

OPINION

Microbial endemism: does phosphorus limitation enhance speciation?

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Abstract | There is increasing evidence for the existence of unique ecosystems that are dominated by locally adapted microbiota which harbour distinct lineages and biological capabilities, much like the macrobiota of Darwin's Galapagos Islands. As a primary example of such a system, we highlight key discoveries from the Cuatro Ciénegas basin in Mexico. We argue that high microbial endemism requires a combination of geographical isolation, long-term continuity and mechanisms for reducing the intensity of horizontal gene transfer (HGT). We also propose that strong phosphorus limitation has an important role in microbial diversification by reducing the intensity of HGT.

Some of Charles Darwin's most important observations in formulating his theory of evolution were of the Galapagos finches, which were broadly similar to the finches on the mainland but had somehow diversified locally to become adapted to their own way of life according to the conditions on each particular island. Darwin's insights into the dynamic tensions between geographical dispersal and local adaptation set the stage for 150 years of studying the evolutionary ecology of macrobiota. In microbiology, developing an understanding of microbial speciation has been challenged by historical difficulties in producing a natural history of microorganisms. However, the molecular revolution changed the playing field, and we might now be able to produce a first sketch of the rules that determine the microbial origin of species¹⁻³. In this Opinion article, we propose that, just as for the finches in Darwin's isolated archipelago, the microbiota in geographically isolated habitats vividly display how evolution has played its most astounding tricks.

Endemism in microbial systems

For microbiologists, the concept of geographical isolation is a recent development. For nearly a century, microbiology has operated under the general paradigm that 'everything is everywhere and the environment selects' (REFS 4,5); in other words, most taxa have extremely broad biogeographical distributions and high levels of gene flow owing to broad dispersal that overwhelms local adaptation. However, this paradigm is coming under sharp scrutiny⁶, as increasingly

sensitive tools are being more widely used in microbial ecology.

As with the macrobiota, the diversity and development of local endemism highlight the roles of isolation and natural selection in microbial speciation. On first consideration, the issue of speciation in microorganisms is paradoxical, as microorganisms reproduce by binary fission and therefore are 'natural cloners'. Thus, every new microbial lineage would seem to be a new 'species'. However, because of the widespread occurrence of horizontal gene transfer (HGT), this is not the case². Although HGT provides an important mechanism for adaptation to local conditions by introducing novel capabilities into evolving lineages, it also potentially homogenizes genes among various populations, and even taxa, that share an environment.

Given the occurrence of HGT, we can begin to think of microbial speciation in the same way as we do for the macrobiota. For high levels of locally unique diversity to arise, there is a requirement for geographical isolation (so that local populations are not swamped by gene flow from large, outside sources), temporal continuity (so that local adaptations can accumulate) and strong selection (so that localized adaptations are environment specific). However, as with the island-specific finches of the Galapagos Islands, high levels of local microbial endemism can also arise through sympatric speciation — in which the populations that are undergoing speciation are not geographically isolated — if conditions arise that can limit gene flow through HGT. Therefore, as for the macrobiota, there remains a

dynamic tension between the promotion of local adaptation (by introducing potentially valuable variations) and its obliteration (by homogenizing the gene pool).

Is there any evidence for local endemism in microbial systems? Although in this article we focus on a particular example in the Cuatro Ciénegas Basin (CCB) in Mexico, other researchers have offered compelling evidence for locally unique microbiota⁶. For example, Demergasso *et al.*⁷ used 16S ribosomal DNA (rDNA) libraries and denaturing gradient gel electrophoresis (DGGE) to document various highly divergent halophilic bacterial and archaeal taxa from saline ponds in the Atacama Desert, South America. In cryptoendolithic samples from Antarctic dry valleys, 51 groups of bacteria and archaea were described with high levels of local patchiness and strong divergence from known taxa⁸. Whitaker *et al.*⁹ documented strong biogeographical structures in hyperthermophilic archaea from Iceland, North America and eastern Asia. Another example comes from a global study of 16S ribosomal RNA (rRNA) and 23S internal transcribed spacer sequences of hot-spring cyanobacteria¹⁰, which showed strong regional and local diversification. Such examples continue to accumulate in the microbiology literature, highlighting the increasing realization that geographical isolation is an important, but generally overlooked, aspect of microbial evolution. Therefore, as for the macrobiota, strong geographical isolation that is maintained for long and continuous periods is expected to increase levels of microbial endemic diversity.

However, although geographical isolation is necessary, it is not sufficient to produce high local endemic biodiversity. Imagine if Darwin had come upon only a single Galapagos Island: without dispersal barriers between the populations that were isolated on different islands, he would probably have found only one or two finch species, not the remarkable number of species that he did.

Genetic diversity and genetic novelty arise in all organisms by various forms of mutation. The role of recombination through HGT is to distribute non-deleterious, new alleles, mainly within a population but also, on occasion, to other taxa that share the same environment. Natural selection will then operate on this new genetic background and environmental context, fixing or deleting this new information. This process acts in a similar way to migration in homogenizing gene pools^{11,12} and thus slows sympatric speciation². HGT occurs by three main mechanisms: transformation (uptake of free DNA sequences); transduction (viral

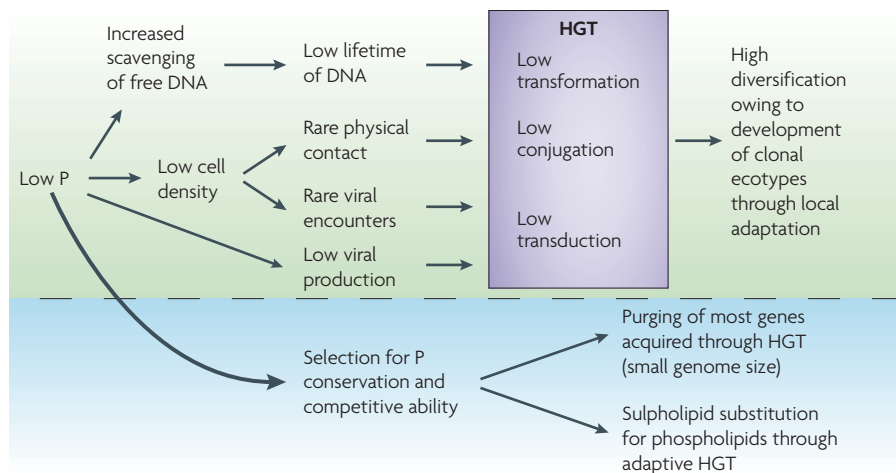


Figure 1 | The influence of phosphorus (P) limitation on horizontal gene transfer (HGT). This conceptual diagram illustrates our proposal for how strong ecosystem P limitation can not only promote local adaptation to increase P competitive ability (blue shading) but, importantly, also reduce gene flow by reducing the intensity of HGT (green shading). Thus, a scarcity of P not only drives major evolutionary change, but also enhances microbial diversification and results in clonal structure and strong local endemism in isolated, P-limited environments. Although some of these mechanisms (for example, low biomass that leads to reduced encounter frequencies) might also operate generally in most oligotrophic ecosystems, we propose that P is of special importance because of its important effects on viral proliferation and nucleotide turnover.

transfer of DNA sequences); and conjugation (cell–cell DNA exchange). What mechanisms might limit the local intensity of HGT such that local endemism can develop in geographically isolated systems?

Unfortunately, the factors that affect the intensity of HGT in particular systems are not well studied. Here, we propose that nutrient limitation, especially by phosphorus (P), plays an essential part in limiting HGT in isolated systems (FIG. 1). It seems clear that the overall densities of microbial cells will probably have major impacts on the intensity of HGT by altering the encounter rates between cells (conjugation) with free DNA (transformation) and with viruses (transduction). Therefore, it would generally be expected that low-nutrient, low-productivity systems would have reduced intensities of HGT, although this effect might be counteracted if the microbial cells become increasingly ‘competent’ (susceptible) for natural transformation as nutrients become more limiting. However, nutrient limitation probably also reduces HGT in other, less obvious, ways (FIG. 1). For example, virus production from P-limited cells can be greatly reduced¹³. Also, under severe P limitation, the turnover rates of labile pools of dissolved organic P (such as free DNA) are extremely high owing to microbial production of phosphatases¹⁴. Thus, the likelihood of HGT through transformation would be lower. Finally, strong P limitation probably places a premium on efficient cellular handling of P and therefore could restrict

the accumulation of ‘extra’ P-rich DNA into the genome by HGT (FIG. 1). Although such effects could also be manifested by other kinds of resource limitation (for example, by N or C) simply by affecting encounter rates under oligotrophic conditions, we argue that the P-rich nature of nucleic acids¹⁵, along with the obvious intimate involvement of nucleic acids in gene exchange, make conditions of severe P limitation particularly likely to reduce the intensity of HGT.

This leads us to propose that the occurrence of strong ecosystem nutrient limitation, especially by P, is important in encouraging local microbial endemism, as it reduces gene flow among locally adapting microbial lineages. For the microbiota in the CCB, resource limitation has the role of the oceanic barriers between Darwin’s Galapagos Islands.

CCB: an archipelago of microbial diversity

The CCB (FIG. 2) is located in the Chihuahuan Desert of Coahuila in north central Mexico. The high mountains that surround the basin expose upper-Jurassic to lower-Cretaceous limestone, sulphate-rich evaporites, sandstones and conglomerates of the San Marcos and Cupido formations^{16,17}. The geological history of the basin can be traced to the end of the Palaeozoic era, when the supercontinent Pangaea fragmented to form two great land masses, Laurasia (north) and Gondwanaland (south). With the separation of Laurentia (North America) from Eurasia during the Jurassic period, the North Atlantic and the

Gulf of Mexico began to open, eventually connecting to the ancient Tethys Sea through the Mediterranean to the Pacific.

In the CCB, a regional uplift that is called Coahuila Island was present throughout the late Jurassic (Tithonian) to early Cretaceous (Neocomian) periods. The sedimentary sequences that were exposed in the surrounding mountains represent fluvial and shallow marine sediments that accumulated along the margins of Coahuila Island and were deposited on older (pre-Jurassic period) igneous and metamorphic basement rocks, entrapping interstitial marine waters¹⁸. The relationship between the microorganisms in this area and the history of the marine connections will be discussed below, but the geological history of the basin suggests that aquatic habitats have had a long and continuous history in this region¹⁹.

Although a substantial, 60-year literature exists on the natural history of the CCB and its environs²⁰, it largely emphasizes the macrobiota. The CCB is thought to have “the highest level of endemic biodiversity in all of North America” (REF. 21), at least on the basis of macroscopic organisms (70 endemic species within 500 km²). As described below, “this high biodiversity appears to extend also to the microbiota” (REF. 18). As a result of the high degree of endemism and unique desert aquatic habitats, the CCB has been designated an area of protection of flora and fauna (Área de Protección de Flora y Fauna) and is administered by the Mexican Ministry for the Environment and Natural Resources (SEMARNAT; Secretaría de Medio Ambiente y Recursos Naturales). The basin has also been designated a high priority site for conservation by Nature Conservancy, the World Wildlife Fund and UNESCO (United Nations Educational, Scientific and Cultural Organization), and has been listed as a Wetland of International Importance within the international Ramsar Convention.

Within the basin, a large number of highly diverse springs (>300), spring-fed streams and terminal evaporitic ponds form an inverse archipelago (FIG. 2), in which aquatic systems are separated by sparse desert vegetation, microbial crusts and salty soils. Geothermal waters are associated with a major north–south active fault that bisects the basin. Spring heads vary in size (surface areas range from <1.0 m² to >1.0 ha) and discharge (seepage can occur at up to ~1.0 m³ per second), temperatures range from 24 to ~40°C, total dissolved solids range from 0.3 to 2.0 mg per litre and the chemistry varies from being CaSO₄ to Ca(HCO₃)₂ dominated. Older travertine hot-spring deposits and

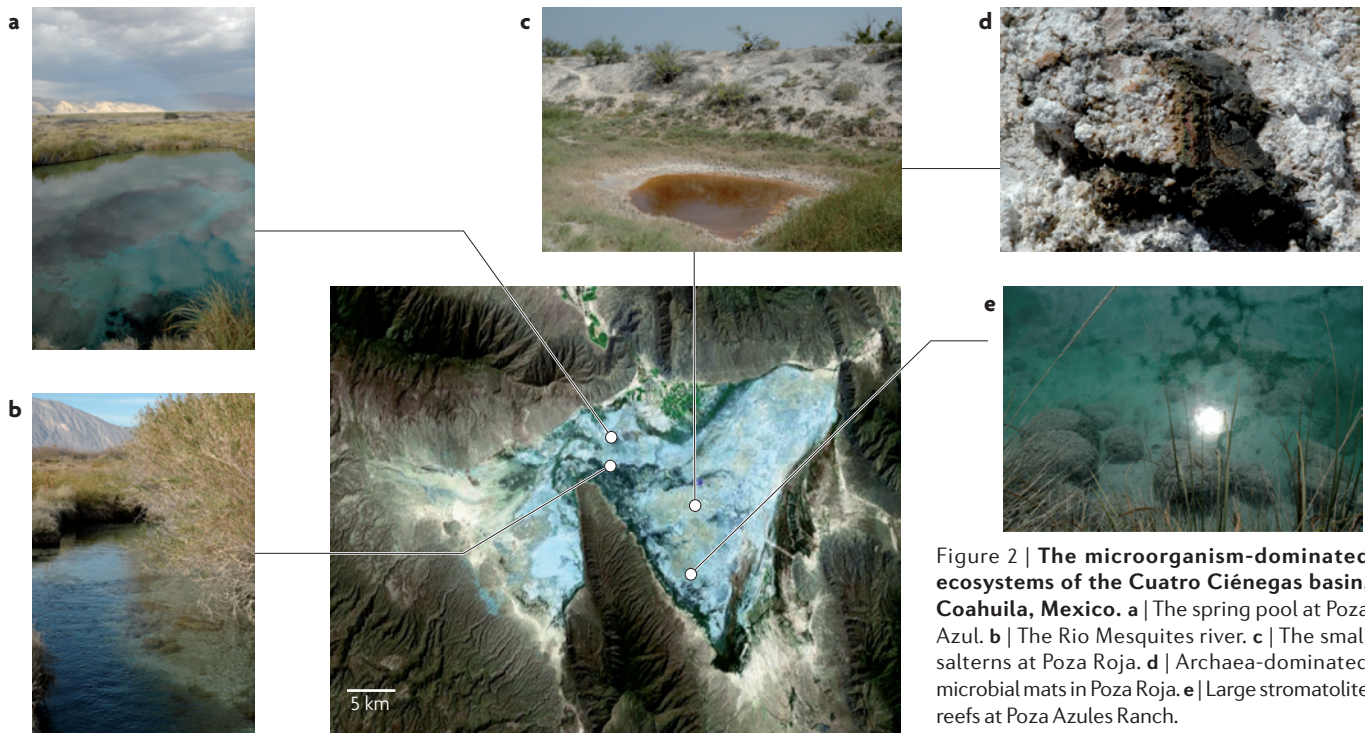


Figure 2 | The microorganism-dominated ecosystems of the Cuatro Ciénegas basin, Coahuila, Mexico. a | The spring pool at Poza Azul. b | The Rio Mesquites river. c | The small salterns at Poza Roja. d | Archaea-dominated microbial mats in Poza Roja. e | Large stromatolite reefs at Poza Azules Ranch.

lower-temperature tufa mounds are found in association with some active springs, but also occur in the older, dry portions of the basin floor, suggesting the long-term persistence of aquatic habitats in the basin²². Within these chemically and thermally heterogeneous habitats, complex microbial communities are found, many of which form thick, often laminated, mats that are composed of cyanobacteria, diatoms, bacteria and archaea. Indeed, the complex morphometries, both microscopic and macroscopic, of these mats (FIG. 2) allow them to be characterized as ‘microbialites’, similar to those that gave rise to stromatolites, the laminated microbial remains that dominate much of the Earth’s early fossil record²³.

We have begun to characterize the diverse head-spring, pond, stream, sediment, soil and subterranean sub-habitats in the basin. The results obtained so far suggest that the CCB is an isolated desert oasis of diverse microbial life that is subject to a range of major environmental stresses, but especially severe P limitation. Below, we review these findings in light of our proposed mechanism for how ecosystem conditions enhance microbial speciation in isolated ecosystems.

Evidence for isolation and continuity

We have already highlighted emerging data from a range of habitats that indicate strong biogeographical structure and thus evolutionary isolation in various groups of

microorganisms. The microbiota of the CCB offer another particularly striking example. We have studied the community composition of cultivable²⁴ and non-cultivable bacteria^{25,26} in the water column at different scales using 16S rDNA¹⁷ (FIG. 3). At the 16S level, all our studies at various scales and in contrasting environments show low dominance, with an equitable distribution of microbial species within communities and the widespread presence of new and locally endemic phylotypes²⁶. Even viral communities exhibit strong patterns of endemism in the CCB²⁷, in contrast to previous studies that suggested cosmopolitan distributions of viral taxa²⁸. These data suggest that isolation by distance and discontinuous distribution is the rule in the CCB, despite the frequent occurrence of high winds that can disperse microorganisms among neighbouring spring-fed pools or ‘pozaz’ (the pozaz range from a few metres to hundreds of metres in diameter). Our data are consistent with those of Noguez *et al.*²⁹ and Horner-Devine *et al.*³⁰, who have shown that microbial communities demonstrate non-random patterns of co-occurrence that are broadly similar to those found in assemblages of macroorganisms. In the CCB, therefore, we have found strong evidence of evolutionary isolation and local adaptation.

But how long has this isolation been in place? Answering this question is difficult, but our molecular data suggest an ancient origin that is connected to the marine ancestry of the

basin. As we were documenting the distribution and abundance of microorganisms in the CCB, we found a large assemblage of 16S data with marine affinity both in cultivable bacteria and in clone libraries from the water column, microbial mats^{18,25,31} and soils. This surprising result has been confirmed by different studies from different research groups, including a phylogenetic analysis of diatoms from the CCB³², genomic sequencing of a new species of *Bacillus* from the CCB³³ and a viral metagenomic study²⁷, all of which identify a strong marine affiliation among the CCB microbiota. Based on this, we suggest that the biota has kept its marine ancestry despite geological indications that marine waters left the valley at the end of the formation of the proto-Gulf of Mexico 90 million years ago. Thus, the CCB has two of the ingredients for hyper-diverse microbial endemism: isolation and long-term continuity. But what about local selection?

Evidence for strong local selection

We have argued that high levels of microbial endemism arise in ecosystems that are geographically isolated for extended periods, as seems to be the case in the CCB. As with macroorganisms, we would also expect to observe extensive development of unique microbiota if local environmental conditions impose strong selection pressures that drive established lineages to develop specialized adaptations to particular sub-habitats. There is abundant evidence in the CCB for a range

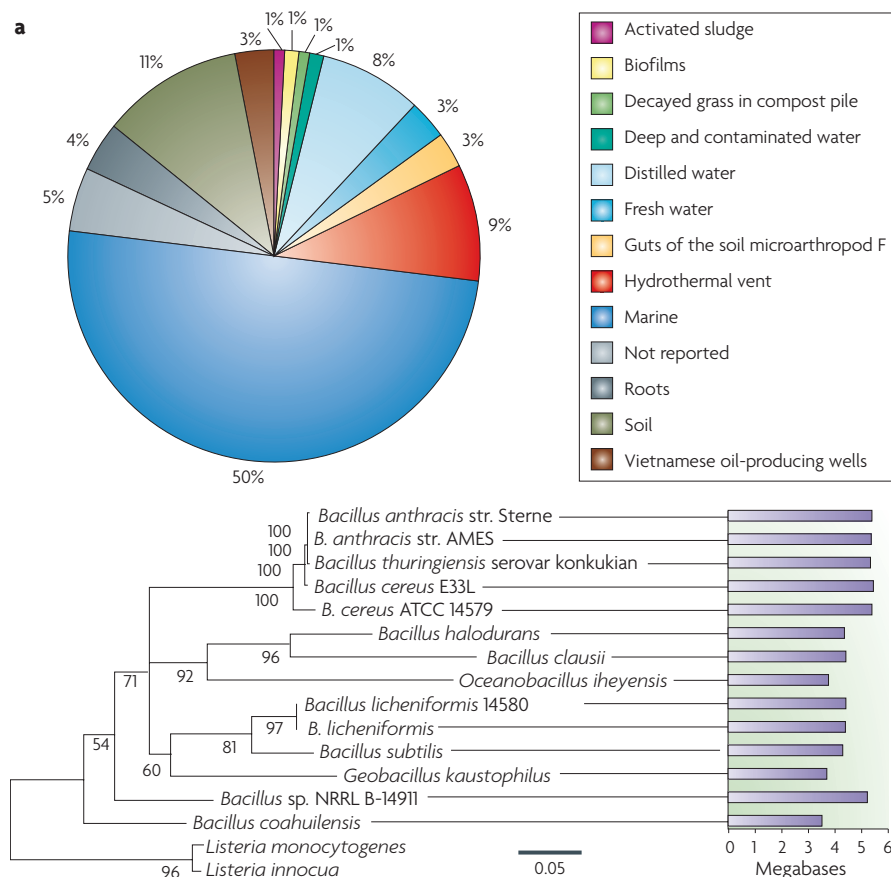


Figure 3 | Microbial diversity and endemism at the Cuatro Ciénegas basin. a | 16S ribosomal RNA (rRNA) identifications of non-cultivable microorganisms obtained in a clone library (160 total clones) from the Cuatro Ciénegas Basin. It can be seen that 50% of the clones are most closely affiliated with marine taxa in GenBank¹⁸. **b** | Phylogenetic analysis based on all sequenced genes in known genomes of *Bacillus* spp. The extreme divergence of *B. coahuilensis*, as well as its small genome compared with other bacilli, is shown.

of strong selection factors, including extreme temperature and desiccation in soil crusts (E. Lopez, unpublished data) and hypersalinity in small evaporative salterns and larger evaporative lagoons^{24,31}. Intriguingly, as discussed below, despite the strong level of microbial endemism that we have documented, HGT seems to have been the source of at least some of the novel adaptive features of the microorganisms in the CCB.

Of particular interest with respect to our proposal regarding the development of barriers to HGT, our sampling and experimentation in the CCB has shown that the ecosystems at this site are severely limited by P, having high concentrations of inorganic nitrogen (as NO₃) but low concentrations of available P (both inorganic and organic forms of P, including phosphonates)^{34–36}. Thus, the N-to-P ratios of total nutrients in these systems are extremely high (>100:1), and are higher than generally observed in other ecosystems for which comparable

data are available³⁷. This scarcity of P is reflected in the biomass of the stromatolitic microorganisms: their C-to-P and N-to-P ratios are extremely high (>1000:1 and 100:1, respectively) and greatly exceed the values that have been observed for autotrophs in other aquatic ecosystems³⁷. P limitation was directly demonstrated in a series of field experiments in which ambient PO₄ levels were enriched in stream-side microcosms that contain oncoid stromatolites (ovoid, irregularly laminated concretions formed by carbonate deposition) from the Rio Mesquites (FIG. 2). After 3–5 weeks of fertilization, stromatolites had greatly decreased C-to-P and N-to-P ratios along with increased rates of primary production and net calcification^{34–36}. P enrichment produced massive changes in the surficial microbial community: DGGE analysis based on 16S rRNA sequences for photoautotrophic microorganisms showed massive increases in the ribotypes associated with diatom taxa but much weaker responses among cyanobacteria,

suggesting that P availability particularly restricts eukaryotic proliferation in the Rio Mesquites and, perhaps, elsewhere in the basin. Overall, P enrichment reduced species diversity in the stromatolites (as indicated by Simpson's diversity index), as the community became dominated by one or two diatom taxa.

Our data indicating strong P limitation of oncoid microbialites are similar to those of Haegele *et al.*³⁸, who studied microbialites in a stream in Germany. Their oncoids bear a striking morphological similarity to those in the Rio Mesquites, yet with a different set of cyanobacteria and diatom species in the photoautotrophic community. Intriguingly, ambient N-to-P ratios in the stream were >115:1, which is similar to that in the Rio Mesquites. We therefore suggest that oncoid stromatolites could be an example of a convergent ecological structure that is driven by severe P limitation. This would also be consistent with the argument of Elser and colleagues¹⁵ for an important role for P limitation during the Precambrian period, when life on the Earth was dominated by stromatolitic microbial mats.

Several lines of evidence from recent molecular studies indicate that the low P conditions in the CCB have resulted in an evolutionary response in its endemic microorganisms. The genome of *Bacillus coahuilensis* has recently been sequenced³³ using a hybrid strategy of high-coverage (29 X) pyrosequencing and low-coverage (6 X) Sanger sequencing. At 3.35 Mb, the *B. coahuilensis* genome is the smallest *Bacillus* genome sequenced to date (including *Oceanobacillus iheyensis* and *Geobacillus kaustophilus*). The reduced genome of *B. coahuilensis* can be interpreted as a response to chronically low P supplies, as nucleic acids are rich in P³⁶. Genome reduction as a P-sparing mechanism has been proposed for autotrophs³⁹, and we argue here that it is also of importance for heterotrophs.

The genomic analysis revealed additional signs of adaptation of *B. coahuilensis* to its environment³³. One of the most striking of these is its ability to produce sulpholipids rather than phospholipids, as it lacks genes for the production of P-rich teichoic acids and the polyanionic teichuronic acids. Facultative production of sulpholipids in place of phospholipids has been observed extensively in a globally important marine clade of cyanobacteria⁴⁰ in response to environmental P limitation. Our data represent the first reported evidence of sulpholipid substitution in a heterotroph. Furthermore, our data indicate that sulpholipid production is 'hard-wired' in the *B. coahuilensis* genome. Finally,

it is interesting that, although *B. coahuilensis* has the smallest and most divergent *Bacillus* genome yet reported, it nevertheless contains nine rRNA operons (*Escherichia coli* contains just seven) and various insertion-sequence families. Therefore, it might be the case that *B. coahuilensis* is conserving P from membranes and DNA for investment in ribosomes to maintain a capacity for rapid growth (FIG. 1). In summary, *B. coahuilensis* seems to have developed niche specialization to a P-limited, oligotrophic environment.

The involvement of HGT

We have now reviewed data showing that the CCB combines geographical isolation, long-term continuity and strong local selection pressures (especially from P limitation), all of which are important in the development of high levels of endemic biodiversity. Extensive HGT provides a means for evolutionary innovation and adaptation, but can obliterate local diversification and inhibit sympatric speciation. We have argued here, however, that strong P limitation can reduce the intensity of HGT through a range of mechanisms. So, what is the role of HGT in evolution in the CCB? How clonal are the microorganisms from the CCB? How large are their populations? And is there periodic selection of clonal ecotypes or natural selection in dynamic genotypes?

Our data demonstrate both the operation of HGT in microbial evolution in the CCB and its ineffectiveness in homogenizing gene pools in locally adapting microbiota. In the genome of *B. coahuilensis*, we have provided strong evidence that key genes involved in its local adaptation have originated from several independent HGT events. For example, the *B. coahuilensis* genome contains genomic islands with a G+C content that is similar to regions found in distant *Bacillus* taxa (*Bacillus subtilis* and *Bacillus halodurans*), which is indicative of HGT among members of the same genus. HGT from more distantly related taxa is also evident. For example, the sulphoquinovose synthesis operon is absent in all known *Bacillus* taxa, but is present in *B. coahuilensis* with a structure that is identical to that found in the unicellular cyanobacterium *Synechococcus*. Additional evidence for HGT comes from the presence of a gene that encodes rhodopsin and can be traced to the filamentous cyanobacterium *Anabaena*. The mechanisms behind these HGT events are not known. However, *B. coahuilensis* has the genetic machinery for transformation, and viral metagenomic data indicate that viral transduction is in operation in the CCB, as a number of specialization genes (for example,

those involved in nucleotide recycling, phosphate starvation, photosynthesis and sulpholipid biosynthesis) have been identified in purified viral fractions from CCB microbialites (M. Breitbart, D. Hollander, A. Hoare, A. Nitti, M. Haynes, L. Dinsdale, R. Edwards, F. Rohwer, J.S. and V.S., unpublished data). This suggests that viruses are vectors for the movement of genes that are crucial for adaptation to the unique environment of the CCB.

These results indicate that HGT is active in microbial evolution in the CCB. But is HGT strong enough to break linkage disequilibrium and therefore prevent microbial speciation? In contrast to data emerging from metagenomic analysis, which show extensive genetic homogenization through HGT in an acid-mine drainage site⁴¹, our emerging data indicate that the answer is no. The contrast between an extreme environment where nutrients are not limiting and an environment that seems to be less harsh but has extreme P limitation can explain these differences. We have completed a population genetic analysis for six *Bacillus* species and five *Pseudomonas* species from the CCB that focused on five core genes (*acnB*, *gyrB*, *recA*, *rpoD* and 16S rRNA). All of the data indicate that each species is highly clonal, has been subjected to strong purifying selection and exhibits a genetic structure that indicates recent population expansion and diversification after a bottleneck. Our data for the CCB therefore resemble those reported for endosymbiotic bacteria⁴², *Mycobacterium tuberculosis*⁴³, *Yersinia pestis*⁴⁴ and some pseudomonad lineages from soil⁴⁵. However, our data for *Bacillus* spp. are unique in that strong local endemism has not been previously reported for this taxon. Importantly, our data reveal that the microbiota of the CCB, much like the finches, tortoises and other macrobiota of the Galapagos Islands, are weaving a rich and diverse web of unique local diversity in response to the particular circumstances of this desert valley. Everything is not everywhere. In some places, everything is special.

The future

Work at the CCB has just begun to unravel the full microbial diversity at this site, but much more work is required both in the CCB and in other isolated microbial ecosystems to fully understand the processes that drive microbial speciation. At the CCB, we are in the process of obtaining more genome sequences for *Bacillus* spp. (similar to *Bacillus horikoshii*) and other taxa (including *Exiguobacterium*, a new *Calothrix*-like genus, and other cyanobacteria) from which we can evaluate the generality of the

evolutionary responses discussed above. We are also developing metagenomic analyses of a microbial mat from a saltern and a stromatolite and are in the process of assessing the timescale of these events by calibrating a set of molecular clocks for five taxa (*B. coahuilensis*, the new *B. horikoshii*-like taxon, two pseudomonads and the new *Calothrix*-like genus) by several methods⁴⁶. These are being coupled to isotopic dating of sediment cores to allow us to assess the temporal stability of particular habitats. Similar studies in other isolated microbial ecosystems are also required.

We need comprehensive biogeographical data to identify the conditions under which local endemism develops. For example, the CCB seems to be isolated and severely P limited. To understand if this is indeed a general phenomenon, we need data from contrasting habitats in terms of geographical isolation and/or biogeochemical templates. The oncoid stromatolites of Germany³⁸ arise in a biogeographically homogenous region and therefore would be particularly interesting to contrast with the oncoid microbiota of the CCB. Studying geographically isolated systems that do not experience severe P limitation would also be informative; our hypothesis predicts that such systems would not present high endemism owing to strong gene flow from HGT.

However, the most conclusive data that are relevant to the ideas we propose in this Opinion would come from controlled field and laboratory experiments⁴⁷ that manipulate the key factors associated with HGT, such as isolation, physical proximity, viral abundance and P supply. For example, we predict that P fertilization should greatly increase the frequency of physical contact among microorganisms, increase the lifespan of free DNA and increase the proliferation of viruses, all of which should increase the frequency of HGT. Furthermore, after P fertilization, genomes should be more receptive to new DNA sequences by HGT because of weakened selection against increased P costs, which would further increase the likelihood of HGT. We are in the process of developing such experiments at the CCB, but other similar studies are needed in other isolated ecosystems.

Worldwide, unique microbial ecosystems are under increasing pressure¹⁸. Although some systems are under effective protection, such as the hydrothermal systems of the Yellowstone basin, elsewhere population expansion, agricultural intensification, poorly planned ecotourism, climate change and groundwater extraction are increasingly placing unique microbiota in jeopardy.

Several of these pressures, including ground-water pumping in support of alfalfa production and unregulated ecotourism, have combined to threaten the unique ecosystems of the CCB. However, increased attention to CCB conservation has allowed an increase in scientific knowledge, and a range of more effective conservation measures are slowly being put into place. Similar efforts are needed elsewhere; for example, in the geyser valleys of Kamchatka, eastern Russia, where only limited scientific data are available but increasing levels of tourist visitation are degrading local habitats. Only sustained efforts in such places will allow us to continue to discover such microbial Galapagos.

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DATABASES

Entrez Genome Project: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj>
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