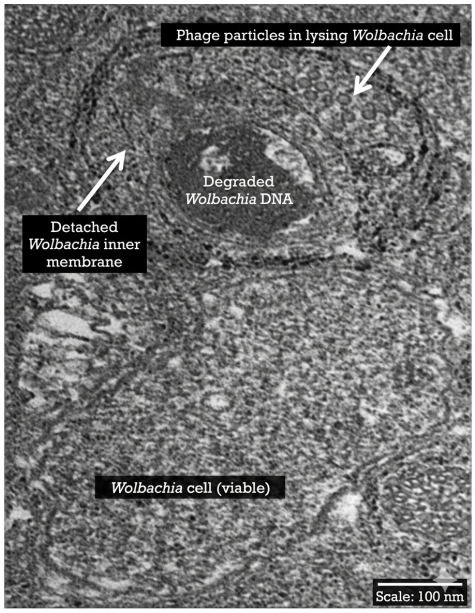
OVERVIEW

For thirty years, the lab has studied the border where the visible life ends and the microbiological world begins. Biology has long taught for centuries to see a plant or an animal as a solitary actor, a lonely island of its own cells and DNA. Yet when we look closer, that island reveals itself to be a metropolis. From the evolutionary gears that turn within an insect's reproductive tract to the gut microbial conversations that dictate human health, our lab's work has helped lead a new reality of the natural world: there is no such thing as a solitary organism. Visible life forms are holobionts - communities where the host and invisible microbes connect into a structural and functional unit of life that will be scientifically databased for the world and quantitatively interrogated (Science 2024).

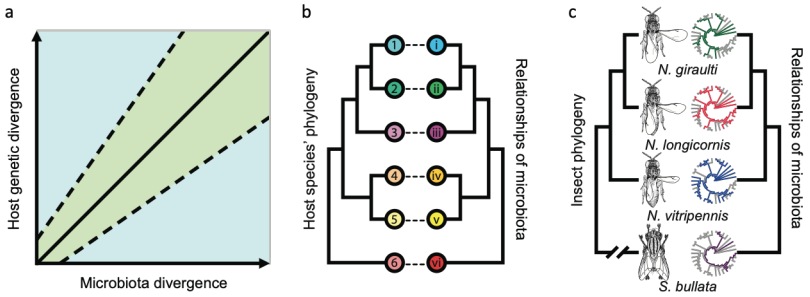
THE MOLECULAR BASES OF A GLOBAL SYMBIOTIC ADAPTATION

In 2017, we solved a seminal quest in the molecular biology of symbiosis: the genetic basis of cytoplasmic incompatibility (CI) in which animal sperm from a symbiotic male cause embryonic death upon mating with an aposymbiotic female. This phenomenon, which dictates the reproductive success of billions of insects worldwide, was a "black box" until we unmasked the causative *cifA* and *cifB* genes (Nature 2017, PNAS 2018). These genes are effectively endpoints of a nested symbiosis - viral stowaways from bacteriophage WO residing within the endosymbiotic bacteria *Wolbachia* that dwell intracellularly in insects worldwide (see figure). This breakthrough had immediate translational impact because *Wolbachia*-mosquitoes are now deployed globally to control dengue and Zika transmission. By solving the genetic levers of this symbiotic adaptation, we provided future avenues to make super *Wolbachia* strains that are more efficacious at fighting dengue and Zika. We further demonstrated that transgenic dual expression of the c*ifA* and *cifB* genes can synthetically recapitulate the symbiont's adaptation - the first case of engineering animal reproduction to depend on phage genes. We meticulously found that the genes reprogram sperm epigenetics, including long non-coding RNA and nucleoprotein complexes, to bestow their paternal-effect lethality and transmission advantage (Science 2024, PLOS Biology 2024). To our knowledge, this is the first discovery of bacteriophage proteins that modulate eukaryotic non-coding RNA. We also found the first male-killing gene from this nested symbiosis; it acts as a surgical tool for sex-specific lethality by *Wolbachia*, causing male embryos to perish during early development (PLOS Pathogens 2019). This work revealed that even a single synonymous nucleotide change in *wmk* can completely ablate the killing phenotype, highlighting the exquisite precision of a silent mutation in inter-domain warfare (eLife 2021). These findings resolved major questions for the symbiosis, entomology, and evolutionary biology fields, and they now provide a molecular blueprint for using symbiotic genes to crash insect vector and pest populations that can halt the global transmission of agricultural and human diseases.



The most common bacterial symbiont in animals – *Wolbachia* - occurs worldwide in arthropods. The image shows an electron micrograph of a *Wolbachia* cell being lysed by its bacteriophage WO. A healthy *Wolbachia* control cell is shown below it. We discovered that bacteriophage WO proteins (CifA/B) uniquely interact with eukaryotic macromolecules to shape a symbiosis that is fundamental to arthropod ecology, evolution, and vector control against major human diseases.

THE SYMBIOTIC BASES OF HOST SPECIATION



We coined the term and formulated "phylosymbiosis" to support a hypothesis-driven and statistical framework for the characterization of a flourishing, cross-system trend in host-associated microbiomes. (a) The central prediction of phylosymbiosis is that divergence in host species is positively correlated with ecological differentiation of the host-associated microbiomes. Thus, more divergent host species will have more divergent microbiomes, reflecting an influence host evolutionary history on microbiome assembly (b) Parallel trees or differentiation between the host phylogeny and the microbiome relationships is a key test of phylosymbiosis that we developed a quantitative tool for (c) Schematic of a real data example from our model study system in which host species evolution recapitulates the relationships of the microbiomes between the host species.

A major line of research in my laboratory has its foundations in Charles Darwin's 1859 *Origin of Species* and Lynn Margulis's late 20th century advocacy for the prominent role of microbial symbionts in host speciation. We took on this challenge when it was left to storytelling, put it to the test, and asked over thirty years how do closely related animals vary in their bacterial symbiont communities? Does host genetic variation affect these differences? And what are the roles of microbial symbiosis in the formation of new host species? We provided the first definitive case and later a second case that microbiomes cause reproductive isolation between closely-related insect species (Nature 2001, Science 2013). This work validated decades-old speculation about symbiosis in speciation, showing members of the microbiome are not passive passengers but active drivers of macroevolutionary processes. We coined and experimentally established the pattern of 'phylosymbiosis' (Nature 2013, see figure above), when host phylogenetic relationships mirror their microbiome relationships. We further showed phylosymbiosis for bacteria, fungi, and viruses across diverse animal systems including insects, mice, humans, and their hominid ancestors (PLOS Biology 2016, 2024). This collective work uncovered a major evolutionary trend, namely that microbiome assembly in hosts is not stochastic but often reflects predictable evolutionary principles that can directly affect the formation of new animal species. Critically, our microbiome transplant experiments between closely-related insect and mammalian species demonstrated that phylosymbiosis has major, functional consequences: mismatched host-microbiome pairings reduce survival and/or performance in half, demonstrating selective pressures can shape these associations (PLOS Biology 2016, mBio 2019).

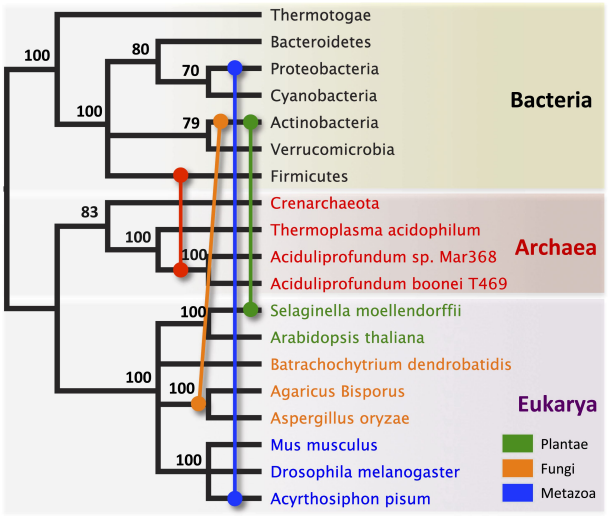
THE HUMAN MICROBIOME AND HEALTH DISPARITIES

In a major shift from our insect holobiont studies, we established over a ten year period that human social lives and lived experiences are recurrently etched into human microbial ecology - providing a biological basis for understanding how social determinants may link with health disparities. Gut and oral variation across human bacteria, viruses, and fungi in the United States persistently link with self-identity and social group (PLOS Biology 2018, 2022, 2023, 2025). These signatures appear as early as three months of age, enduring even after controlling for the traditional effects of diet and geography. Moreover, while bacteria have long dominated the gut microbiome field, my lab also sought to evaluate and elevate the fungal kingdom - the mycobiome - to a new appreciation in human gut biology. We recently uncovered the first ternary relationships between human genetic variation (148 loci), gut fungi, and chronic disease (PLOS Biology 2025). We revealed that human genetic variation exerts precise control over specific residents, such as the yeast *Kazachstania*. We causally linked this fungus to cardiovascular disease risk in a Mendelian randomization analysis, moving the mycobiome from a neglected bystander to an intriguing, new actor in cardiology. Additional analysis using case-control studies and two of the largest repositories of electronic health records globally found that among 900 microbiome-associated genetic variants in the human genome, many have associations with neurological,

metabolic, digestive, and circulatory diseases (PNAS 2022). This body of work represents a transition from my traditional insect symbiont work to human health, social sciences, and microbiology. By mapping the intricate feedback loops between human self-identify groups and multi-kingdom symbioses, we forged a holistic blueprint for more personalized diagnostics of chronic disease that treats the human body as the multi-kingdom ecosystem it truly is, across social and environmental variation.

HORIZONTAL GENE TRANSFERS AND THE FIRST ARCHAEA ANTIBIOTIC

The Universal Tree of Life is often drawn with distinct, bifurcating branches, but my research revealed it to be more of a porous scaffold, breached by the lateral movement of genes with potent innovations. While the exchange of DNA between two domains is a known curiosity, the serial transfer of a single genetic sequence across all three domains of cellular life is a profound evolutionary rarity. It suggests a selective pressure so immense that it trumps the inherent genomic costs of integrating foreign code. We identified such a rarity in an antibacterial lysozyme - a molecular blade that splits open bacterial cell membranes. Our work discovered for the first time that a gene was borrowed independently by archaea, eukaryotes, and even viruses in multiple, independent events (eLife 2014). This is not random genomic noise. These transfers occurred between disparate taxa that share the same intimate ecological niches, suggesting that symbiosis and proximity are the primary catalysts for these cross-domain leaps. The functional logic of this gene theft is clear: survival. We notably characterized the transferred lysozyme as the first antibiotic from an archaeon - a peptide from a hydrothermal vent dweller that kills bacteria with dose-dependent precision. Moreover, when these archaea encounter bacteria, they mount a transcriptional defense, resulting in a measurable increase in Malthusian fitness. This discovery provided a new foundational benchmark for horizontal gene transfer. It proved that a potent antibacterial gene can colonize all three domains of the Universal Tree of Life because non-bacterial taxa are under constant, relentless selection to either cooperate with or, more often, clash with the microbial world that surrounds them.



An antibiotic gene that first arose in bacteria encodes an enzyme that breaks down the outer casing of a bacterial cell: a step that is required for a bacterium to reproduce and divide in two. We found an evolutionary singularity at the time that copies of this gene 'jumped out' of bacteria into all domains of life (bacteria to archaea that live in host, deep sea vents, bacteria to plants, bacteria to fungi, bacteria to animals) at least four times. https://doi.org/10.7554/eLife.04266

Impact: Our collective work exemplifies transformative science that both solves longstanding mysteries and opens entirely new research directions. The discoveries provide mechanistic understanding where only phenomenology existed, demonstrate cross-system generality where only isolated observations were known, and deliver translational applications from fundamental insights. I have been instrumental in establishing holobiont biology as a unifying discipline (PLOS Biology 2015, Science 2024), synthesizing concepts of hosts and their microbial communities as eco-evolutionary and functional units. This theoretical framework, supported by empirical discoveries, affects how we approach problems from agricultural productivity to human disease. Finally, I direct the internationally-awarded One Health Microbiome Center at Penn State (550+ members, 125 faculty from 42 departments) and have been recognized with University, Society, and Journal awards spanning high impact research, education, and citations.