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REVIEW ARTICLE

Hypermutation and stress adaptation in bacteria

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Abstract

Hypermutability is a phenotype characterized by a moderate to high elevation of spontaneous mutation rates and could result from DNA replication errors, defects in error correction mechanisms and many other causes. The elevated mutation rates are helpful to organisms to adapt to sudden and unforeseen threats to survival. At the same time hypermutability also leads to the generation of many deleterious mutations which offset its adaptive value and therefore disadvantageous. Nevertheless, it is very common in nature, especially among clinical isolates of pathogens. Hypermutability is inherited by indirect (second order) selection along with the beneficial mutations generated. At large population sizes and high mutation rates many cells in the population could concurrently acquire beneficial mutations of varying adaptive (fitness) values. These lineages compete with the ancestral cells and also among themselves for fixation. The one with the 'fittest' mutation gets fixed ultimately while the others are lost. This has been called 'clonal interference' which puts a speed limit on adaptation. The original clonal interference hypothesis has been modified recently. Nonheritable (transient) hypermtability conferring significant adaptive benefits also occur during stress response although its molecular basis remains controversial. The adaptive benefits of heritable hypermutability are discussed with emphasis on host–pathogen interactions.

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Introduction

Adaptive evolution by natural selection depends upon the supply of mutations, especially beneficial mutations (reviewed by Sniegowski and Gerrish 2010). Generally, spontaneous mutation rates are very low, of the order of 10^{-10} to 10^{-6} per base pair per cell per generation. Intuitively, one could imagine that elevated mutation rates (hypermutability) could speed up adaptation, especially under stressful conditions when organisms might need multiple mutations for successful adaptation. However, since the majority of mutations happen to be deleterious rather than beneficial (Kibota and Lynch 1996; Boe *et al.* 2000; Imhoff and Schlotterer 2001), elevated mutation rates could be potentially more harmful than beneficial. Therefore a sensible strategy would be to keep mutation rates as low as possible. Thus, several decades ago, Sturtuvant (1937) posed a question 'why does the mutation rate not become reduced to zero?' (see also Sniegowski *et al.* 2000). While mutations are necessary to generate variability upon which natural selection could act, maitaining the integrity of the genome is also essential for the perpetuation of species. Thus, there is a 'fundamental dialectic paradigm of evolution—stability versus variability' (Radman *et al.* 1999). Genome stability and variability ultimately depend on the fidelity of genome replication, rigid fidelity favouring the former and relaxed fidelity favouring the latter. Many mechanisms of error avoidance and error correction exist to ensure rigid fidelity of replication and low mutation rates. The metabolic costs as well as physicochemical constraints of these mechanisms may limit mutation rates from evolving to zero. In any case, organisms do need to have some mutability to adapt to stress and fluctuations in living conditions. A compromise between the indispensability of mutations and risks associated with high mutation rates would be to keep mutation rates as low as possible, but not zero. The literature on the evolution of mutation rates have been reviewed by many authors (Drake *et al.* 1998; Radman *et al.* 1999; Sniegowski *et al.* 2000; Denamur and Matic 2006; Baer *et al.* 2010). Defects in many genes and processes lead to the malfunctioning of the replication fidelity control mechanisms and give rise to cells with elevated mutation rates, called hypermutators, or simply, mutators (for references and review see Jayaraman 2009; in this review the terms mutators and hypermutators will be used interchangeably). In spite of the risks associated with it, hypermutability is quite common in nature, especially among

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clinical isolates of pathogenic bacteria (see below). Mutators have been implicated in many medical problems such as treatment failure in infectious diseases, antibiotic resistance etc. (Giraud *et al.* 2002; Blazquez 2003; Chopra *et al.* 2003; Miller *et al.* 2004; Macia *et al.* 2005; Oliver 2005, 2010; Orlen and Hughes 2006; Oliver and Mena 2010). This review is a sequel to the one published earlier (Jayaraman 2009) wherein the mechanisms of heritable hypermutability were reviewed. Therefore, the emphasis here will be on heritable hypermutability. However, this is not to belittle another, equally or more, important process, namely, transient (nonheritable) hypermutabilty which is associated with stress response. This process will also be reviewed briefly.

Merits and demerits of hypermutability

The evolutionary advantages of hypermutability have been discussed by several authors (Chao and Cox 1983; Taddie *et al.* 1997a; Tenaillon *et al.* 1997; Giraud *et al.* 2001a; Bayliss and Moxon 2002; Itoh *et al.* 2002; Le Chat *et al.* 2006; Oliver 2005; Woodford and Ellington 2007; Marias *et al.* 2008; Marcobal *et al.* 2008). Briefly, hypermutability enables organisms to adapt faster than normo-mutators to fluctuating and stressful environments. When many mutations are available, as will be the case with hypermutators, it is possible that some of them could be adaptively beneficial. For example, during infection, pathogens face many bottlenecks and demand multiple mutations to survive and establish an infection. Hypermutability would enable them to face such challenges. It is, therefore, not surprising that clinical isolates of several pathogens contain sizeable proportions of hypermutators (for references see de Visser 2002; Labat *et al.* 2005; Oliver 2005, 2010; Denamur and Matic 2006; Oliver and Mena 2010). Mutators which are defective in the DNA mismatch repair (MMR) system have an additional beneficial property, namely, homeologous recombination, i.e., recombination between largely homologous but nonidentical DNA sequences, such as, genetic crosses between *Escherichia coli* and *Salmmonella typhimurium* (Rayssiguier *et al.* 1989). This property is a bonus, and useful in adaptation by the acquisition and incorporation of DNA from disparate organisms by horizontal gene transfer. However, in stable, nonfluctuating environments or after successful adaptation to a new environment, hypermutability is not only of no value but may even be a fitness disadvantage which can be mitigated to some extent by the restoration of normal mutability by horizontal gene transfer of good MMR genes from heterologous sources followed by homeologous recombination (for references, see Jayaraman 2009). Besides the possible accumulation of deleterious mutations, mutations which are adaptively beneficial in one environment could prove detrimental in another, a trait called 'antagonistic pleiotropy' (Cooper and Lenski 2000). Funchain *et al.* (2000) showed that growth of a mutator strain for several generations resulted in the loss of many lineages. Similarly, Giraud *et al.* (2001a) showed that 25% of mutators adapted to growth in the mouse gut became auxotrophs whereas only 5% of nonmutators did so. In a recent report, Philippe *et al.* (2009) showed that two *E. coli* lineages from the long-term evolution experiment (LTEE; see below) had evolved mutations in the promoter of the *pbpA* gene, resulting in reduced synthesis of PBP2 (penicillin-binding protein 2). This conferred significant fitness advantage in the medium in which they evolved but a disadvantage, namely reduced salt tolerance, in other media. Thus, hypermutability could be an asset as well as a liability.

Indirect (second order) selection of mutators

Mutator mutations, like mutations in general, are infrequent in a population at the time of occurrence. However, mutators can increase in frequency over time and could get 'fixed' such that the entire population could consist of mutators. This has been studied extensively using theoretical, computer simulation and experimental approaches (Taddie *et al.* 1997b; Tenaillon *et al.* 1997, 2000; reviewed by Sneigowski and Gerrish 2010). Since bacteria is primarily asexual, all mutations are physically linked to one another in the genome; there is no recombination to unlink them. When a mutator cell adapts via a beneficial mutation, it also carries along the mutator allele due to physical linkage between the two. This has been called hitchhiking (Chao and Cox 1983). This indirect selection (also called second order selection) is believed to be the major mechanism for the spread of mutators in a population. Giraud *et al.* (2001a) have provided experimental support to this idea. A *mutS* (mismatch repair defective) mutator mutant, reisolated after adaptation to growth in the mouse intestinal environment, recolonized the mouse gut better than the ancestral (*mutS*$^{+}$) strain even if converted to *mutS*$^{+}$ by transduction. This showed that selection of the mutator allele is indirect, coupled to the beneficial mutation(s) it generates; once adaptation is achieved, it is dispensable. Second order selection has been reviewed by Giraud *et al.* (2001b) and Tenaillon *et al.* (2001). The results of Mao *et al.* (1997) also support the hitchhiking hypothesis. They showed that after two successive selections for antibiotic resistance, almost the entire population of *E. coli* cells were enriched for mutators.

The rise and fixation of mutators in wildtype populations of *E. coli in vitro* have been studied extensively by Lenski and coworkers in their LTEE (Lenski *et al.* 1998; Elena and Lenski 2003; Blount *et al.* 2008; Barrick *et al.* 2009; Philippe *et al.* 2009; other references cited above). A popular and personalized account of the LTEE can be found in Lenski (2011). For these experiments, 12 replicate lineages of *E. coli* were founded from a common ancestral strain and were grown in glucose-limiting-minimal medium for several generations by daily subculture. Samples were removed at every 500 generations of growth and stored frozen to provide a 'fossil record'. Founded in 1988, these lineages have gone through alternate cycles of growth and starvation (cycles of feast and famine) and have grown for more than 44,000

generations as of 2008 (Blount *et al.* 2008; now they have grown for more than 53,000 generations as could be seen from Lenski's Web page (http://myxo.css.msu/edu/ecoli). It should be noted that these cultures were not subjected to any deliberate and hard selection pressure other than growth in glucose-limiting medium. Several genetic and phenotypic alterations have been documented in these evolved lineages relative to the ancestral population (for detailed information and references see Blount *et al.* 2008; Philippe *et al.* 2009; Stanek *et al.* 2009). Of particular relevance to the present discussion is the finding that three (or four; see below) among the 12 populations evolved into mutators in less than 10,000 generations, their spontaneous mutation rates being 10–100-fold higher than their ancestors or the other nine evolved lineages (Sniegowski *et al.* 1997). The authors concluded that mutators got fixed by hitchhiking with other beneficial mutations (Sniegowski *et al.* 1997), a conclusion experimentally shown to be true later (see below) (there is mention of a fourth mutator in Cooper and Lenski (2000), but it does not seem to have been characterized further).

In a subsequent report Shaver *et al.* (2002) showed that the three evolved mutator strains had lesions in the MMR genes. The mutator sweep in these strains was shown to be due to hitchhiking with beneficial mutations rather than direct selection or genetic drift. Two of them showed a significant fitness gain during the mutator sweep. The interesting question is: how do the mutators which were in a minority in the beginning increase in frequency and get fixed eventually, out competing the majority nonmutators? The likely answer could be that the numerical disadvantage of mutators was in some way compensated for by their high mutation rates. Under conditions where multiple beneficial mutations are required for successful adaptation, mutators can generate them successively and ultimately rise to fixation. This has been shown experimentally by Mao *et al.* (1997) and Miller *et al.* (1999). Based on the results of competition experiments between mutators and nonmutators *in vitro* Le Chat *et al.* (2006) have suggested that in infectious diseases wherein the pathogens have to acquire multiple mutations to win over the host, mutators are at an advantage over nonmutators because of their ability to produce successive mutations, even if out numbered by nonmutators. The question of mutator fixation has also been addressed theoretically by Tenaillon *et al.* (1997) and Tanaka *et al.* (2003). The latter authors have introduced the time of appearance of beneficial mutation in (minority) mutators vis-a-vis (majority) nonmutators as a possible and important factor for the success of mutators. In already well adapted populations, mutator subpopulations do not enjoy any advantage of hitchhiking with beneficial mutations. On the contrary, they face the risk of extinction due to the generation of deleterious mutations.

Speed limit to adaptation: clonal interference

Adaptation depends upon a parameter which de Visser (2002) has termed beneficial mutation supply rate (BMSR). BMSR is simply the product of the mutation supply rate (MSR) and the fraction of mutations that are adaptively beneficial. MSR, in turn, is the product of population size and the mutation rate (de Visser *et al.* 1999). In large populations, BMSR is likely to be high such that many beneficial mutations could arise concurrently in different cells of the same population. A clonal population may, therefore, contain many subpopulations, each carrying a beneficial mutation of varying adaptive (fitness) value. Under such conditions the different subpopulations compete among themselves as well as their ancestor, for fixation. The one possessing the fittest mutation ends up as the winner. The others carrying less fit beneficial mutations are eliminated, in a sense wasting the mutations. This effect has been called clonal interference (CI) in asexual organisms and the Hill–Robertson effect in sexual organisms. The CI hypothesis was first formulated statistically by Gerrish and Lenski (1998); many others have elaborated on it subsequently using theoretical or a combination of theoretical and experimental approaches (for references see Fogle *et al.* 2008; Sniegowski and Gerrish 2010). Clonal interference imposes a speed limit to adaptation. Because of their high BMSR, large populations or mutator populations adapt faster than smaller populations, but not in strict linear proportion to their BMSR (less than linear), resulting in 'diminishing returns' (de Visser *et al.* 1999). Small populations in which BMSR is neither high nor low but intermediate benefit a lot by being mutators since a higher BMSR could accelerate their pace of adaptation and also shorten the waiting time between successive beneficial mutations. In addition to population sizes and mutation rates, the state of adaptedness of the populations is also an important determinant of the pace of adaptation. Two important predictions of the CI hypothesis are the simultaneous presence of many clones in an evolving population, each harbouring mutations of varying fitness benefits and long times for fixation of the fittest. Shaver *et al.* (2002) and de Visser and Rozen (2006) have provided experimental evidence in support of the CI hypothesis. Besides, there is extensive theoretical support for clonal interference (see references cited above). Recently, Blount *et al.* (2008) reported the emergence of citrate-utilizing (Cit^+) mutants in one of the evolving populations in the LTEE, between 31,000 and 31,500 generations, but the precise time of their occurrence could not be determined. Their frequency rose from 0.5% at 31,500 generations (the time when they were detected), increased to 15% and 19% after two successive measurements (not specified, but presumably at 32,000 and 32,500 generations) and then declined sharply (1.1% at 33,000 generations). This was attributed to CI whereby the majority Cit^- population acquired some other beneficial mutation(s) and out competed the minority Cit^+ variants, until the latter also acquired mutation(s) which allowed better utilization of citrate and consequently their frequency rose again, i.e., after 33,000 generations.

Another instance of clonal interference was reported by Stanek *et al.* (2009). They detected a mutation in one (only

one) of the 12 evolving populations in the LTEE. This mutation, named BoxG1^{8A}, presumably occurred after 500 generations of growth and resulted in a modest (10%) decrease in the expression of the *glmUS* operon. Stanek *et al.* (2009) suggested that the reduced expression of the *glmUS* operon and consequent reduction in the supply of peptidoglycans could confer a benefit on the evolving population. The BoxG1^{8A} mutation was not found in any of the other 11 cultures even after 20,000 generations of growth. Stanek *et al.* (2009) suggest that during the early periods of the LTTE, other more beneficial, mutations could have eliminated the BoxG1^{8A} mutation (had it occurred) in the other cultures by clonal interference. At later times, mutations with an epistatic effect on the BoxG1^{8A} mutation could have occurred in other genes such that the combined effect would have been neutral or less advantageous or even disadvantageous. If such mutations had occurred in the other cultures the chances of their getting the BoxG1^{8A} lesion would be almost negligible (for a detailed discussion, see Stanek *et al.* (2009)).

The original formulation of the CI model assumed that all beneficial mutations occur only in the wildtype genetic background. In this formulation the less beneficial mutations lose out in the race for fixation and hence are wasted (see above). The possibility that a second (or even a third) beneficial mutation could occur in a cell which already has one, was not considered. It is conceivable that a combination of two (or more), less beneficial mutations can confer better fitness advantage than a single beneficial mutation, even if the latter were fitter than either of the former. Desai and Fisher (2007) and Desai *et al.* (2007) considered this possibility to develop a multiple mutation model. Zeyl (2007) calls the Desai model a 'piggybacking' model wherein additional mutations piggyback on to a cell which already has a less fit one, rendering it more fit and a superior competitor for fixation. The implications of the multiple mutation model has been explained in simple language by Zeyl (2007). It should be noted that the multiple mutation model does not negate the original CI model (also called the one-by-one clonal interference model). Fogle *et al.* (2008) evaluated the influence of clonal interference and multiple mutations on adaptation dynamics in large asexual populations and concluded that both models are applicable but under different situations. When mutations with large beneficial effects are common, the CI model will describe the dynamics better whereas under situations when such mutations are rare the multiple mutation model will do so better. The voluminous literature on clonal interference has been reviewed recently by Sniegowski and Gerrish (2010).

Stress-associated hypermutability and adaptation

Since the benefits of constitutive (heritable) hypermutability could be offset by the inevitable risks of accumulation of deleterious mutations, hypermutation sans risks would be adaptively more advantageous. Two processes accomplish this; one is transient hypermutability which is implicated in the mutagenicity associated with stress responses. This is commonly referred to as stress-induced mutagenesis (since there is some disagreement on the notion that stress could 'induce' mutations (see below), the term 'stress-associated mutagenesis' is used here to avoid any bias). The other process is localized hypermutability in which mutagenesis is confined only to certain regions called the contingency genes. Localized hypermutation, which underlies the phenomena called phase variation and antigenic variation, has been reviewed recently (Jayaraman 2011). Stress-associated mutagenesis will be reviewed briefly below.

The wisdom of modern genetics holds that mutation and selection are independent. This is a legacy of the path-breaking work of Luria and Delbruck, and the Lederbergs in the late 1940s (see Brock 1990). In an interesting but controversial paper, Cairns *et al.* (1988) suggested that some mutations could be 'directed' to occur in response to selection. This triggered many heated debates and arguments in the following years because the idea had clear Lamarckian overtones and did not go down well with strict Darwinian thinkers. However, the notion of directed mutagenesis was eventually abandoned after evidence was obtained to show that cells became generally mutagenized following stress (Foster 1997; Torkelson *et al.* 1997). In its place another idea that stress could induce general, genomewide, transient hypermutability emerged. In addition to the widely used *lac* reversion assays (using the F′ factor FC40) originally used by Cairns *et al.* (1988), and its variations, other systems such as mutagensis in ageing colonies (MAC) of *E. coli,* also called resting organisms in structured environments (ROSE) were developed to study mutagenesis under stress (Taddie *et al.* 1997c; Bjedov *et al.* 2003). The former authors showed that several natural isolates of *E. coli* displayed MAC. Organisms such as *Helicobacter pylori* which lack the major contributor to heritable hypermutability in nature, namely, the MMR system, have also been used to investigate stress-induced mutagenesis (Kang *et al.* 2006). A new experimental system for studying adaptive mutagenesis has also been reported (Zhong and Aoquan 2001). All these resulted in significant advances in our understanding of the occurrence, genetic requirements, possible mechanisms, biological significance etc. of stress-associated mutagenesis (earlier called adaptive mutagenesis). The various mechanisms proposed involve recombination-dependent mutagenesis, switch from high-fidelity to error prone DNA double strand break repair, adaptive amplification, gene amplification preceding stable mutation etc. Due to constraints of space, these mechanisms and their merits and demerits cannot be discussed in detail presently. However, several excellent reviews and reports on this topic have appeared in recent years (Foster 1999, 2007; Wright 2004; Galhardo *et al.* 2007, 2009; Hastings 2007; Gonzalez *et al.* 2007; for more references see Petrosino *et al.* 2009; Storvik and Foster 2010; Frisch *et al.* 2010; Gibson *et al.* 2010). The well-documented genetic requirements for stress-associated mutagenesis include *recA, recBCD, ruvABC, dinB, rpoS,*

rpoH, groE and *ppk* (see the references cited above). Recent additions to this list are *nusA* (encoding a read-through transcription factor; Cohen *et al.* 2009; Cohen and Walker 2010) and *rpoE* which encodes a stress response regulatory sigma factor (Gibson *et al.* 2010).

Although there is a near consensus of opinion on the idea that stress could trigger generalized mutagenesis, there has also been some disagreement, notably from Roth's group who argue that there is no such thing as stress-induced mutagenesis. Instead, they take the position that the so called stress-induced mutants do not arise through postselection (stress induced) mutagenic events but are 'initiated by common, small-effect mutations that preexist selection but grow and improve rapidly under growth limiting conditions' (Roth *et al*. 2006; Wrande *et al.* 2008; Andersson *et al.* 2009; Roth 2010). With respect to MAC, Wrande *et al.* (2008) claimed that there was no mutagenesis in ageing colonies as reported by Bjedov *et al.* (2003), and the observed emergence of rifampicin resistant mutants was due to the faster growth of some Rif^r mutants. However, whether this can be extrapolated to all (or many) of the natural isolates of *E. coli* is uncertain. With respect to the well studied *lac* reversion system, the model of Roth and coworkers proposes suboptimal growth, promoted by amplification of 'leaky' *lac* mutant alleles, as a prelude to the emergence of stable Lac^+ colonies under lactose selection. Some reservations on this model have been pointed out recently by Gibson *et al.* (2010). Jayaraman (2000) reported that increasing the leakiness of an ochre mutation in *argE* (*argE3*) by sublethal concentrations of streptomycin enhanced its reversion under selection for arginine prototrophy and this was abolished in $recA^0$ mutants. This and the earlier reports of the same author (see Jayaraman 2000) highlight the role of allele leakiness in stationary phase (adaptive or stress-associated mutagenesis). Gene amplification preceding the generation of stable adaptive mutations has also been shown in the emergence of antibiotic resistance mutations (Sun *et al.* 2009; see also the reviews by Andersson and Hughes 2009; Lindgren and Andersson 2009). Recently, Pranting and Andersson (2011) reported that a protamine resistant, growth restricted mutant (small colony variant) of *S. typhimurium* escaped from growth restriction by amplifying the mutant gene copy number (partial escape) and acquiring a compensatory mutation, either replacing the original lesion or adjacent to it, in one of the amplified gene copies, followed by segregation to the single gene copy state (full escape) (see also the commentary by Roth (2011)). It will not be surprising if the many mechanisms discovered so far as well as other, yet unidentified ones, may eventually turn out to be not mutually exclusive. While the molecular basis of stress-associated mutagenesis remain(s) controversial, it should be noted that the differences in perception are confined to the underlying mechanisms of the phenomenon but not to its occurrence or biological significance. Irrespective of the differences of opinion, the fact remains that stress-linked hypermutation is as beneficial in adaptive evolution as is constitutive (heritable) hypermutation, perhaps even more, since it is free from the risks associated with the latter. Although hypermutability, whether transient or constitutive, will render cells prone to generate deleterious mutations (J. Roth, personal communication), the likelihood of occurrence of such mutations might be less in the case of transient hypermutation since the organism could escape from the stressful state as soon as a beneficial mutation becomes available and will no longer be hypermutable thereafter. Clinical isolates of many pathogens have a high preponderence of heritable mutators (see above), but nonmutators are also found in equal (or even higher) proportions among them. Since the latter also have survived and prospered in the face of intense selection pressure, they could have been mutators initially but became nonmutators subsequently due to reversion or horizontal gene transfer. Alternatively, they could have adapted through transient hypermutability and enjoyed its risk-free benefits. It has also been shown that constitutive hypermutators, especially drug resistant mutants, acquire compensatory mutations which offset their fitness disadvantage (for a discussion and references see Perron *et al.* 2010).

Adaptive benefits of generalized hypermutability

The adaptive benefits of hypermutability have been mentioned frequently and in general terms in the earlier sections. In this section a few specific examples will be discussed. One of the best documented examples of adaptation by general hypermutabilty is the adaptation of pathogens to their hosts, particularly exemplified by *Pseudomonas aeruginosa* adapting to the airways of patients afflicted with cystic fibrosis (CF; reviewed by Oliver 2010; Oliver and Mena 2010). CF is an inheritable respiratory disorder, characterized by the secretion of hyperosmolar and highly viscous mucus in the airways, which provides an ideal niche for bacterial infections. Such infections could persist chronically for as long as 30 or more years and the infecting pathogens undergo tens of thousands of cell divisions. They provide an excellent *in vivo* experimental system to study adaptive evolution. The most common pathogens associated with CF are *Staphylococcus aureus, Haemophilus influenzae, P. aeruginosa* and *Burkholderia cepacia* complex (reviewed by Harrison 2007). While a high prevalence (30% or more) of mutators has been observed in isolates from CF patients with chronic *P. aeruginosa* infections, only a small fraction of isolates (<1%) from of acutely infected patients were mutators (Gutierrez *et al.* 2004; see also Oliver 2010; Oliver and Mena 2010). Two types of mutators, namely, strong mutators (>20-fold higher mutation frequencies than basal level) and weak mutators (2–5-fold higher) have been isolated from CF patients. While most of the mutators had lesions in the genes involved in DNA damage avoidance and/or repair pathways, a few newer mutator loci have also been discovered in chronic infections with *P. aeruginosa* in CF patients (Weigand *et al.* 2008). These observations imply

that chronic infection which involves long-term adaptation to hosts could be correlated with hypermutability (as pointed out earlier, nonmutator lineages are also present in clinical isolates; these could have adapted by other, possibly stress-associated, mechanisms). Whole genome sequence analysis of two *Pseudomonas* isolates from a CF patient, one isolated at 6 months of age (early infection) and the other at 96 months (chronic infection), showed that the latter had accumulated as many as 68 mutations during a period of 7.5 years (Smith *et al.* 2006). Similarly, Hogardt *et al.* (2007) reported that *P. aeruginosa* isolates from three CF patients during the last 3–6 years of their lives showed a high preponderance of mutators in the late stage isolates, which were also multiply antibiotic resistant. In addition, the late stage isolates had lost many virulence-associated traits as well as the ability to survive in a nonlung environment such as water. Continuing the work of Smith *et al.* (2006); Mena *et al.* (2008) showed that four out of the 68 mutants of Smith *et al.* were hypermutators harbouring the same lesion (R490L) in the *mutS* locus. Moreover, a large fraction (62%) of the 68 mutations were confined to the four mutator variants. Analyses of many isolates from several CF patients showed that mutator mutations enhanced genetic adaptation to the deteriorating lung environment during chronic infection (for details see Mena *et al.* 2008). The loss of virulence traits as well as the inability to survive in a nonlung environment (tap water) of lung-adapted *P. aeruginosa* (Hogardt *et al.* 2007) are perhaps the consequences of overspecialization to survive in one environment (in this case the CF lung) at the risk of losing the ability to survive in others (antagonistic pleiotropy; see above). Mena *et al.* (2007) showed that deletion of the *mutS* gene of *P. aeruginosa* was associated with a reduction in fitness, attenuation of virulence and promotion of long term persistence (chronicity).

Hypermutation is not restricted only to *P. aeruginosa* infections in CF. Isolates of *H. influenzae* and *S. aureus* from CF patients also have high proportion of mutators. For instance, auxotrophic, small colony variants (SCVs) of *S. aureus* have been isolated from a variety of drug resistant, chronic infections including CF (for references see Besier *et al.* 2007). A thymidine-dependent SCV isolated from CF patients has been shown to be a hypermutator and the property was suggested to be involved in chronicity and antibiotic resistance (Besier *et al.* 2008). Similarly, hypermutators are not restricted to CF only. Oliver and Mena (2010) and Oliver (2010) have reviewed the extensive literature on several disease states and causative organisms in which a link between hypermutation and chronicity has been shown. In general, mutators are more prevalent in chronic infections than nonchronic, acute infections. Although measurement antibiotic resistance is often used to assess hypermutability because of its practical convenience, it is not the only trait that is influenced by hypermutability. Long-term adaptation results in several phenotypic changes (see Ciofu *et al.* 2010). A characteristic property of *P. aeuginosa* in CF is mucoid growth and loss of quorum sensing properties due to mutations in *mucA* and *lasR,* respectively. Mucoidy has been shown by some workers (for references see Ciofu *et al.* 2010) to be linked to hypermutators but there is also some disagreement on this. Ciofu *et al.* (2010) showed that mutator mutations occurred in lineages already possessing *mucA* and *lasR* mutations. *P. aeruginosa* grows as biofilms in the lungs of CF patients (Prince 2002). Driffield *et al.* (2008) showed biofilm cells of *P. aeruginosa* are hypermutable (due to down regulation of *katA*, the gene encoding catalase) and consequent hypersensitivity to oxidative DNA damage and mutability. In essence, hypermutability seems to provide a short cut to the multiple adaptations needed for the chronic persistence of pathogens in diseased animals. The adaptations might be of value to the pathogens in their struggle for survival and evolution, but from a human point of view they render the treatment of the diseases more problematic. A knowledge of their existence and mechanisms could help in the development of more effective therapeutic strategies.

Concluding remarks

The success of adaptive evolution depends on how well bacteria sense and adapt to fluctuations in their environments. Bacteria, especially pathogens, encounter several unpredictable threats to survival and are constrained to keep evolving adaptive strategies to escape such threats. On their part, the hosts also come up with more and more strategies to contain and eliminate the invading pathogens. Hence, there is a coevolution of the threat as well as the response. This is euphemistically called the biological arms race or the Red Queen's race, after Lewis Carrol's classic, 'Through the looking glass' ('It takes all the running you can do to keep in the same place'). In simple, layman's language, stress and response could be compared to a predator and its prey, each of which try to outcompete the other by running faster and faster. Evolutionarily speaking, hypermutability (as well as phase and antigenic variation, reviewed by Jayaraman 2011) empower bacteria to 'run faster' to deal with unforeseen and life threatening stresses. The literature reviewed in the foregoing pages illustrates how bacteria exploit the power of hypermutability to evolve counter offensive mechanisms. When a large repertoire of mutations is available, it is possible that some among them could be adaptively useful and they could be taken advantage of to face the threat. Even mutations with small phenotypic effects could facilitate adaptation through successive improvements under selection pressure (Le Chat *et al.* 2006; J. Roth, personal communication). This cascade process could be augmented by hypermutability. However, as has been repeatedly pointed out earlier, there is also the danger of accumulation of deleterious mutations which will lead to a reduction of fitness. A blessing is that such fitness reductions will be perceived in the long run whereas the beneficial mutations could be exploited immediately. The danger of accumulation of deleterious mutations after successful adaptation can be controlled either by the reversion of the mutator allele or

reacquisition of the wildtype allele by horizontal gene transfer. However the probability of occurrence of such events may not be high (see de Visser 2002). This will lead to long-term persistence of the mutator state and could have consequences. Some of the predicted consequences include extensive inactivation of many genes, ultimately leading to their deletion, changes in the base composition of DNA and impairment in holmologous recombination, genetic isolation, impairment in horizontal gene transfer etc. (Marias *et al.* 2008). Andre and Godelle (2006) have shown by analytical modelling that modifications in the environment could favour the mutator state because their benefits could be perceived sooner than their costs (see above). Perhaps this could be one of the reasons for the high prevelance of mutators in clinical isolates of pathogens. Also, the occurrence of compensatory mutations (see above) could favour the persistence of the hypermutable state. However, using analytical models and simulations, Gerrish *et al.* (2007) have predicted that the long term persistence of high mutation rates could result in catastrophic levels of accumulation of mutations and ultimately lead to extinction. In a recent report in Genetics (published ahead of print, on June 24, 2011, as 10.1534/genetics.111.130187), Quinones-Sato and Roth have provided additional data in support of their model of mutagenesis during growth under selection (see text).

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