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Restricted gene flow between *Holothuria scabra* (Echinodermata: Holothuroidea) populations along the north-east coast of Australia and the Solomon Islands

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ABSTRACT: To describe patterns of gene flow for the fished holothurian species *Holothuria scabra* 17 to 141 individuals were collected from 8 populations from north-east Australia, the Torres Strait, and the Solomon Islands. Samples were investigated by allozyme electrophoresis of 7 polymorphic loci. Cluster analyses using Rogers' genetic distance identified 3 distinct groups of populations from the north-east coast of Australia, representing samples from the 3 regions Hervey Bay, Upstart Bay and Torres Strait. Populations in the latter region were closely connected to those from the Solomon Islands. *F*-statistics indicated restrictions in gene flow (average genetic variation between populations, F_{ST} = 0.088). Hierarchical analyses revealed that 94.7% of the variation was within sampling locations. Approximately 77% of the variance among populations was due to differences between regions, and 23% within regions (most of the latter caused by differences between the 2 Solomon Island populations). Mantel's tests indicated that a high proportion of the variation in genetic distances along the north-east coast of Australia was explained by isolation by distance (Mantel's normalised Z = 0.88). This proportion reduced when the Solomon Islands were included (Z = 0.65). The detection of separate stocks along the north-east coast of Australia is an important finding that has significant consequences for the development of sustainable management plans for this species. Low dispersal may significantly reduce recovery of overfished areas if no local refugia are provided.

KEY WORDS: Connectivity · Holothurians · Invertebrate fisheries

INTRODUCTION

Fishing for several aspidochirotide holothurian species (bêche-de-mer) is an important source of income for many developing countries throughout the Indo-Pacific region. However, due to the current high demand in China, over-harvesting can now be considered a global phenomenon in developing countries (examples in Conand 1997, Conand & Jaquemet 2000). Recent data showed that over-exploitation can also be a problem in developed countries such as Australia, with relatively well managed coral reef ecosystems (Uthicke & Benzie 2000a).

Holothuria scabra ('sandfish') is one of the most important species for the tropical bêche-de-mer fishery (Conand 1989a). Despite the commercial importance of *H. scabra*, information on the biology and ecology of this species is sparse. Such information is urgently needed for management of the fishery and to understand the impact of the fishery on the ecosystem. *H. scabra* is one of the few tropical aspidochirotide species which prefers coastal areas to coral reefs (Conand 1989b, 1993). It is often found in seagrass beds, and

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seagrass plays an important function in triggering larval settlement (Mercier et al. 2000). The sandfish burrows into the sediment for part of the day (Wiedemeyer 1993, James et al. 1994, Mercier et al. 1999), and fishing of this species usually takes place by walking at low tides. Sexual reproduction via broadcast spawning occurs in the warm months (December to February) in the southern hemisphere (Harriott 1980, Conand 1989b, 1993). The planktotrophic larvae of this species spend 10 to 14 d in the water column before reaching late pentactula stage and subsequent settlement (Battaglene et al. 1999, Mercier et al. 1999). There is, therefore, potential for larval dispersal between populations.

It has been established that the 2 colour varieties observed for *Holothuria scabra* are conspecific and that shallow and deep populations in 1 area of Australia were genetically undistinguishable (Uthicke & Benzie 1999). The latter finding is consistent with the view that juveniles settle in shallow seagrass beds and then migrate to deeper areas during their life span (Vail 1989, James et al. 1994). Although their function in seagrass ecosystems has not been studied, holothurians have important functions in bioturbation and nutrient recycling in coral reef ecosystems (Uthicke 1999, 2001). Therefore, one may assume that apart from direct effects of fishing on population densities, a reduction in holothurian densities will have secondary ecological effects.

Information on the connectivity of exploited populations is an important pre-requisite for managers to decide on which scale (e.g. local or regional) a fished stock should be managed. Furthermore, such information allows an assessment of the potential geographical range of ecological consequences of over-fishing on the population level and to advise on the usefulness of protective measures such as marine protected areas.

Allozyme markers have proved to be a useful tool for describing gene flow and have been used to describe connectivity of echinoderm populations in the Great Barrier Reef (GBR) (Benzie & Stoddard 1992, Williams & Benzie 1993, Uthicke & Benzie 2000b). These studies focussed on reef species and described high rates of gene flow along the entire length of the GBR, consistent with the general perception that long larval duration leads to high connectivity between populations. Genetic differences between populations were found in 2 holothurian species on the GBR (Uthicke et al. 1998, 1999), but this was attributed to the effects of asexual reproduction, which occurs in these species in addition to sexual reproduction. *Holothuria scabra* is not known to reproduce asexually, and it is not a reef species, but inhabits coastal areas. Therefore, connectivity between populations may differ from that of other echinoderm species investigated on the north-east coast of Australia. The aim of the present study was to investigate geneflow between populations separated by different geographic scales (~20 to 2000 km), along the north-east coast of Australia, Torres Strait and the Solomon Islands, to provide information on connectivity to assist management and add to fundamental knowledge on the biology of this ecologically and economically important species.

MATERIAL AND METHODS

Sampling strategy. Two shallow populations of *Holothuria scabra* were sampled in the area of Hervey Bay (Urangan: 152° 54′ E, 25° 18′ S; Tin Can Bay: 153° 01′ E, 25° 49′ S) in south Queensland during June 1998 (Fig. 1). Individuals from a deeper population in Hervey Bay (18 to 20 m) were obtained during 3 trawl shots (approximate position: 153° 04′ E, 24° 59′ S) using commercial prawn-trawling equipment. One intertidal population was sampled ca. 800 km north (Upstart Bay: 147° 42′ E, 19° 50′ S). (Data from these samples were used in a previous study investigating the relationship between 2 colour morphs and the gene flow between deep and shallow populations: Uthicke & Benzie 1999.) During August 1999, samples were obtained from 2 reefs in the Torres Strait at the northern end of the GBR (Warrior Reef: 143° 01′ E, 09° 45′ S; Dungeness Reef: 142° 57′ E, 09° 55′ S). Two locations in the Solomon Islands, Kohinggo Island (157° 06′ E, 08° 09′ S: hereafter called Solomon Island A) and Kolombangarra Island (156° 59′ E, 08° 04′ S: Solomon Island B), were sampled in December 1999. Because the individuals in the Upstart Bay population sampled in 1998 were exceptionally small, this population was re-sampled in May 2000 to investigate whether gene frequencies and the small size of individuals were stable over time.

Samples from intertidal populations were taken during low tides by walking on the mud flats. During these periods, holothurians in shallow tide pools, usually with at least a sparse seagrass cover, migrate to the surface of the sediment. Since large areas had to be covered to obtain sufficient individuals, no effort was made to obtain subsamples within each of the populations. The length of all individuals was recorded to the nearest centimetre. A subsample of the gut lining (cleaned from sediments) was snap frozen in liquid nitrogen for later analyses.

Allozyme electrophoresis. Approximately 250 mg of frozen gut tissue was homogenised in the same volume of Tris HCl buffer (100 mM Tris adjusted to pH 8.0 with HCl) prior to electrophoresis. Electrophoresis of all enzymes was performed on 12% horizontal starch gels. Seven polymorphic enzyme loci were surveyed: phosphoglucomutase (E.C. 5.4.2.2, *PGM*$^{\bullet}$), hexokinase

(E.C. 2.7.1.1, *HK**) and glucose-6-phosphate isomerase (E.C. 5.3.1.9, *GPI**) as described in Uthicke & Benzie (1999), were analysed on TEC7.9 gels (electrode buffer 135 mM Tris, 32 mM citric acid, 4 mM Na_2EDTA, pH 7.87; gel buffer 8.5 mM Tris, 2 mM citric acid, 0.27 mM Na_2EDTA, pH 7.87, electrophoresis for 16 h at 90 V). Malate dehydrogenase (E.C. 1.1.1.37, *MDH**) and peptidases (E.C. 3.4.11/13) were analysed on TG8.4 gels (electrode and gel buffer 25 mM Tris, 192 mM glycine, pH 8.4, 230 V for 16 h). Different substrates allowed detection of activity in several peptidase loci. The results for these are reported as Peptidase Locus 1 (*PEP-1**, migration distance 6.5 to 7.5 cm, visible on leucyl-glycyl-glycyl, LGG), *PEP-2** (visible on leucyl-proline, travel distance 2.5 to 3.5 cm) and *PEP-3** (visible on LGG and valyl-leucine, migration distance 1 to 2 cm). Full details of staining and electrophoresis methods are given in Ballment et al. (1997).

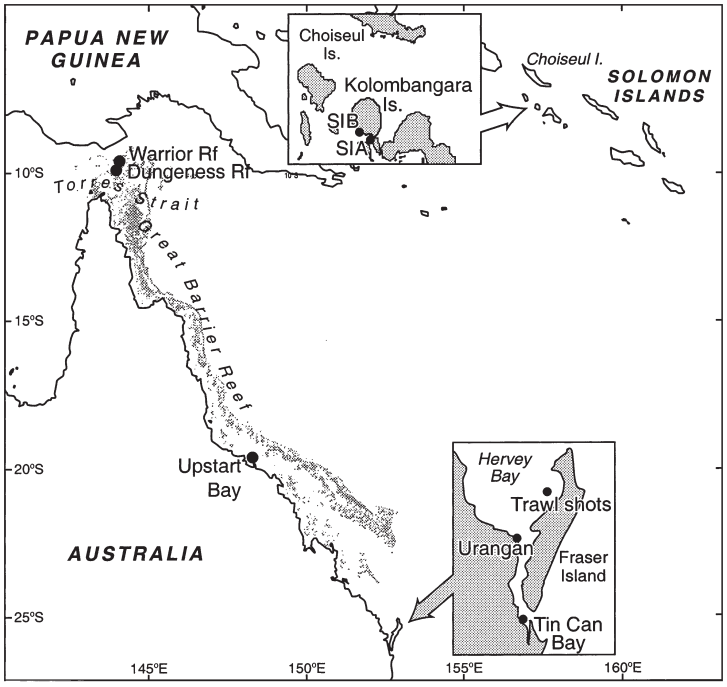

Fig. 1. Locality map of 8 sampling stations on the Queensland coast, Torres Strait, and the Solomon Islands

Statistical analyses. Basic analyses of genetic variability were carried out using programs in the BIOSYS-1 package (Swofford & Selander 1981). *F*-statistics, cluster analyses and tests of conformation to Hardy-Weinberg expectations were performed using the TFPGA package (Miller pers. comm. 1997, computer software distributed by author). Weir & Cockerham's (1984) methods for calculating Wright's *F*-statistics with corrections for unequal sample size were used. Total genetic variation (F_{IT}) was partitioned into that occurring within populations (F_{IS}) and that occurring between populations (F_{ST}). The significance of F_{IS} and F_{ST} values was tested using the χ^2 statistic as described in Waples (1987). In addition, the 95% confidence intervals for the average F_{ST} and F_{IS} values were calculated by bootstrapping across loci. Hierarchical *F*-statistics (Wright 1978) were calculated to partition variation into that occurring within sampling locations, between sampling locations within regions, and between regions (Hervey Bay, Torres Strait and Solomon Islands). The contribution of these variances to the total variance was calculated after Preziosi & Fairbairn (1992). The latter analysis excluded the Upstart Bay sample, because only 1 population had been obtained from that region.

One parameter that had previously proved to be a reliable estimator for the contribution of asexual reproduction to each population was calculated to test whether this mode of reproduction occurs in *Holothuria scabra*, although it has not been reported previously. The maximum input of sexual reproduction in each population was estimated by calculating the maximum number of sexually produced individuals (N*) and dividing this by the sample size (Johnson & Threlfall 1987). The calculation of this parameter is described in detail in Uthicke et al. (1998).

Deviations from Hardy-Weinberg equilibrium for each locus at each reef were tested by an exact-test, using the conventional Monte Carlo method with the default settings in TFPGA. Significance values were corrected for multiple simultaneous tests with a sequential Bonferroni correction described by Hochberg (1988).

To test for evidence of isolation by distance, Mantel's (1967) tests were performed on transformed (log + 1) geographic distance (km) and Rogers' genetic distances (Rogers 1972). The significance of Mantel's normalised *Z* was tested by 10000 random permutations using NTSYS-PC software (Rohlf 1990).

RESULTS

With the exception of *MDH**, all loci investigated for *Holothuria scabra* were polymorphic in all populations, and most alleles (exceptions are PGM^{80}, HK^{90}, $GPI^{113,90}$) were found in all populations (Table 1).

Genotype frequencies were not significantly different from those expected under Hardy-Weinberg equilibrium (exact-test, $p > 0.05$ after corrections for multiple tests), with 1 exception. The *PGM** locus showed significant heterozygote deficits ($p = 0.000$) for only 1 population.

Table 1. *Holothuria scabra*. Allele frequencies at 7 loci in 8 geographical populations. Numbers in parentheses: sample size at each locus

Locus	Urangan	Tin Can Bay	Trawl shots	Upstart Bay 1998	Upstart Bay 2000	Warrior Reef	Dungeness Reef	Solomon Islands A	Solomon Islands B
PGM⋆	(100)	(16)	(136)	(49)	(54)	(41)	(30)	(30)	(23)
100	0.625	0.656	0.629	0.592	0.593	0.646	0.583	0.383	0.674
94	0.080	0.094	0.110	0.092	0.093	0.073	0.083	0.15	0.022
90	0.230	0.219	0.213	0.255	0.250	0.195	0.183	0.100	0.174
86	0.045	0.031	0.037	0.061	0.065	0.085	0.150	0.300	0.130
80	0.020	0.000	0.011	0.000	0.000	0.000	0.000	0.067	0.000
HK⋆	(100)	(17)	(141)	(49)	(54)	(42)	(30)	(30)	(23)
100	0.870	0.912	0.890	0.908	0.889	0.762	0.783	0.467	0.652
94	0.130	0.088	0.110	0.092	0.111	0.226	0.200	0.533	0.348
90	0.000	0.000	0.000	0.000	0.000	0.012	0.017	0.000	0.000
GPI⋆	(100)	(17)	(141)	(50)	(54)	(42)	(30)	(30)	(23)
113	0.000	0.000	0.018	0.000	0.019	0.060	0.067	0.05	0.022
100	1.000	1.000	0.982	0.900	0.898	0.929	0.917	0.950	0.870
90	0.000	0.000	0.000	0.100	0.083	0.012	0.017	0.000	0.109
MDH⋆	(100)	(17)	(141)	(50)	(54)	(42)	(30)	(30)	(23)
100	0.990	0.941	0.993	1.000	1.000	1.000	1.000	1.000	1.000
91	0.010	0.059	0.007	0.000	0.000	0.000	0.000	0.000	0.000
PEP-1⋆	(87)	(14)	(108)	(39)	(54)	(42)	(29)	(30)	(23)
106	0.488	0.464	0.616	0.269	0.352	0.357	0.431	0.433	0.283
100	0.512	0.536	0.384	0.731	0.648	0.643	0.569	0.567	0.717
PEP-2⋆	(100)	(17)	(140)	(50)	(54)	(42)	(29)	(30)	(23)
100	0.835	0.853	0.854	0.9	0.898	0.548	0.552	0.617	0.543
94	0.165	0.147	0.146	0.1	0.102	0.452	0.448	0.383	0.457
PEP-3⋆	(100)	(17)	(141)	(50)	(54)	(42)	(30)	(30)	(23)
100	0.920	0.794	0.926	0.440	0.472	0.631	0.733	0.700	0.630
95	0.080	0.206	0.074	0.560	0.528	0.369	0.267	0.300	0.370

Table 2. *Holothuria scabra*. Summary statistics and parameters describing genetic variability at 7 loci in 8 geographical populations. Standard errors are given in parentheses where appropriate, standard deviations are given for length measurements. Calculations of N_i, N_{go}, and $N^\star$ are only based on individuals for which all loci could be scored

Station	Mean sample size	Mean no. of alleles per locus	% of loci polymorphic	Mean heterozygosity Observed (H_o)	Mean heterozygosity Expected (H_e)	No. of Ind. (N_i)	No. of genotypes observed (N_{go})	$N^\star/N_i$	Average length (cm)
Urangan	98.1 (1.9)	2.4 (0.5)	85.7	0.209 (0.066)	0.247 (0.082)	87	54	1	17.8 (2.5)
Tin Can Bay	16.4 (0.4)	2.1 (0.3)	85.7	0.264 (0.082)	0.274 (0.076)	14	13	1	14.4 (1.8)
Trawl shots	135.4 (4.6)	2.4 (0.4)	100.0	0.216 (0.069)	0.237 (0.078)	105	58	1	26.9 (4.1)
Upstart Bay 1998	48.1 (1.5)	2.1 (0.3)	85.7	0.253 (0.071)	0.287 (0.079)	39	31	1	9.8 (1.5)
Upstart Bay 2000	54.0 (0.0)	2.3 (0.4)	85.7	0.294 (0.075)	0.302 (0.080)	54	43	1	9.1 (2.6)
Warrior Reef	41.9 (0.1)	2.4 (0.4)	85.7	0.291 (0.065)	0.355 (0.078)	41	39	1	19.7 (3.0)
Dungeness Reef	29.7 (0.2)	2.4 (0.4)	85.7	0.333 (0.076)	0.360 (0.081)	29	28	1	19.4 (3.0)
Solomon Island A	30 (0.0)	2.3 (0.5)	85.7	0.352 (0.099)	0.393 (0.097)	30	28	1	20.0 (2.4)
Solomon Island B	23.0 (0.0)	2.3 (0.4)	85.7	0.385 (0.082)	0.373 (0.072)	23	22	1	21.6 (3.3)

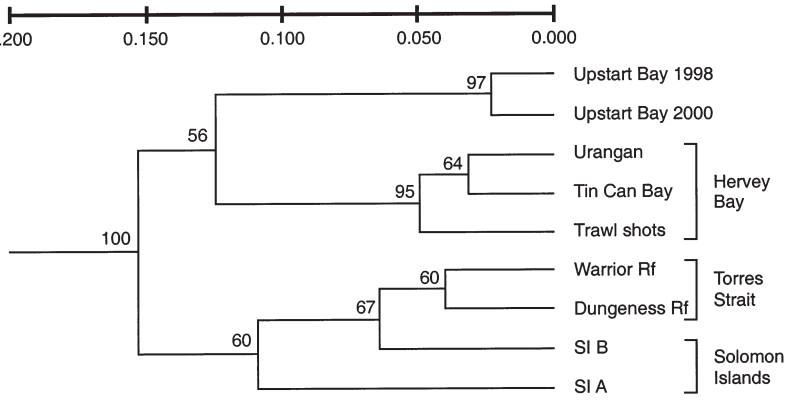

Fig. 2. *Holothuria scabra.* Dendrogram illustrating genetic relationships among 9 samples from the Queensland coast, Torres Strait and the Solomon Islands (SI A, SI B) using UPGMA cluster algorithm and Rogers' (1972) genetic distance. Values above the nodes represent bootstrap values obtained by 1000 permutations. The cophenetic correlation of the analyses was 0.86

There was no apparent difference in genetic variability (expressed as mean number of alleles per locus, % polymorphic loci or heterozygosity) among the populations investigated (Table 2). The number of genotypes observed was distinctly below the number of individuals sampled in populations with large sample size (Urangan and trawl samples). However, calculation of the upper 95% confidence estimate for the number of sexually produced individuals (N*) indicates that these genotypes were likely to be repeatedly produced by sexual reproduction, and the ratio of N^*/N_i was 1 for each population (Table 2).

The average length of *Holothuria scabra* varied at different locations (Table 2). Largest individuals (mean 26.9 cm) were sampled in the deep population in Hervey Bay ('Trawl shots'), and the average length of most other populations ranged between 14 and 22 cm. Individuals sampled in Upstart Bay in 1998 and June 2000 were small (~9 cm) at both sampling times.

Cluster analyses using Rogers' (1972) genetic distance clearly grouped sites by geographical region (Fig. 2). The 3 main clusters corresponded to (1) samples from Upstart Bay, (2) samples from the Hervey Bay area, and (3) samples from the Torres Strait and the Solomon Islands. One sample location from the latter islands appeared relatively distinct from the other population from the Solomon Islands and the Torres Strait samples. An exact test revealed no significant differences ($\chi^2 = 4.89$, df = 14, p = 0.981) between samples from Upstart Bay in 1998 and 2000. Therefore, those samples were pooled for subsequent analyses.

Differences between samples from the 2 locations in the Solomon Islands were significant (exact-test, Table 3). In most other cases, the results from the exact-test confirmed the clusters obtained with UPGMA (Fig. 2). Several comparisons involving Tin Can Bay were not significant, which is likely to have been caused by the small sample size for that population. Table 3 also lists pairwise genetic distances expressed as Nei's (1978) unbiased genetic distances, for comparison with other studies.

There was only a weak, but significant (Mantel's normalised $Z = 0.66$, p = 0.0004) relationship between geographic distances and Rogers' (1972) genetic distance (Fig. 3). When samples from the Solomon Islands were excluded from this analysis, a larger portion of the variation in the genetic distances was explained by geographic separation ($Z = 0.88$, p = 0.003).

Significant F_{ST} values were detected for all loci (Table 4). The confidence interval for the overall F_{ST}

Table 3. *Holothuria scabra.* Nei's (1978) unbiased genetic distances (above diagonal) and p-values of the exact tests for population differentiation (below diagonal) for each pair of populations. Bold print indicates significant (α = 0.05) population differentiation, after sequential Bonferroni corrections for 28 multiple simultaneous tests

	1	2	3	4	5	6	7	8
1 Urangan	–	0.0000	0.0019	0.0496	0.0346	0.0200	0.0616	0.0487
2 Tin Can Bay	0.6620	–	0.0017	0.0238	0.0229	0.0145	0.0626	0.0385
3 Trawl	0.2780	0.2553	–	0.0619	0.0483	0.0292	0.0733	0.0680
4 Upstart Bay	**0.0000**	**0.0005**	**0.0000**	–	0.0336	0.0436	0.0840	0.0413
5 Warrior Reef	**0.0000**	0.0083	**0.0000**	**0.0000**	–	0.0000	0.0311	0.0000
6 Dungeness Reef	**0.0000**	0.0610	**0.0000**	**0.0000**	0.9621	–	0.0245	0.0034
7 Solomon Island A	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0002**	0.0160	–	0.0222
8 Solomon Island B	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.5429	0.2894	**0.0025**	–

Table 4. *Holothuria scabra*. *F*-statistics for 8 populations. *Significant at α = 0.05; **significant at α = 0.01 (after sequential Bonferroni corrections); ns: not significant. Lower part of the table shows results from the hierarchical *F*-statistics. F_{IT}: total genetic variation; F_{ST}: variation between populations; F_{IS}: variation within populations

Locus	F_{IT}	F_{ST}	F_{IS}
*PGM**	0.1711	0.0121*	0.1610**
*HK**	0.1602	0.1015**	0.0654^{ns}
*GPI**	0.0622	0.0446**	0.0184^{ns}
*MDH**	−0.0023	0.0151*	0.0177^{ns}
*PEP-1**	0.1185	0.0556**	0.0666^{ns}
*PEP-2**	0.1750	0.1167**	0.0660^{ns}
*PEP-3**	0.2938	0.2180**	0.0969^{ns}
Average	0.1744	0.0877	0.0950
Upper 95% CI	0.2290	0.1542	0.1315
Lower 95% CI	0.1277	0.0362	0.0580

Hierarchical *F*-statistics:

	Variance	F_{ST}
Location within region	0.027	0.012
Location within total	0.124	0.053
Region within total	0.097	0.041

value does not encompass zero, indicating significant restrictions in gene flow between populations. Hierarchical *F*-statistics showed that 94.7% of the variance was within sampling locations (Table 4). Of the remaining variance, 77.4% were caused by differences between regions and only 22.6% were due to differences between locations within regions. Further analysis showed that the latter variance component was mainly caused by the differences between the 2 Solomon Island populations.

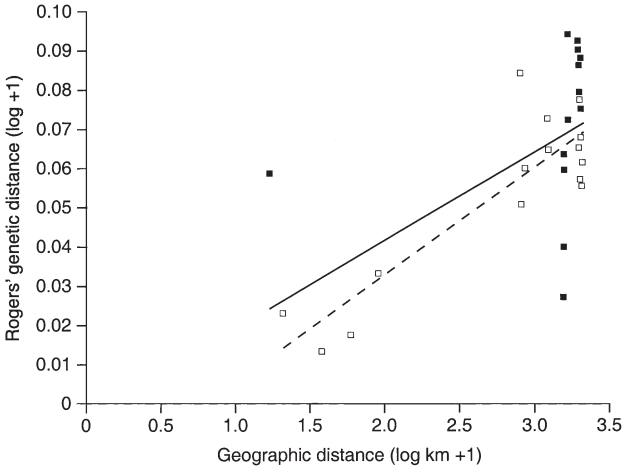

Fig. 3. *Holothuria scabra.* Relationship between Rogers' (1972) genetic distance and geographic distance including all sample locations (□, ■, continuous line) and excluding the Solomon Islands (□, dashed line)

Table 5. Estimates of the expected number of migrants per generation (N_em, calculated assuming a stepping-stone model) between populations derived from pairwise F_{ST} values. (A) Pairwise estimates between populations within regions; (B) populations within regions were pooled to derive estimates for gene flow between regions

	N_em
(A) Populations within regions	
Urangan vs Tin Can Bay	∞
Urangan vs Trawl shots	196.8
Tin Can Bay vs Trawl shots	138.3
Warrior Reef vs Dungeness	∞
Solomon Island A vs B	34.1
(B) Populations between regions	
Hervey Bay vs Upstart Bay	7.6
Hervey Bay vs Torres Strait	12.3
Hervey Bay vs Solomon Islands	7.3
Upstart Bay vs Torres Strait	14.4
Upstart Bay vs Solomon Islands	9.5
Torres Strait vs Solomon Islands	63.7

Given that the data were consistent with the presence of isolation by distance, the number of expected migrants (N_em) between populations were derived from pairwise F_{ST} values assuming a stepping-stone model (Slatkin 1993). Calculations were performed as described in Benzie & Williams (1997), assuming a mutation rate of $\mu = 10^{-7}$ (Nei 1987: p. 30 to 38). Large numbers of migrants were observed between populations within areas (Table 5), with the smallest values being observed between the 2 sample locations at the Solomon Islands. Estimates of N_em between regions were generally more than 1 order of magnitude lower, with highest migration rates observed between the Torres Strait and the Solomon Islands regions. However, all estimates involving Torres Strait yielded relatively high migration rates compared to other pairwise comparisons.

DISCUSSION

This study investigated gene flow in *Holothuria scabra* populations with a view to increasing knowledge on this commercially important species and assisting management. Allozyme analyses have identified that *H. scabra* populations along the north-east coast of Australia can be grouped into at least 3 genetically distinct stocks: (1) southern populations from the Hervey Bay area, (2) one population from the central coast, and (3) populations from Torres Strait. The latter region is closely related to samples from the Solomon Islands.

Although several studies on reef fishes (Doherty et al. 1995), giant clams and foraminiferans (Benzie 1994),

and crown-of-thorns populations on the GBR (Benzie & Stoddart 1992) reported some significant F_{ST} values, these were about 1 order of magnitude less than those reported here. Several other studies on invertebrates of the GBR (Benzie & Williams 1992, Williams & Benzie 1993, Uthicke & Benzie 2000b) did not detect any significant population differentiation between populations separated by distances of up to 1500 km. The only published F_{ST} values of a similar order of magnitude to, or higher than, those observed here are for species which are viviparous (Ayre & Dufty 1994) or which have asexual reproduction (Burnett et al. 1995, Uthicke et al. 1998, 1999). With 1 exception, all loci in all populations were in Hardy-Weinberg equilibrium. The contribution of the Wahlund effect (this may reduce heterozygosity due to population subdivision) to the heterozygote deficits at that locus was calculated to be approximately 2%, suggesting that the source of these deficits is some variation in breeding success among particular genotypes at that site. The maximum number of sexually produced individuals in each population equals the sample size (i.e. $N^*/N_i = 1$). Together with the overall low deviations from Hardy-Weinberg equilibrium, this confirmed the general perception that asexual reproduction is not a feature of the biology of *Holothuria scabra*. Therefore, it can be concluded that differences in gene frequencies do not result from differential asexual reproduction. Although there are differences in the degree of differentiation among populations for different loci, the fact that all contribute to population differentiation suggests that patterns of spatial differentiation in *H. scabra* is not the result of selection either, but of restrictions in gene flow among populations.

Estimates of gene flow are correlated to dispersal ability across a large variety of taxa from terrestrial, freshwater and marine environments (Bohonak 1999). The time span of planktonic larval development is often considered as a factor limiting dispersal in the marine environment (e.g. Hedgecock 1986, Doherty et al. 1995). However, larval development in *Holothuria scabra* takes at least 14 d (Battaglene et al. 1999, Mercier et al. 2000), a time span well within the range of that for other invertebrates which lack or show only marginal population differentiation (see previous paragraph). The main difference to the studies cited above is that the present study investigated a species which is mainly distributed nearshore, not on the GBR proper. With the exception of the samples from Torres Strait, samples were obtained from muddy intertidal seagrass beds directly at the coast. One possible reason for restricted larval dispersal may therefore be a different current regime near the coast compared to the GBR proper. On mid and outer shelf reefs of the GBR, the main current flow is generally south-eastward during summer, the spawning period for most invertebrates on the reef. This results in a large potential for larvae to disperse over the GBR (Williams et al. 1984). Williams et al. suggested that the net drift in summer in nearshore areas will be more restricted due to periodic reversal of water movements in that area. Furthermore, gene flow between populations along coastlines may be restricted by physical and hydrological barriers, or by lack of prey for larvae (Hedgecock 1986). An offshore movement of the East Australian Current was one of the likely reasons for the population subdivision of *Actinia tenebrosa* on the temperate east coast of Australia (Ayre et al. 1991). Similarly, shelf water is transported seaward in an area north of our Hervey Bay samples (Burrage et al. 1996) which may restrict longshore movement of larvae produced in this area towards the north. It is possible that similar hydrological features exist along the north-east coast of Australia, restricting gene flow between other coastal populations investigated here.

The GBR consists of nearly 3000 single reefs, and distances between a reef and its nearest neighbour rarely exceed 15 km. Therefore, even if larvae were not dispersed over long distances, the reefs can act as stepping stones, allowing populations to mix over a small number of generations. This appears not to be the case for coastal species such as *Holothuria scabra*. Finding the populations sampled proved time-consuming. Although it is certain that not all populations on the north-east coast were sampled, it appears that populations of *H. scabra* can be separated by stretches of several 100 km of unsuitable habitat. Long stretches of unsuitable habitat were also recognised as barriers to dispersal for the sea anemone *Actinia tenebrosa* along the south-east coast of Australia (Ayre et al. 1991).

Genetic structures of populations do not necessarily reflect present-day gene flow, but can reflect historical events. For example, inter-oceanic genetic differences in the starfish *Linckia laevigata* (Williams & Benzie 1998) and *Acanthaster planci* (Benzie 1999) were explained by restrictions in gene flow between oceans due to lower sea levels during the last ice-age. Similarly, genetic distances between populations of coastal species may have evolved when there was reduced habitat availability during glacial periods and may be maintained by present-day ecological gradients and currents (Reeb & Avise 1990).

Although only separated by about 16 km, the 2 sample locations at the Solomon Islands were surprisingly distinct from each other. Also, the population from Solomon Island B, showed no significant genetic distance with samples from the Torres Strait located about 1500 km away. The significant genetic distances between the 2 Solomon Island populations and the apparent difference in connectivity with other popula-

tions may be explained by the micro-geography of these 2 locations. The population from Solomon Island A was sampled in a lagoon system which is nearly closed to the ocean, whereas the location at Solomon Island B is open towards the ocean. It appears that the lagoonal population is genetically isolated from other populations, and that the open coastal populations on the Solomon Islands may have nearly unrestricted gene flow with populations from Torres Strait.

Along the north-east coast of Australia, genetic distances increased with geographical separation, suggesting that most of the dispersal may be explained through isolation by distance. This relationship became less distinct when samples from the Solomon Islands were included, mainly because genetic distances between those samples and those from the Torres Strait samples where relatively small given their large geographic separation.

Differences in the nature of dispersal among populations in different parts of their range, or over different spatial scales, has been described before in Indo-Pacific marine species. For example, *Acanthaster planci* showed isolation by distance within regions, but patterns were less distinct when comparisons between the Pacific and Indian Ocean were made (Benzie 1999). Similarly, *Tridacna maxima* in the Pacific Ocean showed isolation by distance within island chains, but patterns consistent with an island model between island chains (Benzie & Williams 1997). In these cases, it was suggested that isolation by distance within regions represented recent dispersal and that differences between regions reflected divergence during times of lower sea level.

Uthicke & Benzie (1999) suggested that the genetic similarity between the deep and shallow populations in Hervey Bay, in conjunction with the large body size of deep-water individuals, supports previous notions that this species settles in shallow seagrass beds and later migrates to deeper areas (Vail 1989, James et al. 1994). The population at Upstart Bay sampled 2 yr apart did not change in allele frequencies, and individuals were small on both occasions. One reason for this may be that they simply did not grow during this period. However, known growth rates for *Holothuria scabra* suggest that the holothurians were not older than 8 mo on both occasions (Shelley 1985, Battaglene et al. 1999). On both sampling occasions, no individual had gonads (data not shown), which suggests that they were juveniles. Therefore, it seems more likely that the population sampled at Upstart Bay consisted of recent recruits at each sampling time. Genetic similarity between the samples suggests that the populations received recruits from the same source of spawners on both occasions, and it is possible that there is a deeper population at Upstart Bay, to which individuals migrate after settlement in the shallow seagrass bed.

The identification of several separate stocks of *Holothuria scabra* along the north-east coast of Australia has important implications for the management of the fishery. If recruitment depends on relatively local sources, one may expect that recovery of overfished stocks is delayed depending on the magnitude of the overfishing. Therefore, it may be important that the *H. scabra* fishery in this area is managed on a local scale, and the provision of local refugia appears vital. An artificial stock enhancement using juveniles produced in hatcheries may be a viable management option (Battaglene et al. 1999). However, the genetic distinctiveness of populations, in the case of the Solomon Islands even on the scale of several kilometres, indicates that care should be taken to use only local sources of spawners for artificial stock enhancement.

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