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REVIEW

Universal soldier: *Pseudomonas aeruginosa*—an opportunistic generalist

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Abstract **The opportunistic pathogen *Pseudomonas aeruginosa* commonly causes chronic and ultimately deadly lung infections in individuals with the genetic disease cystic fibrosis (CF). *P. aeruginosa* is metabolically diverse; it displays a remarkable ability to adapt to and successfully occupy almost any niche, including the ecologically complex CF lung. These *P. aeruginosa* lung infections are a fascinating example of microbial evolution within a "natural" ecosystem. Initially, *P. aeruginosa* shares the lung niche with a plethora of other microorganisms and is vulnerable to antibiotic challenges. Over time, adaptive evolution leads to certain commonly-observed phenotypic changes within the *P. aeruginosa* population, some of which render it resistant to antibiotics and apparently help it to out-compete the other species that co-habit the airways. Improving genomics techniques continue to elucidate the evolutionary mechanisms of *P. aeruginosa* within the CF lung and will hopefully identify new vulnerabilities in this robust and versatile pathogen.**

Keywords *Pseudomonas aeruginosa*, cystic fibrosis, evolution, adaptive radiation, antibiotic resistance, quorum sensing

In ecological parlance, a niche is defined by the species which occupies it. "One species, one niche" is a concept that is entrenched in modern post-Darwinian thinking (Hardin, 1960). However, this does not necessarily mean that one species can only occupy one niche; many examples are known in which a single species diversifies to fill all of the available niches in a habitat. This multiple niche polymorphism is particularly relevant to the "hostile takeover" of the cystic fibrosis (CF) airway microbiota by the opportunistic human pathogen, *Pseudomonas aeruginosa* (Govan and Deretic, 1996; Foweraker, 2009).

When a species invades a new habitat, especially a spatially heterogeneous one, it often undergoes a burst of adaptive radiation to maximize niche occupancy (MacLean, 2005). Adaptive radiation is the diversification (branching) of lineages to fill all of the available niches. However, new habitats rarely represent virgin territory, and the interloper usually has to compete with other species, which themselves are undergoing (or have undergone) genetic diversification. Such intense selective pressures can drive niche partitioning (and therefore, diversification) yet we still know little about how intra-species population diversity impacts on inter-species community dynamics.

With their rapid growth rate, large population sizes and genetic tractability, bacteria are increasingly being recognized as an excellent test-bed for many of the concepts arising out of the Darwinian theory of evolution. In recent years, this realization has spawned a plethora of laboratory studies designed to road-test and extend evolutionary theory through the study of microbial populations *in vitro*, although, few, if any of these studies have tackled the problem of microbial evolution in natural ecosystems. With their intrinsic complexity and versatile selection pressures, such "real-world" environments are a far more realistic evolutionary proving ground than laboratory-based models.

For most bacteria with medium-large sized genomes such as *P. aeruginosa* (the principle protagonist in this review) around 10% of the genome encodes functions that are essential for life per se (Jacobs et al., 2003). The remaining 90% comprises "contingency genes" that enable niche specialization or adaptation to environmental challenges (Mathee et al., 2008). In the case of *P. aeruginosa*, much of this excess genetic baggage encodes latent metabolic and regulatory functions; *P. aeruginosa* is one of the most metabolically-versatile Gram-negative bacteria known. This

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adaptive flexibility endows the organism with an almost unrivalled ability to colonize different habitats (Spiers et al., 2000). Relatively few pathogens can claim such generalist attributes–*P. aeruginosa* is the ultimate opportunist. As pointed out by Buckling et al. (2003), generalists are far more likely to diversify when confronted with an imposed selection pressure compared with specialists. Indeed, in highly differentiated ecosystems, non-uniform resource availability and spatial heterogeneity virtually guarantee that adaptive radiation (and therefore niche differentiation) will occur.

Adaptive radiation in laboratory model systems (reviewed by MacLean (2005))

Perhaps the most ingenious laboratory-based study of adaptive radiation was presented by Rainey and Travisano (1998), who showed that when a *Pseudomonas fluorescens* population is maintained in static culture, nutrient and oxygen gradients are generated that drive adaptive radiation. Remarkably, the pattern of adaptive radiation was strikingly similar in parallel independent experiments, indicating that with a constant selection pressure, evolution exhibits a high degree of convergence. Subsequent work showed that adaptive radiation is often accompanied by a fitness "trade-off" i.e., the evolution of specialist behavior in a lineage does not come without cost (MacLean et al., 2004). However, as long as the selective pressure is maintained, this is offset by compensatory secondary mutations, which act to reduce the overall cost of specialization (MacLean et al., 2004). A corollary of this is that specialization can become a one-way street; once a lineage has committed to this evolutionary pathway, it is often difficult to reverse the trend and the organism may become "locked" into its new niche. [This notwithstanding, we note that specialization has not prevented some "epidemic" strains of *P. aeruginosa* (see below) from moving between patients, although these may be a special case.]

The CF lung as an evolutionary model

In spite of their utility, *in vitro* laboratory microcosms are no substitute for studying microbes in their natural environment. Natural habitats are spatially heterogeneous, chemically complex and are often occupied by multiple co-habiting species competing for the same limited resources. The drivers for niche realization in such environments are therefore far more powerful than those *in vitro*. The chronically-infected cystic fibrosis lung is a clinically-important and well-characterized example of such an *ex vitro* ecosystem. Although CF itself is a genetic disease, most patients also show an exquisite predisposition toward acquiring bacterial infections in their airways (Foweraker, 2009). In their early years, many CF individuals become infected with opportunists such as *Staphylococcus aureus* and *Hemophilus influenzae*, although eventually, around 70%–90% become colonized by *P. aeruginosa*; a species that appears to have a particular affinity for the CF lung environment and which can actively displace earlier colonists. A key theme in the treatment of CF therefore focuses on preventing these infections from taking hold. Early and intermittent *P. aeruginosa* infections can usually be cleared by aggressive therapy with anti-pseudomonal antibiotics (reviewed by Foweraker (2009)). The longer these early *P. aeruginosa* infections can be staved off through treatment, the better the long-term prognosis for the patient.

Despite intensive treatment with combinations of antibiotics, the spectre of antibiotic resistance or re-infection (from external sources or from internal reservoirs) remains a perpetual problem among CF patients. The intrinsically-high antibiotic resistance of *P. aeruginosa*, arising mostly from constitutive high-level expression of the broad spectrum MexAB-OprM efflux pump (Kumar and Schweizer, 2005) is further exacerbated by other factors; the bacteria are thought to reside in the CF lung in antibiotic-insensitive biofilm-like assemblages (Singh et al., 2000), slow-growing persister cells (Mulcahy et al., 2010) and antibiotic-resistant small colony variants (SCVs) (Häussler et al., 1999, 2003; Starkey et al., 2009) often arise, and the CF lung environment harbours anaerobic pockets (Worlitzsch et al., 2002) that reduce the efficacy of aminoglycoside antibiotics (a clinical mainstay for many patients). Moreover, many CF-adapted strains also acquire a hypermutation phenotype (usually through loss-of-function mutations (Stickland et al., 2010) in the mismatch repair machinery (Oliver et al., 2000)). Hypermutation enables evolution to go into overdrive and can accelerate the rate at which the strain becomes resistant to antibiotics. This is where the generalist attributes of *P. aeruginosa* really come into play: the genome houses a large repertoire of normally latent antibiotic resistance determinants, including cryptic multidrug efflux pumps. The expression of many of these determinants can become inappropriately turned on through mutation (Stickland et al., 2010), and this is often cited as a major driver underpinning enhanced antibiotic resistance. However, hypermutation is something of a Jekyll and Hyde phenotype: although hypermutators often show an enhanced ability to survive aggressive antibiotic exposure, two factors mitigate against runaway hypermutation. First, just as resistance-conferring determinants can be hit by mutation, so too can "innocent" genes. This can confer a fitness cost. Second, the activation of normally cryptic resistance determinants is also often accompanied by a fitness tradeoff. For example, mutations in *nfxB*, which lead to activation of the MexCD-OprJ multidrug efflux pump, confer a large energetic burden on the cell and are strongly negatively selected when cocultured with the wild-type (Stickland et al., 2010). Consequently, although hypermutators often arise in *P. aeruginosa* CF populations, they rarely dominate the population ensemble. Another–essentially pathognmonic–phenotype associated with *P. aeruginosa*

isolates from CF patients is the acquisition of mucoidy (Pritt et al., 2007). Mucoidy is a very rare phenotype in environmental *P. aeruginosa* strains, and seems to be driven by the unique microenvironment in the CF lung. Mucoid strains produce exuberant quantities of a polysaccharide called alginate, which coats the bacteria and is thought to provide yet an additional level of protection against immune cell clearance and antibiotic action. The layer of alginate that encapsulates the cells has also been postulated to promote biofilm formation, although not all mucoid strains form robust biofilms (Chung et al., 2012). In non-mucoid wild-type cells, alginate biosynthesis (encoded by the *alg* operon) is suppressed because the sigma factor (AlgT) necessary for transcription of the operon is sequestered by the anti-sigma factor, MucA. Mucoidy arises when the *mucA* gene accrues inactivating mutations, making the encoded MucA unable to bind AlgT, consequently de-repressing alginate biosynthesis. There is a strong negative correlation between the appearance of mucoid isolates in CF sputa and patient wellbeing. Collectively, the factors outlined above mean that intermittent infections almost inevitably become non-resolvable, at which point they become defined as chronic. The onset of chronic infection correlates strongly with an accelerated decline in lung function (Foweraker, 2009).

In many chronically-infected CF patients, it is not uncommon to find 10^8–10^9 cfu/mL *P. aeruginosa* in sputum samples – remarkably high titers, considering that the patient may survive for decades (Govan and Deretic, 1996; Foweraker, 2009). Consistent with a general selection for slower-growing variants in the CF habitat (Yang et al., 2008), the measured doubling time of *P. aeruginosa* in CF sputum is long (~150 min). Consequently, for those adult CF patients who have been chronically-infected for 10 years or more, the *P. aeruginosa* population will have undergone approximately 35000 doublings, more than enough time for selection pressures to sculpt the population structure. The key, and currently unanswered question, is what the end product of this evolutionary grindstone looks like: a single highly-adapted lineage displaying little diversity beyond that arising from genetic drift, or multiple co-existing lineages, each occupying a unique niche. It seems increasingly likely that there will be no simple, or even single answer to this multifactorial problem, which may also be dependent on the type and number of species which co-habit the lung. However, some common trends are emerging.

Brockhurst et al. (2007) have examined the impact of existing niche diversity on adaptive radiation by *P. fluorescens*. These workers found that in a simple monotrophic laboratory ecosystem pre-seeded with *P. fluorescens* specialists, increased size and complexity of the resident community acted to constrain adaptive radiation by an incoming (non-specialized) strain of *P. fluorescens*. However, whether this is also the case in a complex multi-species microcosm such as the CF airway, where inter-species communication may drive the formation of novel "biotic" niches and/or promote niche partitioning, remains to be seen. Moreover, we note that the opportunities for repeated bursts of adaptive radiation in the CF airway are high, since the microbial community has the potential to be regularly remodeled by frequent antibiotic sweeps. Although this possibility has not yet been explicitly tested, it is consistent with the observation that at a phenotypic level, the *P. aeruginosa* population structure in expectorated CF sputum is highly dynamic, even over periods as short as a few months (Mowat et al., 2011). Another emerging area of interest is in the role(s) of bacteriophage in tailoring the population. Genome sequencing has revealed that many CF isolates encode latent prophage, and some antibiotics (e.g., fluoroquinolones) are known to strongly induce transition into the lytic cycle (James et al., 2012). Indeed, it is formally possible that antibiotic-induced phage release (rather than the antibiotics themselves) is the actual driver of population change, although again, this remains to be tested. Equally, the preponderance of prophage in CF isolates may serve a different role; the phage may act as guardians of the niche, preventing super-infection by incoming susceptible competitor strains (a kind of crude "kin selection" mechanism (Chung et al., 2012)).

Community diversity in the CF lung environment

Although *P. aeruginosa* is often the dominant pathogen associated with chronic CF infections, it is rarely the sole species present and usually shares the niche with a wide range of other microbes. Indeed, recent advances in cultivation-independent methodologies have revealed the true vista of microbial species present (Rogers et al., 2003, 2004; Cox et al., 2010; Guss et al., 2011). These co-habiting species can be partitioned into two clear sub-groups, namely; "core" species (found in most CF patients) and "satellite" species (often CF-associated, but with a rather patchy distribution among different patients (van der Gast et al., 2011)). As noted above, the CF lung environment can contain regions that are strongly anaerobic, and consistent with this, anaerobes have been found in high titers in the CF lung and in some cases, in even higher numbers than *P. aeruginosa* (Tunney et al., 2008). Crucially, there appears to be a direct link between community diversity and the severity of respiratory disease; individuals who have better lung function parameters often harbour a more diverse spectrum of bacterial species. Conversely, individuals with poorer lung function carry a much more restricted range of species, often dominated by *P. aeruginosa* (Stressmann et al., 2012; Zhao et al., 2012; Daniels et al., 2013).

Interestingly, although usually efficacious from a clinical perspective, the application of anti-pseudomonal antibiotics often does little more than make a minor dent in the *P. aeruginosa* population. Indeed, it is becoming increasingly clear that such treatments often have a disproportionately greater impact on the non-pseudomonads present, serving to

drive down the overall "species richness" in the lung (Zhao et al., 2012; Daniels et al., 2013). This effect appears to be transient and in the refractory post-antibiotic period, the bacterial community gradually reverts to a state resembling the pre-intervention composition. One possibility is that since "gaps" in the antibiotic-depleted communities do not appear to be stochastically repopulated by other species, the ecological connections which stabilize the microbial community may be remarkably robust. However, this assumes that the same *intra*-species lineages re-occupy the vacated niches, but this is not known. In principle, the surviving lineages could take full advantage of periodic antibiotic scourings by undergoing further rounds of adaptive radiation, enabling them to progressively infiltrate the liberated niches. Consistent with this, the polymicrobial diversity has been found to decline in older CF patients, and *P. aeruginosa* begins to gain the upper hand (Cox et al., 2010). This is a good example of how a multi-niche ecosystem, originally occupied by many species, eventually becomes occupied by just a single species. The question of what happens to the *P. aeruginosa* population diversity as *inter*-species diversity declines is not yet known, although in many late stage CF patients, the organism completes the coup and becomes the dominant, if not sole detectable species.

Overview of *P. aeruginosa* genome structure

Genomic analyses have helped to reveal the reason why *P. aeruginosa* infections are so prevalent among the CF community. The first *P. aeruginosa* genome to be sequenced was that of the type strain, PAO1 by Stover and colleagues in 2000 (Stover et al., 2000). The 6.3Mbp genome was found to encode 5571 ORFs and 106 non-coding RNAs, with the latter number continually being revised upwards as more non-coding regulatory RNAs are discovered (e.g., see Wurtzel et al., 2012); we also note that ORFs of < 50 residues – and the *P. aeruginosa* genome is littered with these – are not normally annotated, yet increasing numbers of small polypeptides of this nature are being found to play pivotal roles in many bacteria (reviewed by Hobbs et al., 2011). Following completion of the PAO1 genome, other genomes soon followed, including that of strain PA14 (isolated from a burns patient, 6.5Mbp, 5977 ORFs), the Liverpool Epidemic Strain (LES; isolated from a CF patient, 6.6Mbp, 5931 ORFs; Winstanley et al., 2009) and the taxonomic outlier, PA7 (a 6.6Mbp non-CF isolate; Roy et al., 2010). Comparative analysis of these sequence data revealed the mosaic and highly plastic nature of the genome. The *P. aeruginosa* genome can be divided into "core" and "accessory" components, with the horizontally-acquired accessory elements (which comprise up to 10% of the overall genome size) often being strain-specific (Mathee et al., 2008). It turns out that most known virulence factors, long considered critical for infection, are housed in the conserved core genome and therefore all *P. aeruginosa* strains are potentially pathogenic. However, although this feature goes a long way toward explaining the exquisite predilection of the organism for the CF airways, another key factor is the ubiquity of the species. Indeed, in spite of controlling patient-to-patient infection routes, many CF patients eventually become infected by unique, environmentally-acquired strains of *P. aeruginosa*. The accessory genome seems to play an important role here because it confers broad environmental adaptability, encoding enhanced metabolic capabilities or other niche-specific functions. This ability to customize its genomic repertoire through discarding or acquiring discrete blocks of DNA means that *P. aeruginosa* can, and does, live almost anywhere. As a consequence, infection (especially of individuals predisposed toward *P. aeruginosa* colonization, such as those with CF) eventually becomes a near certainty. The key issue is whether once a strain establishes itself in the CF lung, it begins to streamline its genomic repertoire as it "beds in" and adapts to its new circumstances.

P. aeruginosa evolution in the CF lung

A number of workers have investigated the evolution of *P. aeruginosa* lineages in CF, although it needs to be stressed that none of these earlier studies have investigated the impact of *P. aeruginosa* population diversity on the other microbial species present or vice versa. Smith et al. (2006) broke new ground by using whole genome sequencing technology to try and capture some of the changes that accompany the early adaptive radiation that follows initial colonization. They sequenced the genome of a single *P. aeruginosa* isolate obtained from a CF individual aged 6 months, and of a second isolate of the same strain from the same patient aged 96 months (Smith et al., 2006). This analysis revealed that during this 7.5-year period, the lineage had accrued mutations in 68 genes, and a strong signal of positive selection was evident. In a later study, Cramer et al. (2011) analyzed the genome sequences of three isolates from each of two patients over a ca. 15-year period (in each case including an isolate that had been harvested at or around the time of initial infection). These workers found that the isolates accrued very few mutations over this period, and concluded that in the absence of hypermutation, the mutation rate is "amazingly low." More recently, Yang et al. (2011) studied genomic changes in longitudinal isolates of *P. aeruginosa* strain DK2. Unlike many CF infecting strains, DK2 is a so-called "epidemic" strain that is transmissible from patient-to-patient. Consequently, the strategy adopted by Yang et al. was to sequence the genome of DK2 isolates that had been harvested and stored from the sputum of several CF individuals over a ca. 35-year period. These authors were able to demonstrate parallel evolution of the same traits in samples from the different patients, suggesting that the selection pressures acting in different individuals are similar. Moreover, Yang et

al., found that DK2 exhibited a burst of diversification shortly after infecting its first CF host, followed by a prolonged period of random genetic drift. During the initial burst of adaptive radiation, they report that a number of pleiotropic regulators were affected, including the main quorum sensing regulator, *lasR* (see below). However, since DK2 (along with other epidemic strains such as the Liverpool strain, the Manchester strain and more recently, also the so-called "Prairie strain" (Workentine et al., 2013)) appears to be genetically pre-configured toward infecting CF patients, it may not necessarily be representative of a typical CF infecting strain. Indeed, a strong signature of negative selection in the more recent DK2 isolates suggests that the organism has reached a major peak in the adaptive fitness landscape. It should be noted here that a clear distinction should be made between the infectivity (transmissibility) of a strain and its ability to produce virulence factors; not all highly-infective strains such as DK2 necessarily produce the full spectrum (or quantities) of known virulence factors, and it is still not clear why these particular strains are so transmissible. Interestingly, and in spite of its large population of CF patients, there are currently no reports of epidemic strains in the USA. More recently, the Danish team showed (using whole-genome sequencing of 45 isolates of DK2, harvested from 16 patients over a 35 year period) that the DK2 genome displays significant degradation upon adaptation to the CF lung environment, with one isolate losing around 8% of its genomic repertoire (Rau et al., 2012). This degradation affected both the core and accessory genome, although there was a bias toward loss of the latter. Degradation rates were very high – ca. 0.62bp/generation (12–36-fold higher than degradation rates observed *in vitro*) and again, there was strong evidence of parallel evolution among different isolates.

Intra-clonal heterogeneity

Although most chronically-infected CF individuals tend to be colonized by unique strains of *P. aeruginosa*, clonally-derived isolates from a single sputum sample often display multiple heritable colony morphotypes and diverse antibiotic susceptibility profiles (Mowat et al., 2011; Workentine et al., 2013). These data strongly suggest that the population may be genetically heterogenous, prompting the current authors to explore this possibility further. We did so through the simple expedient of sequencing the genomic DNA from coeval pairs of *P. aeruginosa* isolates, with each pair being obtained from the sputum of a different CF patient (Chung et al., 2012). The results were striking: the paired isolates from the one patient differed due to only 1 single nucleotide polymorphism (SNP) and 8 short insertions/deletions (indels) in the core genome. However, the paired isolates from a second patient differed due to 54 SNPs and 38 indels, whereas the pair of isolates from a third patient cumulatively differed due to 344 SNPs and 93 indels. The high rate of mutation in the latter pair of isolates could be attributed to a *mutS* mutation which confers a hypermutator phenotype. Furthermore, in two of the pairs of isolates, a different accessory genome composition was noted. For example, an F10-like chromosomally-integrated prophage was noted in isolate 5_S, yet this prophage was absent in the cognate paired isolate from the same sputum sample, 5_M. Thus, the cross-sectional genomic variation at every level of sequence resolution (SNP, indel and accessory genome) among contemporary *P. aeruginosa* CF isolates can be comparable to the variation previously reported to differentiate between paired longitudinally-sampled isolates (Chung et al., 2012). Given that these differences were apparent in just two randomly-chosen isolates from each patient, it seems very likely that the *P. aeruginosa* population as a whole in each patient will display substantial micro-heterogenity.

Klockgether et al. (2013) recently added to the debate by integrating transcriptomic, proteomic and metabolomic analyses with whole genome sequence data. These authors characterized two very closely-related CF strains that differed due to just 7 non-synonymous SNPs and/or indels in the core genome. In spite of their almost identical core genome sequences, the strains differed substantially at the phenotypic and functional genomic level, and concluded that the small number of SNPs and indels may have been responsible for this. Taken at face value, these results indicate that phenotypically-distinct lineages do not necessarily have to be radically-different at the genotypic level. A SNP here, a SNP there – such small changes could therefore make all the difference when it comes to niche expansion. However, and while it is relatively easy to see how some of the SNPs and indels identified by Klockgether et al. might give rise to pleiotropic effects, we note that the two strains also differed due to the presence of a large PAGI-2 like horizontally-acquired genomic island. Presumably, this too could also have contributed toward the observed phenoptypic differences.

Quorum sensing and loss of virulence in CF *P. aeruginosa* isolates

In addition to whole genome sequencing, targeted gene sequencing and phenotypic analyses have also been used to monitor the evolutionary trajectory of *P. aeruginosa* in the CF lung. Several common features have emerged from these studies. One of the most startling is that the genes encoding virulence factors or their global regulators readily accrue loss-of-function mutations (Lorè et al., 2012) and become redundant during chronic infection. This suggests that virulence is a macro-phenotype that is negatively-selected in CF infections (presumably, it is not in the organism's best long-term interests to kill the host). Indeed, it is debatable whether virulence factors play *any* role in chronic CF infections. Critical to the argument here is the role played

by "quorum sensing" (QS) – a mechanism of gene regulation based on intercellular communication (Chugani et al., 2012). QS by *P. aeruginosa* exploits two types of intermolecular signal; *N*-acylated homoserine lactones (AHLs) and a quinolone-type molecule (the Pseudomonas Quinolone Signal, or PQS) (Williams and Camara, 2009). Both types of signal have been shown to positively-regulate virulence factor production and both have been identified in CF sputa (Singh et al., 2000; Collier et al., 2002). However, and although QS defective mutants display greatly-reduced virulence in acute mouse and rat lung infection models, this does not necessarily mean that QS and virulence factors are important in chronic CF infections. For example, mid-late stage CF isolates are frequently found to be defective in both AHL- and PQS-dependent QS (Diggle et al., 2007; Bjarnsholt et al., 2010; Chung et al., 2012). One oft-cited explanation for this is that virulence factors might only be required for the initial infection and are dispensable thereafter. However, and mitigating against this possibility, is the observation that the above-cited DK2 strain lost its ability to carry out AHL-dependent QS in 1979 (Yang et al., 2011) yet still retains full infectivity. This may simply reflect the peculiarities of the DK2 strain. An alternative view is that within the CF airway environment, QS incurs a fitness cost and may even be counter-selected. QS is a metabolically-costly regulatory strategy. The synthesis and secretion of fatty acid-like signaling molecules is inherently wasteful of resources. However, this fitness cost must be offset in environments other than the CF lung (otherwise it would not have evolved) by an additional fitness advantage that is bestowed by QS regulation in that context, an advantage that by inference is absent in the CF lung environment. In this scenario, if there is a selective disadvantage to be had in performing QS, inactivation of the genes involved in QS should be positively selected in the CF airway environment i.e., dN/dS (the ratio of observed/expected non-synonymous:synonymous mutations) should be > 1. [Disruption of QS will also indirectly promote the inactivation of downstream genes; since these are no longer expressed, they effectively become "silent." However, such degradation should be distinguishable due to its neutral genetic signature (i.e., $dN/dS = 1$).] Presumably, since most infecting strains of *P. aeruginosa* are derived from environments where QS may be beneficial, this may explain why the initial infecting isolates generally retain an ability to carry out QS. One final suggestion is that QS mutants arise as a natural consequence of Darwinian evolutionary logic; they are "cheats" which bypass the fitness costs associated with virulence factor production, yet indulge in the nutritional windfall generated by the action of secreted "public goods" (virulence factors) produced by their QS-positive neighbors (Diggle et al., 2007). However, and although some QS-defective CF isolates may well be *bona fide* cheats, logic dictates that the population can only accommodate a certain steady-state level of selfish cheats before the strategy becomes disadvantageous ("the tragedy of the commons"). This may explain why QS mutants are common in CF, yet often co-exist with QS-positive strains. More prosaically, QS molecules like PQS may be produced simply because they are antibacterial (reviewed by Williams and Camara, 2009) and thereby help to protect the niche from competitors.

Conclusions

The picture emerging from recent work, especially genomic and culture-independent analyses, is that CF lung infections are polymicrobial, show dynamic behavior and are likely to be strongly influenced by external perturbations such as antibiotic intervention. The CF lung is initially colonized by a wide range of microbes, and this is supported by the substantial spatial and chemical heterogeneity (= ecological opportunity) in that environment. However, once *P. aeruginosa* gets a foothold in the airways, perhaps aided and abetted by long-term antibiotic intervention, it can out-compete the resident microbiota and become the dominant or even sole species present. By inference, the *P. aeruginosa* in late-stage patients must either occupy the niches vacated by earlier colonists, or have mechanism(s) by which to exclude other species from re-infiltrating these niches. In principle, a generalist such as *P. aeruginosa* could potentially simultaneously occupy multiple niches through differential gene expression (e.g., driven by localized nutritional inhomogenieties in different parts of the lung etc.). However, as pointed out above, in the longer-term, the necessary adaptations eventually become hardwired through mutation, at which point the organism usually becomes irreversibly "committed" to its new home and way of life. That these changes are genomic is reflected by the fact that colonies isolated from CF sputa show heritable phenotypic differences, and indicates that the population is comprised of a mixed bag of co-habiting, clonally-derived genomic variants. A parsimonious explanation is that as other species become displaced, within-species diversity allows the infecting strain to rapidly optimize occupancy of the vacated niches. We note that an additional selective advantage of maintaining a pool of pre-formed microvariants is that the population is always "primed," ready to respond to external challenges (the "red queen" effect). On a practical level, these issues will only serve to frustrate the development of new or improved intervention strategies for this patient group.

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Compliance with ethics guidelines

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Rzhepishevska, Madeleine Ramstedt and Martin Welch declare they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethical committee.

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