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The phylogeography and connectivity of the latitudinally widespread scleractinian coral *Plesiastrea versipora* in the Western Pacific

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Abstract

Whereas terrestrial animal populations might show genetic connectivity within a continent, marine species, such as hermatypic corals, may have connectivity stretching to all corners of the planet. We quantified the genetic variability within and among populations of the widespread scleractinian coral, *Plesiastrea versipora* along the eastern Australian seaboard (4145 km) and the Ryukyu Archipelago (Japan, 681 km) using sequences of internal transcribed spacers (ITS1-2) from ribosomal DNA. Geographic patterns in genetic variability were deduced from a nested clade analysis (NCA) performed on a parsimony network haplotype. This analysis allowed the establishment of geographical associations in the distribution of haplotypes within the network cladogram, therefore allowing us to deduce phylogeographical patterns based under models of restricted gene flow, fragmentation and range expansion. No significant structure was found among Ryukyu Archipelago populations. The lack of an association between the positions of haplotypes in the cladogram with geographical location of these populations may be accounted for by a high level of gene flow of *P. versipora* within this region, probably due to the strong Kuroshio Current. In contrast, strong geographical associations were apparent among populations of *P. versipora* along the south-east coast of Australia. This pattern of restricted genetic connectivity among populations of *P. versipora* on the eastern seaboard of Australia seems to be associated with the present surface ocean current (the East Australian Current) on this side of the south-western Pacific Ocean.

Keywords: gene flow, nested clade analysis, phylogeography, *Plesiastrea versipora*, population genetic structure, scleractinian coral

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Introduction

A major difference between terrestrial and marine communities is the extent of genetic connectivity between populations of the same species (Veron 1995). Whereas terrestrial populations might show genetic connectivity within a continent, marine species may have connectivity stretching to all corners of the planet. Scleractinian corals are no exception, with populations within a single species often ranging from the Red Sea to the eastern Pacific. Genetic variability along these distribution ranges is complex, however, and is the result of accumulative historical events that have occurred over thousands of years (Benzie 1999; Veron 2000).

The task of ascertaining the relationship between dispersal ability and magnitude and genetic differentiation of populations at multiple scales has been crucial for understanding the micro-evolutionary processes of differentiation within coral species (Ayre & Dufty 1994; Hellberg 1995; 1996; Ayre *et al*. 1997; Ayre & Hughes 2000). This has required quantification of how gene flow among populations interacts with genetic drift, natural selection and mutations. To date, gene flow among coral populations has been calculated using *F*-statistics or some variants of them, assuming a model of gene flow (Ayre & Dufty 1994; Benzie

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et al. 1995; Hellberg 1996; Ayre & Hughes 2000). However, inferences about current patterns of gene flow might be confounded by the remnants of historical associations among populations (Ayre 1991; Benzie 1999). At present, there is a need to test the assumed model of population genetic structure used to estimate population-level parameters, such as gene flow. This requires approaches that allow partitioning of the population history from population structure, which cannot be achieved using traditional *F*-statistics as this does not use temporal information on allelic variation.

New statistical procedures can make use of haplotype trees to partition population historical factors from population structure (Slatkin & Maddison 1989; Hudson *et al*. 1992; Templeton *et al*. 1995). Templeton *et al*. (1995) proposed a technique that uses haplotype trees to define a nested series of branches (clades), thereby allowing an evolutionary nested analysis of the spatial distribution of genetic variation. These nested phylogeographical analyses have more power to detect geographical associations than do traditional, nonhistorical analyses (Templeton *et al*. 1992, 1995). More importantly, these nested clade analyses (NCA) can discriminate between phylogeographical associations due to recurrent but restricted gene flow and historical events operating at the population level, such as past fragmentation or range expansion events.

The Faviid coral, *Plesiastrea versipora*, is the focus of this study. This species is the widest distributed coral within the Indo-Pacific Ocean, and one of the few hermatypic corals that has a distribution that extends beyond the physiological-limiting latitudinal borders of 25° S and 25° N defined for tropical hermatypic corals (Veron 1995). The distribution of this species from the upper part of the Japanese archipelago to the southern waters off Australia stands in contrast to most other hermatypic corals which are restricted to the warm, high-light and relatively stable conditions of tropical seas (Fig. 1a). Although the magnitude of larval dispersal for this broadcasting coral species has not yet been assessed, its broadly geographical distribution within the Indo-Pacific Ocean suggests a high capability for dispersion. Elucidation of the phylogeographical patterns and connectivity among the populations of this species at a broadly geographical scale will allow testing of whether there is a correlation between population structure and present day ocean circulation patterns.

We used the Templeton *et al*. (1995) approach of NCA to discriminate between recurrent gene flow and historical events in populations of *P. versipora* along two latitudinal reef gradients on the western Pacific Ocean (the Ryukyu Archipelago and the eastern seaboard of Australia). For this, we used the internal transcribed spacers of ribosomal DNA (ITS rDNA) as molecular markers. We also examined the extent to which the phylogeographical patterns within this species are correlated with oceanographic features within the Indo-Pacific Ocean.

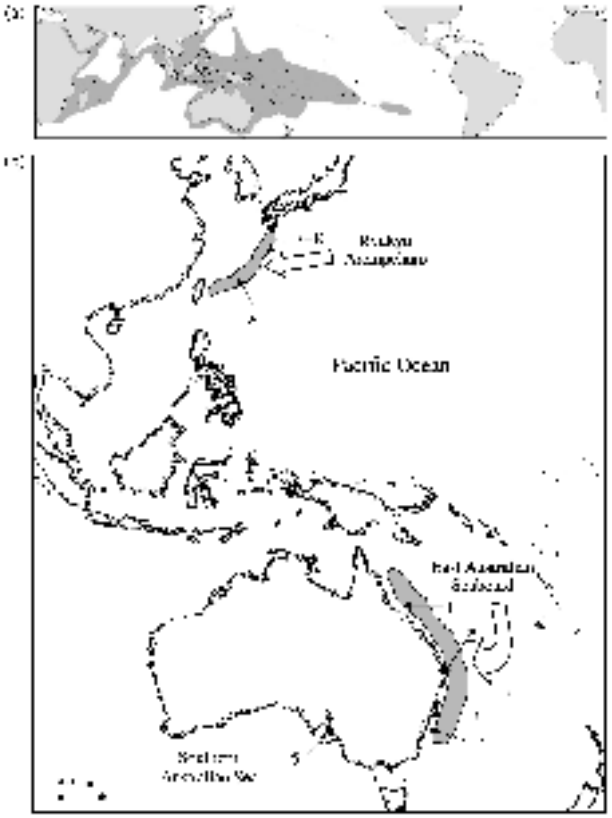

Fig. 1 (a) Distribution of *Plesiastrea versipora* in the Indo-Pacific Ocean (map taken from Veron 2000). (b) Map of Western Pacific Ocean, showing the two latitudinal gradients of coral reef studied and the sampling locations (1, Orpheus I; 2, Moreton Bay; 3, Port Jackson; 4, Batemans Bay; 5, Gulf St. Vincent; 6, Kuroshima I; 7, South-Amami I; 8, East-Amami I).

Materials and methods

Study sites and sample collection

Colonies of *Plesiastrea versipora*, Lamark (1816) were sampled from eight sites along the east and south-east coast of Australia and the Ryukyu Island Arc of Japan (Fig. 1b, Table 1). We sampled colonies that were separated by at least 10 m to minimize the chance of sampling the same genotype more than once. Some of the study sites were represented by small numbers of samples (Table 1), due to the low abundance of this species. Coral fragments of ≈ 4 cm^2 were collected from individual colonies, and the tissue removed from the skeletons using directed high-pressure air jets. The tissue slurry was mixed and preserved with an equal volume of 20% DMSO in 0.25 M EDTA and saturated NaCl (pH 8.0). Samples were stored

Table 1 Latitudes and geographical distances (km) among the populations of *Plesiastrea versipora* used for the nested clade analysis. It also includes the number of samples collected. A = eastern seaboard of Australia; B = Ryukyu Archipelago

A						
Latitude	Location	Orpheus I. (km)	Moreton Bay (km)	Port Jackson (km)	Batemans Bay (km)	Samples collected
18°03′ S	Orpheus I.	—				7
27°11′ S	Moreton Bay	1625				8
33°52′ S	Port Jackson	2478	853			5
35°49′ S	Batemans Bay	2645	1020	167		3
34°57′ S	Gulf St. Vincent	4145	2520	1667	1500	5

B				
Latitude	Location	Kuroshima I. (km)	South-Amami I. (km)	Samples collected
24°14′ N	Kuroshima I.	—		5
28°00′ N	South-Amami I.	650		4
28°07′ N	East-Amami I.	681	31	3

and transported at temperatures ranging from 0 °C to ambient temperature (28–35 °C), or processed immediately in the laboratory.

DNA extraction

Tissue slurries were incubated in 1% SDS at 65 °C for 1 h followed by digestion with Proteinase K (Sigma) in a final concentration of 0.5 mg/mL at 39 °C for 8 h (Sambrook *et al*. 1989). DNA was extracted from the digestion in two steps using equal volumes of phenol/chloroform (25:24 v/v) and chloroform/isoamyl alcohol (24:1 v/v). DNA was precipitated at 0 °C by the addition of 3 M sodium acetate (pH 5.2) and cold isopropanol (1:10 v/v). The precipitate was washed with 1 mL of 70% ethanol, dried and resuspended in 50 µL of sterile MQ water and stored at –70 °C (Sambrook *et al*. 1989).

PCR amplification

The full ITS rDNA introns, including the inserted 5.8*S* region, were amplified from the coral *P. versipora* using the universal primer ITS4 and the 'coral-specific' primer (A18S) designed by Takabayashi *et al*. (1998). The primer mix consisted of an equimolar mixture of both primers, forward (A18S) 5′-GATCGAACGGTTTAGTGAGG-3′ and reverse (ITS4) 5′-TCCTCCGCTTATTGATATGC-3′. All polymerase chain reactions (PCR) contained 0.4 µg of template DNA, 10 µL of 10× PCR buffer (1 M Tris–HCl, pH 8.3), 6 µL of 25 mM $MgCl_2$, 1.5 mM total dNTP, 30 pmol of each primer and 0.5 µL of *Taq* DNA polymerase (5 unit/µL) in a total volume of 100 µL. Amplifications were performed using a DNA thermal cycler (PCR express, Hybaid) with the following thermal cycling regime: 94 °C for 1 min, 55 °C for 2 min and 72 °C for 3 min (30 cycles).

DNA cloning and sequencing

Purification of DNA from the PCR solution of each sample was carried out using a purification kit (GFX™; Amersham Pharmacia Biotech). Approximately 60 ng each of these purified PCR products was ligated into the vector (pGEM-T Easy Vector System I; Promega), and then added and mixed to the competent cells (TOP10F′; Invitrogen Corporation, Carlsbad, CA, USA). After the transformation process, cells were grown on Luria–Bertani agar (Bacto-Agar, Difco) plates with ampicillin at 37 °C overnight. Individual colonies from the plates were taken using sterile toothpicks and placed in tubes with Luria–Bertani broth (Luria-Bertani dehydrated; Difco Laboratories) and ampicillin. These tubes were shaken (180 r.p.m.) overnight at 37 °C. Two millilitres of the overnight culture were centrifuged and the plasmids precipitated and purified (High Pure Plasmid Isolation Kit; Roche, Mannheim-Germany). Four clones per sample were sequenced. Samples of plasmid DNA (with the PCR product inserted) were sent for sequencing in both directions to the Australian Genome Research Facility (AGRF). AGRF utilizes an ABI 377 automatic DNA sequencer and process samples sequenced using the BigDye terminator chemistry. The ABI Prism BigDye terminator cycle sequencing ready reaction kit is used.

Alignment and haplotype network estimation

The full ITS rDNA sequences were aligned using CLUSTAL W (Thompson *et al*. 1994) and saved under nexus format. The algorithm of Templeton *et al*. (1992) was used to estimate the haplotype network trees. This method not only estimates the unrooted haplotype tree but also provides a 95% plausible set for all haplotype linkages

within the unrooted tree. Templeton *et al*. (1992, 1995) provide a detailed explanation of how, using a finite-site model of DNA evolution, haplotype networks can be constructed using parsimony. To construct haplotype networks, TCS α1.13 software developed by Clement *et al*. (2000) was used. This estimation algorithm also allowed checking of the complexities caused by limited recombination, as a high amount of recombination affects the results of the NCA.

Nested clade analysis and test for geographical association

After the 95% plausible set was estimated, the set of plausible cladograms was converted into a nested design in which haplotypes (O-step clades) separated by a single mutation were grouped together in one-step clades, carrying on from the tips to the interior of the network, and then grouping the resulting clades into two-step clades (under the same principle), and so on, until the final level of nesting encompassed the entire tree. All nesting rules used to construct the nested cladogram were as described in Templeton *et al*. (1987, 1995) and Templeton & Sing (1993).

Once the nested design had been determined, an exact permutational contingency analysis of categorical variation was implemented in a nested fashion (i.e. for each step level, the associations between clades and geographical locations were tested). This contingency test was performed using the software GEODIS 2.0 developed by Posada *et al*. (2000) on the clades with more than one haplotype and more than one sample location, following the algorithm given by Templeton & Sing (1993). The advantage of this new software is that it not only detects significant genetic and geographical associations (localizing them within the haplotype cladogram), but also incorporates the geographical distances as clade distance (D_c) and nested clade distance (D_n). Clade distance measures how geographically widespread are the individuals that bear haplotypes from a specific given clade (X) (see Templeton *et al*. 1995 for further explanation in terms of mathematical operations). The second distance (D_n) measures how far individuals bearing clade X haplotypes are from all individuals that bear haplotypes included in the higher step clade (that includes clade X). This analysis should allow us to distinguish between population structure and population history (see examples by Templeton & Sing 1993; Templeton *et al*. 1995; Crandall & Templeton 1996; Templeton 1998; for review and further explanation). The statistical significance of these two measures was estimated by recalculating both in 1000 random permutations. This randomization procedure allowed testing of the null hypothesis of no geographical association within the nested clade design (Templeton *et al*. 1995). Phylogeographical interpretations of significant values for D_c and D_n were inferred using the inference key for the nested haplotype tree analysis of geographical distances included in GEODIS (Templeton *et al*. 1995; Posada *et al*. 2000).

To test the existence of genetic differentiation at large geographical scales between the north-western (Ryukyu Archipelago) and south-western (eastern Australian seaboard) Pacific Ocean, a hierarchical analysis of molecular variance (AMOVA) was also applied. This analysis was performed on a matrix of pairwise genetic distances among all the full ITS rDNA sequences using ARLEQUIN software (Schneider *et al*. 2000). This matrix of distances was calculated using the Kimura 2-parameter model. Comparison of pairs of populations based on the average number of pairwise differences between populations was also performed.

Results

Characteristics of the ITS1-2 regions

A total of 48 complete sequences of the full ITS rDNA introns (409 bp), from 40 colonies of *Plesiastrea versipora* from all locations were amplified, sequenced and aligned. The inserted region, 5.8*S* rDNA, between the ITS1 and ITS2 introns was separated and excluded from the analysis, as the evolutionary rate of this rDNA gene is slower than its flanking introns. Moreover, comparisons (not presented here) of this 5.8*S* region among all samples showed no differences. Nucleotide sequences of ITS haplotypes are deposited in GeneBank (Accession nos AF483778–AF483813) and sequence alignment is available from M. Rodriguez-Lanetty upon request. The main characteristics of both ITS regions of *P. versipora* from all the studied populations are summarized in Table 2. ITS1 is slightly shorter than ITS2, 203 and 206 bp, respectively. However, ITS2 ($3.15 \pm 2.24\%$) was, on average, more variable than ITS1 ($1.53 \pm 0.43\%$). All the mutations observed in all populations were substitutions, however, the ratio of transitions to transversions was higher in ITS1 than ITS2 (2.38 ± 1.7 and 1.54 ± 0.31, respectively; see Table 2). The average GC content in ITS2 (59.22%) was slightly higher than that in ITS1 (53.24%, see Table 2 for information on each population). Overall, levels of intragenomic variation within both ITS regions were low compared with the divergence found among populations. Of the eight populations studied, only three (Gulf St. Vincent, Moreton Bay and Orpheus I) showed some level of intragenomic variation or polymorphism. Among these, the highest intragenomic average variation occurred within the Orpheus Island population (eastern coast of Australia), showing 0.35% (Table 2) compared with 1.00% from the interindividual variation found in the same population (Table 8).

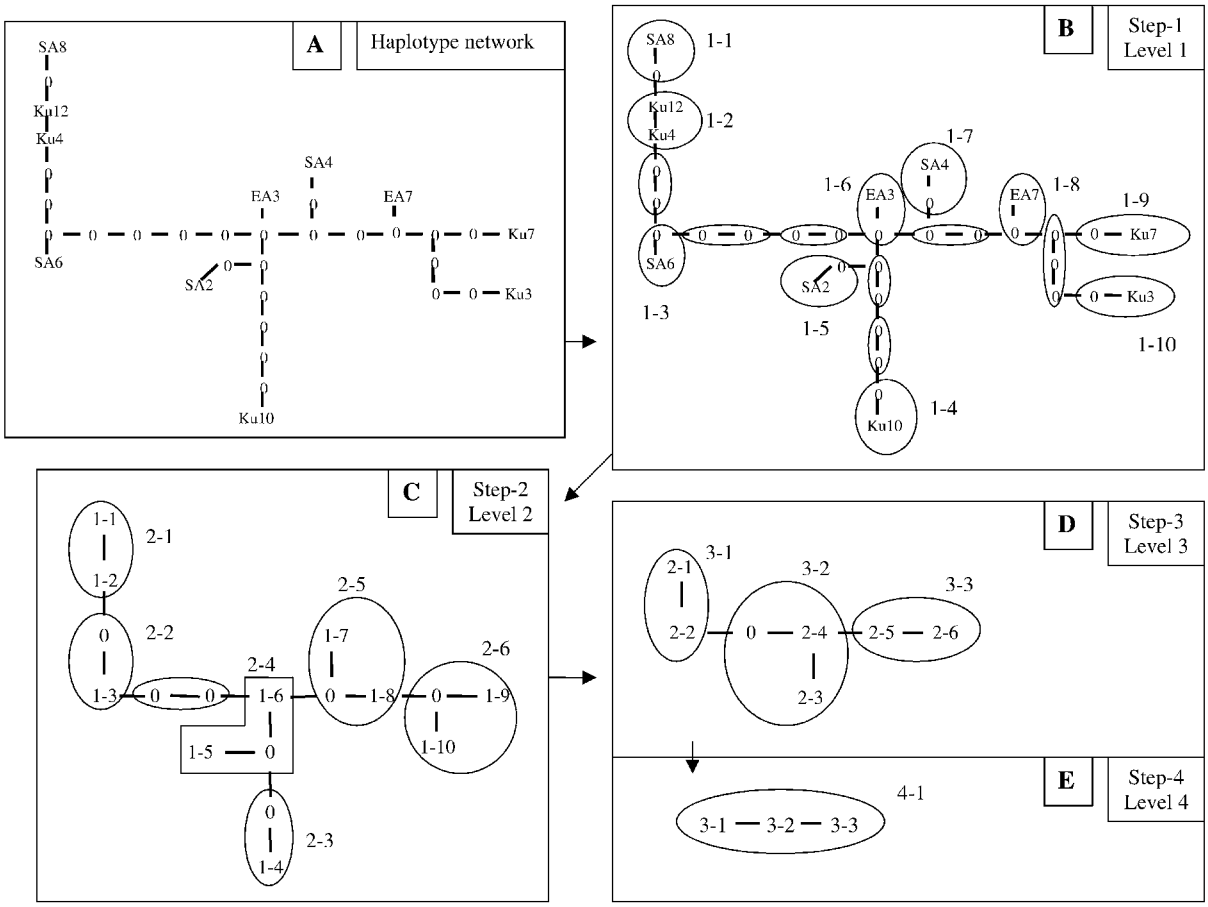

Fig. 2 Clade nesting of the parsimony network of full ITS rDNA haplotypes found in colonies of *Plesiastrea versipora* from populations along the Ryukyu Archipelago (Japan). (a) Unrooted haplotype network, which represents the estimated 95% plausible set of cladograms. The distribution and frequency of the haplotypes within the populations are shown in Table 3a. Zeros (0) within the network, represents missing haplotypes not found within the populations. (b) Step 1 of the clade nesting, grouping within ovals and circles the haplotypes separated by only a mutation, which will compose the clades level 1; (c) step 2, grouping the subclades level 1 (from step 1) separated by one mutation; (d) step 3, subclades level 2 (from step 2) grouped by one mutation distance. (e) Step 4, group together all the haplotypes within the final subclade.

Haplotype network and nested statistical design of the full ITS rDNA introns

Because the intragenomic variation in sequence composition of the full ITS introns from *P. versipora* seems negligible compared with the intercolony variation within populations, this makes these haplotypes quite informative at the population level (D. Posada, personal communication).

The lack of sampling sites within the central Indo-Pacific Ocean could result in phylogeographical misinterpretations, overestimating nested clade distances. To avoid this, populations from the Ryukyu region were analysed separately from Australian populations. However, comparisons of molecular variance between these two large geographical regions were performed, and are shown below. The nesting haplotype structure for the full ITS data set from *P. versipora* for the Ryukyu Islands and south-east Australia are shown in Figs 2 and 3, respectively. Both networks presented no internal ambiguities (Figs 2a and 3a). For these analyses a total of 11 haplotypes for the Ryukyu region and 25 for south-eastern Australia were used. The haplotype frequencies are shown in Table 3. Table 4 presents the results of the nested contingency analysis of haplotype–geography associations for the nested clades of the haplotype networks (shown in Figs 2 and 3). No significant structure was found at any level at which haplotypes were nested within the network from the Ryukyu populations (Table 4). In contrast, significant structure was found at various levels within the haplotype network from the south-east Australian populations.

Several phylogeographical patterns were deduced within the significant results from the clade and nested clade distances (D_c and D_n; Table 5), following the inference key for

Table 2 Molecular diversity indices and nucleotide composition data for ITS1 and ITS2 rDNA regions from eight populations of *Plesiastrea versipora*. Note: (*) Australia; (**) Japan; a = ITS1; b = ITS2

Population	Intragenomic average variation (%) (Full ITS1-2)	Transition/ Transversion Ratio		Substitutions (%)		Nucleotide composition							
						A		T		C		G	
		a	b	a	b	a	b	a	b	a	b	a	b
Gulf St. Vincent (*)	0.22 ± 0.3	—	1	0.98	0.97	21.68	18.82	24.95	21.95	25.31	26.97	28.06	32.26
Batemans Bay (*)	—	6	1.5	3.45	2.43	21.83	18.85	24.87	22.30	25.38	27.21	27.92	31.64
Port Jackson (*)	—	—	0	0.49	0	23.23	19.02	24.95	22.93	25.35	26.83	26.47	31.22
Moreton Bay (*)	0.30 ± 0.7	0.33	1.75	1.97	5.33	21.82	19.06	24.83	21.7	26.29	27.76	27.06	31.48
Orpheus I. (*)	0.35 ± 0.3	2	1.33	1.48	2.91	21.82	18.84	24.87	21.92	26.33	27.40	26.98	31.85
Kuroshima I. (**)	—	2	1.8	1.48	6.31	21.77	18.93	24.40	21.17	26.41	28.68	27.42	31.22
South-Amami I. (**)	—	2	1.4	1.48	5.82	21.64	19.15	24.78	21.22	26.54	28.9	27.04	30.73
East-Amami I. (**)	—	2	2	0.98	1.45	21.74	18.7	24.25	21.63	27.09	27.8	26.92	31.87

Table 3 Frequency of the full-ITS haplotypes within the eight populations of *Plesiastrea versipora*. A = Ryukyu Archipelago; B = Eastern Australian Seaboard

A

Population	Haplotypes										
	EA3	EA7	SA2	SA4	SA6	SA8	Ku3	Ku4	Ku7	Ku10	Ku12
East-Amami I.	2	1	—	—	—	—	—	—	—	—	—
South-Amami I	—	—	1	1	1	1	—	—	—	—	—
Kuroshima I.	—	—	—	—	—	—	1	1	1	1	1

B

Population	Haplotypes																								
	OI 2–5	OI 5–8	OI 7–3	OI 9–1	OI 9–5	OI 10	MB 1	MB 2	MB 3	MB 4	MB 5	MB 6	MB 7	MB 8	MB 9	MB 10	PJ 11	PJ 56	BB 6	BB 7	GSV 1	GSV 2	GSV 3	GSV 4	GSV 5
Orpheus I.	3	1	1	3	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Moreton Bay	—	—	—	—	—	—	2	1	1	1	1	1	1	1	1	1	—	—	—	—	—	—	—	—	—
Port Jackson	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	4	—	—	—	—	—	—	—
Batemans Bay	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	1	—	—
Gulf St. Vincent	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	2	2	1

Haplotype sequences are deposited in GenBank under the following Accession nos: EA3 (AF483803), EA7 (AF483804), SA2 (AF483805), SA4 (AF483806), SA6 (AF483807), SA8 (AF483808), Ku3 (AF483809), Ku4 (AF483810), Ku7 (AF483811), Ku10 (AF483812), Ku12 (AF483813), OI2-5 (AF483778), OI5-8 (AF483779), OI7-3 (AF483780), OI9-1 (AF483781), OI9-5 (AF483782), OI10 (AF483783), MB1 (AF483784), MB2 (AF483785), MB3 (AF483786), MB4 (AF483787), MB5 (AF483788), MB6 (AF483789), MB7 (AF483790), MB8 (AF483791), MB9 (AF483792), MB10 (AF483793), PJ11 (AF483794), PJ56 (AF483795), BB6 (AF483796), BB7 (AF483797), GSV1 (AF483798), GSV2 (AF483799), GSV3 (AF483800), GSV4 (AF483801), GSV5 (AF483802).

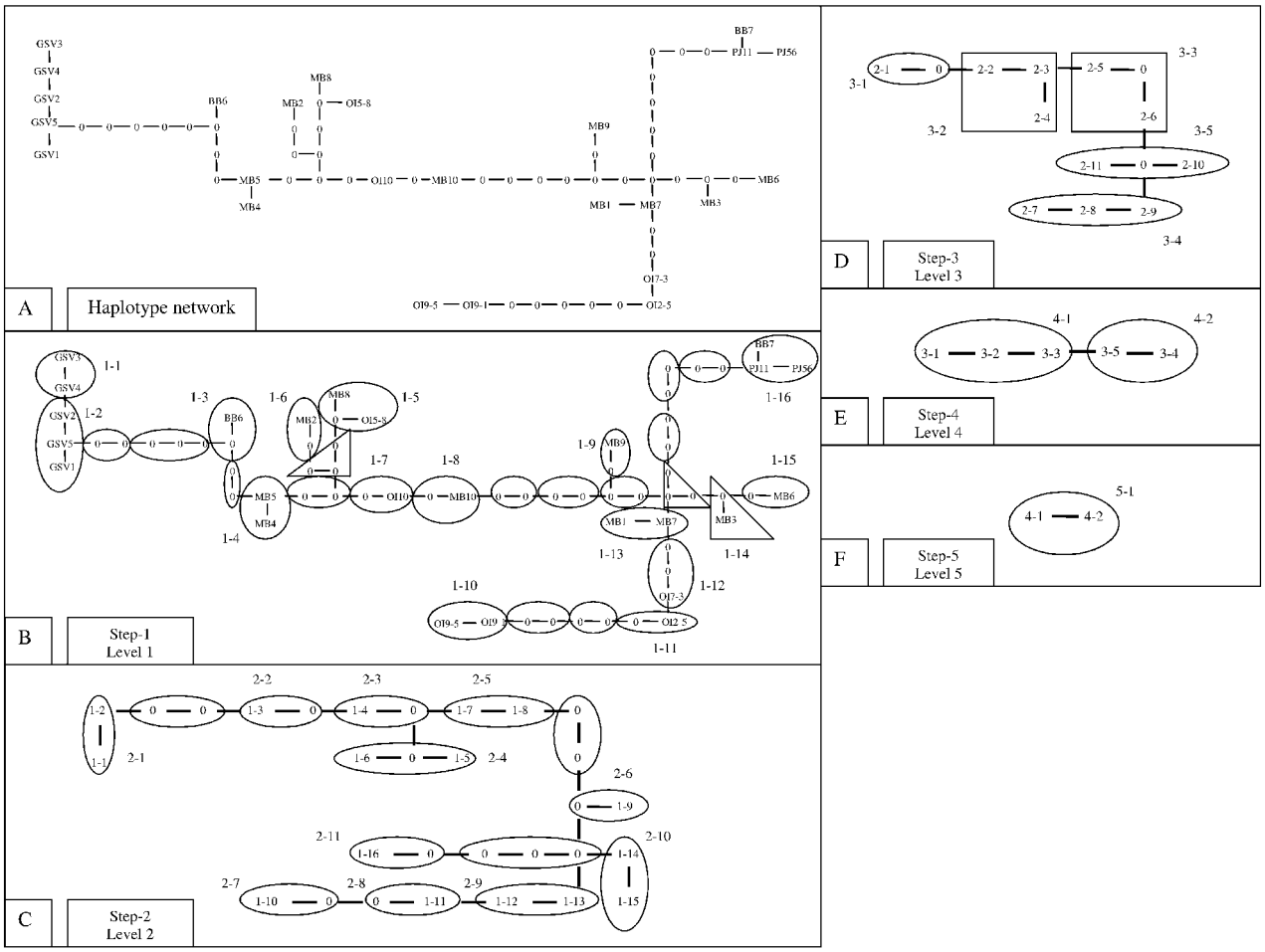

Fig. 3 Clade nesting of the parsimony network of full ITS rDNA haplotypes found in colonies of *Plesiastrea versipora* from populations along the eastern seaboard of Australia. (a) Unrooted haplotype network, which represents the estimated 95% plausible set of cladograms. The distribution and frequency of the haplotypes within the populations are shown in Table 3b. Zeros (0) within the network, represents missing haplotypes not found within the populations. (b) Step 1 of the clade nesting, grouping within circles and triangles the haplotypes separated by only a mutation, which will compose the clades level 1; (c) step 2, grouping the subclades level 1 (from step 1) separated by one mutation; (d) step 3, subclades level 2 (from step 2) grouped by one mutation distance (within ovals and squares). (e) Step 4, group together the subclade from level 3; (f) step 5, group finally together all the haplotypes within the level 5.

NCA proposed by Templeton *et al*. (1995) and Posada *et al*. (2000). The resulting interpretations are shown in Table 6. Significant structuring at the nested clade 3–4 (Orpheus I and Moreton Bay haplotypes) and clade 4–1 (Orpheus I., Moreton Bay, Batemans Bay and Gulf St. Vincent haplotypes) appears to be related to restricted gene flow with isolation by distance. This interpretation is based on the fact that the tip clades are narrowly distributed geographically (i.e. significant small clade distances) and some interior clades are broadly distributed (i.e. significant large nested clade distances). At nested clade 3-5, the outcome was inconclusive as the tip/interior status could not be determined. The reason for this is that all the nested subclades (2–10 and 2–11) are terminal within the network tree (Fig. 3d).

The population within Port Jackson (Sydney Harbour) on the eastern Australian coast appears to have been the result of a range expansion event. This is shown by the geographical association found within clade 4-2 (Table 5). This interpretation is deduced mainly by the fact that the interior clade distance was significantly small, the tip nested clade distance was significantly large, and the interior/tip nested clade distance was significantly small.

Analysis of molecular variance and comparisons of pairs of populations confirm the results obtained from the NCA (Tables 7 and 8). The populations from the Ryukyu Archipelago did not show any genetic difference among them, but were shown to be distinct from each of the populations from the eastern seaboard of Australia. Nevertheless, the differences between the two large geographical regions (Ryukyu Archipelago and eastern seaboard of Australia) were not significant (AMOVA, $P = 0.386$, Table 7), and showed a very low percentage of molecular variation (1.51%).

Table 4 Nested contingency analysis of haplotype–geography associations for the populations of *Plesiastrea versipora* from the two geographical regions (Japan and Australia). The nested designs for the Japan and Australia regions are given in Figs 2 and 3, respectively

Geographical region	Clade	Permutational chi-square statistic	Probability
Ryukyu Archipelago (Japan)	2-1	3.000	0.315
	2-4	2.000	1.000
	2-5	3.000	0.342
	3-1	1.333	1.000
	3-2	3.000	1.000
	3-3	5.000	0.185
	4-1	2.253	0.862
	Entire cladogram		
Eastern Seaboard (Australia)	1-1	0.833	1.000
	1-5	2.000	1.000
	1-16	6.00	0.300
	2-1	0.686	1.000
	2-4	0.750	1.000
	2-5	2.000	1.000
	2-9	4.000	0.250
	3-2	7.000	0.353
	3-3	0.750	1.000
	3-4	7.219	0.040*
	3-5	8.000	0.032*
	4-1	15.938	0.009*
	4-2	14.077	< 0.001*
	5-1	15.962	0.001*
	Entire cladogram		

*Significant at $P < 0.05$.

Conversely populations from the eastern seaboard of Australia were shown to be highly structured and all different (Table 8, $P < 0.05$).

Discussion

The level of intraspecific variation of the full ITS introns detected in our samples from *Plesiastrea versipora* is comparable with the range of variation found in other marine species, including algae (*Heterosigma akashiwo*, 1.7%, Connell 2000), higher plants (0–6%, Ainouche & Bayer 1999), corallimorpharians (2.5%, Chen *et al*. 1996) and *Madracis* corals (3.3–3.5%, Diekmann *et al*. 2001), but is much smaller than that found in acroporid corals (3–29%, Odorico & Miller 1997). The enormous interspecific variation revealed in scleractinan corals from the genus *Acropora* may be related to the high occurrence of interspecific hybridization events occurring in this group (Hatta *et al*. 1999; van Oppen *et al*. 2001). Our data suggest that this is not the case for the scleractinian coral *P. versipora*.

Phylogeographical interpretations based on a nested clade analysis

The results indicate that recurrent restricted gene flow and nonrecurrent historical events at the population level have played a considerable role in determining the geographical association of ITS haplotypes for *P. versipora* along the south-eastern coast of Australia, but not within the Ryukyu Archipelago (Japan).

The null hypothesis that there is no association between the distribution of haplotypes and geographical location within the Ryukyu populations was accepted. This suggests that all the populations under study have sufficient gene flow to be panmictic despite the large distances involved (e.g. as much as 700 km). Comparisons of pairs of populations, based on genetic distances, also confirmed the null hypothesis of no geographical association of the ITS haplotypes within the Ryukyu populations. We propose that this lack of geographical association within the haplotypes among these populations, which are separated by hundreds of kilometres, might also be related to the oceanography of the Ryukyu Archipelago. The strong unidirectional Kuroshio Current is likely to act to connect populations in this area of the world as it flows northward. The core of this current begins on the north of the Philippines (Nitani 1972) and brings water to the Japanese sites via the strait between Taiwan and the Yaeyama Islands (where one of the southernmost studied populations is located at Kuroshima Island). The current moves northwards, without coming across any island on the west side of the main Ryukyu Islands (200 km away) until it hits the Amami island group (where another two of our

Table 5 Nested geographical distance analysis for the *Plesiastrea versipora* full-ITS haplotypes from populations along the eastern seaboard of Australia. The hierarchical structure represented in this table is the same as the one shown in Fig. 3. From the left to right, the different columns indicate increasing nesting levels, from haplotypes (0-step clades) to 5-step clades. Brackets reflect the nesting structure, grouping in a given clade the immediate low-level nesting categories. Clade (D_c) and nested clade (D_n) distances are given for each clade. Detection of significant differences between the observed and the expected distances under a situation of random geographical distribution of haplotypes is indicated with a superscript capital 'S' (for distances significantly smaller than expected) or 'L' (for distances significantly larger than expected). In clades where the interior/tip status can be determined, the result of the interior/tip clade test is indicated by 'I-T' in italic for the corresponding clade. Also, in these cases the interior clade is shown in bold

Haplotypes			1-step clades			2-step clades			3-step clades			4-step clades			5-step clades
Clade	D_c	D_n	Clade	D_c	D_n	Clade	D_c	D_n	Clade	D_c	D_n	Clade	D_c	D_n	Clade
GSV3	993	1020													
GSV4	**0**	**736**	1-1	930	868										
I-T:	–993	–283													
						2-1			3-1	750^S	1324				
GSV2	0	0													
GSV5	0	0	**1-2**	**0**	**499**										
GSV1	0	0	*I-T:*	*–930*	*–368*										
BB6			1-3			**2-2**	**0**	**850**							
MB5	0	0													
MB4	0	0	1-4			**2-3**	**0**	**260**							
									3-2	**711^S**	**1201**				
MB8	0	851													
OI5-8	0	773	1-5	810	823							4-1	1367	1465	
I-T:	*unresolved*					2-4	744	837							
						I-T:	*–744*	*–380*							
MB2			1-6	0	576										
			I-T:	*unresolved*											
OI10			1-7	0	773										
						2-5	810	824							
MB10			1-8	0	851										
			I-T:	*unresolved*					**3-3**	**744**	**2038**				
									I-T:	*–28*	*156*				
MB9			1-9			2-6	0	577							5-1
						I-T:	*unresolved*								
OI9-1	0	0													
OI9-5	0	0	1-10			2-7	0	413							
OI2-5			1-11			**2-8**	**0**	**413**							
									3-4	617^S	1204^L				
OI7-3			1-12	0	1188										
MB1	0	0				**2-9**	**638**	**998^L**							
MB7	0	0	1-13	0	425	*I-T:*	*365*	*334^L*							
			I-T:	*unresolved*											
MB3			1-14	0	0							4-2	995	968	
						2-10	0	830^L				*I-T:*	*unresolved*		
MB6			1-15	0	0										
									3-5	**199^S**	**847**				
PJ11	**0**	**56**													
PJ56	0	56	1-16			2-11	83^S	113^S							
BB7	0	167				*I-T:*	*unresolved*		*I-T:*	*–417*	*–357^S*				
IT:	*0*	*–22*													

Clade	Inference
3-4	Restricted gene flow with isolation by distance
3-5	Inconclusive outcome (tip/interior status cannot be determined)
4-1	Restricted gene flow with isolation by distance
4-2	Range expansion
5-1 (Entire cladogram)	Inconclusive (tip/interior status cannot be determined)

Table 6 Interpretation of nested clade analyses from the *Plesiastrea versipora* full-ITS haplotype network of the eastern seaboard of Australia, following the key path reported by Templeton *et al*. (1995) and Posada *et al*. (2000)

Table 7 Analysis of molecular variance (AMOVA) among populations of *Plesiastrea versipora* using the full ITS-rDNA data. Two hierarchical levels (regions and populations) were implemented. Region 1: the Ryukyu Archipelago. Region 2: the eastern seaboard of Australia. Batemans Bay population was excluded from the analysis owing to a small number of sequences. The significance tests were based on 1023 permutations

Source of variation	d.f.	Sum of squares	Variance component	Percentage of variation	Fixation indices	*P*
Among region	1	15.848	0.067	1.51	0.015	0.386
Among populations within regions	5	84.209	2.317	52.08	0.529	< 0.0001
Within populations	38	78.454	2.065	46.41	0.536	< 0.0001

Table 8 Comparison of pairs of populations. Here is shown the average percentage of pairwise differences between populations (above diagonal) and within population (diagonal elements, in bold). Below diagonal, it is shown the *P*-values and the statistical significance of the genetic differences between the populations. The Kimura 2-parameter distance method was used for the calculations

Population	East-Amami	South-Amami	Kuroshima I	Orpheus I	Moreton Bay	Port Jackson	Gulf St. Vincent
East-Amami	**0.82**	1.42	1.69	1.48	1.24	3.19	2.42
South-Amami	0.315	**1.77**	1.77	1.87	1.61	3.27	2.93
Kuroshima I	0.225	0.810	**2.09**	1.83	1.78	3.42	2.74
Orpheus I	0.018*	< 0.001*	< 0.001*	**1.00**	1.25	2.69	2.37
Moreton Bay	0.009*	0.036*	0.043*	< 0.001*	**1.13**	2.69	2.44
Port Jackson	0.018*	0.009*	< 0.001*	< 0.001*	< 0.001*	**0.09**	3.21
Gulf St .Vincent	0.018*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	**0.44**

*Significant at $P < 0.05$.

populations are situated). The current continues northward toward the south-east coast of mainland Japan as the Kuroshio extension (Veron & Minchin 1992; Xu & Su 1997). *Plesiastrea versipora* is a broadcasting coral species (Dai *et al*. 1993), whose larvae can probably remain in the plankton long enough to be transported hundreds of kilometres by the strong Kuroshio Current. The Kuroshio Current is therefore likely to transport larvae from source populations in the southernmost part of the island arc, where this species is actually quite abundant, to sink populations along the whole Ryukyu Arc. Similar levels of connectivity, using the same molecular marker (ITS1), have also been found for the coral *Stylophora pistillata* (Takabayashi 2000) along the Kerama Islands (west of Okinawa Island). Moreover, a lack of correlation between geographical and genetic distances using allozymes was also found among populations from one of the most common scleractinian corals, *Pocillopora damircornis* along the same geographical range, the Ruykyu Archipelago (Adjeroud & Tsuchiya 1999), suggesting that there was no significant isolation by distance. The results of these previous studies have also been explained on the basis of the general south–north circulation caused by the major oceanic current. We propose here that the interactive effect of larval dispersal and strongly constant movement of ocean currents has maintained high levels of larva dispersal and gene flow of *P. versipora* along the Ryukyu Archipelago reefs from a mainly south–north direction within this region of the north-western Pacific Ocean.

Although the null hypothesis of no geographical association was accepted for the Ryukyu Arc populations, it was rejected repeatedly within the nested clade analysis for Australian populations. General genetic data analysis revealed a strong geographical association among the

P. versipora populations along the south-east coast of Australia. The phylogeographical predictions inferred in this study are based on models of restricted gene flow, range expansion and fragmentation.

Nested clade 3-4, represented by subclades 2–7 and 2–8 (some haplotypes from Orpheus I), and 2–9 (some other haplotypes from Orpheus I and Batemans Bay) show that the genetic divergence among these populations seems to be due to isolation by distance under a model of restricted gene flow. Significant structuring found in nested clade 3-5 represented by subclades 2–10 (some haplotypes from Moreton Bay) and 2–11 (all haplotypes from Port Jackson Bay and one haplotype from Batemans Bay), could not be deciphered or interpreted, as the tip and interior clade status could not be determined due to the fact that both subclades are terminal within the haplotype network. This phylogeographical interpretation was resolved, however, in the subsequent nested clade as observed at clade 4-2. At this nested clade, a range expansion event was detected within the population from Port Jackson (Sydney Harbour).

Significant results obtained from the high nested clade 4-1 suggest that genetic divergence among all the populations (Orpheus I., Moreton Bay, Batemans Bay and Gulf St. Vincent) must have occurred through a process of isolation by distance. The genetic structure among these populations, except Port Jackson, clearly shows a geographical association defined by the distances separating them from each other, and a pattern of connectivity that runs from north to south. However, even though the model of gene flow was tested and confirmed for all populations, excluding that from Port Jackson, it was not possible in this study to apply statistic analogues to F_{ST} to calculate the degree of gene flow, as ITS rDNA introns do not behave as diallelic single-copy loci (Hillis & Dixon 1991). Even so, the identified pattern of restricted genetic connectivity among populations of *P. versipora* on the eastern seaboard of Australia appears, as with the Japanese populations, to be correlated with surface ocean currents on this side of the south-western Pacific Ocean. The east Australian current travels from north of the Great Barrier Reef to the south-eastern latitudes of Australia (Veron 1995). This current seems to bring larvae mainly in one direction and to link north and south populations of *P. versipora* on the eastern Australian coast, probably following a stepping-stone process. Similar phylogeographical patterns (based on a model of restricted gene flow) among the broadcasting corals, *Acropora cytherea* and *A. hyacinthus*, have also been detected within the Great Barrier Reef (Ayre & Hughes 2000). Although substantial genetic variation between reefs separated by 1200 km was found for these species, estimation of *Nem* (> 5) suggests moderate levels of gene flow. Ayre & Hughes (2000) proposed that larval dispersal in these species transported by the along-shelf movement of major ocean currents is sufficient to maintain moderate levels of gene flow along the entire Great Barrier Reef.

Range expansion in the population from Port Jackson Bay seems to be the only clear nonrecurrent historical event occurred in *P. versipora* on the eastern Australian coast. Port Jackson Bay is a quite large and deep branching enclosed body of water, with a small and limited entrance. It may, therefore, be possible that the flow of new larvae coming into the bay has been restricted, and that the earliest colonization episode of this coral within this bay was due to a historical nonrecurrent event. The lack of gene flow after colonization between populations inside and outside the bay, and an independent population history as the generations go by, where mutations are accumulated independently between the populations might have caused the genetic divergence observed in the Port Jackson population. Additionally, the high homogeneity of ITS among colonies from this Port Jackson population support the suggestion that gene flow has been restricted between populations from inside and outside of the bay, giving rise to rapid fixation and homogenization of sequence variants through a process called concerted evolution (Dover 1982; Dover & Tautz 1986).

With respect to the comparison of the population differentiation between the regions of the Ryukyu Archipelago and the eastern seaboard of Australia, our results showed no differences between geographical regions (see AMOVA, Table 7). In fact, genetic differences between the Japanese and Australian populations were of similar magnitudes to those detected among the Australian populations (Table 8). This suggests that even though populations from these two regions present distinct population genetic structuring, there seems to be some degree of genetic connectivity among the populations from these two regions. Futures studies that include locations between these two geographical regions are needed to elucidate the actual connectivity and phylogeographical patterns between them and the central Indo-Pacific region.

Phylogeographical patterns and present ocean currents

It has been suggested that nowadays genetic structure and gene flow of coral reef organisms within the Indo-Pacific Ocean do not correspond with present-day ocean circulation patterns, and that this genetic structure has resulted from highly pulsed dispersal events associated with range expansion during interglacial periods (Benzie 1999). There is, indeed, general agreement that genetic population variability in coral reef species has been the result of accumulative historical events that have occurred over thousands of years (Veron 1995; Benzie 1999). However, there are also well-documented studies of coral genetic populations which show how genetic structure variability is related to present day oceanographic patterns

(Ayre *et al*. 1997; Adjeroud & Tsuchiya 1999; Ayre & Hughes 2000; Takabayashi 2000). This study also shows compelling congruence between the genetic structure of populations from *P. versipora* and the present-day surface circulation within the south and north-western Pacific Ocean. Nevertheless, it also shows that historical events, such as range expansion, have played an important role in shaping the genetic structure of some populations of *P. versipora*.

In summary, this study showed that recurrent (gene flow) and nonrecurrent (past dispersal) events at the population level have played a considerable role determining the geographical association of ITS haplotypes for *P. versipora* within the south-eastern seaboard of Australia, but not within the Ryukyu Archipelago (north-western Pacific). Genetic structures from populations on the eastern Australian coast show a certain degree of geographical association, explained by a model of gene flow restriction (isolation by distance). Finally, the patterns of genetic connectivity among populations of *P. versipora* in the north and south of the western Pacific Ocean seem to be associated with present surface ocean currents around these areas.

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