Evolutionary Instability of Symbiotic Function in *Bradyrhizobia japonicum*

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Bacterial mutualists are often acquired from the environment by eukaryotic hosts. However, both theory and empirical work suggest that this bacterial lifestyle is evolutionarily unstable. Bacterial evolution outside of the host is predicted to favor traits that promote an independent lifestyle in the environment at a cost to symbiotic function. Consistent with these predictions, environmentally-acquired bacterial mutualists often lose symbiotic function over evolutionary time. Here, we investigate the evolutionary erosion of symbiotic traits in *Bradyrhizobium japonicum*, a nodulating root symbiont of legumes. Building on a previous published phylogeny we infer loss events of nodulation capability in a natural population of *Bradyrhizobium*, potentially driven by mutation or deletion of symbiosis loci. Subsequently, we experimentally evolved representative strains from the symbiont population under host-free *in vitro* conditions to examine potential drivers of these loss events. Among *Bradyrhizobium* genotypes that evolved significant increases in fitness *in vitro*, two exhibited reduced symbiotic quality, but no experimentally evolved strain lost nodulation capability or evolved any fixed changes at six sequenced loci. Our results are consistent with trade-offs between symbiotic quality and fitness in a host free environment. However, the drivers of loss-of-nodulation events in natural *Bradyrhizobium* populations remain unknown.

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