at the subsite level, the site level (fusing data within sites), and the locality level (fusing all data within localities). The BIOSYS-1 package (Swofford and Selander 1981) was used to calculate gene frequencies and levels of genetic variability within subsites, sites and localities. Genotypes which occurred more than once per locality, site or subsite were included once only in calculations of gene frequencies. BIOSYS-1 was also used to calculate predicted genotype frequencies under conditions of Hardy–Weinberg equilibrium, χ^2 contingency tables of genetic heterogeneity among subsites within sites, and Wright's (1978) hierarchical F-statistic. Significance of χ^2 tests for deviations of genotype frequencies from Hardy–Weinberg predictions were corrected for multiple simultaneous tests using a simple Bonferroni technique (Miller 1980), and Levene's correction for small sample sizes (Levene 1949) was applied in Hardy–Weinberg analysis of subsite data. Pairwise measures of linkage disequilibrium (σ) were calculated using the multiallelic formula of Hill (1975), and were tested for significance using χ^2.

Values of genetic differentiation between (F_{ST}) and within (F_{IS}) subsites, sites and localities were calculated using the formulae of Weir and Cockerham (1984) which take into account differences in sample size; 95% confidence intervals were calculated by jacknifing over loci, and χ^2 tests for their significance followed Waples (1987).

The number of migrants per generation ($N_e m$) between subsites, sites and localities was estimated using $N_e m = (1/F_{ST} - 1)/4$ (rearranged from Wright 1931) and the regression equation of Barton and Slatkin (1986), in which $\log_{10}(\bar{p}(1)) = a\log_{10} N_e m + b$, where $\bar{p}(1)$ is the mean frequency of all alleles which occur in only one population (Slatkin 1981). Values of a and b were used as appropriate for approximate sample numbers, and estimates of $N_e m$ were corrected for actual sample numbers following Barton and Slatkin (1986); 95% confidence limits for this estimate were calculated by jacknifing over alleles (Johnson et al. 1988).

Results

Asexual reproduction results in the multiple occurrence of genotypes in populations. The index of observed to expected genotypic diversity, G_0:G_E^*, provides an indication of the relative importance of sexual and asexual modes within populations. For *Zoanthus coppingeri* in all sites, G_0:G_E^* was lower than expected under a model of pure sexual reproduction (G_0:$G_E^* = 1$), and many genotypes were observed more than once (N_g:$N < 1$ Table 1). Overall levels of genetic variability were high (Table 2), and the probability of any one seven-locus genotype occurring twice in a particular subsite, site or locality by random assortment (i.e. as a consequence of sexual reproduction) was small ($< 10^{-6}$). It was therefore concluded that physical individuals with identical genotypes represented asexual offspring (ramets) of sexually derived genotypes (genets). The number of detected ramets per genet was generally low (Fig. 2), and most genotypes were detected only once.

Table 1 *Zoanthus coppingeri*. Genotypic diversity of 5 sites [N sample size; N_g number of 7 locus genotypes; G_0 observed genotypic diversity; G_E^* expected genotypic diversity]

Site		(N)	N_g	N_g:N	G_0	G_E^*	G_0:G_E^*
Kissing Point		(52)	35	0.67	14.08	52	0.27
Cockle Bay	A	(97)	75	0.77	47.28	97	0.49
	B	(90)	74	0.82	59.56	90	0.66
Low Isles	A	(83)	72	0.87	60.93	83	0.73
	B	(89)	62	0.70	25.31	89	0.28

In the absence of disturbance, colony fragmentation and growth should result in the formation of aggregations of many ramets of the same genet. However, the spatial distribution of ramets among subsites within sites was not significantly different from the expectations of the Poisson distribution in 3 of the 5 sites examined (Table 3). At the other two, Kissing Point showed more aggregated, and Low Isles A more uniform distributions of ramets than random. There was no overall consistent aggregation of genetically identical ramets within sites, suggesting that ramets of different genets are mixed by disturbance (wave action) over distances of at least 10 m. Few genotypes were found to occur in more than one site, indicating that such mixing does not frequently occur at distances that exceed 50 m. There were only three genotypes common to both sites at each of Cockle Bay and Low Isles. No genotype was found to occur at more than one locality.

Genetic relatedness of subsites, sites and localities was inferred from analysis of gene frequencies. Many population genetic statistics are calculated under the assumption that population subdivision is the consequence of genetic drift alone, and natural selection causes bias of such statistics (Waples 1987). Selection can strongly influence gene frequencies in asexual organisms, due to proliferation of successful clones. To eliminate bias as far as possible and so form a more accurate estimate of levels of gene flow among populations, clonal genotypes which occurred more than once per subsite, site or locality were only included once for each level of population pooling when calculating gene frequencies (Table 4).

Most loci had the same common alleles in all subsites, sites and localities. Kissing Point samples were found to have a high incidence of the **79* allele at the

Table 2 *Zoanthus coppingeri.* Genetic variability at 7 polymorphic loci in 5 sites. Standard errors in parentheses. Locus is considered polymorphic if frequency of most common allele is < 0.99

Site		(*N*)	Mean no. of alleles	% loci polymorphic	Mean heterozygosity	
					Observed	Hardy–Weinberg expected[a]
Kissing Point.		(35)	3.3 (0.4)	100.0	0.429 (0.098)	0.456 (0.072)
Cockle Bay	A	(75)	3.7 (0.6)	100.0	0.404 (0.077)	0.441 (0.069)
	B	(74)	3.9 (0.5)	100.0	0.384 (0.057)	0.452 (0.054)
Low Isles	A	(73)	3.9 (0.6)	100.0	0.356 (0.086)	0.407 (0.080)
	B	(62)	3.3 (0.4)	100.0	0.382 (0.091)	0.411 (0.081)

[a] Unbiased estimate (Nei 1978)

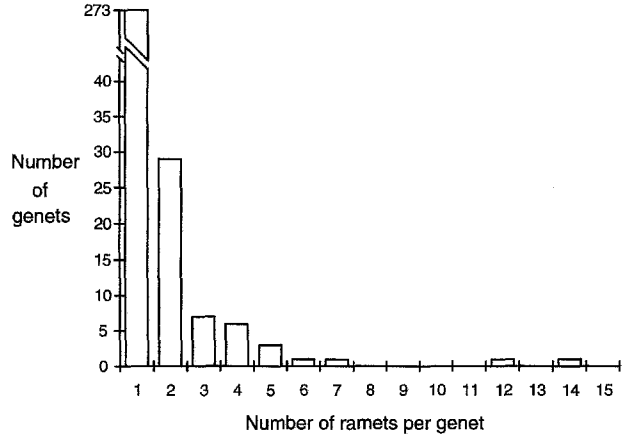

Fig. 2 *Zoanthus coppingeri.* Frequency distribution of number of physical individuals (ramets) detected per genotype (genet) from all populations combined

Table 3 *Zoanthus coppingeri.* Conformation of ramet distribution to Poisson distribution ($*p < 0.05$)

Site		χ^2	*df*	Mean ramets per subsite	Variance
Kissing Point		7.79*	2	0.929	2.43
Cockle Bay	A	1.95	2	0.938	0.77
	B	0.07	2	0.614	0.71
Low Isles	A	4.25*	1	0.563	0.45
	B	4.44	2	0.864	1.79

*ENO** locus, and a corresponding reduction in the frequency of the **61* allele, which was the most common allele in samples from other localities. Less common alleles at all loci showed considerable variation in frequency, and several alleles were unique to particular subsites, sites and localities. Observed genotype frequencies conformed to Hardy–Weinberg predictions for levels of heterozygosity with the exception of the *HK** locus, which showed deficiencies of the **84/*78* and **78/*50* heterozygotes that were significant at all localities ($p < 0.001$). Within sites, these deficits were significant only at Cockle Bay B, Low Isles A and Kissing Point ($p < 0.05$). There were no significant deficits within subsites.

Associations between particular alleles in different loci due to linkage can indicate the existence of cryptic species or unresolved clonal structure. Pairwise tests of linkage disequilibrium (Hill 1975) were non-significant in all subsites, sites and localities (data not shown).

Population structure within and between subsites, sites and localities was examined using *F*-statistics (Weir and Cockerham 1984; present Table 5). F_{ST} (standardised variance among subpopulations within the total data set) was not significantly different from zero among the subsites within each particular site. Significant values of F_{ST} were detected among sites and among localities. F_{IS} (variance among individuals within subpopulations) was significantly different from zero for locality data, indicating the presence of more than one genetic group per locality. F_{IS} within sites and subsites was non-significant. F_{ST} and F_{IS} values suggest that sites are genetically homogeneous, but differ from one another. Hierarchical *F*-statistic analysis (Wright 1978; present Table 6) showed the same variance in gene frequencies among sites within localities as was found among localities, indicating that variation over 50 m contributed to one-half of the total variance seen in the study, which covered 300 km in total.

As a more sensitive test for gene-frequency differences among populations, χ^2 contingency tables for heterogeneity of gene frequencies among localities, sites and subsites were also calculated (data not shown). Results agreed with those from *F*-statistic analyses. χ^2 showed decreasing levels of significance as the degree of population pooling was reduced. Among localities and

among sites within localities, χ^2 was highly significant ($p < 0.00001$, $p < 0.00005$ respectively). Among subsites within sites, χ^2 was non-significant in all cases except between Subsites 1 and 4 at Low Isles Site A ($p < 0.05$). These results confirm that, in most cases, the site formed the most biologically meaningful approximation of population size within the data set.

Estimates of gene flow among subsites, sites and localities were obtained using two independent methods; from F_{ST} (using the equation of Wright 1931) and from the conditional average frequency of "private", alleles that occurred in only one population (Barton and Slatkin 1986). It has been suggested that private alleles provide an estimate of gene flow that is less prone to bias due to selection (Slatkin 1981) than values calculated from F_{ST}, and as such might give an indication of the importance of selection as a cause of population differentiation. The estimated numbers of migrants per generation among populations (N_em) from each method are shown in Table 7. Estimates from the two methods agreed closely when sample sizes were large, both methods predicting N_em of the same order of magnitude in comparisons among sites and among localities. In comparisons among subsites, however, estimates from F_{ST} were consistently much higher than estimates from private alleles. This may reflect the greater vulnerability of the latter to sampling error when the sample size or number of private alleles used is small (Slatkin 1985; Waples 1987; Johnson et al. 1988). For F_{ST}, robustness is more dependant on the number of loci screened than actual sample size. Values of N_em obtained from private alleles were therefore treated with caution in among-subsite comparisons.

Discussion and conclusions

Populations of *Zoanthus coppingeri* showed reduced levels of genotypic diversity relative to expectations under conditions of purely sexual reproduction, consistent with their known habit of asexual reproduction by colony fission. In contrast with some populations of other clonal cnidarians (Shick et al. 1979; Sebens 1982; Ayre 1983, 1984; Neigel and Avise 1983; Stoddart 1984; Shaw et al. 1987; Ayre et al. 1991), the overall number of clones observed in each population was relatively high, and most clones were represented by only a few individual samples. Ninety-four percent of genotypes were observed only once or twice in the data set, and these accounted for 77% of the total number of physical individuals.

The maintenance of genotypic diversity in populations of asexual species may be due to high mutation rates, high recruitment rates of sexual propagules, or high clonal longevity. General conformance of genotype frequencies to Hardy–Weinberg predictions suggested that novel genotypes were of sexual origin rather than the result of mutation in asexual lineages. Furthermore, estimated values of gene flow are low compared with other invertebrates from the GBR examined to date, which suggests that recruitment is relatively reduced in *Zoanthus coppingeri*. Measurements from Caribbean reefs have shown recruitment rates in *Zoanthus* spp. to be low (Karlson 1991). High genet longevity seems the most likely explanation for the large number of genotypes observed. Large numbers of ramets are produced rapidly in other, closely related species (Karlson 1986). Ramet mortality is high because populations on unstable substrata are prone to disturbance (Karlson 1991). Spreading the risk of genet death by fragmentation promotes genet longevity. High rates of ramet mortality also reduce competition for space in spite of high growth rates. There may be a subsequent reduction in the competitive exclusion of some genets by others (Sebens and Thorne 1985).

Ramets of the same genet were found to be randomly distributed within sites in most cases. Randomness is consistent with regular disturbance by wave action, which prevents the formation of large patches of ramets of a single genet by mixing fragments up over small distances. This would not appear to operate regularly over larger distances (> 50 m), as few genotypes were observed at more than one site per locality. Storms of sufficient magnitude to mix fragments over scales of > 50 m must be comparatively rare, but do provide a mechanism for limited gene flow among sites within a particular locality.

Long-lived pelagic larvae have been observed or inferred for all zoanthid species examined to date (Menon 1926; Scheltema 1968; Karlson 1981; Babcock and Ryland 1990; Burnett et al. 1994; W.J.B. personal observation), and a minimum larval duration of ~ 3 wk has been suggested for most species (Babcock and Ryland 1990). Compared with genetic surveys of other GBR invertebrate species investigated to date (Williams and Benzie 1993; Benzie 1994; Burnett et al. 1994), estimated levels of gene flow among populations of *Zoanthus coppingeri* are low. Zoanthids thus provide an interesting comparison to sexual species with similarly long-lived larvae. Gene flow among populations of strictly sexual species in the GBR is believed to be consistently high, e.g. in three species of giant clam with larval durations of 7 to 10 d: $N_em = 20.1$ (*Tridacna derasa*), $N_em = 83.1$ (*T. maxima*), $N_em =$ infinity (*T. gigas*, Benzie 1994). In two species of starfish with larval durations of 14 to 28 d, $N_em = 13.6$ to 75.5. (*Acanthaster planci*, Benzie 1994) and $N_em = 249.8$ (*Linckia laevigata*, Williams and Benzie 1993). Such values contrast markedly with those estimated for *Z. coppingeri* ($N_em = 5.1$ to 6.4 among sites) and another zoanthid with an asexual reproductive mode, *Palythoa caesia* ($N_em = 15.4$; data from Burnett et al. 1994). The priority given to asexual reproduction by *Zoanthus* spp. (Karlson 1988) reduces sexual reproductive effort, and larval output could as a consequence be insufficient for

Table 4 *Zoanthus coppingeri*. Gene frequencies at 7 polymorphic loci detected in 8 subsites (*A1–A4* and *B1–B4*) from Cockle Bay and Low Isles, and 4 subsites (*A1–A4*) from Kissing Point. Repeated (clonal) genotypes are included only once in each estimation of gene frequency (– absent)

Locus/ allele	Cockle Bay										
	Subsite								Site		Locality
	A1	A2	A3	A4	B1	B2	B3	B4	A	B	
(*N*)	(24)	(21)	(21)	(20)	(19)	(21)	(19)	(22)	(75)	(74)	(146)
*ENO**											
79	0.271	0.205	0.190	0.250	0.237	0.405	0.289	0.250	0.227	0.311	0.271
61	0.729	0.795	0.738	0.725	0.763	0.595	0.711	0.750	0.747	0.689	0.716
43	–	–	0.071	0.025	–	–	–	–	0.027	–	0.014
*MDH-1**											
76	–	–	–	–	–	–	0.026	–	–	0.007	0.003
68	–	–	–	–	–	–	–	–	–	–	–
62	0.583	0.455	0.452	0.600	0.368	0.333	0.211	0.364	0.520	0.338	0.432
57	–	–	–	–	–	0.048	–	–	–	0.014	0.007
49	0.417	0.545	0.548	0.400	0.632	0.619	0.763	0.636	0.480	0.642	0.558
*MDH-2**											
136	–	–	–	–	0.026	–	–	–	–	0.007	0.003
118	0.042	0.068	0.024	0.025	0.184	–	–	–	0.047	0.047	0.045
110	0.042	–	–	–	–	0.048	0.132	0.114	0.013	0.074	0.045
68	0.917	0.932	0.976	0.975	0.789	0.952	0.868	0.886	0.940	0.872	0.908
36	–	–	–	–	–	–	–	–	–	–	–
*EST**											
136	–	–	–	–	–	–	–	–	–	–	–
125	–	–	0.024	0.025	–	0.024	0.026	–	0.013	0.014	0.014
100	0.667	0.705	0.714	0.750	0.789	0.786	0.842	0.841	0.727	0.804	0.767
89	–	–	–	–	–	–	–	–	–	–	–
72	0.333	0.295	0.262	0.225	0.211	0.190	0.132	0.159	0.260	0.182	0.219
*HK**											
120	–	–	–	–	–	0.048	–	–	–	0.014	0.007
108	0.042	–	0.048	–	0.105	0.071	0.079	0.068	0.027	0.081	0.055
84	0.188	0.091	0.095	0.175	0.105	0.190	0.237	0.318	0.160	0.230	0.199
80	–	–	–	–	–	–	–	–	–	–	–
78	0.729	0.773	0.833	0.825	0.789	0.595	0.684	0.614	0.753	0.649	0.695
50	0.042	0.136	0.024	–	–	0.095	–	–	0.060	0.027	0.045
*PGM**											
111	–	–	0.048	0.025	–	–	–	–	0.020	–	0.010
109	–	–	–	0.025	–	–	–	0.045	0.007	0.014	0.010
104	0.271	0.250	0.262	0.275	0.211	0.190	0.211	0.205	0.287	0.203	0.250
103	–	–	0.048	0.025	0.026	0.095	–	–	0.020	0.034	0.027
102	0.271	0.295	0.262	0.350	0.184	0.286	0.289	0.295	0.293	0.270	0.281
100	0.417	0.455	0.333	0.300	0.579	0.429	0.447	0.455	0.347	0.466	0.401
60	–	–	–	–	–	–	–	–	–	–	–
55	0.042	–	0.048	–	–	–	0.053	–	0.027	0.014	0.021
*LGG**											
116	–	0.024	–	–	–	–	–	–	0.007	–	0.003
106	0.271	0.286	0.524	0.650	0.447	0.238	0.184	0.227	0.433	0.277	0.353
100	0.729	0.643	0.429	0.275	0.553	0.690	0.632	0.682	0.513	0.642	0.579
92	–	0.048	0.048	0.075	–	0.071	0.184	0.091	0.047	0.081	0.065

reliable recruitment, so reducing realised gene flow. Gene flow in other asexual species from the GBR might also be reduced compared with that seen in species which devote a higher proportion of available resources to gametogenesis.

Low rates of recruitment would make *Zoanthus coppingeri* populations prone to stochastic variation in gene frequencies (genetic drift and founder events) compared to species with high recruitment rates. Additionally, larvae of broadcast-spawning GBR cnidarians are known to be spatially patchy (Willis and Oliver 1990; Oliver et al. 1992), and larval patchiness can result in genetic patchiness in populations of sessile adults (Johnson and Black 1984; Campton et al. 1992;

Low Isles											Kissing Point				
Subsite								Site		Locality	Subsite				Locality
A1	A2	A3	A4	B1	B2	B3	B4	A	B		A1	A2	A3	A4	
25	15	17	23	19	22	14	16	72	62	128	8	10	9	11	31
0.180	0.300	0.176	0.348	0.368	0.455	0.429	0.594	0.247	0.452	0.355	0.750	0.650	0.611	0.909	0.700
0.820	0.700	0.824	0.652	0.632	0.545	0.571	0.406	0.753	0.548	0.645	0.250	0.350	0.389	0.091	0.300
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.020	–	–	–	0.079	–	–	–	0.007	0.024	0.016	–	0.200	0.111	0.091	0.086
–	–	0.029	–	–	–	–	–	0.007	–	0.004	–	–	–	–	–
0.680	0.433	0.500	0.543	0.500	0.386	0.286	0.500	0.541	0.427	0.473	0.375	0.450	0.611	0.455	0.500
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.300	0.567	0.471	0.457	0.421	0.614	0.714	0.500	0.445	0.548	0.508	0.625	0.350	0.278	0.455	0.414
0.060	–	–	0.043	0.053	–	0.036	0.031	0.034	0.032	0.035	0.063	–	–	–	0.014
–	–	–	–	–	–	–	–	–	–	–	0.125	0.050	0.167	0.091	0.114
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.940	1.000	1.000	0.957	0.947	1.000	0.964	0.969	0.966	0.968	0.965	0.812	0.950	0.722	0.909	0.829
–	–	–	–	–	–	–	–	–	–	–	–	–	0.111	–	0.043
–	–	–	–	–	0.068	0.179	0.031	–	0.040	0.020	0.063	–	–	–	0.014
0.020	0.033	0.059	–	0.132	0.159	0.036	0.031	0.027	0.113	0.070	–	–	–	–	–
0.920	0.967	0.794	0.848	0.789	0.773	0.750	0.938	0.870	0.815	0.836	0.875	0.900	0.833	0.955	0.914
–	–	0.059	–	–	–	–	–	0.014	–	0.008	–	–	–	–	–
0.060	–	0.088	0.152	0.079	–	0.036	–	0.089	0.032	0.066	0.063	0.100	0.167	0.045	0.071
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	0.156	–	0.040	0.020	0.125	–	–	–	0.043
0.100	0.233	0.265	0.217	0.079	0.023	–	0.063	0.205	0.040	0.129	0.250	0.450	0.278	0.227	0.286
0.020	–	–	–	–	–	–	–	0.007	–	0.004	–	–	–	–	–
0.760	0.767	0.706	0.761	0.868	0.864	1.000	0.750	0.733	0.855	0.785	0.625	0.500	0.667	0.591	0.614
0.120	–	0.029	0.022	0.053	0.114	–	0.031	0.055	0.065	0.063	–	0.050	0.056	0.182	0.057
–	–	0.029	0.043	0.026	0.023	–	–	0.021	0.016	0.020	–	–	–	–	–
–	–	0.029	–	–	–	–	–	0.007	–	0.004	–	–	–	–	–
0.120	0.233	0.206	0.174	0.263	0.273	0.143	0.156	0.178	0.202	0.191	–	0.100	0.167	0.227	0.129
–	–	–	–	–	–	–	–	–	–	–	0.125	0.150	–	–	0.071
0.240	0.367	0.265	0.435	0.368	0.341	0.321	0.313	0.322	0.355	0.324	0.250	0.150	0.278	0.091	0.200
0.440	0.367	0.412	0.348	0.289	0.273	0.393	0.438	0.384	0.323	0.359	0.500	0.450	0.444	0.500	0.414
–	0.033	0.059	–	–	–	–	–	0.021	–	0.012	–	–	–	–	–
0.200	–	–	–	0.053	0.091	0.143	0.094	0.068	0.105	0.090	0.125	0.150	0.111	0.182	0.186
0.020	–	–	–	–	–	–	0.031	0.007	0.008	0.008	–	–	–	–	–
0.420	0.433	0.529	0.652	0.658	0.545	0.607	0.750	0.514	0.621	0.555	0.813	0.700	0.611	0.864	0.686
0.560	0.567	0.412	0.348	0.342	0.455	0.393	0.219	0.466	0.371	0.430	0.188	0.300	0.389	0.136	0.314
–	–	0.059	–	–	–	–	–	0.014	–	0.008	–	–	–	–	–

Burnett et al. 1994; McMillen-Jackson et al. 1994). Low recruitment rates and clonal longevity is thought to produce long-term stability of gene frequencies in populations of other clonal cnidarians (Hoffmann 1987). Genetic differences among populations of *Z. coppingeri* may be maintained for very long periods.

Natural selection could contribute to genetic differentiation among populations, but it was not considered the major cause of population differences in *Zoanthus coppingeri* for several reasons. Firstly, all loci except *PGM** contributed to the signficance of F_{ST} among sites. It is unlikely that similar levels of selection would occur at 6 out of 7 loci. Secondly, there were no obvious

habitat differences between sites at the same locality that could account for the strong differences seen between them. Sites were chosen (subjectively) to be as similar as possible, having the same exposure, aspect and tidal height, and with no other obvious differences such as amount of freshwater runoff between them. The possible existence of less obvious selection pressures cannot be discounted, however. Thirdly, estimates of gene flow among localities from private alleles agree closely with predictions from F_{ST} values, suggesting the absence of strong selection bias in the latter. We therefore conclude that population structure in *Z. coppingeri* is the consequence of restricted gene flow and genet longevity rather than of selection.

Table 5 *Zoanthus coppingeri.* Mean values of genetic differentiation within (F_{IS}) and between (F_{ST}) populations; 95% confidence limits in parentheses (* $p < 0.05$; *** $p < 0.001$)

Comparison	F_{ST}	F_{IS}
Among all localities	0.039 (± 0.004)***	0.127 (± 0.015)*
Among sites:		
In all localities	0.043 (± 0.004)***	0.118 (± 0.016)
Within Cockle Bay	0.021 (± 0.002)***	0.112 (± 0.035)
Within Low Isles	0.023 (± 0.005)***	0.108 (± 0.036)
Among transects within:		
Cockle Bay Site A	0.018 (± 0.006)	0.102 (± 0.029)
Site B	0.007 (± 0.002)	0.111 (± 0.031)
Low Isles Site A	0.013 (± 0.002)	0.111 (± 0.037)
Site B	0.012 (± 0.002)	0.060 (± 0.019)
Kissing Point	0.011 (± 0.008)	0.072 (± 0.042)

Table 6 *Zoanthus coppingeri.* Hierarchical *F*-statistics (Wright 1978)

Comparison	Variance component	*F*
Subsites within sites	0.04173	0.014
Subsites within localities	0.11384	0.037
Subsites within total	0.18881	0.060
Sites within localities	0.07211	0.024
Sites within total	0.14708	0.047
Localities within total	0.07497	0.024

In conclusion, populations of *Zoanthus coppingeri* consist of large numbers of clones, a consequence of genet longevity and intermediate disturbance rather than high recruitment rates. High energetic investment in somatic growth increases genet longevity but may lower sexual reproductive effort, so indirectly reducing recruitment rates via decreased larval abundances. Gene flow among populations is restricted over as little as 50 m, and genet longevity maintains stochastically-generated variation in allele frequencies among populations. Populations of other clonal species exhibit varying genotypic diversities as a consequence of differing degrees of disturbance, competition among genotypes and other ecological factors. Low genetic turnover is, however, likely to be characteristic of many of these organisms. As a result, many of the more important groups on the GBR, such as the reef building corals, may show considerably more population structuring than has been described previously in strictly sexual species.

Acknowledgements Thanks to A. Chen, J. Walker, M. Deslandis, L. West and P. Bono for their assistance in the field, and to the crew of the R.V. "Sirius" and AIMS marine operations staff. R. Rowan, S. Williams and two anonymous referees made helpful comments on the manuscript. W.J.B. acknowledges the support of NERC/CASE studentship ref: GT4/91/ALS/47. This is Contribution No. 719 from the Australian Institute of Marine Science.

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Table 7 *Zoanthus coppingeri.* Estimates of migrants per generation among populations using two methods, and number of private alleles used; 95% confidence intervals in parentheses (*CAF* conditional average frequency)

Comparison	No. of migrants (from F_{ST})	No. of migrants (from CAF)	No. of private alleles
Among all localities	6.2 (5.5–7.0)	4.5 (3.9–5.1)	9
Among sites:			
In all localities	5.6 (5.1–6.2)	5.8 (5.2–6.4)	8
Within Cockle Bay	11.7 (10.7–12.8)	7.8 (7.0–8.8)	7
Within Low Isles	10.6 (9.1–12.8)	5.2 (4.6–5.9)	8
Among subsites within:			
Cockle Bay Site A	13.6 (10.2–20.6)	4.4 (3.3–6.3)	3
Site B	35.5 (27.5–49.8)	1.1 (1.0–1.3)	8
Low Isles Site A	19.0 (16.4–22.5)	1.5 (1.2–1.8)	8
Site B	20.6 (17.6–24.8)	1.3 (0.4–37.0)	2
Kissing Point	22.5 (12.9–83.1)	1.5 (1.2–1.9)	4

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