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Interspecific hybridization and restricted trans-Pacific gene flow in the Tropical Eastern Pacific *Pocillopora*

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Abstract

Coral reefs in the Tropical Eastern Pacific (TEP) are among the most isolated in the world. This isolation has resulted in relatively low species diversity but comparatively high endemism. The dominant reef-building corals of the TEP are the *Pocillopora* corals, a ubiquitous Indo-Pacific genus commonly regarded as inferior reef-builder. In addition to being the dominant reef-builders in the TEP, the Pocilloporids have undergone a reproductive shift from internally brooding larvae through most of their Indo-Pacific range to free-spawning in the TEP. Using genetic data from the internally transcribed spacer (ITS) regions of the nuclear ribosomal DNA gene cluster, we show here that this apparent reproductive shift coincides with interspecific hybridization among TEP *Pocillopora* species. We document a pattern of one-way gene flow into the main TEP reef builder *P. damicornis* from one or both of its TEP congeners — *P. eydouxi* and *P. elegans*. Our data provide preliminary evidence that trans-Pacific gene flow in *P. damicornis* between the Central and Eastern Pacific is restricted as well (Φ_{ST} = 0.419, $P < 0.0001$). In combination, these results suggest that Eastern Pacific corals exist in relative isolation from their Central Pacific counterparts and interact with each other differently via hybridization.

Keywords: corals, hybridization, ITS, *Pocillopora*, Tropical Eastern Pacific

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Introduction

The Tropical Eastern Pacific (TEP) is one of the most isolated and peripheral biogeographical regions of the world oceans (Chavez & Brusca 1991; Grigg & Hey 1992; Veron 1995). Bordered by the American continents to the east and limited to the north and south by cool ocean currents, the westernmost point of the TEP, Clipperton Island, is separated by 5000 km of open ocean from the Line Islands in the Central Pacific (Fig. 1). This vast expanse, called Eastern Pacific Barrier (EPB; *sensu* Ekman 1953), has been described as 'impassable' for tropical shelf fauna (Darwin 1859; Mayr 1954). Low coral species diversity coupled with relatively high endemism in the TEP testifies to the strength of the EPB as an isolating force. Yet, the occurrence of trans-Pacific species (i.e. species occurring on both sides of the EPB) indicates that the EPB functions more like a filter than a complete barrier (Briggs 1974) by limiting trans-Pacific larval dispersal mostly to species with teleplanic, long-living larvae (Vermeji 1978).

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Considerable debate has surrounded whether the current TEP marine fauna are remnants of historical vicariance due to the closure of the Isthmus of Panama 3 million years ago (Heck & McCoy 1978) or more recent immigrants from the Central Pacific (Garth 1966; Dana 1975). Biogeographical studies indicate that both processes have contributed to the present-day fauna. Geminate species (i.e. species that arose due to the closure of the Isthmus; Starr Jordan 1908) occur in most taxonomic groups (Mayr 1954; Vawter *et al*. 1980; Lessios 1981; Knowlton & Weigt 1998; Rodriguez *et al*. 2005), but trans-Pacific species are common as well (Briggs 1995; Fauchald 1977; Vermeji 1978; Lessios *et al*. 1996; Robertson *et al*. 2004). Genetic comparisons among trans-Pacific species across a variety of taxonomic groups have revealed evidence for both, ongoing trans-Pacific gene flow (e.g. in

fish — Lessios & Robertson 2006; Rosenblatt & Waples 1986; sea urchins — Lessios *et al*. 1998, 2003; starfish — Nishida & Lucas 1988) and historical subdivision (e.g. in fish — Colborn *et al*. 2001; Lessios & Robertson 2006; sea urchins — Lessios *et al*. 1999, 2001; McCartney *et al*. 2000).

Little is known about the origins of TEP reef corals and their potential for trans-Pacific gene flow. Of the 34 TEP coral species, 80% are trans-Pacific species (27 species) and 20% are regional endemics (seven species). Fossil evidence suggests past trans-isthmian connections (Budd 1989), including the occurrence of *Pocillopora* in the Caribbean (Geister 1977), but only one probable geminate coral has been documented (*Siderastrea glynni*, Forsman *et al*. 2005). With respect to trans-Pacific gene flow, Forsman (2003) presented genetic evidence for ongoing trans-Pacific gene flow in the coral *Porites lobata* between the South Pacific and Galapagos (F_{ST} = 0.07–0.25), while other TEP localities such as Panama fell out as distinct clades (see Discussion). For the majority of the TEP corals, nothing is known about the history of gene flow across the EPB and the origins of the TEP endemic coral fauna.

Because of their ecological dominance in the TEP, *Pocillopora* corals are central to the debate about the origins and ongoing trans-Pacific connections in TEP reef corals. Pocilloporids are among the most widely distributed Indo-Pacific corals, ranging from the Red Sea to the TEP. Across most of their ranges, *Pocillopora* species are common, but subordinate framework builders. However, in the TEP, they are the primary reef-building corals (Glynn & Macintyre 1977; Glynn & Wellington 1983; Guzman & Cortes 1993), capable of forming expansive reefs up to 370 ha (Glynn *et al*. 1996). *Pocillopora* corals are well known for their high dispersal potentials (> 100 competent days, Richmond 1987; Harii *et al*. 2002), which may facilitate trans-Pacific dispersal. Pocilloporids are notoriously plastic in their morphologies, which has made morphology-based taxonomy difficult within this group (e.g. Brüggemann 1879; Veron 2000). The only exception is *Pocillopora damicornis*, which is frequently separated from the other species and reliably identified due to its intergrading verrucae and branches (Cantera *et al*. 1989; Glynn 1999). *Pocillopora* corals are also distinctive for their labile reproductive strategies, shifting from internally brooding larvae across most of their ranges to free spawning at some locations including the TEP (Glynn *et al*. 1991; Glynn 1999). *P. damicornis*, in particular, is well known for its ability to produce both sexual and asexual (i.e. parthenogenic) larvae to different degrees across its range (Ayre & Miller 2004; Stoddart 1983, 1988; Ward 1992; Miller & Ayre 2004; Diah Permata & Hidaka 2006).

Here we compare genetic relationships among five TEP *Pocillopora* species, three trans-Pacific (*P. damicornis, P. eydouxi,* and *P. elegans*) and two TEP endemics (*P. inflata* and *P. effuses*) from Panama, Clipperton Island and Hawaii to published sequence data from elsewhere in the Pacific. Using data from the internally transcribed spacer regions (ITS) of the nuclear ribosomal gene cluster (rDNA), we show that *P. damicornis* in the TEP hybridizes and receives genes from its TEP congeners. These genetic data suggest that trans-Pacific gene flow in *P. damicornis* is restricted as well.

Methods

A total of 69 individuals were collected at several locations within Panama, Clipperton Island and Hawaii (Fig. 1, Table 1) and preserved in 95% ethanol for genetic analyses. DNA was extracted using a modified GITC protocol, standard phenol-chloroform extraction and ethanol precipitation

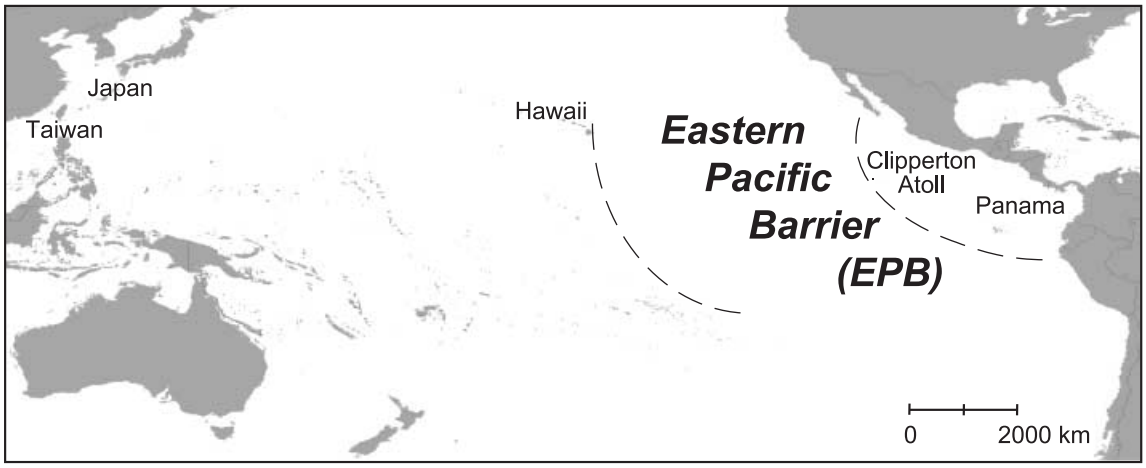

Fig. 1 Map of the Pacific showing the 5000-km expanse — called the Eastern Pacific Barrier — separating the Central and Eastern Pacific, and our collection localities.

Table 1 Summary population genetic statistics including heterozygosity for all five *Pocillopora* species by their geographical location

Species population	*N*	*A*	*h*	*K*	ITS type frequencies I	II	III	Heterozygosity $Hets_{(within/between)}$	H_O	H_E	P_{HWE}
P. damicornis											
Japan	15	30	12	4.72	0.933	0.067	—	1 (1/0)	0.067	0.184	0.002**
Taiwan	1	2	1	—	—	1.000	—	0 (0/0)	0.000	0.000	NA
Hawaii	2	4	2	0.50	—	1.000	—	1 (1/0)	0.500	0.375	0.637^{NS}
Panama	10	20	12	3.98	—	0.400	0.600	6 (4/2†)	0.600	0.640	0.058^{NS}
P. effusus											
Clipperton	4	8	4	4.27	0.250	0.250	0.500	2 (2/0)	0.500	0.750	0.062^{NS}
P. inflata											
Panama	5	10	4	0.47	—	—	1.000	5 (5/0)	1.000	0.500	0.025*
P. elegans											
Panama	10	20	4	0.39	—	—	1.000	2 (2/0)	0.200	0.180	0.725^{NS}
P. eydouxi											
Panama	10	20	4	0.37	—	—	1.000	2 (2/0)	0.200	0.180	0.725^{NS}
Hawaii	1	2	2	1.33	—	—	1.000	1 (1/0)	1.000	0.500	0.317^{NS}

N, sample size; *A*, number of alleles; *h*, number of haplotypes; *K*, average number of differences.
$Hets_{(within/between)}$, no. of heterozygotes (within ITS type/between ITS type); *H*o, observed heterozygosity; H_E, expected heterozygosity
P_{HWE}, *P* value test for deviation from Hardy–Weinberg equilibrium; NA, not applicable; ^{NS}, not significant.
*$P < 0.05$, significant; **$P < 0.01$, highly significant; †includes the type III x recombinant heterozygote.

(Fukami *et al*. 2004). The complete ITS1-5.8S-ITS2 region was amplified under normal PCR conditions using coral-specific primers ITSFCoral (5′-GTCGTAACAAGGTTTC-CGTA-3′) and ITSRCoral (5′-AAAGTGCTTCTCGCAA-CTAC-3′). These primers were designed using published *Pocillopora* sequences (Ridgway 2002; Chen *et al*. 2004), to anneal in the 18S and 28S ribosomal genes. A typical PCR profile included 2 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 30 s at 48 °C and 2 min at 72 °C. Amplified fragments were sequenced directly using PCR primers and ABI cycle sequencing chemistry on an ABI 3100 Automatic Sequencer (Applied Biosystems).

Direct sequencing revealed the presence of multiple, variable copies of the assayed rDNA gene region, which were visible as heterozygous bases and confirmed by cloning of PCR products (pGEM-t cloning kits, Promega) from a subset of samples. Cloned and sequenced PCR products revealed multiple, complex microsatellite and insertion/deletion (indel) regions within ITS1, which impeded reliable direct sequencing with forward primers. Direct sequences were reliably obtained in the reverse direction through ITS2 and 5.8S. Intragenomic rDNA variation was observed in our *Pocillopora* sample but in general was low, with the exception of a few individuals with divergent ITS types (see Results), and thus our data were not confounded the problems associated with high intragenomic variation observed in the coral genus *Acropora* (Vollmer & Palumbi 2004; see also Wei *et al*. 2006). Heterozygous ITS2 sequences were resolved using heterozygous base-calling techniques (Hare & Palumbi 1999), or by cloning and sequencing PCR products in complex sequences. Sixteen additional ribosomal DNA sequences were obtained from GenBank for *Pocillopora damicornis* — one from Taiwan (Chen *et al*. 2004; Accession no. AY722785) and 15 from Japan (M. Hirose, D.W. Permata & M. Hidaka, unpublished; AB214382–AB214396).

Sequences were aligned manually. Indels were coded as single substitutions. A neighbour-joining (NJ) tree was constructed using uncorrected pairwise distances and 1000 bootstrap replicates. Maximum-likelihood and maximum-parsimony searches yielded similar topologies (results not shown). The NJ tree is presented here because recombinant sequences were detected in our sample. A variety of population genetic statistics were calculated in the programs DNASP 4.10.3 (Rozas *et al*. 2003) and ARLEQUIN 2.5 (Schneider *et al*. 2000). Each individual was coded as possessing two rDNA alleles for the purpose of calculating these population genetic statistics including heterozygosity. Although the rDNA gene cluster exists in multiple copies (Ohta 1980), this allele-coding strategy is justified (with caveats) since no individuals were detected with more than two distinct ITS sequences. Analyses of molecular variance (AMOVA), implemented in ARLEQUIN, was used to estimate the levels of genetic differentiation between species and calculate pairwise differences between species by geographical locality (using Φ_{ST}). Deviations from the expected levels of heterozygosity (Nei 1987) were tested as deviations from Hardy–Weinberg equilibrium (HWE) expectations using exact tests (Guo & Thompson 1992).

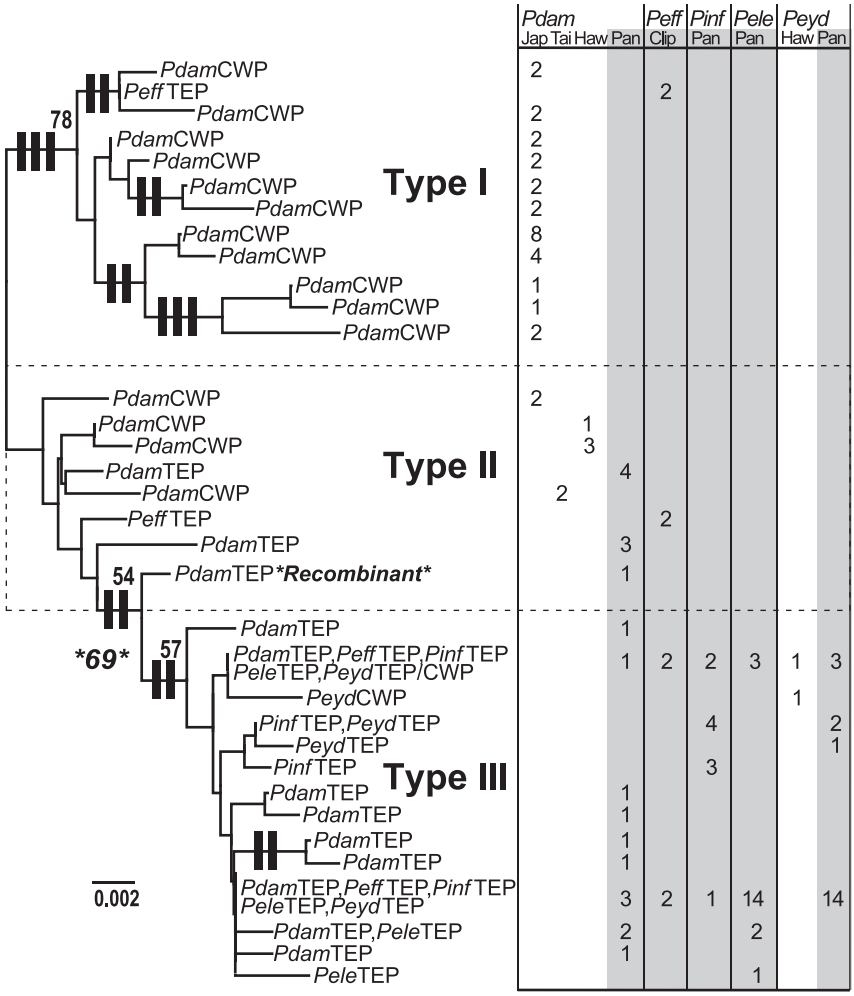

Fig. 2 Neighbour-joining (NJ) tree and allele table by species and collection locality. The three ITS types (types I, II, and III) are labelled on the tree and the OTUs are labelled according to the abbreviated species identity and geographical region. Bootstraps (> 50%) are given for major nodes. The OTU marked *Recombinant* is the sequence identified as a recombinant between ITS type II and type III. Removal of this recombinant sequence increased bootstrap support to 69%, which is shown with asterisks (i.e. *69*). The ITS allele table shows the distribution of alleles by species and geographical locality. TEP populations have a grey background. The Accession numbers for the new sequences are EU314741 to EU314802.

Results

Approximately 1125 bp of the ITS rDNA gene region, consisting of 18S (44 bp), 28S (59 bp) and the complete ITS1- (444 bp) 5.8S- (168 bp) ITS2 (410 bp) region were obtained from a subset of our *Pocillopora* samples (n = 42; Table 1). The cloning and sequencing of the amplified PCR products identified a number of microsatellite and insertion/deletion (indel) regions. ITS1 contained two microsatellite regions, one $GA_{(n)}CA_{(n)}$ repeat at base pair 147 and a $CCAT_{(n)}$ repeat at base pair 269, which impeded direct sequencing in the forward direction through the ITS1 gene region. ITS2 contained one parsimony-informative $CA_{(n)}GGCT_{(n)}$ microsatellite region at base pair 719. Two Japanese *Pocillopora damicornis* sequences (AB214392 and AB214394) contained single indels in 5.8S (10 bp) and ITS2 (34 bp), respectively. Direct sequences were reliably obtained in the reverse direction through a 555-bp region containing the complete ITS2 and 5.8S gene regions. rDNA sequences were obtained for the complete ITS2 and 5.8 s gene regions for a total of 58 individuals from five *Pocillopora* species and five geographical locations (Table 1). We identified 34 unique rDNA sequences within our data set.

Multiple ITS sequences (i.e. copies) were observed in 20 out of 58 individuals (i.e. 34.5% of individuals), both as heterozygous bases in direct sequences and as multiple sequences in cloned PCR products. However, no more than two sequences were observed in any individual. The highest levels of heterozygosity were observed in *P. damicornis* from Panama (H_O = 0.60) and the two TEP endemics — *Pocillopora effusus* (H_O = 0.50) and *Pocillopora inflata* (H_O = 1.00), but only in *P. inflata* (were all five individuals were heterozygotes) was the departure from HWE significant (P = 0.025). *P. damicornis* from Japan had significantly lower than expected heterozygosity (H_O = 0.067, P = 0.002). Two *P. damicornis* from Panama were heterozygous and contained divergent sequences from two distinct ITS lineages (i.e. ITS types II and III, Fig. 2).

The NJ tree for the complete 5.8S-ITS2 region (555 bp) shows three distinct clusters of rDNA variants (labelled types I, II, and III). Each of the three ITS types is separated by a minimum of four substitutions and supported by

Table 2 Pairwise genetic differences (Φ_{ST}) between the *Pocillopora* species and the different subgroups of *P. damicornis* and TEP species

		P. damicornis					*P. eydouxi*	*P. elegans*	*P. inflata*
Species	Location	All	CWP	TEP	TEP_{typeII}	$TEP_{typeIII}$	CP/TEP	TEP	TEP
P. damicornis	CWP	0.052†							
	TEP	0.185‡	0.419‡						
	TEP_{typeII}	0.160†	0.342‡	0.134‡					
	$TEP_{typeIII}$	0.338‡	0.554‡	0.304*	0.726‡				
P. eydouxi	CP/TEP	0.410‡	0.614‡	0.240‡	0.830‡	0.084*			
P. elegans	TEP	0.406‡	0.612‡	0.241‡	0.858‡	0.050^{NS}	0.034^{NS}		
P. inflata	TEP	0.393‡	0.577‡	0.267†	0.819‡	0.300‡	0.334†	0.534†	
P. effusus	TEP	0.130*	0.332‡	0.064^{NS}	0.428‡	0.259‡	0.358*	0.388*	0.384†

*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.
CWP, Central West Pacific (Japan, Taiwan and Hawaii).
TEP_{all}, all Tropical Eastern Pacific *P. damicornis* samples.
TEP_{typeII}, Tropical Eastern Pacific *P. damicornis* samples with type II alleles.
$TEP_{typeIII}$, Tropical Eastern Pacific *P. damicornis* samples with type III alleles.
^{NS}, not significant.

bootstrap values of 69% or higher. One recombinant sequence (labelled PdamTEP**Recombinant** on the NJ tree) was detected in our sample of rDNA alleles, based on the four-gamete test (Hudson & Kaplan 1985; Hudson 1987) implemented in DNASP, which is a recombinant of type II and III sequences and fell between the two ITS types on the NJ tree.

The distribution of the three ITS types differed between the five *Pocillopora* species and showed phylogeographical structure in *P. damicornis* (Fig. 2, Table 1). *P. damicornis* and the TEP endemic *P. effusus* possessed all three ITS types. Only ITS type III alleles were found in the remaining three *Pocillopora* species — *P. eydouxi* (TEP and Hawaii), *P. elegans* (TEP) and the TEP endemic *P. inflata*. ITS type III alleles were shared among all five species, but were only found in *P. damicornis* and *P. effusus* in the TEP and not elsewhere. The distribution of the three ITS types showed strong geographical structure within *P. damicornis* as well (Fig. 2, Table 1). Type I alleles were found at high frequency in *P. damicornis* from Japan (frequency = 0.933). Type II alleles were found in *P. damicornis* from the Central West Pacific (CWP) populations (Japan, Taiwan, Hawaii) and in subset of the TEP *P. damicornis* (frequency = 0.40). The shared type III alleles were found only in the TEP *P. damicornis* and at relatively high frequency (0.60).

High levels of ITS diversity were observed in *P. damicornis* and the endemic *P. effusus* (Table 1) as a result of both species possessing all three divergent ITS types. Average pairwise sequence divergence (*K*) was 4.72 in the CWP *P. damicornis* and only slightly less at 3.98 in the TEP *P. damicornis*. Average sequence divergence within the TEP endemic *P. effusus* measured 4.27. High levels of sequence divergence in the TEP *P. damicornis* and *P. effusus* were elevated by the ITS type III alleles found within these populations. By comparison, the ITS diversity in the two other trans-Pacific species — *P. eydouxi* ($K = 0.37$) and *P. elegans* ($K = 0.39$) — was 10 times lower than the TEP *P. damicornis* sample. Likewise, the ITS diversity within the other TEP endemic *P. inflata* ($K = 0.47$) was almost 10 times lower than the ITS diversity in *P. effusus* ($K = 4.27$).

Hierarchical AMOVA tests comparing all five *Pocillopora* species showed high levels of genetic differentiation between species ($\Phi_{ST} = 0.397$, $P < 0.0001$). Pairwise Φ_{ST} values (Table 2) show that *P. damicornis* is strongly differentiated from the other two trans-Pacific *Pocillopora* — *P. eydouxi* and *P. elegans* ($\Phi_{ST} = 0.410$ and 0.406, respectively) — and the endemic *P. inflata* ($\Phi_{ST} = 0.393$). *P. damicornis* was similar but significantly different from the endemic *P. effusus* from Clipperton Atoll ($\Phi_{ST} = 0.130$, $P = 0.033$), which shared its diverse set of rDNA allele types. Among the three *Pocillopora* species with only type III alleles (i.e. *P. eydouxi*, *P. elegans*, and *P. inflata*), pairwise Φ_{ST} values indicate that the TEP endemic *P. inflata* is genetically distinct from its related trans-Pacific congeners *P. eydouxi* and *P. elegans* ($\Phi_{STt} = 0.334$ and 0.534 respectively). No significant differences were detected between *P. eydouxi* and *P. elegans* ($\Phi_{ST} = 0.034$, not significant).

Among the *P. damicornis* populations, there was strong genetic differentiation between the CWP and the TEP populations ($\Phi_{ST} = 0.419$, $P < 0.0001$). These differences were inflated by the presence of type III alleles in TEP *P. damicornis*, which are potentially of hybrid origin (see Discussion). Genetic comparisons restricted only to type I and II alleles (the putative nonintrogressed alleles) showed strong trans-Pacific genetic differentiation in *P. damicornis*-specific alleles as well ($\Phi_{ST} = 0.342$, $P < 0.0001$).

Discussion

Our results show that *Pocillopora damicornis* possesses three divergent ITS types that are geographically structured across the Pacific. In the Tropical Eastern Pacific, *P. damicornis* shares one of these ITS types (i.e. ITS type III alleles) with its trans-Pacific congeners *P. eydouxi* and *P. elegans* and the local endemics *P. inflata* and *P. effusus*. These shared ITS type III alleles are quite unexpected given the morphological distinctiveness of *P. damicornis* from its TEP congeners (Cantera *et al*. 1989; Glynn 1999), and prior genetic work on the Hawaiian *Pocillopora* (using ITS2) indicating that *P. damicornis* and *P. eydouxi* are genetically distinct (i.e. reciprocally monophyletic) from one another (Flot & Tillier 2006). Our data support the morphological data indicating a close evolutionary relationship between the trans-Pacific species *P. eydouxi* and *P. elegans* (Cantera *et al*. 1989), but could not distinguish these two species from each other statistically (with Φ_{ST}). The data also show that the TEP endemic *P. inflata* (described by Glynn 1999) is closely related but genetically distinct from the trans-Pacific species *P. eydouxi* and *P. elegans* (Φ_{ST} = 0.334 and 0.534, respectively). The second endemic *P. effusus* from Clipperton Atoll has a diverse set of rDNA variation (including all three ITS types), and is highly similar in its genetic make-up to TEP *P. damicornis*.

Peripheral hybridization in TEP

The shared ITS type III alleles observed in the five TEP *Pocillopora* species have two possible sources; they either represent shared ancestral rDNA variation resulting from incomplete lineage sorting, or are due to interspecific hybridization and interspecies gene flow within the TEP. Incomplete lineage sorting seems highly unlikely given that only one of the three distinct ITS types (i.e. type III alleles) is shared between the species, and that the only place these shared alleles occur is in the TEP at the periphery of the species' ranges. In addition, nearly all of the shared ITS type III alleles are identical. Coalescent theory predicts that the expected coalescent time to the most recent common ancestor of n gene copies is $4N(1 - 1/n)$ generations (Kingman 1982; Tajima 1983; Hudson 1990). As a result, complete lineage sorting (i.e. reciprocal monophyly) is typically expected if species divergence times are much greater than four N_e generations (Tavare 1984). Given this relationship between lineage sorting and the effective population size (N_e), it is doubtful that the lopsided pattern of lineage sorting observed here would have occurred among the *Pocillopora* species elsewhere in the Pacific, but not in the small peripheral populations of the TEP.

Instead, the most likely explanation for these shared ITS type III alleles is introgressive hybridization within the TEP *Pocillopora*. Moreover, the pattern of allele sharing is consistent with one-way gene flow of these shared ITS type III alleles into *P. damicornis* from one or more of its TEP congeners *P. eydouxi*, *P. elegans*, and/or *P. inflata*. Thus, ITS type III alleles represent *P. eydouxi*, *P. elegans*, and/or *P. inflata* alleles that are introgressed in *P. damicornis* in the TEP, whereas ITS types I and II alleles found in *P. damicornis* in the TEP and elsewhere in the CWP represent its own 'native' rDNA variation. This one-way interspecific gene flow would be achieved first by hybridization between *P. damicornis* and its TEP congeners within the TEP, and then one-way backcrossing of *P. damicornis* with hybrid individuals.

The genetic differences between TEP *P. damicornis* and its potential hybrid partners *P. eydouxi*, *P. elegans*, and *P. inflata* (Φ_{ST} ranging between 0.240 and 0.267; Table 2) suggest that interspecific gene exchange within the TEP is rare, i.e. less than one introgression event per generation (Wright 1931). In addition, the genetic differences between its three potential hybrid partners (Table 2) are too small to unequivocally identify which species is the most likely hybrid partner of *P. damicornis* within the TEP. Nevertheless, the introgressed ITS type III alleles found in TEP *P. damicornis* are more similar to *P. elegans* (Φ_{ST} = 0.050, ns) than to *P. eydouxi* (Φ_{ST} = 0.084, P = 0.018) (Table 2). This is interesting from an ecological standpoint because *P. elegans* is typically found interspersed within reefs dominated by *P. damicornis*, whereas *P. eydouxi* is mainly associated with rocky habitats where *P. damicornis* is much less common.

The two TEP *P. damicornis* with both ITS type 2 and 3 alleles within their genomes (Table 1) are presumably products of recent hybridization within the TEP and most likely recent hybrids or backcrosses. Both have mixed (i.e. hybrid) genotypes similar to the F_1 hybrids called *Acropora prolifera* in the Caribbean *Acropora* hybridization system (Vollmer & Palumbi 2002) and hybrid *Acropora pulchra* individuals in the Pacific *Acropora aspera* group (van Oppen *et al*. 2002). Multilocus data are needed to establish hybrid/backcross generation of these individuals, and more data from *P. damicornis* are needed to determine the frequency of hybrids on TEP reefs. In addition to these hybrid genotypes, we also detected TEP *P. damicornis* individuals with only type 3 sequences. These individuals are interesting because they have become fixed (or nearly so) for their hybrid partners ITS type. Because ribosomal genes exist in multiple copies and evolve via concerted evolution (Dover 1982; Hillis & Dixon 1991), the near or complete fixation of the rDNA variation in these individuals for their hybrid partners ITS type must have taken multiple generations (Ohta 1980). Thus, these individuals must be many generations removed from the initial hybridization event.

Interestingly, this peripheral introgressive hybridization in TEP *Pocillopora* seems to correspond with a reproductive shift from internal fertilization and brooding of larvae in the CWP to external fertilization and free-spawning in the TEP (Glynn *et al*. 1991; Glynn 1999). This shift in their

reproductive strategy to free-spawning and external fertilization in the TEP would dramatically increase opportunities for interspecific hybridization within the group, especially in *P. damicornis*, which reproduces predominantly by brooding asexual larvae across most of the CWP (Miller & Ayre 2004). Similar reproductive shifts from brooding to free-spawning have also been observed in other peripheral populations of *Pocillopora* including Southwest Australia (Ward 1992), the Red Sea (Shlesinger & Loya 1985), and South Africa (Kruger & Schleyer 1998), but it is not yet known if introgressive hybridization occurs at these locations as well. Evidence for peripheral hybridization has been documented at Lord Howe Island (i.e. the southernmost Australian reef) between *Pocillopora damicornis* and *Stylophora pistillata* (Miller & Ayre 2004), which is a rare case of natural intergeneric hybridization.

Preliminary evidence for restricted trans-Pacific gene flow

The strong genetic differentiation detected within our small sample of *P. damicornis* populations in the CWP and TEP provides preliminary evidence for restricted trans-Pacific gene flow (Φ_{ST} = 0.419). These estimates may be confounded by the signature of introgressive hybridization within the TEP *P. damicornis*. Vollmer & Palumbi (2007) have shown that introgression signatures can bias estimates of gene flow in corals, and thus the inclusion of introgressed ITS type III alleles could inflate estimates of trans-Pacific genetic differentiation in *P. damicornis*. The removal of putative introgressed ITS type III suggests that trans-Pacific gene flow in *P. damicornis* is still quite low (Φ_{ST} = 0.342), approximately equivalent to one migrant every two generations (Wright 1931).

The three genetic types of *P. damicornis* in the TEP —'pure' *P. damicornis*, mixed or recombinant, and 'pure' introgressed — provide additional insights into the immigration history of TEP *P. damicornis*. Within the TEP, *P. damicornis* with only ITS type II alleles have no apparent hybridization history and may be recent TEP immigrants. *P. damicornis* with mixed or recombinant genotypes have histories of past hybridization and must have been in the TEP for at least one generation. Whereas, *P. damicornis* individuals with only type III (or introgressed) rDNA variants may be long-term residents of the TEP since the fixation of these introgressed ITS type III alleles via concerted evolution would presumably take many generations.

Pocillopora damicornis is known among reef corals for its exceptionally high larval dispersal potential (Jokiel 1984; Richmond 1987). Thus, this preliminary evidence for restricted trans-Pacific gene flow in *P. damicornis* suggests that trans-Pacific gene flow might be restricted in other TEP corals as well. Genetic data for the coral *Porites lobata* support this assertion (Forsman 2003). Forsman (2003) presented genetic data indicating varying degrees of trans-Pacific gene flow between the Central Pacific and Galapagos (Φ_{ST} = 0.02–0.12), whereas no gene flow was detected from the Galapagos or other Pacific locations into Panama, which was genetically distinct (i.e. reciprocally monophyletic). Moderate to high levels of population genetic structure in *Pocillopora* corals have been detected elsewhere across the Indo-Pacific as well (Stoddart 1984; Ayre & Hughes 2000, 2004; Magalon *et al*. 2005).

Conclusion

Evidence for peripheral hybridization in TEP *Pocillopora* represents another example of hybridization among corals (reviewed in Willis *et al*. 2006). Peripheral introgressive hybridization detected in TEP *P. damicornis* confirms that the degree to which coral species are exchanging genes may vary across the large geographical ranges of most coral species (Veron 1995; Fukami *et al*. 2004). Moreover, the signature of peripheral introgressive hybridization in the TEP *P. damicornis* coupled with strong trans-Pacific population structure provide preliminary evidence that trans-Pacific gene flow in corals may be highly restricted. Further research is required to establish patterns of trans-Pacific gene flow within reef corals, and identify the importance of introgressive hybridization in *Pocillopora* corals.

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