GENE NETWORK EVOLUTION

Modularity of the life cycle

Life stages in *Bacillus subtilis* are controlled by regulatory blocks that can be kept or lost across species in response to selection in different environments.

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A fascinating feature of life is the diverse set of behaviours and lifestyles encoded in a genome. Over the course of a lifetime, a single genotype can express multiple phenotypes depending on the environmental circumstances. The lifestyles can be so different that it has led to some organisms being considered as two distinct species until their life cycle was deciphered[1]. The expression of lifestyles is controlled by complex gene networks — the ones that in visualizations look like large tangled balls of string (Fig. 1). These gene networks consist of thousands of genes whose interactions and dynamic concentrations somehow translate into discrete phenotypic states like 'moving' or 'sessile'. The task of understanding how such convoluted gene networks give rise to phenotypes is challenging, and even more so is studying how they evolve. Writing in *Nature Ecology & Evolution*, van Gestel and colleagues[2] demonstrate that a life-cycle perspective can improve our understanding of transcriptional regulation and its evolution. The authors uncover blocks of genes that regulate the various lifestyles of *Bacillus subtilis* and show that these blocks are either kept or lost across Bacillale species, giving rise to a mosaic pattern of genetic similarity.

Previous studies into how *B. subtilis* navigates its lifestyles typically investigate single transitions from one life stage to another. van Gestel et al. take a holistic perspective by considering how gene regulation and lifestyle decisions operate in the context of an organism's entire life cycle. Comparing four reconstructions of the transcriptional network, they identify a small number of genes that are responsible for most of the regulatory interactions. These genes, called global regulators, work in concert to regulate each other, and they also activate unique, mostly non-overlapping, sets of genes that correspond to particular functional programmes.

After identifying the main organizational features of the *B. subtilis* transcriptional network, van Gestel et al. next determine how the expression of the global regulators and their targets change over time. They apply a machine learning approach called auto-associative artificial neural networks to a dataset of 252 tiling microarrays, which measured the gene expression of a strain of *B. subtilis* exhibiting different lifestyles in different environmental conditions. A key feature of the machine learning approach is that it maps a multidimensional dataset, a gene expression profile, to a single value. Interestingly, this value also happens to correspond to the relative time, 'pseudotime', when the lifestyle is expressed in the life cycle of *B. subtilis*. What emerges is an ordering of gene expression profiles along a life cycle from germination to sporulation and a modularity to the life cycle in which global regulators are expressed at characteristic times, triggering the expression of associated functional programmes.

The modular organization of the life cycle also has evolutionary consequences. van Gestel et al. use a phylogenetic approach to compare *B. subtilis* with 384 of its fellow Bacillale to investigate life-cycle differences. They find a mosaic pattern in which whole regulatory blocks corresponding to distinct life stages are either kept or lost across species. In particular, the motility and sporulation life stages are repeatedly lost in similar ways across species. Interestingly, the mosaic conservation pattern reflects a modular organization of the life cycle, in which life stages are kept or lost depending on their value in an environment. Since the Bacillale experience very different ecological and environmental conditions, it is curious how they essentially modify (keep or lose) the same basic set of lifestyles. It points to the idea that the organisms themselves are likely collecting multidimensional data about their environment and classifying their situation into a few states with different characteristic behaviours, that is, should I stay or should I go? Is it likely to get better soon or should I sporulate?

Finally, van Gestel and colleagues explore what happens if there is selective pressure in favour of one life stage — the hypothesis being that such selection could lead an organism to suppress a possibly maladaptive life stage. The authors constructed an evolution experiment in which eight Bacillale strains experience conditions that favour colony growth, hypothesizing that mutations that disable sporulation might fix. After a few hundred generations, half of the evolved populations showed a significant reduction in sporulation and 20% of them completely lost sporulation. They identified the responsible mutations in these populations and found that most of the mutations negatively affected the same global regulator, Spo0A, preventing the transition from colony growth to sporulation.

Fig. 1 | A complex genetic network is coloured according to the expression of lifestyles by analysing gene expression profiles. Comparing across populations reveals a mosaic pattern in the evolution of gene networks. Credit: String image, the_guitar_mann/iStock/Getty Images Plus/Getty; mosaic sphere, Alvaro Cabrera/Alamy Stock Vector

The result that global regulators might be likely targets of selection connects well with other experimental evolution work[3,4] and helps to explain the puzzling observation that mutations in well-connected genes are common in experimental evolution studies[5]. Most mutations result in a loss of function. It seems it would be less disruptive to the normal functioning of an organism if it adapts to an environment via mutations in genes that have limited connectivity, that is, knocking out a central gene is likely to have too many downstream consequences. Yet, experimental evolution frequently sees mutations in well-connected genes. The study by van Gestel et al. suggests that because global regulators control large programmes of activity related to lifestyles, they are precisely the ones to target. If a lifestyle is maladaptive then the easiest way to shut it down is via a global regulator. This result demonstrates the important role of genetic architecture in influencing the course of evolution and is another example of some predictability in evolution[6,7].

The multifaceted approach used by van Gestel et al. sheds light on the organization and evolution of a life cycle composed of different lifestyles. They show that lifestyles can be lost as units in response to selection in different environments. However, their study raises tantalizing questions concerning the opposite process of how new life stages evolve. If evolutionary change occurs through small-scale genetic change then how does the large-scale, modular organization of lifestyles come about? Is the modularity a later adaptation or is it a signature of how new adaptive lifestyles are gained? Does the pseudotime axis of their machine learning approach give some indication of where such new lifestyles are likely to originate or how they might be constrained? It is a good time to be studying large tangled balls of string. ❐

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Competing interests

The author declares no competing interests.