Bacterial Evolution and Involution

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Abstract

In biology, the concept of evolution is often linked to an increase in directional complexity driven by particular selective landscapes. In contrast, involution is understood as the loss of adaptive gains achieved through evolution, leading to de-adaptation to the environment and eventually to extinction. However, the microecological heterogeneity of spaces occupied by bacteria necessarily makes evolution a diversifying force, gradually producing more specialized variants. The fit between the environment and adapted bacteria results in the loss of function of genes not required for adaptation, which become a burden on bacterial replication and initiate reductive genetic evolution, which can be considered an involution. Therefore, evolution produces involution. At this stage, involutionary entities start to collaborate with each other, creating novel entities at a higher hierarchical level, as may occur with the microbiome. Alternatively, the involutionary entity exploits higher organisms, such as eukaryotic cells, at the expense of losing its bacterial ontology and being converted into organelles. Therefore, involution produces evolution. Involutory intermediates may give rise to novel evolutionary trajectories. These trade-offs can be studied in experimental evolutionary models.

Evolution versus Involution.

In 1885, in the aftermath of debates surrounding the evolutionary theory, Ezra Z. Kerr published a book titled “Evolution versus Involution”. For the first time, Kerr proposed that both evolution and involution are at work in the world and that we should understand both dimensions to navigate life’s complexities. A year later, an Editorial Comment on this book appeared in a highly prestigious scientific journal, referring to the evolutionary hypothesis, and ended with the words: “….it no longer makes it necessary to try to disprove this growing theory of science” (1, 2).

What we usually have in mind when we refer to evolution concerns entities that emerge, unfold, develop, spread, or appear in a succession or series, implying change over time into different forms 3). Curiously, the term *evolution* does not appear in the "Origin of Species" (1859) probably because of Darwin's reluctance to relate his theory to a developmental, embryonic-like process, rather than to one based on small, independent steps of transmutation. However, in his seminal book, the word *"evolved"* appears as the last word, clearly linked to evolution as a process producing advanced, more complex forms of life: "From so simple a beginning endless forms most beautiful and most wonderful have been, and are being evolved". In contrast, the word *involution* is understood as a backward movement, deterioration, simplification, and eventually loss of what has evolved. Eventually, evolution may cause involution, as, for instance, predicted by Malthusian theory. In bacteria, the drivers of evolution, such as hypermutagenesis or genomic insertions, can also drive involution. In the traditional view, evolution and involution apply to living organisms, but hypothetically, such a dynamic was born before cellular life, in the prebiotic molecular world (4). From the Darwinian perspective on natural selection, the series of changes and dynamics of change are not the effect of stochastic neutral evolution, but arise from ***constraints***, thereby making selection of the best fit the directing cause of evolutionary or involutionary change over time. The question we re-examine in this Perspective is whether adaptive evolutionary changes, including mutational events or the acquisition of mobile genetic elements, *necessarily* create new constraints that may force evolution onto irreversible trajectories, as in the Muller-ratchet metaphor, ultimately culminating in involution and eventually extinction.

Every evolutionary or involutionary process necessarily results from change, but not every change results in evolution or involution. A random change in the spatial position of a particle may occur; no kind of order or directionality is present in this entropic state, as in Brownian random-walk motion (5). This state of continuous change can be designated as *volution*, taking the original classic sense of rolling or twisting to denote a fully stochastic movement without modification of the object's *identity*, only changing position, not ontology. In other words, *volution* is the dynamic movement intrinsically associated with existence, very much in the Heraclitean sense. It is worth noting that the notion of *identity* has peculiarities in the biological world; for instance, a bacterial organism harboring a synonymous mutation is phenotypically identical to the other members of the population and therefore immune to selection. However, its proportion relative to the other members can be distorted by sampling bias. As an exemplary case, it can be captured by chance in the small inoculum transferred daily from one tube to another in long-term evolution experiments (6). In nature, accidental subdivisions of populations may occur during events that lead to overdispersion, thereby offering the possibility of enrichment of non-selectable changes by genetic drift (7). (Such a cryptic synonymous mutation and drift can thus accelerate local evolution and involution by opening access to diverse adaptive peaks (8). However, drift may favor involution through purifying selection, at least in the sense of reducing genome size (9). In summary, evolutionary progress may occur either by evolution or involution (Figure 1).

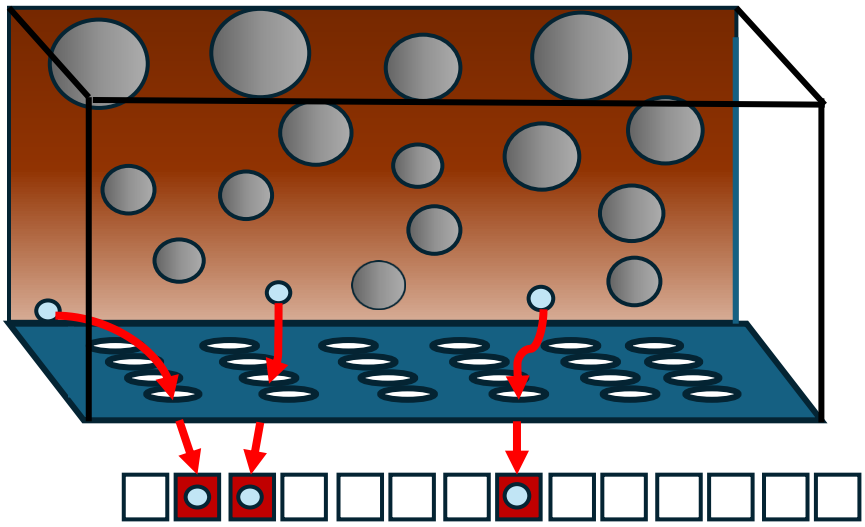

Figure 1. Schematic example showing that evolutionary progress can proceed by evolution or involution. Biological entities, here represented by balls of different sizes, with their volume proportional to their functional capabilities, generally linked to the genome size. The reddish space corresponds to a nutritional gradient; entities evolving to larger sizes may be able to exploit a wider range of nutrients, moving up the evolutionary ladder. Eventually, small blue involutive balls appear that can access novel, highly specific compartments below the blue perforated blade. Invading these spaces, they have access to rich, nutrition-specific environments that are unavailable to their more versatile relatives.

Evolution of Involution: Eukaryotic Cellular Integration

Although gain-of-function mutations that create new evolutionarily advantageous metabolic-ecophysiological potential can, in a few instances, occur by single mutations that, for example, change the specificity of a gene regulator (10) or an enzyme (11). the evolutionary process generally involves multiple genetic changes that increase system complexity and diversification. In such cases, many previous adaptive changes are no longer beneficial and accumulate as hitchhikers, increasing a legacy metabolic burden integrated into a denser ecophysiological system that continues to evolve. Cellular metabolism is highly regulated in most systems most of the time – the cellular bureaucracy that maintains metabolic order and efficiency. Genetic change may perturb one or more processes in metabolic-ecophysiological networks, resulting in reduced process efficiency and, in some cases, trigger a response - a reset of cellular controls - as has been shown by experimental evolution studies utilizing genetic engineering and synthetic microbiology approaches (12). and theoretical evolution studies exploiting systems microbiology-metabolic modelling (13). Importantly, however, genetic change and cellular response to it may create a

barrier to particular evolutionary trajectories, a major constraint on further evolutionary adaptations. This reset may provoke involution, the opposite of evolution, and lead to shrinking, degeneration, and even disorganized collapse. Considering the large population sizes and widespread distribution of bacteria, the involution of a few members of the collective population should not, in principle, influence the evolution of the taxon. Only if a non-gradual, punctuated involutive process is adaptively successful will it become widely propagated. If such a selective advantage is ephemeral, it poses a real risk of collapse of the bacterial population.

However, in the absence of full extinction, during a subinvolution stage, the force of natural selection continues to operate in multiple adaptive dimensions on a profoundly altered biological structure. In this sense, there is a positive secondary selective dynamic, meaning that the constraint serves as a supplier or cause of novel evolutionary direction and change (14). An exemplary case is the drift involution that results in genome reduction in a species of the family Erwiniaceae after being captured by the cells of the aphid *Cinara cedri*, becoming the endosymbiont *Buchnera aphidicola*, and further evolving into a variant with an even shorter genome (15). A kind of evolutionary rescue changes the direction of these organisms' evolution from an involutionary state to a novel ontological level, becoming, as it occurred with mitochondria and chloroplasts, eukaryotic cellular organelles. In these cases, the basic structure of the entity is partially preserved, but at the expense of losing its microbiological ontology and, to a certain extent, its individual evolvability. In fact, their evolvability becomes coupled to that of the host cell. Note that even eukaryotic organelles of microbial origin, such as mitochondria, probably derived from an endosymbiotic *Alphaproteobacterium* in the order *Rickettsiales* (16) are subject to processes of evolution and involution. Here, the driving constraints that change the organelle, such as the mitochondria, depend on the intracellular molecular ecology of the host cell, which is contingent on the cell's functional needs (17). Mitochondrial genome-encoded products must physically assemble with their nuclear-encoded counterparts to form functional respiratory complexes (18). Such adjustment between early cellular hosts and diverse microbial candidates to become organelles occurred very early during eukaryogenesis (19). Ultimately, complete incorporation of all mitochondrial functions into the host genome may occur (20), a total involution of the original bacterial entity in favor of the evolutionary progress of the eukaryotic host.

Evolution of Involution: Integration in Microbiota

There are other evolutionary rescues compensating for involutionary effects resulting from successive constraints. As Stephen Jay Gould recalls, the word “constraint” also has the original positive meaning of *con-stringere*, bringing items into the field of change or compression (14). Genetic accretion during evolution may lead to involution, but involution opens new pathways to evolutionary associative dimensions. Microbes generally exist as members of communities that represent higher-level units of evolutionary selection. Thus, although genetic change generally occurs within a single cell, and is propagated vertically within its lineage (but also, under certain conditions, may be transferred horizontally to multiple cells within and between lineages by epidemic transfer of mobile genetic elements. They contribute to both evolution and involution,

ithe latter case through insertion sequence-mediated gene inactivation (21); consequently, compensatory changes may be selected at the community level rather than in the affected cell itself. In other words, as metabolic order declines within a cell/population/clade, community bureaucracy imposes order at a higher hierarchical level (22, 23). The above example of mitochondria recalls the assembly of interdependent consortia of microbes, associations that form the basis of supra-cellular organizations, such as the microbiota. The absolute number of bacterial amino acid auxotrophs, denoting involution, is much higher in the host-associated microbiota than in isolated species (24). Such loss of individual complexity in the cell is transferred to a new multicellular complexity in a niche-dependent eco-devo process (25).

Bacterial species and lower taxa are not expected to persist indefinitely, either due to extrinsic factors, such as loss of niches during environmental changes, or intrinsic factors, such as irreversible collective involution. However, the core of the species, the genus, may remain much longer, meaning greater resilience to involution, driven by the birth of new species. Therefore, higher taxa serve as a reservoir for evolutionary processes and the resulting outcomes of differentiation. In addition, a significant part of the microbiosphere, e.g., in the subsurface, in air, and in nutrient-poor parts of the ocean, experiences long-term starvation. While starvation in most eukaryotes leads to death, in many microbes it leads to dormancy, an essentially non-active state that is reactivated by favorable growth conditions. The cryosphere also harbors a wealth of dormant microbes that become active as it warms. The dormant microbiosphere represents a kind of microbial diversity seed bank, whose members can become participating evolutionary partners once they become reactivated. On the other hand, successive constraints that lead to involution might also confer survival advantages. Hypermutagenesis and reductive evolution driven by continuous antibiotic exposure promote involution, resulting in long-term stability of *Pseudomonas aeruginosa* in the bronchi of patients with cystic fibrosis (26). There is an advantage to simplicity, reducing functions and thus their energetic costs in streamlined microorganisms where nutrients limit growth (27). This may eventually foster the evolution of new life forms characterized by atypical prototrophy, such as those based on piezoelectric energy, in which mechanical forces are converted into biochemical energy (28).

Evolutionary-Involutionary Dynamics in Experimental Models

Experimental evolution can provide insights into the trade-offs between evolution and involution. In the long-term evolutionary experiments (LTEE) developed decades ago by Richard Lenski, more than 50,000 generations were reached in each one of 12 independent replicate cultures seeded with a single ancestor *E. coli* strain. Full population involution and extinction have not occurred in any of the cultures, despite constant nutritional bottlenecks, the emergence of hypermutators, and clonal competition between mutants. Clonal competition rarely resulted in the extinction of a lineage; however, inside each population, some mutants have gone extinct. This suggests that in a bacterial population, the evolution and involution trade-off tends toward an equilibrium. Evolutionary progress was frequently convergent, that is, similar adaptive features independently occurred in different lineages, involving genes encoding proteins with core regulatory and

metabolic functions (29). Involution can also be convergent; during the LTEE, all populations lost their ability to grow on ribose, and many lost their ability to grow on maltose. However, in general, each population was unique and followed its own evolutionary trajectory, depending largely on the random history of mutational events and their historical contingency. Over longer timescales, mutation-to-mutation interactions, pervasive epistasis, change the magnitude and the sign of the mutational fitness effects, so that some previously advantageous mutations become deleterious and vice versa, an excellent example of the oscillatory dynamics between evolution and involution (30) A particularly interesting example is that organisms resulting from late generations in long-term evolutionary experiments evolve to inhibit their coexisting ancestors, a "killing the father" dynamics (31), allowing new evolutionary starts. In this example, evolution contributed to the involution of the older individuals. We have many other cases of coexistence of evolution and involution, such as the antagonistic pleiotropy evolved in LTEE with pathogens harboring beta-lactamases and cultured on media containing different beta-lactams; in this case, evolutionary gains to increase resistance to a drug resulted in involutory loss of resistance to other drugs of the family (22). Such an effect has been named collateral sensitivity, and has been shown to influence the spread of antibiotic resistance in the clinical setting (32).

Evolution and Involution: Diversification and Homeostatic Strategies in a Changing Environmental Landscape

Evolution, the change in bacteria over time, is contingent on spontaneous environmental changes, whether stochastic and accidental or geocyclic and circadian. The random complexity of fine-grained bacterial microenvironments favors the diversification of selective spaces and, consequently, frequent exposure to changing conditions. An illustrative example is the role of environmental gradients and intersecting gradients of physicochemical parameters, nutrients, inhibitors, etc., where minimal changes in concentration can result in different, often opposing, selective activities. Moreover, as a result of their small size and high metabolic activities, microbes actively create microgradients in their immediate vicinity, both at the single cell and community-biofilm levels. Concentration-dependent selection ensures the evolution of very small phenotypic differences (33). In LTEE, many genes yield mutations that confer minor fitness gains, enabling adaptation to very small changes (29). Tiny changes can, in fact, be highly selective, and their possible cost is easily compensated (34). The rugged evolutive-involutive landscape arising from the interaction of spontaneous bacterial endogenous variation and exogenous random environmental variation, coupled with a multitude of microbial sensors exquisitely sensitive to physico-chemical concentrations and the ability to physically navigate up and down gradients by taxis (35,36) yields a series of successive and unpredictable selective-counterselective landscapes. Bacterial phylogenetic diversification results from the sequential fragmentation of selective spaces (37). Diversification entails specialization in particular niches, at the expense of genomic involution. This creates significant constraints for the survival of specialists. The problem is solved by cooperation between specialists - escalating selection to a higher organizational level - and assuring their persistence by an organismal-like intercellular collaboration (Figure 2).

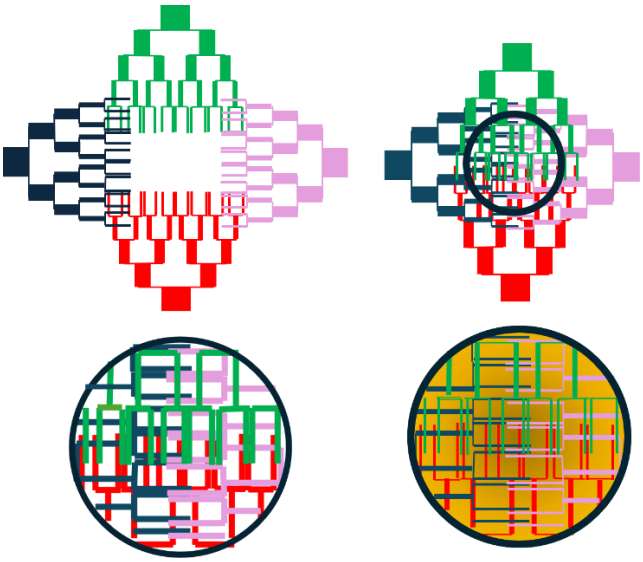

Figure 2. The construction of a multiphyletic entity by involution. Upper left: phylogenies of four different lineages starting with full-size genomic individuals. Phylogenetic diversification progresses with greater specialization and a reduction (thinner rectangles) in genomic information. Upper right: if these phylogenetic trees coalesce, the deficiencies caused by involution are compensated for by the activities of other specialists, producing a collaborative biological space that acts as a unit (black circle). Lower left: detail of the black cycle showing multiple interactions between the involuted extremes of the phylogenies. Lower right: coalescence gives rise to a homeostatic system (yellow ground), ensuring the persistence of the multiphyletic entity.

In addition, complex systems also promote their own homeostasis, the collective stabilization of the common environment, resulting in a collective niche construction. This occurs when there is perpetual physiological interaction between system components, which is necessary for the preservation of each one of them – allostasis - which implies quasi-organismal behavior and system-level selection (22,38). Conceptually, the evolutionary boost may be considered a result of a continuous quest for niches immune to change where the living entity could perfectly fit with a stable and/or predictable environment (39). The paradox of this possibility is that its success would tend to hinder further evolution. But, as involution is inexorably linked to the evolutionary process, and the global environment is constituted of a mixture of homeostatic and imbalanced, fluctuating, and disrupted compartments, evolution never ceases.

Conclusion

Evolution never stops, and involution contributes to the birth of secondary evolutionary trajectories. Evolvability, the capability of a population to adapt, is driven by a constant search for

the optimal niche and comprises evolution and involution. As previously proposed, there is a Hegelian synthesis, a dialectical unity of evolution and involution (40). Something similar was proposed comparing the dynamics of biological aggregation and disaggregation (41). Selective constraints are the motor of evolution and involution. If the number of constraints increases during the Anthropocene, as a result of human activities and resulting changes in natural ecological processes and interactions, we can expect an acceleration of bacterial evolutionary dynamics, with unpredictable consequences for planetary health (42). This suggests that it may be helpful to expand exploration of the interplay of evolution and involution, and its consequences, experimentally through genetic engineering-metabolic design-synthetic microbiology, and through AI-supported modeling.

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