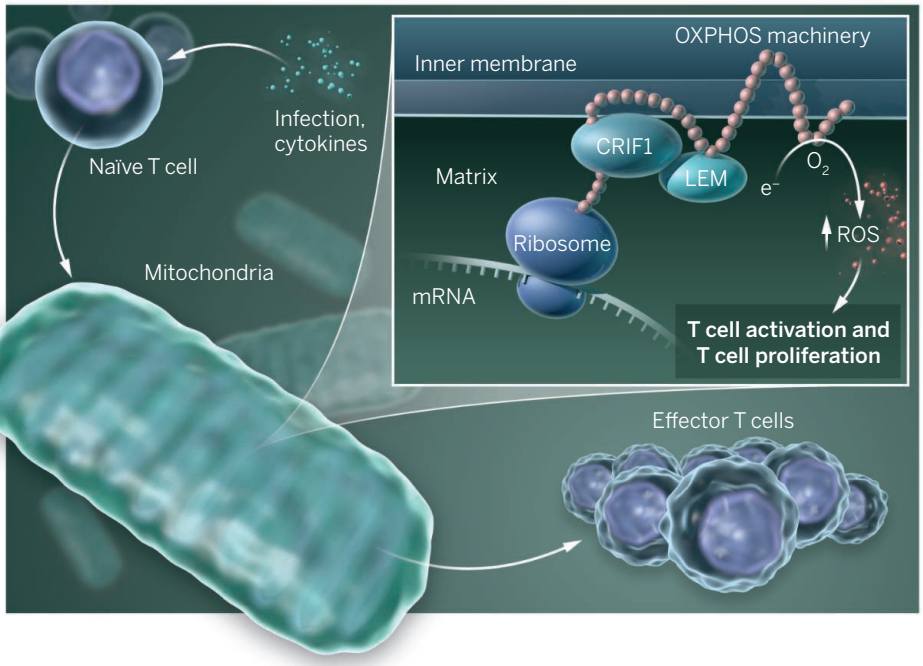

ILLUSTRATION: C. BICKEL/*SCIENCE*

Metabolic reprogramming. Upon activation, naïve T cells undergo a change in metabolism to support proliferation and development into effector T cells. Activation drives LEM expression, and its interaction with CRIF1 enhances OXPHOS and ROS production.

To identify how LEM promotes CD8 T cell proliferation, Okoye *et al.* used yeast two-hybrid screens and immunoprecipitation to pinpoint LEM binding partners. They found that LEM associates with CR6 interacting factor 1 (CRIF1), a protein required for the synthesis and insertion of polypeptides into the inner mitochondrial membrane, which is needed for effective oxidative phosphorylation (OXPHOS) (*6*) (see the figure). Loss of CRIF1 or LEM inhibited T cell proliferation. LEM-CRIF1 interaction increased both the expression of proteins necessary for OXPHOS and the activity of protein complexes of the electron transport chain. Despite the increased OXPHOS in Retro CD8 T cells, no decrease in the extracellular acidification rate, an indicator of aerobic glycolysis, was observed, suggesting a substantial net increase in adenosine 5′-triphosphate (ATP) production. Localization of CRIF1 to the nucleus has previously been linked to negative regulation of the cell division cycle (*7*). Perhaps CRIF1 recruitment to the mitochondria removes a cell cycle block, while concomitantly enhancing OXPHOS.

The production of reactive oxygen species (ROS) derived from OXPHOS is crucial for T cell activation and proliferation (*8*). Okoye *et al.* hypothesized that enhanced ROS production augments proliferation of Retro T cells. On day 8 after LCMV infection, Retro CD8 T cells had increased amounts of ROS, which was reduced by the superoxide dismutase mimetic, MnTBAP. Administration of MnTBAP in Retro mice after LCMV infection also reduced T cell proliferation and elevated LCMV titers. The authors concluded that mitochondrial-derived ROS drives CD8 T cell expansion in Retro mice.

Although this is a plausible hypothesis, further studies are needed to identify a direct link between LEM, mitochondrial ROS, and T cell proliferation. Deletion of CRIF impairs activity of all electron transport chain complexes except complex II, and promotes ROS production (*3*). It is conceivable that LEM-CRIF interactions, in addition to enhancing OXPHOS, may alter the balance in expression and activity of electron transport chain complexes, affecting ROS or redox balance in a way that promotes proliferation. How increased ROS is sustained at day 8 after infection when LEM expression is reduced, and whether the effects of MnTBAP are specific to mitochondrial ROS in CD8 T cells remain to be determined. Also, what role enhanced OXPHOS, and thus a likely increase in ATP production and carbon flux through the mitochondria, has in the observed phenotype is still unclear. Nonetheless, the finding that LEM controls T cell proliferation by metabolic reprogramming further establishes that metabolism can determine biological outcomes in the immune system. ■

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MICROBIOLOGY

Flexible gene pools

Rapid genetic exchange leads to mosaic genomes in cyanobacterial populations

By **Michael M. Desai**[1] *and* **Aleksandra M. Walczak**[2]

Evolution acts to shape genetic variation within populations. However, in microbial communities, it is often surprisingly difficult to characterize what the "population" actually represents. This makes it hard to interpret the diversity often observed in microbial communities. To what extent is diversity within a microbial species (or operational taxonomic unit, typically defined to span ~3% 16*S* rRNA sequence divergence) representative of one population occupying a single ecological niche? Do multiple sequence clusters in a given population represent distinct functionally diverse strains? And by how much does genetic exchange blur the boundaries of strains and species? On page 1019 of this issue, Rosen *et al.* (*1*) show that rapid genetic exchange maintains extensive diversity of mosaic genomes in a cyanobacterial biofilm community, despite the action of selection on many individual loci.

Naturally occurring microbial diversity has traditionally been studied with approaches based on short but conserved genomic regions such as 16*S* ribosomal RNA, or on sequencing a few distinct loci (multilocus sequence typing, MLST). These methods provide a relatively coarse view of the population structure. The results suggest that microbial populations often consist of multiple distinct sequence clusters, each of which contains closely related genotypes. These sequence clusters can be interpreted as functionally distinct "ecotypes" that occupy specific ecological niches (*2*).

Advances in sequencing technology now make it possible to probe microbial diversity at much finer resolution. However, it is difficult to understand the overall structure of genomes based on short-read sequences from natural microbial populations. In particular, it remains unclear whether extensive diversity across a microbial genome in whole-population sequencing data arises from a small number of distinct but coherent strains (see the figure, panel A) or

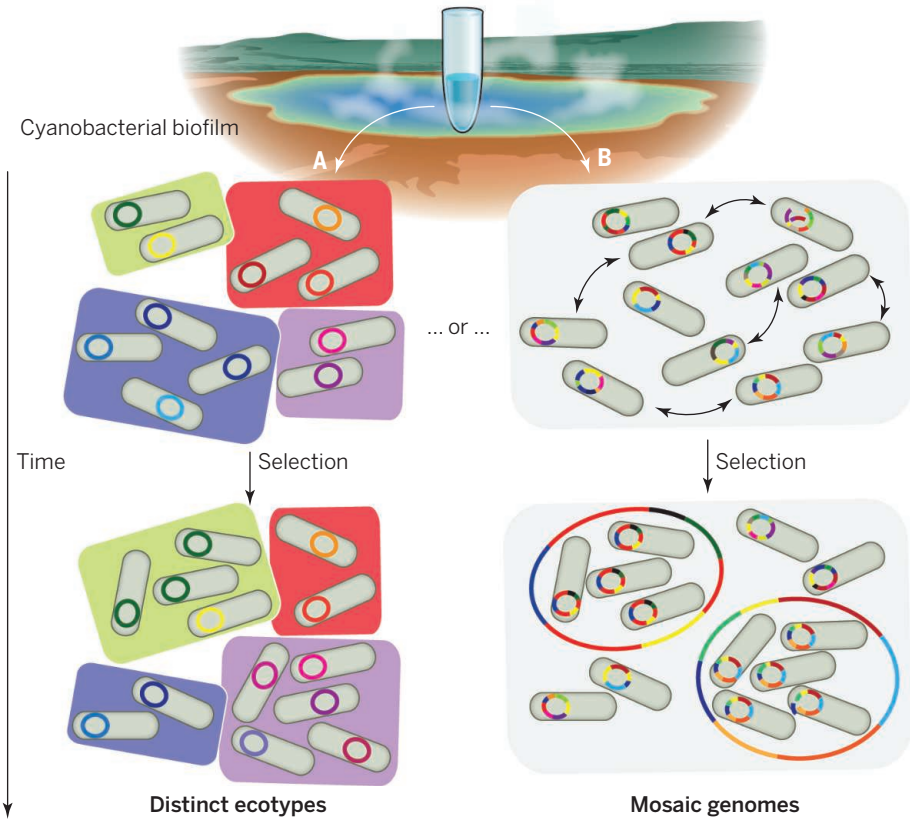
whether there is a more flexible gene pool, with individuals freely exchanging parts of their genome (*3*) (see the figure, panel B).

To address these questions, microbial ecologists have recently used isolate and single-cell sequencing methods, which circumvent the limitations of short-read sequencing of mixed communities. Studies of isolate genomes from *Vibrio* bacteria (*4*) and thermophilic archaea (*5*) show that recombination maintains genome-wide diversity despite selective sweeps that purge genetic variation at individual advantageous loci. A recent study of ~1000 individual *Prochlorococcus* genomes showed that these communities consist of a set of coherent strains. Each strain is characterized by specific alleles of a set of core genes (a conserved "genomic backbone"), along with a smaller group of strain-specific flexible genes (*6*). These strains fluctuate in frequency with time but coexist stably over long time scales, supporting the idea that they represent distinct ecotypes (see the figure, panel A).

Rosen *et al.* now introduce an alternative framework to analyze the structure of *Synechococcus* cyanobacterial biofilms. They begin by sequencing whole-population samples at multiple specific loci, an extension of traditional MLST. By sequencing each locus relatively deeply (sampling many individual cells), the authors can characterize within-locus genetic variation. This deep-sequencing approach is related to methods used in other systems (such as laboratory evolution experiments), but is relatively uncommon in microbial ecology. To analyze the data, the authors first use a traditional measure of correlations between sites, known as linkage disequilibrium, to estimate recombination rates. They then study the joint distribution of allele frequencies at two sites (a less commonly used measure of how a mutation at one site correlates with a mutation at another site) as a function of the distance between these sites.

This combination of data and analysis sheds light on both the structure of the variation in the population and the evolutionary forces that lead to this structure. If the community consists of coherent strains evolving in well-separated niches, allele frequencies among members of the same strain should be correlated even at long genomic distances. Instead, Rosen *et al.* find that these correlations decay rapidly with distance, arguing for a freely recombining gene pool without coherent strains corresponding to stable ecotypes (see the figure, panel B).

[1]Departments of Organismic and Evolutionary Biology and of Physics, Harvard University, Cambridge, MA 02138, USA. [2]CNRS, Laboratoire de Physique Theorique, Ecole Normale Superieure, 75 231 Paris Cedex 05, France.
E-mail: awalczak@lpt.ens.fr



Structures of genomic diversity in a cyanobacterial biofilm. Diversity can be organized into coherent strains, each adapted to a specific ecological niche (**A**). Alternatively, extensive genetic exchange can lead to mosaic genomes despite the action of selection on many individual loci (**B**). Rosen *et al.* show that patterns of diversity in a cyanobacterial biofilm community support the latter view.

To probe the effects of natural selection in these populations, Rosen *et al.* exploit methods for correcting PCR and sequencing errors (*7*) to focus particularly on rare alleles. The idea is that selection acts to rapidly increase or decrease the frequency of mutations, so that alleles are either new and rare or old and common. Frequency correlations between sites will be strong for rare alleles, which are new and have not had time to recombine. Without selection and recombination, we expect the opposite effect: Only high-frequency alleles should be correlated. By comparing site frequency correlations at different distances as a function of allele frequency with neutral expectations, Rosen *et al.* can narrow down the possible evolutionary scenarios.

The results from this analysis show that variable selection pressures act on many individual loci. These selection pressures likely include frequency-dependent or local selection pressures that change the frequencies of individual alleles (*8*, *9*). But instead of creating coherent strains, these selection pressures only structure diversity on local genomic scales, with recombination dominating on longer scales. This leads to a highly diverse "quasisexual" population without distinct genome-wide ecotypes (see the figure, panel B).

This evolutionary scenario is much more complex than is typically envisioned by evolutionary models. Nevertheless, these cyanobacterial communities are ultimately subject to the same laws of population genetics as other systems. Rosen *et al.* show that with appropriate theoretical and experimental methods, it is possible to characterize the evolutionary forces acting in these cyanobacteria. The authors do so with much less raw sequencing data than would be required in the case of single-cell genomics. Their approach shows how carefully designed empirical methods, guided by theoretical expectations, make it possible to tease apart the ecological and evolutionary forces that shape complex patterns of diversity in microbial communities. ■

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