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A SYSTEMATIC AND EVOLUTIONARY STUDY OF OENOTHERA (ONAGRACEAE): SEED COAT ANATOMY

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On the basis of a study of 32 of the 124 species representing the 14 sections of *Oenothera*, we find that the seed coats exhibit considerable anatomical diversity, particularly the exotesta, mesotesta, and endotesta. Features throughout the genus are: (1) endotegmen is one cell thick and persistent and (2) consists of longitudinally elongate, tannin-containing cells; (3) exotegmen consists of longitudinally elongate, thick-walled, pitted, lignified fibers; and (4) endotestal cells contain crystals. Unequivocal relationships are demonstrated for species with essentially identical seed features: (1) between section *Kleinia* and section *Oenothera* subsection *Raimannia*; and (2) among sections *Hartmannia*, *Kneiffia*, *Xylopleurum*, and *Anogra*. A cladistic analysis, using seed anatomy data, indicates that (1) *O. havardii* differs in several characteristics from members of section *Gauropsis* and should be excluded; and (2) seed anatomy of *O. maysillesii* differs in several features from the other species of subsection *Emersonia*; these same differences are shared with sections *Ravenia*, *Contortae*, *Eremia*, and *Pachylophus*, indicating that *O. maysillesii* is their sister group. The analysis indicates an early divergence of *Oenothera* into two major lineages: (1) species with thick endotesta (sections *Contortae*, *Eremia*, *Kleinia*, *Oenothera*, *Pachylophus*, and *Ravenia*); and (2) species with radially flattened endotestal cells (sections *Anogra*, *Gauropsis*, *Hartmannia*, *Kneiffia*, *Lavauxia*, *Megapterium*, *Paradoxus*, and *Xylopleurum*). Subsequent changes in seeds of both lineages include multiplication of the fibrous exotegmen, development of a crushed mesotesta or multiplication of the cell layers of the mesotesta, and specialization of the exotesta involving various radial cell enlargements. Diversification of the first lineage has involved changes in seed anatomy that correlate with ecological shifts, while to a much greater extent the evolution of the second lineage has not. Outer layers of the seed appear to be more open to adaptive modifications than inner ones.

Introduction

Seed coat structures within a genus can be useful for assessing relationships and delimiting taxa (e.g., *Eucalyptus*, GAUBA and PRYOR 1958, 1959, 1961; Asteraceae tribe Mutisieae, GRAU 1980). In some groups of plants, however, they are not helpful (e.g., WAGNER and GOLDBLATT 1984). CORNER's (1976) and BARTHLOTT's (1981) surveys of the angiosperms gave new insights into relationships and ecological significance of variation patterns. In *Oenothera*, the only study of seed anatomy is an incomplete description of the seed coat of a single unidentified *Oenothera* species (CORNER 1976).

Because of the potential value of these characters, patterns of seed coat surface morphology were studied in conjunction with the systematic revision of various sections of the genus (section *Kneiffia*, STRALEY 1977; section *Pachylophus*, WAGNER et al. 1985; section *Oenothera* subsection *Emersonia*, DIETRICH et al. 1985). Our goal was to expand our rudimentary knowledge of the comparative anatomy of seeds to obtain new insight into infrageneric relationships.

Material and methods

We examined 32 of the 124 species in the 14 sections of *Oenothera* (table 1). One or more species of each section were studied to sample the full diversity of seed types in the genus. Selection of the representative seed samples was based on an extensive scanning electron microscopy (SEM) survey of 85 of the 124 species of *Oenothera*, coupled with more limited SEM surveys of seeds of all other genera in tribe Onagreae as well as examination of numerous populations by light microscopy and hand sectioning of nearly 100 species. Seed size and general external shape and morphology are summarized from examination of seeds from many populations throughout the range of each species during the systematic revisions. Seeds of most of the species were obtained from recent collections in the Missouri Botanical Garden herbarium (MO); material from other species was collected from the field, the greenhouses of the Missouri Botanical Garden, or Botanisches Institut der Universität, Düsseldorf.

After being fixed in FAA (5:5:90, formalin:glacial acetic acid:50% ethanol), the seeds were dehydrated through a *t*-butyl alcohol series and embedded in Paraplast, mp 57–58 C, for sectioning. Cross or longitudinal sections, 12–15 μ m thick, were stained with safranin and fast green FCF and mounted in Histoclad; the complete series of microtome sections are retained by H. TOBE. Micrographs from the ongoing SEM survey of *Oeno-*

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TABLE 1

OENOTHERA SPECIES EXAMINED

Section, subsection, and species	Voucher
Section <i>Oenothera</i> (5 subsects., 76 spp.):	
Subsection <i>Emersonia</i> (4 spp.):	
<i>O. macrosceles</i> A. Gray	Cultivated by W. STUBBE from seeds from Mexico: Durango, Wagner & Solomon 4316 (DUSS, MO).
<i>O. maysillesii</i> Munz	Cultivated by W. STUBBE from seeds from Mexico: Durango, Breedlove 18812 (DUSS, M, MO).
<i>O. organensis</i> Munz	Cultivated by W. STUBBE from seeds from U.S.A.: New Mexico, Dona Ana Co.; original source J. STRAUB and S. EMERSON.
<i>O. stubbei</i> Dietrich, Raven & W. L. Wagner	Cultivated by W. STUBBE from seeds from Mexico: Nuevo Leon, Sanders et al. 1203 (DUSS, M, MO).
Subsection <i>Oenothera</i> (13 spp.):	
<i>O. villosa</i> Thunb. subsp. <i>villosa</i>	U.S.A.: Missouri, St. Louis Co., Wagner & Mill 4521 (MO).
Subsection <i>Raimannia</i> (11 spp.):	
<i>O. heterophylla</i> Spach	Cultivated by W. STUBBE from seeds from U.S.A.: Alabama, Pickens Co., 1974, Kral s.n. (DUSS, M, MO).
<i>O. laciniata</i> Hill	Cultivated by W. STUBBE from seeds from U.S.A.: Georgia, Paulding Co., 1968, Hoff s.n. (DUSS, M, MO).
Section <i>Kleinia</i> (2 spp.):	
<i>O. albicaulis</i> Pursh	U.S.A.: Texas, Brewster Co., Powell 3590 (MO).
Section <i>Pachylophus</i> (5 spp.):	
<i>O. caespitosa</i> Nutt. subsp. <i>caespitosa</i>	U.S.A.: Nevada, Humboldt Co., Wagner 4472 (MO).
<i>O. cavernae</i> Munz	U.S.A.: Nevada, Clark Co., Ackerman 30072 (MO).
<i>O. harringtonii</i> W. L. Wagner, Stockhouse & Klein	U.S.A.: Colorado, El Paso Co., 1970, Stockhouse s.n. (MO).
Section <i>Eremia</i> (1 sp.):	
<i>O. primiveris</i> A. Gray subsp. <i>primiveris</i>	U.S.A.: Arizona, Mohave Co., Wagner & Mill 4581 (MO).
Section <i>Contortae</i> (1 sp.):	
<i>O. xylocarpa</i> Coville	U.S.A.: California, Mono Co., 1979, DeDecker s.n. (MO).
Section <i>Ravenia</i> (2 spp.):	
<i>O. muelleri</i> Munz	MEXICO: Nuevo Leon, Cerro Potosi, Lloyd 4081 (MO).
<i>O. tubifera</i> Seringe	MEXICO: Durango, Breedlove 14321 (MO).
Section <i>Megapterium</i> (4 spp.):	
<i>O. brachycarpa</i> A. Gray	MEXICO: General Trias, Wagner & Solomon 4355 (MO).
<i>O. macrocarpa</i> Nutt. subsp. <i>macrocarpa</i>	U.S.A.: Texas, Collins Co., Wagner 4440 (MO).
Section <i>Lavauxia</i> (2 subsects., 5 spp.):	
Subsection <i>Lavauxia</i> (3 spp.):	
<i>O. flava</i> (A. Nels.) Garrett subsp. <i>flava</i>	MEXICO: Durango, Wagner & Solomon 4321 (MO).
Subsection <i>Australis</i> (2 spp.):	
<i>O. acaulis</i> Cav.	CHILE: Aconcagna, Quilpué, 1980, Zölner s.n. (MO).
Section <i>Anogra</i> (9 spp.):	
<i>O. engelmannii</i> (Small) Munz	U.S.A.: Oklahoma, Jackson Co., Goodman et al. 8069 (MO).
<i>O. kleinii</i> W. L. Wagner & Mill	U.S.A.: Colorado, Mineral Co., Wagner 4531 (MO).
<i>O. pallida</i> Lindl. subsp. <i>runcinata</i> (Engelm.) Munz & Klein	U.S.A.: Utah, San Juan Co., Wagner 4533 (MO).
Section <i>Gauropsis</i> (2 spp.):	
<i>O. canescens</i> Torr. & Frem.	U.S.A.: Texas, Lubbock Co., Hunt 140 (DS).
<i>O. dissecta</i> A. Gray ex S. Wats.	MEXICO: Zacatecas, Wagner & Solomon 4224 (MO).
Section <i>Paradoxus</i> (1 sp.):	
<i>O. havardii</i> S. Wats.	U.S.A.: Texas, Presidio Co., Hinckley 3477 (NY). U.S.A.: Arizona, Cochise Co., 1962, Hesperheide s.n. (DUKE).

TABLE 1 (Continued)

Section, subsection, and species	Voucher
Section <i>Kneiffia</i> (2 subsections., 5 spp.):	
Subsection <i>Kneiffia</i> (4 spp.):	
<i>O. fruticosa</i> L. subsp. <i>fruticosa</i>	U.S.A.: Virginia, Sussex Co., <i>Fernald et al.</i> 6657 (MO).
<i>O. perennis</i> L.	U.S.A.: Maine, Aroostook Co., <i>Seymour & Svenson</i> 25838 (MO).
Section <i>Xylopleurum</i> (1 sp.):	
<i>O. speciosa</i> Nutt.	U.S.A.: Texas, 1975, <i>Hoff s.n.</i> (MO).
Section <i>Hartmannia</i> (10 spp.):	
<i>O. epilobiifolia</i> H.B.K. subsp. <i>cuprea</i> (Rose) Raven & Parnell	MEXICO: Distrito Federal, <i>Ventura</i> 8932 (MO).
<i>O. kunthiana</i> (Spach) Munz	MEXICO: Chiapas, <i>Breedlove</i> 33748 (MO).
<i>O. rosea</i> L'Her.	MEXICO: Durango, <i>Wagner & Brown</i> 3960 (MO).
<i>O. seifrizii</i> Munz	COLOMBIA: Sierra Nevada de Santa Marta, <i>Weston</i> 6207A (MO).

thera seeds by WAGNER are included for selected species to illustrate the surface feature of exotestal cells in relation to their anatomy.

For critical comparisons, observations were made on mature seeds, in which the endosperm was entirely absorbed and the embryo occupied the entire embryo sac. The only exceptions were seeds of *O. flava*, *O. dissecta*, and *O. kunthiana*, which were slightly immature. Observations were made strictly on a part of the seed coat that had originally been constructed of both the inner and the outer integuments and at maturity consisted of both tegmen and testa. Terminology of the histological structure of seed coats and of the seed surface pattern primarily follows STEARN (1973) and CORNER (1976).

Observations

The seed coats of species of *Oenothera*, composed of the tegmen and testa (developed inner and outer integuments), exhibit considerable diversity in anatomy, particularly in features of the endotesta, mesotesta, and exotesta.

COMMON FEATURES

Only four anatomical features of *Oenothera* seeds were shared by all species studied: (1) a persistent endotegmen; (2) an endotegmen one cell layer thick with longitudinally elongate, tannin-containing cells; (3) an exotegmen with longitudinally elongate, thick-walled, pitted, lignified fibers; and (4) an endotesta composed of crystal-containing cells.

VARIABLE FEATURES

Many of the anatomical specializations of *Oenothera* seeds are present only in the species of a single section or occasionally a few sections (tables 2, 3; figs. 1–48). Thus, the seeds of most species share many common characters in addition to the four characters above. Most *Oenothera* seeds are

1–3 mm long, arranged in two rows in each of the four locules. The exotegmen is one cell layer thick; the mesotestal cells are sclerotic and pitted; and the exotesta is 10–45 μm thick, with flat or collapsed cells. While the thickness of the endotesta varies in the genus, there are three basic cell types (table 2): (1) radially enlarged, rectangular, or stellately lobed with the inner and radial walls thickened; (2) polygonal with sclerotic and pitted walls; and (3) radially flattened with the inner wall thickened.

TEGMEN

Species of sections *Eremia* and *Pachylophus* exhibit tegmen anatomy different from that of species of all other sections (table 2).

SECTION EREMIA.—The endotegmen of *O. primiveris* subsp. *primiveris* consists of one cell layer in the upper half of the seed close to the micropyle (fig. 13) and two cell layers close to the chalaza in the lower half of the seed.

SECTION PACHYLOPHUS.—*Oenothera caespitosa* subsp. *caespitosa* (fig. 10), *O. cavernae* (fig. 11), and *O. harringtonii* (fig. 12) are characterized by a distinctive thick tegmen not observed elsewhere in the genus except in *O. primiveris*. The exotegmen is usually three cell layers thick or, at places, two to four cell layers thick. This multilayered exotegmen is almost certainly secondarily formed by multiplication of the outer epidermis of the inner integument: in young ovules of *O. caespitosa* subsp. *marginata*, the inner integument originally consists of two cell layers; the outer epidermis develops into the exotegmen; the inner epidermis develops into the endotegmen. All layers of the exotegmen are identical and have longitudinally elongate, thick-walled, pitted, and lignified fibers.

TESTA

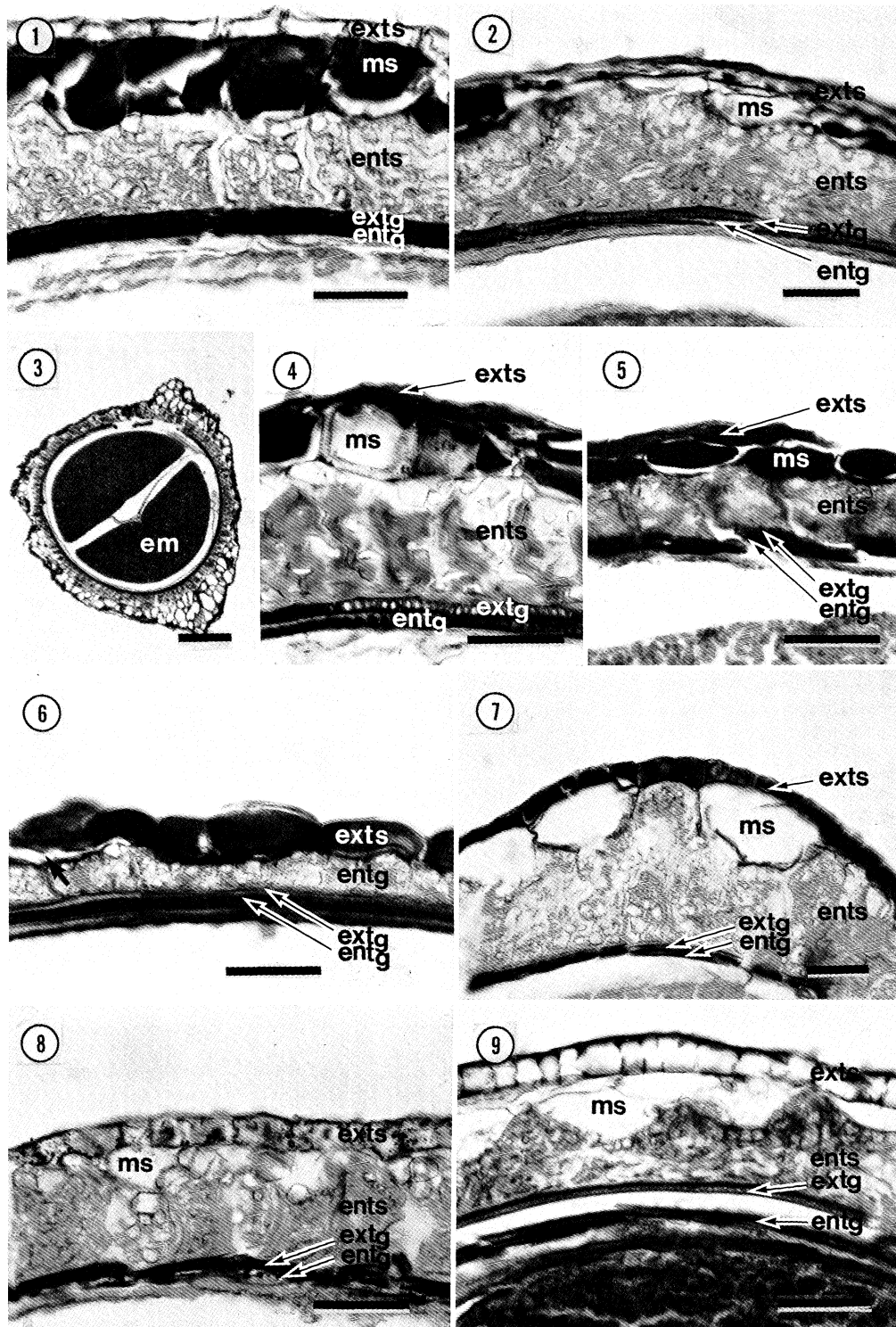
SECTION OENOTHERA SUBSECTION EMERSONIA.—In *O. stubbei* (fig. 1), *O. macrosceles* (fig. 2), *O.*

TABLE 2

COMPARISON OF SEED SIZE AND VARIABLE ANATOMICAL FEATURES OF THE EXOTEGMEN AND ENDOTESTA

	SEED SIZE (mm)		EXOTEGMEN (no. cell layers)	ENDOTESTA		
	RANGE			Thickness (μ m)	Cell	Thickenings
	Length	Width				
Section <i>Oenothera</i> :						
Subsection <i>Emersonia</i> :						
<i>O. macrosceles</i>	1.5–1.8	.7–.9	1	65.5–82.4	Radially enlarged	Inner & radial
<i>O. maysillesii</i>	2.0–3.0	1.0–1.8	1	67.6–88.7	Radially enlarged	Inner & radial
<i>O. organensis</i>	1.5–2.1	1.0–1.2	1	30.0–38.0	Radially enlarged	Inner & radial
<i>O. stubbei</i>	1.2–2.1	.8–1.2	1	50.7–63.4	Radially enlarged	Inner & radial
Subsection <i>Oenothera</i> :						
<i>O. villosa</i> subsp. <i>villosa</i>	1.5–1.7	.7–.8	1	14.8–27.5	Radially enlarged	Inner & radial
Subsection <i>Raimannia</i> :						
<i>O. heterophylla</i>	1.1–1.8	.4–.8	1	42.3–63.4	Radially enlarged	Inner & radial
<i>O. laciniata</i>9–1.8	.4–.9	1	63.4– 126.8	Radially enlarged	Inner & radial
Section <i>Kleinia</i> :						
<i>O. albicaulis</i>	1.0–1.2	.6–.9	1	27.5–52.8	Radially enlarged	Inner & radial
Section <i>Pachylophus</i> :						
<i>O. caespitosa</i> subsp. <i>caespitosa</i>	2.2–3.4	1.1–2.4	(2-)3(-4)	27.5–36.0	Radially enlarged	Inner & radial
<i>O. cavernae</i>	2.5–3.1	1.1–1.4	(2-)3(-4)	21.1–50.7	Radially enlarged	Inner & radial
<i>O. harringtonii</i>	2.1–2.3	1.1–1.3	(2-)3(-4)	42.3–52.8	Radially enlarged	Inner & radial
Section <i>Eremia</i> :						
<i>O. primiveris</i>	3.0–3.5	1.0–1.4	1–2	31.7–44.3	Radially enlarged	Inner & radial
Section <i>Contortiae</i> :						
<i>O. xylocarpa</i>	2.4–3.2	1.3–1.7	1	31.7–35.9	Radially enlarged	Inner & radial
Section <i>Ravenia</i> :						
<i>O. muelleri</i>	3.0–5.0	2.6–3.5	1	38.0–52.8	Polygonal	Sclerotic, pitted
<i>O. tubifera</i>	3.0–4.0	1.8–2.3	1	31.7–52.8	Polygonal	Sclerotic, pitted
Section <i>Megapterium</i> :						
<i>O. brachycarpa</i>	3.0–5.0	1.8–2.2	1	10.6–12.7	Radially flattened	Inner & radial
<i>O. macrocarpa</i> subsp. <i>macrocarpa</i>	3.0–5.0	1.8–2.3	1	19.0–21.1	Radially flattened	Inner & radial
Section <i>Lavauxia</i> :						
Subsection <i>Australis</i> :						
<i>O. acaulis</i>	2.5–3.5	1.1–2.2	1	14.8–19.0	Radially flattened	Inner & radial
Subsection <i>Lavauxia</i> :						
<i>O. flava</i>	1.8–2.5	1.2–1.8	1	8.5–10.6	Radially flattened	Inner
Section <i>Anogra</i> :						
<i>O. engelmannii</i>	1.0–1.5	.3–.5	1	8.5–10.6	Radially flattened	Inner
<i>O. kleinii</i>	2.5–2.8	.5–.8	1	16.9–21.1	Radially flattened	Inner
<i>O. pallida</i> subsp. <i>runcinata</i>	1.2–2.0	.3–.5	1	6.3–8.5	Radially flattened	Inner
Section <i>Paradoxus</i> :						
<i>O. havardii</i>	2.2–2.5	1.2–1.5	1	10.6–12.7	Radially flattened	Inner
Section <i>Gauropsis</i> :						
<i>O. canescens</i>	1.2–1.5	.4–.5	1	4.2–5.3	Radially flattened	Inner
<i>O. dissecta</i>	ca. 1.5	.6–.8	1	5.3–6.3	Radially flattened	inner
Section <i>Kneiffia</i> :						
<i>O. fruticosa</i> subsp. <i>fruticosa</i>	1.4–1.6	.5–.8	1	6.3–10.6	Radially flattened	Inner
<i>O. perennis</i>7–.8	.2–.3	1	4.2–6.3	Radially flattened	Inner
Section <i>Xylopleurum</i> :						
<i>O. speciosa</i>	1.1–1.5	.3–.5	1	8.5–12.7	Radially flattened	Inner
Section <i>Hartmannia</i> :						
<i>O. epilobiiifolia</i> subsp. <i>cuprea</i>	1.2–1.5	.4–.6	1	4.2–5.3	Radially flattened	Inner
<i>O. kunthiana</i>	1.2–1.5	.4–.8	1	4.2–6.3	Radially flattened	Inner
<i>O. rosea</i>7–.9	.4–.5	1	4.2–5.3	Radially flattened	Inner
<i>O. seifrizzi</i>	1.1–1.4	.5–.7	1	3.2–4.2	Radially flattened	Inner

NOTE.—The arrangement of the sections for observations of each section moves from those with more generalized to those with more specialized features.



Figs. 1-9.—Longitudinal (LS) and cross (CS) microtome sections of seed coats of *Oenothera*. *em*, Embryo; *entg*, endotegmen; *ents*, endotesta; *extg*, exotegmen; *exts*, exotesta; *ms*, mesotesta. Scale = 50 μm in figs. 1, 2, 4-9; 0.2 mm in fig. 3. Fig. 1, *O. stubbei* (CS). Fig. 2, *O. macrosceles* (CS). Figs. 3, 4, *O. maysillesii* (CS). Fig. 5, *O. organensis* (LS). Fig. 6, *O. villosa* subsp. *villosa* (LS). Fig. 7, *O. laciniata* (LS). Fig. 8, *O. heterophylla* (LS). Fig. 9, *O. albicaulis* (CS).

maysillesii (figs. 3, 4), and *O. organensis* (fig. 5) the endotesta varies in thickness from one part of the seed to another and among the species (table 2). The mesotesta is persistent, but it may become somewhat compressed and varies in thickness and in degree of specialization (table 3). In *O. stubbei*,

O. macrosceles, and *O. organensis* the mesotesta is consistently one cell thick, whereas in *O. maysillesii* it is two or three, rarely one cell, thick; these layers may be compressed at places (figs. 3, 4). The mesotestal cells of *O. maysillesii* become sclerotic and pitted (fig. 4); in the other species of sub-

TABLE 3
COMPARISONS OF VARIABLE ANATOMICAL FEATURES OF THE MESOTESTA AND EXOTESTA

	MESOTESTA		EXOTESTA	
	No. cell layers	Cell	Thickness (μm)	Cell
Section <i>Oenothera</i> :				
Subsection <i>Emersonia</i> :				
<i>O. macrosceles</i>	1	Thin walled	...	Flattened
<i>O. maysillesii</i>	1-3	Sclerotic, pitted; occasionally compressed	...	Collapsed
<i>O. organensis</i>	1	Thin walled	...	Collapsed
<i>O. stubbei</i>	1	Thin walled	...	Flattened
Subsection <i>Oenothera</i> :				
<i>O. villosa</i> subsp. <i>villosa</i>	0	Crushed	...	Flattened or collapsed
Subsection <i>Raimannia</i> :				
<i>O. heterophylla</i>	1	Thin walled	...	Flattened or collapsed
<i>O. laciniata</i>	1	Thin walled	...	Flattened or collapsed
Section <i>Kleinia</i> :				
<i>O. albicaulis</i>	1	Thin walled	...	Flattened or collapsed
Section <i>Pachylophus</i> :				
<i>O. caespitosa</i> subsp. <i>caespitosa</i>	3-4	Lignified, compressed	...	Irregularly swollen or collapsed
<i>O. cavernae</i>	1-2	Lignified, compressed	...	Irregularly swollen or collapsed
<i>O. harringtonii</i>	1	Lignified, compressed	...	Flattened or collapsed
Section <i>Eremia</i> :				
<i>O. primiveris</i>	2-5	Sclerotic, pitted	...	Irregularly swollen or collapsed
Section <i>Contortae</i> :				
<i>O. xylocarpa</i>	1-2	Sclerotic, pitted	...	Irregularly swollen or collapsed
Section <i>Ravenia</i> :				
<i>O. muelleri</i>	4-8	Sclerotic, pitted	...	Longitudinally elongate, collapsed
<i>O. tubifera</i>	3-5	Sclerotic, pitted	...	Longitudinally elongate, collapsed
Section <i>Megapterium</i> :				
<i>O. brachycarpa</i>	8-16	Sclerotic, pitted	...	Irregularly swollen or collapsed
<i>O. macrocarpa</i> subsp. <i>macrocarpa</i>	6-20	Sclerotic, pitted	...	Irregularly swollen or collapsed
Section <i>Lavauxia</i> :				
Subsection <i>Australis</i> :				
<i>O. acaulis</i>	0	Crushed	38.0-42.3	Enlarged; subcuboidal
Subsection <i>Lavauxia</i> :				
<i>O. flava</i>	0	Crushed	46.5-126.8	Radially enlarged; pillar-like
Section <i>Anogra</i> :				
<i>O. engelmannii</i>	0	Crushed	...	Collapsed
<i>O. kleinii</i>	0	Crushed	12.7-16.9	Enlarged; subcuboidal
<i>O. pallida</i> subsp. <i>runcinata</i>	0	Crushed	19.0-25.4	Enlarged; subcuboidal
Section <i>Paradoxus</i> :				
<i>O. havardii</i>	2-5	Sclerotic, pitted	67.6-99.3	Radially enlarged; papilla-like
Section <i>Gauropsis</i> :				
<i>O. canescens</i>	0	Crushed	74.0-152.1	Radially enlarged; pillar-like
<i>O. dissecta</i>	0	Crushed	46.5-59.2	Radially enlarged; pillar-like
Section <i>Kneiffia</i> :				
<i>O. fruticosa</i> subsp. <i>fruticosa</i>	0	Crushed	38.0-46.5	Radially enlarged; papilla-like
<i>O. perennis</i>	0	Crushed	21.1-31.7	Radially enlarged; papilla-like
Section <i>Xylopleurum</i> :				
<i>O. speciosa</i>	0	Crushed	27.5-38.0	Radially enlarged; papilla-like
Section <i>Hartmannia</i> :				
<i>O. epilobiiifolia</i> subsp. <i>cuprea</i>	0	Crushed	10.6-12.7	Enlarged; subcuboidal
<i>O. kunthiana</i>	0	Crushed	16.9-19.0	Radially enlarged; papilla-like
<i>O. rosea</i>	0	Crushed	23.2-27.5	Radially enlarged; papilla-like
<i>O. seifrizii</i>	0	Crushed	12.7-14.8	Enlarged; subcuboidal

section *Emersonia* they are thin walled and not pitted. In general, the mesotestal cells of these species are contiguous (fig. 1), but occasionally they may become separated at places as a result of radial enlargement of the endotestal cells (fig. 2). The exotestal cells are either radially flattened, as in *O. stubbei* and *O. macrosceles*, or somewhat collapsed, as in *O. maysillesii* and *O. organensis*. The surface pattern is basically reticulate (fig. 37).

SECTION OENOTHERA SUBSECTION OENOTHERA.—*Oenothera villosa* subsp. *villosa* (fig. 6) has a simpler testal structure than the species of subsection *Emersonia*. The endotesta of this species is thin (table 2). The mesotesta is unusual in being nearly completely crushed (table 3). This crushing, which occurs during development, leaves small air spaces between the endotesta and exotesta. The exotesta has irregularly shaped, thick-walled, tannin-containing cells. The surface pattern is similar to that of *O. organensis*.

SECTION OENOTHERA SUBSECTION RAIMANNIA.—*Oenothera laciniata* (fig. 7) and *O. heterophylla* (fig. 8) have features that closely resemble those of *O. stubbei* and *O. macrosceles* but differ from those of *O. maysillesii* and *O. organensis* (tables 2, 3). The exotestal cells are not specialized; they are radially flattened and contain variable amounts of tannin; the surface pattern is reticulate.

SECTION KLEINIA.—The histological features of *O. albicaulis* (fig. 9) are remarkably similar to those of *O. stubbei*, *O. macrosceles*, *O. laciniata*, and *O. heterophylla*. The single cell-layered mesotesta is persistent, thin walled, not pitted, often somewhat compressed, and discontinuous at places (table 3). The surface pattern is scalariform, and the surface has deeper depressions (fig. 38) than in species of section *Oenothera*.

SECTION RAVENIA.—The testal anatomy in *O. muelleri* (figs. 15–17) and *O. tubifera* (fig. 18) is identical (table 2). The endotestal cells are polygonal, uniformly thick walled, sclerotic, pitted, and contain crystals, features unique in the genus (figs. 17, 18; tables 2, 3). The mesotesta is exceptionally thick (3–8 cells) and constitutes a major part of the seed coat (figs. 15, 16). The mesotestal cells are uniformly thick walled, sclerotic, and pitted but differ from the endotestal cells in lacking crystals. The exotesta is composed of longitudinally elongate, collapsed cells (fig. 39); the surface pattern is reticulate to papillate; the papillae are elongated longitudinally and compressed laterally (fig. 39).

SECTION CONTORTAE.—The mesotesta of *O. xylocarpa* (fig. 14) is one to two cell layers thick (table 3). Some of the cells in the outer cell layer are more radially elongate than those in the inner layer. The exotestal cells are thin walled and collapsed and may develop into small papillae; the surface pattern is reticulate to papillate (WAGNER et al. 1985).

SECTION EREMIA.—The mesotesta of *O. primiveris* subsp. *primiveris* (fig. 13) has uniformly thick-walled, sclerotic, pitted cells and is two to five cells thick. The exotestal cells are thin walled and collapsed and may develop into small papillae; the surface pattern is reticulate to papillate (WAGNER et al. 1985).

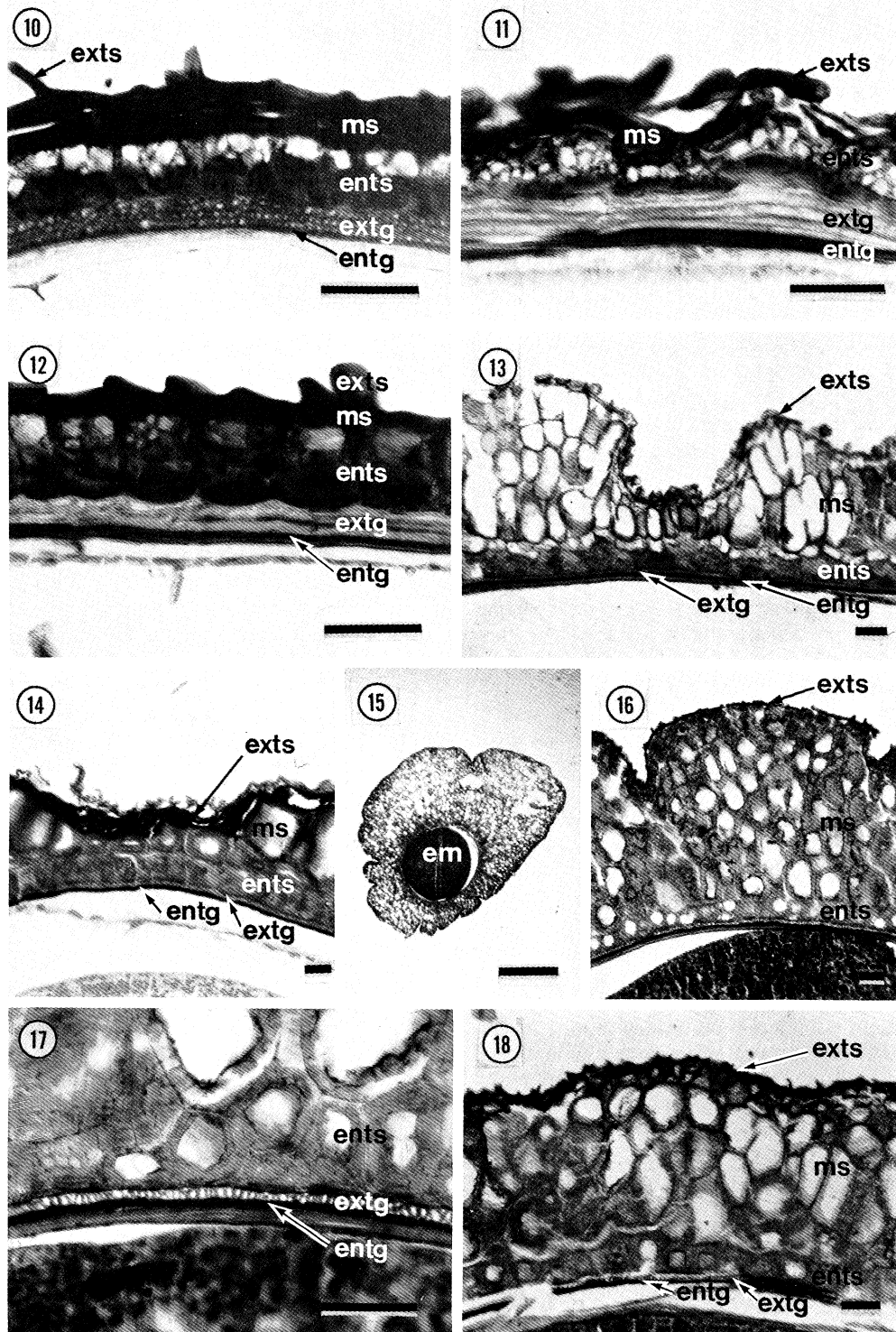
SECTION PACHYLOPHUS.—The mesotesta has thick-walled, highly lignified, compressed cells, a feature unique in the genus (figs. 10–12). The mesotesta is one to four cells thick (table 3). The exotesta has thin-walled cells that are somewhat radially developed or collapsed, and the surface pattern is papillate to reticulate (WAGNER et al. 1985).

SECTION MEGAPTERIUM.—The seeds of *O. brachycarpa* (figs. 19, 20) and *O. macrocarpa* subsp. *macrocarpa* (fig. 21) have a thinner endotesta than those in the preceding sections (table 2). The endotestal cells are radially flattened and contain crystals (fig. 20). In section *Megapterium* and in all of the following sections, the endotestal cells are not prominently enlarged radially in contrast to those in the preceding sections. The mesotesta has sclerotic, pitted cells and is 8–16 cells thick in *O. brachycarpa* and 6–20 cells thick in *O. macrocarpa* subsp. *macrocarpa*. The exotestal cells are thin walled and not specialized; the surface pattern is reticulate or has collapsed papillae (fig. 40).

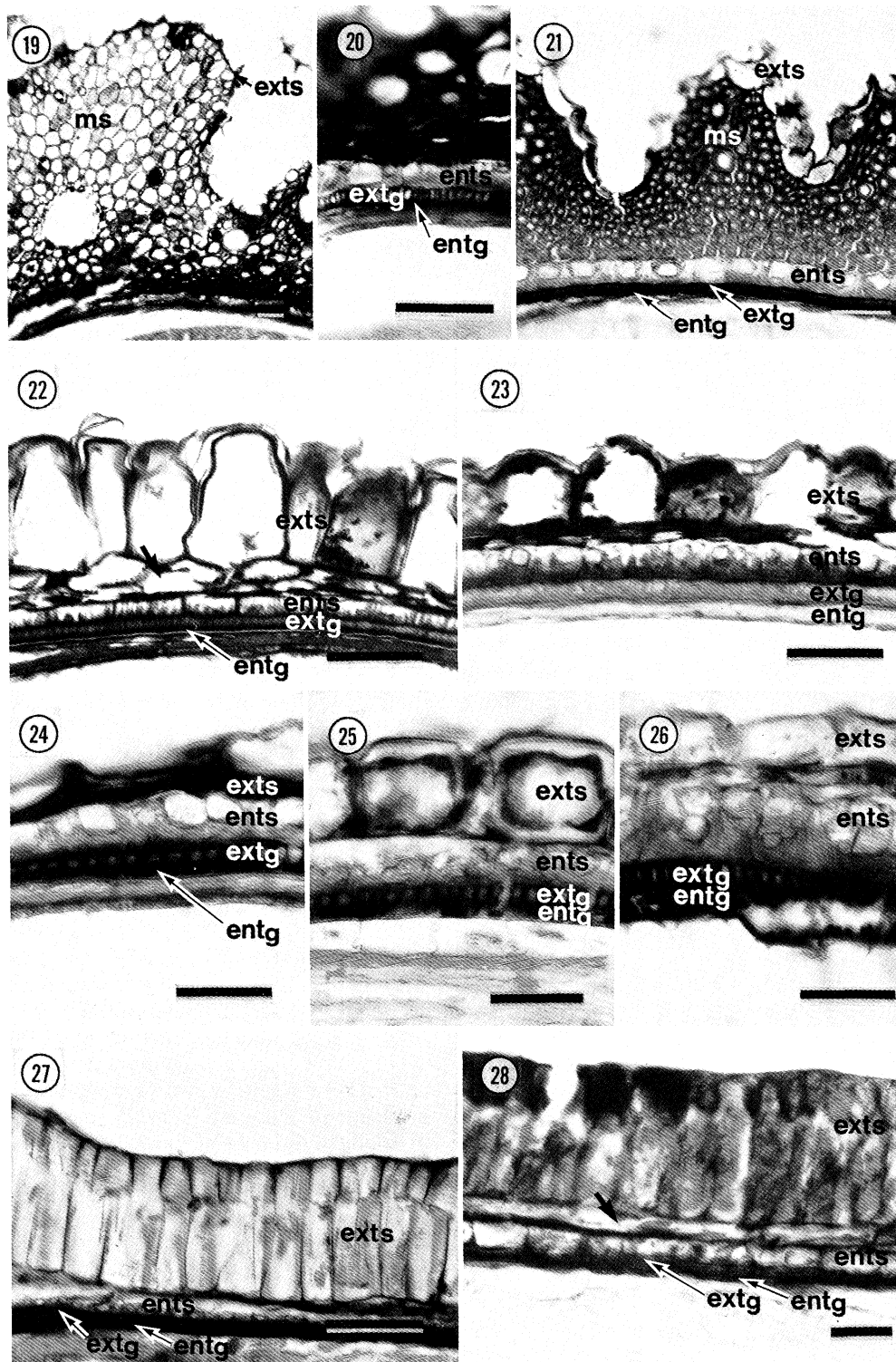
SECTION LAVAUXIA SUBSECTION LAVAUXIA.—*Oenothera flava* (fig. 22), a North American species, has a thin endotesta with thickened inner walls (table 2). The mesotesta becomes crushed at maturity (table 3). The exotestal cells are radially elongated into pillar-like structures. The upper half of each exotestal cell is narrower than the lower half and is separated from the adjacent cells, giving the seed surface a beaded appearance (fig. 41). The cells also have numerous minute papillae on their upper surface.

SECTION LAVAUXIA SUBSECTION AUSTRALIS.—*Oenothera acaulis* (fig. 23), a South American species, has a thin endotesta. The endotestal cells, however, have thickened inner and radial walls, typical for sections with thick endotesta (table 2). The mesotesta is crushed, as in *O. flava*. The exotestal cells are not as enlarged as those of *O. flava* (table 3); they are thick walled and more continuous with one another than in *O. flava*, resulting in a colliculate surface pattern (fig. 42).

SECTION ANOGR.—*Oenothera engelmannii* (fig. 24), *O. pallida* subsp. *runcinata* (fig. 25), and *O. kleinii* (fig. 26) have thin endotesta, with a thickened inner wall and a completely crushed mesotesta (tables 2, 3). The exotesta of *O. pallida* and *O. kleinii* has enlarged subcuboidal cells, making the surface pattern alveolate (fig. 44). In *O. engelmannii*, however, the exotestal cells are collapsed, giving the seed surface a scalariform pattern (fig. 43).



FIGS. 10–18.—LS and CS of seed coats of *Oenothera*. *em*, Embryo; *entg*, endotegmen; *ents*, endotesta; *extg*, exotegmen; *exsts*, exotesta; *ms*, mesotesta. Scale = 50 μ m in figs. 10–14, 16–18; 1 mm in fig. 15. Fig. 10, *O. caespitosa* subsp. *caespitosa* (CS). Fig. 11, *O. cavernae* (LS). Fig. 12, *O. harringtonii* (LS). Fig. 13, *O. primiveris* subsp. *primiveris* (CS). Fig. 14, *O. xylocarpa* (CS). Figs. 15–17, *O. muelleri* (CS). Fig. 18, *O. tubifera* (CS).



FIGS. 19–28.—LS and CS of seed coats of *Oenothera*. *entg*, Endotegmen; *ents*, endotesta; *extg*, exotegmen; *exts*, exotesta; *mt*, mesotesta. Scale = 50 μ m in figs. 19–23, 27; 20 μ m in figs. 24–26, 28. Figs. 19, 20, *O. brachycarpa* (CS). Fig. 21, *O. macrocarpa* subsp. *macrocarpa* (CS). Fig. 22, *O. flava* (CS), an immature stage in which the mesotestal cells are still being crushed. Fig. 23, *O. acaulis* (LS). Fig. 24, *O. engelmannii* (CS). Fig. 25, *O. pallida* subsp. *runcinata* (CS). Fig. 26, *O. kleinii* (CS). Fig. 27, *O. canescens* (CS). Fig. 28, *O. dissecta* (CS).

SECTION GAUOPSIS.—*Oenothera canescens* (fig. 27) and *O. dissecta* (fig. 28) have thin endotesta with a thickened inner wall (table 2). The mesotesta of both species is completely crushed, and the exotesta has remarkably radially elongated cells that are modified into pillar-like structures at maturity (table 3). Unlike those of *O. flava* (fig. 22), however, the pillar-like exotestal cells of these two species are tightly connected with one another along their entire length, making the external surface smooth or inconspicuously reticulate (fig. 46).

SECTION PARADOXUS.—*Oenothera havardii* (fig. 29) has a thin endotesta with a thickened inner wall but has a persistent mesotesta two to five cells thick, with cells that are sclerotic, pitted, and radially flattened (tables 2, 3). The exotestal cells are radially elongated and separated from one another in their upper halves, as in *O. flava*, giving the surface a beaded appearance. They also have numerous minute papillae on their upper surfaces (fig. 45).

SECTION KNEIFFIA SUBSECTION KNEIFFIA.—The seeds of *O. fruticosa* subsp. *fruticosa* (fig. 30) and *O. perennis* (fig. 31) are similar in structure. The endotesta of these species is very thin, with thickened inner walls (table 2). The mesotesta is completely crushed (table 3). The exotesta has papilla-like cells that taper toward the apex; the surface pattern is aculeate (fig. 47).

SECTION XYLOPLEURUM.—*Oenothera speciosa* (fig. 32) is similar to the species of sections *Hartmannia* and *Kneiffia* in having a comparatively thin endotesta and a crushed mesotesta (tables 2, 3). The exotestal cells are papilla-like and are similar to those of section *Kneiffia* and the North American members of section *Hartmannia*. The surface pattern is aculeate (fig. 48).

SECTION HARTMANNIA.—The seeds of *O. kunthiana* (fig. 33), *O. rosea* (fig. 34), *O. seifrizii* (fig. 35), and *O. epilobiifolia* subsp. *cuprea* (fig. 36) have both thin endotesta with thickened inner walls and crushed mesotesta (tables 2, 3). By contrast, these species are diverse in the degree of exotesta specialization. *Oenothera kunthiana* and *O. rosea* have papilla-like exotestal cells that taper in the upper half so that the surface pattern is aculeate (fig. 48). Both *O. seifrizii* and *O. epilobiifolia* are specialized and similar to one another in having subcuboidal exotestal cells, giving a colliculate surface pattern (fig. 42), as in *O. dissecta* and *O. canescens*.

Discussion

SYSTEMATIC CONSIDERATIONS

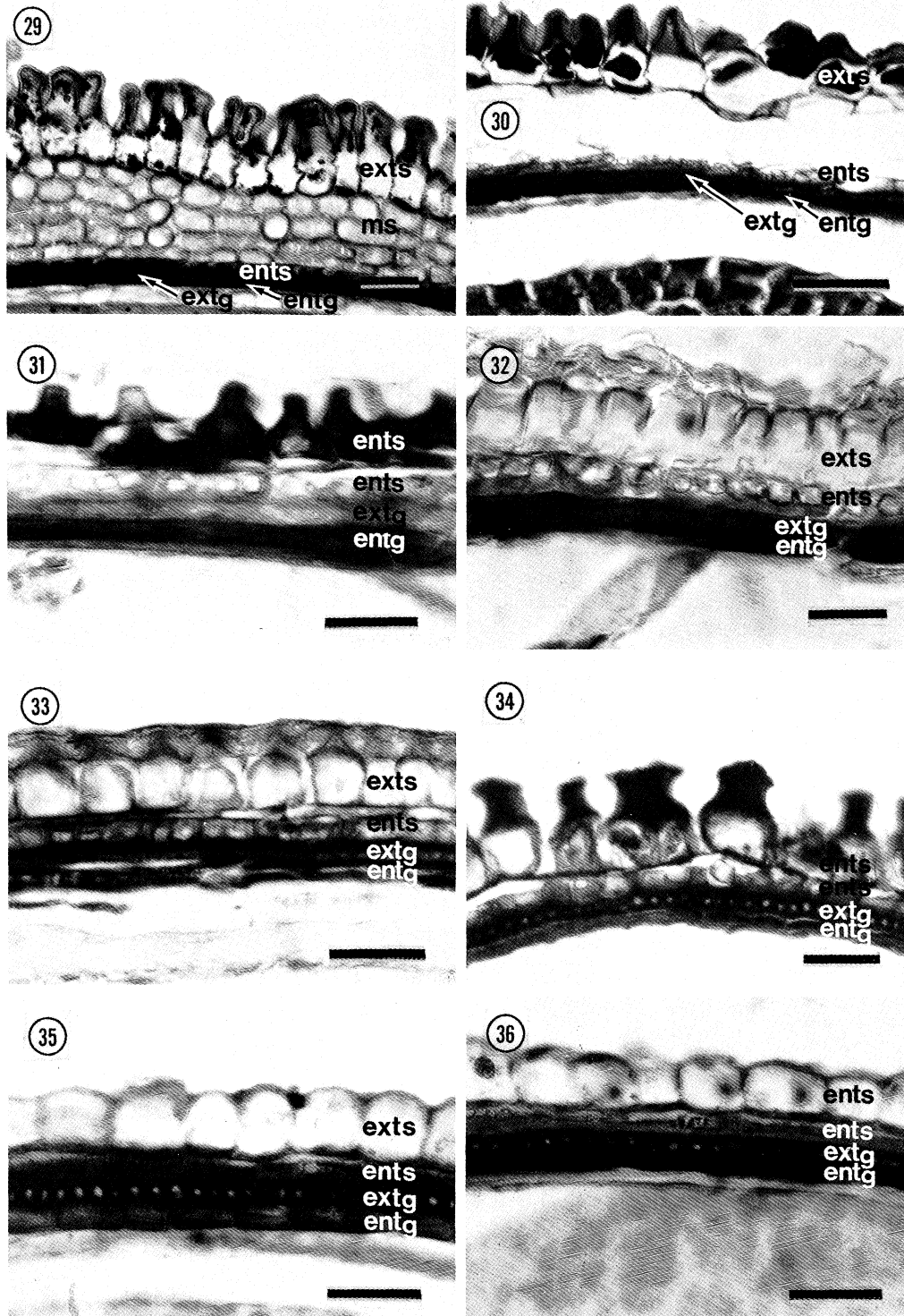
SECTION OENOTHERA.—Based on results of experimental hybridization studies, STUBBE and RAVEN (1979) expanded section *Oenothera* to include five subsections: *Emersonia* (DIETRICH et al. 1985),

Munzia (DIETRICH 1977), *Raimannia*, *Nutanti-gemma* (DIETRICH and WAGNER 1987), and *Oenothera*. A comparison of seed coat anatomy indicates that this section is uniform in having a thick endotesta, persistent mesotesta (except in *O. villosa*, subsection *Oenothera*), and a nonspecialized exotesta. *Oenothera maysillesii* (subsection *Emersonia*) is strikingly different not only from other members of its subsection but also from the other subsections of section *Oenothera*. *Oenothera maysillesii* has a mesotesta one to three cells thick with sclerotic, pitted cells, whereas other species have a one-cell-layered mesotesta with thin-walled cells or no mesotesta at all. The multilayered mesotesta in *O. maysillesii* also occurs in sections *Eremia*, *Contortae*, *Ravenia*, and *Megapterium*. At the same time, crossing experiments and other shared morphological features indicate an undoubted relationship between *O. maysillesii* and the three other species of subsection *Emersonia* (STUBBE and RAVEN 1979; DIETRICH et al. 1985). A multilayered mesotesta appears to be a primitive character for the genus and thus cannot be used to predict relationships.

Oenothera villosa subsp. *villosa* is distinct in section *Oenothera* in having a thin endotesta and a completely crushed mesotesta. Although it has not been determined that these anatomical features of *O. villosa* subsp. *villosa* are typical for subsection *Oenothera*, it appears that this subsection has the most specialized and simplified seed structure among the subsections of section *Oenothera*. Thin endotesta and crushed mesotesta appear to have been independently derived in subsection *Oenothera* and the other species that exhibit these characters.

Both *O. laciniata* and *O. heterophylla* (subsection *Raimannia*) have seed coat structures identical with those of *O. stubbei* and *O. macroscelus* (subsection *Emersonia*). The similarity in seed anatomy between subsections *Raimannia* and *Emersonia*, rather than subsection *Oenothera*, appears to run contrary to results from experimental hybridizations. No seeds were produced in attempted hybridizations between subsections *Raimannia* and *Emersonia* except for pollen-sterile hybrids obtained with difficulty between *O. maysillesii* and *O. drummondii* Hook. subsp. *thalassaphila* (T. S. Brandege) W. Dietrich & W. L. Wagner (STUBBE and RAVEN 1979). By contrast, viable hybrids can readily be obtained in certain plastome conditions between subsections *Raimannia* and *Oenothera* (STUBBE and RAVEN 1979).

SECTION KLEINIA.—*Oenothera albicaulis* and *O. coronopifolia* constitute section *Kleinia* (MUNZ 1965; RAVEN 1970). Based primarily on capsule and seed morphology, MUNZ (1935, 1965) placed the two white-flowered species of section *Kleinia* in his subgenus *Raimannia*, a group of otherwise yellow-flowered species, rather than with other white-



Figs. 29–36.—LS and CS of seed coats of *Oenothera*. *entg*, Endotegmen; *ents*, endotesta; *extg*, exotegmen; *exts*, exotesta; *ms*, mesotesta. Scale = 50 μ m in figs. 29, 30; 20 μ m in figs. 31–36. Fig. 29, *O. havardii* (CS). Fig. 30, *O. fruticosa* subsp. *fruticosa* (LS). Fig. 31, *O. perennis* (LS). Fig. 32, *O. speciosa* (LS). Fig. 33, *O. kunthiana* (LS). Fig. 34, *O. rosea* (CS). Fig. 35, *O. seifrizii* (CS). Fig. 36, *O. epilobiifolia* subsp. *cuprea* (CS).

flowered species, subgenus *Anogra*. RAVEN (1970) considered section *Kleinia* to be intermediate between subgenus *Raimannia* and subgenus *Anogra*. Our data show that *O. albicaulis* has essentially the same seed anatomy as species of section *Oenothera* subsection *Raimannia* and three species of subsection *Emersonia*. The seed coat structure of members of section *Anogra* is entirely different in lacking a mesotesta and in having a thin endotesta composed of cells with a thickened inner wall. This indicates that section *Kleinia* is directly related to section *Oenothera* subsection *Raimannia*. The seed surface pattern, however, differs between section *Kleinia* and subsection *Raimannia* primarily in the deeper depressions on the surface of seeds in the former group (figs. 37, 38).

HECHT (1950) hybridized *O. albicaulis* with species of subsection *Raimannia* but obtained few seeds, which did not germinate. This indicates that, despite the clear relationship between section *Kleinia* and subsection *Raimannia*, considerable genetic divergence may have occurred.

SECTIONS PACHYLOPHUS, EREMIA, CONTORTAE, AND RAVENIA.—Until RAVEN (1970) reviewed the species of these sections, *O. muelleri* had been included in subgenus *Raimannia*, and all others were included in subgenus *Pachylophus* (MUNZ 1965). RAVEN (1970) reevaluated the relationships of these species and suggested that they could be divided into three distinct subgroups: (1) a subgroup of *O. caespitosa*, *O. cavernae*, and *O. brandegeei*, i.e., section *Pachylophus*, which was subsequently expanded to contain two additional species, *O. harringtonii* and *O. psammophila* (WAGNER et al. 1985); (2) a subgroup with *O. primiveris* (now treated as section *Eremia*; WAGNER 1986) and *O. xylocarpa* (now section *Contortae*; WAGNER 1986); and (3) a subgroup with *O. muelleri* and *O. tubifera* (now section *Ravenia*; WAGNER 1986). Biosystematic studies of these species (STOCKHOUSE 1973; WAGNER et al. 1985) strongly supported this treatment. The latter study included SEM observations of seed surface patterns and internal structures of hand-sectioned seeds. In addition, a cladistic analysis (WAGNER et al. 1985) indicated that section *Pachylophus* is a monophyletic group most closely related to *O. primiveris* and *O. xylocarpa*. Our study also strongly supports the conclusion of RAVEN (1970) and is consistent with WAGNER's (1985) cladistic analysis.

The examined species of section *Pachylophus* are distinct in having a persistent, multilayered mesotesta with compressed cells and a multilayered fibrous exotegmen. Both features are unknown elsewhere in the genus except in *O. primiveris* (section *Eremia*), which sometimes has a two-layered exotegmen. *Oenothera primiveris* subsp. *primiveris* may have a two-cell-layered fibrous exotegmen near the chalazal end of the seed, a fea-

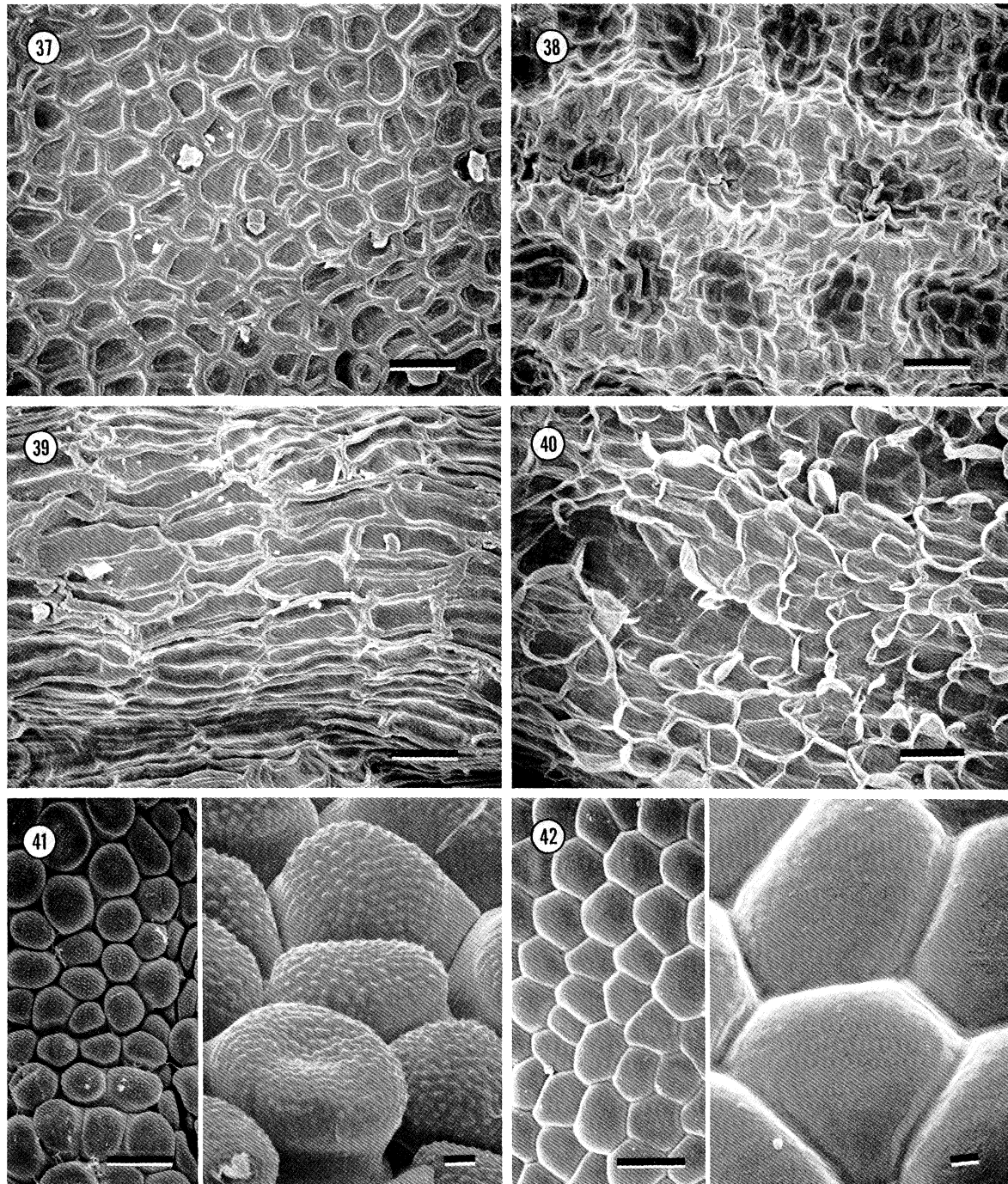
ture that represents a link with section *Pachylophus*. By contrast, *O. primiveris* subsp. *primiveris*, *O. xylocarpa* (section *Contortae*), and *O. muelleri* and *O. tubifera* (section *Ravenia*) have a persistent, multilayered mesotesta with noncompressed, sclerotic, pitted cells and a one-cell-layered fibrous exotegmen. The combination of these characters distinguishes these four species from the members of section *Pachylophus*. Both *O. muelleri* and *O. tubifera* are distinct from *O. primiveris* and *O. xylocarpa* by their unique sclerotic, pitted, polygonal endotestal cells.

Seeds of *O. primiveris* and *O. xylocarpa*, like those of species in section *Pachylophus*, have radially enlarged endotestal cells with thickened inner and radial walls, a feature also shared by members of sections *Oenothera* and *Kleinia*. In this respect, *O. primiveris* and *O. xylocarpa* are more similar to section *Pachylophus* than to section *Ravenia*, as indicated by cladistic analysis (WAGNER et al. 1985). Seeds of *O. primiveris* and *O. xylocarpa* differ from those of *O. maysillesii* (subsection *Emersonia*) only in having a thinner endotesta and exotestal cells that may be irregularly swollen rather than collapsed. *Oenothera primiveris* differs from both *O. maysillesii* and *O. xylocarpa* in its thicker, one- to two-cell-layered exotegmen and thicker, two- to five-cell-layered mesotesta.

SECTION MEGAPTERIUM.—The seed coat anatomy of the two examined members of this section is uniform but distinct from that of other sections. *Oenothera brachycarpa* and *O. macrocarpa* subsp. *macrocarpa* have a persistent, multilayered mesotesta with sclerotic, pitted cells, a feature shared with *O. maysillesii*, section *Oenothera*, and sections *Ravenia*, *Contortae*, *Eremia*, and *Paradoxus*. Although the mesotestal cells are similar in structure to those of these other sections, they are smaller. Species of section *Megapterium* differ from *O. harvardii* in lacking any specialized exotestal cells and from the remainder of the examined species in having thin endotesta. Seeds of sections *Anogra*, *Gauropsis*, *Hartmannia*, *Kneiffia*, *Lavauxia*, *Megapterium*, *Paradoxus*, and *Xylopleurum* have thin endotesta; all of these sections except *Megapterium* and *Paradoxus* have crushed mesotesta.

Thin endotesta, based on the outgroup comparison to *Stenosiphon*, appears to represent a plesiomorphic feature and thus does not indicate relationship. Crushed mesotesta, however, represents a synapomorphy, giving the first unequivocal data that ally these sections to one another.

SECTION LAVAUXIA.—*Oenothera flava* (North American) and *O. acaulis* (South American) differ from each other in the degree of specialization of the exotesta. In *O. flava* the exotestal cells are prominently radially elongated into pillar-like cells, whereas in *O. acaulis* they are less radially elongated and subcuboidal in shape. The resultant dif-



FIGS. 37-42.—SEM of the seed surface of *Oenothera*. Scale = 50 μm in figs. 37-40, 41 (left), 42 (left); 5 μm in figs. 41 (right), 42 (right). Fig. 37, *O. maysillesii* (Breedlove 44288, MO). Fig. 38, *O. albicaulis* (Dueholm 4453, MO). Fig. 39, *O. tubifera* (Stubbe 305, DUSS). Fig. 40, *O. macrocarpa* subsp. *fremontii* (McGregor 32015, MO). Fig. 41, *O. flava* (Wagner & Downs in 1978, MO). Fig. 42, *O. acaulis* (1974, Rodriguez s.n., MO).

ferent seed surface patterns—tuberculate, beadlike in *O. flava* (fig. 41) and colliculate in *O. acaulis* (fig. 42)—support the subdivision of section *Lavauxia* into two subsections (WAGNER 1986).

SECTION ANOGRA.—The three examined species in this section have a relatively simple seed coat

structure, including a thin endotesta, crushed mesotesta, and a relatively unspecialized exotesta. *Oenothera engelmannii* differs from *O. pallida* subsp. *runcinata* and *O. kleinii* in having collapsed exotestal cells. This specialized exotesta probably is related to the encapsulation of each seed in a por-

tion of the capsular tissue, which is dispersed with each seed (WAGNER, unpublished data). The other two species have normal capsule dehiscence, and the seeds are free of the capsule walls.

SECTION GAUOPSIS.—In contrast to the close similarity of seed coat structure between *O. canescens* and *O. dissecta*, that of *O. havardii* is conspicuously different in having both a persistent, multilayered mesotesta with sclerotic, pitted cells and a very specialized exotesta with remarkably radially elongated cells. *Oenothera havardii* not only is distinct from the other two species included by MUNZ (1932, 1965) in his subgenus *Gauopsis* but also from members of all other sections of the genus. These unique differences support the removal of *O. havardii* from section *Gauopsis* to the new monotypic section *Paradoxus* (WAGNER 1984). A pronounced difference in seed size also supports this classification.

SECTION KNEIFFIA.—*Oenothera fruticosa* subsp. *fruticosa* and *O. perennis* are perennial herbs (MUNZ 1965; STRALEY 1977) and have relatively simple anatomical seed coat structures with aculeate seed surfaces formed by papilla-like exotestal cells. According to STRALEY (1977) who studied seed coat surfaces in the five species of section *Kneiffia*, two annual species, *O. linifolia* (the only member of subsection *Peniophyllum*) and *O. spachiana* (subsection *Kneiffia*), have a verrucose seed surface similar to the aculeate surface of the perennial species. Section *Kneiffia* has been placed close to section *Hartmannia* because both sections have clavate capsules with the seeds clustered in each cell (MUNZ 1965). Seed coat anatomy supports this interpretation. Both *O. epilobiifolia* and *O. seifrizii* (section *Hartmannia*) differ from the other taxa in these sections in having enlarged, subcuboidal exotestal cells.

SECTIONS HARTMANNIA AND XYLOPLEURUM.—Among the species examined in these sections, *O. kunthiana*, *O. rosea*, and *O. speciosa* are identical in anatomical structure. They are also similar to the species of section *Kneiffia*. The Central and South American species of section *Hartmannia*, *O. seifrizii* and *O. epilobiifolia* subsp. *cuprea*, differ in having less radially enlarged exotestal cells that are subcuboidal in shape. Based on this evidence, these two species could be separated into their own section or subsection. In further support, both have yellow petals, sometimes with a red spot, while the other species of section *Hartmannia* have white or purple petals. Crossing studies (RAVEN and PARNELL, unpublished data) demonstrated a distinction in crossability among *O. seifrizii*, *O. epilobiifolia*, and other yellow-flowered species of section *Hartmannia* and the remainder of section *Hartmannia*, which has species with white or rose-purple petals.

There is a coincidence in shape of exotestal cells between the yellow-flowered species of section

Hartmannia and section *Lavauxia*: both *O. seifrizii* and *O. epilobiifolia* subsp. *cuprea*, like *O. acaulis* (section *Lavauxia*), have a colliculate seed surface with subcuboidal exotestal cells. This similarity has surely evolved independently in the two groups and perhaps represents a loss of specialization in each lineage brought about by long-distance dispersal.

CLADISTIC ANALYSIS

The relationships among the sections of *Oenothera* have been difficult to analyze. Sectional delimitation was based largely on structure of the capsules and seed arrangement in the fruit (MUNZ 1965). This approach was maintained in subsequent detailed studies of individual sections (DIETRICH 1977; STRALEY 1977; DIETRICH et al. 1985; WAGNER et al. 1985; WAGNER, unpublished data), but it has been primarily useful in delimiting sections, not in determining relationships among them. A cladistic analysis using these new data should thus provide new insights into the relationships among the sections of *Oenothera*. Moreover, hypotheses of relationships based on these data can be tested with other, more diverse data using additional characters.

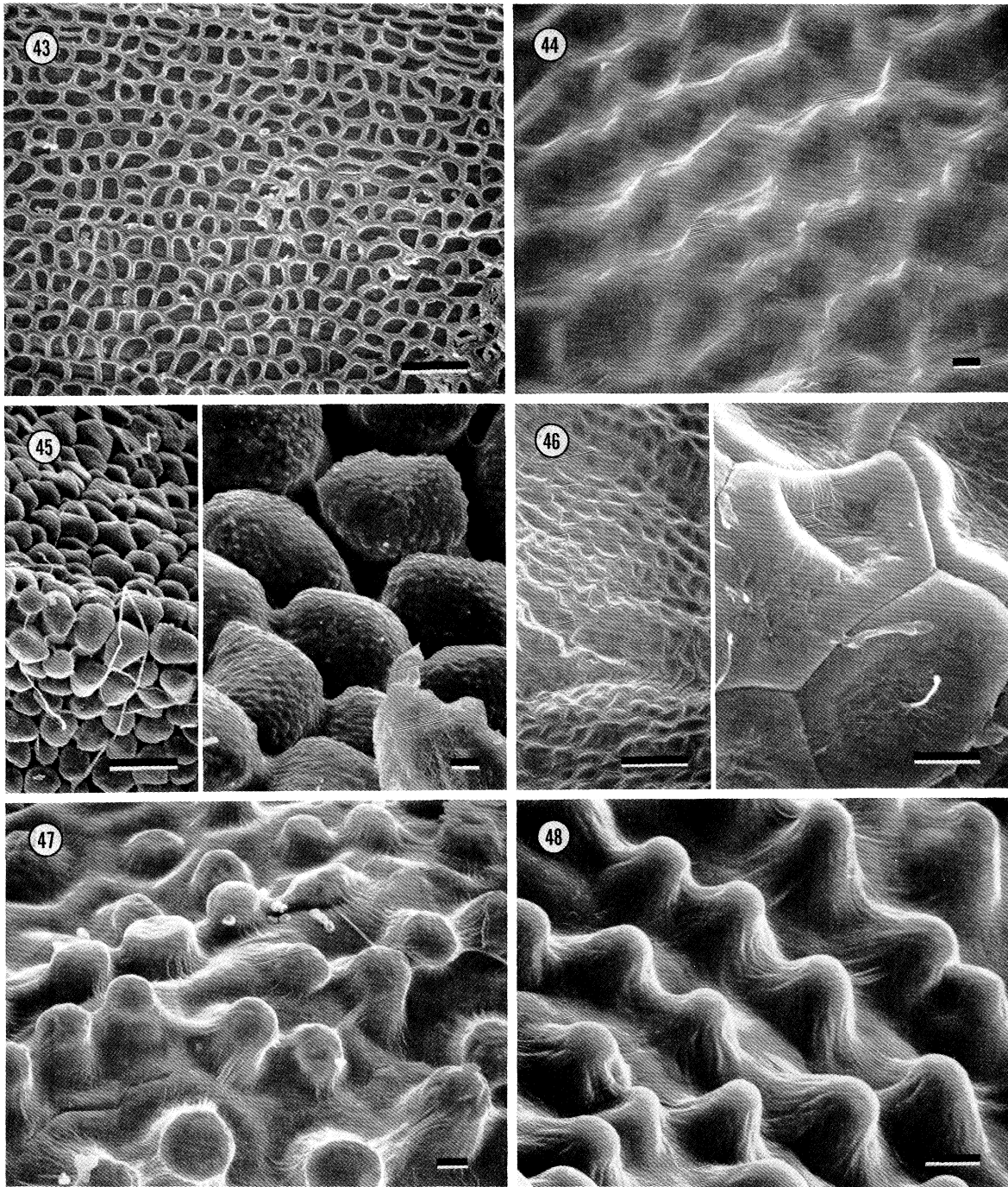
The data were analyzed by the PAUP (Phylogenetic Analysis Using Parsimony) computer program written by DAVID L. SWOFFORD, Illinois Natural History Survey, Urbana, Illinois.

Overall, 20 characters (table 4) were used in the analysis. In the following list the character is given in italics, followed by zero (0) for the plesiomorphic state and one (1) or a higher number for the derived or apomorphic state(s). A brief statement explains the basis of the polarity decision. If the hypothesis of polarity is straightforward, based solely on outgroup comparison to the most closely related genus, *Stenosiphon*, no comment is given.

The monotypic genus *Stenosiphon* was used as the primary outgroup because all of the features observed throughout *Oenothera*—(a) a persistent endotegmen one cell layer thick, (b) consisting of elongate cells; (c) exotegmen with lignified fibers; (d) and crystals in the endotestal cells—also are found in *Stenosiphon* (TOBE, unpublished data). This indicates either that these characters shared by both genera are primitive features of their common ancestor or that *Stenosiphon* was derived from *Oenothera*. It is also the most closely related genus to *Oenothera*, based on specialized stigma morphology (RAVEN 1964).

1. *Seed size (A)*: 0 = 2–3 mm long; 1 = 0.7–2 mm long.
2. *Seed size (B)*: 0 = 2–3 mm long; 1 = 3–5 mm long.

For the polarity determination of characters 1 and 2, a functional outgroup of *O. maysillesii* (section *Oenothera* subsection *Emersonia*) was used. This species was selected because it has the most plesiomorphic character states in the genus. *Stenosiphon* was not used because it has specialized, indehiscent, nutlike fruit in a family that has largely many-seeded capsules or berries. Many-seeded capsules is the plesiomorphic state



FIGS. 43–48.—SEM of the seed surface of *Oenothera*. Scale = 50 μm in figs. 43, 45 (left), 46 (left); 5 μm in figs. 44, 45 (right), 46 (right), 47, 48. Fig. 43, *O. engelmannii* (Goodman, Massey & Lawson 8069, MO). Fig. 44, *O. kleinii* (Wagner 4531, MO). Fig. 45, *O. havardii* (Wagner & Brown 3922, MO). Fig. 46, *O. canescens* (Wagner & Butley 3636, MO). Fig. 47, *O. fruticosa* subsp. *fruticosa* (from plants cultivated from Boufford 21575, MO). Fig. 48, *O. speciosa* (Wagner & Solomon 4089, MO).

for the family. *Stenosiphon* fruit have a single seed that matures. Thus, it is essentially impossible to polarize seed size by the usual method. Moreover, seed size varies among species in tribe Onagreae, of which *Oenothera* is a member. In this situation a functional out-group comparison in the sense of WATROUS and WHEELER

(1981) was thought to be the best possible method for polarizing seed size. This was done by determining the undirected character state that was present in both *O. maysillesii* and other species of *Oenothera*. The state common to both was considered to be the plesiomorphic state.

TABLE 4
DATA MATRIX OF SEED AND CAPSULE CHARACTERS USED IN CLADISTIC ANALYSIS OF OENOTHERA

Taxon		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<i>O. maysillesii</i>	(MAY)	...	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>O. stubbei</i>	(STU)	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>O. macrosceles</i>	(MAO)	...	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>O. organensis</i>	(ORG)	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>O. villosa</i>	(VIL)	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0
<i>O. laciniata</i>	(LAC)	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>O. heterophylla</i>	(HET)	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>O. albicaulis</i>	(ALB)	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>O. caespitosa</i>	(CAE)	0	0	2	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0
<i>O. cavernae</i>	(CAV)	0	0	2	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0
<i>O. harringtonii</i>	(HAR)	0	0	2	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>O. primiveris</i>	(PRI)	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0
<i>O. xylocarpa</i>	(XYL)	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0
<i>O. muelleri</i>	(MUE)	0	1	0	1	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0
<i>O. tubifera</i>	(TUB)	0	1	0	1	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0
<i>O. brachycarpa</i>	(BRA)	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1
<i>O. macrocarpa</i>	(MAC)	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1
<i>O. flava</i>	(FLA)	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	2	0	1
<i>O. acaulis</i>	(ACA)	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1
<i>O. engelmannii</i>	(ENG)	1	0	0	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	1
<i>O. pallida</i>	(PAL)	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1	0	1
<i>O. kleinii</i>	(KLE)	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1	0	1
<i>O. canescens</i>	(CAN)	1	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	2	1	0
<i>O. dissecta</i>	(DIS)	1	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	2	1	0
<i>O. havardii</i>	(HAV)	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	3	0	1
<i>O. fruticosa</i>	(FRU)	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	3	1	0
<i>O. perennis</i>	(PER)	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	3	1	0
<i>O. speciosa</i>	(SPE)	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	3	1	0
<i>O. kunthiana</i>	(KUN)	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	3	1	0
<i>O. rosea</i>	(ROS)	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	3	1	0
<i>O. seifrizii</i>	(SEI)	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1	1	0
<i>O. epilobiiifolia</i>	(EPI)	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1	1	0
<i>Stenosiphon</i>	(STE)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1

3. *Exotegmen thickness (no. cell layers)*: 0 = 1; 1 = 1–2; 2 = (2)3(4).

In addition to *Stenosiphon*, one-layered fibrous exotegmen occurs in *Gongylocarpus* (CARLQUIST and RAVEN 1966), in *Ludwigia* (CORNER 1976; EYDE 1978), and in *Hauya*, *Camissonia*, and *Epilobium* (TOBE, unpublished data).

4. *Endotesta thickness (μm)*: 0 = 5–22; 1 = 30–127.
 5. *Endotestal cell shape (A)*: 0 = Radially flattened; 1 = Radially enlarged.
 6. *Endotestal cell shape (B)*: 0 = Rectangular or stellately lobed; 1 = Polygonal.
 Polygonal endotestal cells are derived and are otherwise unknown in the family.
 7. *Endotestal cell-wall thickenings (A)*: 0 = Inner and radial; 1 = Uniformly thickened (cells also sclerotic and pitted).
 8. *Endotestal cell-wall thickenings (B)*: 0 = Inner and radial; 1 = Inner.
 9. *Mesotesta thickness (no. cell layers) (A)*: 0 = 1–8; 1 = 0 (crushed).

In contrast to the great diversity of mesotesta structure in *Oenothera*, the structure of the integuments is constant throughout the genus (TOBE and RAVEN 1985). BOUMAN and CALIS (1977) postulated that, in dicotyledons, the “subdermally initiated” outer integument is more primitive than the “dermally initiated” one. The term “dermally initiated” integument refers to one in which initiation and growth take place by divisions of dermal cells and of their derivatives of the ovule pri-

mordium. In contrast, a “subdermally initiated” integument is one in which initiation and growth occur by divisions of subdermal cells and their derivatives (BOUMAN 1974). The three genera in Onagraceae with the highest proportion of plesiomorphic features—*Fuchsia*, *Lopezia*, and *Circeae*—have subdermally initiated outer integuments, while other, more specialized genera such as *Epilobium* and *Camissonia* have a dermally initiated outer integument (TOBE and RAVEN 1985). *Oenothera*, although among the more specialized genera, has both subdermally and dermally initiated outer integuments. The dermal initials divide less actively than the subdermal ones, a feature shared only with *Stenosiphon* (TOBE and RAVEN 1985). Thus, the mature outer integument is two-layered in the upper portion and three-layered in the lower. It is not yet possible to determine with certainty which mesotesta type is more primitive. On the basis of present information, it seems best to regard the crushed single cell layer type as a derived feature through a process of simplification in size and number of layers (cf. CORNER 1976, p 57). This hypothesis is supported by outgroup comparison to *Stenosiphon*.

10. *Mesotesta thickness (no. cell layers) (B)*: 0 = 1–8; 1 = 6–20.
 11. *Mesotesta cells (A)*: 0 = Thin walled; 1 = Thick walled; sclerotic, pitted.
 12. *Mesotesta cells (B)*: 0 = Thin walled; 1 = Crushed.
 13. *Mesotesta cells (C)*: 0 = Thick walled, sclerotic, pitted; 1 = Thick walled, lignified, compressed.

This transformation involves only thick-walled cells. The process of becoming lignified and compressed appears to represent terminal, unique developments.

14. *Exotesta thickness* (μm): 0 = 10–45; 1 = 46–152.
 15. *Exotestal cells (A)*: 0 = Flattened to collapsed; 1 = Completely collapsed.
 16. *Exotestal cells (B)*: 0 = Flattened to collapsed; 1 = Irregularly swollen to collapsed.
 17. *Exotestal cells (C)*: 0 = Flattened or collapsed; 1 = Enlarged; subcuboidal; 2 = Radially enlarged; pillar-like; 3 = Radially enlarged; papilla-like.
- Enlarged exotestal cells appear to be a derived feature because they occur only in conjunction with the crushing and loss of mesotestal layers. The only exception is *O. havardii*, which has a multilayered mesotesta. The assignments of 1, 2, or 3 to the derived feature is arbitrary and thus unreliable for predicting relationships. This hypothesis is further supported by the outgroup comparison to *Stenosiphon*.
18. *Seed arrangement in each locule (A)*: 0 = Rows; 1 = Clustered.

Most of the other genera of tribe Onagreae with capsular fruit have seeds arranged in distinct rows. The exceptions appear to represent other derived features for the family, such as the two-seeded capsules embedded within the stem in *Gongylocarpus*, the few-seeded nutlike capsules of *Gaura* and *Heterogaura*, and the one-seeded nutlike capsules of *Stenosiphon*.

19. *Seed arrangement in each locule (B)*: 0 = 2 rows; 1 = 1 row.
- In *Oenothera*, seeds in one row per capsule represent a reduction specialization.
20. *Capsule winged or angled*: 0 = No; 1 = Yes.

Most of the tribes in Onagraceae lack winged or angled capsules. It is difficult to determine the polarity of this transformation series, however, since some genera of the tribe Onagreae (e.g., *Calylophus* and *Clarkia*) have both terete and angled capsules, while others (e.g., *Stenosiphon* and *Gaura*) have angled or winged capsules. Winged capsules are apomorphic in *Oenothera*.

In the first analysis, cladograms were generated for the first 17 characters using the MULPARS and SWAP options of the PAUP program. These options allowed global branch swapping in a search for multiple, equally parsimonious trees (SWOFFORD, unpublished data). This generated 42 equally parsimonious trees, each with 32 steps. Among these trees, only three were topologically different; all the others were arbitrary resolutions of multifurcations. One of the three fully resolved trees is presented (fig. 49). The other two differed only in the relative placement of section *Megapterium* (MAC-BRA) and section *Ravenia* (TUB-MUE). They were placed together as sister groups, either at the position occupied by TUB-MUE or at the present position of MAC-BRA (fig. 49).

Five parallelisms appear (fig. 49): character 2 (large seeds), character 4 (thick endotesta), character 11 (mesotestal cells thick walled, sclerotic, and pitted), character 15 (exotestal cells completely collapsed), and character 17 (exotestal cells radially enlarged; papilla-like). Character 4 is an endotesta apomorphy of the MAC-BRA lineage that also appears on the other principal lineage (fig. 49, below VIL and the remaining species). However, it appears with two other synapomorphies that ap-

pear to define large natural groups. The following questions must be addressed: does section *Megapterium*, *O. macrocarpa* and *O. brachycarpa* (MAC-BRA), belong on the other lineage, or has character 4 evolved independently twice? Similarly, thick-walled, sclerotic mesotesta (character 11) appears in *O. havardii* (HAV) and in the common ancestor to the large group of sections on the right-hand side of the diagram. This character should be studied further since it is grouped with taxa that have a thin-walled mesotesta that is crushed at maturity (fig. 49).

Likewise, the parallelism in character 15 (collapsed exotestal cells) occurs as an apomorphy for *O. engelmannii* (ENG) and as a synapomorphy of section *Ravenia* (MUE-TUB). This most likely represents a true parallelism. In *O. engelmannii* (ENG) of section *Anogra*, this apomorphy appears to be related to the encapsulation of each seed in a portion of the fruit. Character 2 (large seeds) occurs in both the TUB-MUE and MAC-BRA lineages and could represent a true synapomorphy. This interpretation is suggested by the other two diagrams (not presented), which placed these two lineages together.

Five reversals appear (fig. 49): characters 1, 5, 8, 12, and 16. It is not surprising that characters such as 1 (seed size) and 16 (exotestal cells flattened) would exhibit reversals, since size can be modified in a number of ways and surface features appear to be rather easily altered, especially where the difference is a matter of the cells becoming flattened at maturity.

The reversal of character 5 (endotestal cell shape) twice (fig. 49) is puzzling. The shape of the endotestal cells—radially flattened vs. enlarged—is constant within sections of the genus. The hypothesized reversal is not a terminal apomorphy but a synapomorphy that defines a large number of sections on one hand and section *Megapterium* (MAC-BRA) on the other. Perhaps some other cladogram that does not show this character reversal would be a better reflection of the phylogeny of the genus.

The reversal of character 8 (endotestal cells with both inner and radial walls thickened) in *O. acaulis* indicates that the cells of this species should be carefully reexamined. In rechecking the 32 species, we found that, in nearly all species, the character was clearly discernible but occasionally was somewhat difficult to determine. In general, thin, radially flattened endotestal cells were accompanied by a thickened inner wall, and the radially enlarged cells had thickened radial and inner walls. In the radially flattened cells it was sometimes difficult to determine whether the radial wall was thickened, as in *O. acaulis*. Thus, it was not clear whether the thickened radial walls in our preparation of *O. acaulis* actually represented a reversal to conspicuously thickened radial walls of other

characters that exhibit reversals or parallelisms. These exotesta features appear to have been derived more than once within some of these sections, resulting in false hypotheses of relationship. This analysis, however, strongly indicates that sections *Anogra*, *Gauropsis*, *Hartmannia*, *Kneiffia*, and *Xylopleurum* plus sections *Lavauxia* and *Paradoxus* form a monophyletic subgroup in the genus.

Another apparently monophyletic subgroup comprises sections *Pachylophus* (CAV, CAE, HAR), *Eremia* (PRI), and *Contortae* (XYL). In turn, this lineage is related to sections *Ravenia* (MUE-TUB), *O. maysillesii* (MAY) of subsection *Emersonia*, and possibly section *Megapterium* (MAC-BRA). However, since the characters that form the basis of these hypotheses represent parallelisms, the placement of at least section *Megapterium* is unreliable using the present data.

In this initial analysis (fig. 49), a thin endotesta 5–22 μm thick (character 4) is hypothesized to be a derived feature. Because the first analysis showed character 4 to be derived more than once, its polarization was reconsidered. Subsequent examination by TOBE (unpublished data) revealed that thin endotesta 5–20(–27) μm thick was a plesiomorphic feature throughout most of the family Onagraceae. This character is even present in *Ludwigia*, the sister group of the remaining 15 genera of the Onagraceae. The polarity of character 4 was reversed, and the new matrix was run on PAUP. Characters 1, 2, 15, 16, and 17 were omitted from the new analysis because they were considered the least reliable characters for separating homology from parallelism. New nonseed characters 18, 19, and 20 that have commonly been used in the delimitation of presumably monophyletic infrageneric groupings in the past were added to the analysis.

The new data matrix was analyzed as before, using PAUP with SWAP and MULPARS. Several hundred trees were generated, all of which represented arbitrary resolutions of trichotomies and tetrachotomies. The tree in figure 50 was obtained by running the data without the MULPARS option.

The overall topology of the new diagram (fig. 50) is similar in many ways to that in figure 49. Several important differences can be seen. *Oenothera xylocarpa* (XYL) is now grouped with *O. maysillesii* (MAY) because they share the same state for all of the 15 characters analyzed. The placement of *O. havardii* (HAV) is also somewhat different. Species of sections *Lavauxia* and *Anogra*, which previously were split, are now grouped together. The most significant difference, however, is that the MAC-BRA lineage is now adjacent to HAV, rather than to XYL and TUB-MUE.

The cladogram (fig. 50) contains four parallelisms and three reversals. Two of the parallelisms, characters 9 (mesotesta thickness, number of cell

layers) and 12 (mesotesta cells, thin walled vs. crushed at maturity) appear to represent true parallel development. The VIL lineage represents section *Oenothera* subsection *Oenothera*, 13 closely related, specialized North American species. They are adapted to open, early successional habitats and produce extremely large numbers of small seeds. Many of the species have become nearly cosmopolitan weeds. The reduction in mesotesta in subsection *Oenothera* appears to be related to these adaptations. Similarly, the development of characters 9 and 12 in the common ancestor to sections *Gauropsis*, *Anogra*, *Kneiffia*, *Hartmannia*, and *Xylopleurum* appears to represent a parallel but as yet unknown adaptation for smaller seed size and simple anatomical structure. Abundant crossing data support this interpretation, showing that subsection *Oenothera* is closely related to subsections *Raimannia* (HET-LAC) and *Emersonia* (ORG-MAO-STU and MAY). No artificial crosses have ever been successful between any member of these five sections and a species of section *Oenothera*. Morphological evidence further indicates that section *Oenothera* is not closely related to the five sections.

Another parallelism involves character 11 (mesotesta cells thick walled, sclerotic), which appeared twice in the first analysis (fig. 49) but three times in the second—as an apomorphy in both *O. havardii* (HAV) and section *Megapterium* (MAC-BRA) and in the common ancestor to section *Pachylophus* and related sections. As in the initial analysis, the correct placement of the MAC-BRA lineage cannot be reliably determined using these data.


The final parallelism (fig. 50) involves character 18 (seeds clustered in each locule). This feature could conceivably have evolved independently, but with some rearrangements of the cladogram a different tree of the same length can be derived for the taxa involved. In this alternate cladogram, character 18 is a synapomorphy linking sections *Kneiffia* (FRU-PER), *Xylopleurum* (SPE), *Hartmannia* (ROS and remainder), and *Gauropsis* (DIS-CAN). Characters 14 and 20, however, still show the same reversals as in the cladogram (fig. 50), and character 14 further switches back to the derived state in section *Gauropsis* (DIS-CAN). Thus, this new cladogram represents another alternative with equal length and further shows that seed anatomy data are not sufficient to resolve some of the phylogenetic relationships in the genus.


The principal conclusions that can be drawn from the cladistic analysis of seed anatomy data are:

1. Species of section *Oenothera* subsections *Raimannia* and *Emersonia* exhibit seed anatomy that differs from a hypothetical common ancestor of the genus in only one respect: a size increase of the endotesta. This indicates that these species ap-

15 CHARACTERS

LENGTH = 24

 Unique Synapomorphy

 Parallelism


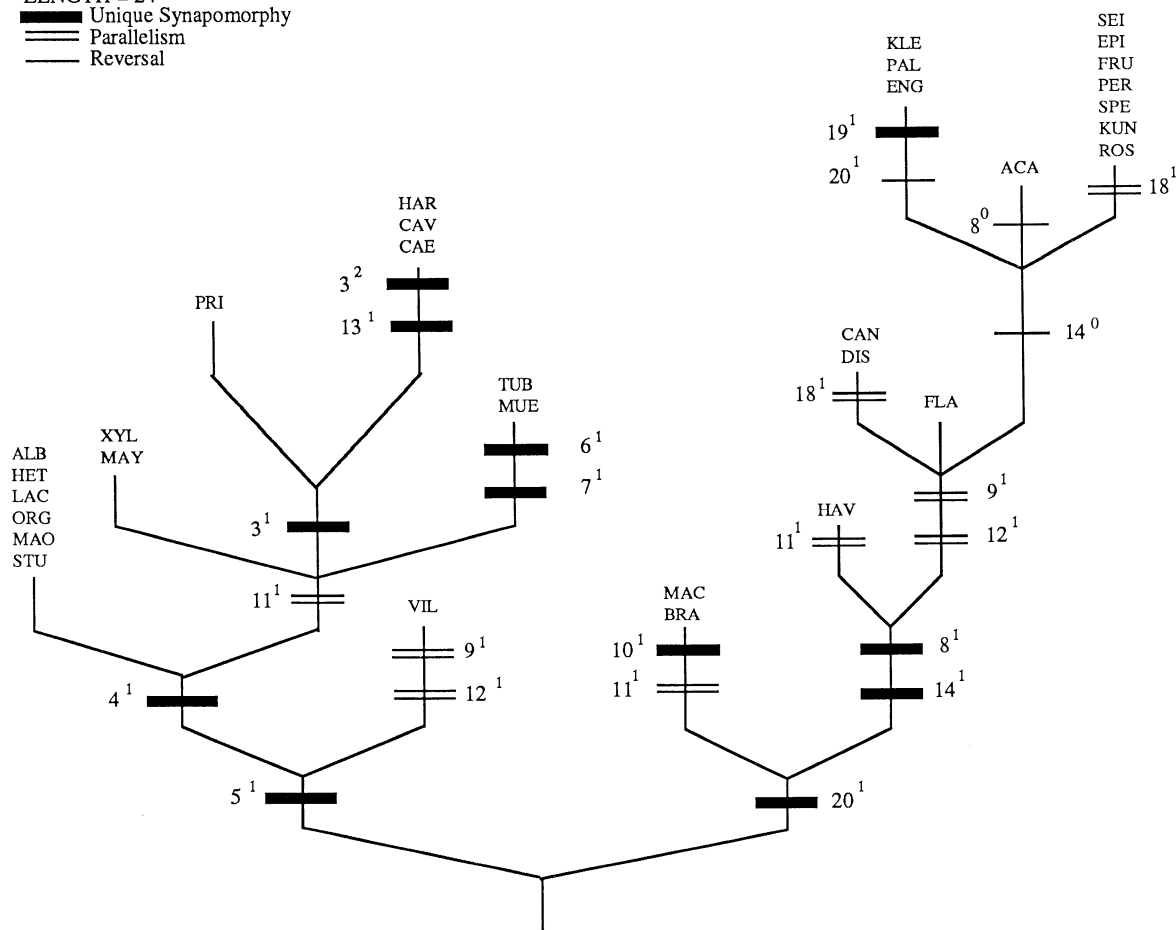
 Reversal


FIG. 50.—Cladogram using characters 3–14 and 18–20 run with SWAP option of PAUP program.

appear to be the closest living relatives of the monotypic genus *Stenosiphon*. *Stenosiphon* thus would be the sister group to *Oenothera*. Alternatively, *Stenosiphon* may represent a very specialized derived lineage that diverged early in the evolution of the genus. Certain members of subsection *Raimannia*, such as *O. rhombipetala*, share a number of morphological features with *Stenosiphon*: habit, inflorescence structure, and petal shape. At present it is not clear whether some of these shared characteristics represent adaptations to the prairie habitat those plants occupy and are thus convergences, or indicate common ancestry. If the latter is correct, then it is not possible at this time to ascertain whether *Stenosiphon* shares a common ancestor with *Oenothera* or represents a very specialized derivative from section *Oenothera* subsections *Raimannia* or *Emersonia*. If the latter were true, then the polarity of several characters may change when another outgroup is selected, such as *Gaura* or *Camissonia*, also of tribe Onagreae.

2. Early in its evolution *Oenothera* diverged into

two major lineages: (1) sections *Oenothera*, *Kleina*, *Ravenia*, *Eremia*, *Contortae*, and *Pachylophus* and (2) sections *Megapterium*, *Paradoxus*, *Lavauxia*, *Anogra*, *Hartmannia*, *Gauropsis*, *Xylopleurum*, and *Kneiffia*. The first lineage can be defined based on the synapomorphy of radially enlarged endotestal cells (character 5) and, except for the derived subsection *Oenothera* (VIL), the synapomorphy of thick endotesta (character 4). Section *Oenothera* subsection *Oenothera* (VIL) is clearly a member of this lineage despite its thin endotesta, which appears to be a secondary reduction. The second major lineage is based on the synapomorphy of winged or angled capsules (character 20). All of the sections in this lineage, except section *Megapterium*, are further linked by having only the inner walls of the endotestal cells thickened (character 8), but it is possibly not reliable. A third synapomorphy, thick exotesta (character 14), is considered to be less reliable since it shows a reversal.

3. Crossing results unambiguously show that the

members of subsection *Emersonia*, especially *O. maysillesii*, share similar genomes and plastomes (STUBBE and RAVEN 1979; DIETRICH et al. 1985). These species also can form hybrids with other species of section *Oenothera*, and *O. maysillesii* hybridizes most successfully with the widest diversity of species (STUBBE and RAVEN 1979; DIETRICH et al. 1985). Despite this indication of close relationship, the seeds of *O. maysillesii* differ from the other species of section *Oenothera* in their larger size and thick-walled, sclerotic, and pitted mesotesta cells. The anatomical features of the seeds of *O. maysillesii* are very similar to those of species in sections *Ravenia*, *Eremia*, and *Contortae*; and *O. maysillesii* appears to be their sister group. The placement of *O. maysillesii* in subsection *Emersonia* greatly increases the heterogeneity of this subsection and does not fully reflect the apparent central and generalized position of this species in the genus. Possibly it should be placed in a new monotypic section or subsection based on overall features, despite its wide crossability with other species of section *Oenothera*.

4. The placement of section *Megapterium* is not clear from the analysis and should be investigated further, using other data.

5. Sections *Kneiffia*, *Hartmannia*, *Xylopleurum*, *Gauropsis*, and *Anogra* have similar anatomical seed features. This provides the first unequivocal evidence that these taxa are related. The characters of seed anatomy by which they differ are essentially external aspects of exotesta. Although exotesta features are valuable for delimiting species and studying relationships of species within sections (WAGNER, unpublished), they are not useful for resolving phylogenetic relationships among sections.

FACTORS RELATIVE TO THE DIVERSIFICATION OF OENOTHERA SEEDS

The early evolution within members of the tribe Onagreae took place in Madrean vegetation of western North America (RAVEN and AXELROD 1978). *Oenothera* is typical of this pattern. Twelve of the 14 sections of *Oenothera* are represented in Texas and Mexico, mostly associated with Madrean woodland or closely related vegetation types. Diversity within the genus very quickly decreases outside this area, although Arizona and New Mexico have representatives of 10 sections. The high sectional diversity in this region and the occurrence in Texas and Mexico of species with the largest number of primitive characters, including seed anatomy features, strongly indicate that *Oenothera* originated in Madrean vegetation in this region, probably by the early Neogene. The genus has subsequently diversified greatly into a wide variety of habitats, ranging from low-elevation hot deserts to montane temperate and subtropical forests, subal-

pine conifer forests, and eastern deciduous forests. They inhabit open, sandy, rocky, or clay sites to occasionally wet soils at stream or bog margins. The range of *Oenothera* has spread throughout most of North America and south through Central America and nearly all of temperate South America. Judging from the patterns of distribution, the origin of the various sections appears to have occurred in conjunction with shifts into new ecological or geographical areas.

Many of the changes in seed anatomy among the sections of *Oenothera* probably occurred in concert with the establishment of new adaptive modes as members of the genus migrated and shifted into new habitats. Two questions concerning seeds can be asked: (1) Has the establishment of new adaptive modes always involved changes in seed anatomy? (2) Does the present analysis show any clear correlations between seed anatomy and adaptations to new habitats? The answer to the first question appears to be no; to the second, yes. Seeds of the specialized sections *Hartmannia*, *Xylopleurum*, *Kneiffia*, *Gauropsis*, *Anogra*, and *Lavauxia* share a great number of derived characters, essentially differing only in features of the exotesta (cell size and shape) and seed size (section *Lavauxia*). Yet the evolution of these sections has involved extensive ecological and geographical changes.

Section *Hartmannia* is centered in Mexico, primarily in Madrean pine-oak forests. Some members of this section have adapted to a variety of high-elevation habitats, up to ca. 4,600 m in the Andes of South America, while *Oenothera rosea* occurs in open, often disturbed sites from 350 to over 4,000 m. Related to section *Hartmannia*, but in drier habitats, is section *Gauropsis*. These plants have shifted to seasonally wet habitats in grassland in the Chihuahuan desert of Mexico and in the High Plains of the United States (WAGNER 1984). The monotypic section *Xylopleurum* grows in open grassland or woodland sites from Texas to Kansas and Missouri, south to northern and eastern Mexico.

Section *Kneiffia* may have originated in more mesic areas from eastern Texas to eastern Oklahoma and Louisiana. Subsequently, members of this section spread nearly throughout the eastern United States and adjacent Canada. Its five species occupy a diversity of habitats from lowland to upland, prairie to forest openings, or stream margins.

All of the species of section *Lavauxia* occur in seasonally wet sites such as arroyo, pond, or stream margins and desiccating flats.

The final section in this assemblage, section *Anogra*, is centered in the western United States. This group probably originated in Madrean forest or woodland in northern Mexico. The section is now very diverse, ranging from the Great Plains grasslands to the Sonoran and Great Basin Deserts.

A reasonable hypothesis for a group of plants that occurs in such a diverse array of habitats is that at least some conspicuous adaptations in the seeds will occur. With respect to the seed anatomical characteristics studied, however, no such variation has been observed.

Contrasting sharply with this lack of anatomical diversity in these *Oenothera* seeds is the pattern observed in the remaining sections of the genus in which the origin of a number of sections, and the attendant shifts in geographical and ecological situations, strongly correlate with modifications in seed coat anatomy. This pattern of correlated change involves section *Oenothera* subsection *Emersonia* and sections *Ravenia*, *Eremia*, and *Contortae*, concluding with the evolution of the specialized section *Pachylophus*.

In this monophyletic lineage, section *Ravenia* is the closest relative to *O. maysillesii* (subsection *Emersonia*). *Oenothera muelleri* and *O. tubifera* occur in cool, mesic to submesic habitats at 2,300–3,200 m, with a patchy and presumably relictual distribution in eastern to central Mexico. The unique, thick-walled endotesta, very thick mesotesta, and exotesta with longitudinally elongate collapsed cells indicate that the seeds of members of section *Ravenia* are very specialized. These features may represent adaptations to the cool montane habitat in which these nonweedy species occur. They appear to represent further specializations on a common ground plan begun in *O. maysillesii*, which occurs at high elevations (2,100–2,600 m in Durango, Mexico).

Based on their occurrence in xeric and submesic vegetation types derived from generalized Madro-Tertiary vegetation, the diversification of the remainder of this lineage may well have been linked to the Pliocene spread of dry climates, especially from the mid-Pliocene onward (WAGNER et al. 1985). During this time, many plant groups radiated from the more southern, warm Madro-Tertiary vegetation into the cooler, drier climates that were rapidly spreading in western North America (AXELROD 1958, 1979; RAVEN and AXELROD 1978).

The more generalized, modally outcrossing, and sometimes self-incompatible subspecies of *O. primiveris* (section *Eremia*) occurs in the northern Sonoran and Mojave Deserts. Its seed anatomy is similar to that of *O. maysillesii* but differs in having the endotesta sometimes two cells thick, a feature found elsewhere only in section *Pachylophus*. There do not appear to be any structural adaptations in the seeds in the evolution of this section other than this feature and a greatly increased seed surface area gained by the numerous enlarged micropapillae on the exposed wall of the exotestal cells, which may function in greatly increasing the adsorptive properties of the seeds of this desert annual (WAGNER et al. 1985).

By contrast, the seeds of the widespread and ecologically diverse section *Pachylophus* are highly specialized, and this probably has contributed to its success. The species of this section (*O. caespitosa* and four closely related ecologically specialized derived species) occur in a wide variety of ecological situations (WAGNER et al. 1985). The four derived species inhabit hot xeric Sonoran Desert sites, mesic foothill grassland sites in Colorado, and the interface between moving sand dune and lava in Idaho. *Oenothera caespitosa* is one of the more widespread species in the genus, ranging from southern Canada to northern Mexico in diverse habitats, including cool to hot deserts, pinyon-juniper woodlands, Great Plains grassland, Rocky Mountain coniferous forest, and subalpine forest.

Specialization in the seeds of members of section *Pachylophus* has almost certainly contributed greatly to the ability of these species to colonize so many xeric to submesic habitats. These adaptations were likely concurrent with the original shift into cooler habitats that became available in the Basin and Range region to the lee of the rising Sierra Nevada, the area in which section *Pachylophus* presumably originated (WAGNER et al. 1985). The seeds of this group are distinct from those of other species of this genus in having persistent, multilayered mesotesta with compressed cells and multilayered fibrous exotegmen. These features are unknown elsewhere in the genus except for *O. primiveris*.

The anatomy of the exotegmen is remarkably constant in the genus (only six of 124 species have any modifications), since it functions as conducting tissue of the seed. The multiplication of the exotegmen in section *Eremia*, and even further expanded and specialized in section *Pachylophus*, is presumably closely tied to the adaptation to xeric habitats. In section *Pachylophus* this development is associated with another unique development: seeds with a hollow chamber ("seed collar") on the adaxial side that constitutes 30%–80% of the seed volume (WAGNER et al. 1985). Directly above the rapial ridge the seed collar is one cell thick and appears as a thin translucent membrane. This chamber quickly absorbs water through the membrane when it becomes available and which it can store, presumably for use in germination after the microhabitat of the seed has again become dry (WAGNER et al. 1985). The modifications in seed anatomy and development of the seed collar appear to be strongly associated with the evolution and adaptive success of section *Pachylophus*.

If one assumes that the anatomical diversity among seeds of *Oenothera* species in this paper represents adaptations to new or changing environments, then the patterns outlined here strongly indicate that the outer layers of the seed, and especially the exotesta, are more strongly open to

adaptive modification than the inner ones. This is borne out by the two detailed examples described above. Essentially, the only modifications noted in the seeds of sections *Hartmannia*, *Xylopleurum*, *Gauropsis*, *Kneiffia*, and *Anogra* are differences in the exotesta. In sharp contrast are the changes in each section of the series, including sections *Ravenia*, *Eremia*, *Contortae*, and *Pachylophus*. Section *Pachylophus* exhibits the only major change in the inner layers of the seed and thus represents a shift to a new complex of seed characteristics.

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LITERATURE CITED

- AXELROD, D. I. 1958. Evolution of the Madro-Tertiary geoflora. *Bot. Rev.* **24**:434–509.
- . 1979. Age and origin of Sonoran Desert vegetation. *Occas. Pap. Calif. Acad. Sci.* **132**:1–74.
- BARTHOLOTT, W. 1981. Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nord. J. Bot.* **1**:345–355.
- BOUMAN, F. 1974. Developmental studies of the ovule integuments and seed in some angiosperms. Ph.D. diss. University of Amsterdam.
- BOUMAN, F., and J. I. M. CALIS. 1977. Integumentary shifting—a third way to unitegmy. *Ber. Deutsch. Bot. Ges.* **90**:15–28.
- CARLQUIST, S., and P. H. RAVEN. 1966. The systematics and anatomy of *Gongylocarpus* (Onagraceae). *Am. J. Bot.* **53**:378–390.
- CORNER, E. J. H. 1976. The seeds of dicotyledons. 2 vols. Cambridge University Press, Cambridge.
- DIETRICH, W. 1977. The South American species of *Oenothera* sect. *Oenothera* (*Raimannia*, *Renneria*; Onagraceae). *Ann. Mo. Bot. Gard.* **64**:425–626.
- DIETRICH, W., P. H. RAVEN, and W. L. WAGNER. 1985. Revision of *Oenothera* sect. *Oenothera* subsect. *Emersonia* (Onagraceae). *Syst. Bot.* **10**:29–48.
- DIETRICH, W., and W. L. WAGNER. 1987. New taxa of *Oenothera* L. sect. *Oenothera*. *Ann. Mo. Bot. Gard.* vol. **74** (in press).
- EYDE, R. H. 1978. Reproductive structures and evolution in *Ludwigia* (Onagraceae). II. Fruit and seed. *Ann. Mo. Bot. Gard.* **65**:656–675.
- GAUBA, E., and L. D. PRYOR. 1958. Seed coat anatomy and taxonomy in *Eucalyptus*. I. *Proc. Linn. Soc. New South Wales* **83**:20–32.
- . 1959. Seed coat anatomy and taxonomy in *Eucalyptus*. II. *Proc. Linn. Soc. New South Wales* **84**:278–291.
- . 1961. Seed coat anatomy and taxonomy in *Eucalyptus*. III. *Proc. Linn. Soc. New South Wales* **86**:96–111.
- GRAU, J. 1980. Die Testa der Mutisieae und ihre systematische Bedeutung. *Mitt. Bot. München* **16**:269–332.
- HECHT, A. 1950. Cytogenetic studies of *Oenothera* subgenus *Raimannia*. *Indiana Univ. Publ. Sci. Ser.* **16**:255–304.
- MUNZ, P. A. 1932. Studies in Onagraceae. VIII. The subgenera *Hartmannia* and *Gauropsis* of the genus *Oenothera*. The genus *Gayophytum*. *Am. J. Bot.* **19**:755–778.
- . 1935. Studies in Onagraceae. IX. The subgenus *Raimannia*. *Am. J. Bot.* **22**:645–663.
- . 1965. Onagraceae. *North Am. Flora, Ser. 2*, **5**:1–231.
- RAVEN, P. H. 1964. The generic subdivision of Onagraceae, tribe Onagreae. *Brittonia* **16**:276–288.
- . 1970. *Oenothera brandegeeri* from Baja California, Mexico, and a review of subgenus *Pachylophus* (Onagraceae). *Madroño* **20**:350–354.
- RAVEN, P. H., and D. I. AXELROD. 1978. Origin and relationships of the California flora. *Univ. Calif. Publ. Bot.* **72**:1–134.
- STEARNS, W. T. 1973. *Botanical Latin*. 2d ed. David & Charles, Newton Abbot.
- STEBBINS, G. L. 1974. *Flowering plants: evolution above the species level*. Belknap Press of Harvard University Press, Cambridge, Mass.
- STOCKHOUSE, R. E. 1973. Biosystematic studies of *Oenothera* L. subgenus *Pachylophus*. Ph.D. diss. Colorado State University, Fort Collins.
- STRALEY, G. B. 1977. Systematics of *Oenothera* sect. *Kneiffia* (Onagraceae). *Ann. Mo. Bot. Gard.* **64**:381–424.
- STUBBE, W., and P. H. RAVEN. 1979. A genetic contribution to the taxonomy of *Oenothera* sect. *Oenothera* (including subsections *Euoenothera*, *Emersonia*, *Raimannia* and *Munzia*). *Plant Syst. Evol.* **133**:39–59.
- TOBE, H., and P. H. RAVEN. 1985. The histogenesis and evolution of integuments in the Onagraceae. *Ann. Mo. Bot. Gard.* **72**:451–468.
- WAGNER, W. L. 1984. Reconsideration of *Oenothera* subg. *Gauropsis* (Onagraceae). *Ann. Mo. Bot. Gard.* **71**:1114–1127.
- . 1986. New taxa of the genus *Oenothera* (Onagraceae). *Ann. Mo. Bot. Gard.* **73**:475–480.
- WAGNER, W. L., and P. GOLDBLATT. 1984. A survey of seed surface morphology in *Hesperantha* (Iridaceae). *Ann. Mo. Bot. Gard.* **71**:181–190.
- WAGNER, W. L., R. E. STOCKHOUSE, and W. M. KLEIN. 1985. The systematics and evolution of the *Oenothera caespitosa* species complex (Onagraceae). *Monogr. Syst. Bot. Mo. Bot. Gard.* **12**:1–103.
- WATROUS, L. E., and Q. D. WHEELER. 1981. The outgroup comparison method of character analysis. *Syst. Zool.* **30**:1–11.