
Some ambiguities in evolution*

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THERE have been marked developments questioning our basic concepts of origin of land plants and later of seed habit. In the history of life on land, it was plant evolution that set the pace for progress. The first step in the process was the “greening of land”, as vascular plants colonized the terrestrial habitat. They made the land habitable for life by providing a shaded moist environment with primary productivity, which the arthropods and later vertebrates invaded.

EVOLUTION OF SEED HABIT

The next step in evolution was the emergence of seed through sexual reproduction, in which a structure like the seed developed with packed food material to give nourishment to the germinating embryo. Hitherto all known seed fossil records show an elaborate pollen trapping mechanism at the seed apex (*Cardiocarpus-ProGymnospermopsida*). Three years back, the seed-like body reported by Geitler and Rowe (1989 cited by Chaloner, 1989), which is just a few million years back than the earliest fossil seeds, shows no pollen trapping structure or evident arrangement for access of pollen to the female gametophytes, though otherwise markedly different from living gymnosperms. This fossil occupies an intermediate position between *Bensonites* and *Archaeosperma*. Where is it? Is it a missing link in the formation of seed habit? If it is so, how was pollination affected? Did the seed fall down on the soil to be fertilized by motile sperm or the pollens were trapped on the outer surface of the seed apex as pollen landed on the stigma of flowering plants? This Montagne Noire Deposit in France has raised important evolutionary questions on the origin of seed habit possibly suggesting polyphyletic rather than monophyletic of seed structure. It is a landmark in the study of ambiguities in evolution.

PLANT-ANIMAL INTERACTION

Another facet of equal importance in evolutionary history is the plant-animal interaction. In fact, the best

example of plant-animal interaction is reflected in the “insect galls”—in plants often noted in Aphids. All through the years, the development of this relationship was considered as a recent event in evolution. A discovery made recently (Moran, 1989) in fossils of Aphid host plant interaction at Eocene nearly 40 million years back, suggests the process to be of great antiquity. The Aphid (*Melaphis*)—Sumac (*Rhus*) fossil may just suggest that the complex life cycle existed during that period and these interacting life histories are the evolutionary commitments of the past. It is an index that plant-animal interaction existed much earlier than hitherto visualized.

CRYPTOGENES

Going into the fundamental material of evolution, the genes and the genetic events, new ideas have come up posing novel issues on the basic concept of the origin of mutations which have affected the plant system to a great extent (Cairns *et al.*, 1988). The usual way through which pathogenic and non-pathogenic bacteria are distinguished, is to note the sugar which they use as source of energy. *E. coli* ferments lactose, whereas the various members of the *Salmonella* and *Shigella* do not.

Certain bacterial species are, however, late fermenters of some sugars which often take a week or more to ferment on different types of sugar. For example, *Sh. sonnei* is classified as a late fermenter of lactose and *E. coli*, a late fermenter of salicin — an aromatic glucoside. Evidently, the genes coding for the appropriate enzymes were there, but they were not easily accessible. Thus bacteria have an extensive armory of “Cryptic genes” which can be called upon for the metabolism of unusual substrates under conditions of stress. The activation is through mutation—insertion, or substitution at the site as well. It is not a case of repression and derepression of genes as without mutation these cryptogenes cannot be activated. In other words, the cells have a system of keeping the genes in them as reserves to be needed under emergent situations, when they can be activated through specific mutations. How are these genes differentiated from the vast array of injurious mutations are the problems unsolved. It poses

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the problem whether evolution is anticipatory or whether the mutation can be directed.

C VALUE PARADOX

The DNA value of the entire haploid genome is termed as the "C" value. On the basis of the fact that DNA is the genetic material, the C value should show an increase corresponding to progressive complexity and differentiation in higher organisms. In nature, however, such correlation does not necessarily exist. For example, lilies have much more DNA namely, 20-300 pgs as compared to man with approximately 7 picograms. This paradoxical situation is otherwise referred to as "C Value Paradox".

It has been noted that in most cases, where such variations of DNA exist, this difference may be attributed to the repeated sequences which exist in large amount. Several genes are present in multiple copies which are termed as "repeated" sequences. Depending on the size and homogeneity, the repeats are termed as "major", "moderate" or "minor". High homogeneous repeats are also known as "satellite DNA". Only a fraction of the total amount of DNA codes for structural proteins whereas the rest is repeats—non-coding or coding for non-specific effects. Such vast amount of non-coding sequences are present in several families of flowering plants and occupy a significant portion of the total amount of DNA. In the human system, a cell contains 7.3 pgs of DNA with approximately 7.3×10^9 base pairs. Even though it can accommodate 6-7 million genes not more than 100,000 essential structural genes have been estimated. Almost 40 per cent of this DNA is repetitive of which 6-10 per cent is highly repetitive. There are also cases as in *Aspergillus nidulans* where 96-97 per cent of DNA is unique and a very small fraction is repetitive. The functions of these amplified sequences are indeed a problem unsolved.

This huge amount of DNA which has been reserved for millions of years contributes to a great extent to the genotypic differences between the species and varieties. Several centres are working on the enigmatic sequences and are trying to unravel their function. It has been demonstrated that they can be utilized as good parameters of genetic diversity (Sharma, 1983; Mukherji & Sharma, 1990). Moreover, their role in conferring adaptability under stress conditions has also been demonstrated. In any case, the conservation of such huge amount of DNA standing rigors of selection, is yet to be fully accounted for in the study of evolution.

INTRONS AND THEIR ROLE IN EVOLUTION

The repeated DNA sequences are additional and mostly conserved DNA and a fraction of such sequences

has the property of amplification and mobility. Their presence in identical sequences in different loci suggests that the dispersion is due to insertion. Evidently, in a number of instances, they have the property of mobility, capable of insertion at different loci.

The fact worth recording is that these amplified sequences which are often called non-essential are present universally in major segments of chromosomes. They are located in introns of genes which are considered as entirely non-essential. These introns are normally cut off during messenger processing. They occupy a significant portion of the gene and are present in multiple copies and capable of insertion at different sites. Introns have been found in all major groups of organisms excepting in Eubacteria.

Though considered as non-essential, these sequences are providing with valuable clues to the study of evolution. The endosymbiotic concept of the origin of cell organelles visualizes the transformation of engulfed prokaryote—either photosynthetic or non-photosynthetic bacteria into chloroplasts and mitochondria. The evidences of the prokaryotic origin of organelles is borne out in their 70s ribosome, circular DNA and stacking pattern of lamellae. However, the later discovery of intron sequences and split gene in chloroplasts and mitochondria posed problems in the acceptance of this concept. Lately, these have been located in cyanobacteria (Xu *et al.*, 1990). The homology of the introns of this group with those of chloroplasts of alga *Anabaena* suggests their relationship and presence of a common ancestor which has been maintained in their genome for at least one billion years. This homology provides further support to the endosymbiotic origin of eukaryotic organelles from prokaryotes, the evidence being provided by non-essential sequences.

These sequences thus seem to be highly conserved and even provide clues to the ancestry of higher organisms. It is known that the land plants originated from aquatic ancestors which were the inhabitants of tidal marks where sexual reproduction could be affected during high tide. Certain forms in order to escape competition established themselves on land where sexual reproduction could be affected only during rains or copious dews. As such, plants had to develop the alternative method of increase of individuals, now known or styled as the sporophyte, through the progressive sterilization of sporogenous tissue. The origin of land plants through algal ancestors though accepted, has suffered from lack of evidences, serving as connecting link between the aquatic and land plants. Just in 1990, intron sequences—so-called non-essential sequences from chloroplast of algae like *Coleochaete* and *Chara* have been homologized with those of *Marchantia* (Manhart & Palmer, 1990). Thus the long awaited gap between algae and land plants could be bridged through

such molecular techniques. The intron sequences of *Magnoliaceous* chloroplasts provide clue to their ancestry. Even the human ancestry owes its evidences to mitochondrial intron sequences.

THE RNA WORLD

Possibly, the greatest discovery in the field of molecular genetics having impact in evolution had been the unravelling of the property of RNA (Hoffman, 1991; Waldrop, 1992). It can act not only as an enzyme by itself—the ribozyme, but it can provide with the template mechanism in the same system for replication as in case of telomeres of chromosomes. All these evidences simply show that simple molecules of RNA are capable of autocatalysis and heterocatalysis. The idea is gradually gaining momentum that with the origin of life, three to four billion years back, the molecule which developed in that anaerobic dark, hot and inhospitable world, was RNA from which later forms of life, even DNA developed. Some of these ideas are undoubtedly conjectural, based principally on circumstantial evidences. But they truly provide with cogent arguments towards unravelling the mechanics of evolution.

INTERKINGDOM TRANSFER IN EVOLUTION

Lately, evidences have also been gathered suggesting the importance of these so-called non-essential amplified sequences in the interkingdom transfer of DNA. *In situ* hybridization which has emerged as a powerful tool in working out homology reveals that the inverted repeat gene sequences of *Agrobacterium* are present in the chromosomes of *Nicotiana*. In fact, sequences of *Agrobacterium* hybridize with the non-infected genome of *Nicotiana* (Furner *et al.*, 1986). In other words, at a certain stage in evolution, these microbial sequences entered into the chromosomes of *Nicotiana*. The results simply indicate that sequences in *Agrobacterium* are

homologous to sequences located in chromosomes of *Nicotiana*. Despite rigorous laws controlling fertilization and compatibility, it appears that there has been transfer of genes from lower to higher organisms under conditions of stress. Further evidences indicate that this process did not happen once but at certain periods in evolution. Several species of *Nicotiana* harbor these intron segments in different degrees. As they have occurred in different phases of evolution, they provide with good parameters of relative antiquity in geological ages. All these are amplified sequences with so-called non-essential property.

The advances of the last few years on concepts of evolution are indeed startling, their implications are far reaching, and in the very understanding of their novelty, lie the beauty and challenge in science.

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