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Review Article

Polyploidy in Prokaryotes: Evolutionary Advantages and Strategy for Survival under Extreme Conditions

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Abstract

Polyploidy is widespread as evident from many species of eukaryotes like animals, plants, and lower unicellular eukaryotes, but in strong contrast, prokaryotes are believed to be monoploid/ haploid and contain a single copy of the genome in the form of the small circular chromosome. There are some exceptions to monoploidy like *D. radiodurans, Borrelia sp.* etc. this phenomenon of polyploidy among these microorganisms is an evolutionary advantage, which makes them able to survive extreme conditions. With accumulating reports of the presence of polyploidy in most of the bacterial and archaeal species, it is being considered that monoploid species are small minorities among bacteria and archaea. The presence of multiple copies of the genome helps survive extreme conditions through various mechanisms which involve resistance to radiation, survival under high temperature and severe desiccation, lowering the mutation rates, intermolecular gene conversion along with the use of part of copies of the genome as a source of nutrients for short term survival and cell multiplication. Not surprisingly polyploidy is also suggested to play an important role in pathogenesis through the production of antigenic variation helping immune invasion, thus ultimately pathogenesis. Polyploid species of extremely halophilic archaea, *Halococcus sp.* are being used as model organisms to study the possibility of survival under Martian conditions and extraterrestrial travel on meteorites. It is alluring to segregate isogenic strains with shifting chromosome duplicate numbers, which would take into consideration efficiently investigating the benefits of polyploidy employing correlation of strains that are indistinguishable apart from their ploidy level.

Keywords: Prokaryotes; polyploidy; extreme conditions; Radiation resistance; Long term survival, Phosphate polymer, evolutionary advantage.

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1. INTRODUCTION

Many eukaryotic species including animals, plants, lower unicellular eukaryotes and many species of protist along with obligate human pathogen Neisseria gonorrhoeae are polyploid. The human body also shows the presence of polyploid cells in the liver and tumour, stating that polyploidy is much more widespread. Many cultivated crops like hexaploid wheat and tetraploid potato are well known to all. The presence of polyploidy in many eukaryotic species is of no wonder but the reason behind accumulating multiple copies of the genome over the single genome and its role in adaptation to various condition and effect on evolutionary success is not yet clear. Over the years everyone believed that prokaryotes especially bacteria are haploid/ monoploid and contains a single circular genome, unlike many other eukaryotic species which is mostly based on the commonly studied bacteria in laboratory-like Escherichia coli. This belief was challenged with the observation that some of the species

of bacteria like Deinococcus radiodurans [1], Borrelia and some species of Streptomyces harbours multiple copies of their typical chromosomes, but they were considered as exceptions. Deinococcus radiodurans was isolated from irradiated meat and is found to be highly resistant to X-ray irradiation and desiccation. D. radiodurans harbours 5-8 duplicates of chromosome and is along these lines can be called as oligoploid dissimilar to cells conveying more than 10 duplicates and are alluded to as polyploid [1]. Deinococcus and Halobacterium can recover total chromosomes from covering parts of seriously dispersed chromosomes, including DNA synthesis homologous and recombination, which is unimaginable for monoploid species, and in this manner, endurance in DNA harming conditions can be considered as a conspicuous developmental favourable position of the oligoploidy. Many archaeal species inhabiting hyperthermophilic environments [2] are shown to have multiple chromosome copy number, while lower mutation rates

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have been observed in Thermus thermophilus [3]. The segregation-protein based chromosome partitioning machinery is found to be less significant for the distribution of chromosomes to the daughter cells in a polyploid bacterium Thermus thermophilus HB27 which showed random and variable chromosome segregation [4]. Many haloarchaeal species are also observed to have varying chromosome copy number during various growth stages. In contrast to microorganisms inhabiting extreme conditions a commonly found gamma-proteobacteria Pseudomonas putida was shown to possess 20 chromosome copies, thus proved to be polyploid [5]. A survey of experimentally proved genome copy number of 11 species of proteobacteria has shown that only three to four are monoploid, thus disproving the general belief that monoploidy is of common occurrence in prokaryotes [5]. The correlation between chromosome copy number and cell size is observed in many prokaryotic and eukaryotic species, but it is not clear how the replication of these multiple chromosomes is regulated. Cyanobacterium Synechococcus elongatus gives away a hint about chromosome replication regulation based on the studies involving dnaA activitybased restricted replication of one or few chromosomes at the time, on the other hand, altering the activity of dnaA intrinsic ATPase through inhibition, increases the number of chromosomes replicating per cell but does not affects cell growth [6].

2. Evolutionary advantages of polyploidy and survival strategies

2.1 Resistance to radiation

Deinococcus radiodurans has long been shown to be oligopoly harbouring more than two copies of chromosomes and is highly resistant to DNAdamaging conditions like X-rays and Gamma rays than other monoploid bacteria. Deinococcus radiodurans carries four to eight genome equivalents, as suggested from DNA renaturation kinetics and terminal genetic marker redundancy calculation, emphasizing its potential significance in extreme radiation resistance [1]. Deinococcus possesses very efficient DNA repair machinery which regenerates its whole chromosome from the homologous shattered pieces of chromosomes [7]. That's why the survival rate of *Deinococcus* in such extreme conditions is very high. Similarly polyploid species of Haloarchaea i. e. Halobacterium salinarum has also been shown to have high resistance to conditions that induce double-strand DNA breaks, i.e., radiation and desiccation [8]. ionizing Like Deinococcus, Halobacterium also possesses a strong DNA repair mechanism helping to survive under such conditions. While studying the radiation resistance of Halobacterium to increasing doses of X-ray irradiation a mutant was isolated having the highest resistance to irradiation of all organisms on earth [9]. These radiation-resistant cells of Halobacterium harbor nearly 30 copies of chromosomes in its cells. The bacterium Deinococcus radiodurans is the most popular

extremophile that can endure very high introductions to parching and ionizing radiation, which break its genome into several short DNA sections which are promptly reassembled once more into the first 3.28-megabase genome [7]. Productive and exact reassembly, overarticulation of the DNA repair measure is the explanation for the bacterium endurance. At least six mechanisms are realized that could, either alone or in some mix, re-join many halfway covering chromosomal pieces, homologous recombination at the fragment non-homologous end-joining; ends; synthesisdependent- strand annealing (SDSA); Intra and interchromosomal single-strand annealing (SSA); break-induced replication; and copy choice (the switching of DNA replication from fragment to fragment). The SSA and SDSA along with the other mechanisms involve Rec-A dependent homologous recombination. Regeneration of а complete chromosome requires the synthesis of new DNA in addition to homologous recombination. This can be clarified by another system of two-stage DNA repair process named 'Extended synthesis-dependent strand annealing' (ESDSA) followed and finished by crossovers hybrids and Rec-A dependent homologous recombination [7]. ESDSA requires at least two genome duplicates and arbitrary DNA breakage. In ESDSA, chromosomal sections with overlapping homologies are utilized both as primers and as templates for rapid synthesis of complementary ssDNA, as happens in a single round multiplex polymerase chain response. freshly synthesized complementary single-stranded overhangs forms 'sticky ends' that anneal together with high accuracy, joining adjoining DNA sections into long, straight, dsDNA strands that require RecAdependent crossovers to develop into circular complete chromosomes that contain dsDNA interwoven patchworks of various DNA blocks synthesized before radiation, associated with DNA blocks synthesized after radiation. This synthesis of single-stranded DNA was proved using Immunofluorescence Microscopy. It involves the use of Monoclonal Antibody which have specificity towards the heavy analogue of Thymine that is 5-Bromo-deoxy-uracile. This antibody binds to the 5-Bromo-deoxy-uracile when incorporated in to singlestranded but not in double-stranded form. Indeed, the cells following exposure to X-ray irradiation started synthesizing single-stranded DNA. After 180 min highest peak intensity was observed in irradiated cells, showing the synthesis of single-stranded DNA, followed, and completed by RecA dependent intermolecular or intramolecular recombination to produce, mature intact copy of the chromosome. In this manner recreating in vitro the DNA recombination fix of D. radiodurans under low- fidelity conditions could give a tool for rearranging genomic sections from the entire biosphere and D. radiodurans can be viewed as a bacterial model of enduring non-dividing neurons. It rouses approaches in anti-ageing research and regenerative medication.

2.1. Survival in high temperature

Prokaryotic species are well adapted to higher temperature conditions and are dominating organisms in these environmental conditions. Many species of Thermus e. g. Thermus acquaticus [10], Thermus thermophilus [11], and other species like Thermococcus kodakarensis [2], Pyrococcus, Sulpholobus, are thermophilic. Besides six subspecies of Synechocystis were found to be oligo and polyploid, exhibiting a higher degree of resistance to temperature in addition to radiation and desiccation [12]. Thermus thermophilus an extreme thermophile was reported to harbor four to five copies of chromosomes along with an equal copy number of mega plasmid pTT27 [11]. This presence of oligoploidy may confer protection, maintenance, and repair of genomic DNA at the higher growth temperature. Besides Thermus thermophilus HB27 also shows random segregation of chromosomes [4]. Heterotrophic anaerobic thermophile Thermococcus kodakarensis is also a polyploid euryarchaeon harbouring seven to nineteen genome copies, which varies depending on the growth phase [2]. Also, a correlation between the presence of histones and polyploidy was observed in this archaeon. Many molecular mechanisms of adaptation to thermophilic conditions are known from previous studies but is there any significant role polyploidy have to play in this or not is not yet clear. Molecular adaptations to higher temperature include differential folding pattern and presence of molecular chaperonin proteins which miss-folding of proteins at elevated prevents temperature and formation of lipid monolayer which is more heat stable compared to bilayer membrane. In addition to this, many other mechanisms exist not excluding the presence of saturated fatty acidcontaining lipids in the cytoplasmic membrane, which forms a stronger hydrophobic environment than unsaturated fatty acids in mesophiles, presence in a high amount of solutes like inositol diphosphate, diglycerol diphosphate and monoglyceride in cytoplasm stabilizes against thermal degradation along with the presence of lipids having branched C40 hydrocarbon chains composed of Penta-isoprenoid compounds bonded by ether linkage which provides stability to membrane against thermal breakage and branching of the hydrocarbon chain decreases membrane fluidity.

2.2. Resistance to Desiccation

Desiccation is the condition of extreme dryness or the process of extreme drying. The presence of polyploidy enhances the resistance to desiccations but the mechanism lying behind is unknown. Polyploid cells of *Haloferax volcanii* carrying 20 copies of chromosomes were shown to have more resistance to desiccation as compared to cell carrying 2 copies [13]. Survival rates were also high comparing monoploids. In *Halobacterium salinarum* inoculated on halite crystal, it was found that even after continuous exposure of cells to 20 days of severe desiccation, survival rate does not fall much and produced a good amount of growth on a halite crystal [8]. This extreme resistance of *Halobacterium* can be seen clearly from cellular growth produced on halite crystal.

2.3. Lower Mutation rates

Mutations are the driving forces behind evolution, but the high frequency of mutation leads to the accumulation of deleterious mutations in an organism's genome. Polyploidy does not only confer resistance to DNA damaging conditions but also reduces the rate of mutation to near order of one. Using *volcanii* as a test organism Haloferax and Phosphoribosyltransferase gene as a reporter [14], it was found that the mutation rate was drastically low as compared to monoploid species. This low rate of mutation may be because of polyploidy, which allows the repair of mutated copies of chromosomes, using wild type chromosomes present in the cell, through homologous recombination. The genomic mutation rate in Thermus thermophilus was comparatively lower than mesophilic counterparts primarily because of a lower rate of base substitution which may be the result of the presence of multiple copies of genomes as reported for other Thermus thermophilus species [3].

2.4. Formation of Heterozygous cells

Heterozygous cells are generally produced and studies in laboratory conditions, but it also occurs in natural conditions. In adverse environmental conditions, polyploid cells make full use of gene redundancy, allowing some of the copies of genes to get mutated in the expectation of producing a heterozygous cell, which will have better survivability over homozygous parental cells. In this way, cells harbor two different genomes having different genes at certain loci forming a heterozygous cell e. g. heterozygous cell selection in Haloferax volcanii [15] which were able to grow as double auxotrophs on the media deficient for both Leucine and Tryptophan. The presence of heterozygous cells was also reported from Methanogenic archaea [16] and several cyanobacteria. Cells fusion act as an alternative mechanism of heterozygous cell formation in nature and it has been proved in various studies. In Haloferax volcanii it was discovered that gene exchange between various auxotrophic cells is conceivable and includes the arrangement of cytoplasmic scaffolds and in all probability the fusion of cells [17]. In a populace of Halorubrum developing in saltern, it was discovered that cells traded hereditary information from different species and taxa without any specificity towards donor cells. In this genetic exchange, linkage equilibrium was so low that, it reached nearly that of sexual reproduction. It indicates that, at least for a while, heterozygous cells form in nature. Cell fusion between the cells of different species of *Haloferax* was reported by [18]. The resulting cells were not stable, but cells with identical genome emerged, that has integrated up to 500kb of another species genome into their main genome. Another study involving Halorubrum has reported that cell clusters can be formed but, the barrier to the genetic exchange between different species is leaky. It indicates that cells fusion and thus the exchange of genetic information might occur in nature at a slow speed.

2.5. Intermolecular gene conversion

Gene conversion is an un-corresponding, intermolecular exchange of hereditary information from a benefactor cell to acceptor cells. Repair of harmed or mutated duplicates of chromosomes utilizing wild data of different chromosomes requires gene conversion. The basic idea behind intermolecular gene conversion and genome equalization is that polyploid species allows mutation in some of the genome copies in adverse environmental conditions, producing a gene or genes cassette, which is required to survive under those conditions. Once the adverse conditions get over and the situation normalizes removing the selection pressure on cells, the genome of these polyploid species gets equalize in the direction of the wild type genome, expelling the mutated copies of the genome from cells. Thus, it is predicted that under certain selection pressure the genome gets equalize in favor of the trait which is required for survival and growth then. This hypothesis has been proved in heterozygous Haloferax volcanii cells containing two different types of chromosomes [15]. In one chromosome leuB gene is present at Leu loci and in another genome trpA gene is incorporated at Leu loci by disrupting the wild type *leuB* gene. Selection for the presence of either of the gene resulted in the equalization of the genome in the direction of respective genes. Significantly without any selection pressure likewise, gene conversion prompted levelling of genome duplicates. A similar wonder has likewise been appeared to happen in methanogenic Methanococcus maripaludis archaea [16]. Methanococcus maripaludis S2 has developed anaerobically in McSe medium containing selenite, Casamino Acids, and acetic acid derivation at 37°C. For the examination of gene conversion, the as of late built M. maripaludis strain SkoD4 was utilized, which harbors various genomes that contain either the selD gene or the pacN gene at the selD locus. The heterozygote equalized its genome in the direction of the gene which was needed in that conditions for survival. In varying concentration of Puromycin the genome got equalized in the direction of genome copy containing the Puromycin resistance cassette and in absence of any selection got equalized in the direction of wild type of chromosome. Around two wild-type duplicates of the genome and above 20 pacN-containing mutated duplicates demonstrated gene conversion without any selection pressure, prompting an amassing of genomes with the local *selD* allele at the *selD* site in an exceptionally quick furthermore, proficient way. Khakhlova and Bock (2006) experimentally proved the presence of gene conversion in the chloroplast. Gene conversion gives a molecular connection between, asexual reproduction, high genome duplicate number and low mutation rates. Biased or one-sided gene

conversion go about as a component for purposeful advancement of gene families, mechanism of antigenic variation or phase variation and can assist with getting away from Muller's Rachet which tells that, "Asexual polyploid species cannot exist, because they would accumulate deleterious mutations".

2.6. Long term survival

Prokaryotes are exceptionally well at surviving over harsh and adverse conditions by employing myriads of mechanisms in addition to the formation of spores and cysts. The interesting question here is, Can prokaryotic species survive over geological times? "The chemical stability of DNA is too small to allow its survival over geological times in intact form", but "Polyploid species like Haloarchaea can survive over a geological time" in the fluid infusions of halite crystals [8], as explained in previous section Haloarchaeal cells can grow in fluid inclusions of halite crystals exposed to severe desiccation conditions. DNA damaging conditions like radiation and desiccation are nearly absent in such halite crystals which increases the chances of survival. Also, the part of the cell population sacrifices itself to keep the remaining population of cells alive. This phenomenon has been proved to happen in *E. coli* cells and known as "Programmed Cell Death" [20]. Recently a group of scientist has isolated haloarchaeal cells in a laboratory which were buried alive many geological years ago [21]. This proves the ability of Haloarchaeal cells to survive for a long time.

2.7. Survival In Extreme Conditions

Extreme conditions like extremely low water activity are very harsh for survival and growth. In such conditions rod-shaped cells divide their cells into 4-5 small spherical cells, increasing the surface area to volume ratio thereby increases nutrient exchange and survivability [22]. But the very phenomenon is not possible in monoploid species, in which only one of the spherical cells formed will receive a genome or chromosome copy. The presence of a potassium pump is another aspect of long term survival strategy in Haloarchaea [23]. These conditions like extreme radiation and extremely low water activity are considered reminiscent of harsh conditions on Mars. Haloarchaeal species able to survive in such harsh conditions are being used as model organisms to study the possibility of survival in Martian conditions. Halococcus dombrowskii cells were subjected to simulated Martian conditions, and it was found that after prolonged exposure also the survival rate of cells was high in fluid infusions in halite crystal [24]. Halococcus sp. cells endure fourteen days in space, showing that extraterrestrial travel on meteorites may be feasible for haloarchaea. Many other species of haloarchaea, Halobacterium salinarum NRC-1 Halococcus hamelinensis. Halococcus morhuae have been tested under simulated Martian conditions of extreme solar space radiation, desiccations etc. [25].

2.8. Genomic DNA as Phosphate Storage Polymer

Prokaryotes are very diverse in their nutritional requirements. There is no natural compound on the planet that cannot be degraded or used as a source of nutrition by one or other bacterial or archaeal species. Many of them stores nutrients in cells in the form of various storage granules like polyphosphate as P storage, Glycogen as starch storage and short and medium-chain polymers of lipids as Polyhydroxyalkanoate (PHA) granules which are used as a nutrient source in nutrient limiting conditions. In contrast to these storage granules, recent studies have proposed that DNA can act as a Phosphorus storage polymer. Haloferax volcanii cells growing in the medium devoid of any Phosphorus source, showed significant cellular growth. On the quantification of chromosome numbers, it was found that chromosome numbers have decreased drastically from 30 per cell to 2 per cells. A fold increase in cell density was much less than a fold decrease in chromosome numbers, these forced us to conclude that cells of Haloferax utilize genomic DNA of its own as a source of phosphorus and other nutrients [13]. One-third of the genome copies were degraded to provide phosphorus to other phosphorus-containing biomolecules like ATP, NADP⁺, phospholipids, Phosphoproteins and phosposugars. No intracellular storage of phosphorus was reported in Haloferax cells. With the increase in cell numbers, the number of ribosomes decreased, but ribosomes were distributed to offsprings and were not degraded to release phosphorus. The group of scientist involved in this study have proposed a very interesting hypothesis which tells that DNA first emerged as a phosphate storage polymer, later on, it evolved its role as genetic information storage polymer, as it was stable compared to RNA and accumulates less frequency of mutation [13].

2.10. Escape from predation

Generally larger celled protist like Amoeba and Paramecium feeds on small-celled bacteria and other prokaryotes. To escape from such a predatory situation, some of the bacteria increase their cell size to form a giant cell, which cannot be engulfed by predators. This increase in cell size is proportional to the number of copies of chromosomes present in the cell. For a giant bacterium which can grow up to a cell size of 600 µm, diffusion of transcripts from one end of the cell to another is too slow. Thus, it requires a larger number of chromosome copies. Indeed the large cell bacterium Epulopsium harbors tens of thousands of copies of its genome [26]. A similar kind of phenomenon can be predicted to operate in another large bacterium Thiomargarita namibiensis, the largest known freeliving bacteria. Its spherical cells are 100- 300 µm wide and large cells have a diameter of 750 µm [27]. Thiomargarita contains a central large vacuole that pushes its active cytoplasm and genomic contents to the periphery near the cytoplasmic membrane. Thus, the presence of polyploidy in prokaryotic cells imparts evolutionary advantages to the host cell.



Fig-1: Evolutionary advantages of polyploidy and survival strategies in extreme conditions

3. Why amplify genome?

As polyploidy is widespread, its direct significance to multicellular organisms is certainly evident from; large size, better growth and survival, adaptation to unconventional environments and tolerance to stress conditions, but in prokaryotes (bacteria and archaea) what exactly leads to amplification of genomes? Amplification of genomes might help rapid growth and cell division, cell compartmentalization, cell specialization, cellular adaptation, repair of genetic aberrations, metabolic adaptation, and support establishment of symbiotic relationship with the host organisms. Many reports hint towards the polyploidy nature of most of the symbiotic bacteria. The proteobacterial symbiont of aphid *Buchnera aphidicola* [28], reported to harbor more than 100 copies of its chromosomal genome. The observed expansion of the genome in B. aphilicola can correlate with increased demand for essential metabolites by host aphids from symbiont. In the case of Rhizobial symbiosis, it is observed that some of the rhizobia undergo genome amplification during differentiation to symbiotic form i.e. Bacteriodes [29]. Therefore, genome proliferation can be linked to selection pressure. Although genome multiplicity is not always linked to cell size, it seems to be the case in Thiomargarita [27] and Epulopiscium [26] DNA distribution in both these bacteria is quite different but, it permits functional compartmentalization and regional specialization. Peripheral localization of genomic constituents may help rapid response to local environmental changes through transcription of the required gene at a distant location in the cell and rapid transport of proteins and metabolites from the site of its synthesis to the site of action [26]. In Epulopiscium the peripheral arrangement of DNA allows for the quick growth of offsprings internally in the central active cytoplasm. A similar phenomenon is also observed in Metabacterium polyspora, [30] which is an endosporeforming bacterium.

4. Research tools to study Polyploidy in Prokaryotes

Eukaryotes being multicellular and large-sized does not hinder the determination of polyploidy or genome copy number, unlike prokaryotes. Determination of genomic multiplicity has come up as a challenge because of small cell size, lower DNA content, smaller genomes and difficulty of studying prokaryotes at the single-cell level. Despite several obstacles, the genome proliferation and copy number in various bacteria and archaea have been quantified.

4.1. Real-time quantitative PCR

The use of real-time quantitative PCR [31,32] assays has come in handy in this regard. Quantification of genome amplification depends mostly on quantification of DNA per unit cell volume and gene copy number of some genes which occurs as a single gene copy in bacterial genome i.e., ftsZ, dnaA, recA. These single-copy genes can be used to represent the unit genome of the bacterium. Along with the singlecopy genes ribosomal RNA gene can also be used to determine gene amplification in bacteria as rRNA gene is found in many copies in some of the bacterial genomes and ribosomal RNA synthesis is the ratelimiting step in many bacteria. Alternatively, any random fragments of known size can be amplified using PCR by using isolated genomic DNA as a template. The fragments are purified using preparatory agarose gel electrophoresis and can be used for real-time PCR after determination of their concentration and molecular weight [5]. Real-time quantitative PCR assay for quantification of genome amplification involves designing the set of forward and reverse primers and a probe for a unique region of single-copy genes,

followed by *TaqMan* quantitative PCR assay. The results are interpreted using suitable software and compared with the standards curve for getting a real picture of gene copy numbers.

4.2. Spectroscopic determination of ploidy level

Spectroscopic determination of the ploidy level of any bacteria involves disruption of cells to prepare the cytoplasmic extract. DNA from the cytoplasmic extract is purified and treated with RNase to free DNA from RNA contamination if any. The spectrum of pure DNA samples can be developed using photometric devices, the most common of which is, Nanodrop. Another data sample is prepared using cell densities and absorption values at 260 nm. The pure DNA photometric spectrum and cell density and absorptions values along with other parameters like absorption values of a known quantity of DNA mean molecular mass of a single base pair and Avogadro number are used to calculate the genome copy number per cell [12].

5. CONCLUSION

Accumulating results supports the view that monoploidy is not exceptions among prokaryotes and polyploidy predominates in nature, but more studies in future are needed stating the polyploid nature of prokaryotes to bolster this hypothesis. The presence of multiple copies of genome or chromosomes in cell imparts a multitude of evolutionary advantages in terms of resistance to irradiation, desiccation, lowering the mutation rates, survival for long terms in hostile environments etc. Most of the halophilic archaea are polyploid and are being used as model organisms to test their survival under Martian conditions and the possibility of interstellar travel of these archaeal species on meteorites is also being tested. The direct purpose of genome amplification or polyploidy in these tiny creatures is being evident from some of the advantages mentioned in previous sections but, many more still needs to unveil. Science is progressing at unprecedented speed but the root cause or factors leading to genome amplification in prokaryotes is still a mystery, which needs to be solved. Prokaryotes paved way for complex life on planet Earth and what we learned so far about it, most of which is credited to the study of these simpler vet more complex creature and there is a lot to be learned.

Author's contribution

Mayur G. Naitam, Dr. Minakshi Grover and Dr. Rajeev Kaushik contributed equally to the manuscript.

Declaration of competing interest

The authors hereby declare that there is no conflict of interest for authorship of the manuscript in the subject matter or materials discussed in this manuscript.

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