Ecological speciation in bacteria: reverse ecology approaches reveal the adaptive part of bacterial cladogenesis

Florent Lassalle [a,*], Daniel Muller [b,c,d], Xavier Nesme [b,c,d,e]

[a] *University College London, Gower Street, London, WC1E 6BT, United Kingdom*
[b] *Université de Lyon, F-69622, Lyon, France*
[c] *Université Lyon 1, Villeurbanne, France*
[d] *CNRS, UMR5557, Ecologie Microbienne, Villeurbanne, France*
[e] *INRA, USC1364, Ecologie Microbienne, Villeurbanne, France*

Abstract

In this review, we synthesise current models and recent comparative genomic studies describing how bacterial species may emerge through adaptation to a new ecological niche and maintain themselves in the same niche over long time periods. We notably consider the impact of genetic exchange with phylogenetically close relatives living in sympatry and how this leads to the heterogeneous evolution of different genes within the bacterial genome. This heterogeneity provides landmarks to recognise genes that determine adaptation to the ecological niche, and we present reverse ecology strategies to unravel ecological properties of bacterial populations.

1. Introduction

The notion of species in prokaryotes is intensely debated [1]. Currently, bacterial species are simply defined by their genomic homogeneity, leading to the conventional division of the bacterial world into genomically homogeneous units, genomic species, that attempt to reflect the natural occurrence of clusters of diversity − though this apparent structure might be due to under-sampling of the extant diversity [2,3]. While this definition of bacterial species is operational, the real challenge is to understand the underlying mechanisms that led to the emergence of this structure of bacterial diversity [4]. This is intimately linked to the current inability of evolutionary microbiologists to find a unifying model of prokaryotic cladogenesis. The classical "Biological Species Concept"

(BSC) [5], which was originally defined for animals, places the sexual isolation of clades as the central condition for their divergence. In this model, sexual isolation can mostly arise through the appearance of prezygotic barriers or geographical separation of lineages. Prokaryotes usually co-occur in the environment, often with no geographical structure of populations, and clonally reproduce, but are subject to intra- and trans-specific sex through homologous recombination and horizontal gene transfer (HGT). In this context, it is impossible to understand the emergence of prokaryotic species in the framework of the BSC [6].

Ecological speciation appears as an alternative, and was proposed to be the major way through which prokaryotes diversify [7]. According to Cohan, within a bacterial population, some mutant genotypes may arise with a new function that changes the definition of their ecological niche by providing a newly accessible resource, or changing the way a previously accessible one is used. When the environment provides the resource in question, these mutants may

* Corresponding author.
   E-mail addresses: florent.lassalle@ucl.ac.uk (F. Lassalle), daniel.muller@univ-lyon1.fr (D. Muller), xavier.nesme@univ-lyon1.fr (X. Nesme).

successfully colonise the new niche, leading to the emergence of an ecotype, an ecologically differentiated lineage. The ecological distinctness of the new ecotype relative to the ancestral population or to sister ecotype lineages allows related ecotypes to co-exist indefinitely without competing, and by evolving along independent paths to form genetically distinct populations, i.e. to speciate [8]. More than any other kind of genomic change such as base substitution, gene duplication or loss, the acquisition of a new gene is likely to bring functional novelty to a genome and thus to open a new niche. HGT therefore appears as a major drive for ecological speciation. Paradoxically, HGT events cause prokaryotic genomes and genes to have (partially) decoupled histories, generating methodological and conceptual problems in the definitions of units of prokaryotic diversity [9,10]. The essence of an ecological definition of species is thus tightly associated with the source of conflict in the appreciation of bacterial diversity in terms of species. Nonetheless, various models of ecotype formation have been proposed to detail the different steps and conditions necessary for ecological speciation to occur in prokaryotes [11,12]. Only a small set of recent studies of nascent ecotype populations can, however, support or refute these theories [13]. Conversely, studies comparing genomes of older taxa provided insights into how variation, notably in gene content, could be linked to the ecology of clades of various depth [14–19], but little theoretical ground has been laid for understanding the evolutionary processes linking ecological speciation events to the advent of these clades.

Here, we will explore the virtues and limits of ecological speciation models in understanding evolution of prokaryotes over large time scales. In the light of comparative genomics and population genomics, we will attempt to reconcile organism/genome-centred and gene-centred notions of adaptation to ecological niches. Finally, we will see how the various predictions of these models might allow us to backtrace ancient events of adaptation of bacterial lineages and how this reverse ecology approach could help microbiologists to bridge the increasing gap between the assessment of genomic diversity and the knowledge of bacterial ecology.

2. Emergence of ecological species

According to Cohan's "stable ecotype" model [20], the acquisition of an adaptive gene (or mutation in general) determining colonisation of a new niche immediately imposes strong selection for the single adapted mutant. Clonal multiplication of the founder mutant yields a nascent ecotype population, and the potential providence in the environment of the newly exploitable resource may lead to its rapid expansion. Even though this scenario can take place in sympatric conditions with the ecotype living amongst non-adapted closely related populations, the latter are not expected to contribute to the genetic pool of the nascent ecotype. Indeed, recombination between closely related bacteria, as estimated in experimental microbial populations [21,22], is thought to be rare relative to the intensity of selection. This causes the founder genotype to spread in the new niche before linkage between the niche-specifying gene and the rest of the genome has had the time to be disrupted [23]. In other words, the ecotype progenitor clonally populates the new niche, founding a population genomically homogeneous where the selected niche-specifying gene is ubiquitous. (Fig. 1, steps 1 and 2). During the clonal reproduction that follows, clonal variants arise, among which those losing the niche-specifying gene are systematically removed from the population occupying this niche. Other variants may prove fitter than the progenitor genotype and may clonally replace it. This selective process causes a purge of genome-wide diversity within the ecotype population, and its recurrent occurrence is called periodic selection [24]. The frequent genotype replacement by any fitter variant is associated with the fixation of all neutral mutations that were linked to the selected one, thus accelerating the divergence of the ecotype from its parent population (Fig. 1, step 9).

However, recent studies showed that divergent ecotypes living in sympatry (i.e. occupying distinct niches in the same micro-habitat) exchange significant amounts of DNA, which makes them maintain a coherent population, with no structure of the genomic variation at loci other than those under ecological selection [25,26]. This conflicts with the classic ecotype model predictions (see Box 1: "Ecotypes: Definitions and concepts"), which can be explained by the departure from the assumption that the ratio of the recombination rate over the intensity of selection for the niche-specifying trait would be low; in fact, recombination rates are likely no higher than previously estimated, but the intensity of selection is probably lower than thought, which could be caused by selective trade-offs experienced by wild prokaryotic populations. Indeed, genomic monomorphy induced by strong selection for the niche-specifying gene could be counterselected to promote the diversity of genomic backgrounds [13]. For instance, there are several reported cases of selection to maintain many rare alleles of cell surface receptors in order to reduce the pressure from predators (phage, amoeba, immune system) on the whole population [27] or to maximise the number of different bacteriocins produced in the population [28]. Under these conditions, periodic selection will instead lead to gene-specific sweeps, meaning that the niche-specifying gene will be conserved within an ecotype population, but not the genomic background, which may recombine intensely with neighbouring populations (Fig. 1, steps 3–5). A group of ecotypes that are still able to recombine frequently with each other form a meta-population, a "maximally inclusive cluster of independently evolving lineages" that is nonetheless structured into several groups of preferential genetic exchange and higher phenotypic similarities [1,12]. Actually, frequent sex within meta-populations can promote the emergence of new ecotypes by transferring generally adaptive mutations between subpopulations, thus preventing their competition on shared niche dimensions [24]. Also, recombination between subpopulations could help ecotypes to more rapidly invent the combination of mutations necessary to reach a more stable adapted state in their respective niches [20].

In the "stable ecotype" model, early sexual isolation is not required for the initial separation of the ecotype lineage, but