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Reefs as Cradles of Evolution and Sources of Biodiversity in the Phanerozoic

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Large-scale biodiversity gradients among environments and habitats are usually attributed to a complex array of ecological and evolutionary factors. We tested the evolutionary component of such gradients by compiling the environments of the geologically oldest occurrences of marine genera and using sampling standardization to assess if originations tended to be clustered in particular environments. Shallow, tropical environments and carbonate substrates all tend to have harbored high origination rates. Diversity within these environments tended to be preferentially generated in reefs, probably because of their habitat complexity. Reefs were also prolific at exporting diversity to other environments, which might be a consequence of low-diversity habitats being more susceptible to invasions.

Systematic differences in evolutionary patterns are well documented among marine environments. On the largest environmental scales these differences are manifested in onshore-offshore patterns, where higher taxa tend to have originated preferentially in nearshore environments and expanded offshore later in their evolutionary history (*1*, *2*), and tropical-extratropical patterns, where more origination took place in the tropics and tropical genera expanded toward extratropical latitudes (*3*). Both patterns suggest that shallow-water and tropical marine environments are not only evolutionary cradles, but are also more prolific at exporting diversity than deeper-water and extratropical habitats and thus represent net sources of biodiversity. The underlying mechanisms of these patterns presumably involve physical disturbance regimes, energy availability, and biotic interactions (*2*, *4*, *5*).

Using fossil occurrence data of benthic marine invertebrate genera from the Paleobiology Database [PaleoDB, (*6*)], we tested the cradle and source hypotheses for biogenic reefs. These largely shallow-water and tropical ecosystems are known for their amazing biodiversity (*7*), but it is disputed whether diversity is mainly generated within reefs (*8*–*12*) or if reefs rather act as ecological attractors for and evolutionary refuges of biodiversity that originated elsewhere (*13*–*15*).

We examined the environments of the geologically oldest occurrences of marine genera and compared sets of (i) reef (R) and nonreefal (NR) ecosystems, (ii) calcium carbonate (C) versus terrigenous clastic (siliciclastic, S) substrates, (iii) tropical (T) versus extratropical (ET) latitudes, and (iv) shallow- (SH) versus deep-water (DP) habitats, applying definitions in (*16*). To reduce the influence of taxonomic errors, we only referred to classified genera for which a species was identified (*17*), and we minimized stratigraphic errors by using only the species occurrences that were firmly assigned to 1 of 74 geological time intervals [supporting online material (SOM)]. If the oldest appearance of a genus fell into several environments, we randomly chose one occurrence and applied resampling to achieve an overall estimate of the most likely environment of origin. Because some environments are genuinely rare or undersampled in particular intervals, we performed subsampling to test if the number of origins was randomly distributed among environments in a uniform subset. We also analyzed the relative proportions of originating taxa that dispersed to other environments during their stratigraphic range.

Of the 6615 benthic invertebrate genera that span more than one geological interval and for which an environment of origin can be assigned, 1426 genera originated in reef environments. This proportion (21.6%) is impressive when considering that only 16.7% of all benthic occurrences in the PaleoDB are from reefs. Probably because of a bias in the taxonomic description of ancient reefs toward the occurrence of reef-building organisms, the proportion of reef originations is much greater for corals and sponges than for reef dwellers such as bivalves and gastropods (Fig. 1). The originations in C, T, and SH are similarly greater than expected by the distribution of sampling (Table 1), but because the relation between sampling and observed originations is nonlinear, sampling standardization is needed to assess the true effect of environmental setting on origination probability.

Subsampling analysis demonstrates that reefs are important evolutionary cradles independent of their preferred habitat in shallow, low-latitude, calcium carbonate environments (Fig. 2). Although the R-NR comparison yields an intermediate origination preference between T-ET and SH-DP, we emphasize that the R-NR pattern is based on a comparison of low-latitude reefs with only those nonreef environments that were also low latitude, carbonate, and shallow water. Per-genus origination probabilities are about 45% greater in reefs than outside reefs. This pattern applies to individual higher taxa as well: The higher probability of reefal origination seen in the raw data is maintained in corals (Fig. 1, Anthozoa), and the sparse reefal originations in bivalves and gastropods are higher than outside reefs when sampling is made uniform (fig. S5).

The C-S, T-ET, and SH-DP comparisons are based on all data and on data excluding reefs. These imply that C, T, and SH are notable

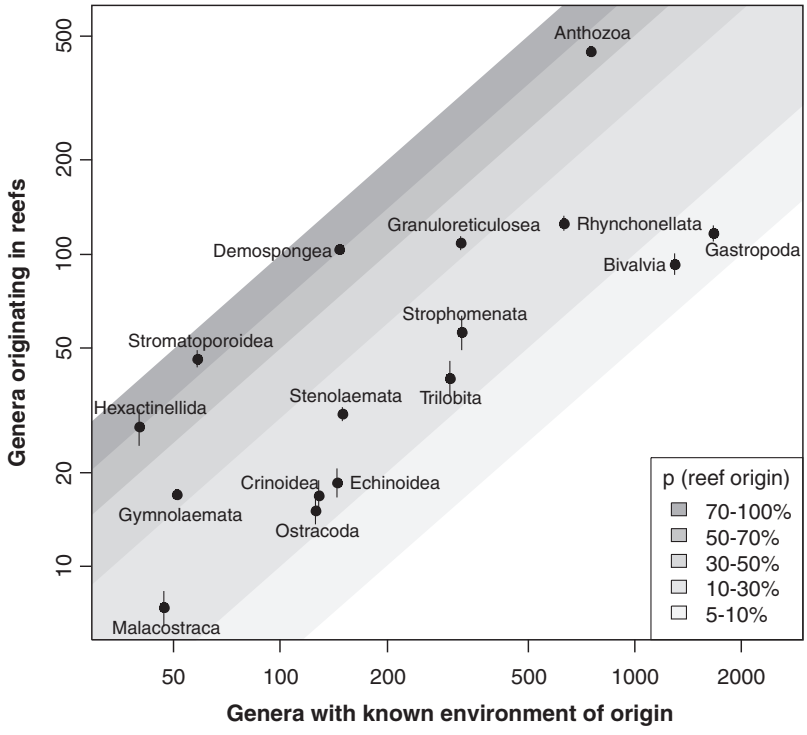

Fig. 1. Phanerozoic originations of marine benthic genera in reefs for the most common classes recorded in the Paleobiology Database. Error bars represent two times the standard deviation of 50 resampling trials of raw data.

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centers of originations independent of reefs, although the SH-DP gradient drops significantly when reefs are excluded. In nonreefal ecosystems, shallow-water environments represent the most important evolutionary cradle, followed by carbonate environments and tropical latitudes. The strong SH-DP signal is somewhat surprising given previous suggestions that the onshore-offshore gradient would only pertain to higher taxonomic levels (*2*). The gradient might be biased by the much better sampling of the spectrum of shallow-water habitats, which cannot be counterbalanced completely by subsampling (SOM). Our results agree with analyses suggesting that particular biologic groups had preferred tropical origins (*3*), although the overall origination preference is comparatively small.

To test if particular environments have also been sources of biodiversity for other environments, we computed the proportions of genera that originated in one environment and are subsequently found in another one. The proportions of these exporters were generally greater for genera originating in R, C, T, and SH than those originating in NR, S, ET, and DP (Fig. 3). The pattern is most prominent in reefs, which have thus been the most significant net exporters of biodiversity. Reefs were ~25% more likely to have exported diversity in the Phanerozoic to nonreefs than were nonreefs to reefs. This is in marked contrast to the SH-DP pair, for which shallow-water environments show a pronounced cradle signal but exported only slightly more of their new genera to deep-water environments than they imported. Offshore-onshore migrations thus may have been proportionally more common than usually assumed (*18*). The T-ET pattern is consistent with the out-of-the-tropics model (*3*), but out-of-the-reefs and out-of-the-carbonates models are equally applicable over the entire Phanerozoic.

Table 1. Global proportions of originations and sampling in raw data and their 95% confidence intervals.

	R/(R+NR)*	C/(C+S)	T/(T+ET)	SH/(SH+DP)
Originations	0.22 ± 0.010	0.53 ± 0.011	0.51 ± 0.011	0.72 ± 0.011
Occurrences	0.17 ± 0.002	0.47 ± 0.002	0.48 ± 0.002	0.68 ± 0.002

*R and NR exclude plankton and nekton.

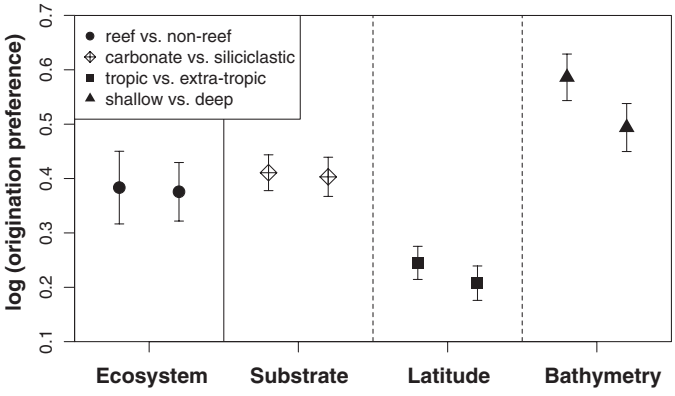

Fig. 2. Sampling-standardized logged origination preferences in pairs of habitats. Ecosystem stands for benthic originations in low-latitude, shallow-water reefs (R) compared with originations in low-latitude, shallow, and nonreef carbonates (NR). Points refer to <30° (left) and <45° paleolatitude (right). The left points in lithology, latitude, and bathymetry comprise all data, whereas the right points exclude reef data. Lithology compares originations on or in carbonate substrates (C) and siliciclastic substrates (S). Latitude weighs tropical (T, < 30° paleolatitude) originations against extratropical (ET) originations. Bathymetry evaluates originations in shallow water (SH, above the storm-weather wave base) versus deeper water (DP). Origination preference is calculated by the risk ratio of per-genus originations in respective habitats (SOM). Error bars represent 95% confidence intervals. All values are significantly above zero, indicating that reefs, carbonates, the tropics, and shallow-water environments represent more important evolutionary cradles than their counterparts.

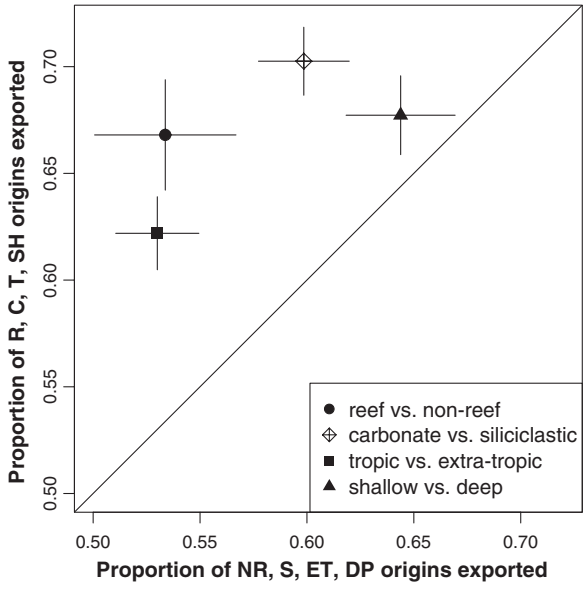

Fig. 3. Sampling-standardized balance of exported and imported diversity in environmental pairs. The plot depicts the proportions of genera originating in R, C, T, and SH that migrate into NR, S, ET, and DP and the proportions of genera originating in NR, S, ET, and DP migrating into R, C, T, and SH. Data for S-C, T-ET, and SH-DP exclude reefs. Error bars indicate 95% confidence intervals.

The strength of the cradle and the source effects varied through the Phanerozoic. Standardized per-genus origination rates were much higher in reefs, carbonates, and tropical latitudes in the Paleozoic than later on (fig. S8). Therefore, either these environments have lost some of their potential to be evolutionary cradles, or their counterparts have increased their potential. The foreign trading balance is less variable (fig. S9). On even finer time scales, the reefal cradle is not clearly related to rebounds from mass extinctions (fig. S10), perhaps because reef ecosystems need to be restructured by organisms before they can act as evolutionary motors.

Our results might be affected by taphonomic biases, geographic aggregation of data, and different stratigraphic ranges of genera. However, none of these potential biases is strong enough to affect the basic results. Sampling probabilities are similar among environments (table S2), geographic clustering does not differ significantly between R and NR (table S3), results are robust with different subsampling methods (figs. S3 and S4), and the slightly different stratigraphic ranges of genera in respective habitats do not substantially influence the source effect (fig. S7).

That a pronounced evolutionary role of reefs is evident even when we explicitly control for correlated factors suggests that an additional ecological aspect must be sought to supplement the energy and disturbance hypotheses, which are usually advocated to explain environmental gradients in evolution. The most likely factor is habitat complexity, expressed in topographic complexity that is generated by the three-dimensional growth of reefs, and ecological complexity generated the many biotic interactions in even low-diversity reefs (*19*). Topographic complexity is known to provide ecological opportunities for species packing of marine fishes (*20*, *21*), and reefal habitat complexity has been suggested to drive the diversification of teleost fishes (*12*). Taphonomic biases inhibit the direct test of a cradle effect for reef fishes, because even undoubted coral reef fish assemblages are usually recovered from off-reef sediments (*22*).

Habitat complexity can only control maximum standing diversity, and there is no evidence that reef diversity increased profoundly over the Phanerozoic (*23*). Without extinctions, we would thus probably not see the pronounced cradle signal in our data.

Indeed, extinction probabilities are as concentrated in reefs as are originations (fig. S13), such that enhanced evolutionary turnover rates might be a reasonable explanation for the reefal cradle, with high origination rates keeping reefs from turning into a museum. This would also explain the stronger cradle signal in the Paleozoic than later on, because turnover rates were generally higher (*24*, *25*). The role of evolutionary turnover has separated reefs from other topographically complex ecosystems such as rocky shores (*26*).

The observation that those settings that tended to have higher rates of origination also tended to export proportionally more taxa to other settings might be a consequence of diversity gradients. Despite conflicting experimental results [reviewed by (*27*)], some theoretical work predicts that high diversity forms a barrier against species invasions by lowering niche opportunities (*28*). This barrier could apply equally to locally evolved genera as well as invaders (*29*), but, compared with immigrants, taxa evolving in a high-diversity regime should more readily occupy vacant niche space that is generated by extinctions. Our study supports the contention that large-scale gradients in biodiversity are at least partially governed by evolutionary history and are not simply due to ecological factors that control standing diversity.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1182241/DC1
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Figs. S1 to S14
Tables S1 to S4
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Therapeutic Silencing of MicroRNA-122 in Primates with Chronic Hepatitis C Virus Infection

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The liver-expressed microRNA-122 (miR-122) is essential for hepatitis C virus (HCV) RNA accumulation in cultured liver cells, but its potential as a target for antiviral intervention has not been assessed. We found that treatment of chronically infected chimpanzees with a locked nucleic acid (LNA)–modified oligonucleotide (SPC3649) complementary to miR-122 leads to long-lasting suppression of HCV viremia, with no evidence of viral resistance or side effects in the treated animals. Furthermore, transcriptome and histological analyses of liver biopsies demonstrated derepression of target mRNAs with miR-122 seed sites, down-regulation of interferon-regulated genes, and improvement of HCV-induced liver pathology. The prolonged virological response to SPC3649 treatment without HCV rebound holds promise of a new antiviral therapy with a high barrier to resistance.

Hepatitis C virus (HCV) infection is a leading cause of liver disease worldwide, with more than 170 million infected individuals at greatly increased risk of liver failure and hepatocellular carcinoma. The current standard anti-HCV therapy, which combines pegylated interferon-α (IFN-α) with ribavirin, provides sustained clearance of HCV in only about 50% of patients and is often associated with serious side effects (*1*, *2*). Therapies that target essential host functions for HCV may provide a high barrier to resistance, and thus could present an effective approach for the development of new HCV antiviral drugs. MicroRNA-122 (miR-122) is a highly abundant, liver-expressed microRNA that binds to two closely spaced target sites in the 5′ noncoding region (NCR) of the HCV genome, resulting in up-regulation of viral RNA levels (*3*, *4*). Interaction of miR-122 with the HCV genome is essential for accumulation of viral RNA in cultured liver cells, and both target sites are required for modulation of HCV RNA abundance (*3*–*5*).

Previously, we reported on potent and specific miR-122 silencing in vivo using a locked nucleic acid (LNA)–modified phosphorothioate oligonucleotide (SPC3649) complementary to the 5′ end of miR-122, which led to long-lasting decrease of serum cholesterol in mice and African green monkeys (*6*). Here, we investigated the potential of miR-122 antagonism by SPC3649 as a new anti-HCV therapy in chronically infected chimpanzees (genotype 1). Baseline measurements were obtained from four chimpanzees for 4 weeks, the last two of which included an intravenous (i.v.) placebo dose of saline. Two animals each were assigned to the high- and low-dose groups (5 mg kg^{-1} and 1 mg kg^{-1}, respectively) and were treated with i.v. injections of SPC3649 on a weekly basis for 12 weeks (Fig. 1A), followed by a treatment-free period of 17 weeks. In the high-dose group, a significant decline of HCV RNA in the serum was detected 3 weeks after the onset of SPC3649 dosing, with a maximum decrease of 2.6 orders of magnitude in HCV RNA levels 2 weeks after end of treatment (Fig. 1A). Analysis of HCV RNA levels in the liver showed a decrease of 2.3 orders of magnitude in the high-dose animals. One low-dose animal achieved a viral decline of 1.3 orders of magnitude; the other experienced fluctuations in HCV RNA levels during dosing that made evaluation of the degree of suppression difficult (Fig. 1A).

We next assessed the in vivo antagonism of miR-122 in chimpanzee liver biopsies. Mature miR-122 was detected in the baseline samples (week –4) from all animals, whereas SPC3649 was detected in RNA samples obtained during treatment and up to 8 weeks after the last dose in the high-dose animals. This coincided with sequestration of miR-122 in a heteroduplex with

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