DNA homology as a tool for determination of divergence of phanerogamic taxa

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The utility of DNA homology as a tool for determination of divergence of phanerogamic taxa is discussed. DNA-DNA hybridization values of living gymnosperms and members of primitive angiospermic families indicate highest homology between *Gnetum* and Magnoliaceae, supporting the gnetalean origin of angiosperms. Conifers like *Thuja occidentalis* show no less homology with primitive angiosperm families than do the cycads. The living monocots and gymnosperms have diverged even farther. The primitive dicotyledonous families revealed fairly high homology among themselves but the other more derived families have diverged appreciably. The DNA homology among the primitive dicots and monocots varied between 45 and 60 per cent. With respect to the more advanced families, the homology values decreased. Families at moderate levels of evolutionary advancement exhibit intermediate values when compared. The two major groups of angiosperms seem to have evolved along parallel lines from a common stock in the remote past.

Key-words—DNA homology, Angiosperms, Gymnosperms, Primitive families, Pteridophytes.

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सारांश

पुष्पोद्भिदी वर्गकों की अपसारिता के निर्धारण हेतु डी-एन-ए सजातीयता का उपयोग

एस० पी० सेन एवं डी० जी० दत्ता रॉय

पुष्पोद्भिदी वर्गकों की अपसारिता के निर्धारण हेतु डी-एन-ए सजातीयता के उपयोग की विवेचना की गई है। वर्तमान अनावृतबीजीयों एवं आद्य आवृतबीजी कुलों के सदस्यों के डी-एन-ए–डी-एन-ए संकरण के मान से **नीटम** एवं मैग्नोलिएसी में घनिष्ठ सजातीयता व्यक्त होती है जिससे यह इंगित होता है कि आवृतबीजीयों की उत्पत्ति नीटेली पौधों से हुई है। कोनिफरी पौधे जैसे **थूजा ऑक्सीडेन्टेलिस** आवृतबीजी कुलों से कम सजातीयता व्यक्त नहीं करते जैसा कि साइकेडी पौधे करते हैं। वर्तमान एकबीजपत्री एवं अनावृतबीजी और अधिक अपसारित हो गये हैं। आद्य द्विबीजपत्री कुल आपस में काफी घनिष्ठ सजातीयता व्यक्त करते हैं परन्तु अन्य और व्युत्पादित कुलों में काफी अधिक अपसारण हो गया है। आद्य एकबीजपत्रीयों एवं द्विबीजपत्रीयों में डी-एन-ए सजातीयता 45 से 60 प्रतिशत तक है। अधिक उन्नत कुलों में यह मान कम होता चला जाता है। ऐसा प्रतीत होता है कि अतीतकाल में आवृतबीजीयों के दो प्रधान समूह समानान्तर रूप से एक ही पूर्वज से विकसित हुए हैं।

CURRENT concepts of evolutionary trends are based largely on comparative biological data on the extant taxa; the available fossil evidence is fragmentary and meagre. The choice of so-called "primitive" and "advanced" characters is rather arbitrary and not always based on reliable documentary evidence. The element of bias has quite often vitiated the deductions leading to controversies which characterise evolutionary biology today. The Adansonian analysis though is free from such bias, yet it also has some limitations since it ignores the inequality of evolutionary rates. The footprints of evolution can, however, be deciphered among the conserved molecules, e.g., proteins such as cytochromes, histones, ferredoxins and plastocyanins or RNA like ribosomal RNA. Since in the ultimate analysis, DNA is the bearer of all hereditary information, it is worthwhile to look for evidence of evolutionary history in the nucleotide sequences of DNA molecules of the living descendants of the primitive forms of micro-organisms, plants and animals. While in micro-organisms like bacteria almost the whole of the genome is expressed, in higher plants only 1-2 per

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cent of the genomal DNA is transcribed (Lewin, 1985).

Consequently, the visible or detectable characters considered by biologists today are limited to the small fraction of the total genomal information, and thus, are of limited value in tracing the origin or phylogeny of any taxon. Suppressed regions of the genome include many nucleotide sequences which once coded for characters not discernible in any living forms. Therefore, DNA homology values may be more useful in tracing evolutionary trends than are comparative biological data. The abundance of repeated sequences in the highly evolved forms complicates the analysis but fortunately, we now have methods by which such sequences can be eliminated and the unique region alone can be utilized for assessing the homology of any two taxa. The success achieved so far, in relation to lower plants like fungi, bryophytes and pteridophytes has been discussed previously (Sadhukhan & Sen, 1979, 1980; Sen *et al.*, 1990). The utility of DNA homology as a tool for determination of the divergence of phanerogamic taxa is the subject of this paper.

The methodology followed has been described elsewhere (Dutta Roy & Sen, 1990). Briefly, young plants of one of the two taxa whose DNA homology is to be assessed are allowed to take up ^{32}P-orthophosphate for three days; the labelled DNA is isolated and purified by the method of Lyttleton and Petersen (1964), sheared and the repeated sequences removed according to Britten and Kohne (1968). The unique DNA fragments are then allowed to hybridize at a cot (concentration × time) value of 10^4 and the extent of hybridization determined with respect to homologous hybridization values.

Table 1—Relationship between some primitive angiosperms and living gymnosperms and pteridophytes as indicated by DNA homology (Dutta Roy & Sen, 1990)

Source of ^{32}P-labelled DNA	Source of unlabelled DNA	% DNA homology after normalising self hybridization to 100% ± SE
Michelia champaka	*Gnetum ula*	83 ± 2.18
Michelia champaka	*Ephedra foliata*	67 ± 2.15
Michelia champaka	*Cycas circinalis*	43 ± 2.13
Annona reticulata	*Gnetum ula*	72 ± 1.83
CD at p = 0.05		4.50
Ranunculus sceleratus	*Gnetum ula*	63 ± 2.17
Ranunculus sceleratus	*Epheda foliata*	53 ± 2.46
CD at p = 0.05		3.36
Cycas circinalis	*Michelia champaka*	47 ± 1.63
Cycas circinalis	*Polyalthia longifolia*	45 ± 2.94
Cycas circinalis	*Piper longum*	40 ± 2.97
Cycas circinalis	*Casuarina equisetifolia*	44 ± 2.83
Cycas circinalis	*Pandanus tectorius*	25 ± 2.93
CD at p = 0.05		5.56
Thuja occidentalis	*Michelia champaka*	55 ± 3.56
Thuja occidentalis	*Casuarina equisetifolia*	48 ± 2.16
Thuja occidentalis	*Polyalthia logifolia*	52 ± 1.63
CD at p = 0.05		2.86
Cocos nucifera	*Cycas circinalis*	21 ± 2.45
Pandanus tectorius	*Cupressus* sp	21 ± 2.16
Ophioglossum reticulatum	*Michelia champaka*	31 ± 2.09
Ophioglossum reticulatum	*Colocasia antiguorum*	21 ± 1.63
CD at p = 0.05		2.86
Adiantum sp	*Michelia champaka*	25 ± 2.16

OBSERVATIONS

Origin of Angiosperms—There are several theories concerning the origin of angiosperms. The pteridophytic origin visualizes derivation of Proto-angiosperms from a pteridophyte like *Isoetes* (Campbell, 1930) or an eusporangiate fern (Engler & Gilg, 1924). Most workers consider that the angiosperms originated from some gymnosperm groups such as Gnetales (Arber & Parkin, 1907; Wettstein, 1935; Muhammad & Sattler, 1983), Cycadales (Sporne, 1971; Meyen, 1984) or the conifers (Engler, 1989). The results of the investigations of Dutta Roy and Sen (1990) are summarised in Table 1. DNA homology of gnetaleans like *Gnetum* and *Ephedra* is highest with the Magnoliaceae among the primitive families of angiosperms. Homology of cycads with members of Magnoliaceae, Annonaceae or Casuarinaceae is much less. The conifers like *Thuja* have a greater homology with the primitive angiosperm families than *Cycas*. Among the pteridophytes the eusporangiate fern *Ophioglossum* shows a DNA homology of only 21-31 per cent with the primitive angiosperms belonging to Magnoliaceae or Araceae.

Krassilov (1977) observed that the monocots might have originated from some gymnospermous ancestors as indicated by fossil evidence in the Early Jurassic Caucacus rocks provided by *Macrotorella* which resembled monocot leaves in plication and apical convergence of veins. This view has been supported by Panigrahi (1984). The DNA homology values examined so far in this respect, however, do not exceed 46 per cent (Dutta Roy & Sen, 1990). The highest homology between monocots and dicots (Table 2) has been detected with the members of Nympheaceae and Typhaceae, the homology being

Table 2—DNA homology of some monocotyledonous and dicotyledonous taxa (Dutta Roy & Sen, 1990)

Source of ^{32}P-labelled DNA	Source of unlabelled DNA	% DNA homology after normalizing self hybridization to 100% ± SE
Piper longum	*Vallisnaria spiraloides*	50 ± 2.16
,,	*Hydrilla verticillata*	46 ± 2.09
,,	*Typha angustata*	58 ± 2.18
Argemone mexicana	*Hydrilla verticillata*	48 ± 2.00
,,	*Vallisnaria spiraloides*	46 ± 2.82
Nymphaea alba	*Typha angustata*	66 ± 2.82
,,	*Hydrilla verticillata*	55 ± 2.16

66 per cent (Dutta Roy & Sen, 1990).

DIVERGENCE OF PHANEROGAMIC TAXA

Gymnosperms

The data presented in Table 3 show that there has been considerable divergence of DNA nucleotide sequences not only among the different groups of gymnosperms but also among the members of the same group. The DNA homology between *Cycas circinalis* on one hand and *Zamia* and *Ceratozamia* on the other is 61 and 66 per cent, respectively. Corresponding values for *Araucaria* (Pinophyta) and *Ephedra foliata* are 56 and 43 per cent, respectively. The DNA homology of *Thuja occidentalis* with *Cryptomeria japonica* and *Araucaria* exceeds 70 per cent. However, *Cupressus* exhibits a lower value. *Cycas circinalis* and *Ephedra foliata* are almost equally divergent from *T. occidentalis* since 58-60 per cent of their nucleotide sequences are shared by *T. occidentalis*.

Table 3—DNA homology among some taxa of gymnosperms

Source of ^{32}P-labelled DNA	Source of unlabelled DNA	% DNA homology after normalizing self hybridization to 100% ± SE
Cycas circinalis	*Zamia* sp.	61 ± 2.13
,,	*Ceratozamia* sp.	66 ± 2.16
,,	*Araucaria* sp.	56 ± 1.63
,,	*Ephedra foliata*	43 ± 2.18
CD at p = 0.05		4.54
Thuja occidentalis	*Cryptomeria japonica*	72 ± 2.12
,,	*Cupressus* sp.	62 ± 2.19
,,	*Araucaria* sp.	75 ± 2.17
,,	*Ephedra foliata*	58 ± 2.15
,,	*Cycas circinalis*	60 ± 2.16
CD at p = 0.05		2.32

ANGIOSPERMS

Divergence of monocotyledonous taxa

Among the monocots DNA homology between *Pandanus tectorius* (Pandanaceae) is highest with *Commelina benghalensis* (Commelinaceae) and *Ananas comosus* (Bromeliaceae; Table 3). Moderate homology of the order of 58 per cent between *P. tectorius* and *Colocasia indica* (Araceae) was detected. DNA homology between *P. tectorius* on one hand and *Cocos nucifera* (Arecaceae), *Musa paradisica* (Musaceae) and *Oryza sativa* (Poaceae) on the other, is less than 50 per cent (Table 4).

Among the Arecaceae, *Cocos nucifera* shows considerable homology with two other members of the family, viz., *Borassus flabellifer* and *Areca*

Table 4—DNA homology among different members of monocotyledonous families

Source of ^{32}P-labelled DNA	Source of unlabelled DNA	% DNA homology after normalizing self hybridization to 100% ± SE
Pandanus tectorius	*Commelina benghalensis*	81 ± 2.58
Pandanus tectorius	*Ananas comosus*	78 ± 2.16
,,	*Allium cepa*	71 ± 2.82
,,	*Vanda roxburghii*	62 ± 2.16
,,	*Musa paradisica*	48 ± 2.13
,,	*Cocos nucifera*	45 ± 1.73
,,	*Oryza sativa*	42 ± 3.55
CD at p = 0.05		4.18
Cocos nucifera	*Borassus flabellifer*	87 ± 1.41
,,	*Areca catechu*	84 ± 0.82
,,	*Ananas comosus*	48 ± 2.16
,,	*Pandanus tectorius*	40 ± 2.83
CD at p = 0.05		3.16
Musa paradisica	*Colocasia antiquorum*	78 ± 2.16
,,	*Commelina benghalensis*	72 ± 2.16
,,	*Oryza sativa*	48 ± 2.82
,,	*Pandanus tectorius*	44 ± 2.68
,,	*Cocos nucifera*	42 ± 2.16
,,	*Allium cepa*	39 ± 2.16
CD at p = 0.05		4.45
Vanda roxburghii	*Colocasia antiquorum*	67 ± 2.16
,,	*Pandanus tectorius*	59 ± 2.83
,,	*Cocos nucifera*	57 ± 2.16
,,	*Musa paradisica*	55 ± 2.16
,,	*Commelina benghalensis*	47 ± 2.16
,,	*Oryza sativa*	42 ± 2.16
CD at p = 0.05		3.17

catechu. Homology values exceed 80 per cent, indicating that the family Arecaceae may be fairly homogeneous. However, DNA homology between *C. nucifera* and *P. tectorius* is only 40-45 per cent. *Ananas comosus* and *Commelina benghalensis* share only 44-48 per cent of their nucleotide sequences with those of *Cocos nucifera*.

The DNA of *Musa paradisica* exhibits highest homology with *Colocasia antiquorum* (Araceae) closely followed by *Commelina benghalensis*. The homology values exceed 70 per cent, indicating that these families are closely related. The homology of *Musa paradisica* with *Oryza sativa* and *Cocos nucifera*, however, is only moderate (42-48 per cent). *Allium cepa* (Liliaceae) has the least homology with *Musa paradisica*.

Orchidaceae, which is considered to be one of the highly evolved families of monocots, shares about two-thirds of its nucleotide sequences with Araceae as indicated by DNA homology values of *Vanda roxburghi* and *Colocasia antiquorum*. *Pandanus tectorius* and *Cocos nucifera* share about 60 per cent of their DNA nucleotide sequences with *V. roxburghii*. About half of the nucleotide sequences of *V. roxburghii* and *Allium cepa* are shared. *Oryza sativa* has the least similarity with *V. roxburghii* DNA indicating that Poaceae and Orchidaceae have diverged considerably from each other.

Divergence of dicotyledonous taxa

In the experiment described in Table 5, the DNA of one member each of seven primitive dicotyledonous families, viz., Magnoliaceae, Annonaceae, Ranunculaceae, Casuarinaceae, Piperaceae, Nymphaeaceae and Papaveraceae, moderately advanced families like Convolvulaceae, and one of the most highly advanced families, Asteraceae, was labelled with ^{32}P and hybridized with one member each of the other dicotyledonous families at different rungs of the evolutionary ladder.

DNA homology values among members of the dicot families at the extremes of the evolutionary scale range from 22 to 82 per cent. The highest homology was detected between *Ranunculus sceleratus* and *Annona reticulata* and least between *A. reticulata* and *Helianthus annuus*. *R. sceleratus* and *Michelia champaka* share 72 per cent of the nucleotide sequences, while the latter has 75 per cent of its sequences in common with those of *A. reticulata*. The three primitive angiosperm families, thus, are closely related to each other. More than 60 per cent of the unique DNA sequences of *R. sceleratus* are common with those of *Polyalthia longifolia* (Annonaceae) and *Casuarina equisetifolia* (Casuarinaceae). However, *Piper*

Table 5—DNA homology among some taxa of dicotyledonous families

Source of ^{32}P-labelled DNA	Source of unlabelled DNA	% DNA homology after normalizing self hybridization to 100% ± SE
Annona reticulata	*Ranunculus sceleratus*	79 ± 2.16
,,	*Michelia champaka*	75 ± 1.63
CD at p = 0.05		2.89
Ranunculus sceleratus	*Annona reticulata*	82 ± 1.63
,,	*Michelia champaka*	72 ± 2.16
,,	*Polyalthia longifolia*	65 ± 1.63
,,	*Casuarina equisetifolia*	62 ± 1.63
CD at p = 0.05		3.39
Nymphaea alba	*Annona reticulata*	65 ± 1.63
,,	*Piper longum*	58 ± 2.16
,,	*Trapa bispinosa*	73 ± 2.16
,,	*Helianthus annuus*	52 ± 2.16
CD at p = 0.05		4.05
Piper longum	*Annona reticulata*	48 ± 1.63
,,	*Trapa bispinosa*	44 ± 0.82
,,	*Ipomea fistulosa*	52 ± 2.45
,,	*Helianthus annuus*	56 ± 2.16
CD at p = 0.05		3.69
Ranunculus sceleratus	*Euphorbia hirta*	59 ± 1.63
,,	*Ipomea fistulosa*	56 ± 2.16
,,	*Solanum torvum*	55 ± 1.63
,,	*Vigna radiata*	52 ± 1.63
,,	*Sida rhombifolia*	51 ± 2.45
,,	*Calotropis gigantia*	52 ± 2.16
,,	*Eclipta alba*	34 ± 2.94
CD at p = 0.05		3.04
Michelia champaka	*Helianthus annuus*	37 ± 2.16
Argemone mexicana	*Michelia champaka*	61 ± 2.94
,,	*Ranunculus sceleratus*	57 ± 1.63
,,	*Ipomea fistulosa*	58 ± 2.38
,,	*Carica papaya*	51 ± 2.16
,,	*Ixora arborea*	49 ± 2.16
,,	*Helianthus annuus*	39 ± 2.94
CD at p = 0.05		4.71
Ipomea fistulosa	*Polyalthia longifolia*	68 ± 2.16
,,	*Casuarina equisetifolia*	64 ± 2.94
,,	*Ranunculus sceleratus*	52 ± 2.16
,,	*Euphorbia hirta*	62 ± 2.16
,,	*Solanum torvum*	60 ± 2.16
,,	*Vigna radiata*	58 ± 2.82
,,	*Calotropis gigantia*	55 ± 1.63
,,	*Sida rhombifolia*	53 ± 2.58
,,	*Eclipta alba*	49 ± 2.94
CD at p = 0.05		4.46
Helianthus annuus	*Leonurus sibiricus*	47 ± 2.1
,,	*Ipomea fistulosa*	43 ± 4.54
,,	*Euphorbia hirta*	41 ± 2.82
,,	*Casuarina equisetifolia*	39 ± 2.82
,,	*Polyalthia longifolia*	36 ± 0.82
,,	*Michelia champaka*	33 ± 2.16
,,	*Annona reticulata*	22
CD at p = 0.05		5.09

longum of Piperaceae shows a homology of 48 per cent with the DNA of *A. reticulata*. *Argemone mexicana* of Papaveraceae exhibits a DNA homology of 61 and 51 per cent, respectively, with *Michelia champaka* and *R. sceleratus* DNA. Surprisingly, the DNA of *Nymphaea alba* of Nymphaeaceae has shown highest homology with *Trapa bispinosa* of Onagraceae, the homology value being 73 per cent; it shares two-thirds of its unique sequences with those of *A. reticulata* and its homology with *Piper longum* (Piperaceae) DNA is 58 per cent. The relationship between *T. bispinosa* and *P. longum*, however, is no more than 40 per cent, indicating that Onagraceae has diverged more from Piperaceae than Nymphaeaceae.

The more advanced families have diverged in varying degrees from the primitive families. Thus, *Euphorbia hirta* (Euphorbiaceae), *Ipomea fistulosa* (Convolvulaceae), *Calotropis gigantea* (Asclepiadaceae), *Solanum torvum* (Solanaceae), *Vigna radiata* (Fabaceae) and *Sida rhombifolia* (Malvaceae) share about 50-60 per cent of their DNA nucleotide sequences with *Ranunculus sceleratus*. About one half of the nucleotide sequences of *Argemone mexicana* (Papaveraceae) is conserved in *Ixora arborea* of Rubiaceae. The DNA of *Ipomea fistulosa* shows highest homology with that of *Polyalthia longifolia* (Annonaceae), followed by *Casuarina equisetifolia* (Casuarinaceae), the respective values being 68 and 64 per cent.

Of the primitive families, Piperaceae has the closest DNA homology value with Asteraceae, one of the most advanced angiosperm families, the homology being 56 per cent. The homology values for *Helianthus annuus* with *Nymphaea alba, Casuarina equisetifolia, Argemone mexicana, Polyalthia longifolia, Michelia champaka* and *Annona reticulata* are 52, 39, 39, 36, 33-37 and 22 per cent, respectively. The more advanced families do not exhibit values higher than 50 per cent with respect to *H. annuus* DNA. Moderately advanced families show a DNA homology of 53-68 per cent with respect to *Ipomea fistulosa* of Convolvulaceae.

The families of dicotyledons thus, have diverged from each other in varying extents.

Divergence among lower taxonomic ranks

Considerable divergence was detected among members of lower taxonomic ranks also. Thus, among Solanaceae, DNA homology varies between 35 and 90 per cent in the tribe represented by *Solanum, Nicotiana, Lycopersicon, Physalis* and *Capsicum*. Within the genus *Solanum* the homology varies between 31 and 49 per cent (Dutta Roy & Sen, 1990; Dutta Rov *et al.*, 1984). There is considerable correspondence between DNA homology values of the non-tuberous species at the interspecific level and their crossability (Dutta Roy *et al.*, 1984).

In the Cucurbitaceae homology at the interspecific level is highest (97 per cent) between *Cucumis melo* and *C. sativus* (Pasha & Sen, 1990). At the intergeneric level Goldberg *et al.* (1972) observed a DNA homology of 51-87 per cent. Among the four genera of the subtribe Benincasineae the homology with respect to *Citrullus lanatus* and *Melothria madaraspatana* is 70-83 per cent. Among the twelve genera of Cucurbitaceae studied variation in homology has been found to be 64-89 per cent (Pasha & Sen, 1990). Cluster analysis of the homology values suggest that *Momordica charantia* and *Luffa cylindrica* probably originated from a common stock which gave rise to Benincasineae, Cucurbiteae and Trichosantheae. *Citrullus* and *Mukia* probably evolved from a stock which gave rise to *Cucumis* and *Trichosanthes* (Pasha & Sen, 1989).

In the subtribe Phaseoleae of Fabaceae homology values range between 64 and 78 per cent. Within the genus *Vicia*, five out of six species exhibit similarities of nucleotide sequences of more than 80 per cent (Chooi, 1971). *Lablab purpureus* and *Phaseolus vulgaris* seem to be equally related to *Vigna radiata* since DNA homology in both cases was 52 per cent. However, the homology between *Vigna radiata* and *Pisum sativum* (Piseae) is only 38 per cent, indicating that these two tribes have diverged considerably (Dutta Roy *et al.*, 1981; Dutta Roy, 1986).

In Poaceae DNA-DNA hybridization studies in 16 species of *Triticum* of various ploidy levels and *Aegilops squarrosa* indicated that there is a high homology between A and D genomes and that the A genomes in some species have undergone considerable modification. The g genome has also departed considerably from the A and B genomes. The common hexaploid wheat, *T. aestivum*, has a DNA homology of 90-93 per cent with respect to the DNA of the tetraploid species studied. These observations indicate that there are both intergenomic and intragenomic variations in wheat (Dutta Roy & Brahmachari, 1983; Dutta Roy, 1986).

Variations in repeated sequences

Repeated sequences are quite frequent in angiosperms (Ranjekar *et al.*, 1988). In the gymnosperms, however, they occur less frequently. These sequences vary between 42 per cent in *Ephedra foliata* and 61 per cent in *Cycas circinalis* (Dutta Roy, 1986). Among the dicots the ratio of repeated to unique sequences show a range of 0.64-

1.43. The ratio is 1.0 or more than 1.0 in all the Monocots studied except *Vanda roxburghii* where the fast repeats are the fewest (20 per cent). In *Cocos nucifera* the fast repeats are most abundant and the moderately repeated sequences occupy 20-38 per cent of the total sequences (Dutta Roy, 1986, 1990). The repeated sequences do not help much in understanding the evolutionary relationship among the angiosperms since no definite trends are observed. Polyploidy, like repeated sequences is also more frequent in pteridophytes and angiosperms than in gymnosperms. Although there is an increase in the frequency of both repeated sequences and polyploidy during evolution from the primitive unicellular to the multicellular higher vascular plants with the exception of gymnosperms (Dutta Roy, 1986), there is no definite evolutionary trend (Sen, 1985).

DISCUSSION

There is considerable agreement between above observations and current views of evolutionary trends but there are also some important differences. Both the approaches towards understanding evolutionary trends—comparative biology and DNA homology—have limitations. Major limitations are lack of knowledge regarding evolutionary and mutation rates, of the susceptibility of the genomal DNA to environmental stress, of DNA repair mechanisms, and of the variations in the factors involved in transcription and translation phenomena.

It appears that nucleotide sequences of DNA of not only different genera but also of different species of the same genus have diverged considerably. In several cases this divergence appears to be more than what a comparison of the phenotypic characters would suggest. Wilson *et al.* (1977) observed that "sequence evolution is primarily a function of time and proceeds as rapidly in phenotypically conservative creatures as in those which have changed radically in phenotype". Unfortunately, only a few conservative groups have been examined thoroughly from the point of view of the evolution of either amino acid sequence of proteins or of DNA or ribosomal RNA nucleotide sequences. It is also very difficult to estimate quantitatively and objectively the phenotypic differences between organisms (Schopf, 1978).

Wilson *et al.* (1977) have further emphasized that the sequence evolution might have proceeded independently of the rate of evolution of the phenotype in two ways: (i) a very small fraction of all evolutionary substitution in genes may be responsible for the major phenotypic changes, (ii) the most significant mutations resulting in extensive phenotypic alterations are regulatory. The former may be more operative in rapidly evolving organisms like the angiospermic herbs and less so in the arch-conservatives like the Cyanobacteria; its contribution to the total rate of substitution events would be too small to enhance appreciably the total rate of evolution of the rapidly evolved organisms. Current evidence strongly favours the view that regulatory evolution is the basis of morphological evolution.

Calculation of the rates of evolution of several so-called conserved protein molecules show wide variations. Thus, while 6-7 million years are required for a 1 per cent difference in amino acid sequence of ferredoxin and plastocyanin, 2-2.5 times this period is needed for a similar change to occur in cytochrome C, for the different histone fractions the time required varies from 8 to 400 million years (Wilson *et al.*, 1977; Ferguson, 1980). Amino acid sequence patterns of cytochrome C are largely in keeping with conventional concepts of classification and phylogeny but there are some important differences as well. According to Ferguson (1980) nucleotide differences estimated from DNA hybridization data are about 5 times higher than those inferred from amino acid sequences. This could partly be due to mutations in the third codon position of the triplet coding for the amino acid which may not affect the protein structure, and the rate of mutation in this locus may be higher than that in other positions. For multigenic characters the situation may be more complicated and the same is also true for various morphological and anatomical characters.

Appreciable homology values between "unrelated taxa" are probably due to the presence of features common to all organisms which include cytochrome C, histones, chlorophylls, RuBP carboxylase and several enzymes concerned with basic metabolism. We may add for comparison that DNA nucleotide sequence dissimilarity between man and chimpanzee is only 1.6 per cent, while that between man and galago is 28 per cent.

There is a controversy regarding whether evolution depends on elapsed time or on the number of generations passed. Wilson *et al.* (1977) have determined the amino acid sequence of twelve polypeptides belonging to 26 pairs of long and short generation length species and concluded that years are more important than generations for sequence evolution. Our studies indicate that considerable nucleotide divergence has taken place in genera and families of not only the primitive orders of plants

but also among different primitive families of both higher and lower plants.

Although we used only the unique sequences for assessment of DNA homology, the repeated sequences also contribute substantially to genome organisation. Ohno (1970) has presented evidence to show that several genes have evolved by duplication of a gene followed by mutation permitting a new functional gene to evolve, while at the same time the original unmutated gene was retained. It is now becoming increasingly evident that some of the reiterated DNA sequences may have some regulatory function. The available information concerning patterns of genome organisation with respect to the short or long period interspersion patterns does not permit a clear understanding of evolutionary trends in so far as structural complexity and functional efficiency are concerned.

Very little work has been done with respect to the conserved nature of ribosomal RNA or the ribosomal proteins of plants. This almost unexplored area, as also the nucleotide sequences of mitochondrial and chloroplastic DNA and tRNA may yield rich dividends, in view of the observations concerning organelle and tRNA homology of a few plants belonging to different taxonomic categories (Newton, 1988; Zurawski & Clegg, 1987; Mubumbila *et al.*, 1980). The development of techniques for the determination of nucleotide sequences (Sanger, 1981; Maxam & Gilbert, 1977) along with the application of restriction eulouncleases have greatly accelerated the rate of progress in this area and the day is not far when we shall know the complete nucleotide sequences of higher plant DNA molecules and their location in specific chromosomes. Determination of evolutionary or taxonomic relationship will then be a simpler proposition with much greater certainty.

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