



Massive gene flow across the world's most potent marine biogeographic barrier

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The 'Eastern Pacific Barrier' (EPB), 5400 km of uninterrupted deep water between the central and eastern Pacific, constitutes the greatest marine obstacle to the dispersal of shallow-water organisms. However, some species are found on both sides of the EPB. These 'transpacific' species are considered by 'dispersal' biogeographers as evidence of invasions through the barrier. 'Vicariance' biogeographers, on the other hand, think that transpacific species are morphologically conservative remnants of previously continuous distributions. We compared nucleotide sequences in a 642 bp region of mitochondrial DNA, and electrophoretically detected alleles in 17 enzymatic loci of central and eastern Pacific populations of *Echinothrix diadema*, an Indo-Pacific sea urchin recently reported from the eastern Pacific. Both types of molecules produced clear evidence of massive, recent gene flow across the EPB. Thus, rather than being isolated relicts of Tethyan distributions, conspecific populations from the eastern and central Pacific are genetically connected. Though the EPB is biogeographically important as a cause of speciation in many groups, it allows genetic connections in others, possibly through larval transport during El Niño events.

Keywords: *Echinothrix diadema*; Eastern Pacific Barrier; mtDNA; isozymes; vicariance; biogeography

1. INTRODUCTION

In addition to land masses, the dispersal of shallow-water marine organisms is impeded by stretches of water so wide that their larvae have little opportunity to traverse them within their competent life times, and so deep that they provide no habitat in which adults can survive and produce a new generation of larvae. The 5400 km of deep water between the Line Islands in the central Pacific and Clipperton Atoll in the eastern Pacific, the so-called 'Eastern Pacific Barrier' (EPB), constitute the greatest such marine biogeographic obstacle (Ekman 1953; Briggs 1961; Grigg & Hey 1992). Because this stretch of open water has existed for a long time—possibly since the beginning of the Cenozoic (Grigg & Hey 1992)—and because there are no islands to serve as stepping stones in migration, relatively few species are represented on both sides of the EPB (Ekman 1953; Briggs 1974; Vermeij 1978, 1987a,b; Veron 1995; Robertson & Allen 1996). These exceptions, the 'transpacific' species, are the object of disagreement between two schools of biogeographers. 'Dispersal' biogeographers (Dana 1975; Glynn & Wellington 1983; Richmond 1990; Vermeij 1991; Grigg & Hey 1992; Glynn *et al.* 1996; Robertson & Allen 1996) interpret transpacific species as resulting from the transport of larvae across the barrier, made easier since the late Pleistocene by the movement of the Line Islands into the path of the North Equatorial Counter-Current, the presumed vehicle of larval transport. 'Vicariance' biogeographers (McCoy & Heck 1976; Heck & McCoy 1978;

Abele 1982; Rowe 1985), on the other hand, view these distributions as the relicts of previously continuous Tethyan distributions that became separated as the EPB developed. A central argument of the vicariance view is that larvae are likely to perish before currents can transport them across such an extensive barrier. Here we report that an Indo-Pacific species of sea urchin found recently in the eastern Pacific (Lessios *et al.* 1996), shows a high degree of genetic identity between central and eastern Pacific populations. The identity is so high, that it can only result from extensive and recent gene flow across the EPB.

Until 1996, echinoids appeared to conform to Darwin's (1872) notion that the EPB is 'impassable'. Mayr's (1954) compilation of the ranges of tropical echinoid species, to deduce patterns of speciation, did not include a single transpacific species, which led him to conclude that 'of the existing barriers, by far the most potent has been that between Polynesia and the American west coast'. Rowe (1985) reviewed records of (non-echinoid) echinoderms with reported amphipacific distributions, and concluded that reports of transpacific species were either the result of inadequate taxonomic study, or represented Tethyan remnants of morphologically conservative self-sustaining separate populations. In 1996, we reported that the fauna of the offshore eastern Pacific islands (Isla del Coco, Clipperton and Revillagigedos) includes four Indo-Pacific species of sea urchins (Lessios *et al.* 1996). Two of these belong to the genus *Echinothrix*, which had never before been reported outside the Indo-west Pacific. One of these species, *E. diadema*, was represented by dense populations that gave every indication of being well-established (Lessios *et al.* 1996). On morphology alone, however, we could not determine whether these populations were long-isolated remnants of an ancient vicariant event, or

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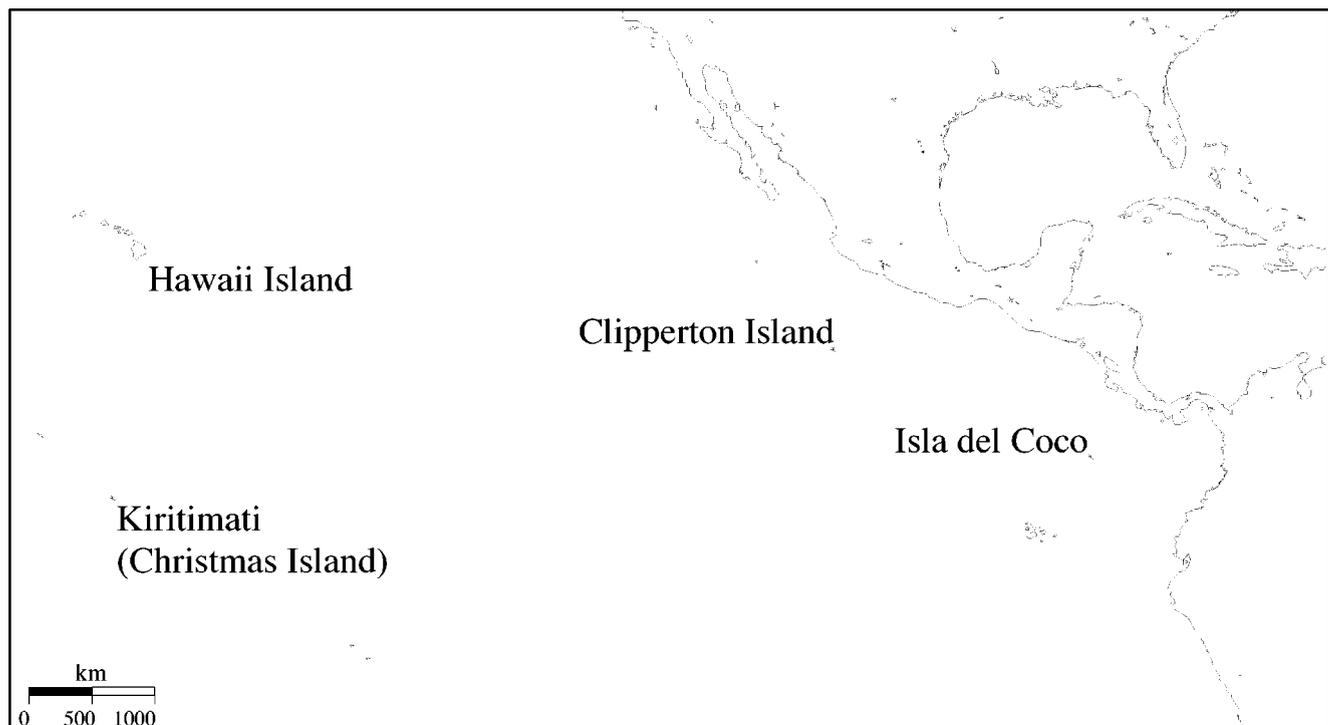


Figure 1. Localities in which *Echinothrix diadema* was sampled on either side of the East Pacific Barrier.

the products of either recent dispersal or continuous larval transport across the EPB. Here, we report the results of a comparison of mitochondrial and nuclear genetic markers of *E. diadema* from the central and eastern Pacific, intended to evaluate these alternatives.

2. MATERIALS AND METHODS

We amplified and sequenced, using polymerase chain reaction (PCR), 642 nucleotides from the cytochrome *b* region of the mitochondrial DNA (mtDNA) of (i) 22 *Echinothrix diadema* from the central Pacific (10 from Hawaii Island, 12 from Kiritimati (former name 'Christmas Island'), in the Line Islands), and (ii) 21 from the eastern Pacific (15 from Isla del Coco, 450 km SW of Costa Rica, 6 from Clipperton Atoll, 1100 km SW of Mexico) (see figure 1). Genomic DNA extractions, PCR amplification, PCR product purification, and DNA sequencing were carried out as described (Lessios *et al.* 1996), with the following modifications: (i) primers for both PCR and sequencing were CB4ef-TTAATCGTCCAAATACTACTGG and CB5er-ATGTGCGGTGGAGTTACCAGGGG; (ii) some PCR products were also purified by running them on 1.2% low-melting point agarose gels and recovering the DNA by digesting the agarose with GelaseTM (Epicentre Technologies, Madison, WI, USA); and (iii) the products of the cycle-sequencing reactions were run on either a 373A or a 377 automatic sequencing machine from Perkin-Elmer/Applied Biosystems, Inc., Foster, CA, USA. All individuals were sequenced at least twice using separate extractions and amplifications, to minimize the possibility of artefacts due to contamination or PCR replication error. DNA sequences have been deposited in GenBank under accession numbers AF012947–AF012991.

We also compared the products of 17 enzymatic loci of 18 individuals from Hawaii and 21 from Isla del Coco, using electrophoresis. The sampled loci were: glucose-6-phosphate

dehydrogenase (*G6pdh*), aspartate aminotransferase (*Got-1* and *Got-2*), isocitrate dehydrogenase (*Idh*), mannose-6-phosphate isomerase (*M6pi*), NAD⁺-dependent malate dehydrogenase (*Mdh-1* and *Mdh-2*), phosphoglucose isomerase (*Pgi*), phosphoglucomutase (*Pgm-1* and *Pgm-2*), triosephosphate isomerase (*Tpi*), L-leucyl-L-tyrosine peptidase (*Peplt*), octanol dehydrogenase (*Odh*), superoxide dismutase (*To*), xanthine dehydrogenase (*Xdh*), fructokinase (*Fk*) and α -glucosidase (*aGlu*). Running buffers are listed in Lessios & Pearse (1996); standard histochemical staining recipes were used to visualize the products of enzymatic reactions.

3. RESULTS

The results show unequivocally that *Echinothrix diadema* populations from the two sides of the EPB have been connected by recent, large-scale gene flow. Although only 3 of the 28 determined mtDNA haplotypes are found both in the eastern and in the central Pacific, the phylogenetic relations between the remaining 25 show no subdivision into east and west clades, as would have been expected if they had been genetically separated for millions of years (see figure 2). Practically all clades contain haplotypes from both sides of the barrier. The mean corrected per cent nucleotide difference across the EPB (0.61%; Kimura 1980) is virtually identical to the mean difference between populations on the same side of the barrier (0.62%) and to the mean intrapopulational difference (0.61%). That this genetic homogeneity is not the result of excessively conservative evolution of this region of mtDNA is evident from the fact that the mean per cent difference between *E. diadema* and the congeneric *E. calamaris*, which was used to root the phylogenetic tree, is 17.09%.

Isozymes also show a high degree of genetic homogeneity between populations on either side of the barrier. Of 17 loci assayed in *E. diadema* populations in both

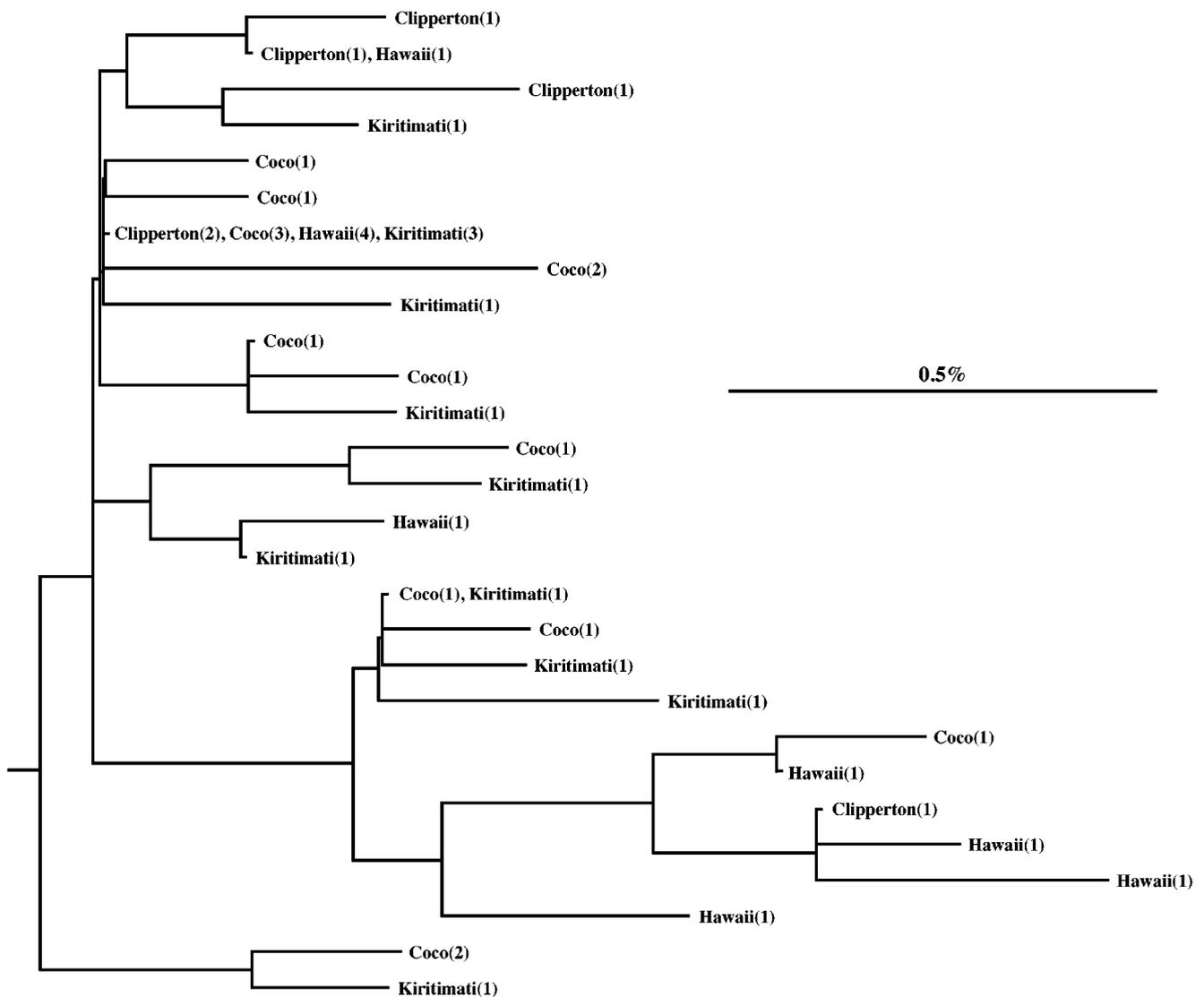


Figure 2. Phylogenetic relations between haplotypes as determined from 642 nucleotides of the cytochrome *b* region of mitochondrial DNA of *Echinothrix diadema* from two localities in the central Pacific (Hawaii and Kiritimati), and two localities in the eastern Pacific (Isla del Coco and Clipperton Atoll). Numbers in parentheses indicate the number of individuals that possessed each haplotype. The tree is based on the 'minimum evolution method' (Cavalli-Sforza & Edwards 1967; Rzhetsky & Nei 1992), employing Kimura (1980) two-parameter distances and calculated using test version 4.0d55 of PAUP* written by David L. Swofford; it is rooted on homologous sequences of *E. calamaris*.

Hawaii and Isla del Coco, 10 were monomorphic. Of the remaining seven, only one locus, *Fk*, showed significant differences in allele frequencies ($p < 0.05$, by Fisher's exact test), due to the presence in the central Pacific, in fairly high frequency, of an allele that is absent from the eastern Pacific (see table 1). If genetic equilibrium is assumed, the number of migrants per generation crossing the barrier, estimated from F_{ST} statistics, range from four to ten (see table 2), numbers far in excess of what is considered theoretically adequate to genetically homogenize subpopulations (Chakraborty & Nei 1974; Slatkin & Maruyama 1975). Four undefined F_{ST} values (three of them in comparisons across the barrier) are the result of higher variability within than between populations. Comparisons of the mtDNA F_{ST} values with those obtained from 1000 replicate reshufflings of the haplotypes with respect to locality (Takahata & Palumbi 1985), indicate that their geographic distribution is not significantly different from random ($p = 0.435$).

4. DISCUSSION

Both proteins and mtDNA demonstrate that populations of *Echinothrix diadema* from the central and the eastern Pacific are linked by high levels of gene flow. Indications of extensive gene flow between Hawaii and the eastern Pacific have also been found in electrophoretic samples of transpacific shorefishes (Rosenblatt & Waples 1986). Two other electrophoretic studies, though not specifically designed to address the question of gene flow in transpacific species, have found little genetic divergence between central and eastern Pacific conspecific populations of a crab (Huber 1985), and a starfish (Nishida & Lucas 1988). The range of Nei's (1978) D values in all three studies is 0.0004–0.060. The Nei's D values between Hawaii and Isla del Coco populations of *Echinothrix diadema* is 0.018. Though it is possible to argue that the apparent high similarity of populations in electrophoretic studies (including our own) is due to the limited resolving

Table 1. *Sample sizes and gene frequencies of electrophoretically detected loci of Echinothrix diadema at Hawaii and at Isla del Coco*

(The following loci were monomorphic: *To*, *Peptl2*, *Odh*, *Xdh*, *Idh*, *Aglu*, *G6pdh*, *Got1*, *Got2*, *Mdh2*. See text for locus name abbreviations.)

locus	allele	Hawaii	Isla del Coco
<i>Fk</i>	<i>N</i>	18	19
	100	0.389	0.000
	110	0.611	1.000
<i>M6pi</i>	<i>N</i>	18	20
	98	0.056	0.000
	100	0.028	0.000
	105	0.167	0.075
	106	0.194	0.225
	112	0.556	0.700
<i>Pgm1</i>	<i>N</i>	18	20
	88	0.028	0.000
	96	0.222	0.425
	98	0.000	0.050
	99	0.667	0.350
	100	0.056	0.100
	106	0.028	0.075
<i>Tpi</i>	<i>N</i>	18	20
	100	1.000	0.975
	105	0.000	0.025
<i>Mdh1</i>	<i>N</i>	18	20
	1.3	0.083	0.000
	95	0.056	0.025
	100	0.750	0.925
	103	0.028	0.000
<i>Pgm2</i>	<i>N</i>	18	20
	88	0.028	0.000
	98	0.111	0.350
	99	0.694	0.450
	100	0.167	0.200
<i>Pgi</i>	<i>N</i>	18	20
	100	0.056	0.075
	105	0.944	0.900
	106	0.000	0.025

power of the technique, the cytochrome *b* sequence data leave no doubt that populations of *E. diadema* from the two sides of the EPB are genetically very similar. Presumably, larvae of this species have been carried by the North Equatorial Counter-Current from the Line Islands to the Clipperton Atoll and Isla del Coco. Under normal conditions, it takes 100–155 days for parcels of water carried by this current to traverse the EPB (Wyrski *et al.* 1981; Richmond 1990). However, during years of strong El Niño flow, the transit time is reduced to 50–81 days (Firing *et al.* 1983; Wyrski 1985; Richmond 1990; Glynn *et al.* 1996). The length of larval life of either species of *Echinothrix* is unknown, but that of the related sea urchin genus *Diadema* ranges from 50 to 90 days in the laboratory (Mortensen 1937; Carpenter 1997). If the larvae of *Echinothrix* can remain competent for a comparable period, it is possible that they could cross the EPB during strong El Niño years.

Despite several zoological expeditions between 1891 and 1941 to the outer eastern Pacific Islands (see Maluf (1988)

for summary), no published records or museum specimens of *Echinothrix diadema* existed from the eastern Pacific until we found it in high abundance at Isla del Coco in 1987 (Lessios *et al.* 1996). This led us to speculate that this species may have invaded the eastern Pacific for the first time during the exceptionally strong 1982–83 El Niño event (Glynn 1988). There are, however, no detectable ‘founder effects’ (Mayr 1970) in either the eastern or the central Pacific populations. A slight reduction in the number of electrophoretically detected protein alleles was seen in Isla del Coco (30 alleles were detected in a sample of 21 individuals), relative to Hawaii (34 alleles detected in a sample of 18 individuals). However, with a sample size of 22 individuals from the central Pacific we detected 16 cytochrome *b* haplotypes; with a sample size of 21 individuals from the eastern Pacific we detected 15 haplotypes. Such slight differences suggest that this is not a case of the establishment of a peripheral population through a one-time transit and settlement of a small cohort of larvae carrying a subsample of genotypes

Table 2. Between-population genetic variance (F_{ST}) and number of migrants per generation ($N_e m$), calculated from protein genotypes and cytochrome *b* haplotypes

(For nuclear alleles, the values were calculated according to Wright (1965); for mtDNA, they were calculated according to Hudson *et al.* (1992), and the $N_e m$ values refer to the effective number of females. Negative values of F_{ST} are the result of higher intra- than interpopulational variability and are considered as undefined (Hudson *et al.* 1992). The F_{ST} value from mtDNA for all populations considered simultaneously is 0.003 ($N_e m = 152.49$.)

	F_{ST}	$N_e m$
proteins		
Hawaii-Isla del Coco	0.064	3.66
mtDNA		
within regions		
Hawaii-Kiritimati	0.063	7.42
Clipperton-Isla del Coco	-0.044	—
between regions		
Hawaii-Isla del Coco	0.048	9.97
Hawaii-Clipperton	-0.061	—
Kiritimati-Isla del Coco	-0.032	—
Kiritimati-Clipperton	-0.004	—

from the source population. Either the invasion was massive, or *Echinothrix diadema* has existed undetected at Isla del Coco and the Clipperton Atoll for a long time, sporadically receiving larvae from the central Pacific. In either case, the most recent episodes of migration must have been large enough to swamp out any genotypes that evolved on the east side of the EPB.

The genetic similarity of *Echinothrix diadema* populations on the two sides of the EPB indicates that larvae can cross it and establish viable and genetically variable populations on the other side. In this (and perhaps other) transpacific species, gene flow across the barrier has been high, and the classification of central and eastern Pacific populations to the same species on the basis of morphology is correct. Contrary to the suggestion of Rowe (1985) about other echinoderms, these are not self-seeding Tethyan remnants, but populations that exchange genes at rates comparable to those of populations on the same side of the barrier. However, this does not mean that the EPB is not a major biogeographic barrier, the establishment of which was an important vicariant event. The dissimilarities in the marine faunas of the eastern and western parts of the Pacific Ocean, remarked upon by Darwin (1872), Ekman (1953), Mayr (1954), Briggs (1974) and Vermeij (1987*a,b*), are real. However, the view of the 'dispersal' biogeographers that the EPB is more similar to a filter, permeable to the larvae of certain species, is much closer to the truth. If larval transit occurs mainly during El Niños, the filter is likely to be haphazard and unpredictable, which would result in many instances of isolation and speciation. However, this episodic transport has also resulted in truly transpacific species, which have either remained unseparated, or have come across from west to east at some point and may continue to receive larval influx after their establishment.

We thank the captains and crew of the RV 'Benjamin' and MS 'Royal Star', as well as G. M. Wellington, for assistance in the cruises that collected the specimens from Isla del Coco and the Clipperton Atoll, and B. J. LeMay, P. Wilder and the Government of Kiribati for making collections at Kiritimati possible. A.

Calderón, L. Calderón, A. Graybeal, C. Luna and M. Soriano helped in the laboratory. E. Bermingham, W. Kooistra, M. McCartney, S. Rehner and S. Williams commented on the manuscript. We are grateful to D. L. Swofford for permission to publish results of phylogenetic analyses produced from the test version of PAUP*. This study was supported by funds from the Smithsonian Molecular Evolution Program, and by Smithsonian Scholarly Studies grant 1234S607 and Baird Fund grant 14404507 to H.A.L., and by Abbott Fund grant 14800513 to D.R.R.

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