Which is the smallest champion?

R. Maheshwari

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According to a radical theory known as serial endosymbiotic theory or SET, advanced by Lynn Margulis, the nucleated eukaryotic cell evolved over millions of years from a series of mergers involving microbes^{1,2}. A wall-less protein-synthesizing archaebacterium capable of existing in warm, sulphur-rich and anoxic environment engulfed a proteobacterium to form the nucleated cell (the first Protist), followed by its engulfment of oxygen-respiring proteobacterium to form mitochondrion, and its engulfment of a cyanobacterium to form chloroplast. Over the course of evolution both host and symbiont evolved to accommodate one another. The engulfed microbial cells transferred most of the genes to host nuclear genome for expression in the host cytoplasm wherefrom the synthesized proteins are imported by the ‘once-upon-a-time bacteria’ that now reside in the cell as endosymbionts or ‘organelles’. Since the formulation of endosymbiotic theory, comparative analysis of sequences of mitochondria and chloroplast DNA from several organisms have revealed these to be strikingly similar to those in bacteria but distinct from nuclear DNA, confirming that all the higher forms of life have descended through specific associations, alliances and mergers with microbes, supporting SET. The endosymbiont in turn may harbor smaller intracellular Protists. Where do these endosymbionts occur, how small have they become, and how small can they become in the continuing process of evolution?

Intracellular symbiosis is very common in insects that feed primarily or entirely on sap which contains sugars but is deficient in essential amino acids and vitamins. For example, the sole diet of most aphids is sap from phloem which has little, if any, of the “essential” amino acids³. Given the great diversity of metabolic capabilities in bacteria, the occurrence of bacterial endosymbionts must be one of the factors in the diversification of hexapods, leading to the famous remark by J.B.S. Haldane as Creator’s “inordinate fondness for beetles”. The intracellular bacteria are found in vacuole, in cytoplasm or within a distinct host cell called bacteriocyte⁴.

Despite endosymbionts being not culturable and hence not identifiable by traditional method, molecular methods are yielding genomic and evolutionary data for symbiont types, allowing new insight into evolutionary innovations and constraints imposed by symbiotic lifestyles, for both bacterial symbionts and their hosts. Total DNA is extracted, the 16S rDNA fragment amplified by PCR using synthetic oligonucleotide primers, purified, cloned and sequenced⁵. The resulting sequences aligned to databases, family trees constructed and the time and distance of diversification and the species identified.

Phylogenetic congruence between host and symbionts indicate their co-evolution and vertical transmission through maternal cytoplasm. The ~200 kilobase genome sequence of the intracellular bacterium *Buchnera aphidicola* has 362 protein-coding genes; this genome represents the minimal known gene set able to support cellular life, but has lost the capacity to synthesize tryptophan. This amino acid is supplied by the secondary symbiont, revealing one of the functions of aphid host – one example how genomics is providing an insight into bacterial adaptation to intracellular life. Most symbionts have a reduced genome. Compared with its symbiont partners, it has lost

R. Maheshwari (✉)
Formerly, Department of Biochemistry,
Indian Institute of Science,
Bangalore - 560 012,
India.
e-mail: ramesh.maheshwari@gmail.com

many functions. A circular genome of *Carsonella ruddii*, a bacterial endosymbiont in all species of phloem sap-feeding insects is only ~160 kb, the smallest discovered so far deriving metabolites (amino acids, nucleotides, cofactors, and lipids) from animal host⁶, beating the previous record of *Nanoarchaeum equitans* (490 kb, 552 genes) that was discovered near a hydrothermal vent off the coast of Ireland, in parasitic relationship attached to another archaeobacterium (Archaea). A theoretical minimal genome capable of independent living is 113 kb and contains 151 genes⁷.

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