Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier

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The ‘impassable’ Eastern Pacific Barrier (EPB), ca 5000 km of deep water separating the eastern from the central Pacific, is the World’s widest marine biogeographic barrier. Sequencing of mitochondrial DNA in 20 reef fish morphospecies encountered on both sides of the barrier revealed cryptic speciation in two. Among the other 18 species only two showed significant differentiation (as revealed by haplotype networks and $F_{ST}$ statistics) between the eastern and the central Pacific. Coalescence analyses indicated that genetic similarity in the 18 truly transpacific species resulted from different combinations of ages of most recent invasion and of levels of recurrent gene flow, with estimated times of initial separation ranging from approximately 30 000 to 1 Myr (ago). There is no suggestion of simultaneous interruptions of gene flow among the species. Migration across the EPB was previously thought to be exclusively eastward, but our evidence showed two invasions from east to west and eight cases in which subsequent gene flow possibly proceeded in the same direction. Thus, the EPB is sporadically permeable to propagules originating on either side.

Keywords: dispersal; marine biogeography; Pacific Ocean; mtDNA; reef fishes; isolation–migration algorithm

1. INTRODUCTION

In The origin of species Darwin (1872) remarked on the ‘impassable’ barriers to the dispersal of shallow water marine species. In addition to the continents, he listed one marine barrier, the 4000–7000 km expanse of deep water without islands that separates the eastern Pacific (EP) from the central Pacific (CP). The effectiveness of this ‘Eastern Pacific Barrier’ (EPB; Ekman 1953) was subsequently documented through the enumeration of shallow water species that were not shared by the two oceanic regions (Ekman 1953; Mayr 1954; Briggs 1974; Vermeij 1978, 1987). Molecular phylogenies of several marine taxa are characterized by the deepest splits between extant species across the EPB (Lessios et al. 1999, 2001; McCartney et al. 2000; Colborn et al. 2001; Collin 2003). The EPB has been in place during the past 65 Myr (Grigg & Hey 1992), but circumglobal gene flow between populations at its two edges was potentially possible until the closure of the Tethyan Sea 11–17 Myr ago (Adams 1981), or perhaps as recently as the closure of the Panama Isthmus, 3.1 Myr ago (Coates & Obando 1996). There is, however, a small number of species, which on the basis of their morphology, are believed to occur on both sides of the EPB. These ‘transpacific’ species have engaged the attention of biogeographers, because of their implications regarding the importance of extrinsic barriers for the process of speciation in the sea (Palumbi & Lessios 2005) and the relative importance of vicariance versus dispersal in establishing biogeographic patterns (Dana 1975; McCoy & Heck 1976, 1983; Heck & McCoy 1978; Glynn & Wellington 1983; Leis 1984; Rowe 1985; Rosenblatt & Waples 1986; Robertson et al. 2004). In the view of most authors, transpacific species exist because of transport of larvae across the barrier (Dana 1975; Glynn & Wellington 1983; Leis 1984; Rosenblatt & Waples 1986; Vermeij 1987, 1991; Grigg & Hey 1992; Robertson et al. 2004), while a minority regards them as long-separated remnants of previously continuous distributions that have not evolved morphologic differences and are thus mistakenly assigned to the same species (McCoy & Heck 1976, 1983; Heck & McCoy 1978; Rowe 1985). We do not yet know whether putative transpacific species are: (i) long isolated relics of an ancient separation event, which have not evolved morphologic differences, (ii) the products of recent invasion across the barrier or (iii) populations that have been resident on both sides of the barrier for varying lengths of time, connected by recurrent gene flow. To distinguish between these possibilities we present evidence from mitochondrial DNA (mtDNA) sequences of 20 transpacific fish species, analysed with traditional population genetics approaches and with a recently developed coalescence method. We also assess the direction of migration through the barrier, which until recently was thought to be almost entirely from west to east (Ekman 1953; Briggs 1974; Dana 1975; Vermeij 1978, 1991; Glynn & Wellington 1983; Rosenblatt & Waples 1986).

2. MATERIAL AND METHODS

Transpacific species are particularly prominent among shore fishes (species with relatively sedentary adult phases that live...
along the shoreline), in which 95 species (roughly 18% of all tropical EP reef fishes) are thought to have resident, locally breeding, populations on both sides of the EPB (Robertson et al. 2004). We studied 20 such tropical species (table 1). We collected 5–25 individuals (mean sample size 15.65) per species in the EP and 6–25 (mean sample size 16.15) in the CP. All but three species were collected from more than one locality in each region (see figure 1 in electronic supplementary material). Genomic DNA was extracted from each individual, and an 842 bp fragment of the ATPase8 and ATPase6 gene regions of mitochondrial DNA was amplified and sequenced. Details of the methods are provided in the electronic supplementary material.

Program TCS v. 1.18 (Clement et al. 2000) was used for the construction of statistical parsimony (Templeton et al. 1992) networks with the confidence of connection limits set at 95%. Program ARLEQUIN v. 2.0 (Schneider et al. 2000) was used for calculations of analysis of molecular variance (Excoffier et al. 1992) and FST statistics. To generate the null distributions for assessing the significance of the FST values, 10,000 permutations of haplotypes between populations were used.

To distinguish recent population splitting from recurrent gene flow and to determine direction of gene flow, we employed Bayesian estimation, based on coalescence, according to the procedure developed by Nielsen & Wakeley (2001) and by Hay & Nielsen (2004). The method uses gene genealogies to estimate effective population size of ancestral and daughter populations, the time since their initial separation (i.e. the time since variance or the last massive invasion) and the migration rate in each direction. We used Program IM (Hay & Nielsen 2004) to estimate the times of separation t (number of generations, scaled by mutation rate, μ) between EP and CP populations, θ = 2Nem (where Ne is the effective population size of the ancestral and the two daughter populations, each estimated separately) and the scaled migration rate m = m/μ (where m is the proportion of migrants arriving into a population per generation) in each direction. As coalescence estimates assume that each population is effectively panmictic, we pooled samples of the same species from different localities within a region only if FST statistics (table 1 in electronic supplementary material) under the island model indicated that they exchanged more than one female per generation (if FST values were less than 0.33 or if they were not significant). Populations with more restricted intra-regional gene flow were compared separately to those on the other side of the barrier. Analyses were implemented assuming that base substitution followed the Hasegawa et al. (1985) model. Details of the IM runs and of the methods used to estimate whether differences between parameter estimates were statistically significant are presented in the electronic supplementary material. As IM makes a number of simplifying assumptions regarding population history and as our data consist of a single locus, we regard the results of this procedure as hypotheses to be further tested with additional data.

From the results of IM, twice the number of females moving through the barrier per generation (M) was calculated as M = 2Neμ = θ/μ/2 (where m is the female migration rate). The time since separation was converted from number of generations scaled by mutation rate to number of years using a mutation rate estimate of the sequenced fragment from six other fish genera, with species-pairs, the members of which were likely to have been separated by the closure of the Isthmus of Panama (see table 2 in electronic supplementary material), 3.1 Myr ago (Coates & Obando 1996). These transisthmian genera were selected on the basis of their similar amounts of divergence in cytochrome oxidase I, as determined by Bermingham et al. (1997). ATPase8 and -6 were amplified and sequenced from a minimum of two individuals per species on each side of the isthmus, with the methods described above, then the divergence in six species pairs was averaged to obtain a rate of 1.3 × 10⁻⁸ substitutions per site per year or a substitution rate per branch for the entire fragment of 5.49 × 10⁻⁶ substitutions per year (see electronic supplementary material).

3. RESULTS AND DISCUSSION

(a) Relationships between eastern and central Pacific haplotypes

Statistical parsimony networks of haplotypes showed that in 18 out of the 20 transpacific species, haplotypes of EP and CP populations were either shared, or separated by only a few mutations (figure 1). Thus, in 18 cases genetic evidence supports the current taxonomy by indicating that populations on the two sides of the EPB have recently exchanged genes and thus belong to the same species. There are, however, two exceptions: (i) the pipefish Doryphramphus excisus shows an extreme degree of divergence not only between individuals from the two sides of the EPB, but also from each locality in the CP. Haplotypes from Midway, Marquesas, Kiritimati and the EP (see figure 1 in electronic supplementary material for locations) could not be joined at the 95% confidence limit and each formed its own network (with one haplotype in Kiritimati being on a separate network). The five clades identified by statistical parsimony are very different from each other (average Kimura two-parameter distance K2 = 8.01%), with the EP clade being only slightly more divergent from the CP clades (mean K2 = 8.33%) than the CP clades are from each other (mean K2 = 7.79%). These large genetic distances are consistent with the limited dispersal potential and high levels of local endemism typically found among Indo-Pacific synangathid fishes (Dawson 1985). (ii) In the hawkfish Cirrhilabrus oxycephalus the haplotypes are joined in a single network. However, EP and CP haplotypes form different clades, separated by six mutations from each other (K2 = 1.22%). Thus, eastern and central Pacific mtDNA sequences of both D. excisus and of C. oxycephalus have sorted out into separate evolutionary units, suggesting that representatives from the two regions are relics of an old separation with no subsequent gene flow and are thus best recognized as separate species or subspecies.

A third case of genetic isolation across the EPB, which partly reflects accepted taxonomy, is indicated by the parsimony networks of the surgeon fish Acanthurus triostegus (figure 1). This species contains three morphologically (Randall 1956) and electrophoretically (Planes & Fauvelot 2002) distinguished subspecies, Acanthurus triostegus sandeensis from Hawaii and Johnston Atoll, Acanthurus triostegus marquesensis from the Marquesas and Acanthurus triostegus triostegus from the rest of the Indo-Pacific. There are no fixed mtDNA differences between the Marquesas, Kiritimati and EP populations, but A. triostegus sandeensis from Hawaii and Johnston Atoll
Table 1. Analysis of molecular variance (AMOVA; Excoffier et al. 1992), comparing variation within and between the eastern (EP) and the central Pacific (CP) and nucleotide diversity within each region. (Values in parentheses in Acanthurus triostegus are from calculations that include populations of A. triostegus sandvicensis from Hawaii and Johnston Atoll. Only one population per region was sampled in Scarus ghobban and in Sectator ocyurus. Nucleotide diversity is based on the Kimura-2 parameter model.)

<table>
<thead>
<tr>
<th>species</th>
<th>percentage variation within populations</th>
<th>percentage variation between populations</th>
<th>percentage variation between regions</th>
<th>(\Phi_{CT}) between regions</th>
<th>(p)</th>
<th>nucleotide diversity EP</th>
<th>nucleotide diversity CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthurus nigricans</td>
<td>48.98</td>
<td>86.14</td>
<td>-35.12</td>
<td>-0.351</td>
<td>0.206</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Acanthurus triostegus</td>
<td>62.32 (18.22)</td>
<td>2.13 (50.99)</td>
<td>35.55 (30.79)</td>
<td>0.365 (0.308)</td>
<td>0.067 (0.018)</td>
<td>0.002</td>
<td>0.018 (0.020)</td>
</tr>
<tr>
<td>Arthron meleagris</td>
<td>63.12</td>
<td>2.33</td>
<td>34.56</td>
<td>0.346</td>
<td>0.066</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Calotomus carolinus</td>
<td>99.60</td>
<td>-0.40</td>
<td>0.80</td>
<td>0.008</td>
<td>0.267</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Cantherinus dumerilii</td>
<td>95.11</td>
<td>22.85</td>
<td>-17.97</td>
<td>-0.180</td>
<td>0.314</td>
<td>0.000</td>
<td>0.001</td>
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<tr>
<td>Cirrhithys oxycephalus</td>
<td>29.35</td>
<td>34.21</td>
<td>36.44</td>
<td>0.364</td>
<td>0.000</td>
<td>0.007</td>
<td>0.004</td>
</tr>
<tr>
<td>Ctenochaetus marginatus</td>
<td>95.29</td>
<td>2.53</td>
<td>2.17</td>
<td>0.022</td>
<td>0.341</td>
<td>0.002</td>
<td>0.002</td>
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<tr>
<td>Diodon holocanthus</td>
<td>55.94</td>
<td>1.69</td>
<td>42.37</td>
<td>0.424</td>
<td>0.202</td>
<td>0.003</td>
<td>0.002</td>
</tr>
<tr>
<td>Dorhyphamus excisus</td>
<td>7.40</td>
<td>43.65</td>
<td>48.49</td>
<td>0.489</td>
<td>0.034</td>
<td>0.010</td>
<td>0.059</td>
</tr>
<tr>
<td>Forcipiger flavissimus</td>
<td>103.82</td>
<td>1.69</td>
<td>-5.52</td>
<td>-0.055</td>
<td>0.859</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>Heteropriacanthus cruentatus</td>
<td>74.60</td>
<td>1.08</td>
<td>24.32</td>
<td>0.243</td>
<td>0.009</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Mullodichthys vanicolensis</td>
<td>86.11</td>
<td>13.84</td>
<td>0.05</td>
<td>0.000</td>
<td>0.479</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>Myripristis berndti</td>
<td>84.27</td>
<td>13.21</td>
<td>2.52</td>
<td>0.025</td>
<td>0.198</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>Novaculichthys taeniourus</td>
<td>83.69</td>
<td>4.34</td>
<td>11.97</td>
<td>0.120</td>
<td>0.094</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Ostracion meleagris</td>
<td>83.53</td>
<td>5.98</td>
<td>10.49</td>
<td>0.105</td>
<td>0.081</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>Scarus ghobban</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.169</td>
<td>0.055</td>
<td>0.003</td>
<td>0.002</td>
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<tr>
<td>Scarus rubrovulaceus</td>
<td>87.46</td>
<td>23.37</td>
<td>-10.83</td>
<td>-0.108</td>
<td>0.824</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Sectator ocyurus</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.120</td>
<td>0.027</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>Stethojulis bandanensis</td>
<td>71.42</td>
<td>47.30</td>
<td>-18.72</td>
<td>-0.187</td>
<td>0.256</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Zanclus cornutus</td>
<td>103.62</td>
<td>-11.71</td>
<td>8.10</td>
<td>0.081</td>
<td>0.095</td>
<td>0.002</td>
<td>0.003</td>
</tr>
</tbody>
</table>
is genetically so distinct from all other populations ($K_z = 3.26\%$), that it cannot be joined in the same network at the 95% confidence level. Thus, *A. triostegus* at Hawaii and Johnston Atoll are isolated not only from the EP, but also from the rest of the CP.

**4) Genetic divergence**

Analysis of molecular variance (AMOVA), which treated populations from each side of the barrier as coming from a separate region, showed that, except for *D. exisitus*, *C. oxycephalus* and *A. triostegus sandvicensis*, all species showed variation between regions that was smaller than variation within populations (table 1). Among the 18 truly transpacific species, inter-regional differentiation ($\Phi_{CT}$ values) was significant only in the glasseye *Heteropriacanthus cruentatus* and the sea-chub *Sectator ocyurus*. Thus, genetic differentiation across the EPB is generally no larger than differentiation within a single locality. Isozyme comparisons (Rosenblatt & Waples 1986) had also found that genetic differences between Hawaiian and EP
Table 2. Times since separation between eastern Pacific (EP) and central Pacific (CP) populations of each species and numbers of females crossing the barrier per generation after separation in each direction ($2N_{ef}m_t$). (Estimates for each parameter were those with maximum Bayesian posterior probabilities, based on the coalescent (Nielsen & Wakeley 2001; Hay & Nielsen 2004). Samples from the same side of the East Pacific Barrier were separately compared to those of the other side when $F_{ST}$ comparisons indicated intraregional restrictions in gene flow. *: significantly different from 0 at $p > 0.05$. Bold: value of $m = 8 m_t$ (where $m$ is the migration rate and $m_t$ is the mutation rate) larger than $m$ in the opposite direction at $p > 0.05$ (see table 3 in electronic supplementary material). Italics: value of $\theta = 2\mu N_e$ (where $N_e$ is the effective population size) in a region significantly larger then $\theta$ in the other region. NC: estimates not possible, because posterior probability curves were flat. Inequalities: posterior probability densities of either $m$ or $\theta$ rise to a plateau, so that all estimates larger than the shown value have the same approximate likelihood. Migration values equal to zero were given as priors, because in these comparisons no haplotypes are shared between regions.)

<table>
<thead>
<tr>
<th>species</th>
<th>comparison</th>
<th>time (thousands of years)</th>
<th>$2N_{ef}m_t$ EP to CP</th>
<th>$2N_{ef}m_t$ CP to EP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthurus nigricans</td>
<td>all CP versus all EP</td>
<td>78.9*</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Acanthurus triostegus</td>
<td>Marquesas + Kiritimati versus all EP</td>
<td>585.1*</td>
<td>17.048</td>
<td>0.030</td>
</tr>
<tr>
<td>Acanthurus triostegus</td>
<td>Hawaii + Johnston versus all EP</td>
<td>2127.9*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arthron meleagris</td>
<td>all CP versus all EP</td>
<td>68.3*</td>
<td>34.907</td>
<td>0.005</td>
</tr>
<tr>
<td>Calotomus carolinus</td>
<td>all CP versus all EP</td>
<td>141.3*</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Cantherinus dumerilii</td>
<td>all CP versus all EP</td>
<td>61.1*</td>
<td>&gt;12.563</td>
<td>0.001</td>
</tr>
<tr>
<td>Cirrhitichthys oxycephalus</td>
<td>mean of 2 pairwise comparisons</td>
<td>746.8*</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Ctenochaetus marginatus</td>
<td>all CP versus all EP</td>
<td>117.8*</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>Diodon holocanus</td>
<td>all CP versus all EP</td>
<td>136.7*</td>
<td>0.011</td>
<td>0.975</td>
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<tr>
<td>Doryrhamphus excisus</td>
<td>mean of 13 pairwise comparisons</td>
<td>5322.8*</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Forcipiger flavissimus</td>
<td>all CP versus all EP</td>
<td>46.5*</td>
<td>70.893</td>
<td>0.011</td>
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<tr>
<td>Heteropriacanthus cruentatus</td>
<td>all CP versus all EP</td>
<td>207.3*</td>
<td>0.179</td>
<td>0.058</td>
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<tr>
<td>Mullolichthys vanicolensis</td>
<td>all CP versus all EP</td>
<td>194.1*</td>
<td>&gt;1420.225</td>
<td>0.025</td>
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<tr>
<td>Myrgrisits berndti</td>
<td>all CP versus all EP</td>
<td>1087.6*</td>
<td>493.992</td>
<td>0.019</td>
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<tr>
<td>Novaculichthys taeniourus</td>
<td>all CP versus all EP</td>
<td>113.4*</td>
<td>0.024</td>
<td>0.024</td>
</tr>
<tr>
<td>Ostracion meleagris</td>
<td>Marquesas + Kiritimati versus all EP</td>
<td>NC</td>
<td>&gt;37.599</td>
<td>0.047</td>
</tr>
<tr>
<td>Ostracion meleagris</td>
<td>Hawaii versus all EP</td>
<td>45.6</td>
<td>0.023</td>
<td>3.006</td>
</tr>
<tr>
<td>Scarus ghobban</td>
<td>all CP versus all EP</td>
<td>308.9*</td>
<td>0.004</td>
<td>0.093</td>
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<tr>
<td>Scarus rubroviolaceus</td>
<td>Marquesas + Hawaii versus all EP</td>
<td>30.1*</td>
<td>0.017</td>
<td>0.014</td>
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<tr>
<td>Scarus rubroviolaceus</td>
<td>Kiritimati versus all EP</td>
<td>59.9*</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Sectator ocyporus</td>
<td>all CP versus all EP</td>
<td>57.4*</td>
<td>NC</td>
<td>0.059</td>
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<tr>
<td>Stethojulis bandanensis</td>
<td>all CP versus Is. Coco</td>
<td>115.7*</td>
<td>0.135</td>
<td>0.036</td>
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<tr>
<td>Stethojulis bandanensis</td>
<td>all CP versus Panama</td>
<td>154.9*</td>
<td>&gt;36.856</td>
<td>0.003</td>
</tr>
<tr>
<td>Stethojulis bandanensis</td>
<td>all CP versus Clipperton</td>
<td>NC</td>
<td>NC</td>
<td>0.024</td>
</tr>
<tr>
<td>Zanclus cornutus</td>
<td>all CP versus all EP</td>
<td>185.4*</td>
<td>120.791</td>
<td>0.023</td>
</tr>
</tbody>
</table>

populations of 11 transpacific shore fishes (including three considered here) were small.

(c) Recent separation versus recurrent gene flow
AMOVA cannot distinguish whether genetic similarity is due to recent separation or to recurrent gene flow after initial separation. Nor can it determine the direction of gene flow. To answer these questions we turned to the coalescence procedure of Nielsen & Wakeley (2001) and Hay & Nielsen (2004). Bayesian estimation of gene flow and time since separation indicated that the close genetic similarity between EP and CP populations was due to different processes in different species (table 2). Estimated time of initial separation in the 18 truly transpacific species ranged from roughly 30,000 to 1 Myr (ago), with no suggestion of simultaneous interruptions of gene flow among the species. Recent (less than 2 x 10^9 years ago) separations of EP and CP populations were estimated to have occurred at various times in 14 species (the surgeon fishes Acanthurus nigricans and Ctenochaetus marginatus, the parrotfishes Calotomus carolinus and Scarus rubroviolaceus, the filefish Cantherinus dumerilii, the porcupinefish Diodon holocanthus, the butterflyfish Forcipiger flavissimus, the goatfish Mullolichthys vanicolensis, the wrasses Novaculichthys taeniourus and Stethojulis bandanensis, the boxfish Ostracion meleagris, the sea-chub S. ocyporus and the moonfish Zanclus cornutus). Older (2 x 10^3 – 10^9 years ago) separation with high subsequent gene flow was seen in two species (the surgeonfish A. triostegus triostegus and the squirrelfish Myrgrisits berndti). In two species (the glasseye H. cruentatus and the parrotfish Scarus ghobban) the estimated time of separation is relatively old and the rate of gene flow is restricted. These are the species with highest divergence between CP and EP populations, as indicated by $\Phi_{CT}$ values (table 1). Thus, the EPB appears to have impeded genetic exchange to different degrees starting at a different time for each species, a pattern consistent with dispersal but not with vicariance.
(d) **Direction of initial invasion**

The traditional approach for inferring the direction of the original invasion across the EPB (Ekman 1953; Briggs 1974; Dana 1975; Glynn & Wellington 1983; Leis 1984; Rosenblatt & Waples 1986; Vermeij 1987, 1991; Robertson *et al.* 2004) has been to consider the region in which populations of a transspacific species are most abundant and widespread as the source and the area in which populations have a more tenuous hold as the target. By this criterion, invasion through the EPB has for a long time been considered as having occurred overwhelmingly in one direction, from west to east. However, a recent detailed consideration of species distributions in each region (Robertson *et al.* 2004) used this criterion to provide evidence that 16 out of 80 transspacific fish species, including *S. oxyurus*, have invaded the CP from the EP. mtDNA sequences can add two other lines of evidence to aid this inference: (i) the location of the ancestral haplotype in the sample and (ii) the comparative levels of genetic diversity in different regions.

In intraspecific phylogenies, the haplotype that is most frequent and has the most network connections to others (the one with the highest ‘outgroup weight’) is generally deemed the oldest (Castelloc & Templeton 1994; Posada & Crandall 2001). When the oldest haplotype is shared between regions, as it is in 13 species we examined (figure 1), the direction of the original invasion is unclear. In *A. triostegus triostegus* and in *H. cruentatus*, however, the ancestral haplotype is in the CP (figure 1). For these two species, (assuming the criterion of outgroup weight roots the network correctly) the genetic data confirm the traditional notion that transspacific distribution is the result of an invasion from west to east. In *A. triostegus* this conclusion is reinforced by the geographic patterns of genetic diversity. In the EP, 14 out of 19 individuals sampled at four widely scattered localities contained the same single haplotype, whereas in the CP molecular diversity was much higher (table 1). This suggests a colonization event into the EP by one or a few females with identical ATPase8 and -6 haplotypes. A second haplotype is shared by the two regions, indicating the introduction of an additional haplotype.

The distribution of genetic diversity indicates eastward invasions in two additional species. In *F. flavissimus* there were nine distinct haplotypes in the CP, but all eight individuals from two localities in the EP had the same haplotype, which they shared with the CP (figure 1). This pattern suggests the recent arrival of this haplotype in the EP, with insufficient time for new mutations to accumulate in this region and is consistent with the short time of separation estimated from coalescence (table 2). In *C. dumerilii* all five individuals at Clipperton possessed the same haplotype, again one that was shared with the CP. We have no samples of this species from anywhere else in the EP, so it cannot be said whether this represents a recent invasion of the entire EP region or just the Clipperton Atoll. However, in the EP this species is found only at the offshore islands and a few scattered places of the mainland (Robertson & Allen 2002), which is consistent with a recent arrival into the CP. Thus, mtDNA evidence suggests four cases of invasion of the EP by propagules that originated in the CP.

There are two invasions that, according to the mtDNA data, have occurred from east to west. In *C. oxycephalus*, in which EP and CP lineages have sorted out to be reciprocally monophyletic, the ancestral haplotype is found in the EP (figure 1). Thus, *C. oxycephalus* appears to have originated in the EP and colonized the CP, with further gene flow blocked by the EPB about 700 000 years ago (table 2). In *D. holocanthus*, in which gene flow between the two sides of the barrier still occurs, the oldest haplotype is also found in the EP, whereas the CP haplotypes (all of them from Easter Island) are derived from a different EP haplotype. Though this could be an artefact of the much larger sample size in the EP, the scarcity of this species in the central parts of the CP (Robertson *et al.* 2004; which caused the paucity of our samples from this area) supports the idea that, in this circumtropical species, the extant populations at Easter Island were derived from a recent invasion, possibly originating in the EP.

(e) **Direction of gene flow**

Coalescence analysis of our data shows that gene flow through the EPB was not necessarily in the same direction as the original invasion (table 2). In eight species (*A. triostegus, C. carolinus, C. dumerilii, F. flavissimus, M. vanicolensis, M. berndti, S. bandanensis* and *Z. cornutus*) a much higher number of females per generation have been crossing the barrier from the EP to the CP than in the opposite direction. Gene flow is low in both directions in *H. cruentatus*, but the few propagules also moved predominantly from east to west. *O. melagris* shows high gene flow from the EP into the Marquesas and Kiritimati and weaker (but asymmetric) gene flow from Hawaii into the EP. There are only two cases of asymmetric gene flow from west to east, those of *D. holocanthus* and *S. ghobban*, but in both species the differences of gene flow in each direction are relatively small. Roughly bidirectional gene flow at low levels is present in two species, *N. taeniourus* and *S. rubrocaviolaceus*. Thus, genes of transspacific species can flow across the EPB in both directions, but in the majority of cases they do so from east to west. Counter-intuitively, the direction of post-invasion gene flow is reverse to the direction of invasion in all four cases for which inferences about both variables could be made (*A. triostegus, F. flavissimus, C. dumerilii*, as well as *D. holocanthus*).

This conclusion must be tempered by the consideration of alternate explanations. As stated previously, in both *F. flavissimus* and *C. dumerilii* the only haplotype in the EP is the common haplotype found in CP (figure 1). The most parsimonious explanation for this pattern would be that, after the original invasion from west to east, there was no subsequent gene flow in either direction. If the possibility of gene flow is admitted, the coalescence reconstruction of the isolation–migration algorithm, that such flow was in the opposite direction, would be intuitively correct, because otherwise additional alleles from the CP should be present in the EP. We have no means of choosing between the alternative parsimony and Bayesian reconstructions.

How do propagules of these species succeed in crossing the EPB? Surface currents flow in both directions, at average speeds that in normal years would result in conveyance times that exceed the competent life times of most shore fish larvae (Robertson *et al.* 2004). However, eastward flow is greatly enhanced during El-Niño
and westward flow during La-Niña events (Robertson et al. 2004; NOAA at http://www.oscar.noaa.gov). Presumably, crossing of a random assortment of long-lived larvae occurs during these extremes, which would account for the paucity of shallow water species that maintain transpacific connections and for the lack of universal patterns of genetic divergence among those that do.

4. CONCLUSIONS

Massive breaching of the EPB has been documented previously by mtDNA sequences in two species of sea urchins (Lessios et al. 1998, 2003), but such isolated cases can only demonstrate that the EPB is not completely impassable. The power of our data from the shore fishes lies in the simultaneous examination of 20 transpacific species. They indicate that, with the exception of vicariance, all alternate hypotheses regarding the presence of the same nominal species on the two sides of the EPB hold true for different species. There is evidence of morphologically similar but long isolated entities with reciprocally monophyletic mtDNA lineages, of recent invasions into either region from the other and of recurrent gene flow in both directions. Ninety percent of the presumed transpacific species we examined show pronounced mtDNA affinities on the two sides of the barrier, showing that morphology is a good, although not perfect, guide in determining the efficacy of the EPB. Thus, the traditional approach in biogeography, of designating provinces by counting number of morphospecies held in common, can, by and large, reach correct conclusions. In the 18 species that are genetically similar across the EPB, the genetic cohesion is not due to a common pattern of historical events. The high scatter of times of estimated separation of the populations indicates that sporadic dispersal through the barrier is the likely cause of the establishment of transpacific populations, or the swamp- ing of their genetic differences. Had a change in physical conditions—such as climatic alteration of current patterns or the sinking of a seamount—interrupted previously recurrent gene flow, we would have expected to see similar levels of genetic divergence in many species. Level and direction of gene flow also vary between the species, as does the direction of invasion, variation which is consistent with dispersal, but not with vicariance.

It should be remembered that the 20 species selected for this study were known to have high morphological affinity across the EPB and are thus exceptional among marine biota. Thus, our data do not indicate that the EPB is an ineffective barrier, but rather that it is a sporadically permeable filter. Though transpacific fishes tend to belong to families with long larval lives (Robertson et al. 2004), the close genetic similarity of their populations on either side of the barrier raises the question of why other species with similar larval durations have been unable to cross the barrier. Differences in the biology of the larvae and in the ecological requirements of the adults are a factor, but the stochasticity of making a successful crossing through this wide stretch of water is also expected to play a major role. Stochasticity of extinction is also important in the initial establishment of transpacific populations. Once substantial resident populations are established on both sides, gene flow across the EPB has a higher probability of being maintained, because larvae that succeed in crossing can encounter mates in the target region. Both initial invasion and subsequent gene flow are dependent on the number of individuals that cross the EPB, but factors affecting post-transit success differ. Whereas successful invasion depends on the availability of suitable habitat on the other side of the EPB (Leis 1986; Robertson et al. 2004), recurrent gene flow in each direction depends on the abundance of conspecifics in the target area. This difference explains why gene flow (if the isolation–migration model is correct) has proceeded in the reverse direction than initial invasion in all four cases in which direction of invasion could be determined. Larger populations have a higher probability of broadcasting propagules, but also provide more opportunity for incoming migrants to find mates. Opportunity for the incoming migrants to propagate their genes to the next generation may also explain why the majority of the fish transpacific species show net gene flow towards the CP, a region in which—assuming equal mutation rates—the majority of species have larger effective population sizes (table 3 in electronic supplementary material). The EPB creates conditions under which not only the establishment of transpacific populations, but also the ability of migrants to encounter mates after crossing are, by and large, stochastic processes with a low probability. Thus, Darwin’s inclusion of the EPB among the impassable barriers continues to be generally justified.

We thank J. McCosker and J. Earle and P. Lobel for help with fish collections, B.D. Kessing for organizing the collection of sequence data, A. and L. Calderon for help in the laboratory and R. Collin, T. Duda, L. Gayer, L. Rocha, S. Vollmer and K. Zigler for comments on the manuscript. The governments of the Republic of Kiribati, French Polynesia and Easter Island provided permission to collect. Funded by the Molecular Evolution program of the Smithsonian Institution, general research funds from STRI, and a grant from the National Geographic Society to D.R.R. (NGS 5831-96).

REFERENCES


Dawson, C. E. 1985 Indo-Pacific pipefishes. Ocean Springs, MS: Gulf Coast Research Laboratory.


Electronic Appendix

Supplementary Materials and Methods

DNA extraction and sequencing: Genomic DNA was extracted from gill, skeletal muscle, or fin tissue either with direct digestion by Proteinase K (Lessios et al. 1996) or with traditional phenol/chloroform procedures. 0.2-0.5 μl of DNA template was amplified in 50 μl volumes containing 1 μl of Master Amp Tfl polymerase (Epicentre®), 5 μl of MasterAmp buffer (with 15 mM MgCl₂), and 2.5 μl each of primers ATP8.2 5'-AAAGCRTYRGCCCTTTAAGC and CO3.2 5'-GTTAGTGGTCAKGGGCTTGGRTC. Amplifications were carried out in one initial cycle of heating to 94°C for 5 sec, followed by 39 cycles of denaturation at 94°C for 30 sec, primer annealing at 50°C for 45 sec, extension at 72°C for 1 min, and ending with 5 min at 72°C. Amplification products were cleaned in agarose minigels, then cycle-sequenced in both directions using the same primers and electrophoresed in either a model 373A or a model 377 automatic sequencer from Perkin-Elmer/Applied Biosystems. The resulting 634 DNA sequences were aligned by eye and trimmed to include only 842 bp between the initiation codon of ATPase8 and the stop codon of ATPase6. They have been deposited in GenBank under accession numbers DQ111069-DQ111703.

Analysis of Data: For the IM analysis, wide limits for prior values for each parameter were selected from initial runs, then multiple runs were carried out for each comparison, starting from simple unheated ones with burn-in intervals of 10⁵ steps, and continuing by increasing the number of steps, the burn-in time, and the complexity of the heating scheme until complete (or nearly complete) posterior likelihood curves were obtained for each parameter.
of interest. Results were accepted if two runs with different random seeds produced estimates for each parameter that did not differ by more than 25% (except for values < 0.1).

The final results for the 39 comparisons (Table 3 in electronic Appendix) were based on runs with number of steps ranging between $1.2 \times 10^7$ and $10^9$, burn-in intervals between $10^5$ and $10^7$ steps, minimum effective sample size (ESS, see J. Hay and R. Nielsen, IM Documentation at http://lifesci.rutgers.edu/~heylab/HeylabSoftware.htm for explanation of terms) among parameters between 201 and $6.6 \times 10^4$, and either no heating, two-step heating, or geometric heating, with 5 to 30 Markov chains. In Doryrhampus excisus, Cirrhitichthys oxycephalus and Acanthurus triostegus sandvicensis, which do not share any haplotypes between regions, prior values of $m$ in both directions were set as equal to zero in order to obtain estimates of time since population separation. In several cases, it was not possible to obtain posterior probability density curves with distinct medians for the estimate of $t$, $\theta$ or $m$, despite extensive heating of the chains, long burn-in times, and a large number of steps, indicating that the data from this single locus may not contain the information necessary to estimation of these parameters. In some additional cases, posterior probability densities rose from a low value to a plateau, so that all estimates larger than the inflection point had the same approximate posterior probability.

Log likelihood ratio tests were used to obtain a rough idea of whether the estimated values of $t$ were significantly different from 0. The maximum likelihood estimate of this parameter ($L_{\text{max}}(t)$) was compared to the likelihood of the smallest value with a non-zero likelihood ($L(t_0)$). The probability that resulted from comparison of twice the likelihood ratio to the $\chi^2$ distribution was divided by 2 as a correction for the boundary conditions of the comparison (Hay and Nielsen 2004). Log likelihood ratio tests were also used to compare $\theta_1$ to $\theta_2$ (the $\theta$
values of EP and CP populations) and $m_1$ to $m_2$ (the scaled migration rates in each direction).

For these comparisons, the maximum likelihood of the parameter with the larger estimated value was compared to the likelihood of the value of the same parameter that was approximately equal to the maximum likelihood estimate of the other parameter. For example, if $m_{1\text{max}}$ is the maximum likelihood estimate of $m_1$, if $m_{2\text{max}}$ is the maximum likelihood estimate of $m_2$, if $m_{1\text{max}} > m_{2\text{max}}$, and if $m_{1x}$ is the value of $m_1$ that is equal to $m_{2\text{max}}$, then the log likelihood value used to test whether $m_{1\text{max}}$ is significantly different than $m_{2\text{max}}$ was $2\ln[\frac{L(m_{1\text{max}})}{L(m_{1x})}]$. A boundary region correction was used in obtaining probability values from the $\chi^2$ distribution in these comparisons as well, because the maximum likelihood estimate of the parameter with the smallest value corresponded almost invariably to one of the lowest values of the other parameter for which the likelihood was larger than 0.

**ELECTRONIC APPENDIX REFERENCES**


Electronic Appendix Fig. 1. Sample localities, and sample size (numbers within symbols) per species per locality. See Table 1 (main article) for symbol corresponding to each species.
Electronic Appendix Table 1. Pairwise $F_{ST}$ values between those populations of each species in which $>2$ individuals were sampled.

(Values in bold are those with $p < 0.05$. See figure 1 in electronic appendix for locations and sample sizes.)

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Electronic Appendix Table 2. Kimura two parameter genetic distance ($K_2$), and substitution rate per branch for the entire 842 bp fragment of ATPase8 and 6, based on the assumption that Atlantic and Pacific members of each pair were separated by the completion of the Isthmus of Panama 3.1 million years ago.

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<td>Mean</td>
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Electronic Appendix Table 3. Values of scaled migration and scaled effective population size for eastern Pacific (EP) and central Pacific (CP) populations of each species.
(Scaled migration: $m = \frac{m}{\mu}$; scaled effective population size: $\theta = 2Ne\mu$ (where $\mu$ is the mutation rate per generation and $Ne$ is the effective population size of females). Parameters are those with maximum Bayesian posterior probabilities, based on the coalescent (Hay and Nielsen 2004). Samples from the same side of the East Pacific Barrier were separately compared to those of the other side when $F_{ST}$ comparisons (Appendix Table 1) indicated intraregional restrictions in gene flow. Values in bold are significantly larger from the corresponding parameter value in the opposite region at $p < 0.05$. NC: Parameters could not be estimated because densities of posterior probabilities were flat. Parameter estimates that appear as inequalities come from posterior probability densities that rise to a plateau, so that all estimates larger than the shown value have the same approximate likelihood. Migration values equal to zero were set as priors, because in these comparisons no haplotypes are shared between regions.)

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