The 2010 Garrod Lecture: The dimensions of evolution in antibiotic resistance: *ex unibus plurum* et *ex pluribus unum*

Fernando Baquero*

Department of Microbiology, Ramón y Cajal University Hospital, IRYCIS and CIBERESP, Ctra de Calmenar km 9,100, 28034 Madrid, Spain; Centre for Astrobiology (CSIC/INTA), National Institute for Aerospatial Technology, Ctra de Torrejón a Ajalvir, km 4, 28850 Torrejón de Ardoz, Madrid, Spain

*Corresponding author. Tel: +34-91-3368832; Fax: +34-91-3368809; E-mail: baquero@bitmailer.net

Received 15 April 2011; returned 26 April 2011; revised 29 April 2011; accepted 4 May 2011

Antibiotic resistance is not only the result of antibiotic-driven selection, as has been frequently considered, but rather the consequence of extremely complex evolutionary processes. These processes act on bacterial populations, but also on populations of subcellular (as plasmids) or supracellular (species, communities) units of evolution. The consideration of the effects of drift and selection provides a first intuition about the dimensions shaping the evolutionary field. We distinguish two alternative, orthogonal dimensions, respectively pushing evolutionary units towards diversification (*ex unibus plurum*) or towards unification (*ex pluribus unum*). Evolution in each one of these dimensions requires alternative evolutionary functional configurations in the evolving unit. These configurations are reached under the influence of evolutionary attractors for diversification or unification, presumably with an oscillatory dynamics. This view illustrates the complexity of possible outcomes in the emergence and evolution of antibiotic-resistant units, and the necessity of applying new evolutionary synthesis approaches to understand and predict human-driven changes in the microbial world.

**Keywords**: drift, selection, diversification, unification

**Introduction**

The major aim of my Garrod Lecture and this accompanying article is to discuss the dimensions used by evolutionary processes involved in antibiotic resistance. The term ‘dimensions’ is used here in the acceptance of coordinates specifying points in a theoretical space in which biological objects are able to change positions; i.e. they are able to evolve. The general mechanisms by which evolving objects (individuals inside populations) move in the evolutionary space are essentially natural selection and drift. These so-called ‘mechanisms’ have also been considered ‘forces’, but I agree with Christopher Stephens that they are not real mechanisms or forces acting on populations, but only ‘pseudo-processes’, statistical by-products of the sequential populations resulting from sequences of events, essentially birth and death rates. The application to collective biological objects of selection and drift can shape evolutionary trajectories within a dimensional space. The central argument of this work is to present here what we can consider as the two main dimensional evolutionary axes in which the biological objects move: along one axis, these objects move towards diversification (*ex unibus plurum*); along the other, towards unification (*ex pluribus unum*), using, as in a recent work, the Saint Augustine (Confessions, IV, 8–13) classical expression. Even if we accept the generality of this view in the living world, evolutionary microbiologists are in a much better position than other scientists to appreciate these fundamental movements. Many microbial and submicrobial objects retain an evolutionary plasticity that has been reduced in more complex forms of life. Once more, we should remark that microbial antibiotic resistance has provided one of the best possible frames to investigate fundamental mechanisms in evolution. By learning about evolution, we have learnt about antibiotic resistance, and by studying antibiotic resistance we have easily slipped into the basic themes of evolution.

The intention of this review was also to honour my esteemed predecessor, Professor Richard Sykes, who last year devoted the Garrod Lecture to ‘The evolution of antimicrobial resistance: a Darwinian perspective’. To a certain extent, this lecture can be considered as a second part of his lecture and therefore an extension of his aim in recognizing the importance of Charles Darwin on the 200th anniversary of his birth, and the 150th anniversary of his seminal book *On the Origin of Species*. The Sykes’ review was more focused on the specific mechanisms involved in the evolution of antibiotic resistance, whereas the present one is more directed to discussing the theoretical dimensions
in which evolutionary trajectories are tracing their complex paths, as a necessary exercise for dealing in the future with the prediction of antibiotic resistance. Both reviews are in line with the feeling that a new evolutionary synthesis is in sight.

What is and what is not natural selection?

Natural selection is Darwinian selection. According to Lewontin, the principles that embody the principle of evolution by natural selection are the following: (i) if there are variant entities in a population; (ii) if these variants experience different reproductive success; and (iii) if the variation is inheritable; then, (iv) the composition of the population will change over time. This can clearly be applied to the case of antibiotic resistance: (i) if there are resistant variants in a population; (ii) if differential reproductive success is experienced in the presence of antibiotics; and (iii) if the variation is inheritable; then, (iv) the composition of the population will change over time in favour of resistant variants.

However, not every change in the composition of a population is necessarily due to natural selection. Of course, natural selection assures the survival and further reproduction of the fittest (Darwinian selection), but there are other possibilities assuring the survival and further reproduction of a group of individuals endowed with specific mechanisms of adaptation to the challenge when others in the same population become extinct.

Survival of the luckiest

This term refers to individuals that overcome a challenging event just by chance, as they are randomly located in a physiological status (such as growth phase) or in a place where the deleterious agent or condition is not lethal. Let us imagine that some of these lucky survivors carry a particular inheritable genetic difference devoid of any adaptive value, e.g. a synonymous substitution in a triplet encoding a particular amino acid. This difference will be amplified in the reconstructed population in comparison with the ancestral one, even though it could be totally unrelated to the deleterious event. Reducing the overall number of cells, antibiotics reduce the diversity of the population, and, therefore, some of the variants that survive just by chance and reproduce after crossing the bottleneck (an event decreasing population size) might appear to have been ‘selected’; however, they are not the fittest to resist the antibiotic, just the luckiest in evading its action. For instance, it is tempting to illustrate the case by mentioning phenotypically antibiotic-tolerant cells that occur transiently and randomly in a bacterial population (as a result of a mere asynchrony in the growth cycle), and which can survive purely by chance.

It should be remembered that the notion of ‘luck’ (either for microbes or humans) refers to something that implies the total equality of chances for all individuals. In the natural world, such deep homogeneity (assuring random sampling) is rare, so that the notion of ‘survival of the luckiest’ is biased according to some circumstantial features. Three of these are discussed here.

Survival of the better placed

‘Survival of the better placed’ refers to individuals that overcome a challenging deleterious event because they are endowed with some characters facilitating their location in particular compartments where the challenge is minimized. For instance, intracellular bacteria might ‘resist’ a number of antibiotics, without any need for special antibiotic resistance mechanisms. The same is probably true for necrotizing bacteria, for organisms adhered to non-vascularized surfaces (e.g. catheters) or for those forming thick biofilms in altered mucosal surfaces, such as in cystic fibrosis. The possible cross-talk between virulence and antibiotic resistance has been reviewed elsewhere.

In some other cases, bacteria might overcome the antibiotic effect just because, by chance, they are positioned in the vicinity of other bacteria that are able to protect them from antibiotic action (e.g. by excreting detoxifying enzymes). These otherwise susceptible organisms are able to survive without natural selection, acting as ‘cheats’ of the protective ‘altruist’ population.

Survival of the best connected

High connectivity opens the door to the fertile adaptive field of horizontal gene transfer. If a bacterial organism belongs to a community of bacteria forming a preferential ‘genetic exchange community’, its possibilities for further adaptation are higher. Probably, just belonging (being connected) to a complex community should increase the survival possibilities when facing difficult times, as complexity tends to favour global robustness.

Survival of the denser

High-organism density is one of the preferred strategies of Nature to assure the non-specific survival of her children. In reality, an important part of the bacterial struggle for life is devoted to obtaining resources for multiplication, as an investment for maintenance during time. To a certain extent, survival of the denser is frequently a pre-condition for assuring survival of the luckiest or survival of the best placed. For instance, high cell density guarantees stochastic access to a protected condition. Most importantly in the case of antibiotic resistance, high density assures availability to random mutations or random gene duplications eventually able to adapt the cell to external challenges, and also to increase high connectivity with other organisms.

Note that in all of these cases the genetic composition of a bacterial population might change over time, because a part of it is spared from a deleterious event; however, the survival of this fraction is not due to the acquisition of any specific adaptive trait to counteract the challenge. In summary, we could differentiate between a situation in which all individuals in the population have equal probability to survive (survival of the luckiest) and others in which some of the individuals have, by reasons unrelated with the change, a higher probability of survival (such as survival of the better placed). The first case fits with the canonical concept of evolution by drift; in the second, there is also drift, but it may be mixed with a certain bit of evolution by selection, this will be discussed later.

Drift and effective population size

A population is changing by drift when there is a change in the frequency of a genetic variant in the population due to random sampling. Sampling means that a fraction of identical individuals
in a population is randomly isolated from the rest, as illustrated by a handful of balls extracted from an urn. Let us imagine that there are only 2 black balls among 200 white balls in the urn, but just by chance 1 black ball is part of a handful of 3 balls extracted from the urn. So, the relative frequency of a hypothetical genotype associated with black balls rises from 0.01 to 0.33. If only two balls are extracted and one is black, the frequency would be even higher, 0.5. But, if a large number of balls could be extracted, let us say 100, and among them was 1 black ball, the proportion would again be 0.01; in this case, the proportion in the sample will be identical to that in the whole urn. The number of balls in the sample required for having a proportion identical to that in the entire urn illustrates the important concept of ‘effective population size’ (Ne). Ne is the expected minimal size of an ideal subpopulation that consistently reflects the diversity of the whole population. Consequently, sampling of populations below Ne are at risk of offering genetic variability that deviates, just through chance, from that of the original population; in fact, the smaller the size of the sample (3 balls instead of 100 out of the urn), the higher the deviation in frequency is expected to be. Of course, drift might reduce bacterial variation; if the number of balls extracted from the urn does not contain any of the rare (black) genotype, the new population will be constituted entirely of white balls. A minority of antibiotic-resistant bacteria might increase in proportion or disappear just by drift. In the first edition of On the Origin of Species, Charles Darwin was already aware of the possibility of fluctuations in the frequency of variations that have no adaptive significance or are otherwise equally fit (and therefore not submitted to natural selection).17

This means that any ‘sampling’ condition resulting in a reduction in the number of cells below the Ne might produce changes in the proportion of a particular variant in a bacterial population that is unrelated with selection, i.e. ‘neutral’. Effective population sizes in bacteria range from Ne ¼ 105 to 109.18 In fact, probably many of the changes in the frequency of particular genetic (and genomic) changes in bacterial genes, including a number of changes that might result in antibiotic resistance, might not be the result of presumed selective events related with antibiotic consumption, but just occur by drift. Note that, most probably, the effective population size for most bacterial populations is very high, in the order of Ne ¼ 108 in Escherichia coli.19 Many circumstances in the microbial lifestyle, including host-to-host transmission bottlenecks, will produce sampling with population sizes below Ne.

Host-to-host bacterial transmission in the community, in hospitals or in infective processes typically starts with a low number of bacterial cells and, therefore, small samples are expected to produce a high degree of drift-based evolution. Drift, indeed, should be proportional to the number of these bottlenecks. The genetic variability of a highly transmissible epidemic clone whose cells are able to surpass many independent bottlenecks should be higher than that of a well-established strain maintained in a stable environment. Similarly, the dilution or migration of a bacterial population in an environment (e.g. sewage soil or water) certainly contributes to its ‘sampling’ in small subpopulations naturally submitted to heavy drift. A sample population of ‘migrants’ might establish a colony far from the site of origin, with a genetic composition eventually biased with respect to that of the ancestral one. For instance, resistance might be rare in the original population and frequent in the derived colony for reasons unrelated to antibiotic selection. This is a ‘founder effect’, as this population might give rise to new secondary populations that might follow the diversification process, but will retain the ‘mark of origin’ of the founder. Coalescent theory has been applied to model the amount of variation expected from such drift-derived effects on populations connected by migration.21,22

Lastly, the use of biocides and antimicrobials also reduces (‘sample’) bacterial populations, which again should increase drift. Persisters cells with phenotypic resistance, able to tolerate exposure to antimicrobials and to reassume growth in their absence, apparently occur randomly in bacterial populations. As previously mentioned, the frequency of genetic traits associated with these persisters will eventually increase locally just by drift. As we will see later, transmission and migration bottlenecks also occur for suborganismal evolutionary objects, such as plasmids, phages or conjugative transposons. Of course, that does not necessarily mean a cumulative and progressive genetic differentiation from the ancestor population, as drift is not expected to result in a directional populational movement, but, most frequently, a kind of random ‘Brownian motion’ in the evolutionary space, not free from extinction events.

The fuzzy line between drift and selection

Even though evolution by drift and evolution by natural selection are frequently considered as alternative forces in evolutionary processes, there are spaces in which both forces are sequentially or coincidently acting on evolutionary objects. A number of steps can be recognized paving the path from drift to selection.

First, we can expect that a single nucleotide variation giving rise to a synonymous codon should be effectively neutral, without any consequence in the protein structure. Therefore, even if this variant could be enriched by drift, nothing will occur in terms of selective adaptation. Second, the nucleotide variation might produce an amino acid change influencing a protein domain but without phenotypic consequences, and so will not be subjected to natural selection. Note that the absence of expected phenotypic consequences (such as an increase in β-lactam MIC) might not necessarily be interpreted by itself as neutrality. For instance, while the change in β-lactamase conformation might not influence hydrolytic efficiency, it might affect the stability of the protein and would therefore comprise a selectable change.23 Third, the nucleotide change might result in a protein change with all appearances of neutrality (i.e. without any functional consequence), but which might influence the effect of other mutations that might occur later, either increasing or reducing the possibility of natural selection (positive or negative sign epistasis).24–26 Fourth, the variant nucleotide might influence the phenotype, but in an extremely subtle way, so that the phenotype might be overlooked by natural selection. This concept was posed (for β-lactamases) as ‘we do not know how small an effect constitutes a selective advantage’.27 It has, however, been shown that very small phenotypic differences are indeed selectable along natural gradients.28,29 In general, the neutrality of the variation in a particular site can be detected by the calculation of the ratio of non-synonymous substitutions (dn) to the number of
synonymous substitutions per site (dS), \((\omega=\text{dN/dS})\). When this calculation gives values of \(\omega=1\), the message is that changes with amino acid replacement are neither selected (positive selection) nor negatively selected (purifying selection), and represent neutral evolution. Indeed, most statistical methods are based on single-locus calculations, disregarding gene–gene interactions in the emergence of phenotypes, a problem that should be urgently addressed.\(^{40}\)

Neutral variation might also occur because of the effect of phenotypic capacitors. Capacitors are proteins involved in cellular networks allowing genetic variation to accumulate in a silent (neutral) state, until it is revealed by environmental stress.\(^{31–33}\) Candidate proteins for effectors of evolutionary capacitance are regulatory genes, networks of chaperones and, in general, proteins with high connectivity with other proteins. It is of note that in several of the above-mentioned cases there is a drift-based unselectable variation that becomes selectable upon a particular event. In these cases, drift evolution is to a certain extent a pre-condition for evolution by natural selection. In a sense, drift can be considered as pre-adaptive. This raises the question as to why rare neutral or pre-adaptive random variations do not disappear in bacterial populations. This can be explained by applying the concept of mutation–selection balance as it was postulated in the 1920s by Fisher and Haldane. Because of the huge number of bacterial cells in most bacterial populations, even very unsuccessful genetic variants are expected to persist for enough time (generations) to reach an area in the fitness landscape in which they could provide a new advantage (e.g. a selective antibiotic concentration), resulting in a new increase in frequency.\(^{34}\)

A central problem in applying the theoretical definition of drift to bacterial evolution is the basic heterogeneity of bacterial populations. In the ‘phenotypically identical balls in an urn’ naïve model (see above), one ball cannot be differentiated from another as they are homogeneously distributed in the urn, resulting in real random sampling. In living bacterial populations, under normal asynchronic growth, every cell has a particular metabolic stage at different phases of the cell cycle; subsets of cells are phenotypically different, gene expression might differ stochastically among cells, mobility might randomly change a cell’s place in the urn and they are submitted to random mutational events, eventually leading to pre-adaptive neutral changes. The problem is that all these random processes are not ‘randomly distributed’ in the natural ‘urns’, where bacterial organisms are at variable distances from nutrients, oxygen or colonizable surfaces (e.g. in the periphery or the core of a biofilm), or are more or less exposed to host immune responses or the effects of antimicrobial drugs. For instance, local stress might result in local increases in the stochasticity (noise) of gene expression.\(^{35–37}\) Different samples might thus contain different types of cells carrying pre-adaptive changes and, therefore, their possibilities of surviving particular challenges might differ; in other words, drift might be biased by selection and vice versa. The relevance of local effects (landscape ecology) on the bacterial evolution of antibiotic resistance has been reviewed elsewhere.\(^{37}\)

The conclusion from the above is that evolutionary trajectories in bacterial organisms, including antibiotic resistance, might be frequently shaped by drift, selection, and the interaction of drift and selection. According to the ‘nearly neutral theory’ of Tomoko Otha,\(^{38,39}\) ‘slightly advantageous’, ‘slightly deleterious’ and ‘in-between advantageous and deleterious’ changes are really important, or at least as important as ‘strongly selectable’ and ‘really neutral’ changes.\(^{40}\) The intensity of changes due to natural selection will produce increases in the standard deviation (\(\sigma\)) of the distribution of values in the population and the intensity of the drift will be inversely proportional to the population size, \(1/N\), so that in large populations genetic drift is a weaker force than selection. In conditions of \(\sigma \leq 1/N\), both drift and selection are playing a role in evolutionary trajectories.

**What is an evolutionary individual?**

Drift and selection, and the interplay of both evolutionary forces, act on individuals that are members of populations. If the individuals are replicators subjected to inheritable variation, and if some of these variants should experience different reproductive success, the composition of the population will evolve over time by natural selection. It is not immediately clear whether the variability of the population will evolve over time by drift. Individuals can therefore be considered as evolutionary units. The critical question now is what is an individual, if ‘lacking individuality, it cannot be a unit of evolution’.\(^{41}\) Interestingly, Darwinian thought has used the concept of ‘selection’ to identify what an evolutionary individual is (expressed in the following syllogism):

M. Only individuals can be units of evolution
m. A unit of evolution is a unit of selection
C. Individuals are the units of selection

**Units and levels of selection in antibiotic resistance**

The units of selection define the evolutionary individuals, but in the case of antibiotic resistance, what is selected? Possible units of selection in antibiotic resistance are discrete genetic sequences, genes, operons, functional genetic modules, mobile genetic elements such as integrons, integrative conjugative elements, transposons, plasmids, or genomes, cells (organisms), clones, clonal complexes, species, communities, and ecosystems. Note that all these possible units belong to different hierarchical levels, ranging from the relatively simple to the complex: resistance genes are part of integrons, the integrons part of transposons, the transposons part of plasmids, plasmids part of cells, cells part of clones, clones part of species, etc. Each unit is a ‘vessel’ for the other, affecting its potential dissemination and also its rate of evolution.\(^{42}\) But, are we right in considering each of these ‘particles’ as a potentially selectable unit? At first sight, selection for higher-level units should force the selection of lower-level units contained in the higher-level particle, but might the opposite also occur and is there room for ‘independent selection’ of the different units? This question about the selection of the different evolutionary individuals is at the root of the debate on the levels of selection and the multilevel selection
Antibiotic resistance is embedded in individual complex systems

We should admit that antibiotic resistance is embedded in complex systems, which are composed of many components, and that they themselves are components in larger systems and networks. The evolution of resistance is therefore the evolution of a complex system. The complexity is due not only to the number of components involved in antibiotic resistance, but to the heterogeneity (self-dissimilarity) of such components, and their hierarchical organization in highly structured networks and spreading at multiple rates. An important part of the difficulties in fighting against antibiotic resistance depends on the robustness that such a complex structure provides to bacterial biological systems. We can try to eliminate (e.g. with a novel antibiotic, a vaccine or an inhibitor of plasmid replication) parts of the system, but we are fighting against a highly robust system.

Robustness is the maintenance of the system characteristics, antibiotic resistance in our case, despite fluctuations in the frequency or behaviour of its component parts that might result from these interventions. In the bacterial world, high robustness at the clonal or species level is based on the extreme redundancy resulting from the huge numbers of individuals. At the level of a single isolated individual cell there is of course complexity and robustness, but still robustness can be overcome by the disruption of cellular processes by unexpected events (such as exposure to a new antibiotic). Similarly, at the other side of the hierarchy, in bacterial communities and possibly in ecosystems, again the number of ‘individuals’ is small, as a community can be considered as a ‘unit’ or ‘collective individual’ and robustness could be overcome by unexpected events affecting all levels (as in so-called ‘forest-fire models’). In these cases, individuals in low numbers under isolation have a real ‘robust, yet fragile’ behaviour. In contrast, if the ‘unit’ is composed of a very high number of component individuals with high connectivity between them (e.g. because of horizontal gene transfer), robustness, tolerance of change, will be high. Inside the conceptual framework of the complexity studies, this behaviour will enter into the class of systems with ‘highly optimized tolerance’. The term ‘highly optimized’ means that the components (pieces) of the system are linked by unique, highly structured, and in a sense, rare patterns that are evolutionary products.

Risk of being simple, risk of being complex

From the preceding paragraph we can conclude that complexity provides stability, inertia to change and robustness to a particular system when confronting changes, particularly predictable (experienced) changes. On the other hand, the internal complexity needed for robustness also constitutes an Achilles’ heel increasing fragility, particularly when confronted with non-predictable changes. Of course, the natural history of most biological entities is the result of exposures to both expected and non-expected changes, acting consecutively or even coincidentally. It is proposed that evolutionary units optimize the risks of such situations by using (or taking advantage of) two evolutionary dimensions, referred to here as the ‘ex unibus plurum’ and the ‘ex pluribus unum’ dimensions.

Two alternative and orthogonal evolutionary dimensions

The ‘ex unibus plurum’ (from one, many) dimension refers to the natural events resulting in the diversification of evolutionary units. The ‘ex pluribus unum’ (from many, one) is its alternative orthogonal (opposite) dimension, leading to the unification of evolutionary units.

The important concept behind this is that both dimensions provide alternative fields of action for the development of different (opposite) evolutionary configurations. Here, we are using the notion ‘field’ and not ‘force’, as the term ‘force’ could represent...
for the reader a process guided by external causes.2 A diversifying field of action results in collections of evolutionary configurations, which are, in fact, highly related manifestations occurring in a unique diversifying field. The opposite configurations occur in the unifying field of action. A number of these alternative evolutionary configurations are listed below. The first term refers to the diversifying and the second to the unifying fields of action. Note that all first terms are highly related and the same applies to the second terms. In fact, we can consider them as perceptible manifestations or topologies of a unique basic evolutionary configuration, which we are differentiating here for heuristic purposes.

(i) Variation versus conservatism. At particular spatio–temporal frames, evolutionary units could either give rise to variants (and these variants to other variants in turn) or, in contrast, they could be stagnant, stably maintaining the previous configuration. For instance, at particular stages, clones, plasmids or transposons could be extremely stable or variable. Variation or stability might result from the relative frequencies of genotypes. Note that for population biologists, conservatism might be understood as preservation under selection of the original polymorphic structure of a population, a stable status of variability, i.e. coexistence of variants, resulting from stabilizing frequency-dependent selection. In contrast, the break of this polymorphic structure results from disruptive frequency-dependent selection.54

(ii) Simplicity versus complexity. Complexity is favoured by stability and exploitation configurations, and vice versa; analogously, simplicity favours exploration and variability. A stable, long-term maintenance of coexistence among evolutionary units increases the connectivity and hence the complexity of the system. For instance, the complexity of the intestinal microbiota favours the spread of resistance genes among genetic exchange communities (see later), assuring the stability of the whole system. In contrast, organisms not integrated (connected) in these networks might be better suited for interhost spread of resistance. In general, the efficiency of acquisition of a unit in a superior level of the hierarchy depends both on the connectivity and the functional compatibility of such a unit with the members already integrated in the higher level. These concepts have been developed in the so-called complexity hypothesis.12,53

(iii) Evolvability versus robustness. Highly variant, simple configurations favour evolvability, whereas more complex, stable configurations tend to perpetuate themselves by increasing their inertia (robustness) when confronted with an environmental change, instead of changing the basic conformation (increasing evolvability).55 This does not mean that complex systems are not subject to evolution, but in general that only occurs to further increase its complexity and robustness, and not to create alternative configurations.

(iv) The fittest versus the flattest. A high-fitness configuration promotes a high replication rate in a particular environment. Nevertheless, such exquisite adaptation might be fragile if the environment changes or the evolutionary unit is altered by new variation. High mutation rates could be extremely beneficial to climb an adaptive hill, but detrimental when the population stays on the top of the hill. In contrast, populations colonizing flatter areas in the fitness landscape reproduce less efficiently, but are more robust to changes and increase their resilience; i.e. they are able to recover from deleterious variation.55

(v) Exploration versus exploitation. Entering the field of exploration, an evolutionary unit might acquire a configuration that is well suited to explore the accessible environment in search of novel niches in which higher fitness is achievable. In contrast, there is an alternative configuration able to optimally exploit a niche in which the evolutionary unit is installed. ‘Explorer’ configurations are good for probing; ‘exploiter’ configurations for leveraging, i.e. obtaining the highest profit from the resource. Variability is favoured by exploration and vice versa. Of course, we can recognize this familiar distinction at all levels of the biological and sociological scales.56,57 For instance, in antibiotic resistance, at particular stages, some resistant clones have high epidemiogenicity, whereas others are well established in particular niches or populations.

(vi) Niche deconstruction versus niche construction. Niche construction refers to the effect of biological units modifying their environments, and consequently modifying local sources of variation and selection.58,59 In reality, this definition implies both niche construction and niche deconstruction. Niche construction could be understood as the possibility of modifying a niche in such a way that it adapts perfectly to the genetic possibilities of an invariant host, resulting in optimal exploitation of the environment. An example (related with altruism—see later) is the creation of environments ‘protected’ against antibiotics by the release of detoxifying substances.60 Niche deconstruction is the conversion of a niche in multiple niches, favouring the emergence of genetic variation in the host, as many niches might host a multiplicity of variants.

(vii) r strategy versus K strategy. All biological units are configured in different configurations that classify them as acting as K strategists or r strategists. The origin of such evolutionary distinctions is rooted in ecological and biogeographical research.61 K strategy refers to the carrying capacity (the maximal number of individuals who can be stably maintained in a particular habitat) and maintenance, implying the absence of degradation of such an environment. K strategists invest energy in ‘quality’, specialized colonization and optimal habitat exploitation, i.e. in efficiency. In contrast, r strategy refers to the maximal intrinsic rate of increase of a population, expressing the individual contribution to population growth. r strategists invest their energy in ‘quantity’, i.e. in productivity.52 r strategists are ‘explorers’, forced to spread, as obviously r might change with population size, such as in the case of density-related effects involving, e.g. exhaustion of resources in the environment.

(viii) Selfishness versus altruism. Altruism tends to preserve unification. A number of examples of altruistic traits can be found in antibiotic resistance. For instance, a limited number of cells might harbour traits that decrease their own fitness but favour the fitness of the surrounding cells, e.g. by releasing detoxifying enzymes.51,63 killing
themselves to release allelopathic agents or by harbouring conjugal plasmids. Altruism enhances the overall population to overcome stressful environments and, most importantly, tends to preserve the original structure of the population. Selfishness, assuring that everything needed for survival is available in a single evolutionary unit, promotes the possibility of dissemination, even in low numbers, and so increases potential variability, including drift effects.

Of course, other heuristic ‘pairs’ could be added to complete the intuition about the evolutionary dimensions that I would like to suggest to the reader, such as freedom versus determinism or disorder (entropy) versus order, but I hope that the main message has already been conveyed.

**Contingency and oscillatory dynamics across evolutionary dimensions**

I alluded above to the risk of being simple and the risk of being complex. We should recall here the Law of Evolution proposed by Herbert Spencer 120 years ago to explain the behaviour of all (biological or physical) systems. The idea is that aggregated complex systems are unstable (fragile) in the long run, as they will be unable to maintain the identity and internal relations of their parts in the face of external perturbations. The question is what might happen with the elements of the disaggregated ex-complex systems.

The notion of cycles of contingency might be applied here. This view is grounded on the developmental systems theory, which considers the ontogeny of a system to be based on contingent cycles of interaction among elements involved in the construction (or deconstruction) of the systems. In my view, contingency should be considered in the context of the logical term. That is to say, the orthogonal configurations considered above may or may not occur, or they might be convenient (true) or deleterious (false) depending on the value imposed by their own structure and the environment in which they are located (valuation). This means that absolute ‘optimal configurations’ are not expected to exist in evolutionary units. There is not a single ‘truth’, but rather optimality is obtained by contingent, transitory configurations oscillating between different optima in one or other evolutionary dimensions, in the path towards diversification (ex unibus plurum) or in the alternative path towards unification (ex pluribus unum). Along all scales of nature, evolutionary entities are organized not in pure fractality (single configurations), but in allometry (alternative configurations).

The concept of cycles of contingency also involves the need for a transition between configurations along the diversification path and the unification path. This probably occurs via complex oscillatory dynamics. A simple approach to this idea, which takes account of pendular dynamics, is that an evolutionary unit reaches a critical level of complexity (unification) in which the advantages of being complex start to be increasingly compromised by increasing disadvantages, and from there starts the path to simplification (diversification). The opposite is also true, where a simple configuration, having reached all accessible advantages of variety and simplicity, might start a complexification (unification) path as the only way to persist or to advance.

Such presumed cyclical behaviour is probably more complicated in nature, for two main reasons. First, for each configuration we do not have a single cycle (unification–diversification–unification–diversification). In most cases, evolution might evolve by hypercycles. Unification steps favour other unification steps, until the critical level that forces diversification is reached; by the same token, diversifying steps might facilitate other diversifying steps, until the need for unification is imposed on the system. Second, most evolutionary units (certainly in the biology of antibiotic resistance) are embedded in each other, as species contain clones, clones contain plasmids, plasmids contain transposons and transposons contain integrons, with each of these units potentially evolving in separate dimensions. For instance, clones could be under diversification from each other, while simultaneously the plasmid could be evolving to better unify with its bacterial host. Coming back to the pendulum metaphor, we should imagine this situation as a double oscillator (a second pendulum attached to the end of the first); if we increase the number of evolutionary units (e.g., transposons or integrons), we have a multioscillator system, in which the movement of each subpendulum influences the movement of the other ones, resulting in the dynamics of the system becoming almost unpredictable (chaotic). Only something like ‘circadian rhythms’ might, to a certain extent, contribute to the periodic harmonization of such complexity.

**The evolutionary attractors for diversification and unification**

Evolutionary attractors are considered here as a finite number of virtual fields in the evolutionary space providing optimal conditions for evolutionary configurations. Such optimization can be conceived of as an attractor for the evolution of evolutionary configurations. As such configurations can be established either in the ‘ex unibus plurum’ or in the ‘ex pluribus unum’ evolutionary dimensions, we can distinguish attractors for diversification and attractors for unification. In both cases, these field attractors act in orthogonal directions and, therefore, as discussed above (‘Two alternative and orthogonal evolutionary dimensions’ section), we have pairs of opposite attractors acting on alternative dimensions, the first being a diversifying attractor and the second a unifying attractor.

**Multiple niche occupancy versus optimal niche exploitation**

Both the occupancy of multiple niches and the optimal exploitation of a particular niche might attract the evolutionary configurations. For instance, diversifying configurations, such as variation, will be attracted by multiple niche occupancy; in contrast, unifying configurations, such as stability, will be attracted by optimal niche exploitation.

**Exploitation of variable versus stable environments**

This is a variation of the former attractor, when a multiplicity of niches occurs in time rather than in the space. Diversifying configurations will favour the exploitation of variable environments.
and unifying configurations will favour the occupancy of stable environments.

**Exploitation of low/medium versus high fitness peaks**

The notion of fitness peaks was presented by Sewall Wright in 1932 during the Sixth Congress of Genetics in his seminal lecture on ‘The roles of mutation, inbreeding, crossbreeding and selection in evolution’. Climbing low/medium fitness peaks provides fewer advantages than climbing high fitness peaks, but there are more low/medium peaks and the energy investment required to climb high peaks could be too high for the evolutionary unit. To stay in a high fitness peak is a dangerous situation, as any small variation will push the individual down in the slope, such a way as enabling the evolutionary unit to keep its configurations stable, whereas variable configurations might overcome smaller risks in lower peaks.

**Avoidance of competition versus efficient local defence**

Avoidance of competition might attract diversifying-type configurations. For instance, the exploratory or niche-deconstruction configurations facilitate access to a variety of possible niches, escaping from those in which competition with others might occur. In contrast, collective defence against potential invaders can act as an attractor for configurations in the unifying-type dimension, such as those related with exploitation or niche construction.

**Resistance to unexpected events versus resistance to expected deleterious events**

Although the development of resistance to expected events is an attractor for highly specific and efficient protection that occurs in unifying-type configurations, it might lower the possibilities of adapting to unexpected challenges. In contrast, broad resistance to unexpected events might attract diversifying-type configurations, priming quantity versus quality, as in $r$ strategy versus $K$ strategy.

**Distribution of costly functions within a consortium versus individual optimality**

The consideration of this pair of attractors reflects the possibility that a particular attractor might influence alternative configurations. On the one hand, the advantage of distributing costly functions among differentiated members of a consortium constitutes a field of attraction for diversifying configurations. On the other hand, such an advantage is only possible when the differentiated members are part of a consortium, a typical case attracting unifying configurations. Individual optimality obviously attracts unifying configurations, as the evolutionary unit collects and optimizes the required function.

**Mechanisms of diversification and mechanisms of unification**

An indicative collection of mechanisms by which the evolutionary objects enter in particular configurations under the influence of evolutionary attractors is listed below. A number of items listed cannot be considered as separate mechanisms, but rather as reflecting different topologies of related mechanisms. However, such overlapping may help the reader to identify the type of action that evolutionary units perform as part of their entry and progression in the diversifying or unifying dimensions.

**Mechanisms of diversification (ex unibus plurum)**

**High replication**

High replication, and hence high density, is frequently but not necessarily a pre-condition for diversification. High numbers assure the emergence of fortuitous changes giving rise to variants or increased connectivity, resulting in the possibility of interactions with other elements that might provide access to novel traits. High replication is the basis of the diversifying $r$ strategy. High replication facilitates high dispersal and high transmission. Interestingly, bacteria tend to increase their replication rate at concentrations of growth-inhibiting substances that are only slightly lower than those that prevent multiplication, but this phenomenon, of potential adaptive interest, has as yet been scarcely explored.

**High dispersal, high transmission**

The efficient dissemination of evolutionary units is a condition for the acquisition of exploratory configurations, attracted by the possibility of multiple niches occupancy. The result is heterogeneous interactions with multiple environments and, therefore, an increase of diversity. As stated earlier, dispersal reduces competition and assures local low numbers, and low numbers increase the emergence of variation by random drift. But, apparently, a neutral character in an evolutionary unit in a particular environment might be converted into a selectable trait in another one that is only gained after effective dispersal. Differences in dispersal occur in any evolutionary unit, e.g. hyperdispersible bacterial clones or broad-host-range mobile genetic elements probably increase the possibilities of diversification.

**Hypermutability, recombination**

Hypermutability essentially depends on an increase in DNA-pairing mistakes during replication or translation and/or a failure in correcting these errors. The expected result is an increase in variability. Nevertheless, hypermutable bacteria increase the possibility of compensatory evolution, reducing the possible cost of mistakes. A similar case occurs for hyperrecombination, which might act either as a generator of variability or as a corrector of changes. But, in general, mutation and recombination should be allocated among generators of diversification. Hypermutable organisms are frequently hyperrecombogenic; in both cases, the increase in variation explains why these strains are more frequently resistant to antibiotics than normo-mutable ones. Gene duplication, which provides the possibility for one of the copies to evolve to a new or expanded function, is a particularly successful way for diversification, once the ‘Ohno dilemma’ (where selection continues acting on the gene with the ancient function) is overcome.
Competition

Competition frequently forces the increase of dispersal and the search for novel niches, which tends to enhance diversity. Competition can be observed at cellular levels as competition for the acquisition of nutrients (as in the 'iron wars'), for surface adhesion and colonization, and, eventually, for the use of allelopathic substances, such as bacteriocins, lantibiotics or microcins. Interestingly, diversification is particularly enhanced in closely related evolutionary units (kin diversification), which share similar biological needs.

Competition assures diversity. A new aspect of kin diversification is based on ancestor inhibition by progeny, assuring transgenerational replacements. At subcellular levels, an example of competition is plasmid incompatibility, where two plasmids sharing similar or identical replication regions are unable to coexist in the same cell. The net result is that one or both plasmids are forced to enter different hosts, which might increase spread and diversification. A similar case occurs for transposons, an example being Tn3-like elements that cannot undergo transposition in genomes carrying similar or identical transposons (transposition immunity), again forcing the spread of these evolutionary units. Indeed, diversification might be driven by anti-unification mechanisms; if the restriction-modification (RM) systems impede the entry of foreign DNA (see next section), anti-RM systems providing evasion from this system are frequently hosted by plasmids, phages or transposons.

Stress responses

Stress responses, including those acting under nutritional starvation (or stationary phase), and also stress induced by antimicrobial agents, increase mutability and recombination in bacterial organisms. For instance, stress-induced up-regulation of error-prone polymerases, such as DinB (DNA polymerase IV), might increase mutational variation, resulting in increased genetic diversification. Stress also increases modularization-based diversification (see below), e.g. new-host or nutrient-shortage stress increase the load of modular mobile elements, such as insertion sequences (ISs). In general, environmental stress increases evolutionary potential.

Modularization

Modularity is an attribute of a system that can be decomposed into a set of repeated, conserved cohesive entities that are loosely coupled. That is to say, they might be present or absent (in this sense, mobile or accessory) in a particular localization of the genome or metagenome architecture and provide no significant pleiotropy for the bacteria. Modularity acts as an increasing-variability process that adds modular units within a given local genetic structure 'isolated' from the functional core of evolutionary units; hence, such recruitment occurs almost without cost. Incremental modularization, the addition of modules to a particular region, might occur because there is a 'module-recruiting' module (e.g. a recombinase), by duplication of a pre-existing module or by insertion of an incoming module. In most of these cases of 'random linkage between modules', modularity increases variability. The concept of 'modular evolution' was first applied to plasmids containing genes encoding antibiotic resistance early in the 1980s. The complete nucleotide sequences of many clones, plasmids, transposons or integrons are now available and have confirmed that these elements frequently have a 'mosaic' structure, with zones of high variability (hot spots) where adaptive modules are collected without harm. Genetic variation frequently depends on ISs, particularly variation seen in resting cells. ISs might act as mechanisms for external gene recruitment and integration in host replicons (such as the chromosome or plasmids), as well as for promoting recombination between these units; thus, generating genomic complexity and diversification. The fact that transposases are the most abundant and most ubiquitous genes in nature indicates the huge biological importance of modularity.

Mechanisms of unification (ex pluribus unum)

Selection

Variation tends to accumulate in different individual members of evolutionary populations in situations where new variants are neutral or quasi-neutral. Such variation is periodically eliminated from the population when one particular member acquires an advantageous trait. In such a case, this successful genotype is selected at the expense of all others (selective sweep of accumulated variation). Sooner or later, individuals of this genotype will start to diversify in their turn and again collect variation, and yet again a new successful genotype will arise to purge the newly accumulated diversity. This phenomenon is expected to occur periodically (periodic selection). In general, selection increases
the density of a particular variant, thereby reducing diversification, as in the case of selection-driven epidemics of particular clones.

**Regulation and reduction in mutation rates**

The rate of mutation is severely controlled in most living beings to maintain self-identity (unicity). As hypermutable asexual organisms, bacteria are expected to reduce their fitness and ultimately collapse as a consequence of the accumulation of deleterious mutations, a situation known as Muller’s ratchet.95 Thus, the regulation of mutation acts in reducing diversity.

A number of effective mechanisms of DNA proof-reading and repair act in the same direction, such as the methyl-mismatch repair system or GO system.96 Also, mechanisms probably exist to reduce or eliminate endogenous oxidative radicals, which induce DNA damage, eventually triggered by the presence of antibiotics.97 Indeed, recombinational events might also restore mutated sequences, in this case acting to reduce linkage disequilibrium. In fact, ‘sexual’ evolutionary units that are able to recombine under genetic and ecological compatibility (as opposed to ‘celibate’ units) might not only diverge,98 but also converge, and thus possibly increase unification.18,99

**Focused mutation and contingency loci**

Concentrating the changes on sequences of adaptive interest might decrease unspecific variation and costs. The mutational event is focused on ‘contingency sequences’ that increase variability in the specific type of genes required for adaptation to a recurrent but not continuous environment.100,101 Under the particular ‘contingency’ requiring a particular bacterial adaptation, there is a high possibility of obtaining adaptive changes at low cost, i.e. without changes in other loci, thereby reducing unwanted diversification. Of course, contingency loci increase ‘local variability’.

**Stress attenuation**

Stress potentially increases genetic variability. The possibility of surviving a stressful situation without stress should conserve genetic structure (unicity). Stress avoidance could be solved by down-regulating stress mechanisms such as alarmones,102 but that is at the expense of reducing a way out of the dangerous situation. A more effective way is stress attenuation, such as that seen during bacterial sporulation or tolerance. One of the reasons for the wide distribution of toxin–antitoxin systems among bacterial genomes might be the possibility of a toxin-mediated entry in a dormant status providing tolerance of stress at the expense of slow or no replication.103

**Restrictive horizontal gene transfer**

Diversification is reduced by mechanisms limiting the spread of genetic material among evolutionary units. For instance, the horizontal transfer of DNA by recombination is hampered by specific competence factors and transforming DNA recognition sequences. This assures that genetic information is kept within limited genetic exchange communities that form a kind of unit, with entry-exclusion and shared-environmental strategies promoting unification. Competence is induced in *Streptococcus pneumoniae* by strain-specific peptide pheromones called competence-stimulating peptides (CSPs). The primary structure of CSPs determines separate pheromone types (pherotypes), so that all bacteria induced to competence by a particular CSP belong to the same pherotype.104,105 At least one-half of all plasmids (the hallmark of ‘mobile’ genetic units) are not mobilizable, and all large plasmids are immobile and kept in domestication (unification) inside the host cell. Domestication means a stable relationship with their hosts, as some of their genes are functionally intertwined with the host’s functions. The known plasmid toxinantitoxin systems programme the death of the bacterial cell that has lost a particular plasmid, therefore forcing a coexistence between plasmid and chromosome replicons (unification).106 Moreover, plasmid conjugation depends on a number of core genes that have evolved in adaptation to specific genetic, physiological and ecological contexts.107 RM systems are able to detect specific DNA sequences in foreign (not-kin) DNA, which will be subsequently cleaved by endo- and exonucleases. Interestingly, these barriers might be relaxed in genetic exchange communities. Antisense RNA regulation has been observed for RM systems.108 Bacteria with clustered regularly interspaced short interspaced repeats (CRISPR) regions carrying copies of the DNA of previously encountered phage and plasmids abort the replication of phage and plasmids with these sequences, thereby reducing connectivity and diversification.80 In mobile elements, not everything is everywhere.

**Modularization**

Modularization can act as a diversifying mechanism and also as a unifying mechanism. When modularization acts to influence random gene ordering, diversification is expected to occur. However, when modularization enhances epistatic interactions between specific loci, optimization of these ‘ensembles’ might happen (epistatic clustering), therefore increasing unification.89

**Defensive allelopathy and colonization resistance**

In the ‘fixed habitat hypothesis’ it has been suggested that in physically structured habitats, where organisms form discrete colonies rather than individual cells, the production of allelopathic substances, such as antibiotics, bacteriocins or even bacteriophages, might have a defensive role against competitors.109 What happens in particular organisms also seems to occur for complex microbial consortia, such as microbiomes, that are extremely refractory to alien invasion.110

**Genetic addiction**

Genetic addiction assures the stable maintenance of an established complex of evolutionary units by programming the death of the system (e.g. a cell) if member(s) of the complex are eliminated. Unification of the complex is assured by this death penalty. Apparently, genetic addiction is particularly advantageous in the presence of a competitor genetic element.111
Towards understanding the multilevel epidemiology of antibiotic resistance

Each one of the units of selection, the evolutionary units of antibiotic resistance, should be taken into account when undertaking surveillance of resistance. A multilayered epidemiological strategy should be applied to deal with the extreme complexity of the oscillating pathways governing the evolution of antibiotic resistance. There is a need for simultaneous characterization of the resistance genes, the genetic platforms in which they are located (such as integrons), the transposable elements harbouring such platforms, the plasmids eventually carrying them, and, at the supracellular level, the bacterial clones, species and communities in which the resistance genes occur. Novel metagenomic high-throughput scanning technologies are awaited for addressing this type of multilevel epidemiology. The first approaches to this goal are already available. Of course, new mathematical modelling and bioinformatic approaches should be applied to deal with this target. Developments in network theory will enable the construction of edge-weighted networks describing the diversity within and across different genetic levels, identified in this theory as ‘genetic worlds’. However, our knowledge about quantitative links among different evolutionary units across different levels is still in its infancy. Probably, we should start by evaluating covariances in the different evolutionary units, e.g. by applying methods possibly based on Price’s equation to data obtained in synthetic microcosms both challenged or not with antibiotics. It should be noted that these parameters might randomly fluctuate according to environmental changes and, therefore, the possible descriptions of the evolution of such complex systems will, at best, be of a probabilistic nature. In this respect, evolutionary graph theories might help in designing evolutionary games on graphs that might at least provide a list of possible events influencing the structure of the antibiotic resistance network. A totally new public health microbiology able to address the key problem of prediction of antimicrobial resistance is urgently awaited, and this novel approach to medical microbiology should be based on the application of evolutionary thinking. The objective of this review has been to provide a conceptual framework for the evolutionary concepts that need to be applied to the study of antibiotic resistance; thus, helping to pave the path towards this goal.

Acknowledgements
The author owes a large debt to many conversations held over the years on the topics covered in this review with Juan Carlos Galán, Bruce Levin, Teresa Coque, José-Luis Martínez, Jesús Blázquez, Susanna Manrubia, Juan-Carlos Alonso, José-Maria González-Alba, Rosa del Campo, Steven Frank, Andrés Moya, Fernando González-Palacios, Antonio Oliver, Marc Lipsitch and Rafael Canton. Ana Moreno-Bofarull and Mary Harper provided substantial help with the manuscript.

Funding
This review is in part funded by the FP7 European Commission PAR (241476) and EvoTAR (HEALTH 2011) Projects.

Transparency declarations
None to declare.

References


The Garrod Lecture


94 Aziz RK, Breitbart M, Edwards RA. Transposases are the most abundant, most ubiquitously genes in nature. Nucleic Acids Res 2010; 38: 4207–17.


96 Muller HJ. The relation of recombination to mutational advance. Mutat Res 1964; 106: 2–9.


