Asexual reproduction and genetic determination of colour patterns within populations of the subtidal sea anemone *Anthothoe albocincta*

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ABSTRACT: Southeastern Australian populations of the dioecious, subtidal sea anemone *Anthothoe albocincta* display considerable spatial variation in the colour patterns of the constituent polyps, and the most visually striking feature of local populations are sets of dense aggregations each comprised of polyps with a single colour pattern. We used a combination of genetic data, observations and manipulative experiments to infer that *A. albocincta* generates these monomorphic aggregations via asexual reproduction and that asexual reproduction plays the major role in the maintenance of established populations. Allozyme electrophoresis of 2 to 5 polyps from each of 20 mapped aggregations (in 2 populations) revealed that in 18 cases (90%) all polyps were electrophoretically identical and always distinct from differently coloured polyps taken from each of 26 adjacent aggregations. Furthermore, the genetic structure of each of 13 populations separated by up to 930 km was consistent with the predicted effects of localised asexual recruitment. We detected 16 heterozygote excesses and 11 heterozygote deficits (p < 0.05) in a total of 66 tests for departures from Hardy-Weinberg equilibria. Moreover, each population contained significantly less (p < 0.005) multi-locus genotypic diversity (G_o) than was expected for a randomly mating population (G_e) (mean G_o:G_e = 0.30) and contained relatively few unique 7-locus genotypes. Some genotypes were found within many aggregations, suggesting successful clones may have extensive and discontinuous distributions within populations. In addition, we observed longitudinal fission of adult polyps in both field and laboratory conditions, and found that over a 6 mo period 82% (i.e. 1469 of 1790) of new recruits into 30 experimentally cleared patches, within 3 populations, were morphologically identical to those within surrounding aggregations. These data, together with our earlier analysis of genetic variation among populations, and observations of gonad development, imply that this species uses fission to maintain populations, consisting predominantly of spatially restricted clones (each with an almost unique colour pattern), but uses sexual reproduction to produce widely dispersed colonists.

KEY WORDS: Cnidarian · Asexual · Sexual · Colour morph · Longitudinal fission

INTRODUCTION

Despite the potential of marine organisms for dispersal over vast spatial scales, recent studies have found that populations of many marine organisms are highly genetically subdivided (e.g. Ayre 1983, 1984, Johnson & Black 1984a, 1991, Burton 1986, 1990, Hoffmann 1986, Grosberg 1987, 1991, Ayre & Dufty 1994). In many of these cases the proximate cause of subdivision has been inferred to be the philopatric dispersal of gametes and/or sexually or asexually produced propagules. However, there is increasing evidence that subdivision may also be reinforced by, as yet poorly studied, fine-scale genotype by environment interactions (e.g. Johnson & Black 1984b, Ayre 1985, 1995, Behrens Yamada 1989, Johannesson et al. 1990) and barriers to gene flow produced by the formation of co-adapted gene complexes (e.g. Burton 1986, 1990, Grosberg 1987, 1991).

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In sea anemones sexual reproduction may involve one of many tactics including self-fertilisation and external brooding of larvae (e.g *Epiactis prolifera*; Bucklin et al. 1984), philopatric dispersal of outcrossed larvae (e.g. *Diadumene leucolena*; Shick & Lamb 1977) or outcrossed, broadcast spawning (e.g *Oulactis muscosa*; Hunt & Ayre 1989) and so have the capacity to either create or overwhelm genetic structuring within populations. Asexual reproduction may occur via processes ranging from fission to parthenogenetic production of larvae (reviewed by Shick 1991), and, in the intertidal, asexual reproduction is usually associated with spatially restricted clonal proliferation leading to highly structured 'clonal' populations (e.g. Shick et al. 1979, Ayre 1983, Shaw 1991, McFadden et al. 1997). The philopatric dispersal of either sexually or asexually generated propagules provides the proximate cause of fine-scale (within population) and meso-scale (within region) genetic subdivision within at least several sea anemone species (reviewed by Shick 1991). Nevertheless, our understanding of the ecological or evolutionary significance of fine-scale genetic structure within local populations of most species remains uncertain and experimental studies are seriously impeded by the need to identify individual genotypes. Such identification typically requires the time consuming assessment of variation in protein or nucleic acid markers.

Local populations of the dioecious, subtidal sea anemone *Anthothoe albocincta* (Stuckey) (Tribe Mesomyaria, Family Sagartiidae) are characterised by striking spatial variation in the colours of the oral discs and tentacles of their constituent polyps (Carlgren 1950, 1954, Thomas & Shepherd 1982, Billingham & Ayre 1996). *A. albocincta* is common along the shores of southeastern Australia, forming large populations (some with 1000s of polyps). Populations extend across a wide vertical range (0 to >24 m) and occur on many substrates (most commonly rock, but also sponges, algae, gastropod shells and ascidians; Billingham pers. obs.). Numerous different colour morphs exist within most populations, although most polyps are found within dense aggregations of identically coloured individuals, which are themselves in close proximity to other aggregations (Billingham & Ayre pers. obs.).

Asexual replication of identically coloured polyps would provide the simplest explanation of the colour variation seen within populations of *Anthothoe albocincta*. Qualitatively similar interclonal variation has been reported for the fissiparous, North American intertidal anemone *Anthopleura elegantissima* (Francis 1973, Ayre & Grosberg 1995). Alternative explanations for the presence of monomorphic aggregations of polyps within populations of *A. albocincta* include the possibility that either: (1) colour is genetically determined and aggregations are produced by the philopatric dispersal of sexually generated but closely related individuals or (2) colour varies simply in response to underlying patterns of environmental variation (phenotypic plasticity). Each alternative explanation would be distinguished by a different pattern of genotypic structuring within populations.

If each monomorphic aggregation consists of a single clone then individual polyps within each aggregation should share the same multilocus genotype and be genetically different to dissimilarly coloured neighbouring anemones. Single-locus genotype frequencies within such 'clonal' populations should differ from expectations for Hardy-Weinberg equilibria, with both excesses and deficits of heterozygotes present (e.g. Ayre 1983). Alternatively, if the limited dispersal of sexually derived progeny gives rise to aggregations then individuals within aggregations would be expected to be genetically similar (though not identical) and fine-scale population subdivision should result in lower than expected numbers of heterozygotes (i.e. Wahlund effect; Hedrick 1985). Finally, if colour has no simple genetic basis but reflects a phenotypically plastic response to fine-scale environmental heterogeneity then any genotypic and phenotypic variation should vary independently.

In this study we used allozyme electrophoresis together with field and laboratory observations, and experimental manipulations to show that: (1) within 2 populations single colour aggregations of *Anthothoe albocincta* result from asexual reproduction via longitudinal fission; (2) successful clones may have extensive and discontinuous distributions within populations; and (3) asexually derived recruitment plays the major role in maintaining each of 13 populations of *A. albocincta* over 930 km of coastline.

METHODS

Collection and electrophoresis. We collected anemones from between 4 and 10 m depth by prising them from the substrata using a small knife. We then transported them live to the laboratory in individual plastic bags ⅓ filled with cool sea water and stored them at −80°C pending electrophoresis. We used horizontal starch gel electrophoresis (12.5% w/v starch) as described in Billingham & Ayre (1996) to determine a multilocus genotype for each sampled polyp of *Anthothoe albocincta*. We assessed the variation at each of 7 variable enzyme-encoding loci, using the electrophoretic buffers and assay conditions described by Selander et al. (1971)—on buffer number 5: glutamate dehydrogenase (GDH; E.C. 1.4.1.3), 6-phospho-

gluconate dehydrogenase (6PGD; E.C.1.1.1.44) and xanthine dehydrogenase (XDH); on buffer number 6: leucine-alanine peptidase (PEP- C; E.C. 3.4.11); and on buffer number 9: glucosephosphate isomerase (GPI; E.C. 5.3.1.9), isocitrate dehydrogenase (IDH; E.C. 1.1.1.42) and phosphoglucomutase (PGM; E.C. 2.7.5.1). All enzymes were apparently monomeric or dimeric (except for GDH which has a higher order multi-subunit structured; Catmull et al. 1987), and isozyme patterns were similar to those described for other species with Mendelian inheritance. At each locus alleles were labelled alphabetically in order of decreasing electrophoretic mobility.

Genotypic diversity within aggregations. In order to use allozyme variation to assess the importance of asexual recruitment in the formation of aggregations of *Anthothoe albocincta*, we first surveyed the 7-locus genotypes of single polyps taken from each of 28 randomly chosen aggregations, representing 13 different colour morphs, on a 23 m length of subtidal rock wall (approximately 100 m^2) at Flagstaff Hill (Fig. 1). This survey provided an estimate of the level of allelic variation within the population and allowed us to identify 10 aggregations in which the sampled polyp was heterozygous for 2 or more loci. In order to test for evidence of asexual replication within aggregations we then assayed an additional 4 polyps within each of the 10 potentially 'heterozygous' aggregations, as well as 3 to 5 differently coloured polyps from areas adjacent to 6 of these aggregations. The remaining 4 'heterozygous' aggregations were themselves neighbours and polyps within 2 of these displayed subtle colour variation. If these aggregations of identically or similarly coloured anemones were generated by asexual reproduction then for all loci at which the first polyp taken from a given aggregation was heterozygous all 5 polyps should be identically heterozygous, whereas aggregations generated by sexual reproduction may be genotypically diverse. Moreover, if colour is genetically determined then adjacent polyps with different colours should be genetically distinct and may display detectably different electrophoretic phenotypes. In addition we tested for asexual replication of genotypes within aggregations by determining the genotypes of 2 polyps/aggregation from each of 12 aggre-
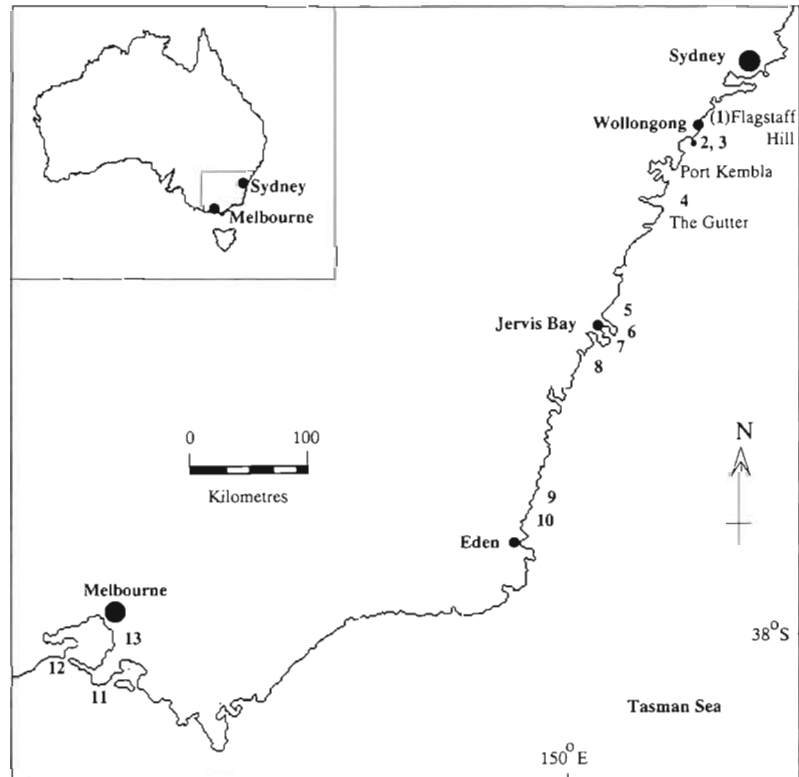

Fig. 1 Southeastern coastline of Australia, showing the study populations; all numbered populations were used for electrophoretic comparisons. The Flagstaff Hill, Port Kembla and Gutter populations were also used in a recruitment study. Other numbered populations: 2, Toothbrush Island (north); 3, Toothbrush Island (south); 4, Gravel Loader; 5, Turtle Row; 6, Bowen Island; 7, Governor's Head; 8, Ulladulla; 9, Tathra; 10, Merimbula; 11, Flinders; 12, Queenscliff; 13, Mornington

gations, representing 10 differently coloured aggregations, on the wooden pier pilings and rubble below the Mornington Jetty (Fig. 1).

Genotypic diversity within populations. In order to test for geographic variation in the relative importance of sexual and asexual recruitment in maintaining entire populations we collected *Anthothoe albocincta* from each of 13 southeastern Australian sites along 930 km of coastline (Fig 1). In most cases we collected a single polyp from each of 15 to 62 randomly selected aggregations within areas of 50 to 700 m^2. However, at Tathra and Merimbula (Fig. 1) we collected relatively few anemones over the smaller areas of 25 and 5 m^2, respectively, as no other anemones could be located at sites inspected in this region. For the northern populations (Flagstaff Hill to Ulladulla; Fig. 1) we sampled anemones from rock walls and boulders. Collections within the 5 southern populations (Tathra to Mornington; Fig. 1) were made from pilings and/or rubble beneath jetties.

Analysis of the genotypic structure of populations. Wright's (1978) fixation index (*f*) was used to describe

the magnitude of departures from the level of hetero-zygosity expected under Hardy-Weinberg equilibria. Positive values of *f* reflect a deficit of heterozygotes whilst negative values indicate an excess of heterozy-gotes. We assessed the statistical significance of such departures using chi-square tests to compare the observed and expected proportions of homozygous and heterozygous individuals within each population.

We used 2 analyses to further estimate the effect of asexual reproduction on the genotypic structure of populations and to compare clonal richness and diver-sity across populations. Firstly, we calculated the observed level of clonal diversity, i.e. the number of unique multilocus genotypes detected in the pop-ulation (N_g), divided by the sample size (N) (Hoffmann 1986). As only a small fraction of the genome was sam-pled electrophoretically, each unique 7-locus genotype may represent more than 1 clone. N_g/N may therefore underestimate the true clonal diversity. Consequently we used the method of Hoffmann (1986) in order to infer whether we had detected all possible clones within each collection, i.e. the number of unique multi-locus genotypes was determined firstly using only each individual polymorphic locus, then all possible 2 polymorphic locus combinations, all possible 3 poly-morphic locus combinations and so on until all loci were included. The average number of multilocus genotypes identified for all 1 locus, 2 loci combina-tions, etc., was then calculated. If all clones were rec-ognized, a plot of number of polymorphic loci included versus average number of multilocus genotypes detected should reach an asymptote that indicates the actual number of clones sampled.

Second, we estimated the effect of asexual recruit-ment on the genotypic composition of populations by comparing the observed level of 7-locus genotypic diversity (G_o) to that of the expected (G_e) under condi-tions of sexual reproduction and free recombination for a population with the sample allele frequency and size (Stoddart & Taylor 1988). Deviations of $G_o:G_e$ from unity provide a measure of the effects of departures from single locus Hardy-Weinberg equilibria and mul-tilocus linkage equilibria. Populations with high levels of asexual input should exhibit relatively low $G_o:G_e$. We evaluated differences between G_o and G_e for each population using an unpaired *t*-test (Stoddart & Taylor 1988).

Recruitment into cleared quadrats. Since the colour of recruits may provide an indication of their source and mode of production we examined recruitment into experimentally cleared quadrats at the Flagstaff Hill, Port Kembla and Gutter sites (Fig. 1) between January and June, 1993. In each population we cleared 10 quadrats (15 × 15 cm) within an area containing 1 or 2 colour morphs. We subsequently monitored recruit-

ment into these quadrats on a monthly basis (weather permitting) recording numbers and colour of all anemones present. All quadrats were in areas densely populated with *Anthothoe albocincta* and were a min-imum of 10 cm from surrounding colour morphs. When the quadrats were cleared, we recorded the colour of anemones removed.

We used the total numbers of original and non-origi-nal coloured polyps present to determine percentage of original polyps present. We also used repeated mea-sures ANOVAs (Glantz & Slinter 1990) within popula-tions to test whether there was temporal variation in recruitment of non-original colour morphs within each site. The number of non-original colour morphs pre-sent was not the same as the number of polyps (e.g. if 3 green oral disc/orange tentacles and 2 brown oral disc/white tentacles anemones were found in a quad-rat, with both colour morphs being non-original, then we assessed the number of new colour morphs present as 2).

Laboratory observations of asexual reproduction. In order to observe the reproduction of *Anthothoe albocincta* directly, we collected 18 individuals from the Flagstaff Hill (Fig. 1) site in September 1992 and observed their development for 16 wk. We kept each anemone in separate 1 l aquaria for 16 wk and fed them with *Artemia* sp. approximately weekly. We replaced the sea water once or twice weekly. We mon-itored the relative size, change in number, and condi-tion of anemones present in each container.

RESULTS

Genotypic diversity within aggregations

Our data show that aggregations of *Anthothoe albocincta* typically consist of genotypically identical groups of putative clones. At Flagstaff Hill we found no genotypic variation within each of the groups of 5 polyps taken from 7 of the 8 uniformly coloured aggre-gations (aggregations A to H; Table 1). We detected 3 multilocus genotypes within aggregation D, however this aggregation also appeared to have been gener-ated in part through asexual reproduction as 3 polyps shared the same multilocus genotype and were het-erozygous at 5 loci (Table 1). The remaining 2 polyps sampled from aggregation D had distinct genotypes, not shared by any other anemones assayed. In all cases the genotypes of polyps taken from these aggregations were electrophoretically distinct from those of visually distinct polyps collected from around their margins (n = 26; Table 1). We detected 2 multilocus genotypes within each of the 2 adjacent aggregations that dis-played subtle colour variation (aggregations I and J;

Table 1. *Anthothoe albocincta*. Comparisons of colour and genotypic variability within sets of 5 polyps from each of 10 aggregations (A–J) at Flagstaff Hill, and genotypic variability within sets of 3 to 6 differently coloured polyps from areas adjacent to 6 of the aggregations. Numbers of polyps displaying each multilocus genotype are given in parentheses

Aggregation	Colour pattern (Oral disc/Tentacles)	No. of multi-locus genotypes (no. of polyps with each genotype)	
		Within aggregation	Within polyps adjacent to aggregations
A	Orange/white	1 (5)	2 (2, 1)
B	Orange/green	1 (5)	4 (2, 2, 1, 1)
C	Orange/orange	1 (5)	6 (1, 1, 1, 1, 1, 1)
D	Orange/white	3 (3, 1, 1)	3 (1, 1, 1)
E	Orange/orange	1 (5)	3 (2, 2, 1)
F	Orange-green/green-white	1 (5)	2 (2, 1)
G	Orange/white	1 (5)	Anemones
H	Orange/orange	1 (5)	from close
I	Orange-green/green-white	2 (3, 2)	proximity
J	Orange-green/green-white	2 (4, 1)	cluster

Table 1). However, 1 multilocus genotype (heterozygous at 3 loci) was common to aggregations I and J (3 and 4 polyps, respectively) indicating that 3 clones were present within these 2 aggregations.

Although aggregations at Flagstaff Hill were typically both monomorphic and apparently monoclonal our surveys also revealed that some of the more common colour morphs occurred within at least several aggregations. For example, the most common genotype that we detected (heterozygous for 5 loci) was common to 2 of the sampled aggregations (D and G; Table 1) and polyps surrounding all the other well separated aggregations (A, B, C, E and F; Table 1). Moreover, this genotype displayed the most common phenotype (orange oral disc/white tentacles) that was distributed along the entire 23 m of rock wall examined. Similarly, the multilocus genotype (heterozygous for 4 loci) of the 2 genotypically identical anemones of aggregation I (Table 1) was the same as an individual surrounding aggregation C and the entire aggregation F (10 m away) all of which had an orange/green oral disc and green/white tentacles. These results are most easily explained by the close aggregation of identically coloured clones with movement of anemones within the population enabling clones to establish additional aggregations.

On the Mornington Peninsula jetty, there was no genotypic variation within 11 of the 12 pairs of anemones collected from aggregations (although 9 of the 11 aggregations were genotypically distinct). The sole exception was a pair of polyps of the most common orange/white colour morph that were separated by 2 m (the largest distance separating a pair of polyps taken from a single aggregation). However, some clones covered distances greater than 2 m. For example, 2 aggregations of the same colour, separated by 3 m and with no differently coloured polyps in between, had the same multilocus genotype (heterozygous at 4 loci),

whilst 2 polyps from an aggregation on one piling shared the multilocus genotype (heterozygous at three loci) and colour as an anemone on an adjacent piling, a total separation of 5 m. Combined, results from these 2 populations indicate that anemones from the same aggregation were electrophoretically identical in the majority of cases (18/20).

Genotypic diversity within populations

Our assessment of the level and patterns of genotypic diversity present within all 13 populations implies that asexual reproduction plays the major role in all recruitment to these populations. Even though our estimates used only 1 polyp/aggregation we found that single-locus and multilocus genotype frequencies were always consistent with the predicted effects of asexual reproduction. We detected a high proportion of statistically significant departures from Hardy-Weinberg equilibria including 16 heterozygote excesses and 11 heterozygote deficits in a total of 66 tests (χ^2, $p < 0.05$; Table 2). Of the 13 populations, 5 exhibited both significant heterozygote excesses and deficits at different loci, and all populations were characterised by both heterozygote excesses and deficits (Table 2). Moreover, these surveys of 15 to 62 polyps per population all showed far fewer putative clones (N_g) than the sampled number of aggregations (N). N_g ranged from 4 within the collection of 50 polyps from Merimbula to 27 in the collection of 50 polyps from Mornington (Table 3). Overall some 30 genotypes were shared by 5 or more individuals and in the extreme a single genotype at Flinders was shared by 30 widely separated polyps. Similarly, values of $G_o:G_e$ were typically very low and ranged from 0.06 at Flinders to 0.49 at Tathra (Table 3). In all cases G_o was highly statistically less than G_e ($p < 0.005$; Table 3)

Table 2. *Anthothoe albocincta*. Departures from single-locus Hardy-Weinberg equilibria, given as Wright's (1978) *f* values using the allele frequencies from groups of individual anemones. Positive values of *f* reflect a deficit of heterozygotes, whilst negative values reflect an excess. N: sample size. Enzyme abbreviations are: GPI, glucosephosphate isomerase; PGM, phosphoglucomutase; IDH, isocitrate dehydrogenase; GDH, glutamate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; PEP-C, leucine-alanine peptidase; XDH, xanthine dehydrogenase. Dashes indicate fixation of 1 allele. Statistical significance of departures from expectations are indicated above each value as *p < 0.05, **p < 0.01, ***p < 0.001. nd: not determined due to small expected value. na: IDH could not be scored for 3 populations

Population	N	Locus						
		Gpi-1	Pgm-1	Idh-1	Gdh-1	6Pgd-1	PepC-1	Xdh-1
Flagstaff Hill	62	−0.348*	−0.225*	−0.440**	−0.159	+0.065	−0.451***	+0.034
Toothbrush Is. Nth	50	−0.451**	+0.196	−	−0.031nd	+1.00***	−	−0.186
Toothbrush Is. Sth	38	+0.722*	−0.298**	−	−	−	+0.221	−0.409*
Gravel Loader	45	+0.524**	+0.021	−0.023nd	−0.059	−0.011nd	−0.134	−0.071
Turtle Row	50	−0.099	+0.689***	−0.351*	−0.500	−1.00nd	+0.645***	+0.232
Bowen Is.	44	+0.162	+0.023	−0.075	−0.062	−0.125	+0.285	−0.081
Governor's Head	23	+0.912***	+0.164	−	−0.045nd	−0.394	+0.151	−0.022nd
Ulladulla	50	−0.087	−0.087	−	−	+0.695***	+0.096	−0.010nd
Tathra	15	−	−0.481*	+0.841**	−0.429	−	−0.556*	−0.304
Merimbula	50	−0.020nd	−0.081	+1.00***	−	−	−	−
Flinders	42	−0.704***	+0.812***	na	−0.953***	−0.953***	+0.876**	−0.063
Queenscliff	49	−0.289	−0.169	na	−0.167	−0.155	−0.361*	+0.191
Mornington	50	−0.147	−0.319**	na	−0.387*	−0.235	−0.262	+0.287

Table 3. *Anthothoe albocincta*. Estimates of the genotypic diversity of 13 southeastern populations. N: number of individuals sampled; N_g: number of unique multilocus genotypes detected; G_o: observed multilocus genotypic diversity; G_e: expected multilocus genotypic diversity, under the conditions of random mating and free recombination. Statistical significance of departures from $G_o:G_e = 1$ given by **p < 0.005 and ***p < 0.001

Population	N	N_g	$N_g:N$	G_o	G_e	$G_o:G_e$
Flagstaff Hill	62	19	0.17	7.4	54.2	0.14***
Toothbrush Is. (Nth)	50	13	0.26	7.1	24.2	0.30***
Toothbrush Is. (Sth)	38	11	0.29	3.2	19.5	0.16***
Gravel Loader	45	18	0.40	8.1	17.0	0.48**
Turtle Row	50	18	0.36	6.5	24.5	0.27***
Bowen Is.	44	24	0.55	13.8	31.4	0.44***
Governor's Head	23	12	0.52	5.2	13.5	0.39**
Ulladulla	50	17	0.34	4.5	20.0	0.23**
Tathra	15	9	0.60	6.4	13.2	0.49**
Merimbula	50	4	0.08	2.1	4.9	0.43***
Flinders	42	5	0.12	1.4	25.6	0.06***
Queenscliff	49	16	0.33	6.7	38.1	0.18***
Mornington	50	27	0.54	17.1	46.1	0.37***
Mean	44	15	0.35	6.9	25.6	0.30

indicating the greater importance of asexual reproduction in the formation, and not just expansion of aggregations within all populations.

The power of genetic data to distinguish between clones is dependent upon the number and variability of available polymorphisms. Here we detected between 3 and 7 loci polymorphic within each population (although IDH could not be scored for the 3 most south-ern populations). Plots of the number of variable loci used versus the number of putative clones (multilocus genotypes) detected were in most cases (8/13) not asymptotic with the number of genotypes detected, suggesting that we had not distinguished all genetically different clones within those samples (Fig. 2). Four plots that appeared to reach an asymptote [Flagstaff Hill, Toothbrush Island (South), Flinders and Mornington] along with 4 that appear to have failed to reach an asymptote (Bowen Island, Governor's Head, Tathra and Queenscliff) are shown in Fig. 2. Importantly, an asymptote was reached for the more intensively sampled Flagstaff Hill and Mornington populations (Fig. 2) indicating that all clones may have been identified in these collections.

Monitoring of cleared quadrats

Over all 3 populations 82% (1469/1790) of polyps reappearing in the cleared quadrats were the same colour pattern as those removed. At Flagstaff Hill and Port Kembla, 89% (472/530) and 90% (717/800) of polyps, respectively, were of the original colour and no temporal variation in colour morph recruitment was found ($F = 1.02$, df = 3, p > 0.05 and $F = 0.137$, df = 2, p > 0.05, respectively). In contrast, at the Gutter, original coloured polyps accounted for only 61% (280/460) of the recruitment into patches and significantly more new colour morphs had recruited into this population in late May and July than in February and March ($F = 3.45$, df = 3, 24, p < 0.05).
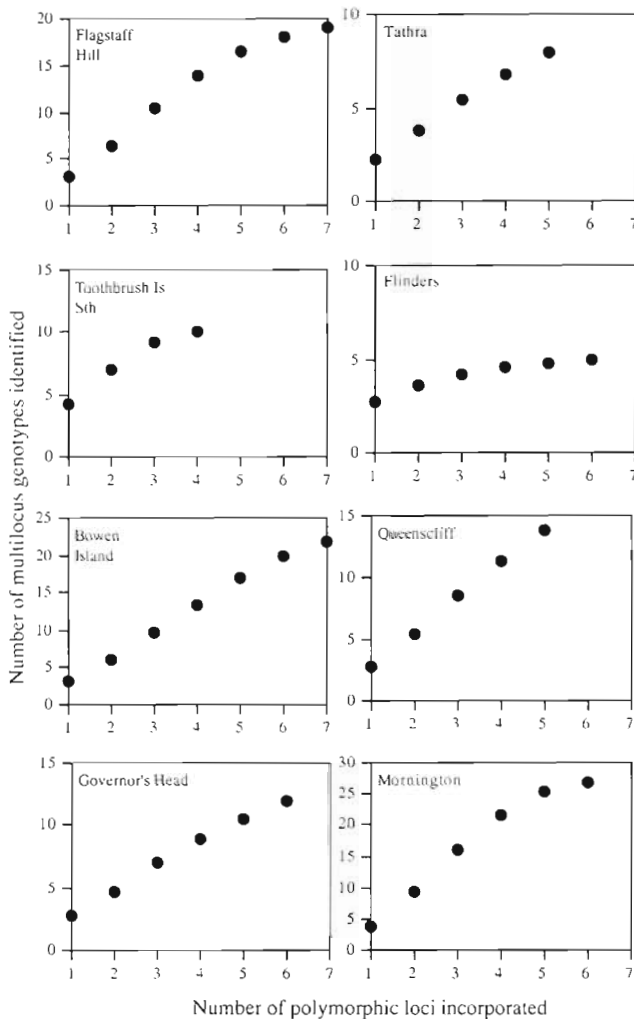
Fig. 2. *Anthothoe albocincta*. Plots of the number of unique multilocus genotypes identified versus the number of polymorphic allozyme loci employed in their detection, within 8 of 13 collections of anemones from southeastern Australian populations

DISCUSSION

Comparative evidence for a wide range of terrestrial and freshwater taxa, with mixed modes of reproduction, suggests that sexual reproduction should be used whenever offspring are likely to encounter changed or uncertain conditions (Bonner 1958; see reviews by Williams 1975, Bell 1982). In the marine environment sexual reproduction is usually associated with the relatively uncertain process of planktonic dispersal and colonisation, whereas asexual reproduction may be used to restock the parental habitat patch (reviewed by Knowlton & Jackson 1993, although see Stoddart 1988). Taken together our data imply that *Anthothoe albocincta* does use longitudinal fission as its primary means of replenishing established populations in southeastern Australia whereas sexual reproduction is used to produce colonising larvae. The resultant formation of clonal aggregations provides a simple proximate explanation for the observed spatial variation in polyp colour but contrasts with the prediction that such local populations should be replenished by sexually derived recruitment (Francis 1988). This population structure is similar to that reported for several intertidal sea anemones (e.g. Shick et al. 1979, Hoffmann 1986) including the sympatric intertidal species *Actinia tenebrosa* (Ayre et al. 1991).

Asexual reproduction and the formation of monomorphic aggregations

Although the genotypic structures of local populations provide the best indication of realised patterns of recruitment, our observations and simple experiments confirmed that *Anthothoe albocincta* can use asexual reproduction to generate pairs of similar sized individuals, with identical colour patterns. Surprisingly we found that, although basal laceration is usual within the Mesomyaria (Shick 1991), *A. albocincta* reproduced asexually through longitudinal fission. Longitudinal fission has however been reported in the congeneric South American, intertidal species *A. chilensis* and may be typical of this genus (Shick 1991). These observations alone are insufficient to indicate the relative importance of fission or any form of asexual reproduction for *A. albocincta*, however, fission is known to generate aggregations of genetically identical polyps in a range of species (Shick 1991).

Our fine-scale surveys of the genotypes of polyps from aggregations within 2 widely separated populations revealed that polyps collected from 90 % (18 of 20) of monomorphic aggregations were genetically identical and were always electrophoretically distinct from neighbouring polyps with different colour patterns.

Laboratory observations

We found that *Anthothoe albocincta* reproduces asexually in laboratory conditions. Fifty percent of polyps (9 of 18) maintained in aquaria for 16 wk reproduced asexually via longitudinal fission. Each division produced a pair of viable and identically coloured polyps. Seven polyps divided on just 1 occasion and each produced 2 polyps of approximately equal size. The other 2 asexually reproductive individuals each gave rise to 4 anemones of equal size, although these divisions were not always symmetrical. Under these conditions fission scars were observed on all dividing anemones and persisted until the end of the observation period (up to 15 wk).

These results strongly support the conclusion that aggregations are formed through fission and contrast sharply with the predicted effects of (1) outcrossed sexual reproduction, which should produce far greater genotypic diversity within aggregations, and (2) self-fertilisation or close inbreeding, which should generate some genotypic variation within aggregations and low levels of heterozygosity. Although the functional significance of the array of colour morphs remains uncertain this variation therefore appears to represent a true genetic polymorphism (Lincoln et al. 1982). Similar, interclonal variation in colour patterns and pigments has been reported for the intertidal sea anemone *Anthopleura elegantissima* (Francis 1973) and the scleractinian coral *Porites compressa* (Hunter 1985)

For *Anthothoe albocincta* the importance of asexual reproduction does not seem limited to the expansion of single aggregations. Even though our surveys of most populations were designed to avoid resampling of aggregations (and so should underestimate the effects of localised fission) our resultant collections of genotypes were highly 'clonal', i.e. we detected both significant excesses and deficits of heterozygotes for each of 5 variable loci, and lower than expected multilocus genotypic diversity ($G_o \ll G_e$) within all populations examined. Indeed, our resultant estimates of $G_o : G_e$ (mean = 30%) for *A. albocincta* are amongst the lowest reported for marine invertebrates that use both sexual and asexual reproduction (e.g. Stoddart 1984, Johnson & Threlfall 1987, Ayre & Willis 1988, Mladenov & Emson 1990, Ayre et al. 1991) and imply that individual clones frequently form multiple aggregations within sites. On average we detected only 15 ± 2 unique multilocus genotypes in our samples of 44 ± 3 polyps. Moreover, although we cannot be certain that it was represented by a single monomorphic clone, the most common multilocus genotype (with the same colour) was found along the entire 23 m of rockwall examined at the Flagstaff Hill and within some populations even rare colour morphs were seen to occupy vast areas of shore (Billingham & Ayre unpubl. data).

Sexual reproduction and recruitment

The highly clonal nature of all of the surveyed populations of *Anthothoe albocincta* may mask the real importance of sexual reproduction for this species. Our observations of recruitment and our surveys of the genotypic diversity of adult populations imply that *A. albocincta* also recruits successfully through sexual reproduction. Moreover, if sexual reproduction is used to colonise new areas then both forms of reproduction should be equally important in the life-cycle of a successful lineage.

During this study most polyps colonising experimentally cleared patches at 3 sites displayed the same morphology as the original and surrounding residents of the quadrat. Such colonists were clearly the products of either current longitudinal fission or had simply walked from the remaining (non-cleared) portion of their aggregation. Nevertheless most *Anthothoe albocincta* polyps produced mature male or female gametes (Billingham & Ayre 1996) and we detected recruitment by a high proportion of non-original colour morphs at the Gutter site in late May and July immediately after the March-April spawning peak (Billingham & Ayre 1996). Many of these recruits displayed colour morphs not found in the vicinity of the quadrats. These data imply that we detected an episode of sexual recruitment at the Gutter site since recruits at the other 2 sites, and to the same Gutter quadrats during February and March, closely matched the morphology of surrounding polyps. Patchy or episodic recruitment has been detected or inferred for a number of anemones (Sebens 1982, Ayre 1984, Hoffmann 1987) and marine invertebrates in general (Loosanoff 1966, Fadlallah 1982, McFadden 1991, Menge 1991) and may in part reflect the potentially great longevity of individuals or clones.

Two additional forms of evidence support the premise of sexually derived recruitment in *Anthothoe albocincta*. First, we have shown previously (Billingham & Ayre 1996) that this species is dioecious and with a high proportion of polyps reaching peak maturity just prior to the inferred recruitment episode at the Gutter site. Second, we have used a detailed analysis of genotypic and allelic variation among populations to infer that sexual reproduction is the primary source of colonists. Most significantly we found that although all populations contained multiple clones, clonal genotypes are not shared by neighbouring sites. Clonal propagules therefore seem unlikely to play a major role in the founding of new populations or in maintaining genotypic diversity within other established populations.

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