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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 arranged 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 relicts of an ancient separation event, which have not evolved morphological 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

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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 F_{ST} statistics. To generate the null distributions for assessing the significance of the F_{ST} 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 vicariance 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, $\theta = 2N_e\mu$ (where N_e is the effective population size of the ancestral and the two daughter populations, each estimated separately) and the scaled migration rate $m = m/\mu$ (where m is the proportion of migrants arriving into a population per generation) in each direction. As coalescence estimations assume that each population is effectively panmictic, we pooled samples of the same species from different localities within a region only if F_{ST} statistics (table 1 in electronic supplementary material) under the island model indicated that they exchanged more than one female per generation (if F_{ST} 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 $M = 2N_e m_f = \theta m/2$ (where m_f 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 *Doryrhamphus 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 $K_2 = 8.01\%$), with the EP clade being only slightly more divergent from the CP clades (mean $K_2 = 8.33\%$) than the CP clades are from each other (mean $K_2 = 7.79\%$). These large genetic distances are consistent with the limited dispersal potential and high levels of local endemism typically found among Indo-Pacific syngnathid fishes (Dawson 1985). (ii) In the hawkfish *Cirrhitichthys oxycephalus* the haplotypes are joined in a single network. However, EP and CP haplotypes form different clades, separated by six mutations from each other ($K_2 = 1.22\%$). 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 relicts 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 sandvicensis* 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 sandvicensis* 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 sandvincensis* 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.)

species		percentage variation					nucleotide diversity	
		within populations	between populations	between regions	Φ_{CT} between regions	p	EP	CP
	*Acanthurus nigricans*	48.98	86.14	−35.12	−0.351	0.206	0.003	0.003
	*Acanthurus triostegus*	62.32 (18.22)	2.13 (50.99)	35.55 (30.79)	0.355 (0.308)	0.067 (0.018)	0.002	0.018 (0.020)
	*Arothron meleagris*	63.12	2.33	34.56	0.346	0.066	0.001	0.001
	*Calotomus carolinus*	99.60	−0.40	0.80	0.008	0.267	0.003	0.003
	*Cantherhinus dumerilii*	95.11	22.85	−17.97	−0.180	0.314	0.000	0.001
	*Cirrhitichthys oxycephalus*	29.35	34.21	36.44	0.364	0.000	0.007	0.004
	*Ctenochaetus marginatus*	95.29	2.53	2.17	0.022	0.341	0.002	0.002
	*Diodon holocanthus*	55.94	1.69	42.37	0.424	0.202	0.003	0.002
	*Doryrhamphus excisus*	7.40	43.65	48.49	0.489	0.034	0.010	0.059
	*Forcipiger flavissimus*	103.82	1.69	−5.52	−0.055	0.859	0.000	0.001
	*Heteropriacanthus cruentatus*	74.60	1.08	24.32	0.243	0.009	0.003	0.003
	*Mulloidichthys vanicolensis*	86.11	13.84	0.05	0.000	0.479	0.002	0.004
	*Myripristis berndti*	84.27	13.21	2.52	0.025	0.198	0.004	0.004
	*Novaculichthys taeniourus*	83.69	4.34	11.97	0.120	0.094	0.002	0.001
	*Ostracion meleagris*	83.53	5.98	10.49	0.105	0.081	0.002	0.003
	*Scarus ghobban*	—	—	—	0.169	0.055	0.003	0.002
	*Scarus rubroviolaceus*	87.46	23.37	−10.83	−0.108	0.824	0.002	0.002
	*Sectator ocyurus*	—	—	—	0.120	0.027	0.001	0.003
	*Stethojulis bandanensis*	71.42	47.30	−18.72	−0.187	0.256	0.001	0.002
	*Zanclus cornutus*	103.62	−11.71	8.10	0.081	0.095	0.002	0.003

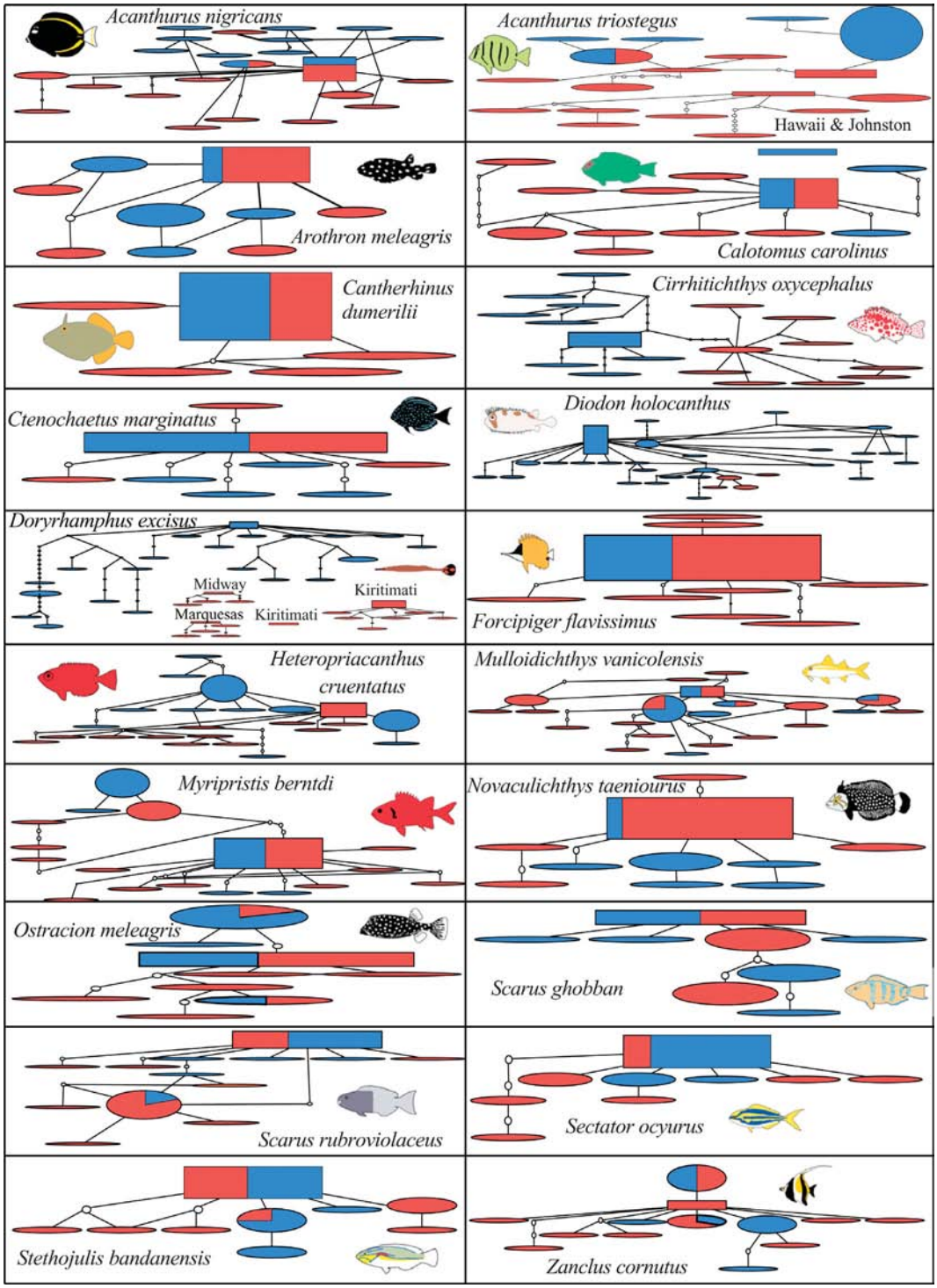

Figure 1. Statistical parsimony (Templeton *et al.* 1992) networks of haplotypes of the 20 species in this study. Area of each shape within each network is proportional to the number of individuals bearing a haplotype, blue shapes indicate haplotypes found in the eastern Pacific, red shapes haplotypes found in the central Pacific. Ancestral haplotypes as determined by outgroup weight (Castelloe & Templeton 1994) are depicted as parallelograms, hypothetical haplotypes as small empty ellipses.

is genetically so distinct from all other populations ($K_2 = 3.26\%$), 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.

(b) *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. excisus*, *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 (Φ_{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 ($2N_{ef}m_f$). (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 $m=m/\mu$ (where m is the migration rate and μ 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 $\theta=2\mu N_{ef}$ (where $N_{ef}$is the effective population size) in a region significantly larger then θ in the other region. NC: estimates not possible, because posterior probability curves were flat. Inequalities: posterior probability densities of either m or θ 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.)

species	comparison	time (thousands of years)	$2N_{ef}m_f$ EP to CP	$2N_{ef}m_f$ CP to EP
Acanthurus nigricans	all CP versus all EP	78.9*	NC	NC
Acanthurus triostegus	Marquesas+Kiritimati versus all EP	585.1*	*17.048*	0.030
Acanthurus triostegus	Hawaii+Johnston versus all EP	2127.9*	0	0
Arothron meleagris	all CP versus all EP	68.3*	*34.907*	0.005
Calotomus carolinus	all CP versus all EP	141.3*	NC	NC
Cantherhinus dumerilii	all CP versus all EP	61.1*	***>12.563***	0.001
Cirrhitichthys oxycephalus	mean of 2 pairwise comparisons	746.8*	0	0
Ctenochaetus marginatus	all CP versus all EP	117.8*	NC	NC
Diodon holocanthus	all CP versus all EP	136.7*	0.011	*0.975*
Doryrhamphus excisus	mean of 13 pairwise comparisons	5322.8*	0	0
Forcipiger flavissimus	all CP versus all EP	46.5*	*70.893*	0.011
Heteropriacanthus cruentatus	all CP versus all EP	207.3*	*0.179*	0.058
Mulloidichthys vanicolensis	all CP versus all EP	194.1*	***>1420.225***	0.025
Myripristis berndti	all CP versus all EP	1087.6*	***493.992***	0.019
Novaculichthys taeniourus	all CP versus all EP	113.4*	0.024	0.024
Ostracion meleagris	Marquesas+Kiritimati versus all EP	NC	**>37.599**	0.047
Ostracion meleagris	Hawaii versus all EP	45.6	0.023	**3.006**
Scarus ghobban	all CP versus all EP	308.9*	0.004	*0.093*
Scarus rubroviolaceus	Marquesas+Hawaii versus all EP	30.1*	0.017	*0.014*
Scarus rubroviolaceus	Kiritimati versus all EP	59.9*	NC	NC
Sectator ocyurus	all CP versus all EP	57.4*	NC	0.059
Stethojulis bandanensis	all CP versus Is. Coco	115.7*	0.125	0.036
Stethojulis bandanensis	all CP versus Panama	154.9*	***>36.856***	0.003
Stethojulis bandanensis	all CP versus Clipperton	NC	NC	0.024
Zanclus cornutus	all CP versus all EP	185.4*	***120.791***	0.023

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 2×10^5 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 puffer *Arothron meleagris*, the parrotfishes *Calotomus carolinus* and *Scarus rubroviolaceus*, the filefish *Cantherhinus dumerilii*, the porcupinefish *Diodon holocanthus*, the butterflyfish *Forcipiger flavissimus*, the goatfish *Mulloidichthys vanicolensis*, the wrasses *Novaculichthys taeniourus* and *Stethojulis bandanensis*, the boxfish *Ostracion meleagris*, the sea-chub *S. ocyurus* and the moorish idol *Zanclus cornutus*). Older ($2\times10^5-10^6$ years ago) separation with high subsequent gene flow was seen in two species (the surgeonfish *A. triostegus triostegus* and the squirrelfish *Myripristis 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 Φ_{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 transpacific 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 transpacific fish species, including *S. ocyurus*, 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 (Castelloe & 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 transpacific 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. meleagris* 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. rubroviolaceus*. Thus, genes of transpacific 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 swamping 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.

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