Title: Uncovering the Hidden Complexity of Multicellular Magnetotactic Bacteria

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Standfirst: New research combines genomics, microscopy, and isotopic labeling to show that Multicellular Magnetotactic Bacteria (MMB) have a surprisingly complex multicellular lifestyle, forming obligately multicellular consortia of genetically-diverse cells with rudimentary division of labor.

Primer: The evolution of multicellularity was transformative for life on Earth, fueling biological innovation and underpinning the origin of novel ecosystems. Bacteria pioneered this major evolutionary transition, first evolving multicellular bodies over three billion years ago [1]. Despite this long history, bacterial multicellularity is often overlooked, as bacteria rarely form the large, long-lived, obligately multicellular bodies we generally associate with multicellular life. However, in recent years, microbiologists have begun to reveal the rich social lives of multicellular bacteria, from the wolf-pack hunting behavior of myxobacteria [2] to bacterial collectives that have evolved adaptive immunity against viruses [3]. In this issue of *PLoS Biology,* Schaible *et al*. use a suite of cutting-edge techniques to provide an unprecedented look into the hidden complexity of a fascinating multicellular bacterium - Multicellular Magnetotactic Bacteria (MMB).

MMB are most often found in low-oxygen, sulfur-rich marine sediment. These bacteria possess the remarkable ability to orient themselves along the Earth's geomagnetic field lines, allowing them to navigate towards their preferred low-oxygen habitats [4]. This behavior is made possible by the presence of specialized organelles called magnetosomes. These lipid vesicles contain either magnetite (Fe_3O_4) or greigite (Fe_3S_4) crystals, which are biomineralized within the

organelles that are arranged in chains along the cytoskeleton of each MMB cell. This creates a magnetic dipole within the cell that aligns it with the Earth's geomagnetic field, aiding navigation. Researchers like Schaible *et al*. also leverage this unique feature and use magnetic enrichment to isolate these otherwise uncultivable organisms from sediment samples.

In this paper, Schaible *et al.* show that, in addition to their incredible range of magnetotactic, aerotactic, phototactic, and chemotactic behaviors [5], MMB are unique in another aspect. They are the first example of an organism that is both obligately multicellular, lacking a unicellular stage, that is also composed of genetically-diverse cells (as opposed to every cell being essentially identical). When MMB cells are removed from their consortia, they die, suggesting that these organisms do not have a persistent single cell life history stage [6]. Multicellularity typically develops one of two ways: obligately multicellular organisms grow when genetically-identical cells stay together after division, and facultative multicellular organisms grow as single cells that form groups via the aggregation of potentially genetically diverse cells [7,8]. To uncover the multicellular nature of MMB, Schaible *et al.* employed a range of cutting-edge techniques.

Using microscopy, the team observes MMB consortium that appear to reproduce by dividing along an acellular region at their center, producing two multicellular daughters (Figure 1). Given that MMB grow via cells 'staying together' after division, one would expect the consortium to be clonal, yet this is not the case. Using metagenomics, Schaible *et al*., uncover a stunning amount of genetic diversity within MMB consortia – up to 100x more diversity than was found within clonal multicellular controls! This suggests that the genetic variation within MMB consortia cannot be explained by *de novo* mutation, but instead must arise from another mechanism, such as between-group cellular exchange.

Cells within MMB also exhibit metabolic differentiation. The authors examined MMB consortia with Nanoscale Secondary Ion Mass Spectrometry (NanoSIMs), a powerful technique for quantifying fine-scale cellular metabolic activity. Within a single MMB consortium, the researchers observed "hot spots" where specific cells metabolized isotopically labeled carbon substrates as well as heavy water at increased rates relative to other cells in the same consortium. The authors then demonstrate that protein synthesis also varies across cells, concentrated near the acellular central region, using Biorthogonal Noncanonical Amino Acid Tagging (BONCAT), an

approach in which consortiums uptake noncanonical amino acids that are used during protein synthesis and are later detected via fluorescence microscopy [9].

What does this metabolic heterogeneity mean? The authors speculate that MMB cells may be metabolically differentiated. For example, certain cells may specialize in the metabolism of specific substrates, like acetate, sharing the resulting resources with other cells via the central acellular space. This type of metabolic specialization is a hallmark of a division of labor, where different cells perform distinct tasks required for overall organismal function. While full-fledged division of labor remains to be demonstrated, this paper provides tantalizing hints that MMB have achieved a remarkable degree of functional integration.

The findings of Schaible *et al.* open up exciting new avenues for exploring bacterial multicellularity. The observation of non-clonal MMB consortia raises the question of how genomic heterogeneity is generated and maintained during MMB replication. What does the complete multicellular life cycle look like? Do groups fuse to one another, and if so, when and how? Being able to bring these organisms into the lab and grow them in stable monoculture may be an important next step, as it would rule out a hidden unicellular phase. Future work should also fully explore the intracellular division of labor - what molecules are being exchanged, and is this dependent on the genetic diversity of cellular constituents?

Perhaps the most important implication of this work is that it expands our conceptual understanding of the potential diversity of basic multicellular life cycles. Until now, we have generally assumed that obligately multicellular organisms would be clonal, and all prior examples support this assumption [10]. This is important: because all other obligately multicellular organisms are also clonal, it has proven difficult to disentangle the role of each of these traits on the evolution of multicellularity. Prior research suggests that both obligate multicellularity and clonality may independently play a role in facilitating multicellular adaptation [10,11], and novel model systems that decouple these traits may prove instrumental in testing these hypotheses.

Overall, recent work has shown that bacterial multicellularity is fundamentally different than what we have come to expect from large, long-lived, functionally integrated multicellular eukaryotes. From the synchronized swimming of magnetotactic bacteria to the coordinated predation of myxobacteria, it is clear that bacteria have evolved a wide range of social behaviors uniquely suited to their specific ecologies. As we continue to explore the frontiers of bacterial

multicellularity with an ever-expanding toolkit, we are likely to uncover even more surprises that will challenge our assumptions about the nature and potential of multicellular life on Earth.

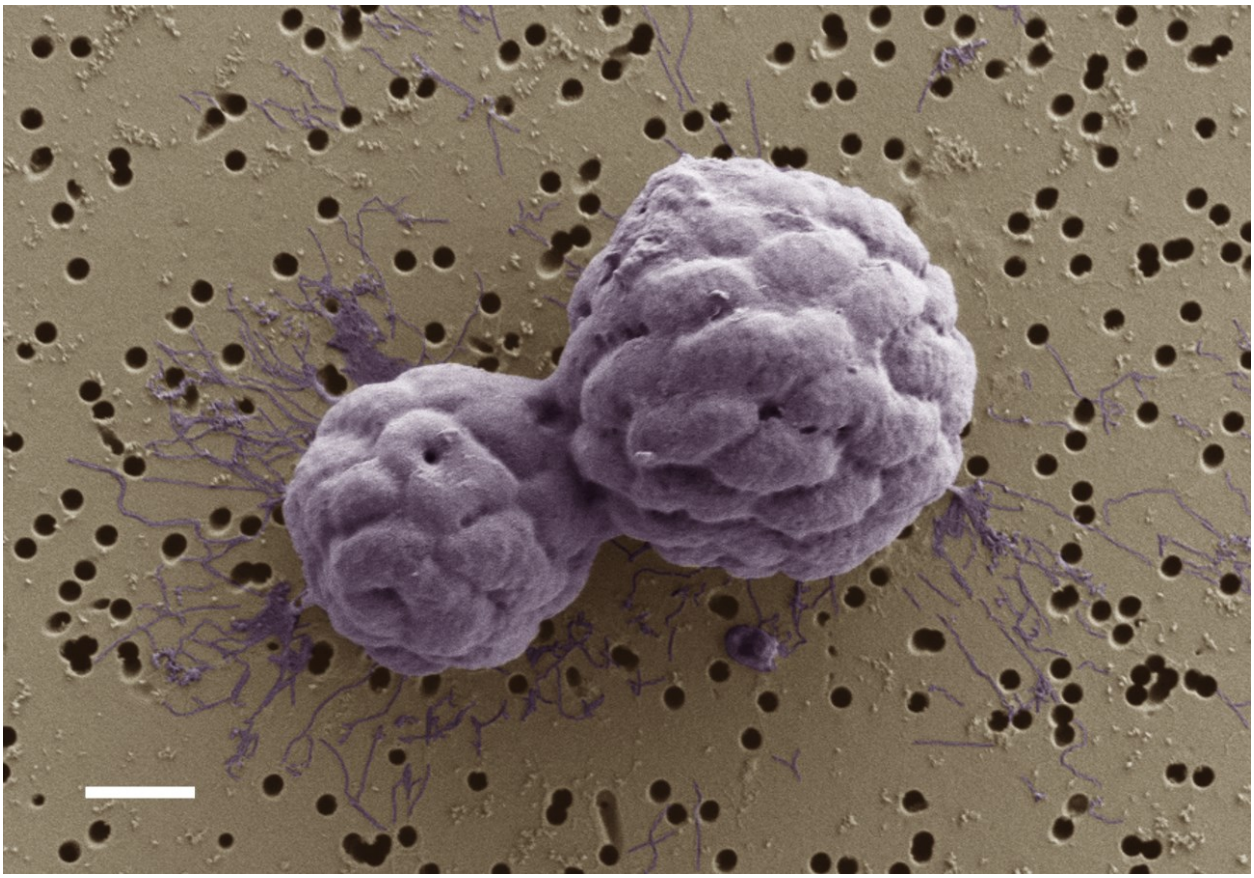

Figure 1. Multicellular Magnetotactic Bacterial collectives, possibly caught in the act of division. Scale bar is 1 μm. Image courtesy of George Schaible, Montana State University.

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