

Culture History and Population Heterogeneity as Determinants of Bacterial Adaptation: the Adaptomics of a Single Environmental Transition

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INTRODUCTION

The adaptive success of bacteria needs explanation. Unfortunately from a reviewer's point of view, the evolutionary advantages of bacteria are diffusely distributed over many structural, metabolic, regulatory, microevolutionary, and genome rearrangement features as well as advantages of growth rate and population size. It is too large a task to integrate all the mechanistic ingredients of success into a single review, but at the same time we wish to reverse the extreme reductionist approach in which individual regulatory and mutational processes of bacteria are commonly considered in isolation. In addition, it is essential to reflect on the full complexity of adaptive possibilities as a note of realism when considering the emerging notion that evolution is predictable at an organismic systems level (251, 308). As a simple test of the science of bacterial adaptation, we wish to consider a single question: what are the ingredients of adaptation of a single bacterial population to a single transition between two environments? To our knowledge, there are no previous attempts to bring together all the population-level changes contributing to this kind of com-

mon transition. Given the current trend toward a systems approach to biological processes, it is important to start to integrate regulatory and mutational adaptation as well as to look at the effects of the inherent heterogeneity of these processes. Indeed the "adaptomic" description of regulatory and mutational responses to a single environmental change is a sensible step toward modeling the outcomes of multistep evolutionary change.

Even considering a single environmental challenge and a single population, there need to be exclusions to limit the scope of the discussion. We exclude very rapid structural, metabolic, or physiological changes (such as ion fluxes or changes in enzyme activity) at one end of the time scale for adaptation. Limiting our dis-

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cussion to a single transition also allows us to ignore ecological specialization and speciation at the other extreme of the adaptational time scale. Thus, we discuss what can be described as regulatory and microevolutionary changes in a single, generic environmental transition. Finally, we also exclude the added complexities of multispecies communities and deal with a monoclonal population. This is less of an exclusion than it may seem, in that the tremendous level of heterogeneity within a single clonal population is one of the main themes in this review.

Three justifications can be offered for discussing regulatory and mutational responses together in a single environmental transition. First, one of the important trends in recent years has been a growing body of evidence linking regulatory and genomic adaptations. Examples include the cellular regulation of DNA repair processes leading to mutation rate variation and effects on evolvability (102, 112, 367) and the stochastic and deterministic effects leading to persister cells (179). Epistatic effects on regulatory circuits and their modulation during mutational adaptation are also increasingly evident as a component of fitness (66, 264). Indeed, the current trend indicates that regulatory adaptations inform the bacterium as to the genomic change that maximizes the chances of adaptation and evolutionary success.

Second, a temporal perspective of adaptation in bacteria indicates the overlapping time frames of regulatory and mutational adaptations, as shown in Fig. 1. The synthesis of new transcripts and average-size proteins can be achieved within 30 s, and the time taken for induction of a large protein such as β -galactosidase is about 2.5 to 3 min (30). However, more complex developmental adaptations such as sporulation take considerably longer (up to 7 to 8 h) (70). Likewise, biofilm formation can be observed within an hour, but mature biofilms can take >24 h (214). Over such time scales, mutational selection can become significant. A mutant present in a large population with a growth advantage in the new environment can become highly enriched depending on its fitness increase (Fig. 1B). Given a large enough fitness difference, mutations in a gene can sweep a population within 24 to 48 h, and this has been demonstrated with regulatory mutations in experimental populations (97). In even more extreme selections, such as for high-level antibiotic resistance, survivors will be solely from the selection and multiplication of resistant mutants over all phases of adaptation. Resistant mutants, depending on their growth rate and other environmental constraints, can become large populations within 24 h.

Third, considering regulation and mutation together is consistent with historical views and definitions of adaptation. For bacteria, Ryan (286) noted 60 years ago: “Bacteria are remarkably plastic in their ability to undergo satisfactory adjustments in new environments. These adjustments are adaptations in the original biological sense of the word whether they are inherited or not, and irrespective of the underlying mechanisms.” Adaptation is thus a comprehensive process, and regulatory adaptations, epigenetic events, and inherited, mutational adaptations all contribute to fitness. We thus adopt the definition of “adaptation” to be “the process of change by which an organism or species becomes better suited to its environment” (however, a range of definitions of this much-discussed term exists [212]).

A further important consideration based on Fig. 1 is that the level of fitness obtained in an environmental transition through a regulated response may not be as high as that potentially available in a mutant present in the population. This was already demon-

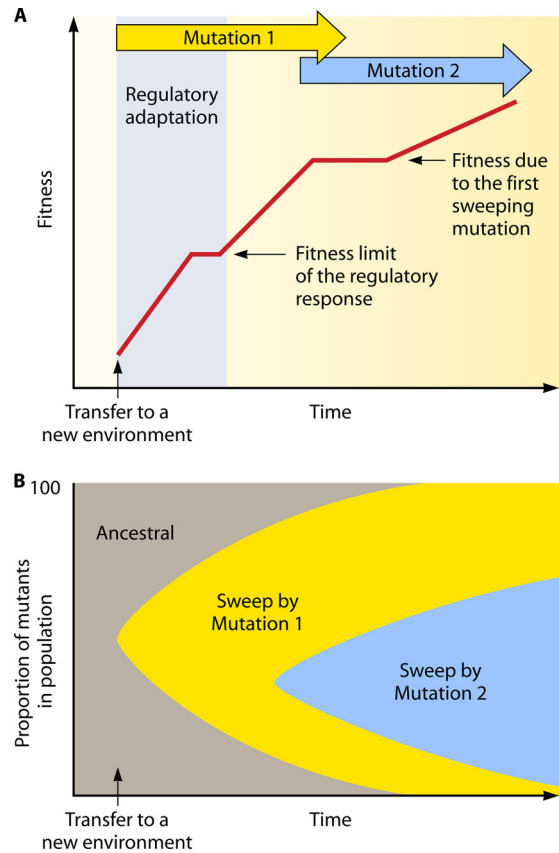


FIG 1 (A) Fitness increases through regulation and mutational processes in a population, relative to the prestressed state, upon a transition to a new environment. “Fitness” in this context is a qualitative measure of adaptation, and true competitive fitness in stress-induced versus uninduced cells is not available or normally measured. (B) The underlying, successive mutational sweeps result in ancestral types (gray) being replaced in the population by initially rare cells containing mutation 1, resulting in increased fitness to above that possible with the ancestor. Mutation 2 is enriched with continued selection and further increases population fitness.

strated in early studies with the classic *lac* system, in that constitutive mutants outcompete wild-type, inducible bacteria in the presence of limiting lactose, resulting in the enrichment of mutants with better β -galactosidase levels than are available from induction; subsequently, gene dosage changes through amplification further increase enzyme levels (240). Hence, as suggested by Fig. 1, the selective advantage through mutation can be an important contributor to adaptation in populations, including in multiple steps if the selection is maintained. Altogether, these considerations suggest that the fitness increase available from a regulatory change fixed by the genetic blueprint is lower than that available from mutational change. Hence, regulation and microevolution through regulatory mutations provide a continuum of possibilities in adaptation (95).

The overall scope of this review is outlined schematically in Fig. 2, which attempts to integrate bacterial adaptive pathways into a generic program during a single environmental transition, at the level of a bacterial population. The new environment in Fig. 2 can either reduce or increase the growth rate; in either case, the response in the new environment will change patterns of gene expression as well as the selection environment for fitter mutants.

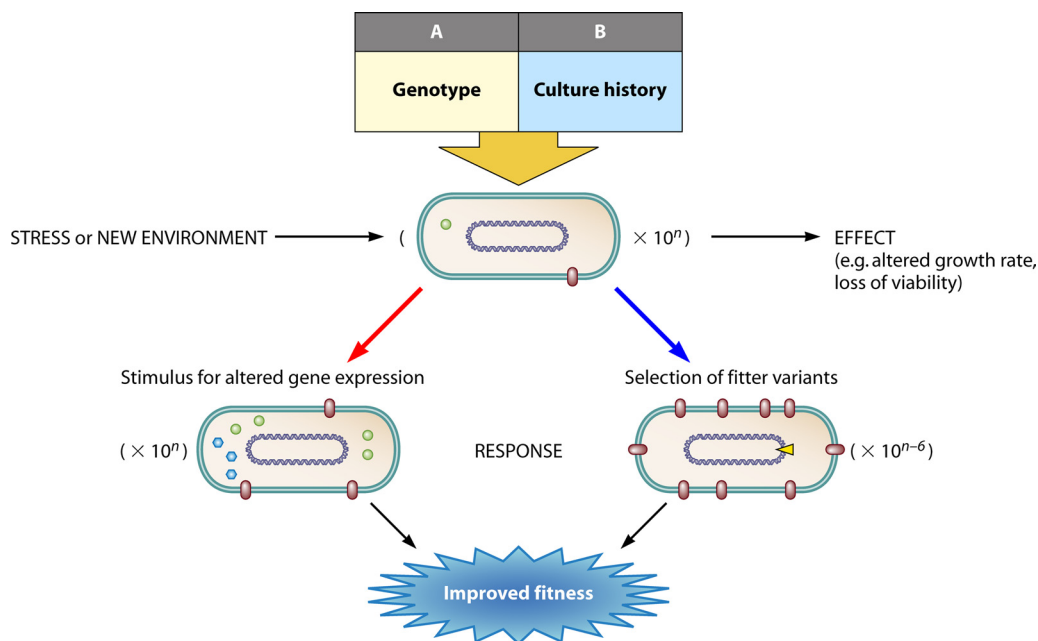


FIG 2 Adaptations from a prior state upon a transition to a new environment for bacteria (rods with DNA circle and cell components) through regulation (red arrow) and mutational processes (blue arrow). The number of bacteria in the population, 10^n , nearly all undertake a regulatory transition, but the number gaining fitness through mutation is environment and function dependent, so the 10^{n-6} shown can vary by orders of magnitude. The effects on cells depend on the genotype of the organism (A) and the history of the population (B), as discussed in the text.

The regulatory response will be elicited in most but not all of the 10^n cells in a population. The heterogeneities in the population arise from many sources (as described below) and complicate the quantitative prediction of the behavior of an organism such as *Escherichia coli* when it is transferred from one environment to another. Effects on both growth rate and viability should be calculable, but the effect of a transition is dependent on many variables in boxes A and B (Fig. 2), as further explained in Importance of the Genotype in Determining the Nature of Adaptive Responses and Importance of Culture History in Determining the Nature of Adaptive Responses below. We are far from being able to quantitatively estimate the contributions of all the inputs in Fig. 2 to 5. These include mutational adaptations, and mutational heterogeneities will certainly be dependent on the size of n in Fig. 2. This review will try to define the extent of this multifactorial problem. Complicating factors include not only the extensive population heterogeneity but also the overlapping interactions between mutational and regulatory responses that enrich the possibilities in adaptive pathways. The inputs A and B affecting the response of a bacterial population to an environmental transition are first discussed in turn.

IMPORTANCE OF THE GENOTYPE IN DETERMINING THE NATURE OF ADAPTIVE RESPONSES

The genetic blueprint of an organism predetermines the adaptive pathways shown in Fig. 2 in two ways. The genome encodes the functional responses of the organism and how the genes are regulated. Bacterial genome sizes vary over a >10 -fold range of DNA content because of various selection pressures (195), so their capacities to encode responses to environments also differ. In addition, the genome structure and the sequence itself influence the mutational possibilities in a chromosome.

The transcription factors and other regulatory components determine the response pattern of a bacterium, and these of course are genome encoded. Complex maps of the roles of the transcriptional regulators and their network organization are now available for some species, including *E. coli* (208). The size of the regulatory network varies from species to species and is related to genome size (219). Variation in regulation is evident not only from species to species, and the identity of genotypes in a population does not mean that transitions are identical in all of the 10^n cells in a population (Fig. 2). The genome itself and the nature of the regulatory networks that it encodes create the scope for transcriptional noise and the possible bistable states in regulation (see “Heterogeneity due to stochastic variations in regulation” below for details). Phenotypic and regulatory variability within populations is thus influenced by several genome-determined processes associated with regulatory networks. Indeed, the ability to switch phenotypes within populations is under evolutionary selection and is also considered in detail below.

In considering regulation, we will focus largely on events and heterogeneities within populations of a single strain. Nevertheless, it is worth stressing that the inter- and intraspecies strain variations in regulation are extremely high according to recent data (52, 90, 151, 317). Although most of the genes involved in regulation are highly conserved, core elements in a genome, they are not necessarily operating in the same way, even in members of a species. The central elements in gene regulation, e.g., the concentrations of sigma factors and alarmones such as ppGpp, vary greatly under the same environmental conditions across a species (98, 304). The instability of such central elements of regulation is particularly evident within the lifetime of a clonal infection in a patient (177). Although variations are genotype specific, they do not always involve polymorphisms in the regulatory gene itself. The

myriad of inputs into most central, high-level regulatory circuits allows many ways of influencing regulator levels. A prime example of this is with the *E. coli* sigma factor RpoS, which controls the expression of over 200 genes (357). The level of RpoS in the cell responds to external and internal stresses and is influenced by over 20 environmental signal inputs (142). The alternative input pathways controlling *rpoS* expression provide degenerate ways of affecting regulation, and indeed many alternative genes can change the particular regulatory circuit controlling RpoS levels (353). Even within a single adapting population, heterogeneities can arise, and regulatory mutations are the most frequent type in experimental evolution studies (52, 264, 353). Hence, it is not surprising that regulation across a species is not uniform.

The genotype-determined phenotypic effects of within-species variation are indeed profound, so one strain of *E. coli* can have a very different response to the same level of stress than another (24, 98) or a very different growth rate or competitive ability in particular environments (157). Genomics efforts have highlighted the relatively small core genome and the relatively large pan-genome within a species such as *E. coli* (193, 348), so it is highly likely that some of the pan-genome differences will affect adaptive responses. Plasmids and prophages add further variability to genomic capabilities (see “External DNA, genetic exchange, phages, and plasmids” below).

On the mutational side, the possibilities for change in a particular genome are influenced by the presence of genomic duplications, unstable elements such as microsatellites, transposable elements, repeats, and sequences contributing to high mutation rates, as discussed in “Genetic Heterogeneity within Populations” below. The great variation in the number and position of insertion sequence (IS) elements within a species must mean that the probability of particular mutations and genome rearrangements is strain specific. For example, the tendency to frequently acquire a mutator mutation in one laboratory strain of *E. coli* K-12 is linked to a specific IS5 movement. *E. coli* B strains do not have an IS at the same position and so do not generate mutators in the same way or with the same high frequency (110). Short repeats and the possibility of slipped-strand errors also differ between genomes (341). Such evolved strain differences could have considerable effects on mutation availability at various positions in the genome within strains. For example, the presence of a run of 8 G bases can potentiate a frameshift in a particular gene (109). Within a single population, we can ignore intraspecies differences, but we will point out where genome properties affect adaptive pathways. These influences are discussed in “Genetic Heterogeneity within Populations” below at a population level, in which mutations result in subpopulations with altered properties and an altered capacity for the transitions shown in Fig. 2.

The genotype also determines the mobility of genomic DNA and how recombinational mechanisms contribute to adaptation. This goes beyond the potential for rearrangements allowed by transposable elements and integrons, especially if the bacterium contains sex determinants such as the F factor or lysogenic phages that can facilitate cell-to-cell transfer of DNA. We consider the role of lateral gene transfer (LGT) in adaptation in “Genetic Heterogeneity within Populations” below.

For most examples, the genome that we will discuss is that of the lab strains of *E. coli*, simply because most is known about it. Nevertheless, we will include adaptations such as competence or sporulation in *Bacillus subtilis* or biofilms in *Pseudomonas aeruginosa*

where these provide better examples of adaptation or its intricacies. Of course readers will have other, possibly better examples of culture effects and population heterogeneity, and we apologize for such omissions.

IMPORTANCE OF CULTURE HISTORY IN DETERMINING THE NATURE OF ADAPTIVE RESPONSES

Besides the genotype, the immediate culture history of a population is an inevitable determinant of the overall effect of an environmental transition. In Fig. 3, we expand culture history effects into seven areas that can strongly influence the scope of the transition shown in Fig. 2. Perhaps surprisingly, the history of the culture is rarely discussed systematically where it comes to understanding adaptation and regulatory responses. Indeed, in laboratory studies when a simple, reproducible baseline is generally preferred, studies of adaptation try to eliminate variations due to history. A baseline population is often one that grows exponentially under near-optimal conditions and excess nutrient levels. What is known about transcriptional changes in model organisms such as *E. coli* is generally based on stresses and transitions applied to exponentially growing bacteria. This of course is extremely artificial, and in nature there is no single baseline; in each environmental transition, the baseline is that provided by the history of a population. In any case, there is extensive evidence that in bacteria grown in the same medium but at different growth rates (e.g., in chemostats) or into different growth states (e.g., exponential- versus stationary-phase or biofilm bacteria), the response to challenges differs greatly. The importance of culture history is widely recognized in food microbiology, where predictive methods for processing need to take into account the variations in bacterial properties, especially stress responses, due to prior culture conditions. It is therefore evident that we cannot hope to predict the adaptomics of a population and the effect of the same stress on the same organism unless we know how it was grown earlier. Therefore, in this section and in Fig. 3, we expand on seven different aspects of history that could or do affect adaptive responses.

Growth Rate before the Transition

Many constituents of bacterial cells as well as the cell size are strongly dependent on growth conditions (148, 196). For *E. coli*, the macromolecular composition is dependent more on the growth rate than on the nature of the growth medium used; media that support the same growth rate result in bacteria with similar macromolecular contents (34). Growth rate effects are not limited to batch cultures containing different substrates but are also found in nutrient-limited chemostats running at different dilution rates but with the same limiting substrate (94). In both situations, growth rate is a major influence on bacterial gene expression and regulation (96, 146, 159).

An underestimated consequence of bacteria having constituents that vary with growth rate is that their adaptive responses have different starting points upon a transition. Indeed, slow-growing bacteria (with doubling times of 3 to 4 h or more) have elevated levels of the alarmone ppGpp and the sigma factor RpoS, stimulating the general stress response as well as other stress proteins dependent on ppGpp (236, 325). The cellular content of stress protectants such as trehalose (whose synthesis is RpoS dependent) is much elevated during slow growth (336), so transitions into new environments with such bacteria are buffered by partly or highly expressed stress responses.

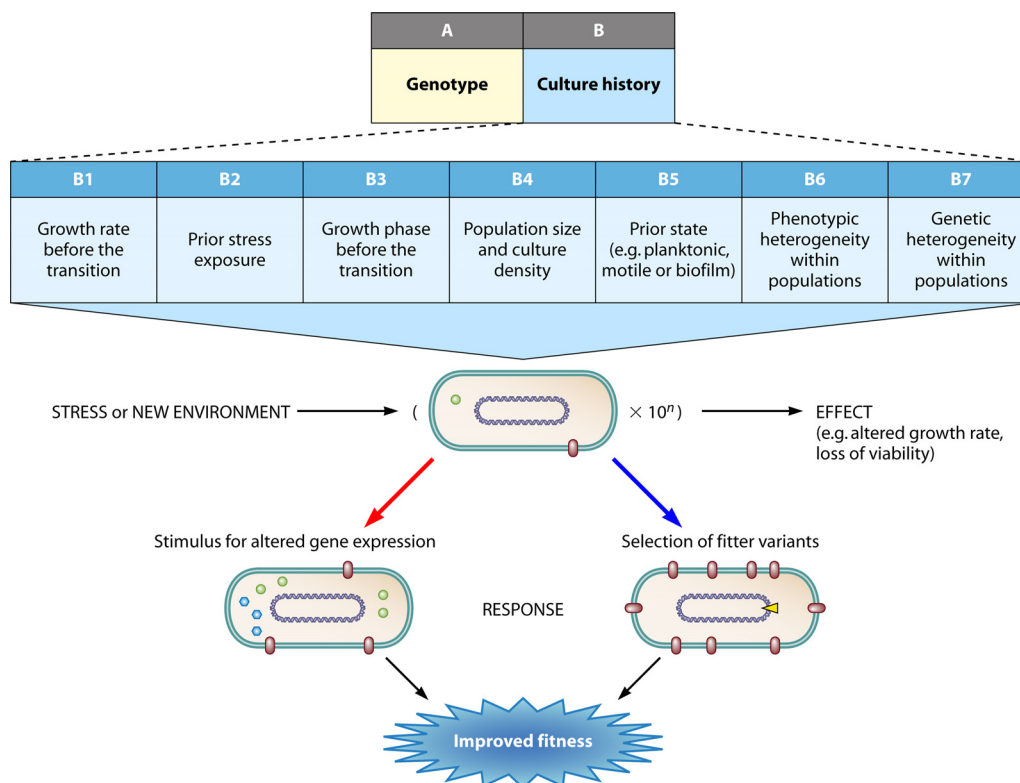


FIG 3 Aspects of culture history relevant to adaptation, expanded upon from Fig. 2 in increasing detail. Each box is further discussed in the text. Other content and arrows are as in Fig. 2.

The ppGpp-controlled stringent response occurs during slow growth or when cells experience a nutritional downshift, e.g., become starved for an amino acid(s), nitrogen, or carbon, or when cells encounter various toxic agents (45). Elevated levels of ppGpp interact with the β and β' subunits of RNA polymerase, affecting promoter binding preference and leading to the inhibition of transcription of stringently repressed genes, and induce transcription of stringently expressed genes (8). This results in a reduction of the synthesis of stable RNA (tRNA and rRNA), which restricts protein synthesis. This is accompanied by repression of other genes involved in normal growth and proliferation and induction of genes to relieve the stress, such as amino acid biosynthetic genes (for a review, see reference 197). *rpoS* is one of the genes that is induced by the stringent response, and many RpoS-controlled genes also require ppGpp for induction, so the general stress response and stringent response overlap each other (165, 332). Among the many changes that ppGpp plus RpoS elicits is a reduction in outer membrane permeability (185), probably accounting for antibiotic susceptibility of bacteria being a function of growth rate (39).

Making high levels of RpoS and ppGpp entails a cost to bacteria, because it diverts resources of the cell used in vegetative growth (304). Natural isolates of *E. coli* vary in growth rate (215), and part of the reason is that there is as much as a 10-fold variation in RpoS and/or ppGpp content between different *E. coli* strains growing under identical conditions. Individual populations are often a mix of *rpoS* mutants and *rpoS* wild-type subpopulations (98, 157). The levels and coexistence of *rpoS* mutant/wild-type subpopulations can reflect a trade-off between stress resistance (enhanced by

RpoS) and nutritional competency (inhibited by RpoS), referred to as self-preservation and nutritional competence (SPaNC) balancing (95). Within a single genotype, the setting of the SPaNC balance is dependent on the growth rate and the culture history of the population (95). Therefore, depending on the strain type and culture history, there will be heterogeneity of RpoS levels within a population which could result in population heterogeneity in RpoS-dependent stress resistance.

Mutational processes are also affected by growth rate and stasis because of RpoS-induced mutagenesis (102, 112). In the same medium, a 6-fold decrease in growth rate in glucose-limited chemostats results in a 30-fold increase in the frequency of neutral mutations, with slow-growing cells having higher mutation rates (239). This is likely to be a regulated difference, and the high level of RpoS can induce error-prone DNA polymerase IV (166). In addition, it was shown recently that among starving cells, a small proportion exhibit heightened genomic instability during which multiple chromosomal structural changes can occur anywhere in the genome (181) (see “Genetic Heterogeneity within Populations” below).

It can also be envisaged that gene dosage varying with growth rate may affect mutational effects and DNA repair. Chromosomal copy number is growth rate dependent (34), with more copies at high growth rates. Although not tested, this could allow more opportunities for mutation elimination through homologous recombination under optimal growth conditions. These aspects will be expanded upon in “Genetic Heterogeneity within Populations” below when we discuss genetic variation.

The final growth rate effect on genomes that we consider is a

question not of DNA change but of evolutionary selection acting on genomes. Adaptation involves not only genetic variation, which is growth rate dependent, but also selection, which is equally growth rate affected. There is mounting evidence that a mutation that is beneficial at one growth rate can be actually deleterious at another (198b, 239). This is again due to the different metabolic-regulatory contexts that a mutation meets in slow- and fast-growing bacteria. The fitness effects of many mutations may indeed turn out to be growth rate affected when further investigated, as also suggested by another example (53).

Prior Stress Exposure

Another aspect of bacterial history that affects the response of bacteria (Fig. 2) is prior exposure to a stress. This is partly for the same reasons to those discussed for growth rate effects in “Growth Rate before the Transition” above. In *E. coli*, the general stress response regulated by the sigma factor RpoS and ppGpp can be stimulated not only by growth rate but also by sublethal levels of acids, high osmolarity, or suboptimal temperatures (142). Induction of the general stress response results in global changes in metabolism that greatly increase the bacterium’s resistance not only to the experienced stress but also to other stresses; e.g., exposure to carbon limitation increases resistance to oxidative stress, high temperature, and low pH (for a review, see reference 17). Induction by individual stresses affords cross-protection to other challenging transitions beyond the inducing stress. The best examples of cross-protection effects come from food microbiology studies on bacterial inactivation (170, 282, 359).

A second aspect of stress effects in the history of a culture is that, commonly, the cellular response may involve a stress-specific component as well as the general stress response. This is illustrated by acid tolerance in *E. coli*, where the defenses used to overcome sublethal levels of acid involve not only RpoS-dependent effects but also additional, acid-specific responses regulated in an RpoS-independent manner (101). This kind of dual regulatory response extends to other stresses, e.g., heat stress and oxidative stress (312). It is therefore an important point that the level of environmental resistance during a new transition as in Fig. 2 is a function of history regarding either specific or general responses or both.

The oxygen content of an environment and transitions from aerobiosis to anaerobiosis (and vice versa) can have a significant effect on adaptation. Oxygen presence potentiates oxygen detoxification mechanisms and elevates catalases and hydroperoxidases (254), and mutational processes are also affected differentially by aspects of aerobic/anaerobic metabolism (299). Hence, a population changing from an anaerobic to an aerobic environment may be more stressed by the same transition than one coming from aerobiosis.

As considered in more mechanistic detail below, high levels of stress exposure can change expression of the SOS response, DNA repair systems, and error-prone DNA polymerases and so can change mutation rates (see “Genetic Heterogeneity within Populations” below). If the stress is brought about by the presence of DNA-damaging chemicals or irradiation, the proportion of mutants in a population may increase and can potentially give different adaptive outcomes upon entry into a new environment because of the increased mutation supply in a population.

Growth Phase before the Transition

Improving survival at the cost of vegetative growth is a common bacterial strategy in response to adverse environments. Indeed, the whole advantage of differentiating into stationary growth phase or toward resistant forms (e.g., spores) is to induce a more generally protective state (160, 211, 345). Several reviews have discussed the properties of stationary-phase bacteria and the regulatory changes involved upon entry into, and maintenance in, stationary phase (100, 143, 160, 187). Microarray studies have revealed the extent of changes between exponential- and stationary-phase bacteria that induce the general stress response (256, 357) as well as other ppGpp-related responses (46). Given that approximately 10% of the genes in *E. coli* are differently regulated in stationary phase and that many of the genes expressed are involved in cell protection, it is evident why resistance to many stresses, such as acid stress or high osmolarity, is very different in exponential- and stationary-phase bacteria. Hence, the transition shown in Fig. 3 has a different baseline and result if exponentially growing or stationary-phase bacteria undertake a transition.

It is also possible to distinguish adaptive differences between early- and late-stationary-phase bacteria. During extended exposure to stationary phase, populations become more heterogeneous and complex. Diversity arises from incomplete sweeps by mutants that compete in the spent medium and have a growth advantage in stationary phase (GASP mutants) (100, 373). Some of these GASP mutants have mutations in global regulatory genes (*rpoS* or *lrp*), so the responses of these subpopulations are likely to be nonidentical if transferred to a new environment. Damaged cells are also a component of late-stationary-phase bacterial populations (241), so these can also be expected to respond differently to additional transitions.

In bacteria that can differentiate beyond stationary phase into spores, such as *B. subtilis*, the population heterogeneity is even more marked after starvation and induction of several coexisting responses and cell types (190). The simultaneous existence of spores, remaining vegetative bacteria, competent cells, toxin synthesis, and cannibalism gives rise to a mix of phenotypes (see “Genotype-determined mechanisms for generating multiple subpopulations” below); how such a mixture behaves in a new environmental transition is likely to be even more complex and is largely unexplored.

Genotypic stability in stationary phase also differ from that in exponential phase. Stationary phase is associated with altered regulation of DNA repair systems, most notably with reduced levels of MutSH (92) and reduced mismatch repair (MMR) (133, 134). GASP mutations are also stimulated by error-prone polymerase (372), which, as discussed in “Genetic Heterogeneity within Populations” below, is partly due to the higher levels of RpoS in stationary phase and elevated mutation rates due to stress-induced mutagenesis (SIM). Cell lysis and DNA release also occur in stationary phase, so the possibility of transformation is also potentially elevated in *E. coli* (249). *Bacillus* behavior upon starvation involves competence for DNA uptake, taking advantage of released DNA available from other lysed cells.

Population Size and Culture Density

Several of the determinants in adaptation are dependent on population size, so it matters whether a population undergoing an environmental transition is small (e.g., 10^3 to 10^4 cells) or large

(10^8 to 10^{10} cells). The number of bacteria in a population determines the spontaneous mutation supply and thus the available range of mutations. The level of regulatory heterogeneity is also strongly affected by population size if dependent on the stochastic effects discussed in “Heterogeneity due to stochastic variations in regulation” below. A good example of how population size affects bacterial survival is that of a pathogen population inside a host cell; large variation in the infectious dose of pathogens is a reflection of the importance of population size in overcoming host challenges.

Adaptations are in some cases impacted by intra- and interpopulation signaling in bacteria, and population density is an important factor in the local concentration and production of quorum signals (153, 315, 356, 362). A broad range of bacterial characteristics, including virulence, are affected by quorum sensing and signal molecules (169, 252), so it is highly likely that a population already subject to quorum signals will behave differently on moving to a new environment than a dilute, signal-less culture. Here again, a complicating factor is that heterogeneity occurs in the extent of the response to quorum signals in populations (259).

Rare beneficial mutations or particular gene duplications are unlikely to be present in small populations, and their fixation is also a function of population size. Adaptation in a small population has fewer sources of available variation, and mutator mutations are of particular secondary benefit in small populations (75). Hence, the same transition shown in Fig. 3 could result in different mutational possibilities with small or large populations of the same organism, simply because the right kind of variation is not present.

So far in this section we have been considering the population size before the environmental transition. If the transition involves a loss of viability (Fig. 2), then survivors may be a small proportion of the ancestral population. This can cause a bottleneck effect, and the full range of variation present in the prestress situation is not available in the survivors. The effective population size determined by the survivors in this scenario can affect the range of possible adaptive outcomes, especially with the rare genetic events considered in “Genetic Heterogeneity within Populations” below.

Prior State (e.g., Planktonic, Motile, or Biofilm)

Exponentially growing, fully mixed bacterial cultures are likely to be a rarity in nature. Bacteria not only frequently starve and enter stationary phase (as discussed above) but also often grow in an unmixed state subject to nutrient or stressor concentration gradients. In nonhomogeneous environments, gradients are sensed by chemoreceptors and chemotaxis is a benefit, but motility is an expensive process (296). Motility is subject to regulation by many inputs from the environment (303), so the response shown in Fig. 3 may depend on the motile state of a population if swimming is an immediate benefit or cost in the new situation. Some bacteria hedge their bets by simultaneously producing both motile and sessile forms from the same genotype (154) (see “Phenotypic Variation within Populations” below).

Bacteria on solid surfaces can swarm and form aggregated cells based on intercellular signaling, so these prior states also set a different baseline for adaptation (294). A bacterium like *P. aeruginosa* is capable of three forms of motility: swimming, swarming, and twitching. Within swarming populations, because of complex signaling between members of a population, cells do not have common patterns of gene expression (333). In turn, this means

that subpopulations in a swarming population have different baselines for further sudden changes of environment. Even more complex are bacterial aggregates that develop into mature biofilms with substructures and internal chemical gradients (191). In such scenarios, patterns of gene expression and cellular composition (especially in surface appendages and flagella) are widely different in subpopulations.

Biofilms show increased resistance to many types of environmental challenges and antibiotics (118). Several resistance mechanisms are involved in the same biofilm (198), and this is consistent with the view that a biofilm is not in a uniform physiological state (310). Antibiotic resistance of the biofilm subpopulations is aided by biofilm-inherent phenotypes, such as persister cells (see “Phenotypic Variation within Populations” below), slow growth, and induction of *rpoS*-mediated stress responses (149). Biofilm subpopulations differ in gross growth characteristics as well (266). The main point here is that subpopulations in the history of a culture before a transition will need to be considered in the response to a transition. If the population in Fig. 3 comes from a biofilm rather than a homogeneous population, the responses to a new environment are inevitably going to be nonidentical in the subpopulations.

Phenotypic Variation within Populations

Importantly, most of our understanding of bacterial behavior is from bulk cultures, averaging results over large populations. The extensive descriptions of bacterial physiology, regulation, and metabolism in reference works such as *Escherichia coli and Salmonella: Cellular and Molecular Biology* (231) and most of our understanding of transcriptional regulatory networks in *E. coli* (15) are based on studied adaptations of averaged populations.

In the past 10 years, though, there has been an increasing interest in heterogeneity as an inherent feature of bacterial growth and behavior (69). As already touched on above, however, the finding of pure-culture heterogeneity is not new, and both growth rate and antibiotic susceptibility variations within populations are long known (26, 155). The kinetics of killing by stresses also pointed to heterogeneities in bacterial populations (31). Further evidence for culture heterogeneities has come from the application of flow cytometry, identifying four or more physiological states in some exponential-phase bacterial cultures (68, 355). With the advent of microfluidics and high-sensitivity detection methods, it is now possible to dissect population effects as happening in individual cells. What is revealed is the high level of noise in gene expression across the whole genome (323). Cell-to-cell variations in macromolecules are high even with abundant proteins, perhaps due to a variable metabolic capacity to produce proteins (337). The reasons underlying noise and heterogeneity are considered in the subsections below. At the single-cell level, the early findings on growth rate heterogeneity have been substantiated in aging bacteria (309), and population diversification of individual cells in spore formation has been identified (345). The single-cell studies of antibiotic persistence have also provided strong indications for population growth rate heterogeneities and dormant cells (12). The evidence for population complexity in any bacterial culture is thus overwhelming.

The way that the distinct subpopulations adapt in new environments is mostly unknown, unless, as in the case of antibiotic persistence, the phenotype of the subpopulation is importantly obvious (see “Heterogeneity in growth rate, persistence, and

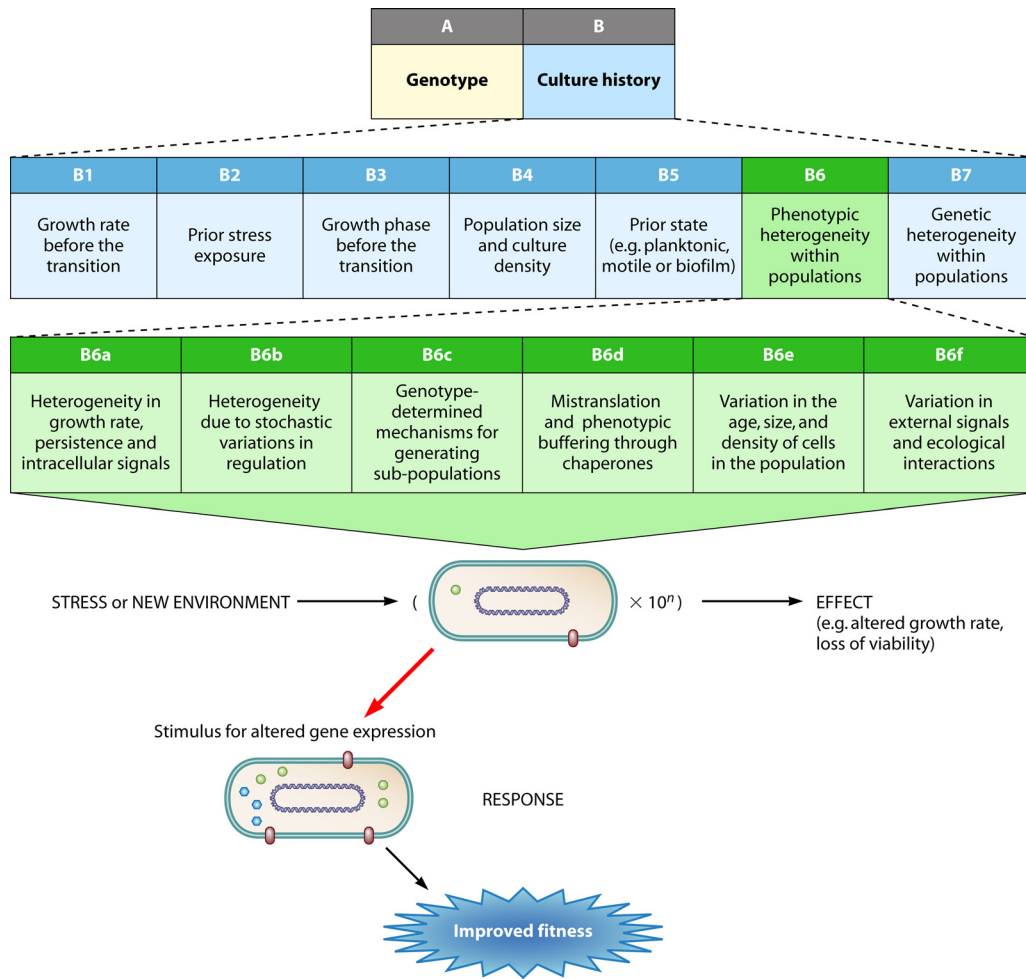


FIG 4 Aspects of phenotypic heterogeneity relevant to adaptation, expanded upon from Fig. 3 in increasing detail. Each box is further discussed in the text. Other content and arrows are as in Fig. 2.

intracellular signals” below). Another factor is that the totality of the heterogeneity is seldom considered; persistent cells coexist with many other subpopulations differing in motility, gene expression, cell size, etc., in the same culture. Thus, the main emphasis in this section is on how culture history influences heterogeneities in a wide range of characteristics. There has been rapid progress in defining the cellular features and evolutionary benefits that contribute to nonuniformities in populations, but it will be a challenge for the future to determine the adaptomics of heterogeneous populations. We can only begin to define the scale of the problem, as shown in Fig. 4.

Heterogeneity in growth rate, persistence, and intracellular signals. As noted in “Growth Rate before the Transition” above, the growth rate affects many characteristics of a bacterial cell. Given this importance in defining cellular characteristics, an important question is whether all the 10^n bacteria undertaking a transition are growing uniformly. Even for exponentially growing cultures, this question has already been answered in the negative; several independent approaches (12, 155, 309) all suggest that resting or slow-growing bacteria are present in growing populations.

The clearest indication that populations contain slow-growing

or dormant subpopulations comes from the phenomenon of bacterial persistence, which is defined as the capacity of some antibiotic-sensitive bacteria to survive lethal concentrations of bactericidal antibiotics (179). This property was initially demonstrated when *Staphylococcus aureus* cultures treated with penicillin always contained a small fraction of survivors, on the order of 10^{-6} or fewer (26). More than 40 years later, Moyed and his group isolated high-persister (*hipAB*) mutants of *E. coli* (29, 223, 224), in which the frequency of persisters after ampicillin exposure could reach as high as 10^{-2} of the population. Another high-persister locus (*hipQ*) was later identified (364). Direct observations of *hip* mutants growing in a specially designed microfluidic chamber identified two kind of subpopulations: the type I persisters, which are a preexisting subpopulation of nongrowing cells generated at the stationary phase, and the type II persisters, which are generated during exponential growth (12). Type II persisters are slow growers but are not as growth arrested as type I persisters. Fluorescence-activated cell sorting and differences in gene expression of inactive cells suggested that a third physiological state, distinct from exponential- and stationary-phase forms, is also possible (293). Therefore, a wild-type culture consists of three or four subpopulations of bacteria growing at different rates.

Persistence is strongly influenced by genetic elements called toxin-antitoxin (TA) modules present in the genomes of prokaryotes, which typically consist of two genes expressed as an operon (114). One of the genes encodes a stable toxin (which inhibits some important cellular functions) and the other an unstable antitoxin which neutralizes the toxin and also acts as an autoregulator of expression. TA modules found on plasmids constitute a maintenance mechanism (115, 140). TA modules are also commonly found in bacterial chromosomes. During experiments on gene expression in persistent cells of *E. coli*, it was observed that some well-characterized TA modules (*relBE*, *mazEF*, and *dinJ/yafQ*) were among the overexpressed genes (156). Overexpression of the plasmid-borne *relE* toxin gene, coding for a translation inhibitor, resulted in a 10- to 10,000-fold increase in persisters (54). Single-cell methodologies revealed that the *hipBA* (TA) module determines the onset as well as duration of a transient growth arrest (281). Successive deletion of all the TA loci of *E. coli* progressively reduced the level of persisters, showing that persistence is a phenotype common to TA loci. These results support a simple model according to which TA loci are activated in a small fraction of growing bacteria, which in turn induces dormancy and persistence (204).

A potential link between the environment, stress, and persistence comes from the role of ppGpp. This alarmone is elevated in stressed cells, but inactivation of the *relA-spoT* gene pair, involved in the synthesis of ppGpp, brought the persister level of the *hip* mutant to that of the *hip*⁺ wild-type strain (162). This defect could be complemented in *trans* by the wild-type *relA* gene. Based on these observations, Korch et al. (162) proposed a model of persister formation invoking a causative role for ppGpp, and indeed the *mazEF* TA is under ppGpp control (207). Another stress-related connection to generating dormant populations comes from the induction of persisters by DNA damage through the SOS-induced TisB toxin (78). Strategies of survival through dormancy and persistent subpopulations may be linked into sensing stressful environments.

The implication of ppGpp in persisters raises another interesting question. If subpopulations with different growth rates occur in any population, then these cells will also have different ppGpp levels. This is because the intracellular concentration of the alarmone ppGpp is central to responding to growth rate changes in the cell (197, 268). The phenotypes of bacteria growing at different rates are significantly different for the reasons already discussed in “Growth Rate before the Transition” above; ppGpp levels are key to the global regulation of ribosome synthesis, cell size, and global gene regulation (197). If a large population has slow-growing bacteria, through either TA effects or stochastic variations such as cells with a temporary deficit of ribosomes or RNA polymerases (see “Heterogeneity due to stochastic variations in regulation” below), then the alarmone levels within a population may also be heterogeneous. Transitions such as nutrient exhaustion also cause very rapid, drastic increase in ppGpp levels; ppGpp was shown to change in *E. coli* within a minute of starvation or addition of glucose (232). This was studied in bulk populations, but the rapidity of change suggests that if a large population has regions of non-constant nutrient levels, then the alarmone levels within a population will be heterogeneous. This is more likely in structured environments such as on an agar plate or biofilms, but it may occur with imperfect mixing in liquid culture.

The likely heterogeneities in ppGpp levels in a population re-

sulting from growth rate differences may be mirrored by other intracellular signaling molecules. Growth rate strongly affects the levels of cyclic AMP (cAMP) in bacteria (23, 237). In turn, cAMP controls a large number of catabolic and anabolic processes and metabolic fluxes (230). Thus, populations with growth rate heterogeneities may have significant effects on metabolism. Experimentally, there was indeed considerable heterogeneity in respiration in bacterial cultures (274); only 90 to 95% are respiration active in growing populations, and heterogeneity increased with starvation. Whether this is indeed due to variation in cAMP or ppGpp remains to be established.

Growth rate can alter other forms of regulation involving small molecules. Intracellular inducer and repressor compounds are responsible for controlling a large number of relatively specific responses encoded within the genome. The majority of regulation in bacteria like *E. coli* involves so-called local transcription factors and inducers/repressors that control a few genes (15). This includes the *lac* system, whose bistability is caused largely by differences in intracellular inducer concentrations between cells due to differences in transporter function (235). A piece of evidence linking growth rates to heterogeneity is the demonstration that the bistable switch responsible for coexisting bacteria with distinct levels of *lac* operon expression is affected by the history of the population and indeed its growth rate (277). Bistability and stochastic variations in populations are considered in the next section, but it is important to realize that the growth rate acts as an epigenetic factor in the generation of population heterogeneity.

The intracellular pool sizes of other regulon inducers such as galactose or maltotriose are a function of growth rate (93), so the expression of genes (additional to those regulated by ppGpp) is also a function of growth rate. There are some important compounds whose variation with growth rate is less well understood; other alarmones such as bis-(3',5')-cyclic dimeric GMP (c-di-GMP) are also important in defining bacterial phenotypes (141). Metabolomics has not yet advanced to a single-cell level except for individual molecules (22), but advances in this area will reveal possible heterogeneities in the pool sizes of inducers and alarmones such as cAMP, ppGpp, and c-di-GMP. However, given the above evidence, it would not be surprising if pool size variations resulted in phenotypic differences due to heterogeneity in growth rates or vice versa. In addition, the effect of prior growth rate on stochastic variation needs more thorough investigation.

Given the importance of environmental signals and stationary phase in persisters, culture history before a transition as shown in Fig. 4 is likely to have a strong influence on the overall population and subpopulation frequencies. The evolutionary advantage of having dormant subpopulations is very evident from clinical settings. For example, *P. aeruginosa* from chronic cystic fibrosis infections shows a much higher frequency of high-persister types in isolates from a patient in later years than in the earlier phase of infection (225). This provides a clear demonstration of the benefit (to the bacterium) of persistence as well as its evolvability. The fitness loss due to reduced growth rate in persisters pays off as a risk-reducing or “insurance” strategy in catastrophic situations (179).

Heterogeneity due to stochastic variations in regulation. As well as the lactose operon bistability and persistence mentioned above, some of the better characterized examples of nonheritable variation in properties include the lysis-lysogeny switch of phage lambda, chemotaxis in *E. coli*, phase variation in a number of

pathogens, and sporulation and competence in *B. subtilis* (9, 69, 85, 277, 300). In all these cases, variation can occur due to the fact that signals are not discrete because of random fluctuations inherent to the biochemical reactions occurring in the cell. This stochastic variation is called “noise.” Phenotypic variability in isogenic populations arises from regulatory mechanisms that exploit this signal noise, particularly when the number of molecules involved in biological processes happens to be small. This signal variability can be transformed into phenotypic variability by utilizing a regulatory network designed to suppress or amplify the effects of noise. For example, negative feedback can dampen the effects of noise (21), whereas positive feedback and other network motifs can exploit biochemical noise to generate population-level variability (85).

Experimental analyses of single-cell expression illustrated the extent of noise in transcription and translation in individual bacteria, resulting in the observed differences in protein concentrations (121, 320, 323). There are two kinds of noise linked to the expression process: the “intrinsic” noise, determined by the gene sequence and the properties of the protein it encodes, and the “extrinsic” noise which describes the fluctuations determined by variation in the number of polymerases and ribosomes in the cell, which are dependent on the metabolic state of the cell, cell cycle phase, or cell age (297, 337). Both sources of noise are hypothesized to contribute to individuality; however, it is not clear which source is dominant, and this will likely vary between regulatory systems (85).

Noise signal modulation can transform a graded response into a binary state, in which cells express a certain gene at low or high levels. At the population level, this switch-like behavior can result in a bimodal distribution in gene expression. It is this kind of pattern that produces two different subpopulations and is called “bistability.” Experimentally, some bistable gene expression patterns rely on positive feedback as well as double-negative feedback (toggle switch) (347).

Not all genes are equally noisy, so some phenotypes are more variable than others. A screen for noisy promoters in *Salmonella enterica* showed that some proteins, especially flagellar gene products, show high stochasticity (105). By broadening the range of characters such as motility and environmental stress resistance across a population, high gene expression noise can increase the likelihood that some cells within the population are better able to endure environmental transitions (9, 31). Experimental results providing support for this hypothesis were obtained in a study that demonstrated a competitive advantage of stress-resistant yeast mutants under high stress due to increased phenotypic heterogeneity (27). Experimental evolution has also shown that selection conditions can favor altered switching in bacteria (20). There is thus increasing evidence that changing noise levels, like other genome-encoded traits, are heritable and evolvable, being subject to selective pressures during the course of adaptation.

However, a high variability in the expression of individual genes does not always confer a selective advantage and may significantly increase the fitness disadvantage under low-stress conditions. The noise would decrease the likelihood of generating viable progeny, as better-fit cells would stochastically change their expression levels and become unfit prior to cell division (104). Consequently, noise reduction mechanisms preserving the fidelity of regulatory signals may evolve in some cellular functions (21, 83).

This is borne out by the difference in noise between genes in a genome (105).

Genotype-determined mechanisms for generating multiple subpopulations. Probably all bacteria can lower risks from environmental transitions by participating in stochastic bet-hedging through creating diversity (347). A particularly clear example of noise-related diversification comes from studies with *B. subtilis*. The complex subpopulation structure of course makes trying to predict the outcome of a single environmental transition with *B. subtilis* even more interesting and complex than with *E. coli*, because *B. subtilis* differentiates into several distinct, specialized cell types, depending on the culture history (189). There are conditions where a culture may consist of four or more subpopulations, each with individual properties and fitness advantages. The topic of *Bacillus* differentiation and the multiple signals and environmental influences affecting it is well reviewed and so will not be repeated here (85, 189, 345, 347), but from the point of view of adaptation and a subpopulation structure, it is instructive to speculate how a population of mixed types undertakes a transition as shown in Fig. 4. Clearly, the proportion of each type and its distinct benefit will determine which of the subtypes emerges with a selective advantage upon a transition.

One form of intrapopulation divergence is in motility. Populations of *B. subtilis* may have coexisting sessile and motile cells even in exponential phase (154). In terms of a single transition, the adaptomics in a new environment will depend on whether motility is an advantage and movement toward nutrients is required.

If *B. subtilis* is starved, about half the cells morph into spores. Heterogeneity of sporulating populations includes at least two cell types: sporulating cells, in which the master regulator of sporulation Spo0A is active, and nonsporulating cells, in which Spo0A is inactive (123). If this mixture ends up in a highly stressed environment, the passive resistance of dormant spores provides a major advantage. Nonsporulating cells can also undertake different pathways to acquiring new properties. About 10% differentiate into competent cells able to accept foreign DNA (85, 189, 347). The competence state allows *Bacillus* to generate diversity through the interchange of genetic information (60). Indeed, using mathematical models, Wylie et al. (371) predicted that if the total population size is approximately constant, cells which stochastically switch between the competent and vegetative phenotypes will prevail in competition experiments against otherwise isogenic cells that either are competence negative or fully commit to competence.

This differentiation into the competent state is a process driven not by genetic differences among cells but rather by a stochastic regulatory mechanism (371). Competence in *B. subtilis* is also a transient process, limited to a short time during the stationary growth phase (347). A complex regulatory network that integrates signals from various pleiotropic regulators controls competence (173) and the expression of all genes that encode the DNA uptake and integration machinery (344). Although subject to various regulatory inputs, the key factor is the stochastic fluctuation in conjunction with the positive-feedback loop that amplifies the signal such that the concentration of the competence regulator ComK exceeds a threshold in some cells, activating the positive loop that drives these cells into the competent state (346).

Another source of heterogeneity in *B. subtilis* is that in some situations, bacteriocins are used to kill members of the same clonal population. Experimental data showed that at the onset of sporu-

lation, bacteria secrete extracellular killing factors that lyse sibling nonsporulating cells, releasing nutrients into the medium on which “cannibal” cells can feed (124). Cannibalism might be an additional way to minimize survival risks by allowing the mobilization of the last nutrients within a niche—the “last supper” (314)—to delay spore formation until the prolonged depletion of nutrients forces the cells to sporulate (124). In *B. subtilis*, killing of nonsporulating cells is controlled by two independent gene clusters that are activated at low concentrations of Spo0A (55). At an early stage of sporulation when the level of activated Spo0A is low, genes involved in auxiliary roles such as cannibalism or building of multicellular aerial structures are turned on (108). Then, if harsh conditions continue, there is a progressive increase in the intracellular concentration of activated Spo0A that promotes activation of genes directly linked to the sporulation (123).

The strategies used by *Bacillus* are also adopted by other bacteria, although the regulatory mechanisms may be different. Competence and DNA uptake are found in taxonomically diverse bacteria (84) and are linked to cannibalistic strategies in *Streptococcus pneumoniae* (128, 139). Cells that are competent for natural genetic transformation lyse noncompetent cells, and virulence factors are released. In contrast to sporulation, nutritional stress does not trigger the development of the competence state in *S. pneumoniae*, which is induced in rich medium during early logarithmic growth phase and develops in response to different environmental signals (58), such as changes in pH or the presence of antibiotics (60, 269). In addition, it has been suggested that fratricide may generate diversity by genetic transformation (57, 59), and experimental evidence strongly supports a role for fratricide in lateral gene transfer (152).

Cannibalism is also associated with yet another subpopulation of *B. subtilis*, namely, that involved in biofilm development (192). Environmental, but not laboratory domesticated, strains of this bacterium form aerial structures in which sporulation occurs (33). In Spo0A-active cells, the cannibal subpopulation involved in killing nonsporulating siblings and delaying the sporulation process promotes at the same time the growth of specialized matrix-producing cells that induce the secretion of these extracellular components (192, 349). This adds yet another ecologically specialized subpopulation to the armory of *B. subtilis*.

In summary, there may be up to six types of cells in a *B. subtilis* population, depending on culture history: motile bacteria, sessile cells, bacteriocin producers, residual vegetative cells, competent cells, and biofilm formers. The behavior of each subpopulation in response to a new transition needs to be considered for the full adaptomic description of such bacteria.

Mistranslation and phenotypic buffering through chaperones. The same genotype can give rise to different phenotypes by a cell making mistranslated proteins from the same coding sequence. Amino acid misincorporation during translation occurs once in every 1,000 to 10,000 codons translated (243, 253), which means that around 15% of average-length proteins will contain at least one misincorporated amino acid. Therefore, exactly replicated genomes are common, but perfectly synthesized proteomes never occur in a bacterium (81). In addition, transcription errors, premature termination, faulty posttranslational modifications, and kinetic missteps during folding are some of the common translational errors over which a plethora of quality control systems act to correct (81, 221). At the same time, the fidelity of protein synthesis is also influenced by intracellular and extracel-

lular factors such as amino acid starvation (221). Therefore, it is important to note that translational error is under a dynamic equilibrium and its alteration directly affects bacterial fitness.

It is intuitively assumed that high mistranslation causes reduced growth rate and fitness because it results in altered proteins with less overall activity. Indeed, the presence of misacylated aminoacyl-tRNA (aa-tRNA) in a protease-deficient strain caused a significant reduction of the growth rate (285). However, under selection pressure, bacteria not only tolerate the presence of misacylated aa-tRNA but also can even require it for growth (285). For instance, *E. coli* can tolerate up to 10% of faulty protein, coping with mistranslation by triggering the heat shock response, which stimulates nonoptimized polypeptides to achieve a native conformation or be degraded (285). In this way, bacterial cells ensure the presence of sufficient functional protein even though at a considerable energetic cost (285). Under certain conditions, increased levels of mistranslation can even be advantageous: in *Acinetobacter baylyi*, substitutions in isoleucyl-tRNA synthetase that allow the mischarging of tRNA^{Ile} with Val confer an increased growth rate compared to that of wild-type bacteria under conditions of limiting Ile and excess Val (11).

In evolution, organisms can theoretically take advantage of beneficial phenotypes generated by error (209, 360). Mistranslation errors may cause individuals in a population to be beneficially prepared for a transition to a new environmental challenge, but advantageous phenotypes caused by mistranslation are not obviously heritable. However, phenotypic diversity generated by mistranslation can potentially allow heritable adaptation. The discovery in *E. coli* and other bacteria of a hypermutagenesis phenotype associated with codon ambiguity, the “translational stress mutagenesis” (TSM) phenotype, provides a potential solution to the problem of heritable transmission of protein translation errors (1–3, 77). Ambiguous codon decoding expands the proteome and generates new phenotypes. Selection of advantageous phenotypes creates positive feedback pressure that maintains ambiguous codon decoding, leading to synthesis of mutant DNA polymerases and DNA repair enzymes and emergence of hypermutagenic clones with an increase in genome mutational load. This accelerates the fixation of advantageous phenotypes and their transmission to the progeny (1–3, 77). Therefore, the high cellular tolerance to mistranslation opens the possibility that evolution of proteomic and phenotypic novelty could be fixed through hypermutagenesis (221). The possible importance of transient mutators, defined as wild-type bacteria that, due to occasional transcription or translation errors, display a mutator phenotype, was already proposed theoretically by Ninio (234), who suggested that transient mutators produce at least 10% of the single mutations and more than 95% of the simultaneous double mutations in an *E. coli* population; this needs experimental validation.

Another source of possible heterogeneity comes from chaperones, or heat shock proteins, that are capable of buffering the negative effect of mutations causing protein defects (202, 329). The level of the heat shock chaperones DnaK and GroEL was increased in lineages that had accumulated many mutations, and experimental overproduction of GroEL further increased the fitness of lineages containing deleterious mutations (329). In transitions such as those shown in Fig. 4, the availability of buffered mutations deleterious in the original environment could lead to the acquisition of neutral genetic diversity but accelerate the rate

of adaptation in a new environment where the available mutations are beneficial.

Variation in the size, age, and density of cells in the population. The fate and response of bacteria are subject to yet other population-level heterogeneities. One of these is in the size of the bacteria, because asymmetry in cell partitioning can generate cells both shorter and longer than the mean (67). Size can affect physical properties relevant to an environment (e.g., protist predation is dependent on size [131]), but other fitness effects are also size dependent. For example, if the environmental challenge is through phage infection, the size of cells in a growing population may intriguingly influence the lysis-lysogeny decision in *E. coli* with phage lambda (313). Also, infection of persister cells and vegetative cells can have different outcomes and produce different decisions in the lytic-lysogenic choice in phage-bacterial interaction (257).

The physical cell density of bacteria in a population growing in liquid culture is also heterogeneous (with up to 15 discrete fractions!) (205). The glycogen content of cells is largely responsible for density differences; the presence of very different glycogen levels in cells in a population is related to the metabolic characteristics of individuals as well as their regulation (363). However, there is no systematic study available to describe the fates of all the possible combinations of age, size, and density subpopulations in a single culture or whether they respond differently to stress.

Of course, heterogeneity is also seen in biofilms, and different cell types can be observed and recovered from biofilms (265, 368). Even colonies on agar plates contain subpopulations with distinct properties (288). In structured environments such as these, a series of local differences (e.g., nutrient gradients and cell-cell signaling, considered in “Variation in external signals and ecological interactions” below) are likely to contribute to this heterogeneity.

Bacterial cell composition is affected by the production of faulty proteins, as discussed in “Mistranslation and phenotypic buffering through chaperones” above. Recent research suggests that aggregates formed by denatured proteins are themselves a signal for cellular divergence. In a normal growing culture of *E. coli*, the evidence suggests that protein aggregation is predominantly at one end of a cell at old cell poles (184, 203). Stewart et al. (309), using cell imaging and tracking technology with dividing *E. coli*, observed that despite the morphological identity of daughter cells arising from symmetrical cell fission, one of the progeny cells inherits preexisting components (old pole) of the mother cell while the other inherits “*de novo*”-synthesized elements (new pole) (309). Notably, cells which successively accumulated old-pole elements showed a reduced growth rate, decreased numbers of offspring, and higher chances of death. Finally, upon reaching around 100 divisions, the older-pole-containing cells ceased to grow (309). Therefore, using an asymmetrical partitioning strategy, some cells would accrue irreparably damaged components, compromising their own reproductive potential in order to create fitter, rejuvenated offspring with maximal cellular capacities (307). The relevance of an asymmetric distribution of bacterial damage in a population in adaptomics is that aged and active bacteria presumably react differently to secondary transitions, as shown in Fig. 4.

Variation in external signals and ecological interactions. The image of bacterial populations as consisting of isolated, noninteracting individuals has given way in recent years to one in which they interact extensively (161). Cannibalism and fratricide, dis-

cussed above in “Genotype-determined mechanisms for generating multiple subpopulations,” are examples of interactive behavior in bacteria, in which groups of genetically identical or closely related organisms synchronize their patterns of gene expression to achieve specific goals that are unattainable for single cells acting on their own. Such collaborative behavior, which depends on cell-cell communication with secreted signaling (quorum-sensing) molecules, is widespread among bacteria.

Intrapopulation signaling through quorum-sensing systems influences many bacterial properties, such as the expression of virulence genes, biofilm formation, antibiotic susceptibility, cellular differentiation, and genetic competence (16, 161, 168). Interactions between bacterial cells involving other diffusible factors, i.e., secreted antibiotics, bacteriocins (44), or toxins (89), may also result in antagonistic interactions, which may kill non-resistant bacteria, nonimmune closely related species, or siblings, respectively (161). Whether quorum signals are present just prior to a transition determines the presence or otherwise of these phenotypes.

An important factor in the production of signal molecules and the local concentration of quorum sensor molecules or autoinducers is the density of the culture (321). As mentioned in “Population Size and Culture Density” above, high-density cultures undertaking an environmental transition may be in a state affected by the presence of signal molecules. For example, in *E. coli*, the response to challenges such as acid stress is modulated by population-level signaling. The release of indole can influence the behavior of neighboring cells (144). This signal molecule also affects antibiotic sensitivity in populations, in which subpopulations release indole (168). The history-dependent changes described in “Growth Rate before the Transition,” “Prior Stress Exposure,” and “Growth Phase before the Transition” above can also be elicited by population density effects. For example, bacterial characteristics similar to those in stationary phase can be elicited by high population density, even when growth is exponential. Bacteria growing at the same rate in chemostat cultures but at high densities exhibit many of the properties of stationary-phase bacteria (186) and so are likely to be more resistant to secondary challenges than low-density cultures. The concentrations of both the cells themselves and released signal molecules will therefore determine the transitions and responses shown in Fig. 3. The above results also suggest that population-level susceptibility, as against individual cell susceptibility, may be different.

Bacteria release a surprisingly wide range of molecules, especially in stationary phase (276). Released compounds include nucleotides and amino acids, and recycling of metabolites and nutrients from lysed cells provides a new ecological background to stationary-phase cultures, as it does in starving *B. subtilis* (see “Genotype-determined mechanisms for generating multiple subpopulations” above). Released nutrients provide new resources to compete for, and in long-term stationary phase, several mutations that enable *E. coli* to better compete for low concentrations of amino acids sweep (378). The heterogeneity of stationary-phase cultures (see “Growth Phase before the Transition” above) is at least partly due to adaptation to this more complex environment. In addition, released compounds such as amino acids can act to signal aggregative or motility-related changes (220). The profile of a culture is thus determined by the presence or absence of these signals and alternative released nutrients.

As mentioned in “Prior State (e.g., Planktonic, Motile, or Bio-

film)” above, chemical signals play an important role in the formation of biofilms. The aggregates themselves create gradients of oxygen and nutrient availabilities, causing differences in gene expression depending on nutrient levels (291). A range of self-produced autoinducers as well as external molecules contribute to biofilm formation (191). In *P. aeruginosa*, along with many other Gram-negative organisms, quorum-sensing systems respond to autoinducers termed acyl homoserine lactones. In *B. subtilis*, surfactin acts as a unidirectional signal so that one subpopulation of cells produces the molecule, whereas other cells respond to it and produce matrix (191). All these kinds of interactions cause heterogeneities in a population.

Bacterial cells also interact by means of contact-dependent systems. As in other processes described above, contact-dependent interactions may have positive or negative effects on bacterial cells. Particularly, sharing of components through contact-dependent structures such as nanotubes within and between species has been suggested (82). The new identification of specific systems involved in contact-dependent activities as well as their wide conservation strongly suggests that contact-dependent interactions are a widespread process and form part of the general interaction of the bacterial cell with its environment (161). A phenomenon called “contact-dependent growth inhibition” (CDI) was described, in which a uropathogenic *E. coli* strain produced a significant reduction in the number of viable *E. coli* K-12 cells (7). Other contact-dependent phenomena in different bacteria have been described (82, 128, 174, 226, 228, 331).

Finally, members of microbial populations are heterogeneous in terms of cheating to obtain commonly produced goods such as siderophores (iron chelators) or externally located enzymes such as β -lactamase (56, 135, 350). Some organisms stop producing the shared product and live off the rest of the population, which still bears the cost of production. The proportion of cheaters in a population is subject to environmental influences (36), so in the context of the single environmental transition that we are dealing with, the proportion of nonproducers and producers of shared resources will depend on culture history and the structure of the population (e.g., biofilm versus planktonic) (37).

Even given our narrow focus on clonal populations and a single transition, the heterogeneities and interactions discussed in “Phenotypic Variation within Populations” clearly define the formidable scale of the problem in trying to assess the makeup of a population at a single time point, before the transfer to a new environment. So far, we have considered mainly phenotypic variation, but we now turn to the added dimension of genomic variation and selection in the adapting population.

Genetic Heterogeneity within Populations

Despite the depth of regulatory solutions available for bacteria to survive a change in environmental conditions, the circumstances that a bacterial population may face in nature are many and can be varied and unpredictable, so it is not possible to have an effective regulatory response to every environment. Mainly due to costs and trade-offs, there are no superfit organisms in nature. In the absence of an appropriate phenotypic response in a new environment, there will be selection pressure to adapt via genetic change, which is the subject we focus on in the following subsections.

In considering how an organism adapts via heritable changes, we are concerned with variations that produce either altered gene expression or expression of an altered gene(s). The way by which

this occurs is through mutational events, which encompasses a diverse array of mechanisms and frequencies of occurrence, including amplifications, deletions, point mutation, insertions, etc. (106). In large populations, as often occur, mutational variation is considerable: in each generation, due to errors in replication, a spontaneous mutation arises on average in 5×10^{-3} of the population, and after n generations, the proportion of ancestral organisms will be, statistically, $0.995n$ (68). If $n = 10^9$ in Fig. 2, then about 5×10^6 bacteria in that population potentially carry a mutation. As discussed below, processes such as amplification and phase variation are even more frequent (5). Adaptation, though, is not just the generation of genomic variability, and we also consider how selection acts upon genomic variation to result in a diversity of fitter offspring. In the following sections we discuss how both genomic variation and selectional processes increase the richness of adaptive outcomes.

In order to maintain genetic stability from generation to generation, the process of DNA replication should be carried out with very high fidelity; this is important since mutations are much more likely to be deleterious than beneficial (79, 260). To this end, the mutation rate per base pair per generation (μ_b) is maintained at a very low level; for example, in *E. coli*, μ_b is estimated to be around 5×10^{-10} (80, 194), although other estimates are lower still (242). The majority of these mutations are neutral or deleterious, while the frequency of beneficial mutations in *E. coli* has been estimated at around 10^{-8} to 10^{-9} per genome (147, 283). This may be an underestimate due to clonal interference (competition in populations between multiple bacteria with independent mutations) that will exist, particularly in larger populations (116). However, the most optimistic estimate in the absence of clonal interference is still only 2×10^{-5} per genome, which is orders of magnitude below the overall mutation rate (260).

The hypothesis that mutations are kept to a minimum in evolution is not altogether convincing, however. A survey of the spontaneous mutations arising in 787 natural isolates of *E. coli* did not indicate a species-wide trend toward a uniform, low rate of spontaneous mutagenesis; in contrast, there was a broad distribution of frequencies of spontaneous mutation to rifampin resistance, with many strains differing by several orders of magnitude (28). A high mutation rate may result in faster adaptation to an environmental change by providing a greater number of alternative genetic solutions from which beneficial solutions can be selected. Of course, the balance between mutation rate and genetic stability represents a trade-off, i.e., the ability to evolve to adapt to change balanced with the need to maintain the integrity of the organism.

There are several ways in which a bacterial genome can change the costs and benefits of this trade-off. In other words, bacteria shift this balance such that when conditions are favorable, the bulk of the population undertakes high-fidelity genome replication (i.e., “stick with what is working”), but when conditions change, there is the capacity within the population to produce new genetic solutions. Several identified mechanisms change mutation rates conditionally. These mechanisms include the following: (i) increasing gene copy number by amplification, which can increase the dosage of a beneficial gene or provide redundancy to increase the target(s) for adaptive mutation while simultaneously decreasing the risk of loss of gene function through deleterious mutation (5); (ii) limiting mutations spatially by possessing contingency loci, i.e., regions of the genome that are more susceptible to mutation, as exemplified by the mechanism(s) of phase variation

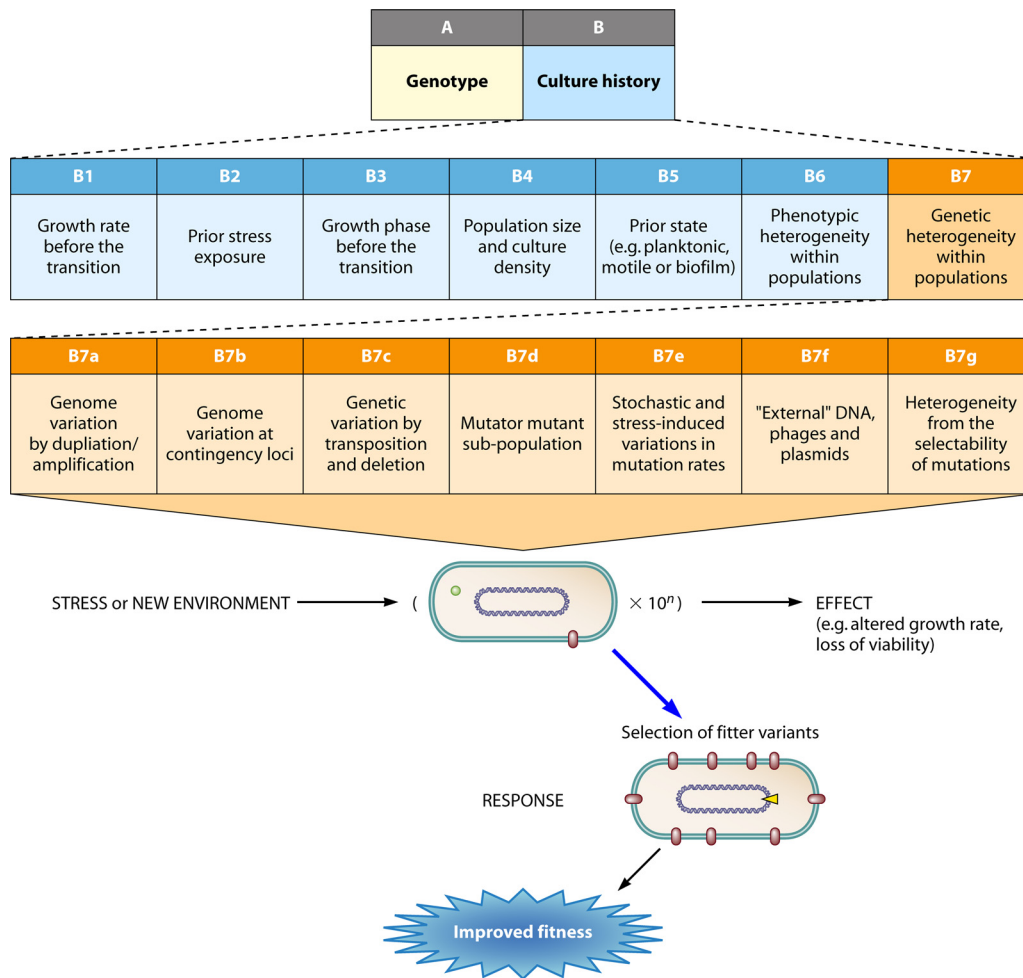


FIG 5 Aspects of genetic population heterogeneity relevant to adaptation, expanded upon from Fig. 3 in increasing detail. Each box is further discussed in the text. Other content and arrows are as in Fig. 2.

(341); (iii) taking advantage of a subpopulation of constitutive mutator mutants with an increased mutation rate that can be enriched by secondary selection in times of need; (iv) limiting mutations temporally, i.e., increasing mutation rates under stress (a process called stress-induced mutagenesis) (28, 102, 112), including increased mutation rate during stationary phase (100); and (v) acquiring new genes by lateral transfer (42). All of the above can provide a great deal of genomic variation within a population that can be exploited to adapt to environmental change. The following sections and Fig. 5 examine each of the above mechanisms in more detail and discuss how they contribute to genetic heterogeneity.

Genome variation by duplication/amplification. Gene duplication/amplification (GDA) is one of the most common types of genome change. It encompasses both simple duplications and higher-level amplifications of up to 40 copies of a gene(s), with sizes of the amplified region of up to several megabases (6, 328). GDA can affect practically any gene and is inherently unstable/reversible. The ease of reversibility of GDA means that the cost of the duplication can be lost in a nonselective environment.

The high frequency of GDA in bacteria means that large populations will be heterogeneous before and during the transitions shown in Fig. 4. GDA occurs in a growing population of bacteria at

a frequency of $>10^{-2}$ to around 10^{-4} per genome (4, 272, 302). From these frequencies and the average size of the amplified regions, it has been estimated that around 10% of all cells in a non-selectively growing culture contain a gene duplication somewhere in their genome (5). If the duplications are spread evenly throughout the chromosome, then around 2×10^4 in a population of 10^9 *E. coli* bacteria contain a duplication of a particular gene. The culture history also influences the frequency of GDA, which can be induced by prior exposure to cellular stressors (136) and may be part of the strategy for stress-induced mutagenesis (138), as further considered below.

Much of our understanding of GDAs had been obtained from studies of *S. enterica*, in which GDAs have been found in all chromosomal regions except the terminus of replication (4). GDA has been proposed to occur by several mechanisms, and it is likely that different ones account for simple duplications and higher-level amplifications (5, 113, 172, 279, 290). The most frequently observed mechanism for formation of simple duplications occurs by RecA-dependent nonequal homologous recombination between regions bounded by directly repeated sequences such as rRNA (*rrn*) operons, transposable elements, and other repetitive sequences (4, 5, 129, 182, 273, 290). However, other duplications

that are both repeat sequence and RecA independent can also occur (290). It has also been proposed that stress might induce repair of broken replication forks to switch from high-fidelity homologous recombination to nonhomologous repair, thus promoting copy number change (137). Higher-level, multiple amplifications can then form from duplications via RecA-dependent recombination between the tandem repeat generated by the initial duplication (273). Multiple amplifications can also form via a rolling-circle replication mechanism in the absence of preexisting duplications (261, 290).

GDA can increase fitness in a selective environment by increasing the copy number (gene dosage) of a beneficial gene, effectively increasing its expression, much like a positive regulatory change. GDA therefore provides a quick and reversible way of adapting, which is in some respects closer to regulatory changes than the more permanent genetic change normally considered heritable. As mentioned above, GDAs are unstable and are easily lost under nonselective conditions by homologous recombination between the repeated amplified sequences (263, 290).

GDA can also act as a facilitator of more permanent genetic changes. Increasing the copy number of a gene permits “prospecting” for beneficial mutations in one replica while still maintaining one or more wild-type copies, abating the risk of loss or reduced gene function because of deleterious mutations. Once a beneficial mutation is acquired, the GDA may be lost (5).

Genome variation at contingency loci. Contingency loci are hypermutable regions of genomic DNA that typically mediate the high-frequency, reversible on-off switching of gene expression, as exemplified by the phenomenon of phase variation. Contingency loci allow an organism to confine an increased rate of change to particular genes whose heterogeneity of expression in a population is of advantage and to reduce the deleterious cost of increased mutation rate throughout the genome. Rates of switching at contingency loci can be much higher than the spontaneous mutation rate, ranging from 10^{-2} to 10^{-5} per genome per generation (222). This, considering the stochastic nature of the switching and the fact that there are often several phase-varied genes in a genome, results in considerable genotypic heterogeneity within a population.

The process of phase variation in bacteria is most studied in bacterial pathogens, where there is heterogeneity in the expression of genes whose products (usually a cell surface component) are visible to the host's immune system. Variation provides a mechanism for bacterial populations to survive clearance by host immune responses (for reviews, see references 222 and 289). There are three common mechanisms for local genome variation, namely, (i) through changes in short sequence repeats (SSRs) in or near contingency genes, (ii) through specific inversions of particular parts of the genome, and (iii) through epigenetic regulation mediated by DNA methylation (341).

Some contingency genes contain SSRs made up of 1- to 6-bp units, either within the promoter region or in the coding sequence of the gene. This facilitates phase variation via the mechanism of slipped-strand mispairing (SSM) (222, 338). SSM occurs during DNA replication or repair requiring strand separation of the parental DNA duplex, giving rise to single-stranded DNA, and synthesis of new daughter DNA, which must then reanneal to the template. The presence of a repeat sequence facilitates the ability of the template and daughter strands to slip in relation to one another, resulting in loss or gain of a repeat unit sequence and

leading to frameshift mutations or changes in promoter sequence spacing, which can then result in altered gene expression. Conversely, the same mechanism can switch gene expression on again following further rounds of DNA synthesis and slipped-strand mispairing (222). SSM is important in many pathogens, particularly *Neisseria meningitidis* and *Helicobacter pylori*, in which 19 and 65 SSM-mediated phase variation loci have been identified, respectively (275, 339).

Another mechanism that can give rise to phase variation of contingency gene expression is site-specific recombination. This involves the inversion of a short DNA segment flanked by sequences recognized by specific recombinases, which contains the contingency gene or its promoter sequence. The gene(s) is then either expressed or not depending on the orientation of the invertible sequence (343). A well-known example of phase variation controlled by site-specific recombination is phase variation of type 1 fimbriae in *E. coli* (343). Flagellar phase variation in *S. enterica* is also controlled coordinately by an inversion event (298). Another related mechanism of phase variation involves the precise excision or incorporation of insertion sequences (343). This phase variation mechanism is restricted to a few IS elements, e.g., IS2561 in *Staphylococcus epidermidis* (343).

Methylation of DNA is involved in a number of examples of phase variations. Unlike the previous two, this mechanism is epigenetic, since the DNA coding is not altered. Methylation can result in phase variation through a transcriptional regulator, as occurs in interdependent expression of fimbrial and flagellar genes in *E. coli* (reviewed in reference 342). Two Dam methyltransferase sites and *pap* DNA methylation patterns are involved in the regulation of the phase variation switch between the on and off states in the synthesis of Pap pili in *E. coli* (32). Dam-dependent phase variation in *E. coli* also accounts for changes in another antigen, Ag43 (130). A third example of a surface component regulated in this way involves *Salmonella* lipopolysaccharide modification genes (35). Hence, a population of bacteria can consist of subpopulations whose methylation pattern and gene expression are determined by epigenetic processes.

As noted in the next section, phase variation may even play a role in regulating the mutation rate, since it has recently been shown that the expression of *mutL*, a gene involved in the mismatch repair system in *S. enterica* serovar Typhimurium, can be reversibly switched on or off via SSM-mediated changes in a number of small tandem repeats (49, 50). Thus, such a genetic switch would enable a subset of a population to readily convert to a hypermutator phenotype, which would be of potential benefit in times of stress, as discussed below. The authors went on to show that the tandem repeat sequences were present in MMR genes from many other bacteria, suggesting that on-off switching of hypermutation may be a prevalent mechanism.

In all of the mechanisms affecting contingency loci, the rate of generation of phase variants is controlled by cellular components, be they methylases, polymerases, or DNA inversion mechanisms. Culture history can change the frequency of switching and contribute environmental regulation to switching rates (18). Stochastic effects discussed above can also contribute to the many factors that affect contingency heterogeneity within a species and a population.

Variation due to transpositions and deletions. In bacterial genomes, transposable elements and especially insertion sequences (IS elements) are widely and diversely distributed (201). Trans-

posable elements in a genome can aid adaptation and fitness (47, 48). Many examples of transposition arise in adapted strains in experimental evolution experiments, and these generate a high level of genetic diversity within *E. coli* (110, 250) and lead to adaptive mutations (375). Several of the IS insertions in experimental evolution studies are beneficial under the selection conditions (110). Movement of IS elements contributes to bacterial adaptation by increasing the mutation supply, in turn enabling the organisms to overcome an environmental challenge (318, 361, 379).

The rates of transposition vary from element to element; for example, under nutrient limitation of *E. coli*, no changes were associated with either IS30 or IS150 but IS1, IS3, and IS5 movements were common (110). The precise numbers of transpositions in any individual population are not thoroughly understood, but methods for quantitating transposition frequency are being developed (248). Nevertheless, based on the high number of transpositions in experimental selections (110, 250), a large population undertaking a transition shown in Fig. 4 undoubtedly includes some genomes with IS movements. The possibility of transpositions in large resistance gene islands on chromosomes and plasmids in multiple drug-resistant isolates is even greater, especially under antibiotic selection (330).

There are examples of organisms in which the frequency of transpositions is also affected by stressful environments, especially when DNA is damaged (87) and perhaps also under extended starvation (227). Thus, culture history may have an effect on these classes of mutation; for example, the prior UV exposure of a population shown in Fig. 3 could affect the proportion of mutations caused by transposable elements.

IS elements also provide a means of obtaining deletion mutations. Repeat copies of IS elements provide homologies permitting deletions to form, as of course do other repeats in genomes (25). An interesting example in *E. coli* is provided by the means of generating mutator mutations through an IS5-mediated transposition followed by deletion of the intervening region (110). Several additional mechanisms of deletion formation have been proposed, and some of these are present in stationary-phase bacteria (13, 14). Hence, this form of mutation, like other forms of mutation discussed in “Mutator mutant subpopulations” below, may be under a culture history influence.

Mutator mutant subpopulations. When a bacterial population faces a change in environmental conditions, it may be worth the risk of raising the mutation rate in order to increase the occurrence of beneficial mutations. One way to achieve this in a population or at the species level is via the existence of subpopulations of constitutive mutator or hypermutator mutants (119).

A major factor in the basal mutation rate is the capacity for DNA repair; mutations in DNA repair genes generally result in increased mutation rates (216). There are many components of DNA repair that influence bacterial mutability (145). The most common and best understood mutations causing a heritable, constitutive mutator phenotype involve genes of the DNA mismatch repair (MMR) system (*mutS*, *mutL*, *mutH*, and *uvrD*), which are responsible for proofreading after DNA replication as well as the inhibition of interspecies recombination events (49, 180, 271, 319). Mutations in the MMR system can elevate mutation rates by more than 100-fold (319).

When starting with a pure nonmutator *E. coli* clone, by the time 10^9 cells are present through division in a population like that shown in Fig. 5, about 10^5 mutator mutants are present in this

population through spontaneous mutations in mutator genes (206). If beneficial mutations are selected in such populations (such as mutations for antibiotic resistance or better growth under nutrient limitation), the MMR mutator cell is 100 times more likely to contain a beneficial mutation. The cell containing a combination of an MMR mutation plus a beneficial mutation is then enriched through selection. Hitchhiking with beneficial mutations allows MMR mutant bacteria to reach high proportions in rapidly evolving cultures (217, 238). This process of secondary selection is most likely responsible for the high proportion of mutators in nature (72, 119).

Indeed, MMR constitutive mutator mutants are present at a surprisingly high frequency in natural populations of pathogenic and commensal *E. coli* strains, making up to 1% or more of the population (167). Many of the mutations in naturally occurring mutator isolates are deletions (164). The frequency of mutators can reach very high levels in uropathogenic *E. coli* (72) or *P. aeruginosa* populations chronically infecting the cystic fibrosis lung, where mutators can make up 40% of the population (91, 244). *P. aeruginosa* cystic fibrosis lung infections are usually lifelong and often are aggressively treated with prolonged antibiotic therapy, and the high frequency may reflect the repeated selection of antibiotic resistance associated with mutators (91, 244, 365).

In addition to increasing the rate of point mutations, a deficient MMR system also results in an increased incorporation of foreign DNA via lateral transfer, owing to the role of the MMR system as an inhibitor of this process (210). *E. coli* mutants without MutS or MutL recombine *Salmonella* DNA into *E. coli* at about 1,000-fold-higher rates than cells with an intact MMR system (305). This is obviously of importance for adaptation as well as the lateral acquisition of antibiotic resistance from other organisms (327). The heterogeneity and mosaicism of mutator gene sequences suggest that changes of mutation and recombination rates by frequent loss and reacquisition of MMR functions occur in *E. coli* genomes (38, 73).

Mutation of the MMR system may be subject to a more deliberate adaptive strategy akin to phase variation. As discussed above, the MMR system is potentially phase variable, perhaps across a range of bacterial species (49, 50). The evidence suggests that repeat sequences present in MMR genes may act as “on-off” switches facilitating the easy conversion to a mutator phenotype to better respond to stressful conditions.

Stochastic and stress-induced variation in mutation rates. In contrast to acquiring a constitutive mutator phenotype via mutation of the MMR system, bacteria can also express a transient elevated mutation rate in two ways, although the two mechanisms may overlap. Temporary mutators or transient mutators (218) can arise for the same stochastic reasons as the phenotypic variation discussed in “Heterogeneity due to stochastic variations in regulation” above. As predicted on theoretical grounds (234), transcriptional and translational errors or cell divisions that result in low levels of MMR components can result in transient mutagenesis. In a large population like that we are considering, all these stochastic events can give rise to subpopulations with elevated mutation rates. The second mechanism causing temporarily increasing mutation rates, known as stress-induced mutagenesis (SIM), is less stochastic and has elements of determinism; several regulatory genes associated with stress resistance are associated with SIM (102, 112, 326). A control mechanism with stress inputs

temporally isolates increased mutation to times when the bacteria are maladapted to their environment.

SIM appears to be a common feature of *E. coli* populations, with up to 80% of isolates of *E. coli* showing a stress-inducible mutagenesis phenotype in aged colonies (28; see reference 369 for an alternative view). This potentially provides a large increase in mutation supply in a population. However, large variations in mutation rate increases exist, ranging from a few fold to >1,000-fold (28), so the extent of SIM appears to be strain specific and may reflect differences in recent evolutionary selective pressures.

Several types of genetic alterations can be involved in SIM, including base substitutions, small deletions and insertions, gross chromosomal rearrangements, copy number variations, and movement of mobile elements (102, 112, 136, 319). A common theme, though, is the requirement for one or more stress responses to induce mutagenesis, e.g., the SOS DNA damage response, the RpoS general stress response, and the stringent response (102, 111, 112, 319, 326). The link between stress and mutagenesis extends to extracytoplasmic stressors (e.g., those causing membrane damage) through another sigma factor, RpoE (117). RpoE, aside from its better-known role in membrane protein protection, is thought to promote double-strand breaks and feed into SIM induction by affecting genome integrity (117). Multiple inputs ensure temporal control of mutagenesis to times of stress, while ensuring genetic stability when cells are well adapted.

The type of stress that is intuitively the easiest to link with increased mutagenesis is one that causes DNA damage, either from exposure to DNA-damaging agents or from uncompleted DNA repair/replication. DNA damage induces the derepression of SOS genes, many of which are involved in DNA repair and recombination (99, 255). The initial SOS response involves induction of enzymes that repair DNA with high fidelity; however, with greater damage, a more mutagenic response is initiated, involving three alternative DNA polymerases, i.e., Pol II, the Y family polymerase Pol IV (encoded by *dinB*), and Pol V (encoded by *umuDC*), which, in contrast to normal replicative Pol III, can replicate past DNA lesions (99). Two of these, Pol IV and Pol V, are both low-fidelity polymerases and therefore replicate DNA in an error-prone manner. In dividing cells, it is the induction of Pol V-dependent replication during the SOS response that is largely responsible for up to a 100-fold increase in chromosome-wide mutagenesis (292, 306, 366). In stationary-phase cells, Pol IV is involved in higher mutation rates (40). RecA also plays a central role, since in addition to upregulating *dinB* and *umuDC* transcription, RecA also interacts directly with Pol IV and Pol V to modulate their mutagenic activity and is involved in the cleavage of nascent UmuD to form functional Pol V (120, 150, 322). The SOS-induced mutagenesis is probably a by-product of a survival-at-any-cost mechanism to overcome otherwise fatal DNA lesions. Nevertheless, it also increases genetic diversity within the population at a time of stress, which may then be of advantage to adaptation.

The population history, as discussed in “Growth Rate before the Transition,” “Prior Stress Exposure,” and “Growth Phase before the Transition” above, has a major influence on mutation rates through a general stress-sensing mechanism; there is strong evidence that the general stress response induces the error-prone Pol IV (40, 166). The induction of Pol IV is mediated by the sigma factor RpoS and occurs when cells enter stationary phase or experience the other stresses discussed above in “Growth Rate before the Transition” and “Prior Stress Exposure.” There are thought to

be two ways in which the general stress response increases the mutation rate, both of which are RpoS dependent. The first is by upregulation of the expression of the error-prone DNA polymerase Pol IV (111, 166), and the second is by downregulating expression of the MMR proteins MutS and MutH (28, 334). The RpoS-dependent nature of both of these mechanisms can give rise to heterogeneity in mutagenesis levels in a population concurrent with heterogeneity in RpoS levels. This indeed explains the heterogeneity in mutation rates across the species *E. coli* (287).

Another player in the control of RpoS/Pol IV mutagenesis is polyphosphate [poly(P)], which consists of chains of phosphate tens to hundreds of residues long and is produced by the enzyme polyphosphate kinase (PpK) (for a review, see reference 163). Poly(P) accumulates in cells entering stationary phase and is required for induction of RpoS and of RecA (295, 335). Thus, poly(P) can influence SIM through its regulation of RpoS; however, poly(P) may also play a more direct role by regulating the activity of Pol IV (316).

The stringent response mediated by ppGpp is a third stress signal that contributes to SIM, partly through RpoS induction (165, 332). This overlap means that the RpoS-induced mutagenesis mentioned above will also occur during the stringent response and, similarly, that heterogeneity in RpoS expression (see “Prior Stress Exposure” above) will result in heterogeneity in stringent response-induced mutagenesis. Like for RpoS, the levels of expression of ppGpp can vary greatly across different strains of *E. coli*, and mutations affecting ppGpp production readily occur (98), which provides further heterogeneity in SIM within a population.

As mentioned above, the stringent response induces transcription of amino acid biosynthetic genes and other genes to relieve stress. Transcription requires the formation of loops of single-stranded DNA, which has been proposed to be more susceptible to damage (370). Potentially, actively transcribed genes can acquire more damage than nontranscribed genes; consistent with this is the fact that DNA repair enzymes are recruited to transcribed genes (213). In particular, error-prone Pol IV associates with RNA polymerase that is stalled at lesions in DNA that it is transcribing, to facilitate translesion DNA synthesis to enable transcription to proceed (62). This provides a potential mechanism by which genes that are being actively transcribed are more prone to mutation via increased error-prone DNA synthesis. This would target mutation to genes that are actually required to overcome stress (370). Indeed, this mechanism has been shown to be involved in SIM in both *lacZ* and *tetR* mutation assay systems, increasing mutagenesis by more than 400-fold (63).

It is tempting to think that SIM is an evolved mechanism to increase evolvability under stress, and evidence is indeed accumulating to suggest that this is the case (112). As mentioned above, the increase in mutation rate may even be targeted to genes that are actively being transcribed, which would target adaptation to genes and pathways that are most likely to lead to an advantage in overcoming a particular stress (370). The mutation rate increase is indeed also restricted to subpopulations of cells, because it appears the hypermutation is mostly restricted to about 1% of stressed cells that have double-strand breaks in DNA (258). Also, the stress-induced GDA changes may be in subpopulations of bacteria that show repeated template switching during DNA replication (181). All this mutational heterogeneity in subpopulations

adds to the rich variation from which adaptive strategies can emerge in bacteria.

External DNA, genetic exchange, phages, and plasmids. As discussed in “Phenotypic Variation within Populations” above, a factor subject to bacterial phenotypic variation is the competence to take up DNA. Besides competence, bacteria have other DNA uptake mechanisms involving conjugation and phage-mediated transduction (10, 43). The ability to access foreign DNA leads to a huge increase in the potential for genetic variation, and indeed the analysis of bacterial genomes points to the importance of lateral gene transfer (LGT) in bacterial evolution (176, 376). The core genome shared by all *E. coli* strains is fewer than 1,000 genes, so even at the species level, a great number of genes get moved around (193). However, in the system we are dealing with, in a clonal population with similar genomes, the possibilities for major variation through LGT are significant only if the new environment has other bacteria as a source of external DNA. In many natural environments, this may indeed be the case. It is extremely difficult to estimate the frequency of such LGT-type events in single transitions, and these will be very variable depending on the environment, sex factors, ability to take up DNA, and ability to recombine foreign DNA. In any case, this lateral source of variation in microevolution is probably rare relative to the other processes discussed here, such as contingency gene changes, GDAs, or even base change mutations. However, it does seem that some species change more often by LGT than others, so the capacity for LGT is also a question of genotype (376).

Nevertheless, there are circumstances when genetic exchange is advantageous even within an *E. coli* population. Experimental evolution studies of pure cultures in the presence of the F plasmid (promoting conjugation in *E. coli*) showed that the fixation of beneficial mutations was enhanced through plasmid-mediated gene transfer (65). Conjugation allows beneficial mutations that arise in different lineages to recombine, hence increasing the number of possibilities in adaptation. Hence, the transition shown in Fig. 2 will depend on the genotype (presence of conjugative plasmids) and the proportion of the population that carries such elements (depending on plasmid stability).

Besides conjugation and genetic exchange, plasmids and phages add another dimension to bacterial variability. The genes carried in extragenomic elements have the potential to alter not only the physiology and metabolism of a cell but also its capacity to generate mutations. The error-prone DNA polymerases can be encoded in plasmids and contribute to mutational adaptation (122, 324). Together with the variation in plasmid content itself as a result of environmental stresses (358), a bacterial population may be heterogeneous because of these plasmid-encoded factors.

The well-known dependence of prophage induction by stresses, coupled to the SOS response in phages such as lambda (278), also has the potential to influence heterogeneity in a population. The basal level of lambda induction, in about 1 in 10^4 cell divisions, is increased by a variety of stresses (41). Hence, in a large population of lysogenic bacteria, heterogeneity and lysis due to released phage is a likely scenario.

Heterogeneity from the selectability of mutations. Selection and within-population competition occur constantly in evolution. With a change of environment as shown in Fig. 2, altered fitness characteristics that can benefit a subpopulation of mutants in the old population are selected. In the above sections we have discussed several mechanisms whereby such fitter mutants can

arise from GDA, LGT, spontaneous mutations, or elevated mutation rates. Still, there can be situations where mutations are limiting; no major adaptive shifts in the population are likely if the beneficial mutations in the particular environment are absent, or particularly rare, or if the population is very small. In these cases of limiting mutation supply, mutational adaptation is unlikely, but elevated mutation rates can overcome this limitation, as discussed above. Selection can then result in changing ratios of subpopulations within the population. There are further scenarios, however, in which the composition of a population may not change appreciably as a result of selection. The fixation of mutations, especially weakly beneficial mutations, is also a limitation, and drift can eliminate these before fixation (245).

More frequently, however, significant population changes can be associated with mutational events. First, a strongly beneficial mutation can sweep through a population in a new environment, replacing the ancestral bacteria. This occurs in lethal selections such as for antibiotic resistance among a sensitive population. Such sweeps result in a new but genetically homogeneous population. Other, more complex, types of population shift can also occur. In large populations or if the mutation supply is high, several different beneficial mutations may be present simultaneously. This scenario is likely to be common in the large populations that occur in nature. In laboratory evolution experiments that use large populations (10^9 to 10^{10} bacteria), the appearance of several beneficial mutations is more the norm. Experiments have revealed a range of conditions under which bacterial diversity is generated in a relatively short period (200, 270, 280). The classic scenario for such divergence is that if the new environment has alternative niches and is itself heterogeneous, then different bacteria may adapt toward the different niches (270). As shown in Table 1, if alternative carbon sources are present upon the transition, then some bacteria can specialize on one and some on the other nutrient (171, 377). This ecological explanation of diversification entirely depends on the new environment shown in Fig. 2. If the new environment results in a biofilm with its distinct zones for example, diversity can later arise in this niche-dependent manner (265).

In recent years, the diversification of populations through mutational processes has also been demonstrated even in new environments that are not structured and without multiple nutrient sources. How diversity arises in microevolutionary adaptation is still not resolved, but it can lead to divergence to the point of individuality in less than 100 generations (200). Heterogeneity obtained in this way, together with the lack of complete elimination of competitors, can have several causes and explanations, as further summarized in Table 1.

It is not yet clear which of the proposed mechanisms in Table 1 has a dominant or even contributing role in the diversification of adapting populations. Some, like coexistence based on trade-offs (127), do contribute to the diversity in *E. coli* chemostat culture populations (199), and negative frequency dependence is also shown by some isolates in the same environment (200). Further experimental evidence for coexistence based on cannibalism or cross feeding also exists (158, 284). Less experimental support (and testing) is currently available for the contributions of more theoretical models (mutation-selection balance, trade-offs, and trade-offs plus frequency-dependent selection in Table 1). Altogether, the evidence is that in large populations a combination of several mechanisms in Table 1 contributes to the overall diversity (198a). None of the mechanisms in Table 1 is disproven, so there

TABLE 1 Proposed models of diversification leading to heterogeneity in populations

Proposed mechanism	Example(s)	Reference(s)
Alternative niches or resources in the new environment	Specialization on one of several carbon sources or one of several locations (e.g., air-water interface) in the environment	171, 377
Mutation-selection balance	Can occur when a high mutation rate and the slow fixation of mutations lead to several coexisting genotypes in a population	74, 374
Negative frequency-dependent selection	The competitive fitness of a type increases as it becomes rarer so does not eliminate competitor	178
Trade-offs	With a SPaNC trade-off, a type with higher stress resistance but a lower growth rate coexists with a variant with less resistance but a better growth rate; trade-offs can also lead to coexistence of rate yield variants	126, 127
Trade-offs plus frequency-dependent selection	Theoretical, no example available	76
Trade-offs plus mutation rate differences	Theoretical, no example available	19
Convergent evolution of equally fit types	Different genotypes result from the same regulatory change and provide the same benefit in the selection environment	125, 353
Niche creation	Cross-feeding between a producer of a fermentation product and an evolved user organism; cannibalism	107, 280, 284

is potentially a rich array of selection pathways available to evolving bacteria. It is tempting to conclude that diversity in bacterial populations arises from the wide pool of selection mechanisms as well as the flexible mutation supply.

CONCLUSIONS ON CULTURE HISTORY AS A DETERMINANT OF ADAPTIVE POSSIBILITIES

A common theme within the many experimental examples described in Importance of Culture History in Determining the Nature of Adaptive Responses above is that culture history changes the responses and heterogeneity of bacterial populations. The predictability of adaptive outcomes with a single environmental transition is hence very much dependent on the baseline population history, for both regulatory and mutational responses. The prior growth rate, growth phase, population density, population structure, prior stress exposure, and all the environmental conditions in the history of the organism (e.g., O₂ exposure) determine the possibilities for further adaptation. The behavior of bacteria upon transition to a new environment, such as resistance characteristics and capacity to use new resources, is certainly dependent on the baseline set by the culture history.

Most existing results on environmental effects come from exposing bacteria growing exponentially in rich medium to a stress. These experiments use a more or less identical baseline for adaptation. We know very little about what happens to bacteria that are not optimally growing when further stressed or transferred to a better medium, except for early upshift experiments on composition (196). Indeed, to fully understand the adaptomics of bacteria, a whole new series of experiments is required, where adaptation

starts not from exponentially growing, unstressed bacteria with excess nutrients but with the many nonoptimal situations and the heterogeneous populations that we discussed above and which are more likely to occur in nature.

The influence of history and many kinds of stresses on mutational processes means that the extent of genetic variation and hence paths to fitness can also differ in stressed and unstressed baseline populations. As shown in Table 2, it is already possible to identify many links between environmental sensing and the mutational processes we have dealt with, and it would not be surprising if more links emerge. Altogether, the cross-interactions between regulation and mutation supply make the adaptomics of populations and microevolutionary outcomes extremely complex and sensitive to small changes in the environment.

CONCLUSIONS ON THE ADAPTOMIC INGREDIENTS OF EVOLUTIONARY SUCCESS IN BACTERIA

Undoubtedly, microbes in general and bacteria in particular are extremely successful in filling the niches available in the biosphere. Here we consider the importance of the adaptive mechanisms that we have discussed to the global success of bacteria.

Dykhuizen (86) argued that a contributing factor to the evolutionary survival of bacteria is an ability to avoid mass extinctions over geological time, owing to their capacity to withstand rapid changes in environmental conditions. In turn, such survival in fluctuating environments requires highly developed adaptive capabilities. As discussed in “Phenotypic Variation within Populations” above, free-living bacteria indeed have a wide range of environmental sensing and regulatory response mechanisms. In

TABLE 2 Cross-interactions between phenotypic and mutational effects in populations

Mutational process or effect	Example(s)	Reference(s)
SIM	DNA damage inducing error-prone polymerases; starvation/stress-mediated increase in RpoS inducing error-prone polymerase	188, 267, 311
Phase variation	Environmental regulation of switching rates	18
GDA	Stress-induced amplification	181
Competence/LGT	DNA uptake regulated by starvation and other stresses	345
Transient mutation	Mistranslation of DNA repair proteins and stochastic variations	218
Buffering phenotypes of mutations	Mutational effects altered by chaperones	202, 329

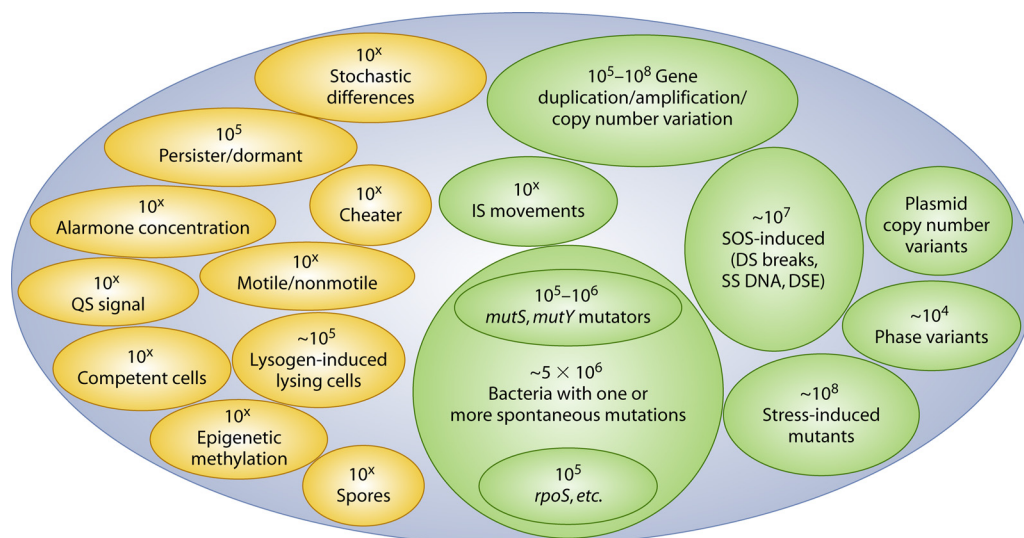


FIG 6 The background shading represents 10^9 bacteria, and the inner shapes represent subpopulations with different phenotypic (yellow) or genetic (green) characteristics. Included are characteristics from several species, so not all bacterial populations will have all subpopulations and the proportions are history dependent, as discussed in the text. In mildly stressed cells (e.g., at the onset of starvation or with mild DNA damage), the numbers in the subpopulations are from the closest published values but are indicative rather than absolute; these too are mostly history and genotype dependent. Where these numbers are extremely variable or unknown, 10^x is used.

addition to this, we have seen in “Genetic Heterogeneity within Populations” above that mutational variation and selection are well developed in populations of bacteria, which ensures that the confined limits imposed by the blueprint of regulatory responses do not limit bacterial survival. The capacities for adaptation defined above indeed allow evolution of microorganisms to occur rapidly, particularly under strong selective pressures. With mutations that can sweep large populations within days (97), there is a convergence of ecological and evolutionary time scales. In this section, we identify various adaptomic principles underlying the contribution of regulatory and heritable adaptations leading to fitness.

Population Heterogeneity as an Ingredient of Evolutionary Success

A number of characteristics of bacterial populations provide sources of adaptability underpinning the long-term evolutionary success defined by Dykhuizen (86). An essential component of bacterial success is diversity itself. Biodiversity in an ecological sense has long been seen as a stabilizing force and may represent a form of biological insurance to more common environmental responses (229). The *Bacteria* and *Archaea* are the most genetically diverse superkingdoms of life (103, 247). Bacteria exhibit not only species diversity but also huge intraspecies variation. In addition to this, populations of individual strains exhibit phenotypic heterogeneity and diversification strategies, as discussed above. Even in genetically similar populations, a large fraction of bacteria exhibit states that differ from that of the majority. The subpopulations may have lower fitness in the extant conditions, even extending to stasis, but may have an advantage upon transition into a new environment. Evolutionary bet-hedging involves a trade-off between the mean and variance of fitness, such that phenotypes with reduced mean fitness may be at a selective advantage under certain conditions (71). Diversity and bet-hedging at every level,

from populations to species to superkingdoms, are inherent ingredients to bacterial success.

Whether population heterogeneities contribute to bacterial fitness or not, the experimental evidence for the contribution of heterogeneity to phenotypes is overwhelming; for example, death curves when bacteria are challenged by stresses or antibiotics indicate nonuniform responses. Generally, these do not follow the kinetics expected from homogeneous populations (31). The heterogeneity and presence of more resistant bacteria in populations affect the shape of killing curves or even the growth pattern under stress, as well as phenomena such as persistence with antibiotics. Another example is with subpopulation dynamics at superoptimal temperatures; irregular growth curves were explained by postulating the coexistence of two subpopulations: a more resistant, growing population and a temperature-sensitive, inactivating population (340). Also importantly, the proportion of the more resistant forms is itself a function of medium composition and culture history (31), so the course of adaptation shown in Fig. 3 to 5 is generally not likely to be a uniform response with different culture histories.

In this review, we have focused on the adaptomics of pure clonal populations. To underline the variation even in these individual populations, it is worth bringing together all the heterogeneities operating even at this taxonomic level of bacterial population biology. In Fig. 6, we attempt to collate all the subpopulations we have discussed. We also include, where available, the proportions of populations likely to exist in the subpopulations. We use a hypothetical, composite organism to emphasize the range of variations. Figure 6 undoubtedly contains inaccuracies, but the likelihood is that we underestimate the extent because of yet-undiscovered subpopulations. Also, we ignore subsubgroups with properties due to overlaps in groupings (e.g., cells with both double-strand breaks and GDA) that would also be possible in large populations.

Based on Fig. 6 and the heterogeneities described above in “Phenotypic Variation within Populations” and “Genetic Heterogeneity within Populations,” a conclusion of this review has to be that adaptation upon an environmental transition can be considered a whole-population change only as a gross simplification. Indeed, an important message from this compilation is that the subpopulations in any large bacterial population need to be considered in adaptomics or other areas of microbiology where population behavior is important. Even if all of the transition rates and frequencies of various states could be measured precisely, it is likely that in adaptomics the best one can hope for is a probabilistic understanding of what will occur in any given transition. For high-frequency transitions (such as sporulation, persisters, etc.), the probability of transitions is likely to be predictable, but for rarer events, such as mutations, a Luria-Delbrück jackpot distribution is more likely. Hence, the results of rare outcomes will have a greater variance and influence how a mixed population like that shown in Fig. 6 behaves.

It is likely that we have not covered all sources of heterogeneity in bacterial populations or indeed the important question whether all the heterogeneity is adaptive. For example, some heterogeneity may be linearly derived in daughter cells from parental cells with differing regulator concentrations or mistranslation errors. We have generally assumed that the heterogeneity is adaptively useful, although the detailed evidence for this is incomplete. A case in point is the mistranslation heterogeneity in cells, which may be nonadaptive accidents of errors in cellular processes. A future need is to obtain evidence for or against the adaptive nature of heterogeneities at every level.

Population Evolvability as an Ingredient of Evolutionary Success

Another identifiable component of bacterial success is evolvability, the capacity of bacteria to change mutational and selectional processes under stress (112, 301). The availability of mutations in populations is far from fixed, and the processes leading to altered mutation rates are increasingly understood. In several examples discussed above, the settings of regulatory processes, determined by the environment, inform and direct the cell toward mutational changes. The theme of diversity or population heterogeneity also impacts evolvability. Mutator subpopulations can represent several percent of many pathogen populations and are a source of beneficial mutations. In addition, some explanations of elevated mutation rates in nonmutator bacteria depend on SIM subpopulations and limiting mutations in space and time (112). The high availability of genome amplifications, both in gene dosage effects and perhaps in generating “adaptive” mutations (262), is another ingredient in generating genetic variation. In any case, mutation supply is not constant across a population that has constitutive mutators, cells with elevated error-prone polymerases, and genome-amplified subpopulations on top of a bulk population with “normal” spontaneous mutation rates. Evolvability can hence vary with the proportion of genome-altered subpopulations. Evolution involves a balance between bacterial integrity maintained by minimizing deleterious mutations under healthy vegetative conditions and switching to greater variation in times of stress.

An essential link in evolvability is the ability to translate regulatory responses sensitive to the environment to the generation of genetic heterogeneity. Mutation supply becomes significant especially in stressed situations when beneficial mutations are most

useful. A clear pattern of regulatory and mutational adaptations being linked emerges. Genetic heterogeneity arising via genome duplications or error-prone DNA replication, as well as the other mechanisms mentioned in “Genetic Heterogeneity within Populations” above, is linked to stress responses in partly defined ways. It really does not matter for bacteria whether “adaptive” mutations during starvation occur via duplications or SOS- plus RpoS-mediated stress responses, but it is environmental inputs that stimulate genetic variation in both.

Metabolic and Regulatory Redundancy as an Ingredient of Evolutionary Success

A considerable evolutionary advantage of adapting bacteria is the degeneracy in function and robustness that bacteria encode (352). It is common for bacteria to have more than one protein with the capacity to influence the same metabolic step or the regulation of the same genes. This degeneracy aids adaptation because the capacity to lose or change by mutation some component of function or regulation does not kill the cell. Redundancy and robustness built into bacteria not only help survival in the presence of deleterious mutations (183, 351, 354) but could actually increase the number of loci for beneficial, adaptive changes (175, 353). In this respect, degeneracy contributes to the selectability of diversity that can evolve during adaptation, as discussed in “Genetic Heterogeneity within Populations” above.

In multicomponent regulatory networks or signal transduction pathways, degeneracy allows mutations in different genes to result in the same phenotype. A good example of this is within the general stress response controlled by RpoS (17) and the cost that this imposes on the SPaNC balance (95). Recent evidence of degenerate mechanisms to obtain mutational adaptive shifts in the SPaNC balance and RpoS levels comes from population changes in adaptation to nutrient limitation in the laboratory (353) or in pathogen populations adapting to an extraintestinal environment (177). A similar phenotype in terms of SPaNC balance can come from mutations in *rpoS* itself, *hfq*, *spoT*, and *rssB* (198a, 353) and others that indirectly influence RpoS levels (177). The mutational degeneracy of changing RpoS levels reinforces the huge diversity in RpoS across the species *E. coli* (51, 98). The similar effect on SPaNC is accompanied by very different phenotypic changes with, for example, *hfq* and *spoT* mutations (353). These secondary differences provide the ingredients for phenotypic diversification seen in populations and across the species.

CONCLUSIONS ON THE PREDICTABILITY OF ADAPTATION: FROM SINGLE TRANSITIONS TO EVOLUTIONARY OUTCOMES

Evolution is deceptively simple in Darwinian terms, consisting of fitness based on genetic variation and selection. For example, these ingredients can be analyzed and modeled by simplifying reality, by assuming particular mutation rates and fixed selection coefficients that allow a description of the fixation of fitter organisms (132). In some cases, adjusting for biological variation by assuming distributions of character values, as done in population biology, is undertaken (245). However, this is also mistaken, as the heterogeneities described in this review are generally not a continuum but often occur with distinctly different patterns (as in bimodal on-off stochastic variation, persister growth/nongrowth, or stress-induced/basal mutation rates differing by orders of magnitude) and with different effects on parts of the chromosome or sets of genes. This consideration of population heterogeneity re-

inforces the problem of “frail hypotheses” in population biology identified by Ninio (233). To further quote Ninio: “I suggest that population geneticists should invest more effort in refining the numerical values of the critical parameters used in their models. They should take into account the recent proposals on how mutations arise. They should also pay more attention to phenotypic variations, and develop criteria to discriminate between proposed evolutionary mechanisms that can actually work, and others that cannot.” We fully concur.

To extrapolate single-transition adaptations to long-term evolutionary outcomes is probably premature. To understand how populations evolve over several rounds of mutation and selection, it needs to be realized that population heterogeneities increase with continued growth and continued selection, even in simple environments. Many studies in the past 10 years have used bacteria in experimental evolution, in which an ancestor is subjected to a particular environment(s) for extended periods (64, 88). These may be through continued subculturing in the same medium or through continuous cultures kept over many days. An unstated assumption in studies of evolution, intrinsic to many experimental evolution studies, including our own (110, 200, 353), is that we start with an ancestor population that is genotypically and phenotypically homogeneous. As discussed above, this overlooks the heterogeneities in bacterial populations. Thus, a more realistic description of experiments started with a clonal ancestral population is that at the start of the evolution experiments, we already start with many distinct subpopulations. The problem is that the mutation rate and benefit of mutations may be different in each subpopulation. There may well be several subexperiments going on, with lineages appearing at very early times in adaptation. This interpretation is entirely consistent with the extremely rapid divergence of evolving populations even in the simplest media (200), which may also depend on a multitude of selection mechanisms (Table 1) that provide alternative pathways to fitness.

Future adaptomics studies will need to investigate whether some of the adaptational divergence seen in effectively all experimental evolution studies of bacterial populations has its seeds in ancestral heterogeneity. Additionally, a deeper analysis of the selection steps in a single environmental transition is needed.

Models of growth and organismic function are targets of systems biology (64, 246), and here again, it is essential to question whether population-level behavior is fully covered by existing models. Heterogeneity and growth history features set challenges in metabolic networks even if stochastic features are incorporated. As in adaptation/evolution studies, systems biology involving mutational or environmental perturbations needs to take into account the history and heterogeneity of the populations studied.

As a final thought, we suggest that progress in the adaptomics of populations can contribute to the science of microbiology in further areas beyond systems biology and population biology. First, our grasp of bacterial behavior will expand by using baseline cultures other than exponential batch cultures as the norm in physiological adaptation studies. Second, research on how heterogeneity contributes to infection processes will influence our thinking about the diagnosis and combating of infectious diseases. Third, the era of “pure culture” microbiology is nearing an end, and heterogeneity is going to be integral to our understanding of microbial processes, not just in ecosystems but also in pure clone experiments. Even in the genomics of microbes, a higher accuracy in sequencing technology and more careful analysis will undoubt-

edly reveal the metagenomics of intrapopulation clonal diversity suggested by Fig. 6.

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