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Invited Article

The Embryology of Angiosperms: Its Broad Application to the Systematic and Evolutionary Study

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Embryology allows one to work with a wide array of characters (more than 50 in general) for each taxon of angiosperms. This paper, while providing a brief review of recent studies on Myrtales and associated families by me and my co-workers, discusses evidence for the general utility of embryological characters for the study of plant systematics. In particular, evidence is given that characters of seed coat anatomy may be best applied to the study of specific and sectional (and even familial) relationships, those of seed appendages as well as of integumentary morphology and histogenesis to the study of generic relationships, and other major characters to the study of familial relationships. Embryology thus provides many features that are complex and, when properly applied along with evidence from other sources, offers good indications of relationships at various taxonomic level, from the ordinal to the specific level. Despite its evident systematic value and increasing need, however, information on embryological characters is still lacking for a majority of genera, and even at the family level, data is lacking or insufficiently available for more than 30% of families.

Key words: Angiosperms — Embryology — Myrtales — Systematics.

The embryology of angiosperms, a research area encompassing investigations on virtually all the events relevant to sexual reproduction (Schnarf, 1929), has contributed much over the last century to an understanding of the structural diversity in micro- and megasporogenesis, the development of micro- and megagametophytes, the fertilization, and the development of embryo, endosperm and seed coat. At various times in the past, an attempt has been made to compile accumulated data and to clarify embryological attributes for each family (e.g., Schnarf, 1931; Davis, 1966). Thus the embryology is at present incorporated in most general publications on angiosperm systematics as one of the major sources of systematic characters (e.g., Cronquist, 1981, 1988).

The systematic value of embryological characters has often been persuasively argued (e.g., Maheshwari, 1963; Maheshwari and Kapil, 1966; Palser, 1975; Herr, 1984). In so doing, authors have usually demonstrated the utility of embryological information in clarifying the position of certain problematic families. Davis (1966)

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and Philipson (1974) have taken a broader view on assessing the systematic value of embryological characters; in particular they have attempted to show a restricted occurrence of character states in angiosperms. The most illustrative evidence for consistent occurrence of certain character states was presented by Dahlgren (1975) in his two-dimensional map, the "Dahlgrenogram", a construct further refined by Gertrud Dahlgren in the XIV International Botanical Congress at Berlin in 1987.

Despite the increasing value of embryological characters in angiosperm systematics, however, studies on embryology have apparently become progressively inactive over the last 20 years. Early in 1960s more than 150 articles on plant embryology were published a year, while early in 1980s only 20–30 papers were published a year. This may have occurred in part because most of the major easily accessible groups have already been studied, and in part because the time-consuming and difficult tasks of embryology, largely based on microtome sections and optical microscopy, are no longer fashionable and have been increasingly replaced by studies using new technologies (e.g., electron microscopy and molecular techniques). However the information obtained by such new technologies is still extremely limited, and our knowledge is still much far from sufficient to allow overall comparisons of any higher rank taxa.

Over the past six to seven years embryological studies in Myrtales and associated groups have convinced me that such investigations are still badly needed for many taxa and that the systematic position and evolutionary relationships assessed by embryological evidence cordinates well with those indicated by other available evidence. Further, even where other evidence is not yet fully available or has failed to resolve disputed systematic problems, embryological characters alone can often provide a sound basis for the resolution of such problems.

This short article is intended to demonstrate how embryological characters can be practically applied to systematic and evolutionary studies and to show which embryological character(s) is (are) most likely to be useful for the studies at a given taxonomic level. The evidence and examples presented here are principally from the results of my own continuing investigations, published over the past six-seven years (and therefore not cited in earlier review papers on embryology) and still in progress. This review may provide some aid both to professionals unfamiliar to embryology and to students who are looking for additional or supplemental approaches to their research subjects.

Characters and Character States

Embryological characters include basically all morphological characters concerned with the development of anthers, ovules and seeds insofar as they show unique character states. Since they have been well discussed in the earlier texts or review articles (e.g., Maheshwari, 1950; Davis, 1966; Palser, 1975; Herr, 1984), it seems unnecessary to describe them again here in detail; it would be sufficient to state that usually more than 50 characters may be investigated for each species. On the left side of Table 1, 48 characters and their respective character states are presented. These characters were selected for discussion on the embryology of Myrtales and associated families but should also represent well major characters for the whole of angiosperm embryology.

Technologies

The paraffin methods are still standard for the study of the embryology. Rotary-microtome sectioning of the materials that were first fixed and preserved in liquid and later replaced with paraffin-wax is the major process prior to observations (see Johansen, 1940). Sections are stained and mounted in various chemicals into permanent preparations and are observed by optical microscopy. Despite this time-consuming process, such methods allow us to examine the whole of the embryological characters, in contrast to the other methods (e.g., electron microscopy) that are applicable only to a limited number of characters as stated below.

In order to cover all the embryological characters, a full range of developmental stages of anthers, ovules and seeds must be fixed (usually in FAA [5 parts stock formalin, 5 parts glacial acetic acid, 90 parts 70 or 50% ethanol]); some emphasis must always be placed on the smallest buds, because they contain many important characters that will not be observed in later stages. It may thus take at least a few months or even a year until a complete set of materials is obtained. However, if once collected, liquid-preserved materials can be studied at any convenient time later. Pollen and seeds from herbarium sheets can also be used to look at cells in mature pollen and anatomical characters of seed.

Recently improved microscopes provided with phase contrast, Nomarsky interference, or fluorescence optics are also used for the examination of particular characters with fresh or non-sectioned materials. For instance, phase contrast and Nomarsky optics can be used for the study of ovule structure and the development of megagametophyte in cleared ovules (e.g., Herr, 1971, 1973), and perhaps for other characters also. Fluorescence microscopy has been used to examine the presence of callose during the micro- and megasporogenesis (e.g., Rodkiewicz, 1967, 1970). In addition, scanning electron microscopy is used for the study of fine surface sculpturing of seeds and other parts, and transmission electron microscopy for the study of the fine structure of gametophytic cells and metabolic changes during their development. Although these microscopies can be applied to only particular embryological characters at one time, they have contributed much to the increase of our knowledge.

Practical Applications of the Embryological Characters to the Study at Different Taxonomic Levels

When we study the embryology of a certain taxon at a certain taxonomic level, the embryological characters do not always have the same systematic value. Their systematic value may differ depending on the taxon as well as on the taxonomic level. Indeed, embryological characters consistent in one family may not be consistent in another family. Nevertheless, any family has at least some characters that are consistent in it, and those characters form a distinctive combination of embryological features. Such is also the case at the ordinal level. As far as an order comprises related families, it also has a distinctive combination of embryological features distinguishable from that of another order. On the other hand, characters that are diverse within a family may be consistent at some lower level (i.e., subfamilial, tribal, generic or sectional level), and those are useful for considering generic or specific relationships.

Table 1, indicating on the left side 48 selected embryological characters and their respective character states, indicates at which taxonomic level each character state was consistent in Myrtales and associated families. Figs. 1-4 present a character state distribution to show how Myrtales can be defined embryologically and be demarcated from associated families, and Figs. 5 and 6 show embryological features characteristic of some of the constituent families.

Application at the ordinal and the familial level

Of the 48 embryological characters selected, 24 are found to be consistent within any of the 14 consituent families of Myrtales* (though Psiloxylaceae and Strephonematoideae of Combretaceae are not yet known embryologically). Among those, eight characters provide a distinctive character state combination to Myrtales. All families (with rare exceptions) share: (1) two-celled pollen at the time of shedding; (2) a glandular anther tapetum; (3) a crassinucellate ovule; (4) a two- layered inner integument; (5) a micropyle formed by both integuments (except in Alzateaceae and Rhynchocalycaceae); (6) no or ephemeral antipodal cells; (7) a Nuclear type of endosperm formation; and (8) exalbuminous mature seed (Figs. 1-4; for details see Tobe and Raven, 1983a, 1984a). Of the above eight features six are apparently plesiomorphic, and two (i.e., features 6 and 8) apparently apomorphic. This characteristic combination of (apomorphic and plesiomorphic) features defining Myrtales was first suggested by my earlier review paper, based on rather insufficient information (Tobe and Raven, 1983a), but has been confirmed through later studies (Tobe and Raven, 1983b, 1984b, c, e, f, 1986, 1987a, b, c, d, e, 1988a).

Importantly a circumscription of Myrtales thus defined embryologically accords well with that suggested by evidence from many other sources (in particular, from wood anatomy) (Dahlgren and Thorne, 1984; Johnson and Briggs, 1984; van Vliet and Baas, 1984). Some other families, like Haloragaceae, Lecythidaceae, Rhizophoraceae and Thymelaeaceae, have also been considered to have close affinities with

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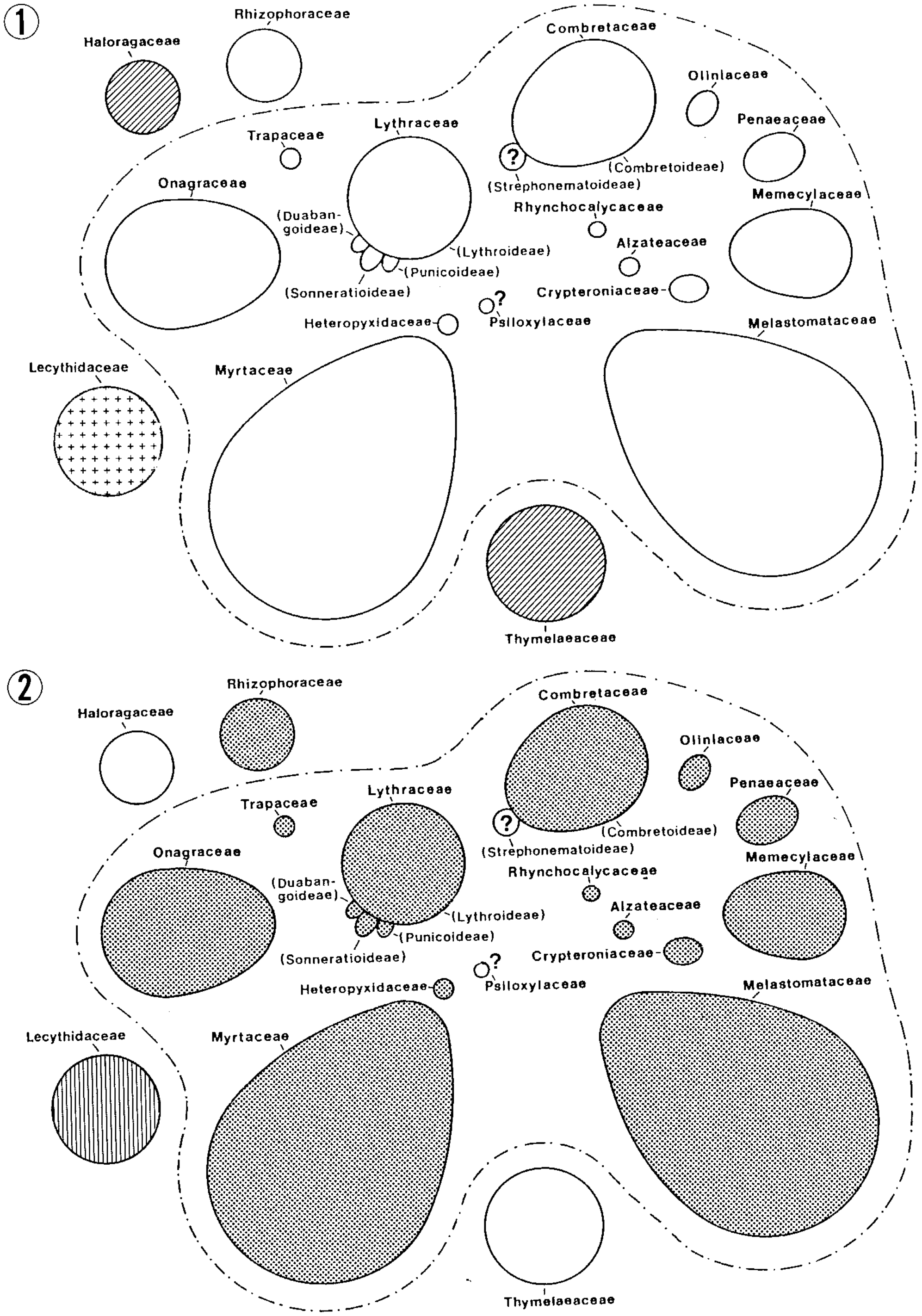
* By the consensus of the participants in the symposium on the order Myrtales, held at the XIII International Botanical Congress, Sydney, Australia, the order is defined to include the following families (with subfamilies in brackets): Onagraceae, Trapaceae, Lythraceae (Lythroideae, Sonneratioideae, Duabangoideae, Punicoideae), Oliniaceae, Combretaceae (Combretoideae, Strephonematoideae), Alzateaceae, Rhynchocalycaceae, Penaeaceae, Crypteroniaceae, Melastomataceae, Memecylaceae, Psiloxylaceae, Heteropyxidaceae and Myrtaceae (Raven, 1984).



Figs. 1 and 2. Distribution of embryological character states (apomorphies) in Myrtales and other families (two-dimensional maps, as well as those in Figs. 3–6, from Dahlgren and Thorne [1984]). 1: Amoeboid tapetum (hatch with cross); 3-celled pollen (oblique-stripe). 2: Tenuinucellate ovule (vertical-stripe); lack of, or ephemeral antipodals (dot).

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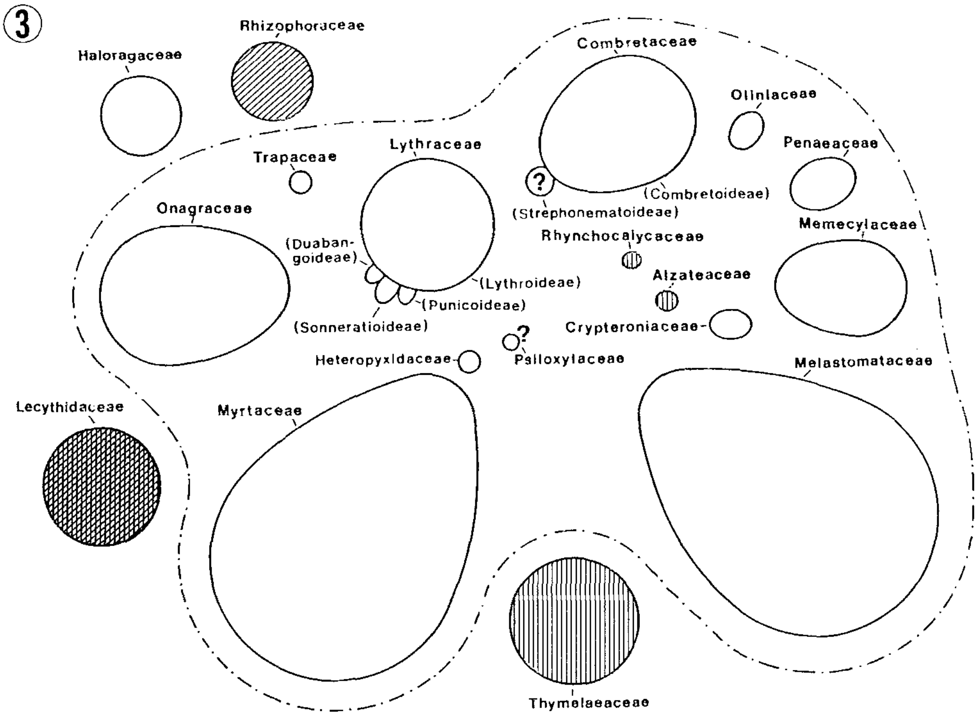

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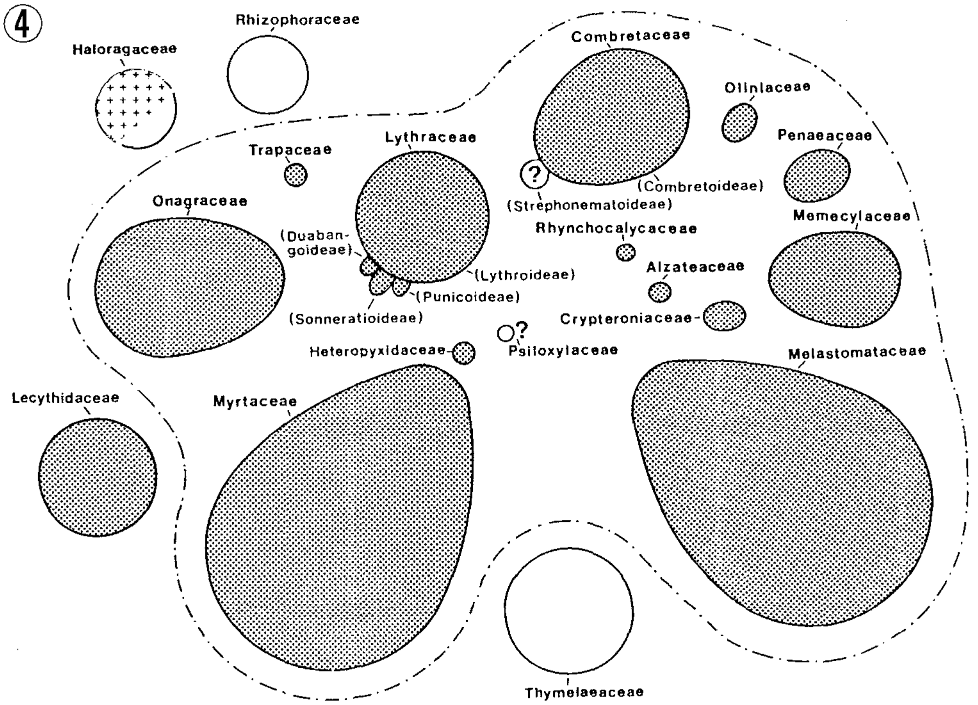

Figs. 3 and 4. Distribution of embryological character states (apomorphies) in Myrtales and other families. 3: Multi-layered inner integument (oblique-stripe); micropyle formed only by the inner integument (vertical-stripe). 4: Cellular endosperm formation (hatch with cross); exalbuminous seed (dot).

myrtalean families and even have been included in the order. However, any one of those families lacks one, two or three of the above eight embryological features. For instance, Thymelaeaceae, which have most often been assigned to Myrtales (e.g., Cronquist, 1981, 1988), clearly differ from the order in having (1) three-celled pollen; (2) a thick, three- to four-layered inner integument; (3) persistent antipodal cells; and (4) albuminous seeds (see Figs. 1–4). These differences indicate that Thymelaeaceae have no close relationships with Myrtales and can be safely excluded from the order (Tobe and Raven, 1983a).

One might suggest that the aforementioned characteristic combination of the eight embryological features can be found in other related or unrelated families. However, even if each individual feature is found elsewhere outside Myrtales, the combination of the eight features is apparently restricted among the angiosperms. When compared with Rosales *sensu* Dahlgren* (1983), an order which is considered most closely allied with Myrtales (Dahlgren and Thorne, 1984), Myrtales are obviously distinct from Rosales in lacking albuminous seeds (for embryological characters of Rosales *sensu* Dahlgren see Tobe and Raven, 1989). Rosales further differ from Myrtales in having an obturator (which is restricted to Combretaceae in Myrtales) and lacking the fibrous exotegmen (which is rather prevalent in Myrtales). Therefore, any order, like Myrtales or Rosales, seems to have a characteristic combination of embryological features distinguishable from that of other orders.

Likewise, individual families also have characteristic embryological features. Within Myrtales, for instance, Onagraceae have a unique Oenothera type embryo sac which is unknown elsewhere in angiosperm (Tobe and Raven, 1987b; see also Tobe and Raven, 1983a, for review); Trapaceae have a nucellar beak and an asymmetrical embryo with unequally developed cotyledons, both of which are unknown elsewhere in Myrtales (Tobe and Raven, 1983a, review); Crypteroniaceae are the only family that has an ephemeral nucellar tissue, so that a developing embryo sac directly borders on the inner integument by the time of fertilization (Tobe and Raven, 1983b, 1987d, e); Alzateaceae (*Alzatea* only) are characterized by having a micropyle formed by the inner integument alone and the bisporic Allium type embryo sac (Tobe and Raven, 1984f); Oliniaceae have a characteristic campylotropous ovule (Tobe and Raven, 1984b); Penaeaceae have a unique tetrasporic Penaea type embryo sac; Melastomataceae have an endothecium which does not develop fibrous thickenings in relation to a characteristic poricidal anther wall dehiscing mode (Tobe and Raven, 1983a, review); Heteropyxidaceae have a unique anther that is tetralocular at anthesis, rather than bilocular as in other Myrtales, and possesses the Allium type embryo sac (Tobe and Raven, 1987c) (for character state distribution see Figs. 5 and 6). Although I do not specify here, all other families of Myrtales also have their own

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* Rosales are rather narrowly defined by Dahlgren (1983) to include Rosaceae, Malaceae (=Pomaceae), Amygdalaceae and Neuradaceae and, with some doubt, further to include Crossosomataceae, Surianaceae and Rhabdodendraceae. Embryological data available from the former three families are summarized as those of Rosales *sensu* Dahlgren (Tobe and Raven, 1989).

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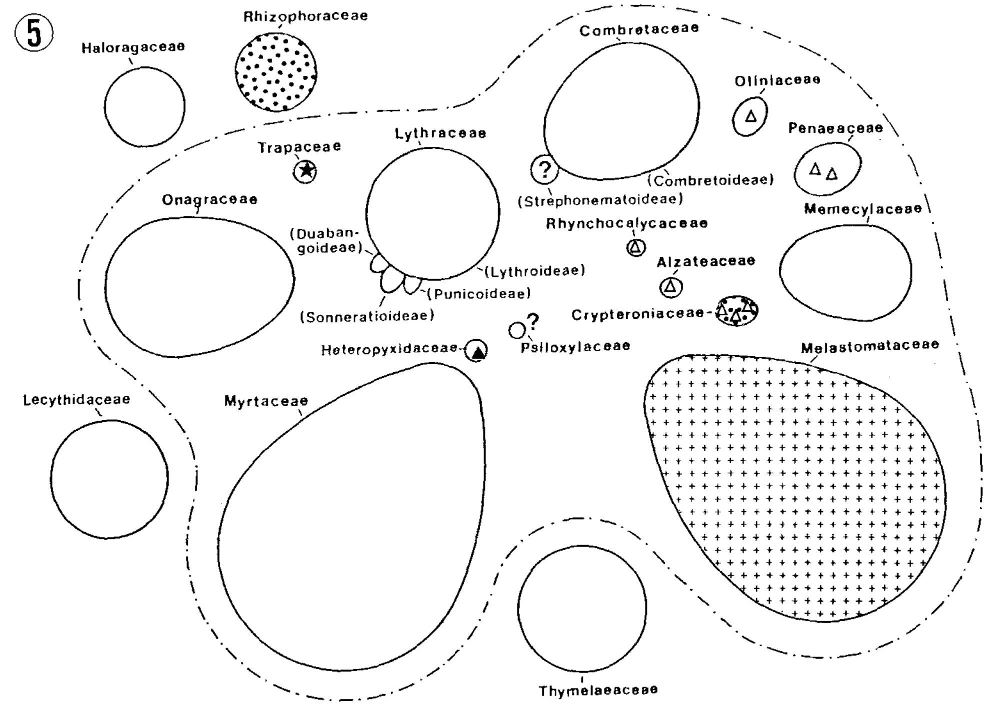

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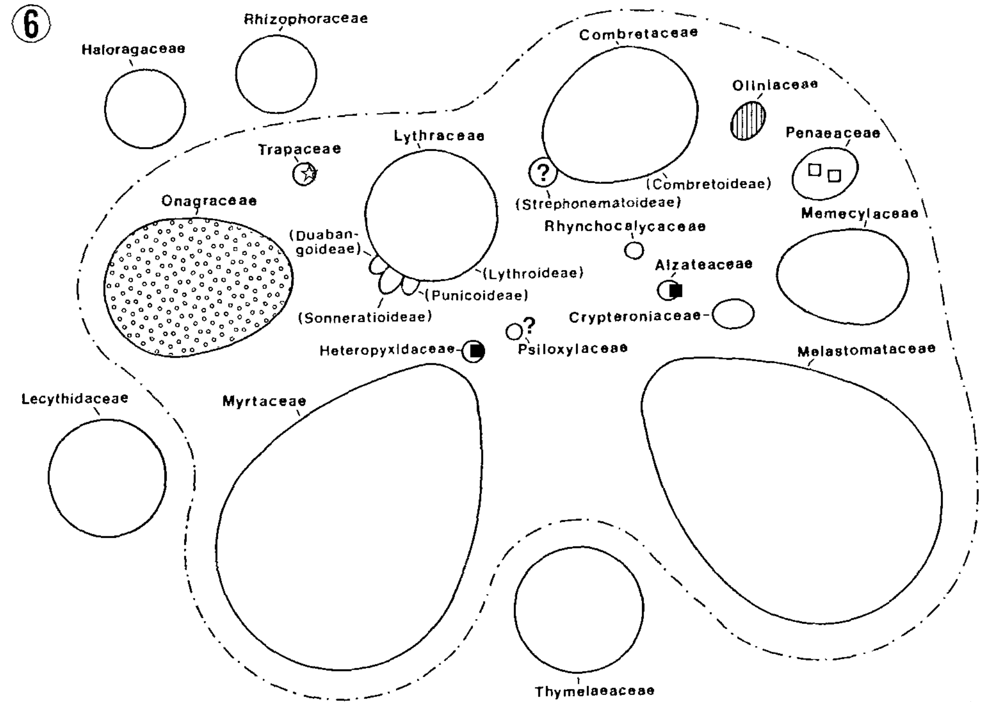

Figs. 5 and 6. Distribution of embryological character states (apomorphies) in Myrtales and other families. 5: Non-fibrous endothecium (hatch with cross); ephemeral nucellar tissue (black dot); nucellar beak (black star); ephemeral endothecium (while triangle); tetralocular anther at anthesis (black triangle). 6: Oenothera type (white dot), Penaea type (white square), and Allium type (black square) embryo sac; asymmetrical embryo with unequally developed dicotyledons (white star); campylotropous ovule (vertical-stripe).

distinct combination of embryological features.

Certain embrylogical characters may further be useful to suggest evolutionary relationships of some families in the order. The endothecium of some large families (e.g., Combretaceae, Lythraceae, Myrtaceae, and Onagraceae), like many other unrelated angiospermous families, develop fibrous thickenings. However, in some other small families (i.e., Alzateaceae, Crypteroniaceae, Oliniaceae, Penaeaceae, and Rhynchocalycaceae) the endothecium degenerates without developing fibrous thickenings (Fig. 5). Each of these families has been controversial with respect to its systematic position and has generally been considered peripheral to one or another of the large families (see their positions scattered in Fig. 1). The shared unique ephemeral anther endothecium (an obvious apomorphy), however, may suggest that they are all derived from a common ancestor of only one large family, although the latter family is uncertain in our present knowledge (for more detailed discussion see Tobe and Raven, 1984c). Thus, this embryological character, which has not attracted much attention so far, may prove valuable to the solution of disputed familial relationships.

Since embryological characters provide a characteristic embryological profile for each family, they may be of use to test the homogeneity of a vaguely defined family and, thereby, to clarify the misplacement of an unrelated segregate. Two examples are presented below, one involving Chrysobalanaceae, and another Anisophylleaceae. Traditionally Chrysobalanaceae had been assigned to Rosaceae as a tribe or subfamily; even if accepted as an independent family, Chrysobalanaceae have nearly always been placed near Rosaceae (for review of the systematic position of Chrysobalanaceae see Prance, 1972; Tobe and Raven, 1984d). However, by the study of the embryology (Tobe and Raven, 1984d) Chrysobalanaceae were found to clearly differ from Rosaceae in having a tenuinucellate ovule, a small nucellus with the tissue soon disintegrating (i.e., an ephemeral nucellar tissue), an endothelium, and an exalbuminous seed. This combination of distinguishing embryological features (all apomorphies) negates any close relationship between Chrysobalanaceae and Rosaceae, and rather suggests, with evidence from other sources, that Chrysobalanaceae be placed in Theales and probably near Theaceae.

Anisophylleaceae had long been assigned as a tribe or subfamily in Rhizophoraceae (for historical survey of the systematic position of Anisophylleaceae see Tobe and Raven, 1987f). Recent embryological studies (Tobe and Raven, 1987f, 1988b, Anisophylleaceae; Tobe and Raven, 1987a, 1988a, Rhizophoraceae), however, showed that Anisophylleaceae are clearly distinct from Rhizophoraceae. For instance, the inner integument is two-layered in Anisophylleaceae (*Poga* and *Polygonanthus*), but multi-layered in Rhizophoraceae; the nucellar tissue persists until early stages of seed development in Anisophylleaceae, but soon disintegrates in Rhizophoraceae (the latter condition, subsequently, is accompanied by the formation of an endothelium except in mangrove genera); mature seeds are exalbuminous in Anisophylleaceae but albuminous in Rhizophoraceae. These embryological differences, with support from other evidence, support a recognition of the separate family Anisophylleaceae. In latest publications, thus, a celastralean affinity is proposed for Rhizophoraceae (Dahl-

Table 1. Embryological characters, and their respective character state and consistency limit at different taxonomic levels estimated from the work with Myrtales and associated families

Characters/character states	Section	Genus	Tribe	Subfam.	Family
Anthers					
Number of sporangia: two or four, or more	+	+	+	+	+
Type of wall development: Basic, Dicotyledonous, Monocotyledonous, or Reduced	+	+	−	−	−
Epidermis: persistent or not	+	+	?	?	?
Endothecium: developing fibrous thickenings or not	+	+	+	+	+
Middle layers: persistent or not	(+)	−	−	−	−
Tapetum: glandular or amoeboid	+	+	+	+	+
Number of nuclei in a tapetal cell: one, two or more	(+)	(+)	−	−	−
Delimitation of microspores: simultaneous or successive	+	+	+	+	+
Predominant shape of microspore tetrads: tetrahedral, isobilateral, or otherwise	+	+	+	+	+
Number of cells in a mature pollen: two or three	+	+	+	+	+
Anther at anthesis: bi- or tetralocular	+	+	+	+	+
Ovules					
Degree of ovule curvature: anatropous, campylotropous, or otherwise	+	+	+	+	+
Number of integuments: one or two	+	+	−	−	−
Thickness of integuments: two- or multi-layered	+	+	−	−	−
Histological origin of outer integument: dermal or subdermal	+	+	−	−	−
Vascular tissue in integuments: present or absent	+	+	−	−	−
Micropyle: formed by inner, outer, or both integuments	+	+	+	+	+
Endothelium: formed or not	+	+	+	−	−
Archesporium: one- or multi-celled	+	+	+	+	+
Nucellus: tenui- or crassinucellate	+	+	(+)	(+)	(+)
Thickness of parietal cells: e.g., two to three or five to six cell-layered, otherwise	+	+	−	−	−
Shape of megaspore tetrads: linear, T-shaped or otherwise.	(+)	(+)	−	−	−
Type of megagametophyte development: Polygonum, Oenothera, Penaea, or other	+	+	+	+	+
Antipodal cells: persistent or ephemeral	+	+	+	+	+

Table 1. Continued

Characters/character states	Section	Genus	Tribe	Subfam.	Family
Shape of mature embryo sac: ellipsoidal, ovoid, or other	+	+	+	+	+
Nucellar cap: formed or not	+	+	+	+	+
Nucellar beak: formed or not	+	+	+	+	+
Hypostase: present or absent	+	+	−	−	−
Obturator: present or absent	+	+	?	?	?
Starch grains in embryo sac: present or absent	+	+	+	+	+
Fatty globules in megaspores and an embryo sac: present or absent	+	+	+	+	−
Seeds					
Path of pollen tube: poro-, chalazo- or mesogamous	+	+	+	+	+
Type of endosperm formation: Nuclear or Cellular	+	+	+	+	+
Endosperm: ruminate or not	+	+	+	+	+
Endosperm in mature seed: present or absent	+	+	+	+	+
Type of embryogeny: Onagrad, Solanad, or otherwise	(+)	−	−	−	−
Characteristics of suspensor: short, massive, haustorial, or otherwise	+	+	+	+	+
Embryo: di- or monocotyledonous	+	+	+	+	+
Nucellar tissue in mature seed: present or not	+	+	+	+	+
Adjuncts to seed: aril, arillode, or other	+	+	(+)	−	−
Exotesta: collapsed or papillate, otherwise	(+)	−	−	−	−
Mesotesta: sclerenchymatous or not	+	−	−	−	−
Endotesta: crystaliferous or not	+	+	+	+	+
Endotesta: thick or thin	+	−	−	−	−
Exotegmen: tracheoidal or not	+	+	+	(+)	(+)
Exotegmen: multiplied or not	+	−	−	−	−

Character state: +, always consistent; (+), consistent depending on a taxon; −, not consistent.

gren, 1988; Tobe and Raven, 1988a), while a rosalean or myrtalean affinity or an isolated position is suggested for Anisophylleaceae (Dahlgren, 1988; Tobe and Raven, 1987f, 1988b, c).

The fact that a family generally shows a characteristic combination of embryological features also makes it possible to search for affinities of genera whose familial or ordinal positions have been uncertain. The study of the embryology, as a part of a multidisciplinary study, has contributed in placing *Empleuridium*, a monotypic southwestern Cape genus (Goldblatt *et al.*, 1985), in the Celastraceae and in recognizing the Fagales as the closest allies for *Ticodendron*, the only genus of a new monotypic family from Central America (Tobe, 1989).

Application at the subfamilial, tribal and generic level

Some characters of seeds and integuments appear to be diverse within a family but consistent at the generic level (Table 1). These can be applied to the study of generic relationships.

Probably in any family it seems worthwhile to investigate whether seeds have an appendage (like an aril, arillode, wing, or other) or not; whether the number of integuments is one or two; whether integuments are multiplicative (multi-layered) or not (2-layered); whether the outer integument is of dermal origin or of subdermal origin*; whether integuments have a vascular bundle(s); and whether the integumentary tapetum (=endothelium) differentiates or not. My studies on those characters often have contributed to an understanding of generic relationships.

In Onagraceae (seven tribes and 17 genera [Raven, 1988]), the ovule is always bitegmic. The genera differ from one another with regard to whether the outer integument is of dermal, subdermal, or both dermal and subdermal origin, and four different developmental types can be recognized: (1) of subdermal origin — *Lopezia* (Lopezieae), *Fuchsia* (Fuchsieae), and *Circaea* (Circaeeae); (2) of both dermal and subdermal origin, with derivatives of the subdermal initials dividing more actively than those of dermal initials — *Oenothera* and *Stenosiphon* (Onagreae); (3) of both dermal and subdermal origin, with derivatives of the subdermal initials dividing less actively than those of the dermal initials — *Hauya* (Hauyeae) and *Calylophus* and *Gaura* (Onagreae); (4) of dermal origin — *Ludwigia* (Jussiaeeae); *Boisduvalia* and *Epilobium* (Epilobieae); and *Cammisonia*, *Clarkia*, *Gayophytum*, *Gongylocarpus*, *Heterogaura*, and *Xylonagra* (Onagreae) (Tobe and Raven, 1985). Importantly, the patterns of relationships suggested on the basis of the developmental mode of the outer integument accord closely with those derived from other lines of evidence.

The number of integuments as well as the presence or absence of vascular bundles in the integument have also been useful in supporting the distinctness of two genera in Myrtaceae, *Eugenia* and *Syzygium*, whose delimitation has been controversial (for historical survey on this controversy see Schmid, 1972). Besides the evidence from floral vascular anatomy (Schmid, 1972), the embryology provided further evidence for distinguishing *Syzygium* from *Eugenia*. In *Syzygium* the ovule has a single integument with vascular bundles, while in *Eugenia* it has two integuments neither of which has vascular bundles as in other Myrtaceae (Tobe and Raven, 1983a). The utility of

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* The terminology of "dermal origin" and "subdermal origin" for integuments follows Bouman (1974). The "dermal origin" is designated to the case in which the initiation and the subsequent growth of the integument takes place by divisions of dermal initials and their derivatives, while in the "subdermal origin" the integument is initiated by the periclinal divisions of subdermal cells and grows by the subsequent divisions of subdermal derivatives. In addition, I used the hybridized term, "both dermal and subdermal origin", for the case in which the integument is formed by derivatives of both the dermal and the subdermal initials.

these integumentary characters was also confirmed in distinguishing *Combretocarpus* (without the integumentary vascular bundles), or both *Combretocarpus* and *Anisophyllea* (with unitegmic ovules), from the rest of Anisophylleaceae (Tobe and Raven, 1987f).

In Rhizophoraceae (four tribes and 14 genera [Tobe and Raven, 1988a]), of ten inland genera four (*Blepharistemma*, *Cassipourea*, *Comiphyton* and *Crossostylis*) have arillate seeds ; three (*Anopyxis*, *Macarisia*, and *Sterigmapetalum*) have winged seeds ; and the remaining three (*Carallia*, *Gynotroches* and *Pellacalyx*) do not have any adjuncts on seeds. This distribution pattern, along with evidence from other embryological characters, explains well evolutionary relationships within Rhizophoraceae (Tobe and Raven, 1988a).

With regard to the value of the thickness of integuments, the possession of a thick, multiplicative inner integument has proved one of the important characteristics distinguishing Rhizophoraceae from Myrtales (Tobe and Raven, 1987a). In addition, within Rhizophoraceae, *Gynotroches* and *Pellaclayx* are regarded as forming a separate group in having a two-layered outer integument instead of the multilayered one found in all other genera (Tobe and Raven, 1988a ; Juncosa and Tobe, 1988).

Thus, seed and integumentary characters, as mentioned above, seem very likely to provide good evidence for intergeneric relationships in other families.

Application at the subgeneric, sectional and specific level

The seed coat, which developed from integuments, usually comprises several histologically distinguishable layers, and its structural details are usually diverse within a genus but rather uniform at the sectional level (and occasionally even at the family level).

In *Oenothera* (14 sections and 124 species) of the Onagraceae, the seed coats exhibit considerable anatomical diversity, particularly in the exōtesta, mesotesta, endotesta, and even in the exotegmen (Tobe *et al.*, 1987). My analysis indicated that the seed coat structure of *Oenothera* is section-specific : in fact, every section has its own distinct combination of seed coat anatomical features. Further, seed coat anatomy is apparently correlated with adaptations to new habitats ; consequently, consideration of these characters allows speculation about evolutionary trends in *Oenothera* (Tobe *et al.*, 1987). The usefulness of seed coat anatomy toward elucidating specific and sectional relationships has further been confirmed in the study of *Ludwigia* (Tobe and Raven, 1987b ; Tobe *et al.*, 1988), and in studies of other genera in Onagraceae now in progress. It seems likely that seed coat characters will prove useful for systematic studies at the generic level wherever they are applied.

It has also been suggested that differences in the size and shape of ovule, megasporocyte, or gametophyte may be useful for comparisons at the specific or higher level (Palser, 1975 ; Herr, 1984). Herr (1984) reviewed a few recent quantitative or statistical studies on growth of the developing female gametophyte (*Cornus*, Smith, 1975 ; *Glycine* and *Phaseolus*, George *et al.*, 1979), which involved the size measurements of the length and width of functional megaspores or embryo sac at the two-, four- and eight-nucleate stages. However, if the purpose of the study lies in the solution of disputed taxonomic relationships and not in the nature of the ovule and/or gametophyte development *per se*, I would recommend the study of seed coat structure. The latter allows the use of dried herbarium specimens and makes possible overall comparisons, which would be very difficult with developmental studies that essentially require fresh or preserved materials.

Summary Discussion and Prospects

As discussed above, the seed coat structure appears most likely to provide a reliable evidence for specific and sectional relationships within a genus ; likewise, the seed appendage as well as the integumentary morphology (i.e., number, thickness, presence or absence of vascular bundles, etc.) and histogenesis (i.e., how it is formed, dermally versus subdermally) may afford evidence for generic relationships and for grouping genera into higher rank categories within a family. Further, other major embryological characters may contribute to visualizing familial relationships and, hence, to defining orders and ordinal relationships. Embryological characters may have evolved independently many times in unrelated taxa, and therefore they have not equal systematic value. Characters relevant to the seed coat structure, for instance, may not afford evidence as clear in other taxa as in *Oenothera*. However, results from the study of *Oenothera* suggest that it is worthwhile to investigate seeds of all taxa. A continuing study on the seed coat anatomy of Onagraceae is indicating many significant differences in every genus examined. Similarly, if less variable characters are chosen, embryology can be expected to provide good indications of relationships at the family level as shown in work on the Myrtales and in many other examples presented in earlier review papers and individual papers.

One with interest in the study of embryology may question the size of samples examined. I suggest that, if the taxon is coherent, one species (preferably two for the safety) may afford sufficient information on the major embryological characteristics for each section, genus, or family. In a comparative study of the embryology in *Ludwigia* on the basis of seven sections and 11 species, which were selected so as to cover the whole variation of the genus, I did not see any essential differences within the genus except for certain differences in endotestal structure among sections. This result indicates that the study of one species per genus may be enough to show major embryological characteristics of the genus. A more critical conclusion will be drawn on this subject when my overall study of Onagraceae is finished in near future.

Although the embryology affords a wide array of characters that are undoubtedly of systematic value at various taxonomic levels, many families and genera are still unknown embryologically or known only to a limited degree. In particular, information on integuments and seed coats in individual genera is extremely limited, with information totally lacking in a majority of genera. With regard to major characters that are less diverse within a family, Johri (pers. comm. in 1988) suggested that 58 families (following Melchior, 1964) are unknown. Beyond this, many other families that have already been studied to some extent often lack information on some significant characters or on important genera (which take a key position for understanding familial characteristics). I would estimate that more than 30% of the families (173 of 467 families *sensu* Dahlgren ([1983]) need a thorough or supplemental study.

The study of embryology is apparently changing from a purely descriptive phase to one involving synthesis; the latter involves not only compiling the accumulated information, but a critical evaluation of characters. Through the process of synthesis, it will become apparent what families or genera and what characters need further investigation. Effective selection of species for investigation, together with an (re-) evaluation of synthesized data, seems to be one of the best ways to visualize the whole picture of systematic relationships among the angiosperms or among any lower rank categories.

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