Evolutionary Criteria in Thallophytes: A Radical Alternative

Abstract. *The classical assumptions, upon which all previous phylogenies for the lower plants (Thallophytes) have been based, are claimed to be erroneous. An alternative view, that the eukaryotic cell arose in the late Precambrian from prokaryotic ancestors by a specific series of symbioses, is referred to here. Mutually consistent phylogenies, one for the prokaryotes, another for the lower eukaryotes, can be constructed on the basis of the symbiotic theory. The resulting prokaryote phylogeny is presented here; it is claimed to be more consistent with cytological data, measured DNA base ratios, and the fossil record than the several classical partial phylogenies for Thallophytes recently published.*

Klein and Cronquist have recently assembled data relevant to the possible phylogenetic relationships among the lower organisms (*1*). It is evident from their presentation of at least 14 different, and often mutually exclusive, "partial phylogenies" (*1*, figs. 20 and 22, a and b; scheme A, B, and C, p. 26, for example) that these new data do not clarify evolutionary relationships in the group as a whole. Taxonomic schemes should help us to make predictions. When we are told that a giraffe is a mammal, we infer that the female suckles her young. Without knowing anything else about *Acer pseudoplatanus* except that it is dicotyledonous, one can deduce that it photosynthesizes and that it has true leaves, roots and stems, flowers, and many other traits. These concepts, so obvious to the great evolutionists such as Simpson (*2*), have been often ignored by many new "biochemical evolutionists" [for example (*3*)] who tend to disregard whole organisms —the objects upon whose populations selection in the natural environment acts.

A new approach to phylogeny of the Thallophytes is obviously needed, and one such approach is suggested here. In Table 1 the principles upon which it is based are compared with the conventional ones of Klein and Cronquist (*1*). They have been discussed in much greater detail elsewhere (*4*). On the basis of these alternative principles a single, unified phylogenetic tree for almost all prokaryotic and eukaryotic organisms can be devised. The basic concept of the origin of eukaryotes from prokaryotes by a series of specific symbioses is outlined in Fig. 1. Details of possible derivations of various well-known and presumably natural prokaryotic groups are shown in Fig. 2. The scheme illustrating evolution of the various eukaryotic lines has already been published. For the details of the right side of Fig. 1, see fig. 1, p. 228 of (*4*). No attempt has been made here to use any but common names. Although, no doubt, there are errors in the details of the scheme, there is no datum known to the author that contradicts the idea. This is true of both the geological record (*5*) and modern biochemical data. [For example, see (*6*) for relationships between plastids and blue-green algae; see (*7*) for relationships between bacteria and blue-green algae, and (*8*) for a possible phylogenetic status of the mitochondrion.] The fact that the DNA base ratios (*6*, *9*) can be easily superimposed on the chart (Fig. 2) lends credence to the idea that the concept is correct.

If the genus *Cyanidium* (*1*, p. 219)

Table 1. Evolutionary criteria in Thallophytes.

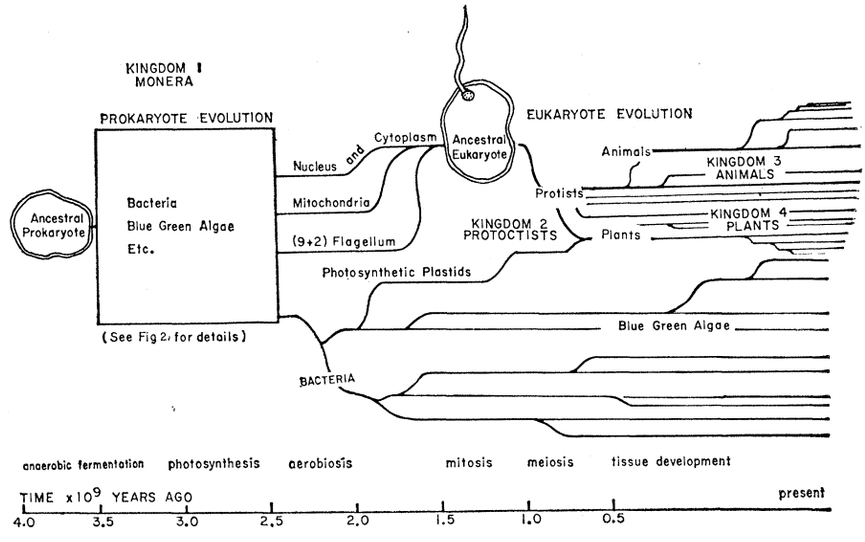
Assumptions of Klein and Cronquist (*1*)	Alternative assumptions (*4*)
1. The basic dichotomy between organisms of the present-day world is between Animals and Plants.	The basic dichotomy between organisms of the present-day world is between Prokaryotes and Eukaryotes.
2. Photosynthetic eukaryotes (higher plants) evolved from photosynthetic prokaryotes (blue-green algae, "ur-algae").	Photosynthetic eukaryotes (higher algae, green plants) and nonphotosynthetic eukaryotes (animals, fungi, protozoans) evolved from a common nonphotosynthetic (amoebo-flagellate) ancestor. There is not now, nor was there ever, an "uralga."
3. The evolution of plants and their photosynthetic pathways occurred monophyletically on the ancient earth.	The evolution of photosynthesis occurred on the ancient earth in bacteria and blue-green algae; higher plants evolved abruptly from prokaryotes when the heterotrophic ancestor (2 above) acquired plastids by symbiosis.
4. Animals and fungi evolved from plants by loss of plastids.	Animals and most eukaryotic fungi evolved directly from protozoans.
5. Mitochondria differentiated in the primitive plant ancestor.	Mitchondria were present in the primitive eukaryote ancestor when plastids were first acquired by symbiosis.
6. The primitive plant differentiated the complex flagellum, the mitotic system, and all of the other eukaryote organelles.	Mitosis evolved in heterotrophic eukaryotic protozoans by differentiation of the complex flagellar system.
7. All organisms evolved from a primitive ancestor monophyletically by single steps.	All prokaryotes evolved from a primitive ancestor by single mutational steps; all eukaryotes evolved from a primitive eukaryote ancestor by single mutational steps. Eukaryotes evolved from prokaryotes by a specific series of symbioses.
8. Morphological, biochemical, and physiological characters are useful in classification of Thallophytes.	Only total gene-based biochemical pathways resulting in the production of some selectively advantageous markers are reliable "characters" in classification; morphology is useless in most prokaryotes (Fig. 2).
Result of Foregoing Assumptions	
Nothing predicted; no consistent phylogeny possible, many predicted organisms not found, for example "uralgae"; no correlation with fossil record possible; no presentation of phylogeny as a function of time elapsed is possible.	Major biochemical pathways predicted; consistent phylogeny constructed; biological discontinuity at Precambrian boundary predicted.

Table 2. The Four Kingdom Classification modified after Copeland (*16*).

Kingdom	Examples of organisms	Approximate time of evolution (millions of years ago	Major traits that environmental selection pressures acted on to produce	Most significant selective factor
Monera	All prokaryotes; bacteria, blue-green algae, actinomycetous fungi, and so forth	Early-Middle Precambrian (3000–1000)	Photosynthesis and aerobiosis	Solar radiation, increasing atmospheric oxygen concentrations
Protoctista	All "higher" (eukaryotic) algae: green, yellow-green, red and brown, and so forth; all protozoans, phycomycetous fungi, ascomycetes, basidiomycetes and so forth	Late Precambrian Early Paleozoic (1500–500)	Classical mitosis and meiosis: obligate recombination each generation; more efficient nutrition	Depletion of organic nutrients
Animalia	Metazoa: all animals developing from zygotes	Paleozoic (600 on)	Tissue development for heterotrophic specializations	Transitions from aquatic to terrestrial and aerial environments
Plantae	Metaphyta: all green plants (above green algae)	Paleozoic (600 on)	Tissue development for autotrophic trophic specializations	Transitions from aquatic to terrestrial environments

had in fact neither mitochondria nor endoplasmic reticulum, it might have represented an inexplicable "uralgan" contradiction, for the theory (*4*) predicts that no plastid-containing organisms without mitochondria ever evolved. However, recent electron micrographs (*10*) shows that *Cyanidium* is in these respects a typical eukaryote. That some eukaryotic algae may have lost their originally symbiotic plastids, and later reestablished new symbioses with somewhat different forms of blue-green algae, is indeed to be expected. Such anomolous symbioses could include not only *Cyanidium* but also *Cyanophora paradoxa* and *Glaucocystis nostochinearum* (*11*).

The amassed data fit the proposed phylogeny as well as they do any of the several schemes presented by Klein and Cronquist (*1*). Furthermore, the symbiotic theory enables one to make many predictions [for example, that the usual pathway of the Krebs cycle and cytochrome electron transport oxidations will be found lacking in detail in prokaryotic chemo- and photoautotrophs, although present in all eukaryotic photoautotrophs; that all cells containing chloroplasts also have membrane-bound nuclei and can produce steroid derivatives; that all cells with the complex



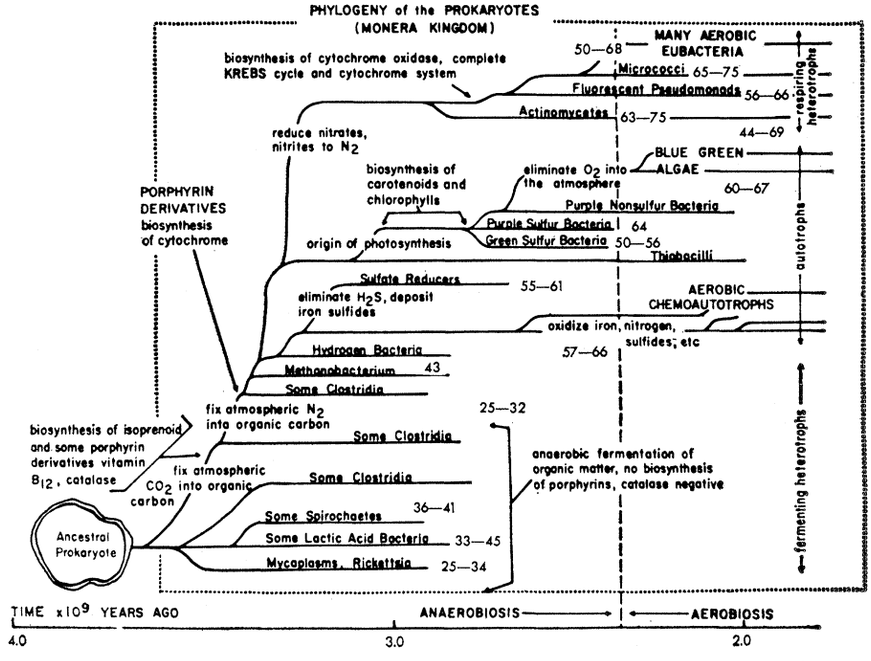


Fig. 1 (right, middle). Summary of the symbiotic theory of the origin of cells (*4*).

Fig. 2 (right, bottom). Summary diagram of the evolution in prokaryotes, based on principles discussed by Margulis (*4*). The numbers represent the ranges of DNA base ratios expressed in percentage of guanine plus cytodine.

(9 + 2) flagellum of eukaryotic cells (*12*) must also contain cytochrome oxidase and other mitochondrial enzymes (*13*); that all cells with the "higher chromosomes" seen in classical mitosis (for example, red algae and ascomycetes) had a (9 + 2) flagellated ancestor and retain the relevant DNA of that "protoflagellum" (*4*) even if they lack visible (9 + 0) centrioles and basal bodies (*14*); that all eukaryotes potentially form the colchicine-sensitive protein of the microtubules (*15*); that all eukaryotic plant cells contain at least three different nonnuclear ("satellite") DNA's; and that steroid and flavonoid derivatives will be found only in relatively young sediments—much younger than those which first contain photosynthetically reduced carbon]. By challenging the students of the enormously diverse Thallophytes to find contradictions to the theory proposed here, perhaps some appropriately focused research will be stimulated. If it proves generally acceptable, the division of living organisms into four kingdoms proposed by Copeland (*16*) logically follows (Fig. 1 and Table 2).

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Lysergic Acid Diethylamide: Mutagenic Effects in Drosophila

Abstract. *d-Lysergic acid diethylamide causes a significant increase in recessive lethal mutations in the X chromosome of* Drosophila *males after intraperitoneal injection of massive doses.*

An increase in chromosomal abnormalities has been detected cytologically in cultured leukocytes from *d*-lysergic acid diethylamide (LSD-25) users (*1*), as well as in leukocytes exposed in vitro to LSD-25 in doses ranging from 0.001 to 10 μg per milliliter of culture medium for 4, 24, and 48 hours (*2*). However, other workers detected no increase in chromosomal abnormalities in leukocyte cultures from humans exposed to recent heavy doses of LSD-25 (up to 150,000 μg) (*3*); nor could damage be detected in leukocytes of children receiving therapeutic doses of LSD (*4*). We now report on genetic damage in *Drosophila*, caused by LSD-25.

The testes of 75 virgin 6-day-old males of composition of *y* sc^{81} *In49* sc^{8}/sc^{8}*.Y.BS* were bathed (by intraperitoneal injection) in approximately 0.3 μl of a fresh solution containing 10 mg of LSD-25 per milliliter of saline (Sandoz, batch No. 43032). Since the average weight of a fly is 0.8 mg, this dose corresponds to about 4000 μg per gram of body weight and is roughly 2000 times that of the highest human dose referred to above. Only 15 of the males survived, and ten were fertile. They were mated individually to fresh, virgin females of genotype *y ct* (yellow body, cut wings) every 3 days for five broods. The offspring that hatched from brood 1 came from fully differentiated sperm cells at the time of the injection, and the later broods represented germ cells successively younger at the time of treatment. Thus the offspring from later broods come either from dividing gonial cells or germ cells in various stages of maturity during their exposure to LSD-25.

The sperm cells of each of the ten fertile males from brood 1 were tested for recessive lethals by crossing daughters of each male individually to their *y ct* brothers and looking for the absence of ct^{+} (noncut) sons. The number of sperm cells sampled from each male ranged from 28 to 47, with a mean of 37.8. Lethals were found among the progeny of six of the ten treated males, two each being found among the progeny of two of the males and one each among the other four. This distribution of lethals rules out the possibility that the lethals represent a cluster originating from a spontaneous lethal arising in the early germ track (in which case they would have all come from the same male). The distribution also indicates reasonable uniformity of mutagenic response of individual males to treatment. An overall recessive lethal mutation frequency in the X chromosome of 2.1 ± 0.7 percent was found for brood 1.

No flies were injected with saline alone because large-scale experiments in our laboratory had shown that injected saline solution has no mutagenic effect. The spontaneous lethal mutation frequency in the inbred stock furnishing the treated males was measured simultaneously, only one lethal being found among 2303 tested chromosomes.

Only mature sperm are appreciably affected by the drug so far as the production of recessive lethals is con-

Table 1. Recessive lethal mutations in the X chromosome and the loss of both markers from the Y chromosome among the offspring of *Drosophila* males according to the stage of maturity of the germ cells at the time of injection with LSD-25. The numbers in italics represent the overall mutation frequency (percent); that is, the ratio of the number of lethals to the number of chromosomes tested.

Brood	Time after injection (days)	Lethal tests			Loss of Y chromosome markers		
		Chromosomes tested	Lethals	Lethals (%)	Offspring examined (No.)	$y\,B^{+}$ (No.)	Males* (%)
1	3	378	8	2.12	808	2	0.25
2	6	238	1	0.88	530	1	.19
3	9	256	1	.39	795	1	.13
4	12	112	0	0	246	1	.41
5	15	119	0	0	471	0	0
6	18	96	0	0	209	0	0
Total		1,199	10	*0.84*	3,059	5	*0.16*
Spontaneous		2,303	1	*0.04*	23,249	9	*0.04*

* These males all proved to be sterile.