

Unique tetrads of *Epilobium luteum* (Onagraceae: Onagreae) pollen from Alaska

JOHN J. SKVARLA¹, JOHN R. ROWLEY², PETER C. HOCH³, AND WILLIAM F. CHISSOE⁴

¹ Department of Botany-Microbiology and Oklahoma Biological Survey, University of Oklahoma, Cross Hall, Norman, Oklahoma 73019, USA; e-mail: jskvarla@ou.edu

² Botany Department, Stockholm University, SE-106 91, Stockholm, Sweden; e-mail: rowleyj@botan.su.se

³ Missouri Botanical Garden, P. O. Box 299, St. Louis, Missouri 63166, USA; e-mail: peter.hoch@mobot.org

⁴ Samuel Roberts Noble Electron Microscopy Laboratory, University of Oklahoma, Norman, Oklahoma 73019, USA; e-mail: williamchissoe@cox.net

Abstract. Aperture morphology of tetrad pollen of *Epilobium luteum* (Onagraceae: Epilobieae) from three Alaskan collections is highly variable. The first collection appears to lack apertures altogether and is presumed to consist of immature pollen grains in a genus known to achieve mature size before the apertures become distinctly protruding. A second collection has tetrads with 3- and 4-apertured grains, the apertures in the latter are often irregularly spaced and not in apposition with the apertures of neighboring members. The third collection consists of the more typical 3-apertured members that characterize the majority of *Epilobium* pollen grains. In all of these collections individual pollen grains (monads) are interspersed among the tetrads. The variations in the number of apertures emphasize the importance of having a comprehensive understanding of the stage of development of the pollen (taxon) examined when describing pollen collections. In the first collection this would mean the recognition that in Onagraceae apertures occur in the later stages of microspore ontogeny. In the latter two collections a thorough background of the literature of the pollen morphology on this largest Onagraceae taxon is useful for the understanding of the significance of a range of aperture numbers on *Epilobium* pollen grains.

Key Words: Apertures, *Epilobium luteum*, monads, Onagraceae, tetrads, viscin threads.

Beer (1905) noted that the striking features of the pollen grains of the Onagraceae have attracted the attention of botanists from a very early date. The pollen grains of *Epilobium* are represented by tetrads arranged in a tetrahedral configuration. Typically, each monad in the tetrad has three apertures, each apposed to a corresponding aperture from the neighboring tetrad component according to Fischer's rule (Fischer, 1890). Tetrad cohesion is maintained by two mechanisms (Ting, 1966; Skvarla et al., 1975): 1) the intermeshing of

individual monad surfaces at points of aperture contact, and 2) by short bridges in a zone immediately beneath the thickened equatorial region.

The pollen morphology of *Epilobium* L. (Onagraceae: Epilobieae), a genus of approximately 170 species, has been extensively documented (see Praglowski et al., 1994, for literature citations). Pollen from three Alaskan collections of *E. luteum* Pursh (Section *Epilobium*) showed variability in aperture number varying from seemingly none to four.

We show that interpretation of the “inaperturate grains” is a direct function of microspore development. We also review the distribution of the unusual arrangement of pollen with four apertures. Lastly, the presence of monad pollen grains mixed with tetrad pollen offered an opportunity to assess this phenomenon.

Methods

Anthers were removed from three collections of *Epilobium luteum* (Alaska: Elfin Cove, Chicagof Island, *Williams 2061*, BYU; Alaska: Maybeso Forest, Prince of Wales Island, *Packer 7*, ALA; and Alaska: Aleutian Islands, Akutan, *Jones 9379*, DS), acetolyzed (Erdtman, 1960), and the residues processed for scanning electron microscopy (SEM) as described elsewhere (Skvarla et al., 1975). Secondary electron imaging and photography was done with International Scientific Instruments (ISI) Super II and JEOL 880 scanning electron microscopes (SEMs), the latter equipped with a lanthanum hexaboride gun. With the former, secondary images were recorded on Polaroid P/N film while the latter used digital imaging.

For transmission electron microscopy (TEM), fresh pollen of *Epilobium montanum* L. in (Fig. 1H) was fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (4°C, pH 7.4) for 24 h and post-fixed in 2% OsO₄ (same buffer and conditions) for 1 h. Dehydration was done through a series of graded acetone and then embedded in Araldite-Epon resin (Electron Microscopy Sciences, Fort Washington, Pennsylvania, USA; Mollenhauer, 1964). Ultrathin sectioning was done with a diamond knife and sectioning staining included exposure to a solution of 2% potassium permanganate for 5 min, saturated aqueous uranyl acetate for 5 min followed by exposure to a solution of lead citrate (Hanaichi et al., 1986) for 3 min. TEM observations and micrographs were made with a Zeiss EM-10A TEM.

Distilled water mounts of unacetolyzed pollen grains (Figs. 1G, 2E) were examined and photographed with light microscopy. All SEM, TEM and LM negatives and prints were scanned at 600 dpi and processed using Adobe Photoshop 7.

Results

Pollen grains from all collections of *Epilobium luteum* occur as either tetrahedral tetrads or as monads mixed with tetrads (Figs. 1A, 2A–B, 2F–K). All monads, whether originally a part of a tetrad, or formed as single grains, show distinct polar asymmetry, with the proximal face highly convex and the apertures located in prominently thickened equatorial regions (Figs. 1A, 1D, 2C, 2K). In the *Williams* and *Packer* collections, this equatorial thickening appeared greater (Figs. 1A–B, 1D–E, 2F–K) than in the *Jones* collection (Figs. 2A–C). The TEM of a young developing microspore of *E. montanum* (Fig. 1H) illustrates the early formation of the thickened equatorial exine on the sides of a protruding apertural vestibule.

Aperture number and shape varied notably in the three collections. The *Williams* collection (Figs. 1A–B, 1D–E, 1G) had circular to oval monad members that appear to lack apertures. The *Jones* collection had a mix of typical 3- apertured (Figs. 2A, 2C) and atypical 4- apertured (Fig. 2B) pollen. The *Packer* collection had pollen consisting of at least four apertures that were often irregularly distributed on the equatorial thickened exine (Figs. 2F–K). Tightly compound viscin threads (Skvarla et al., 1975) were present on all proximal faces (Figs. 1B–C, 2C, 2K).

Tetrad cohesion is maintained primarily by exine bridges located directly beneath the apertures (Figs. 2C–E, 2G, 2J) and linking adjacent monad members or, in the case of the *Williams* collection, directly beneath the points of monad confluence (Figs. 1A–G). The bridges consist of bundles of short to elongate viscin threads. Secondary tetrad cohesion, while less apparent, occurs by means of the intermeshing of exine surfaces at the aperture protuberances (Figs. 2A–B, 2E–J).

In all collections, monad pollen is also commonly mixed with tetrads. Often monads can be reconciled to parent tetrads by the presence of disrupted bridges (Figs. 1B, 1F, 2C). In other cases reconciliation is not possible and these pollen grains may actually

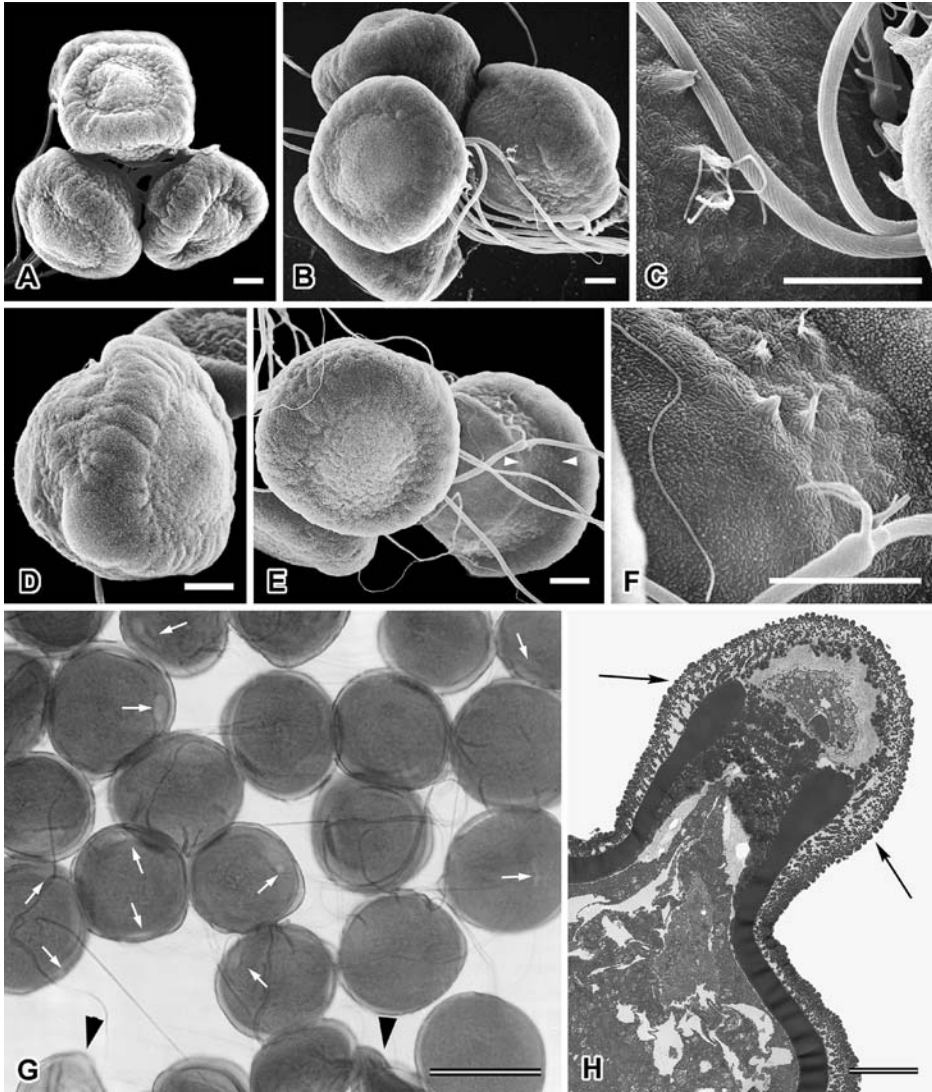


FIG. 1. Scanning electron micrographs (A–F) and light micrograph (G) of *Epilobium luteum* pollen (collection of *Williams*) and transmission electron micrograph of *E. montanum* pollen (H). **A.** Tetrahedral tetrad (the fourth monad protrudes at the rear). The top monad is almost rectangular in shape, due to the early development of the slightly protruding apertural cones. **B.** A tetrad with several viscin threads emerging from the distal portion of the tetrad. Three separated bridges between members of the tetrad are shown in Fig. C. **C.** An enlargement of the viscin threads and three of the separated bridges between monads as in Fig. B. **D.** Monad pollen grain showing thickened exine of the equatorial region in which the apertures develop. The globular shape of these grains is illustrated in this lateral view. The equatorial thickening forms early in exine development and long before apertures form. **E.** The prominent monad at the left emphasizes the absence of the apertural cones typical of mature pollen in the family. There are many viscin threads and attachment sites in this micrograph (region outlined by arrowheads). **F.** An enlargement of region in Fig. E showing several problematic bridge fragments and a compound viscin thread at lower right with several less compound threads. **G.** Photomicrograph of unacetolyzed pollen. The pollen grains are confluent with exines of neighboring grains and are not isolated monads. Many of the grains show early development of pores (arrows) without the protruding apertural cone that typically forms later in mature *Epilobium* pollen. Some of the grains are distorted or appear empty of cytoplasm (arrowheads). **H.** *Epilobium montanum*. TEM of a protruding aperture. The apertures of *Epilobium* grains develop in the early-formed thickened equatorial exine evident on the sides of the protruding apertural (vestibule) cone (arrows). Scale bars=10 μm (A–F); 50 μm (G); 1 μm (H).

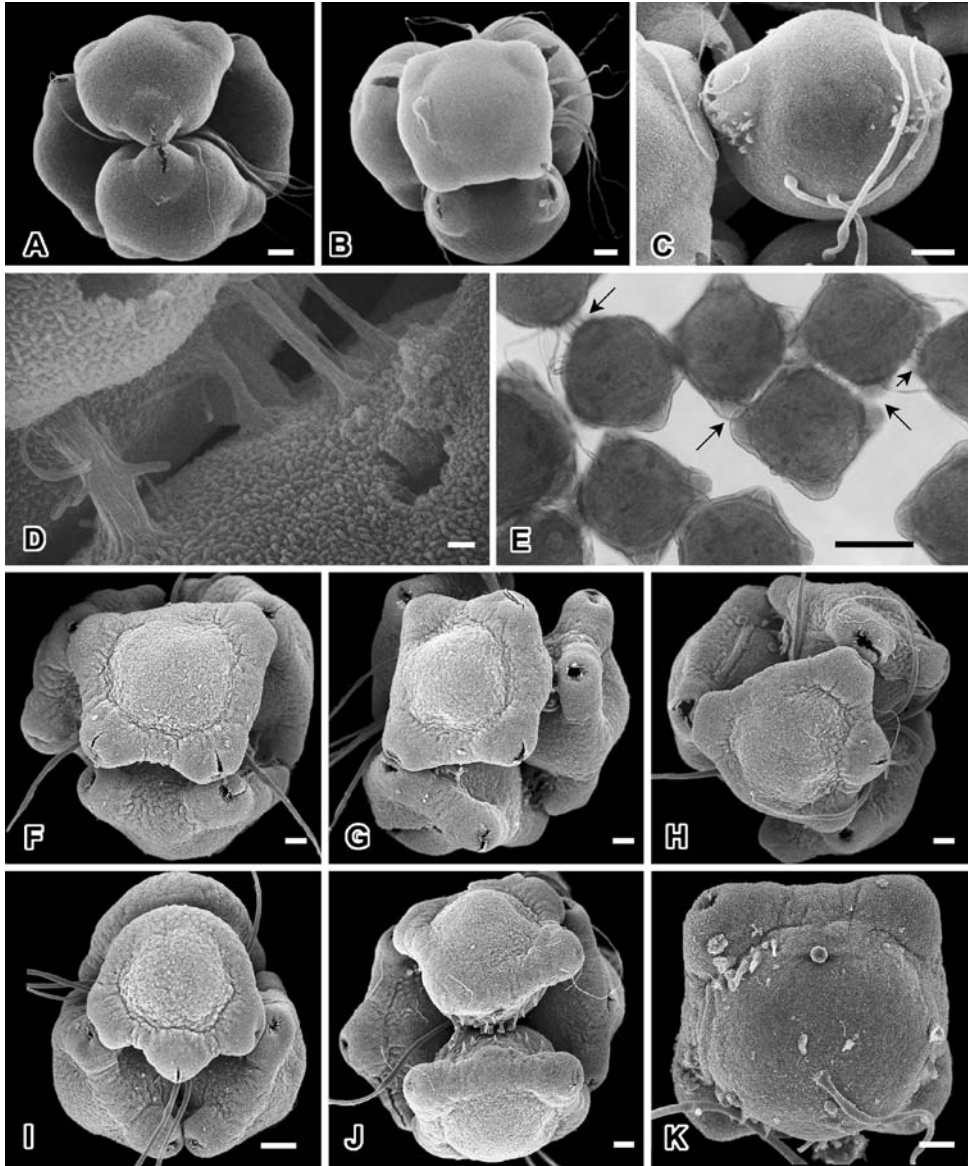


FIG. 2. Scanning electron micrographs (A–D, F–K) and light micrograph (E) of *Epilobium luteum* pollen. A–D. Collection of *Jones*. **A.** Typical tetrahedral tetrad with individual monad members consisting of three apertures that are linked to apposed monad members beneath their respective apertures. **B.** Similar to A but with individual monad members consisting of four apertures. **C.** The proximal faces of two partially disassociated monad members showing exine bridges beneath aperture regions and numerous viscin threads with inflated (multiple) attachments to the exine surface. At left is part of a 4-apertured pollen monad member of a nearby tetrad. **D.** Exine bridges that are intact beneath aperture regions in two monad members. E–K. Collection of *Packer*. **E.** Light micrograph of unacetolyzed pollen. The apparent monad grains are joined with neighboring pollen by short bridges and confluent exine surfaces (arrows). F–K. SEMs of the *Packer* collection. **F.** Tetrad with 4-aperturate monads; all in approximate apposition. **G.** The aperture on the monad to the right does not have a counterpart in the top tetrad. **H.** Aperture to right of top monad without an aperture counterpart. **I.** Unusual arrangement of apertures on top monad. It is clear that all apertures are, however, positioned on the enlarged regional exine. **J.** Numerous connecting exine bridges account for tetrad stability as compared with other collection (see Fig. 1B). **K.** Proximal polar face of isolated monad, note square outline as compared to isolated monad from other collection (Fig. 1E). Scale bars=10 μm (A–C, F–K); 1 μm (D); 50 μm (E).

be individual monads separated from a tetrad early in development (Fig. 1D).

Discussion

Relationship of developmental stage to aperture formation.—The stage of pollen development, whether in grains obtained from herbarium collections, liquid preservation or elsewhere, is frequently unknown. As illustrated in Figs. 1A–G, this can present difficulties when attempting to characterize the morphology of mature pollen grains.

Developmental studies in pollen of *Epilobium montanum* and *Chamerion angustifolium* (L.) Holub (formerly placed in *Chamaenerion* or *Epilobium*), a species related to *E. luteum*, also show the absence of external protruding aperture components until late in microspore development (Rowley & Skvarla, 2004, 2006). During the earliest part of exine formation a thickened equatorial region develops in microspores of *Epilobium* and *Chamerion* (Rowley & Skvarla, 2004: Plates V, Fig. 3 and VI, Fig. 4). It is this equatorial thickening that accounts for the inflated feature of the pollen in Figs. 1A–B, 1D–E and 2F–K. Well-formed aperture components in microspore stages without any indication of aperture protrusion or pores are described in Rowley and Skvarla (2004: Plates XI, Figs. 2, 3 and XII, Fig. 1). Apertures begin to protrude at a slightly later stage of early development (Rowley & Skvarla, 2004, Plates XII, Fig. 2, and XIII). Medium to late microspore stages with protruding apertures are shown in Rowley and Skvarla (2006: Plates III, Figs. 2, 3, 4 and VII, Fig. 3). As further evidence of retarded development of apertures in these species, the fairly well-developed grains illustrated in Rowley and Skvarla (2006, Plates III, Fig. 3 and VII, Fig. 3) have not yet developed an intine.

Although it appears that pollen of the Williams collection is inaperturate, we believe that it represents very immature grains, with apertures that have not become protuberant or that have only begun to protrude, as noted in Fig. 1A. Since the grains in the photomicrograph in Fig. 1G do not show a central vacuole, it seems likely that they were in a late microspores stage or early pollen grain

stage when collected. They show that there is some development of non-protruding aperture components (see arrows in Fig. 1G).

To our knowledge, inaperturate monad members of tetrads are relatively uncommon in plant families. Erdtman (1971, Fig. 157D) diagrammed apertureless tetrads of *Hedycarya arborea*, and Sampson's (1977) elegant light and scanning electron micrographs of Monimiaceae pollen showed tetrads without apertures and tetrad integrity maintained by the fusion of internal papillae of adjacent monad members. Apertureless tetrad pollen was also mentioned in Kapp's revised manual (2000), but specific taxa were not identified. In Onagraceae, this is the first report of inaperturate pollen.

Variation in aperture number.—In earlier publications, variation in the numbers of aperture pores was considered to be an important feature in the Onagraceae. In his study of Onagraceae pollen, Brown (1967: 163) stated: "other workers have correlated the number of pores and phases of ploidy for certain species. Pore number is not an absolute indicator of polyploidy." He emphasized that "deviation from the typical number suggests meiotic irregularities that occur so frequently in this family. Obviously, the 1-pored grains seen in many slides are aborted."

While 3-aperturate pollen is characteristic of the family, pollen with less and more than three apertures has also been reported frequently (Mitroiu, 1961–62; Ting, 1966; Brown, 1967; Skvarla et al., 1978; Praglowski et al., 1994; Punt et al., 2003). In specific reference to pollen grains with four apertures, Ting (1966) presented statistical evidence showing that while Onagraceae commonly have 3 apertures "4-aperturate grains seem to be a normal occurrence." He observed that in some collections of *Chamerion angustifolium* (reported as *Epilobium*) as many as 16.2% of the pollen is 4-aperturate. It should be noted that *C. angustifolium* subsp. *circumvagum* (Mosquin) Hoch is a widespread tetraploid ($n=36$), which may account for the higher percentage of 4-aperturate pollen in some collections. There are no verified tetraploid chromosome counts for any of the 160 species of sect. *Epilobium*, of which *E. luteum* is a member. This is the first report of 4-apertured monad members of

tetrads in *E. luteum*; however, Punt et al. (2003) included an SEM (Plate 16, Fig. 2) of individual monad members aligned with neighboring 4-apertured monads in *E. ciliatum* (as *E. adenocaulon*). Apart from the Williams and Packer collections examined in this study, no other reports of pollen in *E. luteum* suggest variation in the number of apertures. This includes pollen of *E. luteum* we examined earlier from the United States (Praglowksi et al., 1994): Washington, *Hoch 1071*; and an undescribed collection from Washington, *Seavey M623-4*. These two collections had typical tetrahedral tetrads with normal 3-aperturate monad members.

Monad pollen mixed with tetrad pollen.—The occurrence of mature single (monad) pollen grains in samples with tetrad pollen of *Epilobium* is well known (Praglowksi et al., 1994) and similar documentation has been made for *Ludwigia* (Praglowksi et al., 1983). It is important to recognize the early commentary by Ting (1966: 9–10) on tetrad stability in the Onagraceae: “The pollen grains of Onagraceae were shed either as tetrads or as single grains. According to Raven (1963), tetrads occur in most species of *Ludwigia* (including *Jussiaea*)... (and) all of *Epilobium* except sect. *Chamaenerion* and *E. paniculatum*.” Since Raven’s study of 1963, many of the taxa analyzed by Ting (1966) have been merged into *Epilobium*, yet Ting’s observations are remarkably accurate, especially since he did not have the benefit of electron microscopy (SEM, TEM). Brown (1967: 177) also attempted to explain the mixture of monads with tetrads, indicating that at least 50 species of *Epilobium* “... have their pollen in tetrads or in some instances as single grains or a mixture of both. It is probable some tetrads disarticulate more readily than others which may be the reason for mixtures or separation as the result of processing.” Further corroboration of processing as the cause of tetrad breakup was most recently provided by Punt et al. (2003: 107) who noted that “the adhesion between the pollen grains is weak and that tetrads usually fall apart into monads, especially after acetolysis.” While we agree that tetrad disruption may often be the result of processing, most likely acetolysis (Erdtman, 1960), we

also are impressed by large numbers of tetrads that have remained intact and undistorted after processing (Skvarla et al., 1975). It would seem that if acetolysis accounts for disaggregation of tetrads into component monads then the breakdown should be high. That this is not the case is clear from Ting’s comments about tetrad permanency. In our experience, many Onagraceae tetrads that have been processed by acetolysis, etc., including those with weak bridges or even those without bridges, do not always disaggregate into monads (Skvarla et al., 1975; Patel et al., 1984; Praglowksi et al., 1994). Tetrads of *Camissonia cardiophylla* became disassociated into monads when viewed on glycerin jelly slides after light pressure from affixed coverslips (Skvarla et al., 1975). Except for glycerin jelly, where light pressure is necessary for preparation, great care was given to prevent any manipulative damage to tetrads. Similarly, the light microscope preparations of water mounts of the Williams (Fig. 1G) and Packer (Fig. 2E) collections also indicated at least partial tetrad separation into monads. We have concluded that it does not seem likely that “true” monads exist in these tetrad collections because this would imply that at least two different mechanisms of pollen wall formation occur within an anther to accommodate “true” monad and tetrad pollen grains.

Rowley (unpublished) found that mature tetrads of *Epilobium montanum* were mostly intact after acetolysis, whereas many of these same tetrads were disrupted by transfer from the acid to a water washing. His interpretation is that disruption of the tetrad condition may be due not to contraction of the tetrads in acid (acetolysis), which does not seem disruptive in itself, but rather due to expansion of these tetrads when exposed to the water rinse.

The studies of Maheshwari (1950) and Sampson (1977) showed that it is normal for tetrad pollen to have all four monad members at the same stage of development. Levan (1942) found that the tetrad condition in mature pollen of *Petunia nyctangifolia* was determined by presence of a recessive gene in a homozygous condition. When this gene was not homozygous the grains formed as monads. Since true monads do not exist in the collections

used in our current report, and since members of the Onagraceae (except *Chamaerion*) have retained tetrads, it suggests that the Onagraceae may, like Levan's *Petunia*, have a recessive gene in a homozygous condition for tetrad retention.

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