Embryology of Onagraceae (Myrtales): characteristics, variation and relationships

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Abstract

Tobe, H.[1] and Raven, P.H.[2] ([1]Department of Natural Environmental Sciences, Faculty of Integrated Human Studies, Kyoto University, Kyoto 606, Japan; [2]Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166, U.S.A.) 1996. Embryology of Onagraceae (Myrtales): characteristics, variation and relationships. Telopea *6(4): 667–688.* Here we report features of the embryology of 14 genera of Onagraceae, as a companion study to our earlier report on *Ludwigia* alone. We found that the 4-nucleate *Oenothera* type embryo sac that sharply distinguishes Onagraceae from all other Myrtales is common to all genera. Shared features of the nucellus and seed coat structure, however, indicate that Onagraceae more closely resemble Lythraceae than other Myrtalean families. Differences between onagraceous genera are found in 1) the mode of anther wall formation (the Basic or the Monocotyledonous type), 2) the number of cells in the ovule archesporium (one-celled or multi-celled), 3) the nature of early development of the inner integument (retarded or not retarded), and 4) the thickness of the parietal tissue in the nucellus (thin or thick). Based on comparisons in these and other embryological characteristics, we have concluded that: 1) *Ludwigia* (Jussiaeeae) differs sharply from the rest of the family in having a one-celled archesporium in its nucellus; 2) *Hauya* (Hauyeae) and eight of the genera of Onagreae (except *Gayophytum*) closely resemble one another but differ from the other genera of the family in their markedly thick parietal tissue in the nucellus; 3) *Gayophytum*, unlike other Onagreae, resembles *Epilobium* (now including *Boisduvalia*) in having retarded early development of its inner integument and in having thin parietal tissue; 4) *Clarkia heterandra* (formerly segregated as the monotypic genus *Heterogaura*) differs from other species of *Clarkia* and from other Onagreae (except *Gayophytum*) in its nucellar histology.

Introduction

Onagraceae are a well-defined plant family, comprising seven tribes, 16 genera and about 650 species (Raven 1979, 1988; Hoch et al. 1993). The family belongs to the order Myrtales (Dahlgren & Thorne 1984; Johnson & Briggs 1984; Chase et al. 1993), but is quite isolated, marked as monophyletic by at least five autapomorphies (for review, see Raven 1988). Leaf, wood and floral anatomy have been studied extensively; chromosome numbers are known for most taxa and chromosome morphology for all major groups; and breeding systems and pollinators, flavonoids and palynology have been investigated for much of the family (see Raven 1988; Hoch et al. 1993). Recent molecular analyses of relationships in the family, summarized in Conti, Fischbach & Sytsma (1993), while not entirely consistent with one another, nevertheless have provided phylogenetic models within which to examine comparative data from other sources.

Regarding the embryology of the family, about 100 publications are available from a bibliography compiled by Davis (1966: for publications until 1965) and Nagendran & Dinesh (1989: for articles published between 1965–1985). Most of the works published from the 19th century to the middle of the 20th century had described micro- and megasporogenesis and megagametogenesis (i.e., embryo sac formation) using light microscopy. In these works, the distinctive pattern of megasporo- and megagametogenesis named the 'Oenothera' type, which was reported by Geerts (1908) in *Oenothera glazioviana* (*'O. lamarckiana'*) for the first time, was confirmed in 12 of the 16 genera. More recently, many studies have used fluorescence or transmission electron microscopy to investigate

the megasporogenesis of *Oenothera* and *Epilobium* in relation to polarity and the competition between megaspores in a tetrad, or their megagametogenesis to form the 4-nucleate Oenothera type embryo sac. Until recently, relatively little attention has been paid to other embryological characters, and many of more than 50 characters that we discuss in this paper have remained unstudied, including such features as the development of anthers, ovules (the integuments and nucellus in particular) and seeds. We have presented analyses of certain embryological characters in the whole family, specifically, on the histogenesis of integuments (Tobe & Raven 1985) and on the divided (or septate) sporogenous tissue of anthers (Tobe & Raven 1986a). Subsequently, we examined some 40 embryological characters in 11 species of *Ludwigia*, representing seven of its 23 sections (Tobe & Raven 1986b). As a result, except for the above characters of the anther and integuments, we now can summarize the present level of knowledge as follows: *Ludwigia* is thoroughly known; *Oenothera*, *Clarkia* (now including *Heterogaura heteranda*; Lewis & Raven 1992), *Chamerion* and *Epilobium* (now including *Boisduvalia*; Hoch & Raven 1992) are relatively well known; *Circaea*, *Lopezia*, *Camissonia*, *Gayophytum* and *Stenosiphon* are known to a limited degree; and *Fuchsia*, *Gongylocarpus*, *Hanya*, *Xylonagra*, *Calylophus* and *Gaura* are little known or unknown (for references to individual genera, see footnotes in Tables 2–4).

The purpose of this paper is to clarify embryological attributes of all genera of Onagraceae in order to provide a basis for comparison with other families and within the family, and, on the basis of additional embryological evidence, to discuss familial and generic relationships. For this purpose we have investigated one or more species of 14 onagraceous genera (Table 1). Subsequent to the specimen examination and data collection for this study, it has been demonstrated that *Chamerion* should be segregated from *Epilobium* (Baum, Sytsma & Hoch 1994; Hoch, unpublished data). No collection of *Chamerion* was included in our specimens, however, there are several reports for it in the literature (Lebègue 1948b, among others). Because there is no evidence of embryological differences between these two groups in the available reports, we have not treated *Chamerion* separately in this report. Our previous study of *Ludwigia* (Tobe & Raven 1986b) indicated that most embryological features (except for those of embryogenesis and seed coat anatomy) do not vary within a genus. This relationship allows us to use one or a few species as representative of the general embryological features of each genus in the absence of other information. We have also incorporated previously published information about embryology of Onagraceae, evaluating it and presenting it along with our own results.

Materials and methods

Thirty-three species representing 14 genera were investigated. All three major components of embryology — i.e., anthers, ovules and seeds — were examined in each species for which sufficient material was available (Table 1). Samples of flower buds and fruits in various stages of development were fixed in FAA (5 parts stock formalin; 5 parts glacial acetic acid; 90 parts 70% ethanol). Observations were made using serial microtome sections, except that the number of cells in a mature pollen grain was observed using whole pollen grains stained with 1% aceto-carmine (Tobe & Raven 1984). Methods for preparing microtome sections are presented elsewhere (Tobe & Raven 1986b).

We have made a sufficient number of observations to determine the type of embryogenesis in only a few genera, although some features of embryogenesis are reported for most of our samples. Likewise, we report here only limited information about seed coat structure, which nearly always varies within individual genera (e.g., *Oenothera*, see Tobe, Wagner & Chin 1987; *Ludwigia*, see Tobe & Raven 1986b; Tobe, Raven & Peng 1988), because our focus in this paper is on generic relationships.

Table 1. Species examined, collection information, and reproductive parts examined. Key: +, examined; (+), partially examined; –, not examined.

Species	Collection	Parts examined		
		Anthers	Ovules	Seeds
Tribe Fuchsieae				
Fuchsia jimenezii Breedlove, Berry, & Raven	Costa Rica, Monteverde, *Haber, Baker & Baker 434* (MO)	+	+	–
F. paniculata Lindley	Mexico, Chiapas, *Breedlove 42742* (MO)	+	+	–
F. radicans Miers	Brazil, São Paulo, Campas de Jordao, *Ramamoorthy 676* (MO)	+	+	(+)
Tribe Circaeeae				
Circaea alpina L. subsp. *pacifica* (Asch. & Magnus) Raven	Cult., Univ. British Columbia Bot. Gard. (UBC)	+	+	+
C. cordata Royle	Cult., Missouri Bot. Gard. #762431; plants from USSR, Vladivostok, *Raven in 1975* (MO)	+	+	+
Tribe Lopezieae				
Lopezia langmaniae Miranda	Mexico, Chiapas, *Breedlove 32300* (CAS)	–	+	–
L. racemosa Cav. subsp. *racemosa*	Mexico, Chiapas, *Breedlove 7030* (CAS)	+	+	+
Tribe Hauyeae				
Hauya elegans DC. subsp. *elegans*	Mexico, Chiapas, *Breedlove 42631* (MO)	(+)	+	(+)
H. heydeana Donn.Sm.	Mexico, Chiapas, *Breedlove 15669* (MO)	+	+	(+)
Tribe Onagreae				
Gongylocarpus fruticulosus (Benth.) Raven & Breedlove	Mexico, Baja California, Magdalena Is., *Verity 037* (MO)	–	+	–
G. rubricaulis Schldl. & Cham.	Mexico, Chiapas, *Breedlove 41880* (MO)	+	+	+
Gayophytum humile A. Juss.	U.S.A., Oregon, Jefferson Co., *Chambers 4834* (OSC)	+	+	+
G. ramosissimum Torrey & A. Gray	U.S.A., Oregon, Deschutes Co., *Chambers 4817* (OSC)	+	+	+
Xylonagra arborea (Kellogg) Donn.Sm. & Rose	Mexico, Baja California, *Verity, Nakai, & Angel in 1979* (MO)	+	+	+
Camissonia californica (Nutt. ex Torrey & A. Gray) Raven	Cult., UCLA Bot. Gard., *Verity s.n.*, no voucher	+	+	(+)
C. ovata (Nutt. ex Torrey & A. Gray) Raven	U.S.A., California, Marin Co., *Raven & Raven 26148* (MO)	+	+	+
Calylophus hartwegii (Benth.) Raven subsp. *fendleri* (A. Gray) Towner & Raven	U.S.A., Texas, Jeff Davis Co., *Powell 3621* (MO)	–	–	(+)
C. lavandulifolius (Torrey & A. Gray) Raven	U.S.A., Nevada, Lincoln Co., *Tiehm & Williams 6572* (MO).	+	+	–
C. serrulatus (Nutt.) Raven	U.S.A., Kansas, Pecos Co., *Brooks 15533* (KANU)	+	(+)	–
Gaura boquillensis Raven & Gregory	U.S.A., Texas, Brewster Co., *Powell & Powell 3608* (MO)	+	–	–
G. longiflora Spach	U.S.A., Missouri, Jefferson Co., *Wagner, Mill, & Tobe 4522* (MO)	–	(+)	–
G. mutabilis Cav.	Mexico, Mexico, *Rzedowski 34992* (ENCB)	+	+	+
Oenothera flava (A. Nelson) Garrett subsp. *flava*	Mexico, Durango, *Wagner & Solomon 4321* (MO)	+	+	+
O. fruticosa L. subsp. *fruticosa*	Cult., Missouri Bot. Gard. #M1908; plants from U.S.A., North Carolina, Pender Co., *Boufford et al. 21575* (CM)	+	+	–

Table 1 *(continued).*

Species	Collection	Parts examined: Anthers	Ovules	Seeds
O. villosa Thunb. subsp. villosa	U.S.A., Missouri, St. Louis Co., *Wagner, Mill, & Tobe 4519* (MO)	–	+	–
Stenosiphon linifolius (Nutt.) Heynhold	U.S.A., Oklahoma, McClain Co., *Sullivan 1038* (OKL)	+	+	+
Clarkia dudleyana (Abrams) J. F. Macbr.	U.S.A., California, *Gottlieb 129*	+	+	+
C. heterandra (Torrey) H. Lewis & Raven [Syn: *Heterogaura heterandra* (Torrey) Cav.]	U.S.A., California, Tuolumne Co., *Gottlieb in 1977* (MO)	+	+	+
C. tenella (Cav.) Lewis & Lewis subsp. *tenella*	Chile, Malleco, Curacao, *Marticorena & Ovezada 1669* (MO)	–	+	–
Tribe Epilobieae				
Epilobium canum (Greene) Raven subsp. *canum*	Cult., Univ. Calif. Bot. Gard. (Berkeley), UCBG 58.996 (UC)	+	+	–
E. ciliatum Raf. subsp. *watsonii* (Barbey) Hoch & Raven	U.S.A., California, Marin Co., *Sharp in 1967* (MO)	+	+	+
E. concinnum (D. Don) Hoch & Raven [Syn: *Boisduvalia subulata* (Ruiz & Pavón) Raimann]	Chile, Nuble, *Cheese & Watson 4405* (K)	+	+	+
E. pygmaeum (Speg.) Hoch & Raven [Syn: *Boisduvalia glabella* (Nutt.) Walp.]	U.S.A., California, Yolo Co., *Crampton 9212* (MO)	(+)	+	–

Results

Most embryological characters examined were constant within the entire family (Tables 2–4). In these tables, features reported earlier are indicated with an asterisk (*); all other features are reported here for the first time. All features mentioned in the following discussion are common to the entire family, as far as known, unless differences between genera and species are mentioned specifically.

Anthers and microspores (Table 2): In general, the anther wall varies from five to six cell-layers thick, but it is often three or four cell-layers thick in *Gayophytum* and four cell-layers thick in *Clarkia heterandra*. The anther wall is basically composed of an epidermis, an endothecium, two or three middle layers and a tapetum. In most genera the middle layers share their histogenetic origin with both the endothecial and the tapetal cells (i.e., Basic type; Fig. 1), although both or either of the middle layers may be lacking in *Gayophytum*. However, in *Hauya*, *Calylophus*, *Gaura* and *Clarkia*, the middle layers have a common histogenetic origin with the tapetal cell (i.e., Monocotyledonous type; Fig. 2). In *Calylophus* and *Gaura*, the Basic type also occasionally occurs, but it is not the predominant condition.

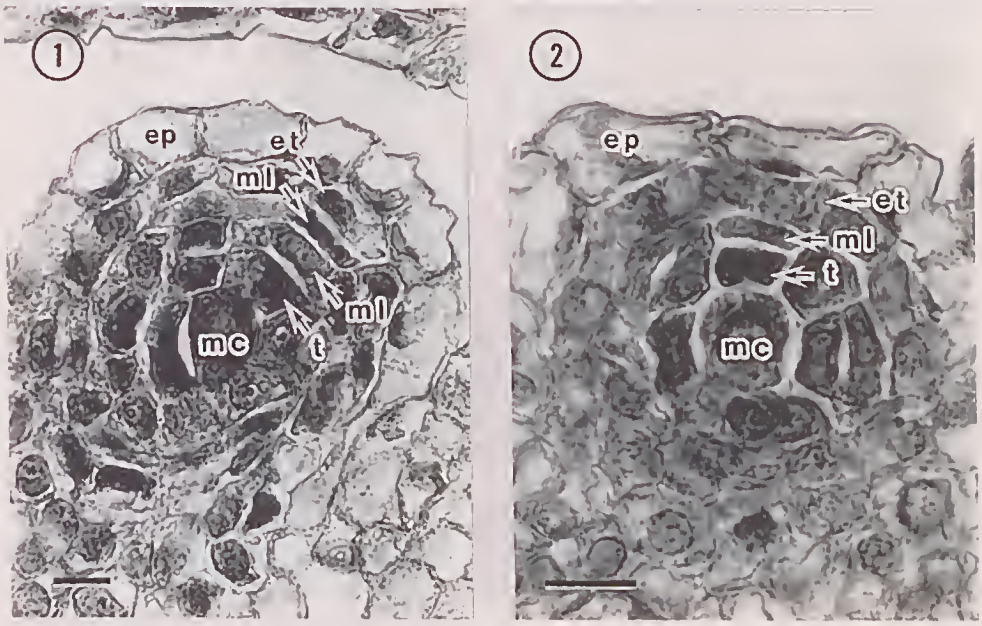
As the anther develops, the middle layers are completely crushed. The epidermis is basically persistent until the time of anther dehiscence, although it may collapse locally. The endothecium always develops fibrous thickenings. The tapetum is glandular and its cells become two-nucleate. We did not see any tapetal cells with more than three nuclei in the species we examined, although Geerts (1909) has reported two- to four-nucleate tapetal cells in *Oenothera glazioviana* ('*O. lamarckiana*').

Meiosis in the microspore mother cells was accompanied by simultaneous cytokinesis in the material we examined. The arrangement of microspores in a tetrad is mostly

tetrahedral. Decussate or isobilateral arrangements also occur, but at low frequencies. Pollen grains are two-celled when shed.

Ovular orientation and integuments (Table 3): The ovule is anatropous and bitegmic, and the micropyle is formed by both integuments. For *Stenosiphon*, Johansen (1930b) reported that 'the inner integument is prolonged into a beak-like process,' and presented drawings of ovules showing the micropyle formed by the inner integument alone (1930b: 319, Figs 6, 7 [sterile ovule]). However, we have confirmed that the micropyle of *Stenosiphon* is also formed by both integuments. The micropyle appears to be formed largely by the endostome of a highly prolonged inner integument, but the outer integument is also prolonged and its tip exceeds that of the inner integument. A more or less prolonged inner (and outer) integument of this kind was observed in other genera of tribe Onagreae (e.g., *Calylophus*) and is not restricted to *Stenosiphon* as a characteristic feature. At any rate, its appearance accounts for Johansen's misinterpretation.

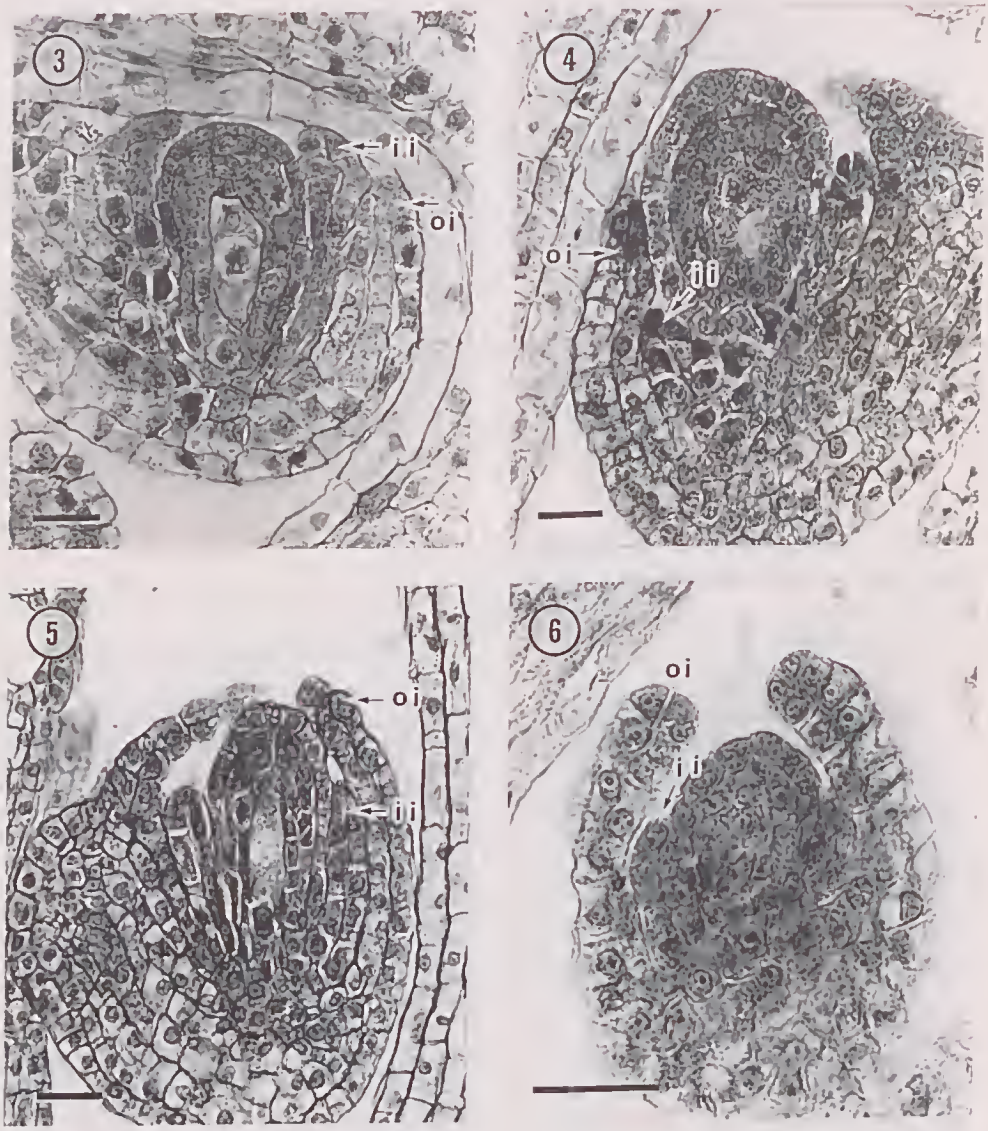
Early in ontogeny both the inner and the outer integument arise almost simultaneously, or the inner integument arises a little earlier than the outer one, from an ovular primordium (see Tobe & Raven 1985: 452, Figs 1A, B; 459, Fig. 4A). In subsequent stages of development in most genera (generally up to the megaspore mother cell stage), the two integuments grow together, so that the tip of the inner integument exceeds that of the outer integument or reaches the top of nucellus earlier than the latter (Fig. 3). However, in the species examined of *Epilobium* (Figs 4, 5) and *Gayophytum* (Fig. 6) the development of the inner integument is extremely retarded. For instance, at the megaspore mother cell stage, the inner integument is much shorter than the outer one, whereas the tip of the outer integument reaches near the top of the nucellus. In a few ovules of *Oenothera flava*, the inner integument was somewhat shorter than the outer one, but most samples of



Figs 1 and 2. Transverse sections of young anthers showing two different types of wall formation. **1.** Basic type (photo from *Oenothera fruticosa*). **2.** Monocotyledonous type (photo from *Clarkia dudleyana*). See text for explanation. Abbreviations: et, endothecium; ep, epidermis; mc, microspore mother cell; ml, middle layer; t, tapetum. Scales equal 10 μm.

this species and all those of other species of *Oenothera* examined do not have shorter inner integuments. An obturator is absent.

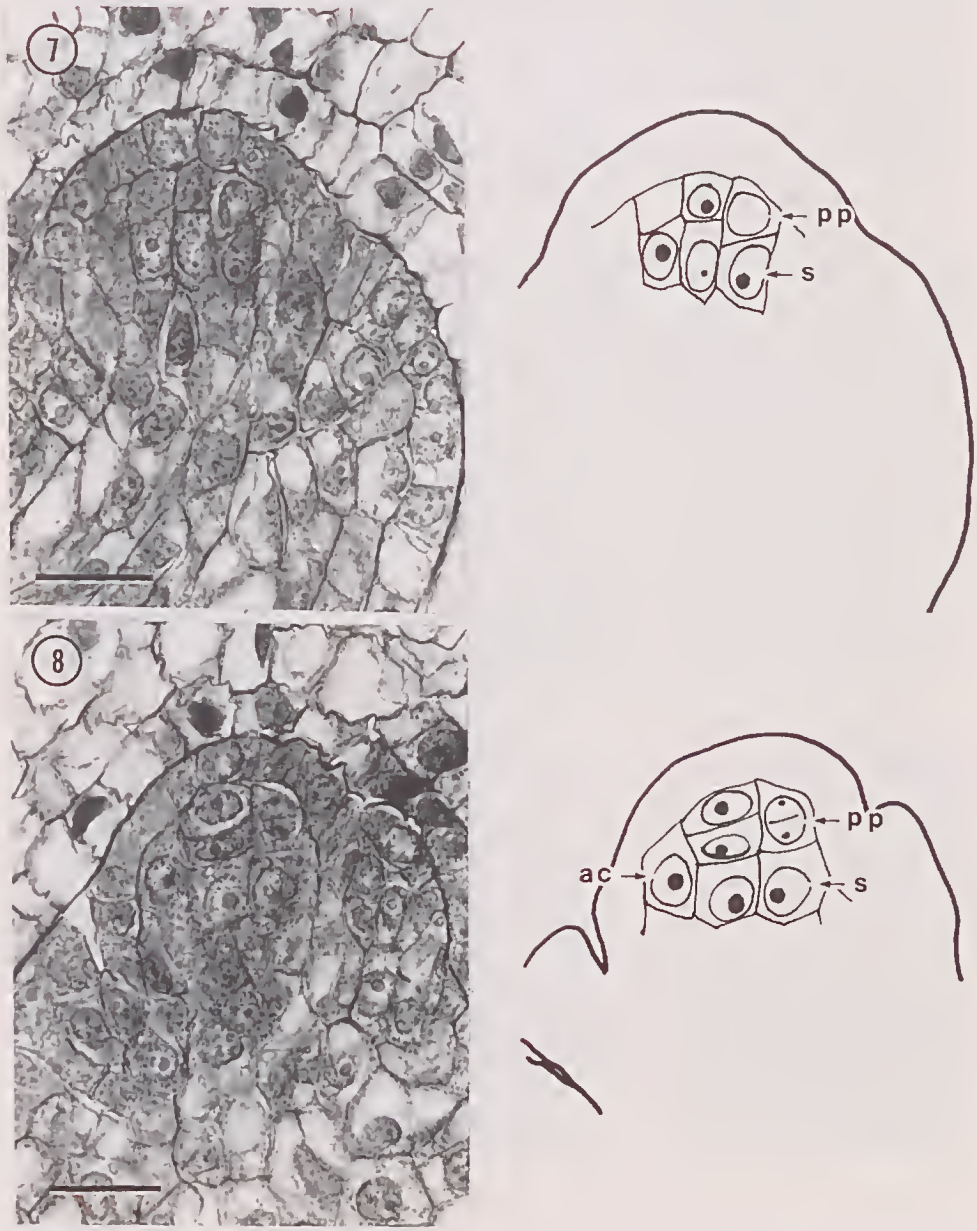
Megagametophyte and nucellus (Table 3): The archesporium in Onagraceae is multi-celled, comprising three to five cells. Archesporial cells are usually distinguished from somatic cells by their larger size, denser cytoplasm and more prominent nucleus (Maheshwari 1950). However, it was very difficult to determine the number of archesporial cells, because the difference is not conspicuous between the archesporial and the somatic cells. We often confirmed the presence of a multi-celled archesporium



Figs 3–6. Longitudinal sections of young ovules showing early development of integuments. 3. *Lopezia racemosa*. 4. *Epilobium canum* subsp. *canum*. 5. *Epilobium concinnum* (*Boisduvalia subulata*). 6. *Gayophytum ramosissimum*. In contrast to that of most genera (for example, as in Fig. 3), the inner integument (ii) of *Epilobium* and *Gayophytum* (Figs 4–6) is extremely retarded in early development and is much shorter than the outer integument (oi). All scales equal 20 µm.

by looking at periclinal divisions of several hypodermal archesporial cells that give rise to the primary parietal and the sporogenous cells (Figs 7, 8: *Fuchsia radicans*).

Most earlier authors have not specified the number of archesporial cells, but have implied that the archesporium is one-celled (see Davis 1966; Seshavataram 1970). Nevertheless, the occurrence of a multi-celled archesporium has occasionally been reported in various species and genera of Onagraceae, although without reference to



Figs 7 and 8. Longitudinal sections of two young ovules of *Fuchsia radicans* showing a multi-celled archesporium. Plural archesporial cells (ac) are differentiated, and most of them divide periclinally to give rise to the primary parietal cell (pp) and the sporogenous cell (s). Scales equal 20 µm.

its relative frequency, for example, in *Epilobium* (Michaelis 1925), *Lopezia* (Täckholm 1914) and *Oenothera* (Hulbary & Rao 1959; O'Neal 1923; Subramanyam & Govindu 1948). In his study of *Oenothera tetraptera* ('*Hartmannia tetraptera*'), Johansen (1929: 289) corrected his early notes on the archesporium, from 'probably several archesporial cells [exist]' to 'a single archesporial initial [exists],' stating that 'cells adjoining the archesporial initial may often simulate the functional appearance of the latter.' However, all samples of Onagraceae we examined had plural archesporial cells in conformity with a few earlier reports of the occasional occurrence of plural archesporial cells (e.g., Hulbary & Rao 1959; Michaelis 1925; O'Neal 1923; Subramanyam & Govindu 1948), as well as of plural megaspores (derived from different archesporial cells) and embryo sacs (e.g., Langendorf 1930; Renner 1914; Täckholm 1915). These earlier reports also seem to suggest that the multi-celled archesporium is prevalent. We cannot confirm the existence of a one-celled archesporium in any Onagraceae.

Usually a sporogenous cell derived from one of the plural archesporial cells increases its volume and becomes a megaspore mother cell. The megaspore mother cell undergoes meiosis, forming nearly always a linear tetrad of megaspores (Figs 9–12) and very rarely an oblique linear tetrad. A triad of megaspores may also occasionally occur, resulting from the suppression of homotypic division in the lower cell of the dyad (for the frequencies of triads in certain genera, see Rodkiewicz & Sniezko

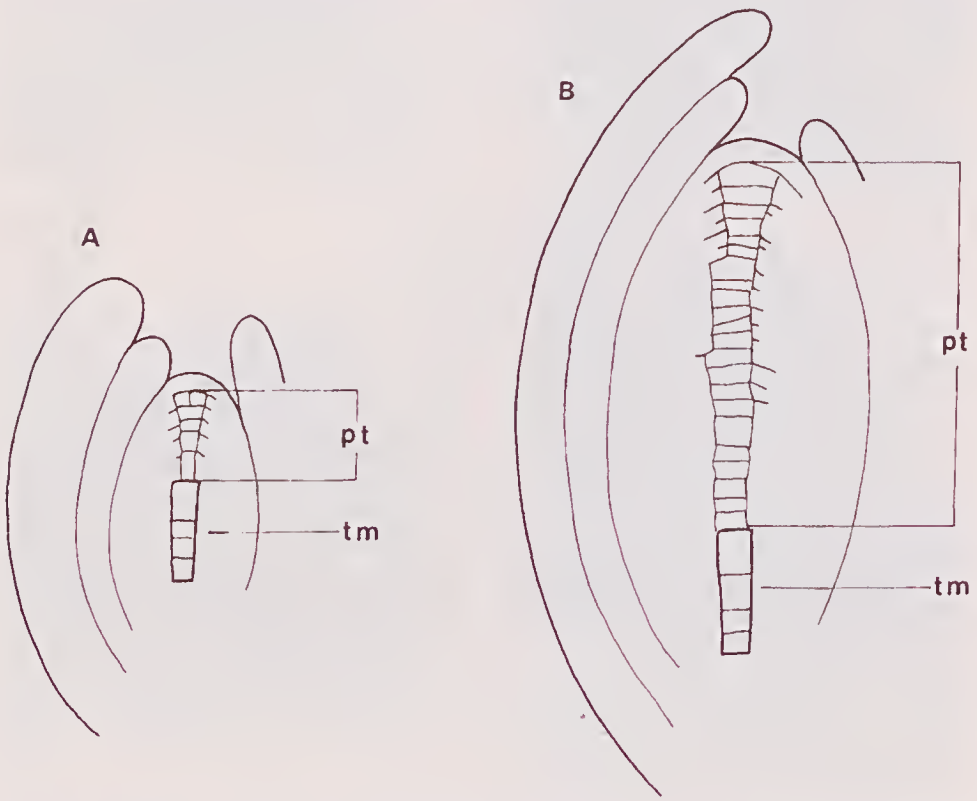
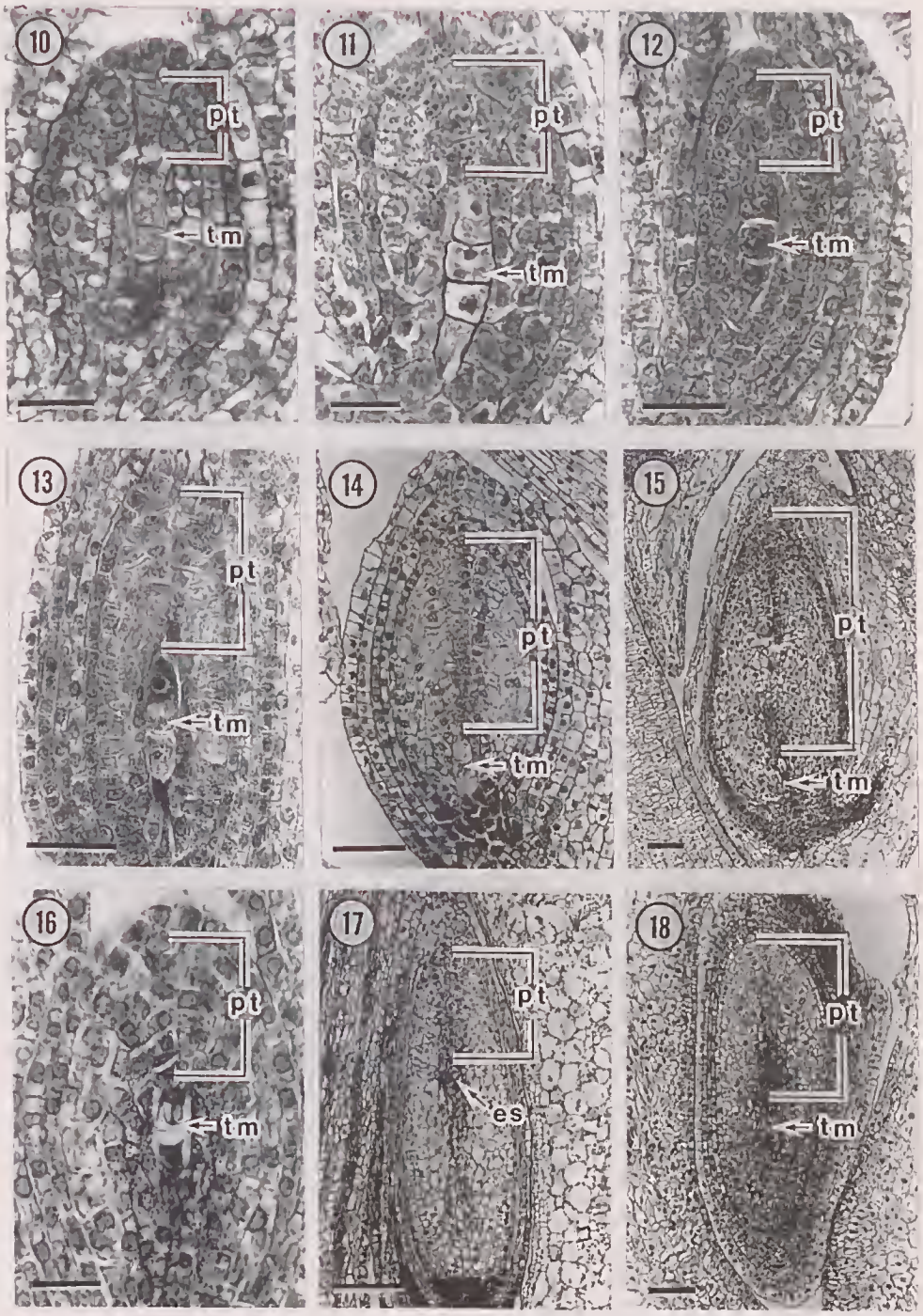


Fig. 9. Diagrams illustrating two contrasting ovules with respect to the thickness of parietal tissue (pt) lying above a tetrad of megaspores (tm). a. Ovule with a thin parietal tissue (5 cells thick). b. Ovule with a thick parietal tissue (22 cells thick).



Figs 10–18. Longitudinal sections of ovules of some genera at the megaspore tetrad stage (Figs 10–16 and 18) and at the 2-nucleate embryo sac (es) stage (17), showing the thickness of parietal tissue (pt) and the position of a tetrad of megaspores (tm). **10.** *Fuchsia jimenezii*. **11.** *Lopezia racemosa*. **12.** *Epilobium ciliatum* subsp. *watsonii*. **13.** *Gayophytum ramosissimum*. **14.** *Clarkia tenella*. **15.** *Hauya elegans*. **16** and **17.** *Clarkia heteraudra* ('*Heterogaura*') **18.** *Gongylocarpus fruticulosus*. See text for explanation. Scales equal 20 μm, 20 μm, 20 μm, 20 μm, 50 μm, 100 μm, 100 μm, 20 μm and 50 μm, respectively.

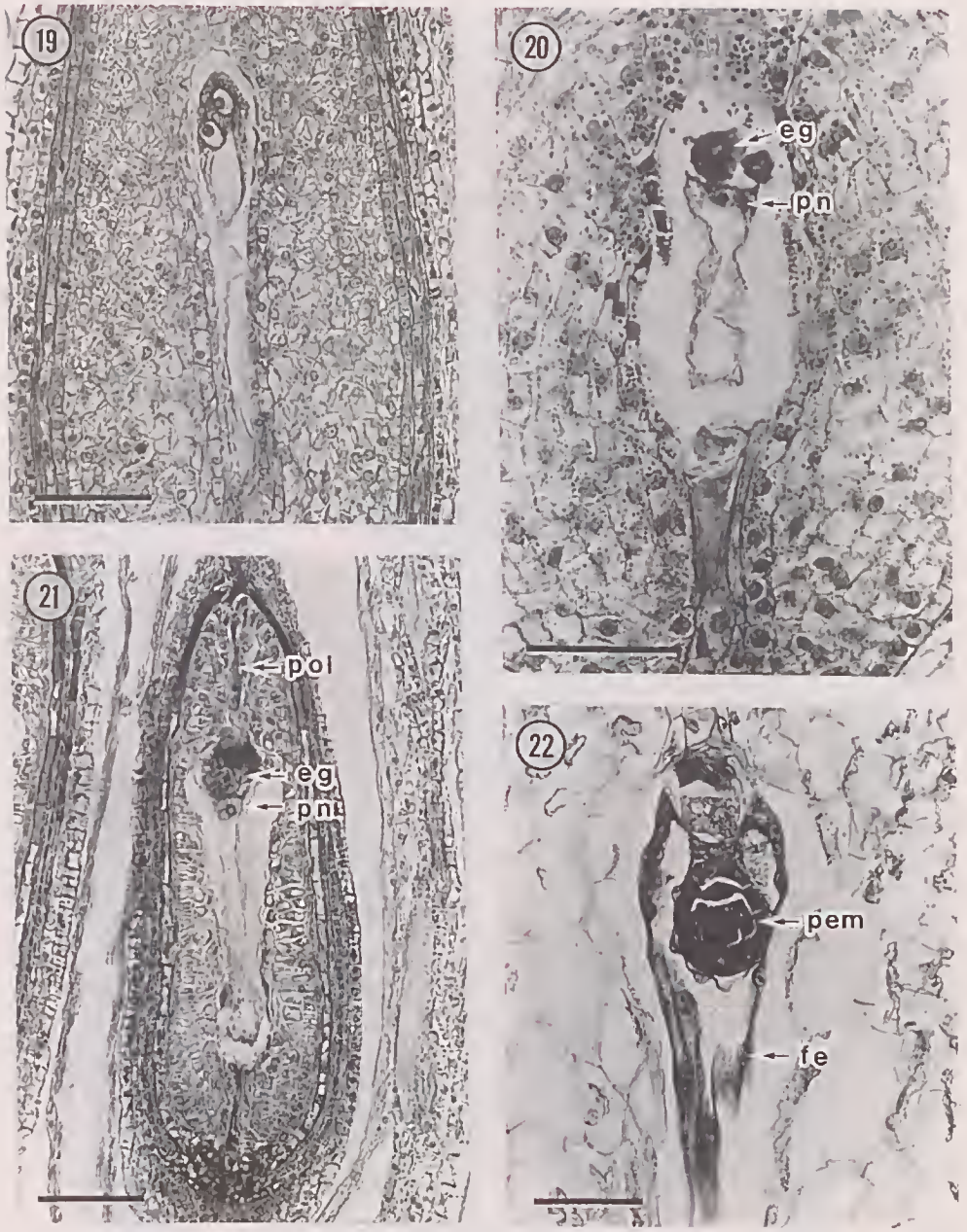
1978). A micropylar megaspore in the tetrad is functional and enlarged (Fig. 13), while the three remaining megaspores degenerate. A chalazal megaspore, instead of the micropylar one, or both the micropylar and chalazal megaspores may rarely appear to be functional, but we have never observed the chalazal megaspore to develop into an organized embryo sac. The nucleus of the functional micropylar megaspore always is located on the micropylar side of the cell and, following its mitotic divisions, develops into a two- (Fig. 19) and a four-nucleate (or -celled) Oenothera type embryo sac (Fig. 20). An organized embryo sac has one egg cell, two synergids and one polar nucleus.

Some genera of Onagraceae differ markedly in the thickness of their nucellar tissue, particularly in the parietal tissue derived from archesporial cells (for two contrasting ovules, see Figs 9a, b). The primary parietal cell (see pp in Figs 7, 8) derived from the archesporial cell divides periclinally, and the two daughter cells continue to divide periclinally. Consequently, above the linear tetrad of megaspores lies a relatively thin layer of parietal tissue, about five to eight cells thick (Fig. 9a), as found in *Fuchsia* (Fig. 10), *Circaea*, *Lopezia* (Fig. 11), *Epilobium* (Fig. 12) and *Gayophytum* (Fig. 13), or a relatively thick layer, about ten to 20 cells thick (Fig. 9b), as found in *Clarkia* (Fig. 14), *Hauya* (Fig. 15), *Gongylocarpus*, *Xylonagra*, *Camissonia*, *Calylophus*, *Gaura*, *Oenothera* and *Stenosiphon*. In most genera the tetrad of megaspores is deeply buried in the nucellus and, except in *Clarkia heteraudra* ('*Heterogaura*') and *Gongylocarpus*, is positioned at or a little above the bottom of the nucellus. Compared to other species of *Clarkia*, *C. heteraudra* is unusual in that the divisions of the parietal cells are retarded and that a tetrad of megaspores is positioned in the middle of the nucellus (Fig. 16). At this stage, *C. heteraudra* has a thin layer (about five to eight cells thick) of parietal tissue above and a somewhat thicker nucellar tissue below the megaspores (Fig. 16). In later stages of development, the cells of both tissues above and below divide rapidly, increasing their respective thickness, so that the embryo sac comes to be positioned in the center of the nucellus. At the two-nucleate embryo sac stage, the parietal tissue is up to 16 to 20 cells thick (Fig. 17). *Gongylocarpus* is similar to most other genera of the tribe Onagreae in having thick parietal tissue (18 to 20 cells thick) above the tetrad of megaspores, but less nucellar tissue than the other genera below the tetrad, so that the tetrad is positioned between the bottom and the center of the nucellus (Fig. 18).

A nucellar cap derived from the nucellar dermal cell by its periclinal division is two or three cells thick if present, but is relatively insignificant and poorly defined. For these reasons, we do not consider it to be a useful feature for making comparisons between genera.

Starch grains, whose functional aspects have sometimes been discussed in relation to the polarity of megaspores in a tetrad (e.g., Rodkiewicz & Bednara 1974; Rodkiewicz & Sniezko 1978; Sniezko & Harte 1984a), are abundant in the nucellar cells, megaspores and embryo sac before fertilization, particularly on the micropylar side (Fig. 20). They become less conspicuous in the post-fertilization stages, however. Ishikawa (1918: 311) gives a comparison among some genera, noting that starch grains in the nucellus are 'many' in species of *Oenothera*, *Gaura* and *Circaea*, 'very few' in *Chamerion angustifolium* ('*Epilobium*') and 'none' in *Clarkia* ('*Godetia* sp.') and *Fuchsia macrostemma*. However, we have confirmed the presence of starch grains in the nucellus and the embryo sac in all species examined of all genera, including *Epilobium*, *Clarkia* and *Fuchsia*.

A hypostase, which is distinguished by accumulation of densely staining tannin-like substances in its cells and the thickening of those cell walls, is always formed although its differentiating stage is different from species to species. According to Johansen (1928), *Oenothera*, *Gaura*, *Clarkia* and *Circaea* possess a definite hypostase, whereas



Figs 19–22. Longitudinal sections of ovules and seeds in various stages of development, showing general embryological features in older embryo sacs and nucelli. **19.** Ovule with two-nucleate embryo sac (photo from *Gaura mutabilis*). Note two nuclei positioned on micropylar side. **20.** Ovule with organized embryo sac composed of an egg apparatus (eg) and a single polar nucleus (pn); arrowheads indicate scattered starch grains in nucellus (photo from *Fuchsia radicans*). **21.** Ovule with embryo sac just fertilized showing porogamy (photo from *Gayophytum ramosissimum*). See pollen tube (pol) penetrating into the nucellus on micropylar side. **22.** Seed with embryo sac containing free endosperm nucleus (fe) and a globular proembryo (pem) (photo from *Clarkia heteraudra*). All scales equal 50 µm.

the majority of species of *Epilobium* and *Fuchsia* are characterized by its absence. However, we have confirmed the presence of a hypostase in all species examined of *Epilobium* and *Fuchsia*, as well as in the other genera for which information on the hypostase has not been available earlier. It appears, therefore, to be common to all species of Onagraceae.

Fertilization, endosperm and embryo (Table 4): Fertilization is porogamous (Fig. 21). Johansen (1934) reports irregularities with respect to the path of pollen tube in ovules of *Circaea alpina* subsp. *pacifica*, but we were not able to confirm his observations in the material of this taxon that we examined. Endosperm formation is of the Nuclear type (Fig. 22). The endosperm is scanty throughout the process of seed development. Even in a nearly mature seed, a limited amount of cellularized endosperm is present on the periphery of the embryo sac and particularly on the chalazal side (Fig. 28). The mature seed completely lacks endosperm (e.g., in *Fuchsia*, Fig. 23, and *Gongylocarpus*, Fig. 26).

Earlier, the Onagrad type of embryogeny has been reported in species of *Circaea* (Souèges 1946), *Oenothera* (Souèges 1920), *Chamerion* (Lebègue 1948b) and *Epilobium* (Lebègue 1948a). We observed the Onagrad type in *Gayophytum humile*, *Camissonia ovata* and *Clarkia heteraudra*. The embryo in a mature seed is straight and dicotyledonous with a short suspensor (Fig. 26).

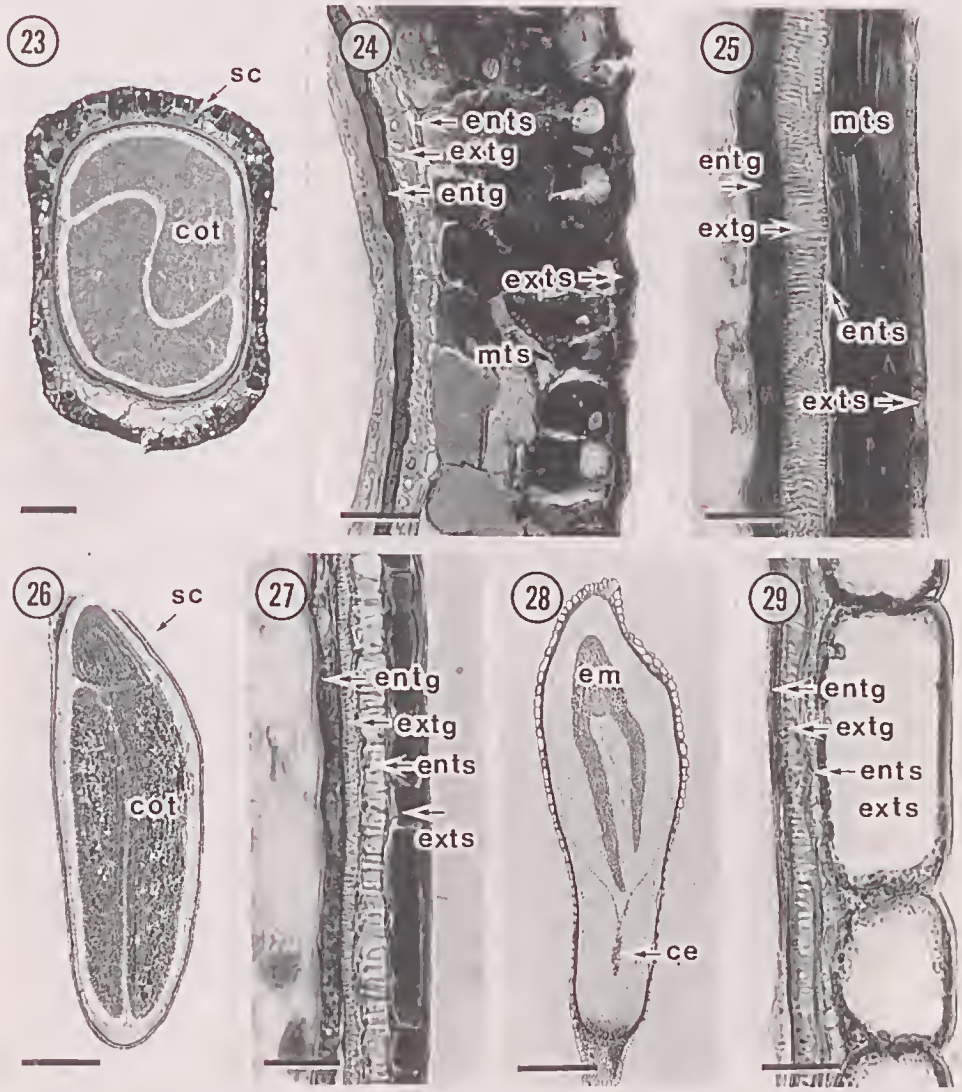
Seed appendage and seed coat (Table 4): Except in *Hauya*, *Xylonagra*, *Chamerion* and *Epilobium*, the seeds of Onagraceae have no conspicuous appendages. Both *Hauya* and *Xylonagra* have a wing on the chalazal side. The wing is flat along the axis of raphe to antiraphe. All species of *Chamerion* and all but a few species of *Epilobium* (especially species of the former *Boisduvalia*) have a coma on the chalazal end of the seed that is composed of a tuft of unicellular trichomes.

The mature seed coat basically comprises the exotesta, endotesta, exotegmen and endotegmen. In addition, a mesotesta is present in *Fuchsia* (Figs 23, 24), *Circaea* (Fig. 25), *Lopezia*, *Oenothera* (some species only) and *Stenosiphon* (Tobe, Wagner & Chin 1987), whereas *Gongylocarpus* (Figs 26, 27), *Xylonagra* (Figs 28, 29) and all other genera lack a mesotesta. As mentioned previously, we did not examine the variation of seed coat structure within particular genera, which is often extensive, for the purposes of this paper. The following are common features characteristic of the whole family. The endotesta, which may be relatively less conspicuous in some species of various genera than usual for the family, is crystaliferous, and its cells are variously thick-walled; the exotegmen is composed of longitudinally elongate fibrous (or tracheoidal) cells; the endotegmen comprises longitudinally elongate, tanniniferous cells. Carlquist & Raven (1966) described the seed coat histology of *Gongylocarpus rubricaulis* as entirely different from that of *G. fruticulosus*, but we found them to be similar (*G. rubricaulis*, Fig. 27); both species share a crystaliferous endotesta and a fibrous exotegmen.

Discussion

In 1983, on the basis of the limited information then available, we discussed the relationships of Onagraceae with the other families of the order Myrtales. We concluded that 'none of the embryological attributes of Onagraceae suggests a particular relationship either to Lythraceae or to any other family' (Tobe & Raven 1983: 86–87). As a result of our subsequent studies, (see Tables 2–4; Tobe & Raven 1986b), however, we have confirmed that most embryological features such as the Oenothera type embryo sac, are common to the entire family Onagraceae. With respect to the relationships of the family, we offer the following observations. First, the Oenothera type embryo sac distinguishes Onagraceae from all other myrtalean families. Second, the presence of the multi-celled ovule archesporium (except in *Ludwigia*), as well as the presence of starch grains in the

nucellus, suggest close resemblance between Onagraceae and Lythraceae (for the presence of multi-celled ovule archesporium and starch grains in Lythraceae, see Hubert, 1896; Joshi & Venkateswarlu 1935a, b, 1936; Venkateswarlu 1937). Third, the combination of a crystaliferous endotesta and a fibrous exotegmen indicates relationships of Onagraceae with Lythraceae and Sonneratiaceae, but not with other families including Myrtaceae and Melastomataceae. Thus we now would conclude that embryological features do support the traditional view that Onagraceae are directly related to Lythraceae. We shall



Figs 23–29. Transverse (23 and 24) and longitudinal sections (25–29) of seeds showing seed and seed coat structure in various genera. **23–27.** Mature seeds. **28–29.** Immature seeds. **23–24.** Seed and seed coat of *Fuchsia jimenezii*. **25.** Seed coat of *Circaea cordata*. **26–27.** Seed and seed coat of *Gongylocarpus rubricaulis*. **28–29.** Seed and seed coat of *Xylonagra arborea*. Note that the seed coats of *Fuchsia* and *Circaea* are thick and have mesotesta (mts), whereas those of *Gongylocarpus* and *Xylonagra* are thin and lack mesotesta. Additional abbreviations: ce, cellular endosperm; cot, cotyledon; em, embryo; entg, endotegmen; ents, endotesta; extg, exotegmen; exts, exotegmen; sc, seed coat.

continue to consider the relationships of Onagraceae as additional information on the embryology of other families of Myrtales becomes available.

Relationships within Onagraceae: A comparison among the genera of Onagraceae indicates that the following four embryological features differ significantly within the family. (1) Anther wall development is predominantly either the Basic or the Monocotyledonous type; (2) the ovule archesporium is one- or multi-celled; (3) the inner integument is retarded in development or not; and (4) the parietal tissue lying above the tetrad of megaspores is either thin or thick. The distribution of features of these four characters within Onagraceae is presented in Table 5, along with that of features of integumentary histogenesis (Tobe & Raven 1985) and of divided microsporangium (Tobe & Raven 1986a), features that we found earlier to differ within the family.

Ludwigia is the only genus that characteristically has a one-celled ovule archesporium, in contrast to the multi-celled archesporium in all other genera. *Ludwigia* now appears unambiguously to be a sister group to the remainder of the family, particularly on the basis of evidence from floral morphology and anatomy (Eyde 1981; Hoch et al. 1993) and from molecular analyses of both ribosomal DNA (Bult & Zimmer 1993) and chloroplast *rbc*L data (Conti, Fischbach & Sytsma 1993). For example, *Ludwigia* has floral nectaries on the gynoecium, instead of at the gynoecium and floral tube junction as in all other genera, and both central and transseptal bundles for ovule supply, instead of only transseptal bundles as in all other genera (Eyde 1981). The difference in the number of archesporial cells in an ovule supports the hypothesis that *Ludwigia* does indeed represent an evolutionary branch separate from the rest of the family.

Hauya (Hauyeae) and all genera of the tribe Onagreae except *Gayophytum* agree with one another in having markedly thick parietal tissue in the ovule. *Hauya* further agrees with *Calylophus*, *Gaura*, and *Clarkia* of Onagreae in having the Monocotyledonous type anther wall formation, instead of the Basic type common to all other genera. Coincidences between *Hauya* and members of Onagreae have already been indicated by other embryological evidence. For instance, *Hauya* shares with *Calylophus* and *Gaura* a distinctive histology of the outer integument (Tobe & Raven 1985), and also shares with *Calylophus*, *Gaura* and *Clarkia* the apparent apomorphy of microsporogenous tissue divided by septa composed of parenchyma and tapetum into many small packets (Tobe & Raven 1986a). This evidence, thus, suggests a close relationship of *Hauya* with tribe Onagreae, particularly with *Calylophus* and *Gaura*, and probably also with *Clarkia*. However, several molecular analyses using both nuclear (Crisci et al. 1990; Bult & Zimmer 1993) and chloroplast DNA (Sytsma, Smith & Hoch 1991; Conti, Fischbach & Sytsma 1993), contradict this placement of *Hauya* near Onagreae, instead supporting a close relationship of *Hauya* to *Fuchsia* and *Circaea*. Ongoing molecular analyses that include all relevant taxa and 'total-evidence' analysis of the studies already available may resolve this controversy, and provide a robust hypothesis within which to interpret the evolution of these embryological characters. The chalazal seed wing shared by both *Hauya* and *Xylonagra* seems to represent a parallel (homoplasious) evolution, since no other evidence supports a close relationship between them.

In *Gayophytum*, unlike other members of the tribe Onagreae, but like *Epilobium* (tribe Epilobieae), the early development of the inner integument is retarded. In addition, *Gayophytum* resembles *Epilobium*, rather than other Onagreae, in having thin parietal tissue in the ovule. This rather surprising suggested relationship appears to be supported by sequence data from analysis of the internal transcribed spacer region (ITS) of the nuclear ribosomal gene (Baum & Sytsma, unpublished data). This suggests that Onagreae may not be monophyletic because Epilobieae appears to be nested within it; the two tribes together, however, appear to form a monophyletic group.

Some comments seem in order regarding the embryological features of *Clarkia heterandra*, which until recently has been segregated as the genus *Heterogaura*, but is now unambiguously assigned to *Clarkia* as a monotypic section closely related to *C. dudleyana* and the other species of section *Peripetasma* (Lewis & Raven 1992). Its relationships were first revealed by evidence from restriction enzyme analysis of chloroplast DNA (Sytsma & Gottlieb 1986a, b) and subsequent analysis of nuclear rDNA (Sytsma & Smith 1988). Embryologically, however, *C. heterandra* differs from all other species of *Clarkia* in the histology of its nucellus. All other species of *Clarkia* and of the entire tribe Onagreae (except for *Gayophytum*), as well as *Hauya*, have markedly thick parietal tissue, so that the underlying tetrad of megaspores is deeply buried and positioned nearly at or a little above the bottom of the nucellus. In contrast, in *C. heterandra* the parietal tissue is thin at the megaspore tetrad stage, and the tetrad of megaspores is positioned at the middle of the nucellus. Later both the parietal cells above and the nucellar cells below the megaspores divide rapidly to form a massive nucellus. This marked difference in nucellar histology clearly distinguishes *C. heterandra* from other species of *Clarkia* and other genera of Onagreae. Since the macromolecular evidence of its close relationships with and probably derivation from *Clarkia* section *Peripetasma* is unequivocal, however, we conclude that the unusual embryological features of *C. heterandra*, like its distinctive morphological and anatomical characteristics, were derived within its evolutionary line after its separation from other species of *Clarkia*.

Table 2. Embryological characteristics and variation of anthers and microspores in Onagraceae.

Genus	Thickness of anther wall basically 5–6 cell-layered (–) or otherwise (+)	Type of anther wall development Basic (–) or Monocotyledonous (+)	Anther epidermis persistent (–) or collapsed (+)	Endothecium fibrous (–) or otherwise (+)	Tapetum glandular (–) or amoeboid (+)	Tapetal cell always or predominantly 2-nucleate (–) or otherwise (+)	Delimitation of microspores simultaneous (–) or successive (+)	Predominant shape of microspore tetrad tetrahedral (–) or otherwise (+)	Pollen grain when shed 2-celled (–) or 3-celled (+)
Fuchsia	–	–	–	–	–	–	–	–	–
Circaea	–	–	–	–	–	–	–	–	–
Lopezia	–	–	–	–	–	–	?	–	–
Hauya	–	+	–	–	–	–	–	–	–
Gongylocarpus	–	–	–	–	–	–	?	–	–
Gayophytum	+	–	–	–	–	–	–	?	–
Xylonagra	–	–	–	–	–	–	–	–	–
Camissonia	–	–	–	–	–	–	–	–	–
Calylophus	–	+	–	–	–	–	–	–	–
Gaura	–	+	–	–	–	–	–	–	–
Oenothera	–	–	–*	–*	–*	–*	–*	–*	–*
Stenosiphon	–	–	–	–	–	–	–	–	–
Clarkia	–	+	–	–	–*	–*	–*	–*	–*
C. heterandra	+	+	–	–	–	–	?	–	–
Epilobium	–	–	–	–	–	–	–*	–	–*

* Character reported in earlier literature and confirmed in this study. References for particular genera listed as follows; for *Oenothera*: Beer (1905), Davis (1909), Gates (1911), Geerts (1909), Hulbary & Rao (1959), Pagni (1958), Rudloff & Schmidt (1932); for *Clarkia*: Håkansson (1925); for *Epilobium*: Håkansson (1924). See also Brewbaker (1967) for the number of cells in mature pollen in *Oenothera*, *Clarkia* and *Epilobium*.

Table 3. Embryological characteristics and variation of ovules and gametophytes in Onagraceae.

Genus	Ovule anatropous (–) or otherwise (+)	Inner integument not retarded (–) or retarded (+) in early development	Micropyle formed by both inner & outer integument (–) or otherwise (+)	Archesporium multi-celled (–) or one-celled (+)	Ovule crassinucellate (–) or tenuinucellate (+)	Parietal tissue thin (5–8 cells thick) (–) or thick (10–20 cells thick) (+)	Type of embryo sac formation Oenothera type (+) or otherwise (–)	Starch grains in nucellus scanty (–) or abundant (+)	Hypostase absent (–) or present (+)
Fuchsia	–	–	–	–*	–*	–	+*	+	+
Circaea	–*	–	–	–	–*	–	+*	+*	+
Lopezia	–*	–	–	–*	–*	–	+*	+	+
Hauya	–	–	–	–	–	+	+	+	+
Gongylocarpus	–	–	–	–	–	+	+	+	+
Gayophytum	–	+	–	–	–	–	+*	+*	+*
Xylonagra	–	–	–	–	–	+	+	+	+
Camissonia	–	–	–	–*	–	+	+*	+	+*
Calylophus	–	–	–	–	–	+	+	+	+
Gaura	–*	–	–	–	–	+	+*	+	+
Oenothera	–*	–	–*	–*	–*	+	+*	+*	+*
Stenosiphon	–	–	–	–	–	+	+*	+	+
Clarkia	–*	–	–*	–*	–*	+*	+*	+	+*
C. heterandra	–	–	–	–	–	+	+	+	+
Epilobium	–*	+	–*	–*	–*	–*	+*	+	+*

* Character reported in earlier literature and confirmed in this study. References for particular genera listed as follows; for *Fuchsia*: Ishikawa (1918), Rodkiewicz (1973), Täckholm (1915), Werner (1915); for *Circaea*: Ishikawa (1918), Johansen (1934), Modilewski (1909); for *Lopezia*: Täckholm (1914); for *Gayophytum*: Johansen (1933); for *Camissonia*, Johansen (1931a); for *Gaura*: Hofmeister (1858), Ishikawa (1918); for *Oenothera*: Geerts (1908, 1909), Haberlandt (1927), Halac (1980), Halac & Harte (1977), Hofmeister (1858), Hulbary & Rao (1959), Ishikawa (1918), Johansen (1929, 1931b), Jalouzot (1978), Langendorf (1930), Modilewski (1909), O'Neal (1923), Pagni (1958), Renner (1921), Rudloff & Schmidt (1932), Subramanyam & Govindu (1948), Rodkiewicz, Bednara & Pora (1971), Sniezko & Harte (1984a, b), Werner (1915); for *Stenosiphon*: Johansen (1930b); for *Clarkia*: Håkansson (1925), Hofmeister (1858), Täckholm (1915), Werner (1915); for *Epilobium*: Bednara (1977), Gachechiladze (1974, 1975), Gachechiladze & Gvaladze (1970), Modilewski (1909), Rodkiewicz (1973), Rodkiewicz & Bednara (1974), Täckholm (1915), Werner (1915).

Table 4. Embryological characteristics of the endosperm, embryo and seed in Onagraceae.

Genus	Path of pollen tube porogamous (–) or otherwise (+)	Type of endosperm formation Nuclear (–) otherwise (+)	Endosperm in mature seed present (–) or absent (+)	Type of embryogenesis Onagrad (–) or otherwise (+)	Seed without (–) or with wing (+)	Mesotesta present (–) or absent (+)	Endotesta crystaliferous (–) or otherwise (+)	Exotegmen fibrous (–) or otherwise (+)	Endotegmen persistent and tanniferous (–) or otherwise (+)
Fuchsia	–	–	+	?	–	–	–	–	–
Circaea	–*	–*	+	–(*)	–	–	–	–	–
Lopezia	–	–*	+	?	–	–	–	–	–
Hauya	–	–	+	?	+	+	–	–	–
Gongylocarpus	–	–	+	?	–	+	–	–*	–
Gayophytum	–	–*	+	–	–	+	–	–	–
Xylonagra	–	–	+	?	+	+	–	–	–
Camissonia	–*	–*	+	–	–	+	–	–	–
Calylophus	–	–	+	?	–	+	–	–	–
Gaura	–	–	+	?	–	+	–	–	–
Oenothera	–*	–*	+*	–(*)	–	+/–*	–*	–*	–*
Stenosiphon	–*	–*	+	?	–	–	–	–	–
Clarkia	–*	–	+	?	–	+	–	–	–
C. heterandra	–	–	+	–	–	+	–	–	–
Epilobium	–*	–*	+	–(*)	–	+	–	–	–

* Character reported in earlier literature and confirmed in this study. (*) Character known only in literature. References for particular genera listed as follows; for *Lopezia*: Hofmeister (1858); for *Circaea*: Johansen (1934), Souèges (1946), Werner (1915); for *Gongylocarpus*: Carlquist & Raven (1966); for *Gayophytum*: Johansen (1933); for *Camissonia*, Johansen (1931a); for *Oenothera*: Haberlandt (1927), Hofmeister (1847), Hulbary & Rao (1959), Langendorf (1930), Modilewski (1909), Renner (1914), Souèges (1920), Tobe, Wagner & Chin (1987), Werner (1915); for *Stenosiphon*: Johansen (1930b); for *Clarkia*: Hofmeister (1847), Johansen (1930a); for *Epilobium*: Lebègue (1948a, b), Michaelis (1925), Modilewski (1909), Täckholm (1915).

Table 5. A comparison in selected embryological characters among genera of Onagraceae.

Genus	Type of anther wall development	Ovule archesporium	Inner integument in early development	Parietal tissue in ovule	Type of septa dividing microsporogenous tissue [1]	Histology of outer integument[2]
Tribe Jussiaeeae						
Ludwigia[3]	Basic	1-celled	Not retarded	Thin	Tapetal/parenchym.	Dermal
Tribe Fuchsieae						
Fuchsia	Basic	Multi-celled	Not retarded	Thin	Tapetal	Subdermal
Tribe Circaeeae						
Circaea	Basic	Multi-celled	Not retarded	Thin	Tapetal	Subdermal
Tribe Lopezieae						
Lopezia	Basic	Multi-celled	Not retarded	Thin	Tapetal	Subdermal
Tribe Hauyeae						
Hauya	Monocot.	Multi-celled	Not retarded	Thick	Parenchym.	Partially subdermal I
Tribe Onagreae						
Gongylocarpus	Basic	Multi-celled	Not retarded	Thick	Tapetal	Dermal
Gayophytum	Basic	Multi-celled	Retarded	Thin	Tapetal	Dermal
Xylonagra	Basic	Multi-celled	Not retarded	Thick	Tapetal	Dermal
Camissonia	Basic	Multi-celled	Not retarded	Thick	Tapetal	Dermal
Calylophus	Monocot.	Multi-celled	Not retarded	Thick	Parenchym.	Partially subdermal I
Gaura	Monocot.	Multi-celled	Not retarded	Thick	Parenchym.	Partially subdermal I
Oenothera	Basic	Multi-celled	Not retarded	Thick	Tapetal	Partially subdermal II
Stenosiphon	Basic	Multi-celled	Not retarded	Thick	Tapetal	Partially subdermal II
Clarkia	Monocot.	Multi-celled	Not retarded	Thick	Parenchym.	Dermal
Tribe Epilobieae						
Epilobium	Basic	Multi-celled	Retarded	Thin	Tapetal	Dermal

[1] Data from Tobe & Raven (1986a). Tapetal = tapetal septa; Parenchym. = parenchymatous septa.

[2] Data from Tobe & Raven (1985). Dermal = the outer integument (oi) is formed only by derivatives of dermal initials of an ovule primordium, no derivatives of subdermal initials contributing to the formation of oi; Partially subdermal I and II = derivatives of subdermal initials participate in the formation of the oi but are restricted to the basal part of oi (I) or reach more than half as much as (but less than) the whole length of oi (II); Subdermal = derivatives of subdermal initials participate well in the formation of the oi and reach up to its tip.

[3] Data from Tobe & Raven (1986b).

Acknowledgements

This study was supported by U.S. National Science Foundation grants to PR, most recently as BSR-8906848. We are grateful to Peter C. Hoch for his comments on the manuscript, and to many people who have collected plant materials for this study, as noted in Table 1.

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Manuscript received 27 November 1995
Manuscript accepted 31 January 1996