

REPORT

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Population genetics of the fissiparous holothurian *Stichopus chloronotus* (Aspidochirotida) on the Great Barrier Reef, Australia

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Abstract Population genetic structure was studied in one nearshore and two offshore populations of *Stichopus chloronotus*, a common holothurian species on Indo-Pacific coral reefs. Genetic variation at five polymorphic loci was examined using allozyme electrophoresis. The nearshore population consisted almost exclusively of male individuals, and more males than females were found in all populations studied. Deviations of heterozygosity from that predicted under Hardy-Weinberg equilibrium indicated that asexual reproduction occurred in all populations. Estimates of the level of asexual reproduction using the ratios of the number of sexually produced individuals to sample size, observed genotypic diversity to expected genotypic diversity, and number of genotypes to sample size confirmed that this reproductive mode was more important at the nearshore reef compared to the two offshore reefs. There were large differences in genotypic frequencies between males and females. F-statistics on clonal genotypic frequencies were not statistically significant between populations for neither females or males, suggesting high dispersal of larvae between reefs. A higher mortality of females during larval or early post-settlement stages, or reduced dispersal capability of female larvae are the most likely reasons for biased sex ratios.

Key words Holothurian · Population genetics · Asexual reproduction · Sex ratio · Electrophoresis

Introduction

Stichopus chloronotus (Brandt) is one of eight aspidochirotide holothurian species (listed in Mladenov 1996; Uthicke 1997) which reproduce asexually by transverse fission and which are conspicuous members of the coral reef community (Franklin 1980; Uthicke 1997, 1998). All these species are facultatively clonal and also reproduce sexually via long-lived planktonic trophic larvae. The fissiparous holothurians are thus distinguished from the majority of benthic clonal species, which generally have short-lived sexual propagules (Jackson 1986). Sexual reproduction in marine invertebrates is an important means of recruitment into new areas and provides a “genetic link” between populations (e.g. McFadden 1997), whereas asexual reproduction may be important in maintaining local population size where sexual recruitment is low or mortality is high (Chao et al. 1993; Uthicke et al. 1998). The ability to undergo fission and broadcast combines the advantages of both reproductive strategies: favourable habitats can be rapidly occupied by asexual reproduction and sexual reproduction provides a potential to colonise new habitats (Mladenov and Emson 1984). Nearly all fissiparous holothurian species are broadcast spawners and have a widespread distribution over the Indo-Pacific region (Clark and Rowe 1971) and reach high local abundances (e.g. Bonham and Held 1963; Massin and Doumen 1986; Uthicke 1994).

Asexual reproduction has a strong impact on the genetic structure of marine invertebrate populations. Many of these populations show extreme deviations from genotypic frequencies expected under Hardy-Weinberg equilibrium (e.g. Black and Johnson 1979; Stoddart 1984; Kwast et al. 1990) and genotypic diversity is usually much lower than that expected under panmixis (e.g. Black and Johnson 1979; Johnson and Threlfall 1987; Mladenov and Emson 1990; Ayre and Dufty 1994; Burnett et al. 1995). Additional effects of cloning on the population structure may be the

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prevalence of small individuals (Mladenov and Emson 1988; Chao et al. 1993; Mladenov 1996; Uthicke et al. 1998) and biased sex ratios (Garrett et al. 1998). Although in most aspidochirotide holothurian species investigated, females and males occur in similar numbers (Franklin 1980; Harriott 1982; Conand 1993), deviations from a 1:1 sex ratio have been reported from some asexually reproducing populations of *Holothuria atra* (Harriott 1980; Uthicke et al. 1998).

The fission frequency in holothurians may vary strongly between locations and between seasons (Conand and De Ridder 1990; Chao et al. 1993; Emson and Mladenov 1987; Uthicke 1997). In populations of *Holothuria atra* and *Stichopus chloronotus* investigated on the Great Barrier Reef (GBR), many more recently split individuals were observed on nearshore reefs compared to midshelf reefs. Both species in this region have a distinct period during the colder months when fission is more common (Uthicke 1997).

Stichopus chloronotus is a highly specialised sea cucumber which occurs throughout the Indo-Pacific region. This species is one of the few aspidochirotides with strictly diurnal feeding, and it hides in crevices during the night (Yamanouti 1939; Uthicke 1994). Feeding in this species is highly selective towards sediments with high organic content (Moriarty 1982) and high microalgal biomass (Uthicke 1999). The peak of asexual reproduction in this species on the GBR alternates with sexual reproduction and spawning, which occurs between December and March (Franklin 1980; Uthicke 1997). This is not necessarily a general pattern in holothurians, since periods of asexual and sexual reproduction in species of the genus *Holothuria* overlap (Uthicke 1997). Gonads of all stages of development can be found in *Holothuria* species (Uthicke 1998; Chao et al. 1994) whereas *S. chloronotus* contain only very small, immature gonads (Uthicke 1998). These data suggested that individuals of *Stichopus chloronotus* which split in winter may take part in the spawning season in the following summer.

In a previous study only male individuals were found in a population of *Stichopus chloronotus* with a high degree of asexual reproduction on a nearshore reef (Great Palm Island) in the central section of the GBR (Uthicke 1997). This finding prompted us to investigate the genetic structure of this population using allozyme electrophoretic markers and to study the genetic differentiation between this population and two neighbouring midshelf reefs, in order to obtain information on the roles of asexual and sexual recruitment to these populations.

Material and methods

Sampling strategy

We collected *Stichopus chloronotus* (Brandt) at water depths between 1 and 2 m at one nearshore island reef (Great Palm Island, 18°41'

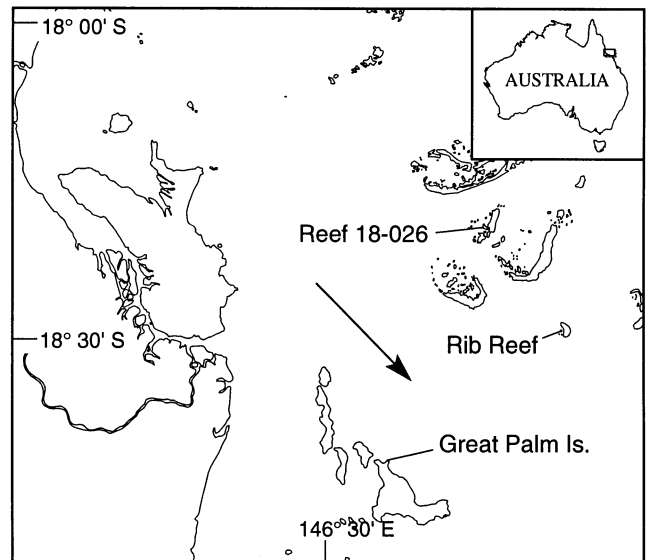


Fig. 1 Locality map of the three populations sampled for *Stichopus chloronotus* on the Great Barrier Reef. The arrow indicates the prevailing current flow

S 146°35' E) and two midshelf reefs (Rib Reef, 18°29' S 146°53' E and Reef 18-026, 18°21' S 146°45' E) in the Great Barrier Reef (GBR, Fig. 1). The population on Great Palm Island exhibits a high incidence of asexual reproduction whereas asexual reproduction on Rib Reef occurs less frequently (Uthicke 1997); no previous information on asexual reproduction was available for Reef 18-026. To our knowledge, the *S. chloronotus* population on Rib Reef is the closest to that of Great Palm Island with a distance of about 40 km. Reef 18-026 is located approximately 44 km NNE of Great Palm Island. Two stations approximately 500 m apart (measured with a Motorola global positioning system) were sampled on each reef. At each station, 30 to 40 individuals were sampled within a radius of 30 m, in early November 1996. Before processing, animals were kept for at least 24 h in 60-l containers with flowing sea water to allow for voidance of the gut contents. Animals were dissected, and a sample of their intestines was snap frozen in liquid nitrogen after removal of remaining sediment and later stored at -75°C . Gonads were removed and frozen (-20°C) in separate zip-lock bags for later sex determination.

Sex determination

The sampling time in November was chosen to increase the likelihood of finding mature gonads in the sampled animals. *Stichopus chloronotus* on the GBR has been reported to have high gonad indices during November (Franklin 1980), and spawning in the sampling area had been observed from December to March (Uthicke unpublished data). However, all gonads were relatively small and showed no macroscopic difference in colour or shape between the sexes. All gonads had to be examined microscopically at 200 times magnification to identify sex. Gonads of both sexes were maturing to mature (phase III to IV according to Franklin 1980). Ovaries were easily distinguished by the occurrence of mature oocytes, although some females contained only previtellogenic oocytes. Testes of *S. chloronotus* were medium to densely packed with spermatoocytes. The sex of a few individuals (<5%) could not be determined, and these individuals were not included in the analyses.

Electrophoresis

In an initial screening of 39 enzyme systems (Ballment et al. 1997) interpretable variation was detected in five polymorphic enzymes in *Stichopus chloronotus*, and these allozyme systems were used in the present study. Variation in hexokinase (E.C. 2.7.1.1; *HK**), mannose-6-phosphate isomerase (E.C. 5.3.1.8; *MPI**), phosphoglucomutase (E.C. 5.4.2.2; *PGM**), triose-phosphate isomerase (E.C. 5.3.1.1; *TPI**) and peptidase using valylleucine as substrate (E.C. 3.4.11/13; *VL**) was determined.

Approximately 500 mg of frozen intestine tissue was homogenised in the same volume of Tris-HCl buffer, pH 8. Electrophoresis was performed for 15 h at 280 V on horizontal, starch gels using Tris-glycine (pH 8.4; TG) buffer. For details on staining and electrophoresis methods see Ballment et al. (1997). Migration distances of the alleles at each locus were measured and alleles were labelled according to their migration distance relative to that of the most common allele, which was assigned a value of 100. For ease of discussion, alleles also were given alphabetical labels, in the sequence from faster-migrating to slower-migrating alleles.

Data analyses

Genotypic frequencies and basic statistics of genetic variability were carried out using programs in the BIOSYS package (Swofford and Selander 1981). In all tests comparing genotypic frequencies or testing conformation to Hardy-Weinberg expectations, the significance values used were Bonferroni-corrected for multiple simultaneous tests (Miller 1966).

Several parameters that were considered to provide estimates of the contribution of sexual reproduction to the population were calculated. First, the observed genotypic diversity (G_o) was calculated as $1/\sum g_i^2$ where g_i is the frequency of the i^{th} multilocus genotype in the population. The expected genotypic diversity assuming random mating (G_e) was calculated following Stoddart and Taylor (1988). The ratio of G_o/G_e provides a measure of single locus and multilocus equilibrium. Values of G_o/G_e much smaller than one indicate the occurrence of clonal reproduction.

Second, the number of genotypes in each population (N_{go}) was used as a measure of sexual reproduction. The ratio N_{go}/N_i , where N_i is the sample size, estimates the minimum input of sexual reproduction (Johnson and Threlfall 1987), whereas N_i/N_{go} gives the average number of individuals per genotype, or, in other words, the average size of the clone or genet (McFadden 1997). N_{go}/N_i is the simplest estimator of sexual input, but is likely to be an underestimate because repeated production of the same genotype through sexual reproduction is ignored. This effect can be corrected for by calculating the maximum number of replicate individuals of a genotype expected from sexual reproduction (Johnson and Threlfall 1987) and summing across loci to give N^* . The calculation of this parameter is described in detail in Uthicke et al. (1998).

F -statistics were used to partition genetic variation into that occurring within populations (F_{IS}) and that occurring between populations (F_{ST}) using equations which take account of differences in sample size between populations (Weir and Cockerham 1984). The significance of F_{IS} and F_{ST} values was tested using the χ^2 statistic. For tests of F_{IS} , χ^2 equals $N(F_{IS})^2 (k - 1)$, with degrees of freedom (d.f.) equal to $k(k - 1)/2$, where N is the total number of individuals sampled and k the number of alleles at the locus. For tests of F_{ST} , χ^2 equals $2N(F_{ST})^2 (k - 1)$ with d.f. = $(k - 1)(s - 1)$, where N and k are defined already and s is the number of populations sampled (Waples 1987). The value of F_{ST} will be inflated by any differential clonal production between populations, and any estimates of dispersal of sexually produced larvae between these stations would be underestimated. Therefore, we compared clonal genotypic frequencies obtained by retaining only one individual of each multilocus genotype in each population.

F -statistics for the total population calculated using these data were considered to provide an order of magnitude indication of the levels of dispersal between populations by sexually produced larvae.

Results

In all three populations more male individuals were found than females. The sex ratio differed significantly from 1:1 at Reef 18-026 ($\chi^2: 5.39$, $P < 0.05$) but the difference at Rib Reef was not statistically significant ($\chi^2: 1.25$, $P > 0.05$). The greatest deviation from equity in the sex ratio was observed at Great Palm Island, where only one female was found in a sample of 59 individuals.

No significant differences in genotype frequencies between stations within each reef were detected using χ^2 analyses, whether data for sexes were pooled or treated separately, with one exception (results not shown). In the exception, the two stations on Reef 18-026 were significantly different at the *PGM** locus, principally because of a high number of *BC* genotypes (a heterozygote excess) at one station and a preponderance of *BB* genotypes and no *BC* heterozygotes at the other station. The difference was mostly attributable to genotype differences in males. However, since no other loci showed significant differences at that reef, and overall genotypic frequencies were similar, stations within each reef including Reef 18-026 were pooled for further analyses.

Single-locus genotype frequencies differed significantly between females and males at Rib Reef and Reef 18-026 (Tables 1 and 2). Due to the low number of females, a test for any difference at Great Palm Island was not possible. Data for females and males were analysed separately, therefore, in subsequent analyses, unless stated otherwise.

All populations had several significant deviations in genotypic frequencies (both heterozygote excesses and heterozygote deficits) from those expected under Hardy-Weinberg equilibrium (Table 3). These deviations were most distinct in the male population of Great Palm Island, where three out of four polymorphic loci showed strong heterozygote excesses.

In the total sample, we detected 28 multilocus genotypes (Table 4). Several of these genotypes were highly replicated in local populations. On Great Palm Island 79% of the male population consisted of one clone (genotype number 1, Table 4). This location shared only one multilocus genotype with each of the two midshelf reefs. Between Rib Reef and Reef 18-026 four multilocus genotypes were shared in the female population and three in the male population. On each of the two midshelf reefs only four multilocus genotypes occurred in both female and male animals.

The mean number of alleles and the percentage of polymorphic loci were slightly less in the nearshore

Table 1 Allele frequencies for female and male individuals of *Stichopus chloronotus* from three reefs on the GBR

Locus/Allele	Great Palm Island		Rib Reef		Reef 18-026	
	Female	Male	Female	Male	Female	Male
<i>N</i>	1	58	28	37	24	43
<i>HK</i> *						
102(A)	–	–	–	–	0.021	0.058
100(B)	1.000	1.000	1.000	1.000	0.979	0.942
<i>MPI</i> *						
110(A)	–	0.491	0.375	0.027	0.188	0.314
100(B)	1.000	0.509	0.625	0.973	0.813	0.686
<i>PGM</i> *						
103(A)	–	0.397	0.179	0.378	0.167	0.058
100(B)	1.000	0.603	0.821	0.473	0.688	0.663
94(C)	–	–	–	0.149	0.146	0.279
<i>TPI</i> *						
109(A)	–	0.431	0.143	0.203	0.083	0.291
100(B)	1.000	0.569	0.857	0.797	0.917	0.709
<i>VL</i> *						
100(A)	1.000	0.922	0.125	0.068	0.375	0.779
93(B)	–	0.078	0.875	0.932	0.625	0.221

N is the number of animals in each category. Alphabetical assignments of alleles (A, B or C) are indicated for each locus

*HK**: hexokinase, *MPI**: mannose-6-phosphate isomerase, *PGM**: phosphoglucomutase, *TPI**: triose-phosphate isomerase, *VL**: peptidase using valylleucine as substrate

Table 2 χ^2 tests of genotype frequency differences between males and females of *Stichopus chloronotus*

Locus	Great Palm Island	Rib Reef	Reef 18-026
<i>HK</i> *	NT	0(0)	1(1) ^{NS}
<i>MPI</i> *	NT	33.8(1) ^{***}	3.27(2) ^{NS}
<i>PGM</i> *	NT	14.9(2) ^{**}	12.98(3) [*]
<i>TPI</i> *	NT	1(1) ^{NS}	10.79(1) [*]
<i>VL</i> *	NT	3.34(1) ^{NS}	13.8(2) [*]
Total	NT	52.96(5) ^{***}	41.9(9) ^{***}

NT indicates no test was performed. Significance levels (individual alpha levels were Bonferroni corrected for 12 simultaneous tests): *: $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant. Numbers in brackets are degrees of freedom

*HK**: hexokinase, *MPI**: mannose-6-phosphate isomerase, *PGM**: phosphoglucomutase, *TPI**: triose-phosphate isomerase, *VL**: peptidase using valylleucine as substrate

population at Great Palm Island compared to the midshelf reefs (Table 5). The third parameter measuring genetic variability, the observed genotypic diversity (G_o), was distinctly lowest in the male population on the nearshore reef. With the exception of the female population on Reef 18-026, mean heterozygosity was always higher than the expected heterozygosity in each population. Although the estimate of this parameter is subject to large standard deviations, this trend confirms the findings of the more specific tests on deviations in genotypic frequencies (see earlier).

The ratio of G_o/G_e was less than 0.63 in all populations, but was particularly small on Great Palm Island. The number of observed genotypes at each location

was usually less than that expected. The ratio of N_{go}/N_i , which serves as a minimum estimate for sexual input to each population, was lower than 50% in each case, and lowest on the nearshore reef. The average clone size (N_i/N_{go}) for males at Great Palm Island was almost 10 individuals, and ranged from 3.1 to 3.9 for other male populations. For the female populations on the two midshelf reefs, average clone size was between 2.4 and 4 individuals. The maximum input through sexual reproduction, N^*/N_i , is lowest on Great Palm Island, with a maximum of 32% of all male individuals likely to be the products of sexual recruitment. The highest value was observed for the female population on Reef 18-026, where 100% of individuals were likely to be the result of sexual reproduction. On both midshelf reefs the estimated maximum input of sexual reproduction was higher in females than in males. In contrast, the number of multilocus genotypes (N_{go}) was higher for males than females at all three populations which might suggest a higher survival of sexually produced male recruits. However, these differences were only marginally significant at Great Palm Island ($\chi^2: 3.57$, $0.1 > P > 0.05$) and not significant at Rib Reef ($\chi^2: 1.32$, $P > 0.1$) and Reef 18-026 ($\chi^2: 0.05$, $P > 0.1$). N^*/N_i is the maximum input by sexual reproduction, thus $1 - N^*/N_i$ gives the minimum input of asexual reproduction. This parameter was 68% on Great Palm Island and between 0 and 38% on the midshelf reefs.

F-statistic analyses revealed several high negative F_{IS} values, resulting from large excesses of heterozygotes at those loci, and several large positive values

Table 3 χ^2 tests of conformance of genotypic frequencies of *Stichopus chloronotus* to frequencies expected under conditions of Hardy-Weinberg equilibrium for females and males in each of three reefs in the GBR

Locus	Great Palm Island		Rib Reef		Reef 18-026	
	Female	Male	Female	Male	Female	Male
<i>HK*</i>	χ^2	-	-	-	0.01 ^{NS}	0.2 ^{NS}
	D	-	-	-	0.021	0.062
<i>MPI*</i>	χ^2	-	54.1***	10.1*	0.03 ^{NS}	1.3 ^{NS}
	D	-	0.966	0.600	0.028	0.231
<i>PGM*</i>	χ^2	-	25.1***	1.3 ^{NS}	0.2 ^{NS}	2.5 ^{NS}
	D	-	0.657	0.217	0.238	-0.042
<i>TPI*</i>	χ^2	-	33.3***	0.8 ^{NS}	2.4 ^{NS}	0.2 ^{NS}
	D	-	0.758	0.167	0.254	0.091
<i>VL*</i>	χ^2	-	0.4 ^{NS}	0.6 ^{NS}	4.7*	5.2*
	D	-	0.084	0.143	-0.357	-0.467

D values expressing the extent of deviations from Hardy-Weinberg equilibrium are given (positive values indicate an excess of heterozygotes, negative values indicate a deficit of heterozygotes). Significance levels (individual alpha levels were Bonferroni corrected for 22 simultaneous tests): *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, NS: not significant. Each test has one degree of freedom *HK**: hexokinase, *MPI**: mannose-6-phosphate isomerase, *PGM**: phosphoglucomutase, *TPI**: triose-phosphate isomerase, *VL**: peptidase using valylleucine as substrate

Table 4 Frequencies of multilocus genotypes in three populations of *Stichopus chloronotus*. Alphabetical assignments of alleles are as indicated in Table 1

Genotype Numbers	Locus					Great Palm Island		Rib Reef		Reef 18-026	
	<i>HK*</i>	<i>MPI*</i>	<i>PGM*</i>	<i>PTI*</i>	<i>VL*</i>	Female	Male	Female	Male	Female	Male
1	BB	AB	AB	AB	AA		46				
2	BB	AB	BB	AB	AA		1				
3	BB	AB	BB	BB	AA		1				
4	BB	AB	BB	BB	AB		6				
5	BB	AB	BB	AB	AB		3	3			
6	BB	BB	BB	BB	AA	1	1				9
7	BB	AB	AB	AB	AB			4			
8	BB	BB	AB	AB	BB				10		
9	BB	BB	AB	BB	BB			5	4		
10	BB	BB	AC	BB	AB				1		
11	BB	BB	AC	BB	BB				9		
12	BB	BB	BB	AB	BB				3		
13	BB	AB	AB	BB	BB			1	1	1	
14	BB	AB	BB	BB	BB			12	1	5	
15	BB	BB	AB	AB	AA				1		1
16	BB	BB	BB	BB	BB			2	4	5	1
17	BB	AB	BB	AB	BB			1		1	4
18	BB	BB	AB	BB	AB				1	3	
19	BB	BB	AC	AB	BB				1		1
20	BB	BB	BB	BB	AB				1	2	
21	AB	BB	BC	BB	AB					1	5
22	BB	AA	AB	BB	AB						1
23	BB	AA	BB	BB	AB						1
24	BB	AB	AB	AB	BB						1
25	BB	AB	BC	AB	AA					2	18
26	BB	BB	AB	BB	AA						1
27	BB	BB	AC	AB	AA					1	
28	BB	BB	AC	BB	AA					3	
Number of clones						1	6	7	12	10	11
Total number of individuals						1	58	28	37	24	43

*HK**: hexokinase, *MPI**: mannose-6-phosphate isomerase, *PGM**: phosphoglucomutase, *TPI**: triose-phosphate isomerase, *VL**: peptidase using valylleucine as substrate

Table 5 Summary statistics and parameters describing genetic variability and diversity at five loci for populations of *Stichopus chloronotus* from the GBR. Numbers in brackets are standard deviations

	Mean number of alleles per locus	% of loci polymorphic	Mean Heterozygosity		No. of Ind. (N_i)	No. of genotypes	
			Direct-count	Expected		Observed (N_{go})	Expected (N_{ge})
<i>Female</i>							
Great Palm Island	1.0 (0.0)	0	0.000 (0.000)	0.000	1	1	1
Rib Reef	1.8 (0.2)	80	0.329 (0.121)	0.250 (0.077)	28	7	14
Reef 18-026	2.2 (0.2)	100	0.258 (0.074)	0.295 (0.088)	24	10	16
<i>Male</i>							
Great Palm Island	1.8 (0.2)	80	0.559 (0.200)	0.325 (0.106)	58	6	27
Rib Reef	2.0 (0.3)	80	0.259 (0.143)	0.226 (0.113)	37	12	16
Reef-18-026	2.2 (0.2)	100	0.409 (0.112)	0.359 (0.066)	43	11	29

are indicative of heterozygote deficits (Table 6). The F_{ST} values estimated from the whole data-set showed consistently higher values for males than females at each locus and in the average value. F_{ST} values for females were significant at only two loci, and the average was not significant. Since F_{ST} values may be inflated by differential clonal reproduction we also calculated F_{ST} values from clonal genotypic frequencies (Table 6), and the average F_{ST} value for females and males were then not significantly different from zero.

Pairwise F_{ST} values calculated separately for males and females using total genotypic frequencies demonstrated no significant values for the females between any pair of reefs, but all three contrasts were significant for the males (Table 7). If clonal frequencies are used to calculate pairwise F_{ST} values, the differences between the reefs for males become non-significant. However, values were higher between Great Palm Island and Rib Reef than between the other two combinations of reefs.

Discussion

Occurrence of asexual reproduction

In all three populations of *Stichopus chloronotus* deviations of heterozygosity from that expected under Hardy-Weinberg equilibrium were observed. The fact

that several genotypes were found in high numbers, suggested the occurrence of clonal reproduction by transverse fission on all reefs. In conjunction with the deviations from Hardy-Weinberg equilibrium, the more specific parameters assessing asexual reproduction (G_o/G_e , N_{go}/N_i , N^*/N_i) leave little doubt that asexual reproduction is more important in the nearshore population on Great Palm Island compared with both midshelf reefs. The population of *Stichopus chloronotus* on Great Palm Island had the lowest number of genotypes of the three populations. Only six multilocus genotypes were detected in this population and estimates of N^* indicated that a maximum of 19 of the 58 individuals of the male population were derived by sexual recruitment. The fact that even in the highly fissiparous population of *S. chloronotus* nearly all individuals had gonads suggests that sexual and asexual reproduction is undertaken by the same individuals.

This pattern confirms an earlier study on observations of fission frequency in *Stichopus chloronotus*, where a minimum of 31% of individuals were found to undergo fission every year at Great Palm Island, whereas this estimate was only 19% for Rib Reef (Uthicke 1997). A similar trend with increasing fission frequencies towards the mainland was observed in *Holothuria atra* on the GBR (Uthicke 1997; Uthicke et al. 1998) and in Réunion (Conand 1996). A potentially higher food availability in sediments in habitats close to the mainland was hypothesised to sustain the larger populations created by higher fission rates (Conand 1996; Uthicke 1997). As higher fission rates were observed at Great Palm Island compared to Rib Reef it is

Table 5 (contd.)

N_{go}/N_i	N_i/N_{go}	Observed genotypic diversity	Expected genotypic diversity	G_o/G_e	N_*	N^*/N_i
1	1	1.0	—	—	—	—
0.25	4	3.9	8.9 ± 2.3	0.44	23.9	0.85
0.42	2.4	7.2	11.9 ± 3.1	0.61	24.0	1
0.10	9.7	1.6	17.5 ± 3.0	0.09	18.8	0.32
0.32	3.1	6.0	9.5 ± 2.2	0.63	27.0	0.72
0.25	3.9	4.1	21.5 ± 4.1	0.19	26.7	0.62

Table 6 F -statistics for females and males in three populations of *Stichopus chloronotus* calculated from genotypic frequencies of the total population (total) and from the clonal genotypic frequencies

Locus	Total			Clonal			
	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{IS}	
Females		N: 53				N: 18	
<i>HK</i> *	-0.007	-0.015 ^{NS}	0.008 ^{NS}	-0.031	-0.073 ^{NS}	0.038 ^{NS}	
<i>MPI</i> *	-0.338	0.073*	-0.445**	-0.296	0.022 ^{NS}	-0.324 ^{NS}	
<i>PGM</i> *	0.014	0.022 ^{NS}	0.037 ^{NS}	-0.041	0.000 ^{NS}	-0.040 ^{NS}	
<i>TPI</i> *	-0.124	-0.011 ^{NS}	-0.111 ^{NS}	-0.202	-0.060 ^{NS}	-0.135 ^{NS}	
<i>VL</i> *	0.423	0.119***	0.279 ^{NS}	0.474	0.212*	0.333 ^{NS}	
Average	-0.006 (0.124)	0.038 ^{NS} (0.028)	0.046 ^{NS} (0.118)	-0.019 (0.133)	0.020 ^{NS} (0.051)	-0.026 ^{NS} (0.108)	
Males		N: 138				N: 29	
<i>HK</i> *	0.003	0.052**	-0.052 ^{NS}	-0.003	-0.011 ^{NS}	0.007 ^{NS}	
<i>MPI</i> *	-0.278	0.229***	-0.658***	0.108	0.104*	0.004 ^{NS}	
<i>PGM</i> *	-0.279	0.110***	-0.432***	-0.064	0.049 ^{NS}	-0.119 ^{NS}	
<i>TPI</i> *	-0.458	0.056**	-0.544***	-0.258	-0.032 ^{NS}	-0.219 ^{NS}	
<i>VL</i> *	0.765	0.667***	0.294***	0.512	0.285***	0.319 ^{NS}	
Average	-0.049 (0.217)	0.229*** (0.116)	-0.278* (0.176)	0.059 (0.128)	0.079 ^{NS} (0.057)	-0.002 (0.091)	

Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant. The standard error for the averages is given in brackets. *HK**: hexokinase, *MPI**: mannose-6-phosphate isomerase, *PGM**: phosphoglucosyltransferase, *TPI**: triose-phosphate isomerase, *VL**: peptidase using valylleucine as substrate

likely that both higher rates of asexual reproduction and reduced sexual recruitment contribute to the low level of genotypic diversity at that reef. In a study of *H. atra* some evidence for a restricted supply of larvae to nearshore reefs was inferred (Uthicke et al. 1998). Similarly to *H. atra*, the highest fission frequencies in *S. chloronotus* were observed at the border of the species' distribution towards the mainland. We are not

aware of an *S. chloronotus* population closer to the coastline than Great Palm Island.

Biased sex ratios

Biased sex ratios in echinoderms may serve as circumstantial evidence for asexual reproduction (e.g. Crump

Table 7 Pairwise F_{ST} values for three populations of *Stichopus chloronotus* on the GBR. Values above the diagonal are for the females, below the diagonal for the males. Values are derived from original genotypic frequencies, and the F_{ST} calculated from clonal genotypic frequencies is given in parenthesis

	Great Palm Island	Rib Reef	Reef 18-026
Great Palm Island	–	0.125 ^{NS} (0.153 ^{NS})	–0.124 ^{NS} (–0.118 ^{NS})
Rib Reef	0.276*** (0.239 ^{NS})	–	0.052 ^{NS} (0.021 ^{NS})
Reef 18-026	0.067** (0.007 ^{NS})	0.216** (0.044 ^{NS})	–

Significance levels: *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, NS: not significant

and Barker 1985; Garrett et al. 1998). In sea cucumbers, biased sex ratios, generally towards males, have been observed in the southern (Harriott 1982) and central sections (Uthicke et al. 1998) of the GBR. The population on Great Palm Island is not exclusively male, as originally thought, but females are extremely rare, and only one female was found among 59 dissected individuals. Most individuals had maturing to mature gonads, although larval production must be extremely low with so few female individuals in the Great Palm Island population. A mass spawning of only male individuals was observed at several occasions during the summer of 1995 and 1996 (Uthicke 1997; Uthicke unpublished data). In 1997 (17-December, between 19.00 and 20.00) we observed 208 male individuals and no females spawning at Great Palm Island. These observations, in conjunction with the genetic data, indicate that the low number of females has been a feature of this population for at least a number of years. Since all three populations had an excess of male individuals, the population on Great Palm Island may be an extreme example of a situation that is normal for the area of the GBR we investigated.

If it is assumed that gender is genetically determined in *Stichopus chloronotus*, reduced numbers of females may result from (1) a higher mortality of adult females, (2) a higher fission rate in male individuals, or (3) lower sexual recruitment or higher mortality of female larvae. A higher mortality in adult females cannot be excluded, but individual weights were evenly distributed between males and females over all samples (data not shown), suggesting a similar age and survival pattern in adult males and females. However, a situation like that on Great Palm Island would require an extremely high mortality of females. Disregarding Great Palm Island, there is no consistent trend in the number of individuals per clone (N_i/N_{go}) between the male and female populations on the two mid-shelf reefs, suggesting that differences in fission rate between males and females are not the reason for biased sex ratios.

It is not possible to infer gender-specific dispersal capabilities from our data. Thus, a higher mortality of females in the larval or early post-settlement phase or a reduced dispersal capability of female larvae are the most likely explanations for the bias in sex

ratios towards males. Although not statistically significant (except marginally at Great Palm Island), the occurrence of more male multilocus genotypes than female ones in all three populations, is supporting evidence that male larvae or juveniles have a lower mortality than females. A higher mortality of females prior to recruitment into the adult population was also thought the most likely explanation for biased sex ratios in *Holothuria atra* (Uthicke et al. 1998). Further experimental studies will be required to determine the mechanisms leading to a higher mortality or reduced dispersal capability of female larvae, and whether this is a general pattern for aspidochirotide holothurians.

Connectivity between the reefs

Dispersal of *Stichopus chloronotus*, as in other aspidochirotide holothurians, occurs via planktotrophic larvae. The length of the larval life of this species is not known, but we assume that it is in a similar range (20-40 d) to that reported for other tropical aspidochirotides (reviewed in Smiley et al. 1991). Panmixis over large areas in the GBR seems to be more the rule than the exception for many invertebrate species, such as *Linckia laevigata* (Williams and Benzie 1993), *Tridacna gigas* (Benzie and Williams 1992) or outbreaking populations of *Acanthaster planci* (Benzie and Stoddart 1992). However, a higher degree of separation between reef populations in the central area of the GBR was observed in the viviparous coral *Seriatopora hystrix* which has a shorter larval life than the invertebrates mentioned (Ayre and Dufty 1994).

F_{ST} values for females and males of *Stichopus chloronotus* using total genotypic frequencies indicated high gene flow among females but deviations from panmixis for the males. In a purely sexual species one would not expect significant differences in genotype frequencies between males and females, and F_{ST} values calculated from either males or females should give the same result, and this should be the same as for males and females combined. Thus it is likely that genotype frequency differences and differences in F_{ST} values between males and females are the result of asexual

reproduction. Using clonal genotypic frequencies, to remove the influence of asexual reproduction, reduced F_{ST} values for males, providing similar estimates for males and females which do not deviate significantly from zero.

Overall F_{ST} calculations can mask differences between population pairs, but pairwise comparisons showed no significant F_{ST} values when calculated from the clonal genotypic frequencies. However, relatively higher F_{ST} values between Rib Reef and Great Palm Island were observed. This pattern is consistent with current flows in the region. Currents flow mostly in a south-easterly direction in the central section of the GBR (Williams et al. 1984) and are likely to move larvae from Reef 18-026 to Great Palm Island, but take larvae from Rib Reef further south. Hydrodynamic models for the GBR predict a limited and unidirectional exchange of neutrally buoyant larvae between midshelf and nearshore reefs (Dight et al. 1990a, b). Sexual output of the population at Great Palm Island will be extremely low, and the larvae are unlikely to be dispersed to other reefs, suggesting that gene flow is unidirectional from the midshelf reefs to Great Palm Island. There was also evidence for restricted gene flow of *Holothuria atra* populations between the midshelf reefs and nearshore reefs in the same area (Uthicke et al. 1998).

In summary, the population of *Stichopus chloronotus* at Great Palm Island was confirmed to consist almost exclusively of male individuals which undergo asexual reproduction by transverse fission at a high rate compared to populations on two midshelf reefs. F_{ST} values indicate that the population on Great Palm Island is not totally isolated, and that a considerable amount of genetic exchange exists with both of the midshelf reefs we investigated. A differential mortality of larvae or post settlement juveniles, or differences in the dispersal capabilities of female and male larvae were inferred as the most likely explanations for differences in sex ratios, as was also suggested in a study of *Holothuria atra* (Uthicke et al. 1998). Further work will be required to elucidate mechanisms that could cause differences in female and male larval or juvenile mortality in *S. chloronotus*. The present study suggests the phenomenon is not restricted to *H. atra*, and provides an intriguing problem in holothurian biology.

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