Rhizobia: Tractable models for bacterial evolutionary ecology

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Much of what we know about the evolution of bacterial populations comes from cultured experimental populations in the lab. These experiments have contributed immense knowledge of bacterial metabolism, adaptation, coevolutionary dynamics, recombination, and mutation (reviewed by O'Malley et al., 2015). We have more to learn from experimental evolution, and great strides continue to be made by harnessing microbial populations in the lab, including quantitatively linking metabolic mutations to population and community dynamics (Harcombe et al., 2014), simulating the evolution of multi-cellular life (Ratcliff et al., 2015), exploring meta-population dynamics (Nahum et al., 2015), and revealing the dynamics of the mutational process that is ultimately the source of all genetic variation (Blount et al., 2012; Tenaillon et al., 2016).

In recent years, however, the field of microbial evolution has stepped out of the lab and into the field – a shift facilitated by efficient and affordable whole-genome sequencing. Sampling microbial populations from the field and analyzing them in an explicit population genomics framework is just beginning to resolve several long-standing issues in microbial evolution (Krause and Whitaker, 2015). For example, when examined closely, everything is decidedly *not* everywhere (Whitaker et al., 2003; Vos and Velicer, 2008; Reno et al., 2009; Hahn et al., 2016). Like eukaryotes, Bacteria and Archaea speciate into cohesive phylogenetic groups that are united by recombination and differentiated from other ecologically-distinct groups (Shapiro et al., 2012; Cadillo-Quiroz et al., 2012). Finally, locus-specific sweeps (versus genome-wide or clonal sweeps) during bacterial adaptation to the environment may be common (Shapiro et al., 2012; Epstein et al., 2012; Bendall et al., 2016; Klinger et al., 2016).

*Rhizobia as models of mutualism coevolution:* Rhizobial bacteria, which fix atmospheric nitrogen (N) in the roots of their host legumes, have enjoyed a particularly long history of interest in agriculture and ecology (Fred et al., 1918). In part, this rich history exists because of the importance of legume-rhizobium symbiosis to N-cycling in both managed and natural ecosystems (Vitousek et al., 1997; Graham and Vance, 2003). Perhaps just as important, though less-appreciated, is the ease with which rhizobia can be sampled and (roughly) identified to species in nature. Because these symbionts can be found predictably within the charismatic root nodules of their host plants, they can be easily cultured in isolation from the rest of the soil community.

Thus populations of rhizobia may be fairly easily sampled from their hosts, and with less interference by the rest of the community, even without sophisticated selective media. Despite dramatic increases in the affordability and ease of community sequencing, the simplicity of sampling a taxonomically-limited set of rhizobium lineages gives rhizobia an advantage, compared to most other soil bacteria, which are relatively difficult to isolate given the diversity of species in the community. Other systems in which population genetics is more tractable include animal and plant pathogens (*e.g., Pseudomonas*; reviewed by Guttman and Stavrinides, 2010; McCann et al., 2012; Stukenbrock and Bataillon, 2012; Darch et al., 2015; Caballero et al., 2015), social bacteria (Wielgoss et al., 2016), and relatively dilute or less diverse aquatic communities (Shapiro et al., 2012; Cadillo-Quiroz et al., 2012; Garcia et al., 2015). These systems are united by the relative ease with which a particular bacterial species can be efficiently isolated from other community members and thus studied in a population genetics

framework, which requires studying tens to hundreds of individuals from a species, sampled within and/or among populations.

In the past two decades, rhizobia have experienced newfound attention after a handful of seminal papers highlighted the utility of these tractable systems for addressing fascinating questions about mutualism evolution (Parker, 1999; Burdon et al., 1999; Denison, 2000; Simms and Taylor, 2002). These papers served to focus a new generation of evolutionary ecologists on legume-rhizobium symbioses as models of interspecific cooperation. For example, selection appears to favor less-beneficial "cheater" rhizobia, confirming a conflict of interest between mutualist partners (Porter and Simms, 2014). Nevertheless researchers have found that host mechanisms act as agents of selection favoring beneficial rhizobium genotypes – stabilizing benefits and helping resolve the evolutionary paradox of cooperation (Kiers et al., 2003; Simms et al., 2006; Oono et al., 2009; Heath and Tiffin, 2009; Gubry-Rangin et al., 2010). Despite the predictions of classical mutualism models, rhizobium researchers have found little evidence for local adaptation between hosts and symbionts from the same population (Wilkinson et al., 1996; Heath, 2010; Barrett et al., 2012) – begging the questions of whether these model coevolutionary interactions are currently coevolving at all, and what forces drive spatial variation.

*Rhizobia and the new era of bacterial population genomics*: Populations of rhizobia offer a rich source of species and population variation with which to address bacterial evolution in nature. By studying these bacterial genomes in the ecological context of their host plants and other environmental factors, population genomic approaches are allowing

our field to address unresolved facets of bacterial evolution, such as the genes underlying rhizobial adaptation to environmental change (Porter et al., 2016; Klinger et al., 2016) and the forms of selection on host-symbiont signaling loci (Kimbrel et al., 2013) and recently horizontally-transferred genes in general (Epstein et al., 2014).

In this issue of *Environmental Microbiology*, Pérez-Carrascal and colleagues leverage natural populations of rhizobia to address fundamental questions about bacterial evolution. Using Illumina whole-genome sequencing of 33 strains of *Rhizobium* collected from the roots and rhizospheres of 6 common bean plants from a field in Mexico, they find five evolutionarily-distinct clusters ("species" defined at 95% nucleotide identity). Three are largely comprised of symbionts of common bean, which possess the symbiosis plasmid (pSym) complete with genes for nodulation and N-fixation, while the other two contain only non-symbiotic, rhizosphere strains that lack the pSym. Analyses of nucleotide variation and recombination implicate a combination of rampant horizontal gene transmission (HGT) and strong selection at the pSym. While phylogenetically-distinct groups are resolved for the chromosome and accessory plasmids (the "core" genome), the pSym is genetically depauperate, cannot be cleanly separated into phylogenetic groups, and does not track the core tree. Thus selection and/or recombination are distinct enough for the pSym that its evolutionary history has become entirely decoupled from the historical and/or contemporary speciation processes at the rest of the genome.

Recent rhizobium studies support this view; Parker (2012) and Kumar (2015) both found evidence for abundant HGT of symbiosis genes across diverse chromosomal backgrounds in both *Bradyrhizobium* and *Rhizobium*. On the one hand, it is fascinating

that different genome components evolve so differently; bacterial genomes certainly stretch eukaryotic models of genome evolution and speciation (Polz et al., 2013; Shapiro and Polz, 2014; Krause and Whitaker, 2015). On the other hand, the fact that evolutionary processes vary along the genome at either large (*e.g.,* chromosomal, large inversion) or small (*e.g.,* gene) scales is well-known in contemporary population genomics, and in fact forms the basis of outlier models intended to detect loci under selection (Nielsen, 2005; Storz, 2005; De Mita et al., 2013; Whitlock and Lotterhos, 2015). Perez-Carrascal and co-authors also find conserved plasmid-borne gene regions (CPRs) that track the chromosomal phylogeny but are evolutionarily-labile in their genomic location; CPR3, for example, sits on a single large plasmid in one species but is spread across two plasmids in another. These changes are somewhat analogous to eukaryotic chromosomal mutations (*e.g.,* fusions, translocations). It will be interesting to learn more about whether these changes are common, and whether they have important roles in speciation and adaptation, as they are well-known to in better-studied eukaryotic systems (Hoffmann and Rieseberg, 2008).

Another implication of the results of Pérez-Carrascal *et al.* is the potential importance of a community perspective in understanding the evolution of the legume-rhizobium mutualism. The local coexistence of multiple, reproductively-isolated chromosomal lineages that all form symbiosis with the same host species flies in the face of one-to-one models of mutualisms and coevolution often explicitly or implicitly invoked when trying to understand the persistence of cooperative traits (e.g., N-fixation; Kiers et al., 2003; Simms et al., 2006) or the geographic patterns of trait or gene variation (Parker, 1999; Heath, 2010; Barrett et al., 2012). A long history of empirical and

theoretical work on diffuse coevolution has taught us that third-parties can dramatically alter the evolutionary outcomes of biotic interactions (Janzen, 1980; Iwao and Rausher, 1997; Strauss et al., 2005; Johnson and Stinchcombe, 2007; Thrall et al., 2007; TerHorst et al., 2015). In addition to well-known mechanisms of diffuse coevolution (reviewed by Iwao and Rausher 1997; TerHorst et al., 2015), the transmission of selected genes across species boundaries suggests that HGT is an important but unrecognized mechanism of diffuse coevolution in bacteria.

Important questions remain, including whether the diversity and recombination regimes are generally representative of ongoing coevolution between legumes and rhizobia in established natural populations. Pérez-Carrascal *et al.* study the rhizobia associated with relatively recently-introduced host (common beans) in an agricultural setting, with several implications. First, the rhizobium population might be relatively new; second, human-managed agricultural host populations cannot evolve. Perhaps early host-symbiont establishment or response to a rapid environmental change is characterized by rapid HGT of the symbiosis module followed by within-lineage coevolution at the pSym and elsewhere. This is a testable hypothesis, but one that would require long-term studies, more genome-sequencing, and multiple natural systems to address in a robust way.

*Looking ahead:* As whole-genome sequencing data, informatics, and molecular population genetic analyses become more easily integrated into lab groups working on ecological and evolutionary questions, we have the potential to use natural populations of rhizobia to address important questions in symbiosis and bacterial evolution more generally. Many visible, easily-measured traits of host plants are truly symbiotic

"extended phenotypes" controlled in part by genes in the rhizobium genome (*e.g.,* nodulation, plant N content) – making rhizobia excellent systems for mapping the genetic basis of quantitative traits in bacteria. The phylogenetic, functional, and genomic diversity of rhizobia (reviewed by Remigi et al., 2016) allows us to ask questions about how these axes of variation influence adaptation. For example, rhizobia vary in the location of the symbiosis genes, with some residing on genomic islands of the chromosome (*e.g., Bradyrhizobium*) while others reside on conjugative symbiosis plasmids (*e.g., Ensifer/Sinorhizobium*). How these differences in genome architecture affect the evolutionary dynamics (recombination, HGT) of these critical gene regions and thus the evolution of symbiosis remains little-studied. Rhizobia offer diverse and ecologically-rich empirical systems for evolutionary biology, and the work of Pérez-Carrascal and colleagues highlights the ways that rhizobium population genomics can be used to study symbiosis as well as the dynamics of bacterial populations in nature.

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