

Review Paper

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Martin's peripheral embryo – unique but not a phylogenetic 'orphan' at the base of his family tree: a tribute to the insight of a pioneer seed biologist

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Abstract

As a tribute to A.C. Martin's classic work on embryos in seeds, we have attempted to gain a better understanding of the peripheral embryo, which puzzled Martin. The peripheral embryo is strongly curved and in contact with the inner surface of the seed coat, and Martin placed it at the base of his family tree of seed phylogeny and called it a 'phylogenetic orphan'. We evaluated ovule/seed development, kind of embryo and occurrence of perisperm in families with and without a peripheral embryo. All families with a peripheral embryo occur in the Caryophyllales. Seeds with a peripheral embryo have a low cotyledon width:radicle width ratio that coincides with Martin's (full-sized) linear embryo. The peripheral embryo develops in campylotropous and/or amphitropous ovules and is pushed to the side of the seed as the perisperm develops. Linear-full embryos and perisperm are widely distributed across extant angiosperms but are rarely found together, except in core Caryophyllales. The non-core Caryophyllales with endosperm and various kinds of embryos, including the linear-full, diverged before the core Caryophyllales. Thus, the ancestral linear-full embryo appears to have been retained when the core lineage developed campylotropous and/or amphitropous ovules and perisperm. Seeds with a peripheral embryo merit a position on Martin's family tree; however, the position should be a side branch ('orphan') slightly above (more advanced than) his linear embryo and not at the base. We conclude that Martin had great insight into the relationships between the kinds of embryos and rightly questioned the position of the peripheral embryo.

Introduction

Alexander Campbell Martin was born in 1897, and he obtained a PhD from George Washington University in Washington, DC (USA), presumably in 1944; his doctorate thesis was submitted in December 1943. His PhD work was published in 1946 in *The American Midland Naturalist*, and the title was 'The comparative internal morphology of seeds'. This paper contains drawings and information on embryos in 1287 genera of seed plants. Interestingly, Martin was 49 years old when this paper was published. While Martin worked on his PhD research, he was employed at the Patuxent Research Refuge of the USA Fish and Wildlife Service near Laurel, Maryland. He worked at this Refuge from 1940 until his retirement in about 1963. As we approach the 125 year anniversary of his birth and the 75th year since the publication of his classic paper on the internal morphology of seeds, we think it is appropriate to reflect on Martin's contributions to seed science.

The major contributions of Martin's 1946 paper, include (1) development of a comprehensive database on embryo morphology of seeds, (2) a family tree of seed phylogeny based on morphological traits and (3) discussion and questions about some of the kinds of embryos, e.g. the peripheral embryo, that still provide challenges for seed biologists today. Martin was not the first person to pay attention to seed anatomy (see Netolitzky, 1926). However, his comprehensive survey gave him the background information with which to contemplate relationships between kinds of embryos and allowed him to construct the first family tree of seed phylogeny.

The information in Martin's 1946 paper has been widely used by seed biologists/ecologists (e.g. Keeley, 1991; Finch-Savage and Leubner-Metzger, 2006; Vyshenskaya, 2006; Baskin and Baskin, 2014). His work has played a role in studies of seed anatomy (Vaughan and Whitehouse, 1971; Gunn, 1974; Boesewinkel and Bouman, 1995; Werker, 1997), development (Floyd and Friedman, 2000), evolution (Grushvitskii, 1961; Forbis *et al.*, 2002; Eriksson and Kainulainen, 2011; Willis *et al.*, 2014) and technology/physiology (Lang, 1965; Grabe, 1970; Justice, 1972; Bass *et al.*, 1988). In addition, Martin's paper has been cited in studies on plant taxonomy (Reeder, 1957; Takhtajan, 1980, 1997), embryology (Raghaven, 1986;

Vyshenskaya, 2006), evolution (Carlquist, 1961; Dahlgren and Rasmussen, 1983; Thorne, 2000), propagation (Hartman and Kester, 1975) and floristics/taxonomic revisions (Palser, 1951; Fryxell, 1978; Hodgson and Mackey, 1986; Harley *et al.*, 2004). According to Google Scholar (29 July 2019), Martin's (1946) paper has been cited 846 times.

Martin had a productive career. However, except for a book co-authored with William D. Barkley (Martin and Barkley, 1961) entitled '*Seed Identification Manual*' he did not publish any more work on seed embryos. He published several papers dealing with the food habits of waterfowl and a book (Martin *et al.*, 1951) entitled '*American Wildlife and Plants*', which had 500 pages and 300 illustrations of the genera of plants used as food by wildlife. Martin with co-authors Neil Hotchkiss, Francis Uhler and William Bourn (Martin *et al.*, 1953) published a paper entitled 'Classification of wetlands in the United States', which was the first attempt to classify wetlands in the USA. He also authored or co-authored four books (*Weeds, Trees, Flowers and Wildflowers*) in the 'A Golden Guide' series of books on natural history (published by St Martin's Press).

One of the intriguing things about Martin's 1946 paper is that he was very frank about the aspects of his family tree of seed phylogeny that puzzled him. One of these puzzles is the peripheral embryo. Martin described the peripheral embryo as a 'phylogenetic orphan' and wrote several comments about it, two of which are provided here:

'Peripheral division – Embryo ordinarily elongate and large, quarter to dominant, continuous in part at least to the testa and often curved; endosperm (actually perisperm) conspicuously starchy; central or in a few instances lateral. Cotyledons narrow or expanded. Dicots – but in several cases one of the cotyledons is abortive.' [p. 519]

'Peripheral division seems to resemble a blind alley leading nowhere beyond itself; though expanded cotyledons in some Peripheral families show at least superficial resemblance to the Foliate subdivision it appears to be a case of "parallelism" in fundamentally distinct groups. Even if the distinct kind of endosperm [perisperm] did not present an insuperable barrier it still would seem almost inconceivable that a peripheral embryo, with its characteristic position surrounding the endosperm [perisperm] could ever have given rise to central-embryoed members of the Axile group.' [p. 526]

Not only did Martin consider the peripheral embryo to be a 'phylogenetic orphan', but he placed it at the base of his tree, showing it as a branch that originates from the side of something that looks like a tree stump (Fig. 1). Much research on seed morphology and on the phylogeny of angiosperms has been conducted since 1946, but the results of these studies have not been evaluated in terms of Martin's conclusion with regard to the peripheral embryo. In attempting to understand the origins and evolutionary relationships of the various kinds of embryos in seeds of angiosperms, it is important to know if the peripheral embryo is truly a phylogenetic orphan, or is it related to other kinds of embryos? As a tribute to Martin's pioneer work in seed phylogeny, we have revisited his peripheral embryo. We have carefully studied the details about the peripheral embryo provided in Martin's 1946 paper and the results from relevant kinds of research conducted since 1946. We can conclude that Martin had great insight with regard to the peripheral embryo, but more can be added to the story, which we think would have fascinated him.

From studying the shape and location of the peripheral embryo in the seed, as illustrated in Martin (1946), we hypothesized that seeds with this kind of embryo have distinct morphological and developmental characteristics. Furthermore, based on the variation within Martin's families with peripheral embryos with regard to cotyledon morphology, his comments about the peripheral embryo and expansion of our knowledge of seed morphology and plant taxonomy/phylogeny since 1946, we hypothesize that the peripheral embryo does not belong at the base of Martin's family tree of seed phylogeny.

To address these hypotheses, the literature was reviewed to find answers to various questions: (1) What families of angiosperms have a peripheral embryo? (2) What traits do families with peripheral embryos have in common, and are any of these traits found in families that do not have a peripheral embryo? (3) What kind of ovule/seed development and cotyledon morphology occurs in taxa with peripheral embryos? (4) What is the phylogenetic position of families with peripheral embryos and of families that share traits with those known to have peripheral embryos?

Plant families with peripheral embryos

Martin (1946) illustrated a peripheral embryo in seeds of 11 families and listed them in the approximate order 'of extent of expansion of their cotyledons': Aizoaceae, Portulacaceae, Cactaceae, Caryophyllaceae, Chenopodiaceae, Frankeniaceae, Scleranthaceae, Amaranthaceae, Phytolaccaceae, Polygonaceae and Nyctaginaceae. All these families belong to the Caryophyllales (Takhtajan, 1997); thus, we need to investigate the characteristics of this order.

Caryophyllales

Plant families with free-central or basal placentation, mostly campylotropous ovules (i.e. curved embryos) and perisperm in the mature seed have long been regarded as a natural unit of classification (Bittrich, 1993). This group of families has been called the Curvembryae, Centrospermae and Caryophyllales (Harms, 1934; Takhtajan, 1997). Harms (1934) listed 12 families in the Centrospermae: Achatocarpaceae, Aizoaceae, Amaranthaceae, Basellaceae, Caryophyllaceae, Chenopodiaceae, Dysphaniaceae, Gyrostemonaceae (now in Brassicales), Nyctaginaceae, Phytolaccaceae, Portulacaceae and Theligonaceae (now in Gentianales). As various kinds of research have been conducted, the list of families in the Centrospermae (now Caryophyllales) has been modified.

By the late 1980s, 12 families were included in the Caryophyllales: Achatocarpaceae, Aizoaceae, Amaranthaceae, Basellaceae, Cactaceae, Caryophyllaceae, Chenopodiaceae, Didiereaceae, Molluginaceae, Nyctaginaceae, Phytolaccaceae and Portulacaceae and about 10,000 species (Cronquist, 1988). However, studies on the phylogenetic relationships of angiosperms using gene sequence data have provided much new insight on angiosperms in general (e.g. Downie *et al.*, 1997; Soltis *et al.*, 1999, 2000; Hilu *et al.*, 2003; Moore *et al.*, 2010) and on Caryophyllales in particular (Giannasi *et al.*, 1992; Retting *et al.*, 1992; Morton *et al.*, 1997; Cuénoud *et al.*, 2002; Greenberg and Donoghue, 2011; Yang *et al.*, 2015; Smith *et al.*, 2018). In some cases, the molecular studies have yielded some surprising results, e.g. Droseraceae belongs in a clade with Caryophyllidae and Nepenthaceae (Williams *et al.*, 1994). The number of families presently recognized in the Caryophyllales is 38 (APG IV, 2016).

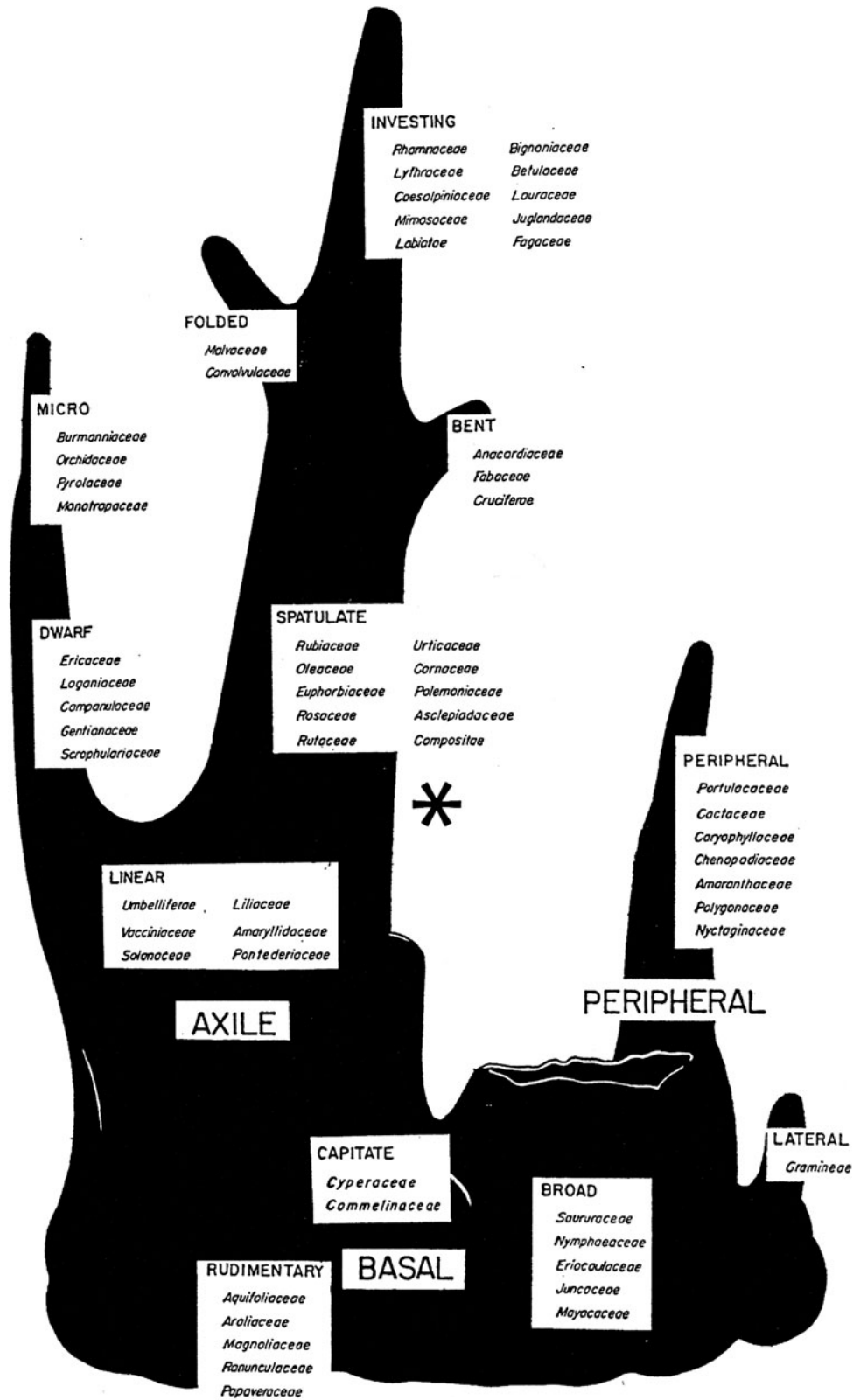


Figure 1. Martin's (1946) family tree of seed phylogeny. *Proposed position on Martin's tree for the peripheral embryo. Reproduced with permission.

Hernández-Ledesma *et al.* (2015) recognized Amaranthaceae and Chenopodiaceae as separate families and thus have 39 families on their list, while APG IV (2016) combined these two families into Amaranthaceae, resulting in 38 families. The number of

species in the Caryophyllales is about 12,500 (Hernández-Ledesma *et al.*, 2015).

According to placement of the Caryophyllales on the angiosperm phylogeny diagram (APG IV, 2016), the Caryophyllales

along with Berberidopsidales and Santalales make up the Superasterid clade, which is sister to the Asterids. As such, the Caryophyllales are placed between the Malvids and Campanulids on the phylogenetic diagram.

Another result of the molecular phylogeny studies is that the Caryophyllales have been divided into two major clades: Caryophyllales I (core) and Caryophyllales II (non-core) (Cuénoud *et al.*, 2002; Hilu *et al.*, 2003; Soltis *et al.*, 2005; Brockington *et al.*, 2009; Endress, 2010; Lee *et al.*, 2013; Walker *et al.*, 2018). The core Caryophyllales consist of the families traditionally included in this order, along with the Agdestidaceae, Barbeuiaceae, Gisekiaceae, Halophytaceae, Lophiocarpaceae, Petiveriaceae, Sarcobataceae and Stegnospermataceae (Table 1). The noncore families include the Ancistrocladaceae, Droseraceae, Drosophyllaceae, Dioncophyllaceae, Frankeniaceae, Nepenthaceae, Plumbaginaceae, Polygonaceae and Tamaricaceae (Table 1).

The Asteropeiaceae, Physenaceae, Rhabdodendraceae and Simmondsiaceae are included in the core Caryophyllales; however, the seeds of these families do not have a peripheral embryo and only scant endosperm and no perisperm, except for scant perisperm in Rhabdodendraceae (Table 1). According to Cuénoud *et al.* (2002), the Simmondsiaceae is sister to the core, and Rhabdodendraceae is sister to the noncore Caryophyllales. Carlquist (2010) followed Cuénoud *et al.*, (2002), but Crawley and Hilu (2012) considered these three families to be sister to the core lineages. According to Brockington *et al.* (2009) and Magallón *et al.* (2015), Rhabdodendraceae and Simmondsiaceae are sister to the core, and Asteropeiaceae and Physenaceae form a clade that is sister to the rest of the core Caryophyllales. In studies of the one-seeded fruits of the core Caryophyllales, Sukhorukov *et al.* (2015) found that the early-diverging lineages, i.e. Asteropeiaceae, Physenaceae, Rhabdodendraceae and Simmondsiaceae, have fruit and seed coats that were different from those of other Caryophyllales. Fruits of these four families have a thick pericarp that is divided into several distinct zones, and the seed coat also is divided into several layers.

Chemotaxonomy research on the Caryophyllales has revealed that members of the Aizoaceae, Amaranthaceae, Basellaceae, Cactaceae, Chenopodiaceae, Didiereaceae, Halophytaceae, Nyctaginaceae, Phytolaccaceae, Portulacaceae and Stegnospermaceae have the pigment betalain, while the Caryophyllaceae and Molluginaceae have anthocyanin (Mabry *et al.*, 1963; Mabry, 1976, 1977; Mabry and Dreiding, 1969) (Table 1). Subsequent lineage-specific gene radiation studies have shown that betalain-specific isoforms in the *CYP76AD1* and *DODA* lineages were lost or down-regulated in Caryophyllaceae and Molluginaceae (Brockington *et al.*, 2015), resulting in production of anthocyanin instead of betalain. Betalains have been found only in the Caryophyllales (Mabry and Dreiding, 1969; Stafford, 1994; Brockington *et al.*, 2011; Thulin *et al.*, 2016).

Another chemical compound of interest in our consideration of the Caryophyllales is ferulic acid, which is ester-linked to polysaccharides in the nonlignified (primary) cell wall. Ultraviolet fluorescence microscopy is used to examine primary cell walls, and if ferulic acid is present the cell walls autofluoresce blue at low pH and green at high pH (Harris and Hartley, 1980). Hartley and Harris (1981) examined the primary cell wall of 251 species in 150 families and 46 orders of basal angiosperms and eudicots and found ferulic acid only in core Caryophyllales (Table 1). In the Commelinid monocots and core Caryophyllales, the concentration of ferulic acid is >3.5 mg (g cell wall)⁻¹, but in eudicots such as *Arabidopsis thaliana*,

Daucus carota, *Medicago sativa* and *Populus trichocarpa* the concentration is <3.5 mg (g cell wall)⁻¹ (Harris and Trethewey, 2010).

Some families in the Caryophyllales have a unique kind of sieve-element plastid in which there are ring-shaped bundles of filaments that contain protein; these plastids are called P-type plastids (Behnke, 1972). There is another basic kind of sieve-element plastid in which starch is accumulated, and it is called an S-type plastid (Behnke, 1972, 1976, 1991; Behnke *et al.*, 1974). The non-core families in the Caryophyllales have S-type sieve-element plastids, and the core families have P-type, except the Asteropeiaceae, Physenaceae and Simmondsiaceae, which have S-type (Table 1).

Peripheral embryo

Development

To understand the relatively long length of the peripheral embryo and its position in the seed, information about the ovule in which it develops is required. An ovule is a sac-like structure that contains the megasporangium in which the egg and eventually the embryo are produced. The oldest known ovules are those of *Elkinsia polymorpha* (Late Devonian, 365 ma) (Gillespie *et al.*, 1981; Rothwell *et al.*, 1989). The ovule of many orders of angiosperms has two integuments (bitegmic) and a relatively large amount of nucellus (crassinucellate) (Bouman, 1984; Endress 2011a). In some angiosperms, e.g. the asterids, the ovule has one integument (unitegmic) and a thin nucellus (tenuinucellate), but ovules can be bitegmic and tenuinucellate, e.g. various families in the Rosiidae and Dilleniidae (Philipson, 1974). Furthermore, there is much diversity in crassinucellate and tenuinucellate ovules (Endress, 2011a). The Caryophyllales have ovules that are bitegmic and crassinucellate (Eckardt, 1976).

The amount of curvature of the ovule that has occurred by the time the embryo sac has formed varies greatly, and the most common kinds of ovules are orthotropous (atropous), anatropous, campylotropous, hemianatropous and amphitropous (Fahn, 1974; Endress, 2011a); there are variations of these five common kinds of ovules. An orthotropous ovule is straight with the micropyle opposite the funiculus, while an anatropous ovule is bent so that the micropyle is adjacent to the funiculus. The anatropous ovule is the most common kind in extant angiosperms (Endress, 2011a), and it is probably the most ancestral (Endress, 2011b). The hemianatropous ovule is bent so that the micropyle is at a 90 degree angle in relation to the funiculus. In orthotropous, anatropous and hemianatropous ovules, the nucellus and embryo sac are straight (Endress, 2011b). Campylotropous and amphitropous ovules are bent, and the micropyle is pointed toward the base of the funiculus. The campylotropous ovule has a bulge on the side, while the amphitropous ovule has a bulge on top. Thus, in campylotropous and amphitropous ovules the nucellus and embryo sac are curved, which results in a curved embryo (Rau, 1940; Takaso and Bouman, 1984). Furthermore, there is more space in campylotropous and amphitropous ovules for the embryo to elongate than in the other kinds of ovules. In campylotropous ovules, the embryo can be up to two times as long as the seed.

Taxonomic occurrence

With the exception of the Asteropeiaceae, Physenaceae, Rhabdodendraceae and Simmondsiaceae, the core families of the Caryophyllales have seeds with a peripheral embryo, while

Table 1. Families in the core and non-core Caryophyllales and information for each family for various seed and plant characters (P, protein; S, starch)

Family/taxon	Embryo ¹⁻⁶	Ovule ^{2,6}	Endosperm or perisperm ^{5,7-13}	Anthocyanin or betalain ^{7,14-18}	Ferulic acid ^{19,20}	Sieve-element plastids ²¹⁻²⁵
Core						
Rhabdodendraceae	Investing	Campylotropous	Scant endosperm and scant perisperm	Betalain absent	No data	P
Simmondsiaceae	Investing	Anatropous	Scant endosperm	Anthocyanin	No data	S
Asteropeiaceae	Spiralled or linear-full	Epitropous (hanging)	Scant endosperm	Betalain absent	No data	S
Physenaceae	Spatulate	Campylotropous	Scant endosperm	Betalain absent	No data	S
Macarthuriaceae	Peripheral	Campylotropous	Perisperm	Anthocyanin	No data	P
Microteaceae	Peripheral	Campylotropous	Perisperm	No data	No data	P
Caryophyllaceae	Peripheral, straight or spiralled	Hemitropous to campylotropous	Perisperm	Anthocyanin	Yes	P
Achatocarpaceae	Peripheral	Campylotropous	Perisperm	No data	No data	P
Amaranthaceae	Peripheral, annular or spirally twisted	Campylotropous	Perisperm	Betalain	Yes	P
Stegnospemataceae	Peripheral	Amphitropous	Perisperm	Betalain	No data	P
Limeaceae	Peripheral	Campylotropous	Perisperm	Anthocyanin	No data	P
Lophiocarpaceae	Peripheral	Campylotropous	Perisperm	Betalain	No data	P
Hypertelis (Molluginaceae)	Peripheral	Campylotropous	Perisperm	Anthocyanin	No data	P
Kewaceae	Peripheral	Campylotropous	Perisperm	Anthocyanin	No data	No data
Barbeuiaceae	Peripheral	Campylotropous	Perisperm	Betalain	No data	P
Aizoaceae	Peripheral	Campylotropous to almost anatropous	Perisperm, scant endosperm	Betalain	Yes	P
Phytolaccaceae	Peripheral	Anacampylotropous	Perisperm	Betalain	Yes	P
Petiveriaceae	Peripheral	Campylotropous	Perisperm	Betalain	No data	P
Gisekiaceae	Peripheral	Campylotropous	Perisperm	Betalain	No data	P
Sarcobataceae	Peripheral	Campylotropous	Perisperm	Betalain	No data	P
Nyctaginaceae	Peripheral or straight	Anatropous or campylotropous	Perisperm	Betalain	Yes	P
Molluginaceae	Peripheral	Campylotropous to almost anatropous	Perisperm	Anthocyanin	No data	P
Halophytaceae	Peripheral	Campylotropous	Perisperm	Betalain	No data	P
Montiaceae	Peripheral	Campylotropous or amphitropous	Perisperm	Betalain	Yes	P
Didiereaceae	Peripheral	Campylotropous	Scant perisperm	Betalain	Yes	P
Basellaceae	Peripheral or annular to cochleate	Anatropous to campylotropous	Perisperm	Betalain	Yes	P
Talinaceae	Peripheral	Campylotropous to amphitropous	Perisperm	Betalain	Yes	P
Anacampserotaceae	Peripheral or slightly curved	Campylotropous to amphitropous	Scant perisperm	Betalain	No data	P
Portulacaceae	Peripheral	Anacampylotropous	Perisperm	Betalain	Yes	P
Cactaceae	Peripheral	Campylotropous to rarely anatropous	Perisperm	Betalain	Yes	P
Non-core						

(Continued)

Table 1. (Continued.)

Family/taxon	Embryo ¹⁻⁶	Ovule ^{2,6}	Endosperm or perisperm ^{5,7-13}	Anthocyanin or betalain ^{7,14-18}	Ferulic acid ^{19,20}	Sieve-element plastids ²¹⁻²⁵
Droseraceae	Spatulate	Anatropous	Endosperm	Anthocyanin	No data	S
Nepenthaceae	Linear-full (minute)	Anatropous	Endosperm	Anthocyanin	No data	S
Drosophyllaceae	Spatulate	Anatropous	Endosperm	Anthocyanin	No data	S
Ancistrocladaceae	Spatulate	Hemitropous	Endosperm	Anthocyanin	No data	S
Dioncophyllaceae	Spatulate (discoid-obconic)	Anatropous	Endosperm	Anthocyanin	No data	S
Frankeniaceae	Linear-full (peripheral)	Anatropous	Endosperm	Anthocyanin	No data	S
Tamaricaceae	Spatulate	Anatropous	Scant endosperm and thin perisperm	Anthocyanin	No data	S
Plumbaginaceae	Spatulate	Anatropous	Endosperm	Anthocyanin	No data	S
Polygonaceae	Linear-full, folded or spatulate (peripheral)	Orthotropous	Endosperm	Anthocyanin	No data	S

1. Martin, 1946; 2. Baskin and Baskin, unpublished embryo database; 3. Eckardt, 1976; 4. Mabberley, 2008; 5. Shepherd *et al.*, 2005; 6. Johansen, 1950; 7. AP (Angiosperm Phylogeny) website; 8. Woodcock, 1914; 9. Houk, 1938; 10. Liao and Wu, 2000; 11. Anderson, 1975; 12. Ayele *et al.*, 2010; 13. Grayum, 1991; 14. Iwashina, 2013; 15. Brockington *et al.*, 2011; 16. Brockington *et al.*, 2009; 17. Watson and Dallwitz, 1992 onwards; 18. Thulin *et al.*, 2016; 19. Hartley and Harris, 1981; 20. Hakki, 2013; 21. Behnke, 1972; 22. Behnke *et al.*, 1974; 23. Behnke, 1976; 24. Behnke, 1991; 25. Fay *et al.*, 1997

non-core families mostly do not have a peripheral embryo (Table 1). Seeds of Frankeniaceae and Polygonaceae (listed by Martin as having a peripheral embryo) are non-core, but they have endosperm (Takhtajan, 1997). The Frankeniaceae have a long narrow embryo (Martin, 1946), and the Polygonaceae have embryos that are long and narrow, spatulate or folded in appearance (Stevens, 1912; Martin, 1946). With the exception of Asteropeiaceae, Simmondsiaceae and Stegnospermataceae, the core families of Caryophyllales have campylotropous ovules (Table 1). All the non-core families have anatropous ovules except the Polygonaceae, which has orthotropous ovules.

To gain a better understanding of the relevance of ovule morphology in our consideration of the peripheral embryo, information on family occurrence of campylotropous and amphitropous ovules was obtained from Johansen (1950) and Takhtajan (1997) and plotted on the APG IV (2016) phylogenetic diagram (Fig. 2). We found campylotropous and/or amphitropous ovules in 77 families, including 26 families in the Caryophyllales. Campylotropous ovules are widely distributed phylogenetically, and their occurrence ranges from the Austrobaileyales (an ANA grade angiosperm) to the Boraginales (a Lamiid) (Fig. 2). The occurrence of amphitropous ovules ranges from the Fabales (a Fabid) to the Gentianales (a Lamiid). Thus, clearly ovules with the capacity to produce seeds with elongated embryos occur throughout the extant angiosperms.

Perisperm

Development

A young angiosperm ovule consists of integuments, nucellus and female gametophyte. The cells of the nucellus represent the remnants of the megasporangium, and they surround the female gametophyte (embryo sac) and contain stored food that is used for embryo growth. In many plant species, as the ovule grows the amount of nucellus increases (Maheshwari and Chopra, 1955; Kellman-Sopyła *et al.*, 2017). After fertilization and formation

of the embryo and endosperm, embryo growth occurs at the expense of the endosperm (Werker, 1997; Burrieza *et al.*, 2014), and the endosperm may transfer food from the nucellus to the embryo (Mohana Rao *et al.*, 1988). Depending on the species, all the stored food in the nucellus may be consumed before or after fertilization or during germination (Mohana Rao *et al.*, 1988; Werker, 1997). Following fertilization, developing seeds of *Chenopodium quinoa* simultaneously accumulate (starch) and use food in the nucellus (López-Fernández and Maldonado, 2013). If any nucellar tissue remains after seed development is completed, it is called perisperm (Burrieza *et al.*, 2014; Jiménez-Durán *et al.*, 2014). Development of perisperm takes place in the ovule, and it helps to ensure a good supply of food and water for the developing seed (Wilms, 1980; Mohana Rao *et al.*, 1988). As the perisperm in campylotropous ovules develops on the lower side of the embryo sac (above the chalaza), the embryo is pushed to the periphery of the ovule as the mass of the perisperm increases (Gibbs, 1907; Buell, 1952; Wilms, 1980; Mohana Rao *et al.*, 1988; Zheng *et al.*, 2010).

Taxonomic occurrence

Except for Asteropeiaceae, Phytolaccaceae and Simmondsiaceae with scant endosperm and Rhabdodendraceae with scant endosperm and scant perisperm, seeds of families in the core Caryophyllales have copious starchy perisperm (Table 1). However, a small amount of endosperm may occur around the radicle end of the embryo in seeds of some core Caryophyllales, e.g. Amaranthaceae (Kajale, 1940, 1954; Pal *et al.*, 1990), Caryophyllaceae (Buell, 1952; Wagner and Teng, 1993; Kellman-Sopyła *et al.*, 2017) and Lophiocarpaceae (Hakki, 2013). Seeds of the non-core Caryophyllales families have endosperm, and those of Tamaricaceae have a thin layer of perisperm in addition to scant endosperm.

To gain a better understanding of the family occurrence of perisperm in seeds, we obtained information from Woodcock

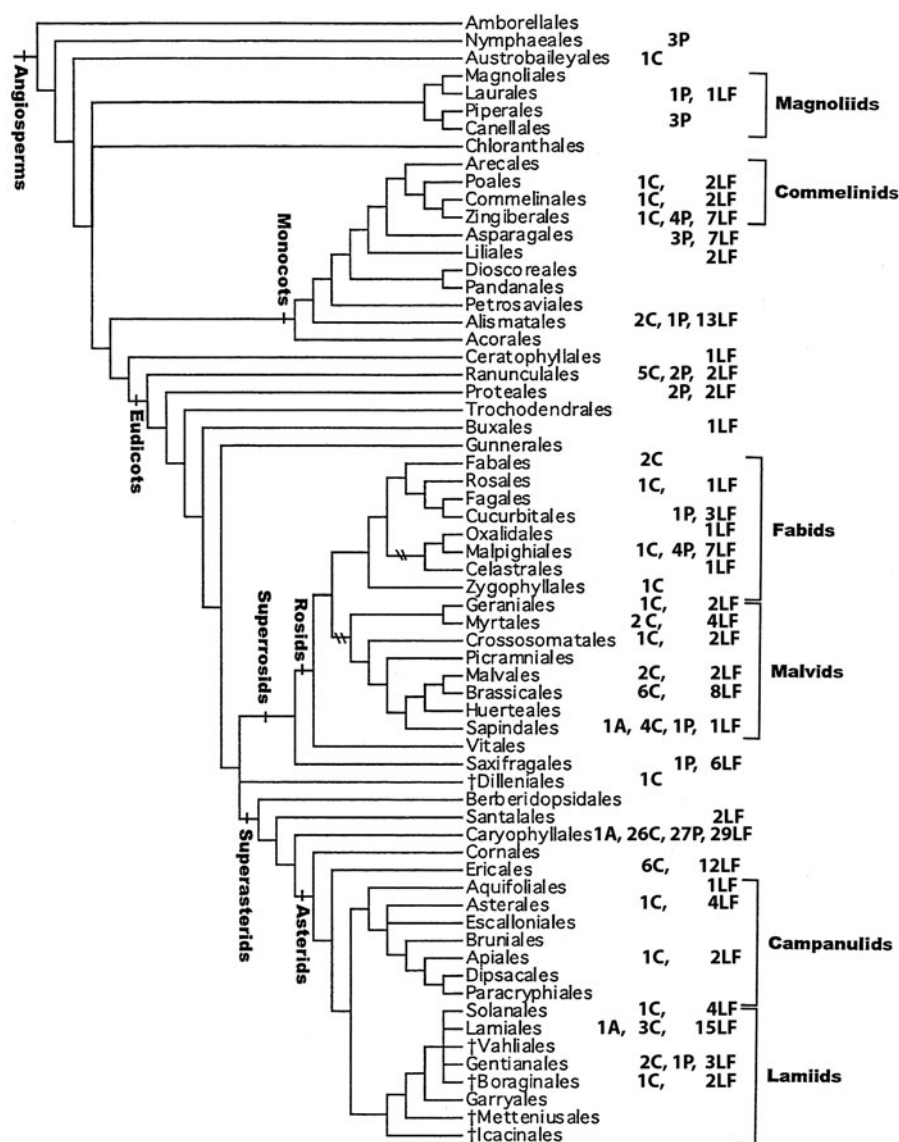


Figure 2. Angiosperm phylogenetic diagram showing number of families with campylotropous (C) and amphitropous (A) ovules, perisperm (P) and linear-full embryos (LF) (modified from APG IV, 2016).

(1914), West *et al.* (1995), Takhtajan (1997), Lu (1985), Goldberg (1986, 1989) and Plakhine *et al.* (2012) and plotted it on the APG IV (2016) phylogenetic diagram (Fig. 2). Perisperm is widely distributed in the angiosperms and ranges from the Nymphaeales to Gentianales. However, the amount of perisperm varies from copious in seeds of Nymphaeales, Piperales, Zingiberales and Caryophyllales (Goldberg, 1986, 1989) to a single layer of cells in Brassicales, Malvales and Lamiales (Werker, 1997; Plakhine *et al.*, 2012). The only families, in addition to those in the Caryophyllales, with both campylotropous and/or amphitropous ovules and perisperm are the Marantaceae, Papaveraceae, Ranunculaceae, Rubiaceae and Simaroubaceae.

Embryo morphology/shape

Aside from the peripheral embryo being located adjacent to the seed coat, how can we describe/characterize it? For various other kinds of embryos in Martin's (1946) paper such as the bent, folded, investing, linear and spatulate, relative width of the radicle and cotyledons is a useful identification character. For all the drawings of members of the Caryophyllales in Martin's

paper, we measured the width of the radicle and cotyledons and calculated the cotyledon (C):radicle (R) ratio. A C:R ratio of 1 represents a long, narrow embryo with the radicle and cotyledons having the same width, whereas a C:R ratio >1 means the cotyledons are wider than the radicle. Furthermore, we determined the C:R ratio for seeds with bent, folded, investing, full-sized linear and spatulate embryos illustrated in Martin's paper.

Mean C:R ratio for the Aizoaceae, Amaranthaceae, Cactaceae, Caryophyllaceae, Chenopodiaceae, Frankeniaceae, Nyctaginaceae, Phytolaccaceae and Portulacaceae, which are in the core Caryophyllales, ranged from 1.0 to 1.82, and the mean C:R ratio for full-sized linear embryos was 1.06 (Table 2). As small linear embryos grow inside the seed prior to germination and full-sized linear embryos do not, we refer to them as linear-underdeveloped and linear-fully developed (linear-full), respectively (Baskin and Baskin, 2007). All families included by Martin that belong to the core Caryophyllales have embryos that easily fit his full-sized linear embryo. All families in the core Caryophyllales, except Asteropeiaceae, Physenaceae, Rhabdodendraceae and Simmondsiaceae, have long thin linear or peripheral embryos (Table 1).

Table 2. Mean (mm \pm s.d.) and range of cotyledon (C) width:radicle (R) width ratios calculated from measurements made on drawings of embryos in Martin's (1946) paper.

	Mean	Range
Martin's Caryophyllales (peripheral)		
Aizoaceae ($n = 6$)	1.30 \pm 0.15	1.1 to 1.5
Amaranthaceae ($n = 6$)	1.37 \pm 0.13	1.2 to 1.5
Cactaceae ($n = 12$)	1.02 \pm 0.06	1.0 to 1.2
Caryophyllaceae ($n = 19$)	1.49 \pm 0.33	1.0 to 2.0
Chenopodiaceae ($n = 18$)	1.34 \pm 0.44	1.0 to 2.7
Frankeniaceae ($n = 1$)	1.0	0
Nyctaginaceae ($n = 5$)	1.82 \pm 0.22	1.5 to 2.0
Phytolaccaceae ($n = 4$)	1.23 \pm 0.31	1.0 to 1.75
Polygonaceae ($n = 13$)	2.56 \pm 1.25	1.0 to 4.7
Portulacaceae ($n = 8$)	1.16 \pm 0.13	1.0 to 1.4
Other embryo types from Martin's work		
Bent ($n = 36$)	3.69 \pm 1.42	1.5 to 6.5
Folded ($n = 32$)	4.25 \pm 2.31	1.0 to 7.75
Investing ($n = 23$)	6.11 \pm 2.11	3.0 to 11.5
Linear-full ($n = 35$)	1.06 \pm 0.13	1.0 to 1.5
Spatulate ($n = 53$)	2.98 \pm 1.04	1.25 to 6.0

However, the mean C:R ratio for the Polygonaceae (2.56) most closely matches the mean C:R ratio (2.98) for spatulate embryos. Families in the non-core Caryophyllales have a diversity of embryo types, including peripheral [Polygonaceae and Frankeniaceae (Martin, 1946)], linear-full, spatulate and investing, with spatulate being the most common (Table 1).

As the majority of Martin's families with a peripheral embryo have linear-full embryos, we queried our embryo database (Baskin and Baskin, unpublished) for other families with linear-full embryos. Linear-full embryos occur in 153 families, including 29 in the Caryophyllales, throughout the extant angiosperms from Laurales (Magnoliids) to Boraginales (Lamiids) (Fig. 2). In addition to the Caryophyllales, ≥ 5 families in the Alismatales, Asparagales, Brassicales, Ericales, Lamiales, Malpighiales, Saxifragales and Zingiberales have linear-full embryos. Linear-full embryos occur in one or more families in all orders with campylotropous and/or amphitropous ovules, except the Austrobaileyales, Dilleniales, Fabales, Saxifragales and Zygophyllales; however, some families in the Saxifragales have linear-underdeveloped embryos (Baskin and Baskin, unpublished embryo database).

On Martin's family tree of seed phylogeny, spatulate, bent, folded and investing embryos are placed above the linear-full (and in this order) (Fig. 1). Thus, in families with campylotropous and/or amphitropous ovules there is a strong association between linear-full and spatulate embryos, but linear-full also may occur with folded, investing and bent embryos. That is, the linear-full embryo is found in various families with campylotropous and/or amphitropous ovules along with the most advanced kinds of embryos (*sensu* Martin, 1946).

Twelve kinds of embryos have been recorded in the 29 families with perisperm, with some families having as many as four kinds for a total of 46 embryo records. The importance of the kind of embryo in families with perisperm is bent (2.2%), broad

(6.5%), capitate (8.7%), cupulate (4.3%), folded (4.3%), investing (13.0%), linear-full (19.6%), linear-underdeveloped (13.0%), rudimentary (4.3%), spatulate (17.4%), spatulate-underdeveloped (2.2%) and undifferentiated (organless) (4.3%).

Evaluation of hypotheses

Hypothesis 1: Seeds with a peripheral embryo have distinct morphological and developmental characteristics

With the exception of Asteropeiaceae, Physenaceae, Rhabdodendraceae and Simmondsiaceae, families in the core Caryophyllales have long narrow embryos that match the characteristics of Martin's linear-full embryos. These linear-full embryos develop in campylotropous and/or amphitropous ovules, and they encircle perisperm in the centre of the seed. This combination of characters (i.e. linear-full embryo, campylotropous and/or amphitropous ovules and perisperm) not only distinguishes the peripheral embryo but helps to characterize most of the core Caryophyllales. Individually, these three characters are widely distributed throughout the extant angiosperms, but they occur together in only seven orders (Alismatales, Gentianales, Lamiales, Malpighiales, Ranunculales, Sapindales and Zingiberales) in addition to the Caryophyllales (Fig. 2). However, in these orders (except for Caryophyllales), the three characters do not usually occur together in the same family. In addition to various families in the core Caryophyllales, this combination of characters is known to occur only in the Marantaceae and Rubiaceae. The Marantaceae have a linear-full embryo that is curved but located in the centre of the seed, and the Rubiaceae have folded, investing, linear-full, spatulate and spatulate-underdeveloped embryos (Martin, 1946).

The combination of linear-full embryos, campylotropous and/or amphitropous ovules and perisperm supports our hypothesis that seeds with peripheral embryos have distinct characters. In addition, P-type sieve-elements, betalain pigment and ferulic acid in the primary cell wall are strongly associated with the core, but not the non-core, Caryophyllales (Table 1), further supporting Martin's (1946) contention that the peripheral embryo is unique among the angiosperms.

Hypothesis 2: The peripheral embryo does not belong at the base of Martin's family tree of seed phylogeny

We now have to ask the difficult question: where does the peripheral embryo belong on Martin's family tree of seed phylogeny? The answer lies in the embryo itself. We queried our embryo database (Baskin and Baskin, unpublished) and asked what kinds of embryos occur in the non-Caryophyllales families with campylotropous and/or amphitropous ovules. Among the 50 non-Caryophyllales families with campylotropous and/or amphitropous ovules, there are 11 kinds of embryos, with some families having as many as five kinds of embryos. In total, we have 116 embryo records for these 50 families: capitate (0.9%), bent (9.5%), folded (12.9%), investing (14.7%), linear-full (20.7%), lateral (0.9%), linear-underdeveloped (8.6%), rudimentary (6.0%), spatulate (22.4%), spatulate-underdeveloped (2.6%) and undifferentiated (0.9%). Fourteen of the families have linear-full as well as other kinds of embryos (31 records). In these 14 families, linear-full occurs with bent (9.7%), investing (16.1%), linear-underdeveloped (9.7%), spatulate (38.7%), spatulate-underdeveloped (3.2%) and undifferentiated (3.2%) embryos. In three families (Lecythidaceae, Ranunculaceae and Rutaceae), linear-full

occurred with folded, investing and spatulate, and in the Myrtaceae it occurred with bent, folded, investing and spatulate. In the Rubiaceae, the linear-full embryo occurred with folded, investing, spatulate and spatulate-underdeveloped embryos. Thus, in the 14 families with linear-full as well as other kinds of embryos, there is clearly a strong association between the linear-full and spatulate embryos. Also, we ask what families have seeds with a linear-full embryo and perisperm? This combination of seed characters occurs only in seven monocot families (Asparagaceae, Araceae, Cannaceae, Marantaceae, Strelitziaceae, Tecophilaceae and Zingiberaceae) and three eudicot orders: Proteales (Proteaceae), Gentianales (Rubiaceae) and Caryophyllales (26 families; Table 1) for a total of 35 families. Thus, the combination of a linear-full embryo and perisperm is most common in the core families of the Caryophyllales.

Seeds of the core Caryophyllales with perisperm also have a linear-full (peripheral) embryo, except those of Rhabdodendraceae, which have scant perisperm, scant endosperm and an investing embryo (Table 1). In the non-core families, spatulate embryos and endosperm are the most common kind, but linear-full and endosperm are found in the Nepenthaceae, Frankeniaceae and Polygonaceae. Seeds of Tamaricaceae have seeds with spatulate embryos, scant perisperm and thin endosperm. Thus, seeds of the Caryophyllales may have endosperm and/or perisperm, and the embryo varies from linear-full to spatulate to investing.

The order Caryophyllales is very old. According to Magallón *et al.* (2015), the median time of divergence of Berberidopsidales and Caryophyllales was 117.9 Ma, and the core and non-core Caryophyllales diverged 107.1 Ma. Within the non-core Caryophyllales, the clade that includes Frankeniaceae, Plumbaginaceae, Polygonaceae and Tamaricaceae diverged from the one that includes Ancistrocladaceae, Dioncophyllaceae, Droseraceae, Drosophyllaceae and Nepenthaceae 99.3 Ma. Within the core Caryophyllales, Rhabdodendraceae diverged 108.9 Ma and Asteropeiaceae/Physenaceae 96.2 Ma. Among the core Caryophyllales, the clade consisting of Achatocarpaceae, Amaranthaceae and Caryophyllaceae diverged 89.5 Ma; the clade consisting of Aizoaceae, Barbeuiaceae, Gisekiaceae, Lophiocarpaceae, Nyctaginaceae, Phytolaccaceae and Sarcobataceae 79.6 Ma; and the one consisting of Basellaceae, Cactaceae, Didieraceae, Halophytaceae, Montiaceae, Portulacaceae and Talinaceae 42.8 Ma (Magallón *et al.*, 2015). Thus, the non-core families with endosperm and various kinds of embryos, including spatulate, investing and linear-full, and endosperm diverged from the other Caryophyllales 107.1 Ma, while the core families with peripheral embryos and perisperm did not diverge from the Asteropeiaceae with spiralled linear embryos and endosperm until 96.2 Ma. From 89.5 Ma, when Achatocarpaceae, Amaranthaceae and Caryophyllaceae diverged from the other Caryophyllales to 28.8 Ma, when Cactaceae and Portulacaceae diverged, 20 families of core Caryophyllales become distinct, and all have peripheral embryos and perisperm.

What we can surmise from this information about the age of divergence of Caryophyllales families is that those with peripheral embryos and perisperm are younger than those with endosperm and linear-full, spatulate or investing embryos. As the peripheral embryo is a linear-full embryo that is pushed to the side of the seed by the developing perisperm (Mohana Rao *et al.*, 1988), we propose that the basal stock for the peripheral embryo had a linear-full embryo and endosperm. In seeds with a peripheral embryo, perisperm became the primary food-storage tissue, but

the linear-full embryo is still present. Concomitantly with production of copious perisperm, it is conceivable that bending and bulging of the ovule may have occurred, i.e. formation of campylotropous and/or amphitropous ovules. According to Bouman (1984), the long embryo in campylotropous ovules is a derived character, and the resulting seedling is large and highly developed when it emerges from the seed and thus has an increased possibility of survival. Following this line of thinking, it is worth noting that some species of Amaranthaceae (subfamilies Chenopodioideae and Salicornioideae) with long coiled embryos have very fast germination, i.e. seeds imbibe and germinate in <24 hours (Parsons, 2012; Liu *et al.*, 2013; Parsons *et al.*, 2014). Nineteen of the 28 angiosperms reported to have very fast germination belong to the Caryophyllales, and 12 of them are in the Amaranthaceae. We suggest that the advantage gained by having seeds with highly developed embryos (some of which also had very fast germination) may have played a role in the high diversification of the core Caryophyllales and even in occupying extreme habitats such as saline areas and deserts.

For (an) unknown reason(s), perisperm is scattered throughout the extant angiosperms: Nymphales, Magnoliids, monocots, Fabids, Malvids, Superasterids and Lamiids (Fig. 2), which suggests that it has become the main food-storage tissue in seeds at different times in various lineages. If perisperm developed in ancestral stock with linear-full embryos and endosperm, it might help explain the presence of endosperm around the radicle in various core families today. The presence of perisperm *vs* endosperm in seeds is of interest to theoretical ecologists who argue that 2n perisperm (2 maternal:0 paternal) gives the maternal parent complete control over resource distribution to the developing seeds. However, 3n endosperm (2 maternal:1 paternal) gives the paternal parent some control over resource allocation to the developing seeds (Westoby and Rice, 1982; Queller, 1983; Haig and Westoby, 1989; Pires, 2014; Povilus *et al.*, 2018). We do not know what, if any, role a shift from endosperm to perisperm played in the evolution of the Caryophyllales. Nonetheless, after divergence of the lineage with perisperm much diversification occurred, as evidenced by the 25 families known today in the core Caryophyllales that have linear-full embryos and perisperm.

The embryo in the core Caryophyllales with perisperm is long, and both the cotyledons and radicle are narrow, resulting in a low C:R ratio. Interestingly, this embryo is very similar to the curved linear-full embryo in seeds of many species of Solanaceae that have endosperm (Martin, 1946). The fact that the peripheral embryo is pushed to the side of the seed by the developing perisperm seems to have diverted attention away from the fact that it is a linear-full embryo. According to Martin (1946), the foliate embryo division (i.e. spatulate, bent, folded and investing) was derived from ‘...erect or nearly straight-embryoed Linear Dicots...’. Thus, we conclude that the peripheral embryo is unique and that it deserves recognition, i.e. a position on Martin’s tree. The evidence we have collected reveals that the peripheral embryo is a curved linear-full embryo and as such should be placed on Martin’s tree near his linear embryo. We propose that the peripheral embryo be depicted as a side branch (‘orphan’) from the right branch of Martin’s tree and that it be located above the linear embryo and below the spatulate embryo (Fig. 1). Martin’s peripheral embryo in the Frankeniaceae and Polygonaceae, both of which have endosperm, are best represented by his linear and/or spatulate embryos that are already on the tree.

Tribute

Martin's insight into the relationships between the kinds of embryos in seeds came from his analysis of morphological traits across many plant families and of many taxa in some of the families. To his great credit, Martin recognized that the families with peripheral embryos had a unique combination of characters, and thus he raised concerns about this kind of embryo. In light of all the seed- and phylogeny-related research conducted since 1946, the uniqueness of the families with peripheral embryos still holds today, i.e. a 'phylogenetic orphan'. What we can add to Martin's puzzle about the peripheral embryo is that it is related to the linear-full embryo. However, without Martin's original concern about the peripheral embryo we might never have given this unique kind of embryo/seed the attention it deserves.

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