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Cindy E. Morris, Marie-Anne Barny, Odile Berge, Linda L. Kinkel, Christelle Lacroix. Frontiers for research on the ecology of plant pathogenic bacteria: Fundamentals for sustainability: Challenges in bacterial molecular plant pathology. *Molecular Plant Pathology*, 2017, 18 (2), pp.308-319. 10.1111/mpp.12508 . hal-02624126

HAL Id: hal-02624126

<https://hal.inrae.fr/hal-02624126>

Submitted on 26 May 2020

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Frontiers for research on the ecology of plant pathogenic bacteria: Fundamentals for sustainability

Cindy E. Morris^{1*}, Marie-Anne Barny², Odile Berge¹, Linda L. Kinkel, Christelle Lacroix¹

¹INRA, UR0407 Plant Pathology Research Unit, 84143 Montfavet, France.

²Sorbonne Universités, UMR1392 INRA, UPMC Univ. Paris 06, CNRS, IRD, Diderot Univ. Paris 07, UPEC Univ. Paris 12, Institut d'Ecologie et des Sciences de l'Environnement de Paris, Case 237, 4 place Jussieu, 75252 Paris, France.

³University of Minnesota, 1991 Upper Buford Circle, 495 Borlaug Hall, St Paul, Minnesota, United States, 55108-0010.

*Corresponding author:

cindy.morris@inra.fr

Plant Pathology Research Unit 0407, INRA-PACA at Avignon

67 Allée des Chênes, CS60094, 84143 Montfavet cedex, France

Phone 33 (0)4 32 72 28 86

Fax 33 (0)4 32 72 28 42

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/mpp.12508

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Abstract

Methods to assure the health of crops owe their efficacy to the extent to which we understand the ecology and biology of environmental microorganisms and the conditions under which their interactions with plants lead to losses in crop quality or yield. But in the pursuit of this knowledge, notions of the ecology of plant pathogenic microorganisms have been reduced to a plant-centric and agro-centric focus. With increasing global change, i.e. changes that encompass not only the climate but also biodiversity, geographic distribution of biomes, human demographic and socio-economic adaptations and land use, new plant health problems will emerge via a range of processes influenced by these changes. Hence, knowledge of the ecology of plant pathogens will play an increasingly important role in anticipating and responding to disease emergence. Here we present our opinion about the major challenges facing the study of the ecology of plant pathogenic bacteria. We argue that the discovery of markedly novel insights into the ecology of plant pathogenic bacteria is most likely to happen in a framework of more extensive scales of space, time and biotic interactions than those that currently guide much of the research on these bacteria. This will set a context that is more propitious for discovery of unsuspected drivers of survival and diversification of plant pathogenic bacteria, of the factors most critical for disease emergence, and will set the foundation for new approaches to sustainable management of plant health. We describe the contextual background, justification for, and specific research questions for the following challenges:

- Develop terminology to describe plant-bacterial relationships in terms of bacterial fitness.
- Define the full scope of the environments where plant pathogenic bacteria reside or survive.
- Delineate pertinent phylogenetic contours of plant-pathogenic bacteria and name strains independent of their presumed life style.
- Assess how traits of plant pathogenic bacteria evolve in the overall framework of their life history.
- Explore possible beneficial ecosystem services that plant pathogenic bacteria contribute.

Running title: Challenges in phytobacterial ecology

Key words: ecology, metapopulations, disease control, disease emergence

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INTRODUCTION

All strategies of plant disease management are founded on fundamental understanding of the ecology and biology of plant pathogens. Crop protection methods include surveillance and quarantine, deployment of host resistance, use of biocides and of biocontrol agents, agricultural practices to limit the contact of crops with pathogens, and means to forecast disease. All of these methods and the estimations of their durability are based on knowledge of the genetic and phenotypic diversity of plant pathogens, their means and extent of dissemination, the extent of their host range and of habitats in which reservoirs of inoculum are found, their mechanisms of virulence to plants, and on how these processes are regulated by the biotic and abiotic environment (Fig. 1). However, research on the biology, ecology and evolution of plant pathogens – bacteria, fungi and viruses alike - has been markedly agrocentric, leading to concepts of disease cycles and pathogen life histories that are focused on agricultural settings at time scales corresponding mostly to cropping seasons and plant developmental stages

Here, we present what we consider to be the most important challenges for understanding the ecology of plant pathogenic bacteria. We argue that markedly novel insights into the ecology of plant pathogenic bacteria are most likely to happen in a framework of more comprehensive scales of space, time and biotic interactions than those that currently guide much of the research on these bacteria. Likewise, the vocabulary we use to communicate discoveries and to reconstruct frameworks can bring depth to, or conversely, can set limits to these frameworks. Therefore, as part of the challenges presented here, we raise issues about vocabulary and terminology. This is meant to help the audience understand and interpret written and spoken information about bacterial ecology more comprehensively. We have not proposed specific changes in terminology because this is a problematic process that will require extensive debate. Despite notable limitations to existing terminology, here we will refer throughout this manuscript to “plant pathogenic bacteria” as bacteria known to cause plant disease, to have the potential to cause plant disease or to be sufficiently related to such bacteria as to bear the same name – until proven otherwise. As will become apparent below, this definition likely includes a spectrum of bacterial lineages, including ones also commonly known to be involved in synergistic interactions, with varying capacities for influencing plant health. We also consider that this term can be interpreted to mean bacteria that have not yet been recognized to have the potential to cause plant disease but

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will be recognized in the future via research discoveries and/or via new disease emergences. The word “disease” will be used here to mean a physiological condition of a plant that is deleterious, either in terms of the plant’s usefulness for humans or for its fitness in an evolutionary context, and that is caused by another organism.

In the context of global change, knowledge of the ecology of plant pathogens will play an increasingly important role in anticipating and responding to disease emergence. Global change encompasses not only climate change but also all of the resulting changes in biodiversity, in the geographic distribution of biomes, as well as human demographic and socio-economic adaptations and their consequences for land use. Hence, numerous aspects of the interaction of plant pathogens with their environment will change as a consequence. In this light, diseases will arise via increasingly varied modes. These can include i) resurgence of “old” pathogens in new contexts where the climate has become favorable, ii) changes in geographic zones of contemporary pathogens with increases in movement of plant materials or dissemination with extreme storms, iii) expansion of host range of old or contemporary pathogens due to genetic modifications in the pathogen and/or to increases in susceptibility of plants under environmental stress or from the planting of new crops, iv) new genetic lines of pathogens from unsuspected reservoirs and v) novel pathogens resulting from hybridization and/or genetic exchange among newly-coincident bacteria. The challenges for ecology that we present below will contribute to deciphering these different modes of disease emergence and therefore to improving how we avoid, anticipate or respond to disease emergence.

CHALLENGES

For each challenge, we present the conceptual context, the state of the art, our recommendations for the types of approaches that could be taken to address these challenges, and what can be gained by confronting these challenges. In some cases, we also present more details about certain important, yet complicated, concepts and specific research questions.

Challenge 1. Develop terminology to describe plant-bacterial relationships in terms of bacterial fitness.

To manage plant health and assure food security, there is a very practical need to have utilitarian vocabulary such as “pathogen” and “disease”. However, this vocabulary can be

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cumbersome for communicating about the ecological strategies of bacteria that could cause disease to plants at some stage in their life. It is also an obstacle to a comprehensive understanding of their ecology. For example, what is the best language to describe a bacterium that has a high level of fitness in association with a plant without engendering any apparent syndrome or disease symptoms? Is it an epiphyte/endophyte, a symbiont, a commensal, or something else? Should the vocabulary be the same or different for a bacterium that has high fitness in association with a plant all while provoking marked alterations in plant health? What if these descriptions pertained to the same bacterium as in the case of *Ralstonium solanacearum* in tomato depending on the cultivar and plant part (roots, collar or stems) (Grimault & Prior, 1993), for example? These two situations will have the same impact on bacterial population dynamics and could have the same evolutionary outcome if the fitness is the same. Yet the current vocabulary is fundamentally plant-centric, and highlights the differences in apparent relationship of the bacteria with plants. The first situation might be considered as the commensal phase of a saprophyte or a latent phase prior to symptom expression, whereas the second example would be considered as disease caused by a pathogen. The colonization strategies and molecular signaling deployed by diazotrophic endophytes and vascular pathogens (Arencebia *et al.*, 2006, Reinhold-Hurek & Hurek, 1998), for example, are strikingly similar yet our vocabulary is based on the apparent outcome for the substrate (plant) that they colonize. From the perspective of epidemiology plant colonization without or with symptoms could lead to the establishment of equally potent reservoirs of inoculum but in the former case this reservoir might not be recognized if the apparent state of the plant was described as healthy. Furthermore, plant-centric terminology depends on the ability to describe the health of the plant, the causal relationships between health and the incriminated bacteria and the stability of these relationships. A recent report illustrates the varying growth-promoting and growth-inhibiting effects of phyllosphere bacteria on tree seedling depending on N and P fertility of soils (Griffin *et al.*, 2016) making one wonder what plant-centric terminology would be appropriate for these bacteria. These examples highlight the significant limitations of using plant-centric terminology for describing a bacterium.

We suggest that vocabulary should be developed that focuses on bacterial fitness, viz. the number of offspring (cells) that a bacterium produces under a defined set of conditions. Vocabulary that focused on bacterial fitness would highlight the ecological, evolutionary and

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epidemiological similarities of the situations illustrated above. It might also facilitate new questions and viewpoints about the selective processes that drive the life history of bacteria having plant pathogenic phases. Clearly, the terms endophyte, epiphyte or saprophyte are pertinent to bacteria such as *Xylella fastidiosa*, *Xanthomonas* spp., *Pseudomonas syringae*, *Burkholderia cepacia*, during certain phases of their life and perhaps even throughout a major fraction of the life of the cells of these organisms. Furthermore, the use of the term pathogen is sometimes accompanied by the adjective “opportunistic” to imply that disease causation is a relatively rare event happening under very particular conditions whereas a ‘pathogen’ without an adjective is inferred to cause disease commonly. This vocabulary persists in spite of ample evidence that specific biotic conditions (e.g. susceptible host, absence of antagonistic microflora) and abiotic conditions (temperature, availability of water, presence of nutrient resources and absence of inhibitory chemicals, etc.) are required for any pathogen to cause disease. These divisions contribute to a compartmentalization of how we perceive life history.

The vocabulary to describe bacterial-plant relationships has another important limit in that it is mostly restricted to two-partner couples: a bacterial clone with a given plant genotype. Synergistic interactions among bacteria (or with other types of microorganisms), for example, that lead to gains in fitness – and eventually to alterations in plant health - fall outside the scope of current vocabulary. For example, strains of plant pathogenic bacteria with certain deficiencies (such as missing or defective effectors) can maintain fitness by free-riding on the capacities of the competent strains with which they co-exist. This has been illustrated under experimental conditions by i) the rescue of epiphytic fitness of a strain of *P. syringae* with a defective *hrpJ* gene by a strain with a functional *hrpJ* gene (Hirano *et al.*, 1999) and ii) the rescue of *in planta* fitness of an ‘*hrpXc*’ defective *X. campestris* pv. *campestris* by a wildtype strain (Kamoun & Kado, 1990). Mutants that each have defects in pathogenicity factors can also complement each other to rescue virulence as in the case of *Erwinia amylovora* where defective in their Type 3 Secretion System (T3SS) and those defective in production of exopolysaccharides complement each other to restore virulence to pear (Zhao *et al.*, 2009). Bacteria inhabiting the same plant tissue can also interact synergistically to increase disease severity. Olive knot cankers, for example, can be colonized by bacteria from various genera including *Erwinia* and *Pantoea*, in addition to *Pseudomonas savastanoi* pv. *savastanoi* that is considered to be the causal agent of the disease. Disease

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severity on olive is greater in the presence of certain saprophytes such as *Erwinia toletana*, *Pantoea agglomerans*, and *Erwinia oleae*. These bacteria contribute to quorum sensing signal production and exchange, important in the expression of *P. savastanoi* pv. *savastanoi* virulence factors and, via their enzymatic activities, they might also help demolish accumulation of host defenses including salicylic acid and various phenolic compounds (Buonauro *et al.*, 2015, Passos da Silva *et al.*, 2014). Co-occurring bacteria can also interact antagonistically. For example, a current epidemic line of *P. syringae* pv. *actinidiae* was co-inoculated into kiwifruit with closely related genotypes of *P. syringae*. This led to reductions in the fitness of all strains compared to when they were inoculated alone and led to attenuated reactions of the plant relative to the intensity of symptoms caused by the epidemic line alone (Bartoli *et al.*, 2015). Furthermore, microbial succession during the development of disease symptoms reveals that many microorganisms can reap the fitness benefit of disease. For example, during the development of soft rot symptoms, *Pectobacterium atrosepticum*, the bacterium that incites the disease process, is rapidly overgrown first by various γ -Proteobacteria including *Enterobacter* and *Pseudomonas* and then later by anaerobic pectolytic Clostridia. The ensemble of these bacteria contribute to the maceration process (Kõiv *et al.*, 2015).

These situations illustrate that bacteria with no or with defective pathogenicity factors can have important fitness gains equivalent to those of bacteria that make energetic investments in maintaining pathogenicity factors. Likewise, they also illustrate that full fitness gains from pathogenicity factors might depend on help from other members of the plant microflora. This point of view could contribute to a better assessment of the evolutionary significance of pathogenicity. In more practical terms, determining how the ensemble of biotic and abiotic environmental factors defines the fitness of plant pathogenic bacteria is not only quintessential ecology but it will also provide a more robust foundation to conceive sustainable methods to constrain their fitness.

Challenge 1 research questions. The preceding observations conjure the following research questions: “What are the contexts under which plant pathogenic bacteria attain the greatest fitness?” and “Are these contexts always associated with symptom expression or loss of productivity in plants?” These questions evoke not only the different drivers of evolution of plant pathogenic bacteria but they are also closely related to the notions of disease resistance and tolerance in plants. Understanding this is also crucial for disease

epidemiology because situations of optimal fitness of plant pathogenic bacteria, and especially those with no obvious symptoms, can lead to build-up of inoculum for other – perhaps more susceptible - plants. The fitness of bacteria in plants or in other environmental contexts is highly dependent on both the abiotic and biotic conditions of the environment. The biotic context has been overlooked but might soon be coming to light with increasing interest in plant microbiomes. The design and pertinence of controlled experiments to assess the impact of the biotic environment on fitness will benefit from more quantitative characterization of the ensemble of microorganisms associated with plants (diseased or otherwise) and other environments suspected of giving a fitness boost to plant pathogenic bacteria (such as leaf litter for *P. syringae* (Monteil *et al.*, 2012), for example).

Challenge 2. Define the full scope of the environments where plant pathogenic bacteria reside or survive.

Until recently, agriculture *sensu stricto* has been the primary context of study for plant pathogenic bacteria. This has greatly influenced how strains are sampled (i.e. from symptomatic plants, and predominantly crop plants, ornamentals and managed forests) most probably leading to under-estimation of the genetic and phenotypic diversity of plant pathogen bacteria. Yet, only a few species or groups of plant pathogenic bacteria are strictly obligate parasites and most have some capacity to live as saprophytes in diverse settings.

Defining the full scope of the environmental habitats of plant pathogenic bacteria challenges the basic assumptions we have about the life history of these bacteria, the extent of their dissemination and their capacity to play other roles or interact with other organisms in the environment at large. As argued above, the label “plant pathogen” is in itself an obstacle to the creation of hypotheses about complex life history. It sets bacteria into a limited playing field and defines a specific role. Addressing this challenge will require intellectual exercises to forget about pathogenicity to plants and envision other, broader environmental roles that these bacteria play, including those involving interactions with other prokaryotes and with eukaryotes such as insects; mediation of gene expression in plants, animals, or other microbes; as competitors for nutrients or space with organisms in diverse habitats; or in important Earth system cycles such as those of water, nitrogen or carbon. There have been efforts to explore the interaction of plant pathogenic bacteria with eukaryotes such as fungi (Wichmann *et al.*, 2008) and insects (Hendry *et al.*, 2014, Ordax *et al.*, 2015, Stavrinos *et*

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al., 2009). Direct environmental evidence for these associations are awaiting, but these experimental efforts are based on the presence of genes with putative roles in virulence to insects or fungi in the genomes of these bacteria (Feil *et al.*, 2005, Smits *et al.*, 2010) and on well-known and *bona fide* associations such as that of *Pantoea stewartii* with its insect vector (Correa *et al.*, 2012). Initiatives to explore the association of *P. syringae* to the water cycle and its role in rainfall have also gained force over the past decade (Morris *et al.*, 2013).

The ultimate goal of this challenge is to give balanced consideration to the significance of diverse ecosystems and their biotic and abiotic contexts within which the complex life history of plant pathogenic bacteria is played out.

Defining the full scope of habitats within which pathogens successfully survive and reproduce will provide clues about the importance of other drivers of selection for distinct fitness traits and population diversification. The importance of habitats beyond agriculture as selective forces and drivers of diversification will become evident from estimations of the relative abundance of a plant pathogen and its comparative diversity in these habitats relative to that within agriculture. This will suggest novel hypotheses about biotic and abiotic conditions to which plant pathogenic bacteria adapt and enlarge the suite of organisms with which plant pathogenic bacteria are likely to interact. Research related to this challenge can also reveal how ecological contexts other than those of agriculture might reinforce the acquisition and/or maintenance of virulence factors that have dual uses – both in the interaction with plants and elsewhere (Lee *et al.*, 2010, Morris *et al.*, 2008).

This is critical information for estimating the durability of disease resistance, biocides, biological control and other methods to protect plant health. Although plant pathogens might evolve in response to the deployment of these methods for plant protection, pathogen populations outside of agriculture might have already diversified such that they are capable of overcoming these disease control methods. This is a hypothesis that has not been considered in plant pathology and has considerable importance for sustainability of disease management practices.

Challenge 3. Delineate pertinent phylogenetic contours of plant-pathogenic bacteria and name strains independent of their presumed life style.

This challenge sounds like an oxymoron, but the reader should keep in mind - as mentioned above - the progressively expanding scope of what “plant pathogenic bacteria” will come to

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mean in the future. The previous sections raised the point that vocabulary and terminology can constrain and distort understanding in communication. Indeed, the names that we use for plant pathogenic bacteria can be obstacles to more comprehensive understanding of their ecology. Names such as *Agrobacterium tumefaciens*, *Burkholderia cepacia*, *Pectobacterium carotovorum*, or the *Ralstonia solanacearum* species complex, for example, refer to bacteria within a certain range of genetic, and perhaps functional, diversity. There are descriptions of the phylogenetic diversity of many plant pathogenic bacteria leading to what becomes the recognized phylogenetic boundaries or “contour” of these groups. This contour sets the context for creating the tools and criteria used for detection, identification and quantification which in turn influence the range of diversity that is considered to be pertinent to etiology, epidemiology and future emergences. Names are part of a dialectical process where they define genetic and functional scope that in turn defines the robustness of identification tools that in turn defines these scopes. If phylogenetic analyses focus on a limited scope of genetic diversity, then detection and identification are likely to be biased. The names we attribute to bacteria are also charged with meaning because they imply traits and behaviors that will influence decisions about control strategies including those governed by official regulatory procedures and decrees. Therefore, names that are derived from insufficient scopes of diversity can result in misleading assumptions and inappropriate decisions about the control measures to enact. Nevertheless, there is an urgent need at present for specific definition of the contours of pathogens that should be regulated and less of an urgent need for breaking the linguistic barriers to the exploration of ecology. In this context we suggest that the diagnostic criteria, and not the names of organisms, should define the scope of regulatory decrees. We discuss this in more detail in the Conclusion.

This challenge is closely linked to the previous challenge. At present, the phylogenetic contour of any given plant pathogenic bacterium has been defined mostly from strains collected from diseased plants. The naming of plant pathogenic bacteria has also given significant importance to pathotypes, pathovars and races. This nomenclature creates considerable difficulty for communicating about bacteria that are within the phylogenetic contour of a plant pathogen but that have been isolated from habitats other than a diseased host plant, and likewise for strains that attack and cause similar symptoms to a same host but belong to distinctly different genetic lines. Furthermore, practices for comparative host range testing with standard sets of plant species/cultivars and clear rules for naming

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pathovars have not been defined thereby increasing the ambiguity of such designations – even though they are part of the vocabulary whereby specific quarantine measures are imposed. A plant-centric approach, in which the ecology and evolutionary biology of a bacterial lineage is implicitly, and perhaps explicitly, defined by the bacterium's effect on a plant host fundamentally limits our perspective on the complex life history of the other bacterial isolates within that lineage. It does not leave room for the possibility that within the same phylogenetic contour, there are strains that represent a range of possible relationships with plants (or other organisms) or that the lifestyles of individual strains vary with time or environmental conditions. Practically, host-oriented names can also create considerable obstacles for development of technologies where these bacteria have beneficial effects for plants or the environment. Debate on how to create *sans a priori* naming systems has been launched (Marakeby *et al.*, 2014, Baltrus, 2016) but needs to be intensified.

Redefining the phylogenetic contours of bacterial plant pathogens will obviously entail sampling from a more comprehensive range of habitats and ecosystems as described for the previous challenge. But, it will also require revisiting the criteria currently used to target plant pathogenic bacteria. Expanding the depth of the phylogenetic description of the *P. syringae* group, for example, brought to light how misleading were the traits and characteristics (i.e. in particular the LOPAT system of biochemical and physiological properties and induction of a hypersensitive response) used to screen for bacteria in this group (Berge *et al.*, 2014). The revision of the phylogenetic contour of *P. syringae* from 7 to 13 clades has led to the development of a molecular marker that is generic across the full range of the currently described *P. syringae* diversity. This marker along with the development and use of an associated PCR protocol allows an objective and more reliable detection and identification of bacteria within this group (Guilbaud *et al.*, 2016).

The goal of expanding the phylogenetic contour of 'species' or 'species complexes' of bacteria is to access comprehensive information needed to understand the role of the different motors of evolution and ultimately disease emergence. It is not about the definition of species, but rather it concerns the ecological origins and diversity of what underlies a group to which a name is attributed. Whereas knowledge about the geographic distribution and the environmental conditions of habitats are crucial to assessing gene flow and to understanding how natural selection operates, a robust phylogenetic context is

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needed to assess the importance of recombination, mutation rates and evolution of traits in the ecology and evolutionary biology of a bacterial lineage. This knowledge, and the parameters in which it is built, also provide the fundamental basis for assessing structures of populations and of the overall metapopulation. In the case of *P. syringae*, for example, we sought to outline a phylogenetic contour that surpassed the phylogroups likely to be directly involved in future disease epidemics. Because of the placement of phylogroups 12 and 13 in the phylogenetic tree and the incapacity of strains in this groups to induce hypersensitivity in tobacco (Berge et al., 2014), they could have been considered as impertinent to disease caused by members of the *P. syringae* complex. On the other hand, they are readily detected with the new PCR primers for the *P. syringae* complex (Guilbaud et al., 2016), a tool that has subsequently revealed the presence of phylogroup 13 in plant tissue in co-existence with strains from other phylogroups of *P. syringae* (Borschinger et al., 2015). Very recently, bacterial lineages close to phylogroup 13 have been shown to be pathogens of citrus (Beiki et al., 2016). Hence, our approach has been to define a broad contour within the limits of technical feasibility for quantitative analyses of population dynamics so as to test hypotheses about evolution, origin, and diversification.

Such hypothesis testing can, nevertheless, be very complicated in light of the metapopulation that underlies broad phylogenetic contours. For example, the recently revealed diversity of the *P. syringae* group illustrates how a metapopulation can be composed of many genetic lines that are relatively rare and a few genetic lines of epidemiological importance that have greater dominance in the overall metapopulation (Berge et al., 2014, Morris et al., 2010). Such structure reflects the mechanisms that preserve the overall diversity of the metapopulation and thus raises questions about what exactly these mechanisms are. Furthermore, it also illustrates clearly why it is difficult to find direct evidence that a newly emerged bacterial pathogen comes from a given environmental reservoir: the frequency of the parent strain in any given environmental reservoir is likely to be rare and below detection levels in contrast to its abundance and dominance in diseased tissue. Therefore, it points to the need to develop experimental designs, quantitative modeling approaches, and molecular tools that can provide this evidence.

Challenge 3 concepts. Here, we explain more fully the structure of metapopulations and how they contribute to the perception of disease epidemiology and processes underlying emergence. A metapopulation refers to the ensemble of spatially separated populations of

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the same species that interact in some way. We will apply this definition to the *P. syringae* group without hampering the discussion by the debate of whether this group is or is not a species. Currently, studies of *P. syringae* provide some of the only quantitative environmental data that can be used to speculate intelligently about the size and underlying structure of the metapopulation of a plant pathogenic bacterium. From samples of headwaters on several continents, the frequency of haplotypes of the *cts* housekeeping gene can be calculated from a core collection of 235 randomly sample strains (Morris et al., 2010) revealing a skewed frequency distribution of haplotype abundance (Fig. 2A). These data represent only a partial assessment of the genetic diversity of *P. syringae*. According to rarefaction analyses of the most diverse collection of strains available, numerous clades (and hence even more numerous haplotypes) have yet to be discovered suggesting the enormous underlying genetic diversity of the *P. syringae* metapopulation (Berge et al., 2014). In spite of its approximate representation of the total diversity, this frequency distribution illustrates a typical feature of biodiversity observed for many types of organisms: there are numerous groups with low abundance and few groups with relatively high abundance (Pielou, 1969). It also illustrates how the dominant group can contribute to a relatively small fraction of the total metapopulation size whereas the cumulated multitude of rarer groups can account for the major part of the population size (Fig. 2B). This common property of the structure of species and communities is what gives rise to the phenomenon of the so-called “rare biosphere” (Pedrós-Alió, 2012). This structure leads to difficulty in representing the diversity of a population when sample size is small relative to the size of the total metapopulation. It also skews the estimation of diversity when the spatial distribution of the different groups is not homogenous.

This structure leads to two specific biases in understanding the etiology and emergence of diseases caused by *P. syringae*. First, it contributes to the idea that diseased plants are colonized by single clones. Clearly, certain strains of *P. syringae* have important gains of fitness during the progress of disease that would allow them to dominate populations in diseased tissue. Hence, they attain dominance that is spatially aggregated. However, most diagnosis of plant diseases has involved isolation of a single or few strains from diseased tissue and often by procedures that are not quantitative but, rather, let the dominant strains grow out on culture media from a piece of infected tissue. These isolation procedures have clearly contributed to the notion that single, clonal lines are responsible for epidemics.

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Secondly, the multitude of rare haplotypes especially in environmental samples means that there is a very low likelihood of finding in the environment the same clone that is causing an epidemic. Therefore obtaining direct evidence for the contribution of environmental strains to disease emergence is improbable without massive sampling efforts.

The skewed metapopulation structure also raises questions about the relative importance of pathogenicity to crops in the diversification of a species of plant pathogenic bacterium. The emergence of the kiwifruit pathogen, *P. syringae* pv. *actinidiae*, is a useful illustration. In light of the intensity of the widespread epidemic of bacterial canker of kiwifruit, it could be argued that the clonal line of *P. syringae* implicated in epidemics across the production regions in the Northern and Southern hemispheres in 2010, at the peak of this pandemic, is the most dominant clonal line of *P. syringae*. Based on the kiwi production rates of the countries affected by the disease in 2010 we can estimate that the total number of kiwifruit trees in these regions is about 10^7 . If we assume an extreme situation where all kiwifruit trees were infected and that each harbored 10^{10} cells of the clonal line of this bacterium, then the total population size of this clonal line would be 10^{17} cells. Although this approximation represents an extreme condition of disease incidence, it illustrates nevertheless the small fraction that this clone is likely to constitute of the estimated *P. syringae* metapopulation of 10^{22} cells (Morris et al., 2010) that are composed of hundreds or perhaps thousands of distinct genetic lines many of which are in non-agricultural habitats. Furthermore, few other genetic lines of *P. syringae* are likely to benefit from the combined effects of a widespread homogenous host and dissemination by vegetative propagation of the host. In this light, pathogenicity to plants might be simply a means for certain lines to gain a temporary enhanced fitness without any real impact on the overall metapopulation diversity. An exciting challenge would be to estimate the frequency and abundance of genetic lines that are of epidemic importance and the sum of their overall contribution to the total metapopulation of *P. syringae* or other plant pathogenic bacteria. This would provide novel insight into the benefits of attaining epidemic proportions.

Challenge 3 research question. A clearer picture of the metapopulation will conjure the following research questions: “What group of plant pathogenic bacteria has the largest population size on Earth?” “Is this group also the most widely-spread of the bacterial plant pathogens in terms of the range and geographic distribution of the habitats that it occupies?” The first question will point to the group (or species) of plant pathogenic bacteria

that has the greatest fitness at present, and could amusingly provide another theme in the series of *Top 10 pathogens* fostered by this journal. Overall, these questions illustrate the practical value of assessing the size, structure and distribution of bacterial metapopulations.

They are pertinent because the surface of land being exploited for crop production continues to increase. Climate change is also leading to shifts in the geographic ranges of crops. Furthermore, in response to the pressures of global change, totally new contexts for crop production are being conceived for urban and peri-urban areas and the recycling of resources such as water and organic matter, for example, are increasing. New insight into human nutrition and trends in food preferences are leading to the introduction of new plant foods into human diets and therefore to the cultivation of these new crops. These changes in crop production can create opportunities for crops to be exposed to new pathogens, new reservoirs of inoculum or to new means for pathogen dissemination in ways that might be considered the “New Worlds” of the New Millennium. One factor that will influence disease emergences in these New Worlds is the likelihood of encounter of pathogens with crops. Knowing the total number of a given group of bacteria and their whereabouts is crucial to assessing this risk. For example, is *Acidovorax avenea* more or less abundant than *Pectobacterium carotovorum*, *Ralstonia solanacearum* or *Clavibacter michiganensis*? What is the extent of their respective geographic and habitat distributions? What reservoirs do they occupy that could have increasing contact with agriculture under the influence of global change? Such questions present an exciting challenge for microbial ecology and plant pathology.

In addition to illustrating the practical value of quantifying the metapopulation size and structure of bacterial groups and the reservoirs they inhabit, these questions also illustrate why phylogenetic scope needs to be broad enough to account for lines of bacteria that could be responsible for future emergences but have not yet attained known epidemiological importance. The phylogenetic scope will also need to be on a somewhat similar scale among the bacteria being compared so as to avoid irrelevant comparisons of the metapopulation size of a single clonal line of current epidemiological importance with that of a species complex, for example.

Challenge 4. Assess how traits of plant pathogenic bacteria evolve in the overall framework of their life history.

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Co-evolution between plants and their pathogens is the framework in which nearly all research on the origin and maintenance of virulence factors is set. There are a few examples where studies of the selective forces that contribute to bacterial survival, such as resistance to UV light, to desiccation, osmotic stress and temperature fluctuations, for example, do not concern situations where bacteria are likely to cause plant disease or where they are necessarily in contact with plants. Otherwise, nearly all of the questions about the origin and maintenance of factors that confer virulence to plants set bacteria into direct interaction with plants. In spite of calls to expand the paradigms about the evolution of parasitic fitness in plant pathogens to include roles for dual use factors or exaptation, well-known to contribute to the evolution of virulence in human pathogens (Morris *et al.*, 2009), little progress in this regard has been made for plant pathogenic bacteria.

Among the few studies in this regard, one revealed the surprising diversity of effector alleles in strains of *P. syringae* from snowpack and streams from sub-alpine regions of the southern French Alps and from pristine regions of New Zealand's south island that were closely related to the current epidemic strains of *P. syringae* pv. *tomato* (Pto) (Monteil *et al.*, 2013). Pto relatives from non-agricultural habitats were pathogenic on tomato in laboratory tests but also were more aggressive on other hosts than Pto strains from disease epidemics. Furthermore, although the strains from snowpack and streams had effector repertoires that were similar to Pto strains from disease epidemics, they had remarkably greater variability in the repertoire of alleles of the effector genes and revealed higher rates of recombination for numerous genes, including genes for virulence factors, than strains from tomato crops. It is difficult not to be intrigued about why and how this diversity is maintained and about the fitness that these effectors could provide to strains when they are not in contact with plants. Such observations beg questions about the relative importance of agriculture vs. other environments as drivers of pathogenic diversification.

This challenge depends on the other challenges because it requires a framework for finding a comprehensive spectrum of reservoirs where unbeknownst diversification could be occurring independent of the processes that have, to date, been perceived as overriding. It also will involve abating the hegemony of research on the T3SS and effector repertoires to allow more inquiry into other traits that could underlie fitness in plants. The absence of the T3SS in plant pathogenic Gram-positive bacteria clearly illustrates how toxins, for example, can be sufficient for parasitic fitness and pathogenicity. For example, pathogenicity in

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Streptomyces spp. is conferred by a pathogenicity island that harbors genes for the toxins thaxtomin and tomitinase, for a necrosis inducing protein and for a plant fasciation operon (Kers *et al.*, 2004). When mobilized and acquired by non-pathogenic strains of *Streptomyces* this pathogenicity island confers virulence in a manner similar to the manner in which *Ochrobactrum*, *Rhizobium* and *Sinorhizobium* spp. became pathogenic to tomatoes and cucumbers after they naturally acquired the Ri plasmid from *Agrobacterium radiobacter* under production conditions in commercial greenhouses (Weller *et al.*, 2004). Bacterial plant pathogens offer excellent models for microbial ecologists to validate theories about community dynamics and evolution. Ecologists interested overall in terrestrial and aquatic ecosystems could be encouraged to embrace plant pathogens as models to benefit from the comparisons that could be made with human pathogens (Box 1.) about unsuspected but analogous aspects of life history and drivers of population dynamics and diversification.

Addressing this challenge will contribute substantially to anticipating disease emergences and to conceiving sustainable disease management practices. It will lead us to confront novel questions such as i) the possible existence- in the environment - of lines of plant pathogenic bacteria already capable of overcoming a new line of disease resistant plant that is currently being introduced, or ii) how the management of forests or wild-life reservations that are linked to agricultural regions through waterways could affect pathogen evolution, or ii) how the use of recycled biological materials from outside of agriculture could be a driver of pathogen evolution.

Challenge 5. Explore possible beneficial ecosystem services that plant pathogenic bacteria contribute.

Many of the bacteria that are capable of causing plant disease are free living organisms able to survive and multiply outside of host plants. It is likely that for much of the life span of cells of such bacteria, they are not involved in causing plant disease. Outside of the context of plant disease, could any of the interactions of these bacteria with their environment be considered as beneficial for the environment? For example, what is the contribution of the pectinolytic enterics to carbon cycling? Do bacterial pathogens help the plant pass through its phenological stages by favoring release of seeds from fruit or the abscission of leaves during senescence? Could bacterial pathogens regulate plant population dynamics or contribute to successional changes or to the co-existence of multiple plant species in dense

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plant communities – thereby promoting biodiversity - as has been reported for many fungal pathogens (Gilbert, 2002)? With growing concern about the negative impacts of plant production practices on the environment, microbial ecology needs to address the possible loss of ecological services when we attempt to eradicate plant pathogens. Such insight is also important to revealing the potential conflicting consequences of managing parks and natural plant stands vs. managing crops. Ultimately, we need to consider if there are any negative trade-offs to controlling plant disease beyond the toxicity of pesticides.

P. syringae is currently the most illustrative example of the potential for conflicting dual impacts on the environment. Its negative impact on crop health and the potential for its beneficial role on precipitation have been the subjects of many sensitive debates. Nevertheless, recent results on the movement of this bacterium into the atmosphere and into clouds, on rates of ice nucleation of lofted strains and on their potential to participate in setting off the formation of rain or snow (Joly *et al.*, 2013, Möhler *et al.*, 2008, Morris *et al.*, 2013) has contributed to understanding the multitude of factors involved in the increasing scarcity of rainfall and snowpack accumulation in certain regions of the world. Based on current estimates from cloud modeling, 10 particles that are ice nucleation active (INP) at -8°C per m^3 of a rain cloud could set off a cascade of freezing and aggregation of cloud drops that could transform cloud droplets into falling raindrops (Crawford *et al.*, 2012). The concentrations of *P. syringae* observed in clouds can sometimes be this large (Joly *et al.*, 2014, Joly *et al.*, 2013). However, *P. syringae* is more likely to be just one of the diverse types of particles that make up the total abundance of INP in clouds. Nevertheless, these INP generate a certain amount of precipitation as they fall through a column of cloud and collect the roughly 500 μL of water dispersed in cloud droplets in each m^3 of cloud. This leads to a paradoxical question: if *P. syringae* could multiply on plants and be disseminated into clouds at a rate that would generate rainfall sufficient for rain-fed agriculture (given the appropriate meteorological conditions) would those quantities of bacteria have a negative impact on plant health - or not? To answer this question would require knowledge of the fraction of *P. syringae* cells that are ice nucleation active at -8°C , the fraction of those that actually get to cloud height and the amount of rainfall generated. It would also require knowledge of any contexts where *P. syringae* itself could play a real role in rainfall generation relative to the abundant other types of INPs. The increasing research effort of the Earth sciences is contributing knowledge useful for answering these questions at a pace that might rival the

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speed at which we can deploy knowledge of epidemiology and of pathogenicity mechanisms to control plant disease.

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Conclusion

The biological cycle of a wide array of environmental microorganisms generally includes growth in some substrate, the dissemination through different compartments of landscapes - natural and cultivated - that differ in their biotic and abiotic characteristics, and survival in the same or other substrates. As particles that are readily lofted into the atmosphere, bacteria are likely to make long distance voyages and encounter extreme conditions at some phase of the life of their cells. Agriculture is generally a very open system with numerous inputs from varied sources including the so-called natural environment. Therefore, the above scenario of movement across space is pertinent to most plant pathogenic bacteria. As a result, plant pathogenic bacteria can sequentially achieve cycles of multiplication in a short time frame on multiple individual hosts from a wide diversity of plant species and can encounter a wide range of substrates to inhabit other than plants. Pathogens can be faced with extreme fluctuations in the diversity of plants they encounter due to human activities (monocultures, weeding, harvest, etc.) and in the associated abiotic conditions. Furthermore, plants, as well as other environmental substrates, harbor complex suites of microorganisms thereby setting the stage for a variety of possible intra- and inter-specific interactions with other microorganisms. In this framework, the options for novel research questions about the ecology plant pathogenic bacteria are overwhelming. Here we have focused on the need to understand roles or interactions other than those directly related to disease causation or where pathogenicity to plants is not the main driver of diversification. Importantly, we make these propositions in the interest of managing plant health and productivity. The path of science to knowledge is dialectical and involves the creation and elimination of hypotheses. Plant pathology overall has focused on the processes of pathogenicity *per se* as the driver of pathogen diversification to the detriment of other perspectives.. This context is not conducive to formal hypothesis testing because of the lack of alternative hypotheses about other drivers of diversification. The novel points of view on bacterial life history that we propose here might be wrong or impertinent for certain plant pathogenic bacteria. But they can inspire more robust hypothesis testing to identify the most important drivers of survival and diversification of plant pathogenic bacteria and the factors most critical for disease emergence. This is pertinent not only for bacteria that cause plant disease but also for other pathogens and pests of plants.

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In this article we have also raised issues about the pertinence of vocabulary such as “plant pathogenic bacteria” and “pathovars” without proposing options for alternatives. Changes in such vocabulary will require a debate about how we assess and describe reactions of plants to microorganisms and how to develop robust and useful indicators of these reactions. We propose two initiatives that could help the transition to other ways of communicating about plant pathogenic bacteria. Firstly, the descriptions of pathogens that fall under the auspices of legislation could be clarified. In Europe, for example, diseases for which quarantine measures will be applied are specified in European directives according to the Latin name of the implicated pathogen including pathovar designation where appropriate. In parallel, the European and Mediterranean Plant Protection Organization issues standards on diagnostic procedures for organisms that are named in these directives (<https://www.eppo.int/QUARANTINE/quarantine.htm>). We suggest that it be clearly specified, in the directives if necessary, that the objects of quarantine correspond strictly to the official diagnostic tests independent of what the Latin name might suggest. We suspect that this is not the current understanding. An illustration is the recent work to give a new pathovar name to variants of *P. syringae* that only cause leaf spots but not severe canker to kiwifruit. This was done to avoid that these fall under the auspices of quarantine control of *P. syringae* pv. *actinidiae* (Cunty *et al.*, 2015) even though these strains do not correspond to the diagnostic standards for the kiwifruit canker pathogen. Whereas the language used to describe the objects of specific control measures should be oriented to clearly delimit the nature of the pathogen, we propose that the language used in scientific publications about the biology, ecology and evolution of plant pathogenic bacteria be much less constraining. For example, a statement such as “*Ralstonia solanacearum* is a soil-borne plant pathogen” could be otherwise stated as “*Ralstonia solanacearum* has been implicated in severe wilting of plants during which its population densities are 10- to 100-fold greater than in absence of symptoms. This bacterium is also known to have an important capacity to survive in the soil. Its ability to induce symptoms long after it colonizes vascular tissue also suggests that it can persist in plants as an endophyte but generally at population densities about 1/10th of that in diseased plants. From here on we will refer to it as a soil-borne plant pathogen but the reader should keep in mind that little research has been dedicated to aspects of its life history outside of its role in plant disease.”

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We see an ideal future where research questions and vocabulary meld i) reactions of plants to microorganisms that are described in terms of alterations to important target phenotypes – phenotypes that underlie the reasons why we cultivate plants - such as yield of certain raw materials, of protein content, of organoleptic quality, etc. or that underlie plant evolution such as rates of reproduction ii) descriptions of bacteria in terms of the required fitness to alter these phenotypes of plants and iii) specification of multiple dimensions of the environmental context in which bacterial fitness was assessed. This will allow us to clearly set studies of plant pathogenic bacteria into an anthropogenic context with the goal of managing how they impact the productivity of crops, and in an evolutionary context with the goal of advancing fundamental understanding of bacterial populations.

Acknowledgements. We thank the editors for soliciting us to write this article and for the opportunity to express our points of view. We thank the two anonymous reviewers for their helpful comments. The authors declare no conflict of interest.

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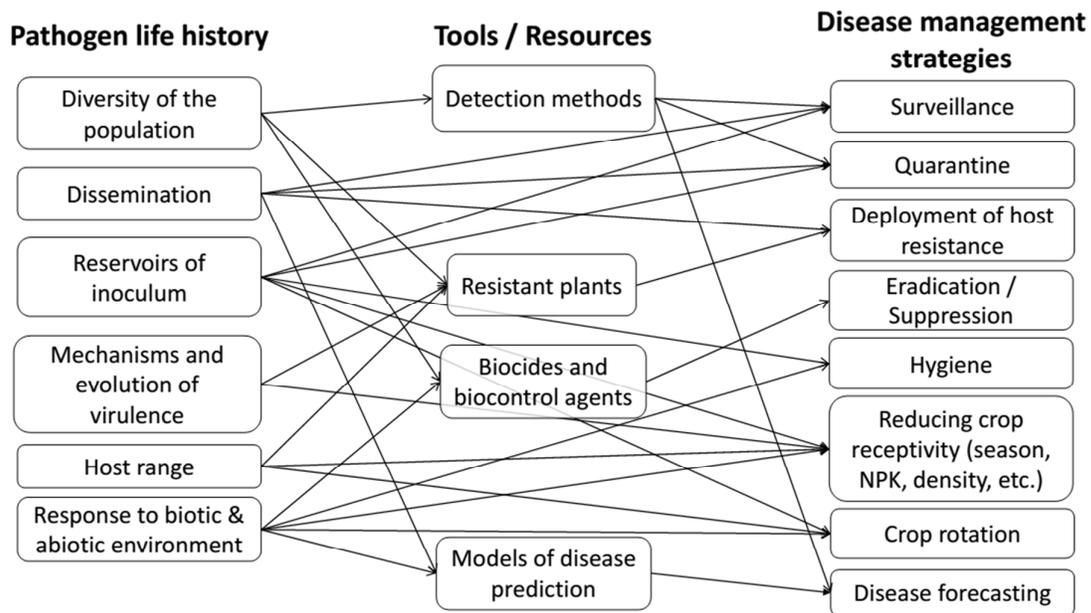
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Figure 1. The links between strategies for the management of plant diseases, the tools and resources that support the deployment of these strategies, and knowledge on the biology and ecology of plant pathogens. Knowledge about pathogen ecology in particular (diversity, dissemination, reservoirs of inoculum and how pathogens respond to the biotic and abiotic environment) underlie the development and deployment of all disease management strategies.

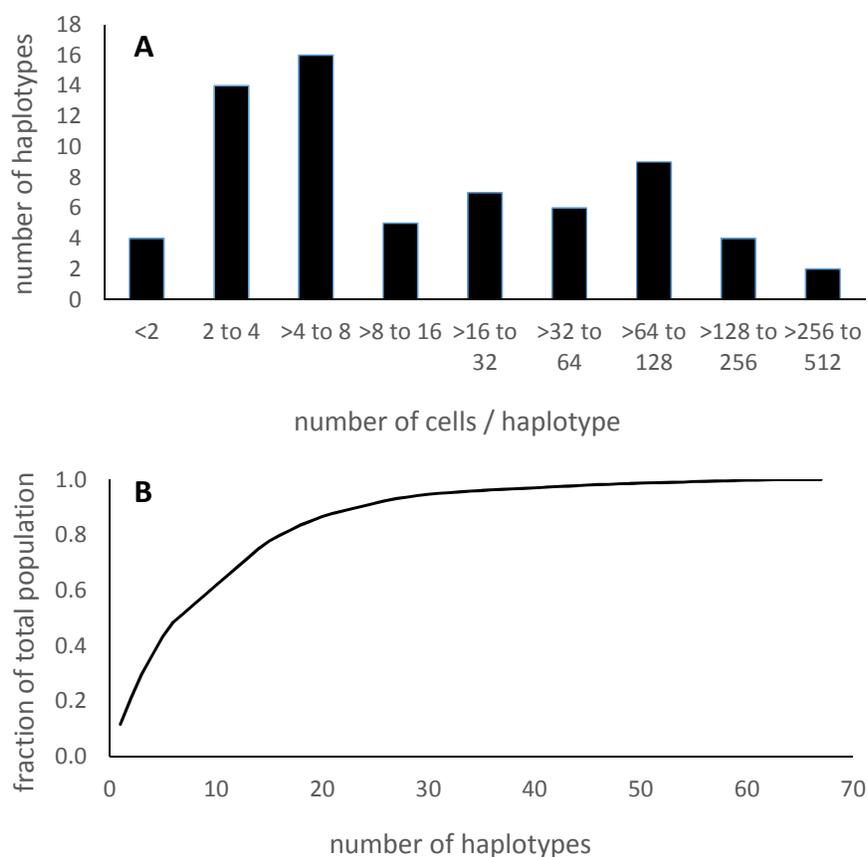


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Figure 2. **(A)** The number of haplotypes of *P. syringae*, based on the partial sequence of the *cts* gene, in terms of the number of cells of each haplotype in headwaters and **(B)** the fraction of the total metapopulation of headwaters accounted for by accumulation of haplotypes from the most dominant to the rarest. The abundance of each haplotype was calculated from a random sample of 235 strains from headwaters. A composite population of 3000 *P. syringae* cells accounting for the relative abundances of the 67 haplotypes detected in 13 different headwaters was constructed to calculate abundances of these haplotypes. Data for this figure were from Morris et al (Morris et al., 2010).



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791

Box 1. The ecology of human pathogens: the example of *E. coli*

792

793

Non-medical environmental reservoirs are increasingly recognized for their roles in the emergence of human pathogens. This has led to changes in the paradigms about the life history of these pathogens¹. Work on *Escherichia coli* embodies this shift in perception. The primary habitat of *E. coli* is the intestinal track of humans and other vertebrates where it mostly resides as a commensal. However *E. coli* is a versatile species and various lines recognized for different pathogenic capacities such as verotoxigenic, enterohaemorrhagic, enteroinvasive, uropathogenic and extraintestinal pathogenic *E. coli* have been described. The history of research on *E. coli* has revealed that understanding what makes it a devastating pathogen has required knowledge of its ecology as a commensal. Characterization of commensal strains from humans and various animals revealed a weak association between host species and the prevalence of strains from the main phylogenetic group among the seven phylogenetic groups of this species¹. By extending sampling to wild life vertebrates, a high genetic diversity with a host-dependant population structure was revealed². More generally, a decrease of diversity was observed among strains isolated from animals with increasing contact with humans³.

E. coli is not restricted to its hosts and can survive in various other habitats such as soil, manure, irrigation water, seeds or on plants⁴. In these habitats, it faces dramatically fluctuating conditions of temperature, carbon and water availability, osmolarity, oxygen level, and pH. Although the conditions outside of intestines are less favourable to the survival of *E. coli* than those in the intestinal track, *E. coli* was nevertheless found to survive for days and even years in these habitats. The ability to survive in these fluctuating environments is likely to be rather similar between various strains because the genes involved in reaction of carbon and energy limitation or in stress protection are well conserved between strains. This persistence in the open environment is a threat to humans as has been observed with food-borne disease outbreaks due to the extraintestinal pathogenic strain O157:H7.

At the genomic level, the presence of several regions encoding traits implicated in virulence such as adherence, colonization, invasion, secretion of toxic compounds and siderophore production contributes to the notion that virulence is a multigenic process⁵. However, most of these traits are also present in commensal strains and could be viewed as simply enhancing bacterial fitness in the intestinal track. This suggests that virulence is a coincidental by-product of commensalism⁶.

¹ Morris C.E. et al. 2009. *PLoS Pathogens*, 5(12), e1000693. doi:1000610.1001371/journal.ppat.1000693

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³ Skurnik D. et al. 2016. *Mol. Biol. Evol*. 33:898-914.

⁴ van Elsas J.D. et al. 2011. *ISME J*. 5:173-83.

⁵ Touchon M. et al. 2009. *PLoS Genet*. 5(1):e1000344.

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