

1991

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Recommended Citation

Jensen, Uwe (1991) "Steps Toward a Natural System of the Dicotyledons," *Aliso: A Journal of Systematic and Evolutionary Botany*: Vol. 13: Iss. 1, Article 7.

Available at: <http://scholarship.claremont.edu/aliso/vol13/iss1/7>

STEPS TOWARD THE NATURAL SYSTEM OF THE DICOTYLEDONS: SEROLOGICAL CHARACTERS¹

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ABSTRACT

Nucleic acids and proteins provide useful characters for the elucidation of evolutionary relationships. Those protein molecules, e.g., legumins, which are subject to less influence of selection processes, have proven to be most valuable as diagnostic characters in phylogenetic studies. Three systematic investigations conducted using comparative serological legumin analyses are discussed as contributions aiding botanists' understanding of a natural system of classification.

Key words: serology, taxonomy, legumin, dicotyledons.

INTRODUCTION

Molecular data have proven to be valuable for the reconstruction of the natural system that represents the phylogenetic relationships of organisms including the dicotyledonous spermatophyta, for which fossil data are often fragmentary. In addition to regulatory mutations, point mutations, which are translated into amino acid exchanges within the proteins, are especially important in molecular evolution. Over extended evolutionary periods, the rate of amino acid exchange within a protein is believed to be more or less constant and the sequence dissimilarity proportional to the time of divergence between two taxa. This so-called evolutionary clock, however, does not tick equally for all DNA molecules or their translated proteins, nor regularly through time for any single macromolecule as proclaimed by proponents of the neutral theory. According to this theory (Kimura 1982) changes in molecular characters occur by chance without being affected by selection processes. This is, however, not strictly true. Proteins are produced to fulfil a specific function in a plant. For this function special properties are necessary, e.g., the three-dimensional structure of the enzymatic site, or the quaternary packing. For the rubisco (ribulose 1.5 biphosphate carboxylase-oxygenase) molecule, the molecular basis of these two properties is already evident in the prokaryotes, and remains largely unchanged during the course of evolution, i.e., over a period of ca. two billion years. Thus, an enzyme, e.g., rubisco, requires a relatively stable molecular conformation to maintain its function, which means a relatively unchanged sequence. Those mutations are incorporated that do not alter the enzymatic site or other important properties. For example, in the Compositae only ca. 50% of all positions of the plastocyanin enzyme show variability (Boulter, Gleaves, Haslett, Peacock, and Jensen 1978). Additionally, only certain amino acid exchanges can occur at these variant positions. Because of these limitations in sequence variability, the number of accomplished mutations will be few in enzymes, especially in those of low molecular weight. For angiosperm cytochrome c, the average number of variant residues is 8.6 out of ca. 111, excepting two

members of the Caryophyllidae with which this value increases to 14.7 (Ramshaw, Richardson, Meatyard, Brown, Richardson, Thompson, and Boulter 1972).

Thus, proteins which are (a) less constrained and not enzymatic, and (b) faster evolving, present more useful characters for the construction of phylogenetic trees. Such a protein, known as legumin, is the major storage protein in seeds. Presently we do not know the phylogenetic origin of this protein, but it is presumed to be associated with a storage function already present in the early land plants. In any case leguminlike proteins are produced in *Ginkgo biloba* L. seeds (Jensen and Berthold 1989), conifers (Jensen, unpubl.; Gifford 1988) and usually in all angiosperms (Jensen and Büttner 1981; Jensen and Grumpe 1983; Jensen 1984). For angiosperms the legumin $\alpha_6\beta_6$ conformational structure has been demonstrated (Pernollet and Mossé 1983).

For legumin, some mostly incomplete sequences are known, and no useful sequence comparison has yet been obtained. At this point of knowledge, therefore, the serological characters become important. Each character, i.e., an antigenic determinant (there are several such sites per polypeptide), reflects about 5 to 10 amino acids, and a single mutation which leads to a different structure for the determinant site alters its serological property. These serological properties can be compared by employing relatively simple techniques. Many investigations have revealed data which have proven to be very useful in taxonomic studies. It should be stressed that most of the plant serotaxonomic data obtained during the last four decades involved extracts from seed proteins consisting of their major protein, legumin.

The following is a review of legumin data revealed from serological comparative analyses of seed extracts. These data have enhanced our knowledge concerning the natural system of the dicotyledonous plants.

RESULTS AND DISCUSSION

The Main Phylogenetic Pathways in the Evolution of Dicotyledonous Plants

To aid in the evaluation of the origins of the dicots, the legumin of the gymnospermous *Ginkgo biloba* has been selected since this taxon shares a common ancestor with the dicots. *Ginkgo biloba* seeds are rich in legumin with only limited variation of the subunits, as revealed by SDS electrophoresis, in contrast to the legumins of many dicotyledonous species. Weak positive cross-reactions between *Ginkgo* antiserum and dicot antigens (e.g., *Aconitum* and other Ranunculaceae; *Mimulus*) have been observed. However, for comparison experiments this *Ginkgo* antiserum was not suitable because of weak cross reactivity titer. Therefore, the results from a *Magnolia* antiserum experiment (Jensen and Greven 1984) will be discussed.

If one assumes that *Magnolia* legumin represents a relatively primitive dicotyledonous legumin structure corresponding with the primitive state of the morphological characters of the genus, then serologically similar legumins should similarly indicate primitively structured legumins. Thus, they might be used as valid indicators of ancient pathways of dicotyledonous evolution. Therefore, serological cross-reactions of a *Magnolia* legumin antiserum with the legumins of many dicotyledonous taxa were made. They revealed that in addition to *Magnoliiflorae* taxa, members of two distinct dicotyledonous groups produce a legumin

similar to that found in *Magnolia* and thus might be designated as primitive members within the dicotyledons, i.e., Hamameliflorae (esp. family Betulaceae); Corniflorae (esp. families Gentianaceae, Caprifoliaceae, Cornaceae, Roridulaceae, Ericaceae, Nyssaceae, Hydrangeaceae, Valerianaceae). This discovery has been interpreted by Jensen and Greven (1984) and Frohne and Jensen (1985) for the Hamameliflorae and Corniflorae in a way which corresponds with Huber's (unpubl.) two "Hauptgruppen" hypotheses. According to Dilcher (1979), Retallack and Dilcher (1981), and Dahlgren (1983), a stock of ancient angiosperms should have existed representing the genetic potential expressed in the Hamamelididae, i.e., wind pollination, small and unisexual anemophilous flowers, as well as that of Magnoliidae, i.e., spiral arrangement of dialypetalous flowers and follicular fruits. From this stock two main dicotyledonous lines could have evolved, the Magnoliidae, as well as the Hamamelididae plus Cornidae (Fig. 1), all three representing a primitive stage in serological and several morphological characters (for the Cornidae especially vessel characters).

The Rosidae-Dilleniidae Problem

It was Rolf Dahlgren who consequently questioned the Cronquist-Takhtajan separation of the Rosidae and Dilleniidae subclasses. This separation has been further weakened by recent findings concerning the centripetalous versus centrifugous androecium (Ehrendorfer 1983). The results of our serological studies (Küchler, unpubl.; Vogel 1986; Gerstberger, unpubl.) using legumin from the Euphorbiaceae, Brassicaceae, and Tropaeolaceae, correspond with this critical interpretation. Our data (Fig. 2) have lead to two conclusions:

(I) The Dilleniidae and Rosidae sensu Cronquist and Takhtajan are probably not the most natural groupings. According to the serological data the Malviflorae and Rutiflorae seem to be particularly closely related. Chemically, this fact is reflected by the production of glucosinolates and/or cyanogenic compounds in scattered taxa, and in the occurrence of proanthocyanidins, myricetin and ellagic acid derivatives.

Setting aside the proposals arising from two generations of taxonomists and going back to Wettstein (1935), we see that he had already postulated the connections between the Malviflorae and Rutiflorae. The "Gruinales" "schließen sich zweifellos an die "Columniferen" an und dürften von dieser, beziehungsweise Vorläufern derselben abzuleiten sein" (Wettstein 1935). He also considered the differences between the "Gruinales" (=Geraniales, Tropaeolales, and Polygalales p.p. sensu Dahlgren [1983]) and the "Columniferae" (=Malvales sensu Dahlgren [1983]) as being essentially restricted to the nature of the androecium and the evolution of herbaceous habit. He considered taxa with zygomorphic flowers to be derived.

Wettstein was as well convinced that the "Terebinthales" (=Sapindales, Rutales, Polygalales p.p., Balsaminales sensu Dahlgren [1983]) were closely related to the Celastrales and Rhamnales, although he relied on the technologically flawed and dubious serodiagnostic results (Bärner 1927). Additionally Scholz (1964) in Engler's Syllabus supported the indicated relationships by the incorporation of the Euphorbiales into the Gruinales. Connections between the Tropaeolaceae (and Limnanthaceae), Euphorbiaceae, Rutales, and Sapindales are also supported by the existence of only one tenuinucellate ovule per locule and frequent occurrence

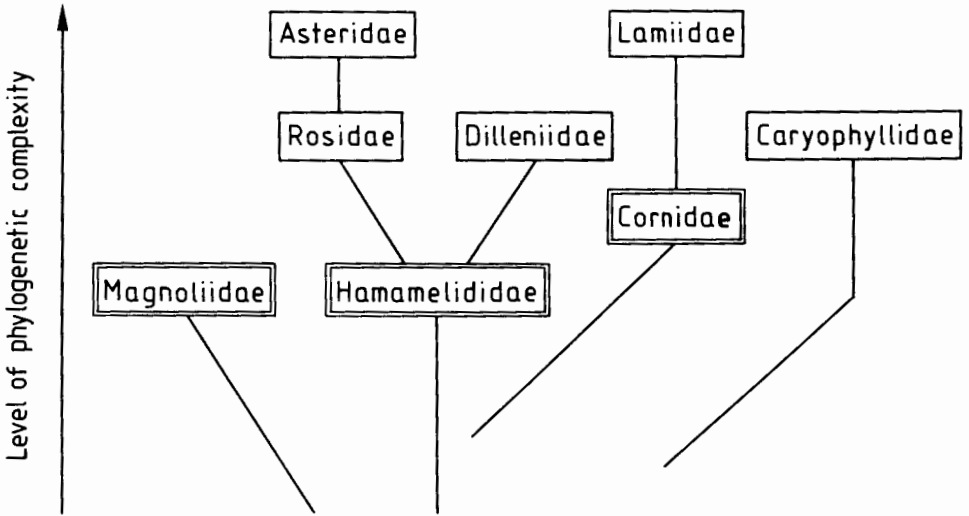


Fig. 1. Suggested phylogenetic arrangement of the subclasses of the dicotyledons. From Frohne and Jensen (1985).

of pachychalazal seeds, with the seed coat being produced from the chalaza (Huber, unpubl.).

The Violales ("Parietales"), especially the Flacourtiaceae, are considered to be a progenitor group for the Malviflorae and Rutiflorae. They possess bitegmic, crassinucellate ovules, nuclear endosperm formation, and frequently copious endosperm in the seeds. The trimeric gynoecium of the Violales is common also in the Euphorbiales and some Malvales. Crystalline phloem-protein bodies are frequent in the Violales and many Malviflorae taxa (Behnke and Barthlott 1983).

(II) None of these characters mentioned for the Violales are shared with the Capparales, although the Violales in common opinion are strictly connected to the Capparales (e.g., Dahlgren 1983), since the relationships between Capparales and Papaverales ("Rhoadales") have been surrendered. Our legumin data do not support the incorporation of the Capparales into the Violiflorae, rather they support a separate superorder "Cappariflorae." Thus, the production of glucosinolates does not indicate a closely related, monophyletic (or paraphyletic) group; rather, at least two distantly related phyletic groups are indicated. Independent genesis of glucosinolate synthesis is conceivable; it could have arisen repeatedly from the phyletically old and widespread process of cyanogenic glycoside synthesis (Rodman 1981; Gershenzon and Mabry 1983).

The Classification of the Compositae

The natural classification of the composite tribes is not sufficiently clear. Recent data regarding pollen morphology, seed morphology, and chemical constitution favor a separation into two groupings (Wagenitz 1976; Dittrich 1977; Frohne and Jensen 1985). In an older unpublished serological study, Schumacher (1966) used crude seed extracts (which included legumin) to test the protein similarities between the composite tribes. Although protein mixtures were compared and the

"Dilleniidae"

"Rosidae"

Malviflorae

- Malvales
- Urticales
- Euphorbiales
- Thymelaeales
- Rhamnales
- Elaeagnales

Violiflorae

- Violales
- Cucurbitales
- (Salicales)
- (Tamaricales)
- Capparales
- Salvadorales

Theiflorae

- Dilleniales
- Paeoniales
- (Theales)

Primuliflorae

- Primulales
- (Ebenales)

excl. Corniflorae

Rosiflorae

- (Trochodendrales)
- (Cercidiphyllales)
- Hamamelidales
- (Geissolomonatales)
- (Balanopales)
- Fagales
- Juglandales
- Myricales
- Casuarinales
- Buxales
- (Cunoniales)
- Saxifragales
- (Droserales)
- (Gunnerales)
- Rosales

Myrtiflorae

- (Haloragales)
- (Rhizophorales)
- Myrtales
- (Chrysobalanales)

Fabiflorae

- Fabales

Rutiflorae

- Sapindales
- Rutales
- (Polygales)
- Geraniales
- Balsaminales
- Tropaeolales

Santaliflorae

- Celastrales
- (Vitales)
- (Santalales)

Araliiflorae

- (Pittosporales)
- Araliales

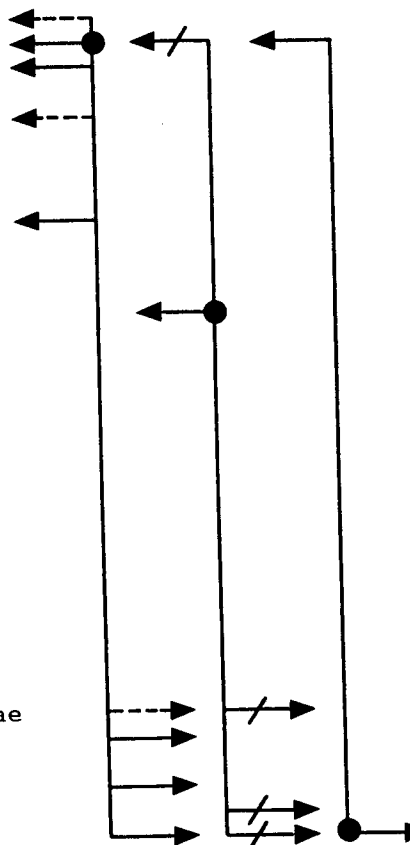


Fig. 2. The main superorders and orders of the Dilleniidae and Rosidae, following Dahlgren (1983). Data from Küchler (unpubl.) and Gerstberger (unpubl.). ● taxa used as reference systems (antisera produced), → prominent serological reactivity, -→ weak serological reactivity, -/→ definitely no serological reactivity. For those orders placed in brackets no species were tested serologically.

techniques were less sophisticated than those used today, Schumacher found the Cichorieae (Liguliflorae) not clearly separated from the other composite tribes (Tubuliflorae), and reported serological connections to the Cynareae. Boulter et al. (1978) reported similar results using plastocyanin sequence data.

Recently, H. Fischer (Fischer and Jensen 1990) studied composite tribes comparing the legumin similarities obtained from serological investigations. These studies mainly evaluated the tribe Cynareae sensu Bentham (1873). Through the use of four antibody reference systems, i.e., *Xeranthemum annuum* L., *Centaurea cyanus* L., *Silybum marianum* Gaertn., and *Echinops sphaerocephalus* L., six groups of differently reacting genera could be separated. All incorporated taxa belong to the Cynareae, while only *Lactuca* was added as a marker from another tribe. The results indicate that the Cynareae should be considered phyletically heterogeneous. The heterogeneity is best demonstrated by the observation that *Echinops* legumin has greater similarities with the legumin of *Lactuca* than with the legumin of many other Cynareae species tested. Also, within the Carlininae, *Xeranthemum* might be segregated. The subtribes Carduinae and Centaureinae, however, revealed the expected close relationship.

These investigations will be extended to other composite tribes; however, two findings should already be evaluated when developing the natural system of the Compositae:

(a) The Cynareae s.l. are heterogeneous, and the Cardueae (including the Carduinae + Centaureinae), Carlineae, and Echinopeae should be treated as separate tribes in agreement with the classifications of Wagenitz (1976), Dittrich (1977), and Takhtajan (1987). The separate position of *Xeranthemum* has to be considered independently and this has already been proposed by Cassini (1819).

(b) The Lactuceae (=Cichorieae) tribe is phenetically similar to the Cynareae s.l. tribes, as revealed by legumin comparison. This is in agreement with the plastocyanin similarities (Boulter et al. 1978), pollen data (Wagenitz 1976), and chemical and morphological data (Frohne and Jensen 1985). However, to make definitive conclusions based upon the legumin affinities, additional taxa from Compositae tribes have to be compared.

To summarize, the serological comparison of legumins is based on comparable molecules ordinarily present in all higher plants. This protein provides new and valuable data. The similarities between the legumins obtained from different taxa probably are generated by a relatively small effect of selection processes. Thus, they can be used as phylogenetic criteria to stepwise ascend the ladder leading to the development of a natural system of the dicotyledonous plants. My unforgettable friend Rolf Dahlgren has put in place many of the stable rungs upon which we are climbing. Without him, the ascent will be much more difficult, and one would feel quite lonely in the high level of taxonomic interpretations.

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FOOTNOTE

¹ Based on a presentation given at the XIV International Botanical Congress in Berlin, 1 August 1987, as part of the Rolf Dahlgren Memorial Symposium entitled "Steps toward the natural system of the dicotyledons," chaired by R. F. Thorne and H. Huber.