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Shifts from intracellular to extracellular symbiosis in attine gut symbionts

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Metagenome sequencing has revolutionized our understanding of symbiotic bacteria of ants and other social insects, but almost nothing is known about the interaction mechanisms with host cells and the interaction dynamics during ontogeny. Recent work has revealed that both *Wolbachia* and specific Entomoplasmatales are obligate symbionts of *Acromyrmex* leaf-cutting ants, associated with the cytoplasm but also free-living in the gut lumen. We used confocal and electron microscopy to analyze the spatial localization and detailed ultrastructure of these bacteria in *Acromyrmex echinatior* and discovered that both symbionts are present in many tissues of adult workers (brain, muscles, digestive system, fat body), except for the midgut. Both *Wolbachia* and the Entomoplasmatales species change their ultrastructural organization when they shift between an intracellular and extracellular lifestyle. Bacteria with dense cytoplasm and without an outer membrane of host origin were observed in the intestinal lumen of workers and they were also found by confocal microscopy in several tissues of larvae. As the epithelium of *Drosophila* larval guts is known to completely degenerate and be replaced by imaginal cells during the prepupal stage, we expect that pupal metamorphosis is a critical transition stage towards extracellular colonization of the intestinal lumen of workers. We assume that further advanced electron microscopy will allow us to gain better understanding of the mechanisms that allow these transitions in bacterial life-style. Both *Wolbachia* and Entomoplasmatales are likely to be mutualists of leaf-cutting ants, so that electron microscopy may also offer further insight in the adaptive function of these bacteria and in possible constraints in expressing these functions. For example, the presence of a thick (ca. 1 µm) cuticle layer at the luminal side of the hindgut epithelium suggests that the bacterial shift from an intracellular to an extracellular niche may be inhibited in some parts of the digestive system.