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Opinion

Scanty microbes, the 'symbionelle' concept

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Mutualistic symbiosis occurs when two different species interact closely with each other and benefit from living and working together. However, not all symbiotic associations are of mutual benefit because there are also forms of parasitism (when one organism benefits but the other is adversely affected) and commensalism (when only one of the organisms involved in the association benefits, but the other is not affected); notwithstanding, the very fact that specific entities can exist together means that natural selection may guide them to live with each other. Endosymbiosis is a special case of symbiosis in which one partner, generally a prokaryote symbiont, lives sequestered inside specialized eukaryotic cells called bacteriocytes.

The notion of microbes becoming organelles of eukaryotic systems through evolution has been widely accepted because Lynn Margulis put forward her serial endosymbiotic theory of eukaryotic cell evolution (Margulis, 1993). Indeed, this is the origin of mitochondria and chloroplasts. There is compelling evidence to support that these two eukaryotic organelles are the product of symbiotic events between prokaryotes and primitive eukaryotes (Latorre *et al.*, 2011). Their original alpha-proteobacterial (mitochondria ancestor) and cyanobacterial (chloroplast ancestor) genomes have been drastically reduced, with a portion of the protein-encoded genes and even RNA genes being transferred to the eukaryotic nuclear genome. Other genes have simply been lost, and their function replaced by the hosts. Since the proposal of these two canonical endosymbioses, symbiotic associations between prokaryotes and unicellular and multicellular eukaryotes have been documented in practically every major branch of the tree of life, which reinforces the role played by symbiosis in the emergence of evolutionary innovations (Moya *et al.*, 2008).

Endosymbiosis in insects is a captivating example of the aforementioned phenomenon. Insects are particularly well suited to establishing intracellular symbiosis with bacteria, which provide them with the metabolic capabilities they lack and enable them to live in almost any environment. At present, there are a number of well-documented cases of insect endosymbionts at different stages of symbiotic integration (Fig. 1). Insect endosymbiosis commonly consists of an obligate mutualistic association, where bacteria produce essential nutrients that are absent in the insect's diet, and the insect, in turn, provides the bacteria with a safe environment and a permanent food supply (Baumann, 2005). These endosymbiotic bacteria are vertically transmitted across host generations. Their metabolic role is renowned, and most insect endosymbiotic systems are largely convergent towards these functions regardless of the lifestyle or genomic repertoire of their free-living ancestor (López-Sánchez *et al.*, 2008; McCutcheon *et al.*, 2009; McCutcheon and Moran, 2010; Sabree *et al.*, 2013). A new symbiotic relationship, which represents a source of novel complexity, has to overcome the obvious problem posed by the fact that both partners must be able to survive together despite differences in biology, particularly generation times and reproduction. Moreover, considering that these organisms generally possess different population genetics and are under different evolutionary pressures, they need to establish a certain trade-off to acquire the evolutionary novelty represented by their stable coexistence (Delaye and Moya, 2010; McCutcheon and Moran, 2012). Thus, important genetic and biochemical modifications are required in these bacteria compared with their free-living state. The eukaryotic host, on the other hand, must develop ways of controlling the bacterial population, engulfing them in specialized cells –the aforesaid bacteriocytes – and/or changing immune responses to recognize these bacteria as non-pathogenic.

One of the most important and well-known features of endosymbiotic bacteria is that they provide extreme examples of genomic shrinkage by undergoing a process called the 'genomic reduction syndrome'. Hence, prokaryotic genomes of endosymbionts are examples of a particular type of naturally evolved minimal cell, with insect

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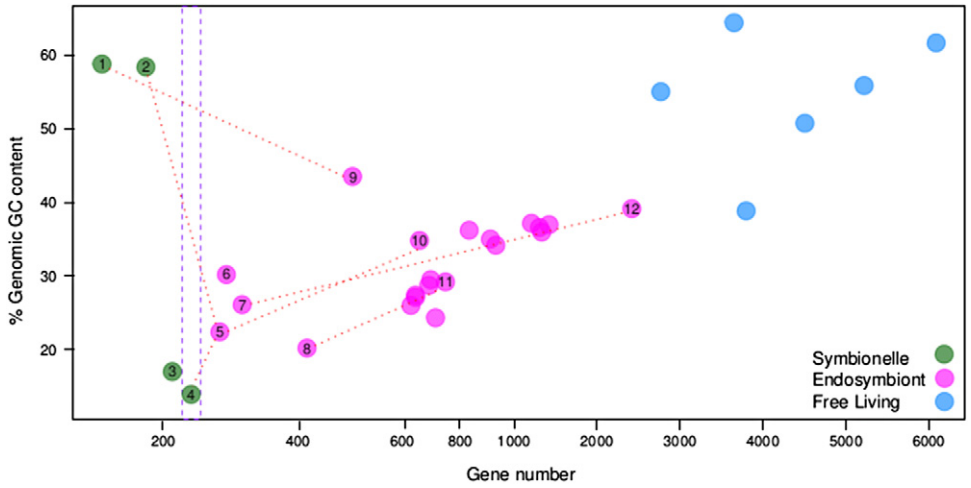

Fig. 1. Genomic GC content (%) versus gene number in several symbionelles, endosymbionts and free-living bacteria. The two dashed purple vertical lines delimit the minimal gene set between 223 and 244 genes. The dotted red lines indicate pairs of symbiotic associations. Symbionelles: (1) '*Ca.* Tremblaya princeps' PCVAL, (2) '*Ca.* Hodgkinia cicadicola' DSEM, (3) '*Ca.* Carsonella ruddii' PV, (4) '*Ca.* Zinderia insecticola' CARI. Endosymbionts: (5) '*Ca.* Sulcia muelleri' GWSS, (6) '*Ca.* Uzinura diaspidicola' str. ASNER, (7) '*Ca.* Portiera aleyrodidarum' BT-QVLC, (8) *B. aphidicola* BCc, (9) '*Ca.* Moranella endobia' PCIT, (10) '*Ca.* Baumannia cicadellinicola' str. Hc, (11) *S. symbiotica* str. Cc and (12) '*Ca.* Hamiltonella defensa' 5AT.

endosymbionts being those with the smallest genomes reported to date (Fig. 1). In fact, as much as 90% of their genetic matter can be lost in advanced or extreme cases of symbiotic integration.

It is remarkable to mention that the previously described genome reduction process correlates with the time period of the symbiotic association. Furthermore, many of the typical genome features of free-living bacteria, like *Escherichia coli*, such as the presence of pseudogenes and mobile elements, as well as a constant exchange of genetic material by recombination or horizontal gene transfer events, are lost in intracellular endosymbionts. As they inhabit a stable and nutrient-rich environment, they lose genes that are redundant or non-essential because they are provided by the host. This situation leads to the irreversible loss of bacterial genes and, thus, unnecessary metabolic capabilities strictly following a 'use it or lose it' tendency in evolution (Wernegreen, 2005; Allen *et al.*, 2009). Finally, it is worth noting that a rather limited number of bacteria is vertically inherited by the host compared with the number that can be achieved during insect development. These dynamics are translated into systematic bottlenecking, which determines an ample effect of genetic drift with respect to natural selection in the evolution of bacterial endosymbionts (Moran *et al.*, 2009; Delaye *et al.*, 2010).

Although organelles and endosymbionts with extremely reduced genomes present some commonalities, there are several important differences. Both share their extremely reduced genome sizes, are maternally inherited and are completely dependent on their host for survival, mutually providing essential functions. Regarding differences, organelles have a double membrane enforced by engulfing the primitive cells inside the host cell, and genes of the organelle-becoming bacteria are transferred to the hosts' DNA, which acquires sophisticated transport mechanisms to transfer protein products from host to organelle and vice versa. Additionally, the host cell takes over the regulation of the organelle's division, synchronizing it with the cell's own division (Gould *et al.*, 2008; Keeling and Archibald, 2008), leaving the organelle without cell status. In contrast, endosymbionts appear to lack a cell wall and possibly depend on host-derived membranes (or membranes synthesized by another symbiont present in the consortium), and to date, there is no evidence of horizontal gene transfer to the host nuclear genome. Moreover, endosymbionts have evolved together with multicellular eukaryotic organisms, plausibly making their cellular status less questionable than in the case of organelles.

In this opinion article, we propose the 'symbionelle' concept for endosymbionts that have partially loss their symbiotic role as a consequence of strong genome shrinkage and go beyond what is theoretically considered a minimal cell (Luisi *et al.*, 2002; Gil *et al.*, 2004; Gil, 2013). A minimal cell is formed by a core minimal genome of protein-coding and RNA genes, which guarantee the following three major functions: (i) genetic machinery composed of virtually complete DNA replication and translation apparatus, and a simple DNA repair system; (ii) an energetic and intermediary metabolism, in which energy is obtained via substrate-level phosphorylation and the basic elements are provided by the environment to synthesize the essential cell components; and (iii) a cell envelope that encloses the genetic and metabolic machineries, controlling interac-

tion with the environment, and growing and dividing to allow the formation of daughter cells. According with these functions, Gil (2013) proposes that the minimal gene-set machinery is composed of 188–206 protein-coding genes and 35–38 RNA genes. Figure 1 demarcates a zone ranging from between 223 and 244 genes (the strict theoretical minimal genome), which separates endosymbionts having minimal cell status (on the right of the dotted line) from symbionelles, a term coined to describe bacteria that fail to reach the minimal gene set (shown either on the left or inside the zone). According to this criterion, *Candidatus* Tremblaya princeps (155 genes), '*Ca.* Hodgkinia cicadicola' (189 genes), '*Ca.* Carsonella ruddii' (213 genes) and, probably also, '*Ca.* Zinderia insecticola' (232 genes) are examples for the term duded here as symbionelles. By contrast '*Ca.* Sulcia muelleri' (264 genes), '*Ca.* Uzinura diaspidicola' (272 genes), '*Ca.* Portiera' aleyrodidarum (292 genes), *Buchnera aphidicola* BCc (402 genes) and *Moranella endobia* (481 genes) meet the minimal-cell criteria (Pérez-Brocal *et al*., 2006; McCutcheon and Moran, 2012; Santos-Garcia *et al*., 2012; Sabree *et al*., 2013).

Manifestly, both endosymbionts and organelles live in the very rich intracellular medium of their host. This type of heterotrophic environment has also been used to define the chemical environment of a minimal cell and contains glucose, phosphate, fatty acids, nitrogenous bases, amino acids, nucleotides, vitamins, inorganic ions and several cofactors (Gil, 2013).

Another interesting feature of endosymbionts with reduced genomes is the formation of consortia among two or more symbiotic bacteria, whereby they complement each other metabolically to fulfil their symbiotic role. However on occasions, as previously mentioned, some endosymbionts (the newly defined symbionelles), go beyond the minimal cell state, and while there are cases of the coexistence of two minimal cells or a minimal cell with a symbionelle, there is no evidence of two symbionelles living together. Examples can be found in the metabolic complementation among two minimal endosymbiotic cells in the sharpshooter *Homalodisca coagulata*, in the cicada *Diceroprocta semicincta* and in the spittlebug *Clastoptera arizonana*. All three insects have the endosymbiont '*Ca*. Sulcia muelleri' (minimal cell), which needs to be complemented by *Baumannia cicadellinicola* (minimal cell), '*Ca.* Hodgkinia cicadicola' (symbionelle) and '*Ca*. Zinderia insecticola' (symbionelle), respectively (McCutcheon *et al*., 2009). It is worth mentioning that '*Ca*. Sulcia muelleri' might lie on the boundary dividing a minimal cell from a symbionelle, as it has lost some genes that would be considered 'essential', like missing genes encoding aminoacyl-tRNA synthetases (McCutcheon and Moran, 2007). Another intriguing case is the nested endosymbiosis of mealybugs of the subfamily Pseudoccinae, such as *Planococcus citri*, where the co-endosymbiont, *Moranella endobia* (minimal cell) is located inside '*Ca*. Tremblaya priceps' (symbionelle). In this case, the complementation involves not only metabolic but also informational functions, as '*Ca*. Tremblaya princeps' appears to be a mere factory for amino acid synthesis and translating proteins, using precursors provided by *M. endobia*, including those for informational proteins (López-Madrigal *et al*., 2011; McCutcheon and von Dohlen, 2011).

'*Ca*. Carsonella ruddii' and '*Ca*. Uzinura diaspidicola', endosymbionts of the psyllid *Pachypsylla venusta* and the armored scale insects (family Diaspididae), respectively, are endosymbionts with highly reduced genome sizes living alone in their respective hosts. However, whereas the genome content of '*Ca*. Uzinura diaspidicola' seems to meet the requirements to be considered a minimal cell fulfilling its symbiotic role (Sabree *et al*., 2013), the case of '*Ca*. Carsonella ruddii' (symbionelle) is striking as it lacks not only the genes necessary for its symbiotic role but also several important genes involved in DNA replication, transcription and translation (e.g. a ligase activity; Nakabachi *et al*., 2006; Tamames *et al*., 2007). It has been hypothesized that the host has taken over the role of the missing genes or that some of the genes have evolved novel functions (Sloan and Moran, 2012).

Endosymbionts of white flies and aphids also reveal additional cases of metabolic complementation between bacterial genomes having the cellular status. '*Ca.* Portiera aleyrodidarum' (292 genes), the endosymbiotic minimal cell of the white fly *Bemisia tabacci*, always coexists with other endosymbionts harbouring higher gene numbers (Santos-Garcia *et al*., 2012). *Buchnera aphidicola* BCc (402 genes), primary endosymbiotic minimal cell of the aphid *Cinara cedri*, has conserved all the necessary genes for its own replication, transcription and translation, as well as a simplified metabolic network to produce energy. This particular *Buchnera* strain has partially lost its symbiotic role and complements with '*Ca.* Serratia symbiotica' (772 genes), which is considered to be a co-endosymbiont (Pérez-Brocal *et al*., 2006; Gosalbes *et al*., 2008).

It is noteworthy that metabolic complementation does not exclude the minimal cell status. A minimal endosymbiotic cell can survive if it is complemented by another minimal cell, a symbionelle or by the host, which provides a function it cannot produce. This is not the case, however, of symbionelles, which are completely dependent on at least one other minimal cell.

In summary, symbionelles represent extreme cases of genome reduction in bacterial endosymbionts and cannot be considered as minimal cells. No rich heterotrophic environment can be envisioned where such symbionelles can survive without the help of an additional cell. However, other endosymbionts present a number of genes and basic functional categories that enable them to be included in the

theoretical definition of a minimal cell. Organelles and symbionelles represent, up to a point, a case of evolutionary convergence, although their evolutionary scenarios are completely different because organelles evolved before multicellularity appeared, and symbionelles evolved later, particularly in insect evolution.

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