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ORIGINAL ARTICLE

Getting a grip at the edge: recolonization and introgression in eastern Pacific *Porites* corals

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ABSTRACT

Aim To infer species identity, population isolation, and geographical variation in inter-specific hybridization among corals of the genus *Porites* from the central and eastern tropical Pacific, with a focus on the timing of separation between populations of *P. evermanni* and *P. lobata* divided by the Eastern Pacific Barrier.

Location Hawaii, American Samoa, Panama and the Galapagos Islands of Ecuador.

Methods Maximum likelihood gene trees were obtained for mitochondrial DNA (COI), the internal transcribed spacer (ITS), and 5 single-copy nuclear (scn) gene regions. Allelic networks were used to group multi-locus scn data into species clusters despite some allele sharing. Coalescent analyses (IMa2) of the 5 scn markers were used to estimate the time of population divergence and test for introgression between *P. evermanni* and *P. lobata*.

Results Allelic networks based on scn gene sequences agreed with mtCOI and ITS designations. Divergence times between Hawaiian and eastern Pacific populations are consistent with an early Pleistocene recolonization of the eastern Pacific by *P. evermanni* followed by a more recent arrival of *P. lobata*. The two species were fully isolated in Hawaii/American Samoa populations, but introgression from *P. evermanni* into *P. lobata* was evident in the eastern Pacific.

Main conclusions These results are consistent with a scenario where a bout of introgression with *P. evermanni,* an early-arriving colonizer of the eastern Pacific suited to marginal environmental conditions, facilitated the later colonization of the more sensitive *P. lobata*.

Keywords
coalescence, coral reef, Eastern Pacific Barrier, introgression, marginal population, Pleistocene

INTRODUCTION

Understanding how marginal populations withstand conditions that border their physiological tolerances may both foreshadow how they will deal with climatic change and provide insights into mechanisms that allow them to persist in environmentally challenging settings. Marginal populations occupy unique habitats that may favour the mixing of lineages (Cruzan & Arnold, 1993; Strelkov *et al.*, 2007). The effects of such hybridization in marginal settings could range from providing genetic variation for adaptive change (Seehausen, 2004; Hedrick, 2013) to introgression leading to the extinction of one lineage (Rhymer & Simberloff, 1996).

The waters of the Eastern Tropical Pacific (ETP) present a marginal habitat to reef corals, both geographically and environmentally. The ETP is physically isolated by the Eastern Pacific Barrier, which separates it from the central Pacific by over 5000 km. Darwin (1880) saw this barrier as absolute for shallow water species. Recent genetic work suggests that while some reef inhabitants experience ongoing gene flow across this span (echinoids: Lessios *et al.*, 1998; teleosts:

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Lessios & Robertson, 2006), the corals that build reefs do not (Combosch *et al.*, 2008; Baums *et al.*, 2012).

Just how long these populations have been isolated has been a matter of long-standing controversy among coral biogeographers. McCoy & Heck (1976) viewed ETP corals as relictual populations derived from pan-Tethyan, proto-Caribbean species. Divergence between central and eastern Pacific reef corals would thus date back over 4 Ma. Dana (1975) reasoned that the eastern Pacific corals had to re-establish themselves since the early Pleistocene following a regional extinction of reef builders (*c.* 2 Ma). Paleontological and ecological data reviewed by Cortés (1986) favoured Dana's view. Molecular data would seem suited for resolving the date of divergence, but to date any such efforts have been slowed by the slow rate of nucleotide substitution in coral mtDNA (Hellberg, 2006) and the lack of calibration for single-copy nuclear markers.

Environmental conditions in the ETP are marginal for reef coral growth, with limited habitat, seasonal upwelling that creates temperature fluctuations, high sedimentation rates and low aragonite saturation states - all different from those prevailing over most of the Indo-Pacific (Cortés, 1997; Kleypas *et al.*, 1999). Just a few areas accumulate significant reef growth (Glynn & Wellington, 1983) and major die-off events in the present (Glynn *et al.*, 1983), loose reef cementation (Manzello *et al.*, 2008), and historical gaps in net reef accretion (Toth *et al.*, 2012) are testament to the tenuous hold reef corals have in the ETP. Decreasing aragonite saturation levels associated with ongoing climatic change should reach critical low levels in the ETP sooner than most other reef-growing regions (Hoegh-Guldberg *et al.*, 2007; Rixen *et al.*, 2007), with increasing bioerosion enhancing the negative impact (Barkley *et al.*, 2015).

Living under the marginal conditions of the ETP may promote genetic exchange between species that may facilitate their survival if such hybridization is adaptive (Rieseberg *et al.*, 2003; Choler *et al.*, 2004). Fossil data suggest that populations at the edge of coral distributions show high levels of evolutionary novelty (Budd & Pandolfi, 2010) due to the fusion or mixing of distinct lineages. Hybridization appears to be most likely when differentiated lineages come into first contact, as would have been the case immediately after recolonization of the ETP following a pre-Pleistocene die-off. Gaskin *et al.* (2009), for example, found extensive hybridization among three congeneric tree species that have recently been introduced to Florida, even though the three are genetically distinct in their native Australia. Genetic evidence from one of the ETP's two major reef building genera, *Pocillopora*, suggest introgression among species (Combosch & Vollmer, 2015), although coalescent analyses that can reveal past exchanges and date divergences between populations (Pinho & Hey, 2010) have yet to be applied.

Corals of the genus *Porites* are one of the principal reef builders in the ETP (Glynn & Wellington, 1983). Their genetic diversity (Baums *et al.*, 2012) and species richness (Glynn & Ault, 2000) are lowest in the ETP, steadily increasing to a maximum in the west central Pacific. A phylogenetic analysis based on mitochondrial cytochrome oxidase I and multi-copy ITS sequences (Forsman *et al.*, 2009) found much of the described morphospecies diversity in this genus to be genetically intermingled with the widespread *P. lobata* and suggested that morphologically diagnosed *P. lobata* harboured a cryptic species within eastern Pacific populations. Microsatellite work (Boulay *et al.*, 2014) resolved two ecologically and genetically differentiated species in the eastern tropical Pacific: one the nominal *P. lobata* (Dana 1846) and the other *P. evermanni* (Vaughan 1907), a species earlier thought to be endemic to Hawaii, where it is clearly distinguished from *P. lobata* by its more columnar growth form. The Eastern Pacific Barrier (Fig. 1) isolates populations of *P. lobata* to either side (other than perhaps between the central Pacific and Clipperton Atoll) (Baums *et al.*, 2012).

Here, we extend these findings by dating the isolation of eastern Pacific populations and testing for signs of past interbreeding between *P. lobata* and *P. evermanni*. We: (1) revisit species delineation and phylogeny of common *Porites* species

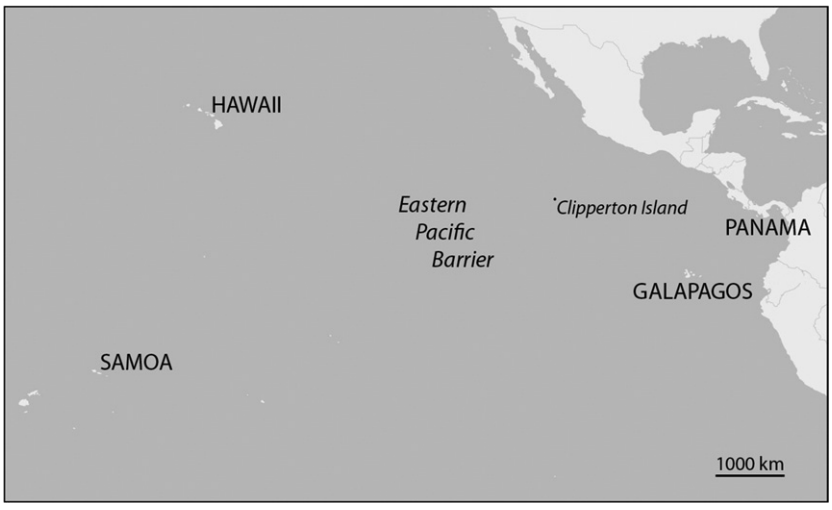

Figure 1 The tropical eastern Pacific Ocean, with the four collecting sites and the Eastern Pacific Barrier that divides them indicated.

from central and eastern Pacific previously explored by Forsman *et al.* (2009) with mtDNA and ITS, (2) employ recent calibrations of single-copy nuclear DNA (scnDNA) data to estimate how long populations of *Porites* in the eastern tropical Pacific have been isolated, and (3) determine whether introgression between *P. lobata* and *P. evermanni* is greater in their marginal geographical range than more central Pacific locales.

MATERIALS AND METHODS

Sampling

We collected genetic data from 47 individuals drawn from 12 nominal Pacific species of *Porites* (Fig. 1, Appendix S1). Thirty-one of these individuals (including representatives of all 12 species) were analysed previously by Forsman *et al.* (2009) using mitochondrial COI and a portion of the nuclear ribosomal internal transcribed spacer (ITS). These 31 individuals were identified using a combination of consultation with regional experts and direct comparison to type specimens. Sample identifiers used here are cross-referenced to those in Table 1 of Forsman *et al.* (2009), although their '*Porites* sp. 2' has since been taxonomically described as *P. randalli* (Forsman & Birkeland, 2009).

We added 11 new samples of *P. lobata* from Hawaii, Galapagos and Panama from the Pacific-wide survey of Baums *et al.* (2012), along with three individuals of *P. evermanni* from Panama. *P. evermanni* and *P. lobata* co-occur in the eastern Pacific as morphologically near-identical colonies; the samples used here have been genetically screened as belonging to either taxon based on multi-locus clustering of 11 microsatellite markers (Boulay *et al.*, 2014), which separated them into two distinct genetic clusters. The two remaining new samples were of *P. lutea* and *P. compressa* (Appendix S1).

Genetic markers

Individuals were sequenced at five single-copy nuclear markers. Three were developed for work within *Porites*: MM32 and MM100 encode proteins while PorAn1 is from an anonymous non-coding region (Prada *et al.*, 2014). ATPsß encodes an intron; primers have been modified to amplify more consistently in *Porites* (Prada *et al.*, 2014). A fifth gene region, MM271, an intron (and short bits of flanking exon) was identified from cDNA from *P. astreoides* (Kenkel *et al.*, 2013), and primers were developed (MM271f2: 5′-CGAGGGATGTCAACAACTTC-3′, MM271R: 5′-AGCATTCCCTCCATTTCCTT-3′).

All individuals were also sequenced for mitochondrial COI and selected individuals for ITS for comparison to gene trees in Forsman *et al.* (2009) using the same primers as those authors. Because ITS is multi-copy and its many copies may vary within individuals, ITS amplicons were cloned and positive clones were sequenced using the amplification primers.

Table 1 Evidence ratio and information theoretic statistics for both regions based on likelihood scores derived from IMa2. The first three letters in each model represent the three population sizes (ancestral and two descendants); identical letters indicate no difference in these parameters. The last two letters represent migration to *P. lobata* and to *P. evermanni,* respectively; zero (0) here indicates one-way migration. *k* = number of parameters in the model, model likelihoods = relative likelihood of the model given the data; wi = model probabilities; evidence ratio = difference in probabilities between the proposed model and the best model. Models with probabilities < 0.5% are not shown.

Model	*k*	Model Likelihoods	wi	Evidence Ratio (best/model)
Eastern Pacific				
ABC DE	5	1	0.695	
ABB DE	4	0.138	0.096	7.26
ABC D0	4	0.138	0.096	7.26
AAA DD	2	0.027	0.019	36.88
ABA DE	4	0.025	0.017	40.23
ABB DD	3	0.023	0.016	42.70
AAC DD	3	0.022	0.015	46.16
AAA DE	3	0.015	0.010	67.35
ABC DD	4	0.013	0.009	79.97
ABA DD	3	0.012	0.008	84.68
AAC DE	4	0.011	0.007	93.14
AAC 00	2	0.007	0.005	139.0
Hawaii/Central Pacific				
ABB 00	2	1	0.404	
ABC 00	3	0.411	0.166	2.43
ABB DD	3	0.368	0.148	2.72
ABC 0D	4	0.151	0.061	6.62
ABC DD	4	0.151	0.061	6.62
ABC D0	4	0.151	0.061	6.62
ABB DE	4	0.135	0.055	7.39
ABC DE	5	0.056	0.022	17.98
ABA 00	2	0.036	0.014	27.97
ABA DD	3	0.013	0.005	76.05

Cloning was also used to resolve both alleles for heterozygous single-copy markers when one of the two alleles was not identical to identified homozygotes.

Alignment and gene genealogies

All marker regions surveyed included indels except for mtCOI and MM32. Sequences were aligned using the phylogeny-aware algorithm of Löytynoja & Goldman (2008) implemented in the program PRANKSTER, which has been shown to outperform other multiple sequence alignment algorithms when indels are plentiful, as expected for loci encoding introns and non-coding regions. The open reading frame of marker MM100 also included indels; these were aligned in amino acid translation and then returned to nucleotide sequences. The number of variable sites and informative sites with and without gaps was determined using DIVEIN (Deng *et al.*, 2010).

Gene genealogies for each marker were constructed to establish relationships among alleles and reveal alleles from single individuals that fell into different clades. Gene

genealogies were constructed using maximum likelihood analyses implemented in RAxML (Stamatakis, 2014) on the CIPRES portal (Miller *et al.*, 2010). Resulting gene trees were rooted with *P. hawaiiensis* which, along with *P. lichen*, consistently fell out as sister to the other Pacific *Porites* sampled here (Forsman *et al.*, 2009). Gene trees were also reconstructed using Bayesian inference as implemented by MrBayes v3.2.1 (Ronquist *et al.*, 2012) using models of evolution as predicted from jModeltest or the closest alternative (Posada, 2008). We used default chain heating parameters and ran 10 MCMC chains for each dataset. We inferred parameters from a run of at least 20 million steps sampled every 20,000 steps after discarding 30% as burnin. All runs exhibit effective sample sizes (ESS) greater than 200, and average standard deviation of split frequencies across runs lower than 0.05.

Network analyses

To quantify the overall genetic similarity of surveyed individuals, including some that may house divergent alleles, we used a network approach. The POFAD algorithm (Joly & Bruneau, 2006) is somewhat analogous to a haplotype network in that it visualizes genetic similarity with few assumptions, but can do so for multiple loci and is thus especially useful for detecting reticulate evolution. The dataset we analyse here is similar to that for which the analysis was initially developed (9 congeneric nominal species, 39 individuals and 3 scn loci). POFAD averages the genetic distance among individuals while accounting for allelic variation within individuals. We used the simple indel coding method (Simmons & Ochoterena, 2000), which counts contiguous gaps as a single change, implemented in SeqState 1.4.1 (Müller, 2006). Genetic p-distances among alleles were then calculated using paup* 4.0 b10 (Swofford, 2003). Gaps were treated as a fifth base because they are a common source of divergence between closely related taxa (Britten *et al.*, 2003) and are phylogenetically informative (Joly & Bruneau, 2006). Genetic distances between individuals across loci were calculated with the program pofad 1.07 (Joly & Bruneau, 2006) and graphed with SplitsTree 4.13.1 (Huson & Bryant, 2006).

Isolation with migration analyses

Migration rates between species and locations were performed using the program IMa2 (Hey, 2010). IM (Won & Hey, 2005) and its software descendants (IMa, IMa2) were designed to distinguish between ongoing genetic exchange and the sorting of ancestral variation following recent isolation; it estimates population divergence time plus five demographic parameters (the sizes of ancestral and both extant populations, and asymmetrical migration rates), or a subset thereof, in the process. The marginal likelihood for each of the model's parameters are estimated by simulation using a Markov Chain Monte Carlo analysis, evaluating values over an array of possible gene genealogies.

Priors for the IMa2 analysis were established during preliminary 24 h runs varying all parameters. After estimating prior values and mixing properties of the chains, we set up three runs per comparison. Each run had a burn in of 5,000,000 followed by at least 10,000,000 steps. Parameter trend lines were visually inspected for proper mixing and convergence. To compare among different runs, we used the scaled mutation rate $2Nm = 4N\mu(M/\mu)/2$. It combines the migration rate ($m = M/\mu$) scaled by the population mutation rate parameter $4N\mu$.

Two datasets were analysed initially: a 'strict' dataset in which only individuals initially identified as being *P. evermanni* or *P. lobata* (either by morphological criteria or, for Panamanian samples, based on microsatellite data from Boulay *et al.*, 2014) and a 'broad' dataset in which all individuals clustering with either *P. evermanni* or *P. lobata* in the POFAD analysis were included (essentially assuming that morphospecies like *P. annae*, whose members fall into both clades depending on geography, have no detectable genetic meaning). Results for both datasets were quantitatively similar, so here we discuss the broad dataset consisting of 14 *P. evermanni* and 34 *P. lobata* alleles (a sampling similar to that employed in one of IM's foundational papers; Won & Hey, 2005) for five nuclear genes. We used the HKY mutation model, having removed indels from our sequences.

We first used IMa2 to test whether a model that included migration between the two 'populations' (either geographical regions or species) was significantly better than one based on strict isolation using model ranking and information theory (Anderson, 2008; Carstens *et al.*, 2009), which uses Akaike information criteria to determine whether the data warrant including all five demographic parameters or if simpler subsets suffice. Once a role for divergence was established (see Results), the time of divergence was estimated for each species using locus-specific rates (mean = 0.138%/Myr) derived for nuclear genes from a fossil-calibrated phylogeny of Caribbean species of *Porites* (Prada *et al.*, 2014). The mean of these values is consistent with those inferred for thousands of nuclear loci from *Acropora* corals (Voolstra *et al.*, 2011). Asymmetrical levels of migration between the eastern Pacific and populations to the west of the Eastern Pacific Barrier were also estimated in terms of 2Nm, the effective number of gene copies per generation. Finally, separate estimates of 2Nm between *P. evermanni* and *P. lobata* were obtained for Hawaiian samples and those from the eastern Pacific (Galapagos and Panama) to test whether the more marginal eastern Pacific populations showed evidence for higher levels of introgression.

RESULTS

Genetic variation, gene trees and congruency

The single mitochondrial marker employed here (COI, 660 bp, no indels) showed low levels of variation, exposing just seven haplotypes among all samples (see Appendix S2).

Some of these haplotypes were specific to recognized taxa (*P. randalli*, *P. lichen* + *P. hawaiiensis*, *P. rus* + *P. monticulosa*) or a geographical locale (2 *P. lobata* from Hawaii). All *P. evermanni* shared a single common haplotype that included Samoan samples of *P. annae* and *P. lutea* as well.

ITS (878 bp aligned), the other marker used by Forsman *et al.* (2009), was far more variable (497 sites) and informative (332 sites including gaps, 108 without). While the gap placement and weighting schemes used between our analysis and Forsman *et al.* differed, the ITS trees (see Appendix S3) agree: *P. lobata* and *P. evermanni* (including samples from Panama) fall into separate clades, *P. rus* and *P. monticulosa* (sometimes placed in their own subgenus, *Synarea*) are monophyletic and closer to the *P. evermanni* clade, and individuals morphologically identified as *P. annae* fall into both the *P. lobata* and *P. evermanni* clades. We extended the previous ITS data by obtaining sequences for multiple gene copies from 20 individuals. Multiple sequences from a single individual were often grouped closely on the tree, forming clades with other intra-individual sequences and perhaps those of one other individual from the same sampled location (e.g. *P. evermanni* PanamaA and *P. lobata* GalapagosB). This was not the rule, however, as some copies from a single individual fell several nodes away from other intra-individual samples (e.g. *P. lobata* GalapagosB_4, HawaiiB), although never into both the *lobata* and *evermanni* clades.

Variation at the five single-copy nuclear markers was closer to that seen for ITS than for mtDNA. Overall, 666 of the 1795 total aligned sites were variable, with 591 of these potentially informative. Variation was highest for marker ATPaseβ. This marker was rich in indels, as was the other intronic sequence, MM271. We obtained genotypes for all individuals for the 5 scn markers with the following exceptions: *P. brighami* HawaiiA (for An1), *P. evermanni* HawaiiC and PanamaC and *P. lutea* SamoaA (for ATPaseβ), *P. brighami* (HawaiiA & B), *P. lichen* (SamoaA & B), *P. lutea* Haw and *P. monticulosa* SamoaA (for MM271).

Gene trees from the single-copy markers (Fig. 2) showed less species monophyly than mtCOI and ITS and more variation in topology, although certainly generalities still hold. The outgroup taxa (*P. hawaiiensis, P. lichen and P. randalli*) are monophyletic here, although where they fall topologically varies among markers. The alleles from the subgenus *Synarea* pair *P. rus* and *P. monticulosa* usually fall together, although they are intermingled with the *P. evermanni* clade for An1 and with *P. duerdeni* alleles for ATPaseβ. One sample of *P. lutea* (SamoaA) tends to fall quite distantly from the other alleles, although some of its alleles fall within small clades consisting mainly of *P. lobata* alleles. Two other samples identified as *P. lutea* fall closer to *P. lobata* or *P. evermanni* alleles. Samoan samples identified as *P. annae* fall with *P. evermanni* alleles, while those from Hawaii group with *P. lobata* samples.

Alleles from *P. lobata* and *P. evermanni* are usually distinct and generally fall into different clades, as for the ITS and mtCOI data; however, exceptions are more numerous. Only for one single-copy marker (MM271) are the two reciprocally monophyletic. For the four other markers, at least one allele is either identical to (ATPaseβ, MM100, MM32) or falls within a clade otherwise consisting of (An1) the other species. Such phylogenetic mixing of alleles could arise due to incomplete lineage sorting or interspecific hybridization. Geographically restricted hybridization should be marked by more mixing within a particular population, a pattern that can be seen using phylogenetic networks based on the genetic distances between individual genotypes.

Genotype networks and the resolution of *P. evermanni* and *P. lobata*

Networks based on four nested datasets (see Appendix S4) consistently revealed four divergent taxa (*P. hawaiiensis, P. lichen, P. randalli, P. lutea* SamoaA), a distinct branch setting apart the *P. rus*/*P. monticulosa* pairing, plus two major clouds of genotypes consisting of individuals allied with either *P. lobata* or *P. evermanni*. Removing the four most divergent taxa, inspection of the networks (Fig. 3) suggests geographical subdivision within the *P. lobata* clades, with genotypes from Hawaii and the eastern Pacific (Galapagos and Panama) separated from each other. *P. evermanni* samples likewise appear subdivided, with those from Hawaii close to *P. annae* samples from Samoa, while those from Panama spread themselves across the gap towards the *P. lobata* samples. This intermediate position for just the *P. evermanni* samples from the single-site eastern Pacific site (Panama) where they were collected in sympatry with *P. lobata* is consistent with introgression at this geographically peripheral locale.

Introgression and divergence times

Levels of introgression between *P. evermanni* and *P. lobata* appear to be higher in the eastern Pacific than in Hawaii and American Samoa. Our model ranking suggest the full model, which includes terms for non-zero asymmetrical migration, best explains genetic variation in the eastern Pacific, with little reason to consider an alternative one (odds ratio > 7, Table 1). For Hawaii/Central Pacific, in contrast, models with no-migration in either way between species are best: the two best models (ABB00, ABC00) have zero migration in both ways and four out of the first six models have migration equal to zero in at least one way (Table 1).

The probability densities for IMa2 estimates of one-way migration further support the isolation of *P. evermanni* and *P. lobata* in Hawaii and the central Pacific (Fig. 4). Gene flow from *P. lobata* into *P. evermanni* in the eastern Pacific had a peak posterior probability near zero (Fig. 4), although with a broader probability distribution than for either of the central Pacific exchange estimates. Effective gene copies from *P. evermanni* into *P. lobata* in the eastern Pacific, however, show a distinct peak (at $2Nm = 0.29$), consistent with one-way introgression.

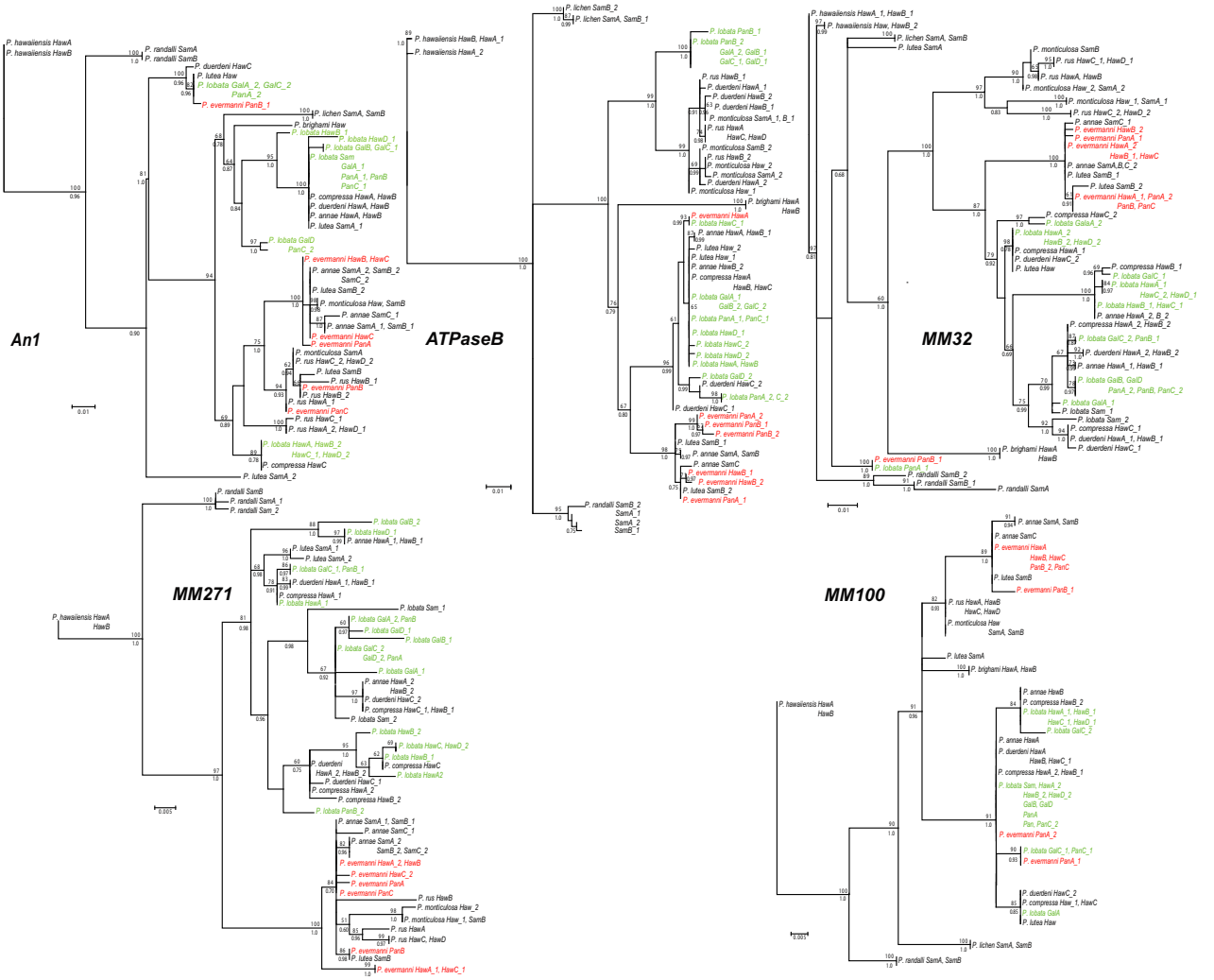

Figure 2 Maximum likelihood gene trees for five single-copy nuclear genes from Pacific species of *Porites*. Labels indicate the nominal morphospecies, following by their location of origin and an individual sample identifier (Appendix S1). Alleles from *P. lobata* are in green, those from *P. evermanni* are in red. _1 and _2 arbitrarily label the different alleles found in heterozygotes. Samples without these suffixes were homozygous. Bootstrap values above 60% are shown above nodes; Bayesian posterior probabilities below.

Estimates of migration across the Eastern Pacific Barrier are roughly similar for the two species (Fig. 5). More gene copies have moved from the eastern Pacific towards the west (peak 1.46) in *P. evermanni* than in the opposite direction. *P. lobata* shows the same pattern, although differences between the peaks are less distinct.

Estimates for the divergence time between eastern Pacific and more central Pacific populations (Fig. 6) had a maximum likelihood of 1.002 Ma for *P. evermanni* and 0.137 Ma for *P. lobata*. Although the probability distributions for these estimates were broad (Table 2), the chances of *P. evermanni* arriving more recently than 500 ka is < 5%. The distribution function for *P. lobata* is well defined between 0 and 500,000 years, with a sharp peak at *c.* 137 ka. Ninety-five per cent highest probability distributions for both species included the beginning of the Pleistocene, *c.* 2 Ma.

DISCUSSION

Previous genetic data show that (1) morphospecies identification is an inconsistent predictor of genetic affinities within *Porites* (Forsman *et al.*, 2009), as is the case for many coral morphospecies (Eytan *et al.*, 2009; Stefani *et al.*, 2011; Prada *et al.*, 2014; Paz-García *et al.*, 2015), and (2) eastern Pacific populations of *P. lobata* are genetically isolated from other regions of the tropical Pacific (Baums *et al.*, 2012). Genetic data from these studies (mtDNA and ITS in Forsman *et al.*, 2009; microsatellites in Boulay *et al.*, 2014) also reveal that colonies morphologically diagnosed as *P. lobata* include a cryptic species (*P. evermanni*). However, given the low divergence of the former and the multi-copy intra-individual variation in the latter, these genetic markers cannot resolve the long-standing biogeographical controversy of the timing of the split of eastern Pacific reef corals either (Lessios, 2012).

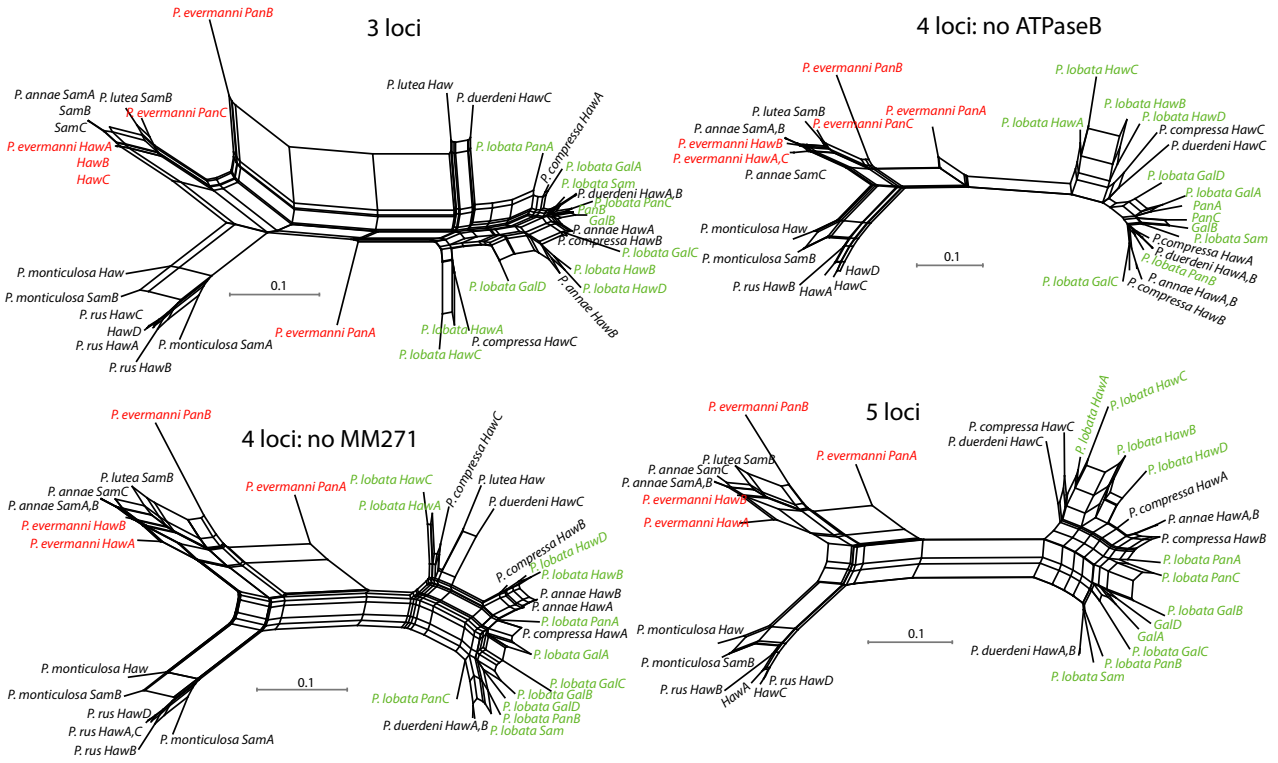

Figure 3 Phylogenetic networks representing relationships between *Porites* samples obtained from the combined POFAD analysis of five nuclear gene sequences. Four divergent outgroup taxa (*P. hawaiiensis, P. lichen, P. randalli, P. lutea* SamA) have been removed to emphasize relationships within and between the *P. evermanni* and *P. lobata* clusters. Analyses for 4 and 5 loci include fewer samples because of incomplete datasets for loci ATPaseβ and MM271.

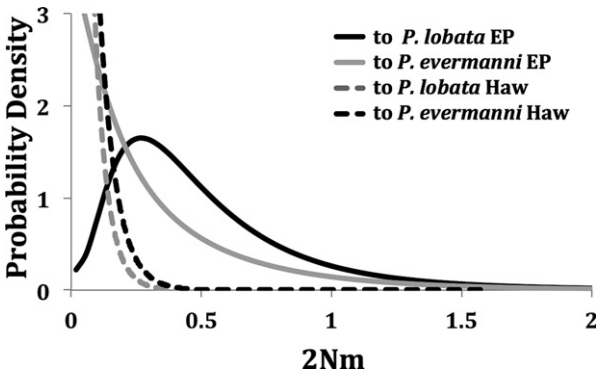

Figure 4 Estimates of genetic exchange between *Porites evermanni* and *P. lobata* in eastern Pacific and Hawaii from IMa2 based on five single-copy nuclear loci.

Here, we used sequences from one mitochondrial and five single-copy nuclear genes to build gene trees from samples of 12 morphospecies of Pacific corals of the genus *Porites*.

Gross inspection of the gene trees (Fig. 2) and genotypic networks (Fig. 3, Appendix S2, S3, S4) that we obtained were broadly consistent with previous phylogenetic analysis (Forsman *et al.*, 2009). The two species we had chosen as outgroups (*P. randalli* and *P. hawaiiensis*) were distinct from each other and distant from all other species, while a clade of the two species placed in the subgenus *Synarea* (*P. rus* and *P. monticulosa*) by Verrill 1864 also formed a group distinct from all others. The remaining samples, representing colonies identified morphologically as belonging to six different species, generally fell into two clusters (Fig. 3, Appendix S4): one aligned with *P. lobata* and the other with *P. evermanni*. Having established that our nuclear gene sequences show the same major divisions as previous work based on mitochondrial and multi-copy ITS sequences (Forsman *et al.*, 2009), we proceeded to analyse colonies from the *P. lobata* and *P. evermanni* clusters to address question concerning the history of reef corals in the eastern Pacific.

Colonization of the Eastern Tropical Pacific dates to the Pleistocene

The ETP has long been seen as offering marginal conditions for reef coral growth, but the extent and duration of its isolation has been more contentious. Three possible time frames for connections to the central Pacific have been posited: residual relatedness dating back to the early Pliocene (McCoy & Heck, 1976), recolonization of the ETP during the Pleistocene (Dana, 1975; Cortés, 1986), and potentially ongoing migration (Richmond, 1990). For *Porites lobata*, the latter possibility was ruled out by Baums *et al.* (2012) based on Bayesian clustering of microsatellite data, which showed eastern Pacific (except Clipperton Island) populations to be

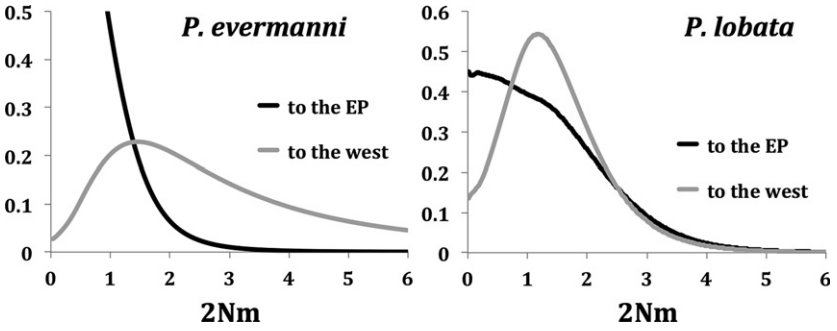

Figure 5 Migration estimates between populations in the eastern Pacific and further west (mainly Hawaii) for (left) *Porites evermanni* and (right) *P. lobata* from IMa2 based on five single-copy nuclear loci.

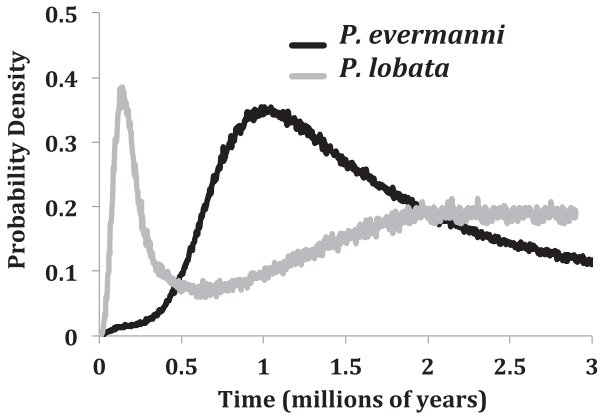

Figure 6 Divergence time estimates for eastern Pacific populations of *Porites evermanni* and *P. lobata* estimated from IMa2 based on five single-copy nuclear loci.

Table 2 Divergence time (years) estimates between eastern and central Pacific populations

	P. evermanni	*P. lobata*
High point probability distribution	1,001,824	137,013
95% Lowest probability distribution	511,695	53,363
95% Highest probability distribution	3,919,072	2,883,035

isolated from those to the west. This study can now eliminate the first possibility for *P. lobata* and *P. evermanni* as well. While the probability distributions for estimates of divergence time were large for both species, they categorically exclude any divergence pre-dating 4 Ma, with the highest probabilities falling firmly within the Pleistocene.

While some species do show patterns of genetic variation consistent with ongoing gene flow across the Eastern Pacific Barrier, the range of initial isolation (=recolonization) times we found are broadly consistent with those species showing such isolation (Lessios & Robertson, 2006). Moreover, the direction of any inferred post-isolation migration is also consistent with other studies: for both two species of reef fish (Lessios & Robertson, 2006) and a cone snail (Duda & Lessios, 2009), migration went westward from the eastern Pacific, as seen here for *P. evermanni* (Fig. 5).

Unexpectedly, the IM analysis (Fig. 4, Table 2) further suggests that *P. evermanni* and *P. lobata* did not arrive in the eastern Pacific simultaneously. *P. evermanni* appears to have arrived substantially earlier than its congener. While both species occur in the eastern Pacific, *P. evermanni* is more common on reefs growing immediately along the continental shoreline (Boulay *et al.*, 2014) and more generally in environments with greater upwelling and cooler, more productive waters (including Isla Española in the Galapagos). *P. lobata*, in contrast, dominates the warmer, clearer waters surrounding most islands.

The different inferred divergence times might also reflect differences in the persistence of each species in the eastern Pacific. Reef cores from Panama (Toth *et al.*, 2012) revealed a *c.* 2 kyr gap beginning *c.* 4 ka with no evidence of growing *Pocillopora damicornis*, the dominant reef builder in that area. A similar episode occurred in Costa Rica (Cortés *et al.*, 1994) on reefs that also included corals that were morphologically assigned to *Porites lobata*. Both of these gaps in reef accretion have been attributed to deteriorating environmental conditions.

Given the cooler temperatures (Dana, 1975) and fluctuating sea levels (Cortés, 1986) that prevailed in the Pleistocene, the difference in estimated arrival times for the two species is consistent with differences in their ecological tolerances: the species (*P. evermanni)* whose present-day distribution suggests that it is better suited to marginal conditions either arrived first or was better able to persist during episodes unfavourable to reef growth. But did the establishment of *P. evermanni* facilitate the arrival of its more narrowly tropical kin?

Introgression in the Eastern Tropical Pacific

Hybridization can introduce adaptive variation into populations (Arnold, 1997; Rieseberg *et al.*, 2003). That hybridization may occur in marginal marine populations is supported by two general observations. First, peripheral populations commonly harbour less genetic variation than more central ones (Hellberg, 1994; Johannesson & Andre, 2006; Nunes *et al.*, 2009), although this has been based on observations of presumably neutral genetic markers rather than at demonstrably adaptive loci. Second, hybridization occurs frequently in disturbed or stressful habitats and when one species is more common than the other (Hubbs, 1955; Fogarty *et al.*, 2012).

Our results suggest that *P. evermanni* and *P. lobata* have exchanged genes in the eastern Pacific, but not in Hawaiian/central Pacific populations. Best-fit models for isolation and migration include a term for inter-specific exchange in eastern Pacific populations, but not central Pacific ones (Table 1). Peaks for the probability distributions for estimates of exchange in Hawaiian/CP populations approach zero (Fig. 5), as does that (albeit broader) for movement from *P. lobata* into *P. evermanni* in the eastern Pacific. A deeper sampling of complete mitochondrial genomes and RAD markers shows no evidence of hybridization between *P. lobata* and *P. evermanni* in Hawaii (ZHF and RJ Toonen, unpub. data).

Given the difficulties in matching morphospecies to genetic entities, other species, including some not sampled here, may have intermixed with *P. evermanni* and *P. lobata*. Opportunities for such broader introgression should be more plentiful in the central Pacific, where many more free-spawning *Porites* species co-occur, than in the eastern Pacific, where just the two studied here are found. The IM analyses showing less introgression in Hawaii and Samoa than the ETP (Fig. 3), however, opposite the prediction based on opportunities for interspecific mixing. A third free-spawning *Porites* (*P. rus*) has been reported from Costa Rica (although not since the 1982–83 El Niño; Cortés & Guzman, 1998), and two colonies of *P. evermanni* share alleles with *P. rus* at one marker locus (An1; Fig. 2). Those two colonies came from Panama, however, not from Hawaii where *P. evermanni* co-occurs with *P. rus*.

Hybridization is now recognized as being more common in the sea than previously thought (Gardner, 1997) and seems to be especially prevalent in isolated populations (Yaakub *et al.*, 2006), including those at range margins (Strelkov *et al.*, 2007; Marino *et al.*, 2013). Among corals, reticulate evolution has been suggested (Veron, 1995) and hybridization has been reported from the hyper-diverse genus *Acropora* (van Oppen *et al.*, 2001; Ladner & Palumbi, 2012). Outside of *Acropora*, however, most reports of hybridization come from marginal populations. For example, the three species of *Montastraea* (*Orbicella*) are more morphologically distinct and genetically isolated in Panama than in the more marginal Bahamas (Fukami *et al.*, 2004). In the eastern Pacific, Combosch *et al.* (2008) inferred hybridization between *Pocillopora damicornis* and a couple of close relatives using ITS sequences, a pattern consistent with later surveys of pooled RAD-Seq data (Combosch & Vollmer, 2015). Pinzón *et al.* (2013), using ITS, microsatellites and a mitochondrial marker on a broader sampling of *Pocillopora*, saw more sharply defined species but still reported the greatest degree of introgression to be in the eastern Pacific (they saw a hint of mixing in Hawaii as well).

The gene exchange seen in the eastern Pacific appears to move in just one direction, from *P. evermanni* into *P. lobata* (Fig. 5). The relative densities of the two species during their initial contact at recolonization may explain this pattern. Low abundance of potential mates can promote heterospecific pairing (Maruska & Peyton, 2007; Montanari *et al.*, 2014) and numerical imbalances have long been associated with one-way introgression in marine fish (Hubbs, 1955; Yaakub *et al.*, 2006). If *P. evermanni* were already well-established when *P. lobata* arrived, then it would have been outnumbered at first contact, an ecological pattern consistent with the inferred direction of genetic exchange. Numerical imbalances aside, hybridization appears to be especially likely immediately after recolonization (Lancaster *et al.*, 2006) and theory predicts that introgression should proceed from the locally established population into the invading one (Currat *et al.*, 2008), as seen here.

While we found evidence for hybridization between *P. evermanni* and *P. lobata* here, no strong signal for introgression or F1 hybrids emerged from microsatellite data (Baums *et al.*, 2012; Boulay *et al.*, 2014) for > 650 ETP *Porites* colonies. This disagreement is consistent with a scenario where most introgression occurred soon after initial contact between the species in the eastern Pacific, but also with differences in the data and analyses employed. Processes that sort to equilibrium values rapidly will be sensitive to recent demographic change, but at the expense of destroying information on older conditions. Nuclear sequence markers, for example, can reveal older historical patterns of population growth than mitochondrial ones due to differences in the effective population sizes of the two genomes (Eytan & Hellberg, 2010). Similarly, the coalescent approach underlying IM will reflect migration averaged over long periods of time (Marko & Hart, 2012), whereas STRUCTURE, resting in part on linkage disequilibria among loci, will be sensitive to population mixing over far shorter time scales (Rosenberg *et al.*, 2001).

CONCLUSIONS

The genetic data presented here are consistent with the view that reef corals resettled in the eastern Pacific following an early Pleistocene extinction (Dana, 1975; Cortés, 1986). More surprising are the dynamics of that recolonization. First, two species of the genus *Porites* did not establish simultaneously, but rather the species whose present-day distribution suggests greater tolerance to marginal conditions (*P. evermanni*) did so first. Second, establishment of the second, more environmentally sensitive species (*P. lobata*) was accompanied by introgression from its resident congener. Future, more loci-rich work should test whether particular genes were transferred from *P. evermanni* to *P. lobata* and facilitated survival of the latter in eastern Pacific (as did restricted transfers to modern humans from Neanderthals, Sankararaman *et al.*, 2014, and among *Heliconius* butterflies, Pardo-Diaz *et al.*, 2012).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 *Porites* samples analysed in this study.

Appendix S2 Haplotype network for mt COI from 11 Pacific morphospecies of *Porites*.

Appendix S3 Maximum likelihood gene tree for ITS sequences from Pacific species of *Porites*.

Appendix S4 Phylogenetic network representing relationships between all *Porites* samples obtained from the combined POFAD analysis of five nuclear gene sequences.

BIOSKETCHES

Michael E. Hellberg's research interests focus on speciation formation in the sea and the evolution of genes facilitating population adaptation and species divergence.

Carlos Prada is interested in studying genetic variation in marine invertebrates along environmental gradients.

Author contributions: M.E.H. and I.B.B. developed the project; Z.H.F. and I.B.B. collected the samples; M.E.H., C.P. and M.H.T. collected the genetic data, with the latter making the key observation suggesting introgression in the ETP; M.E.H. and C. P. analysed the data; and all authors contributed to the writing.

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