# 1 Double Fertilization – A Defining Feature of Flowering Plants

*The expression fertilization may be used in an abstract or a concrete sense. In the abstract it denotes the process by which characters from two individuals are transmitted to a single organism in the succeeding generation. This phenomenon is almost universal throughout the animal and vegetable kingdoms, and its effects have been observed by many successive generations of breeders both of animals and of plants. In this way a considerable body of evidence has accumulated, and it has been found that certain laws are universally true of organisms which thus spring from a double stock. Such an organism passes through its complete life history, which may include more than one cycle of development. It exhibits a combination of characters drawn from both parents. The offspring of the same pair differ from each other: some resemble one parent, some the other, and those of mixed appearance may lean to either side. But a balance is maintained in each generation between the two stocks, so that neither parent has on the whole greater weight than the other.*

***E. Sargant 1900***



This book is about post-fertilization reproductive development in the most evolutionarily successful and wonderfully diverse group of plants on the face of the earth: angiosperms or flowering plants. Angiosperms, along with four different groups of living representatives of gymnosperms, namely, cycads, Ginkgoales (which includes the monotypic *Ginkgo biloba*), conifers, and Gnetales, are also known as seed plants. Seeds of angiosperms are enclosed within a fruit instead of being produced as exposed units on the surface of sporophylls or similar structures as they are in gymnosperms. Although study of the reproductive biology of angiosperms has a long history, sustained cellular and molecular investigations of this topic constitute a modern development.

Fertilization, besides its obvious role in genetic recombination, essentially denotes the fusion of the egg and sperm to form a zygote and, as will soon become clear, the word does not capture the full scope of events that occur in flowering plants. The traditional setting for fertilization in flowering plants is the sanctum sanctorum of the female gametophyte – more popularly known as the embryo sac – which itself is wrapped in several layers of cells of the nucellus and integuments constituting the ovule. A typical embryo sac initially has two groups of four haploid nuclei embedded within it, one at the micropylar end and the other at the opposite, chalazal end. The demarcation of groups of three nuclei at each end, each nucleus surrounded by its own cytoplasmic domain as a distinct, compartmentalized, membrane-bound cell, is the primary determinant of form of the mature embryo sac. The three cells at the micropylar pole are organized as the egg apparatus, consisting of a large egg cell flanked on either side by a cellular synergid. The three cells at the opposite pole become the antipodals. The main body of the embryo sac remaining after the egg apparatus and antipodals are cut off is the central cell consisting of the two orphaned nuclei from either pole, which may remain separate, side-by-side, as unfused haploid nuclei, or fuse to form a diploid polar fusion nucleus. The mature embryo sac is thus a seven-celled, eight-nucleate supercell in which fertilization occurs (Fig. 1.1). This type of embryo sac development, which is prevalent in about 70% of angiosperms, is known as the 'normal' type, and, because it was first described in *Polygonum divaricatum* (Polygonaceae), it is conventionally designated as the 'Polygonum' type (Maheshwari 1950). In the context of fertilization, the term female germ unit has been proposed for the egg apparatus and the central cell (Dumas et al. 1984), but it is not widely used.

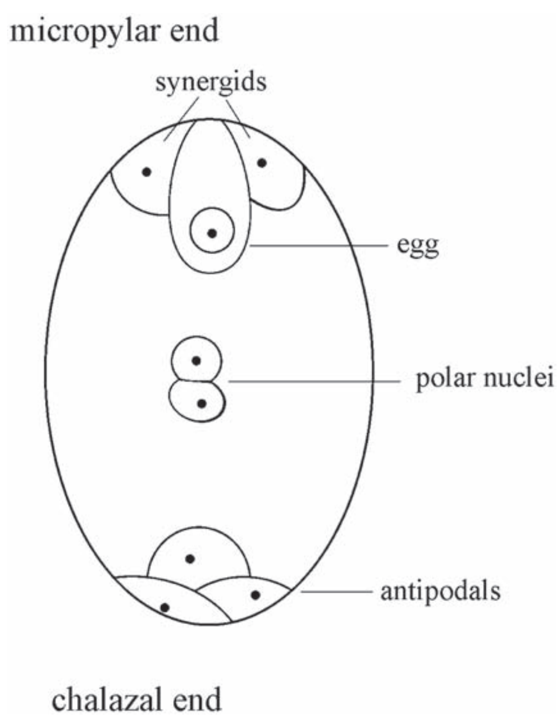
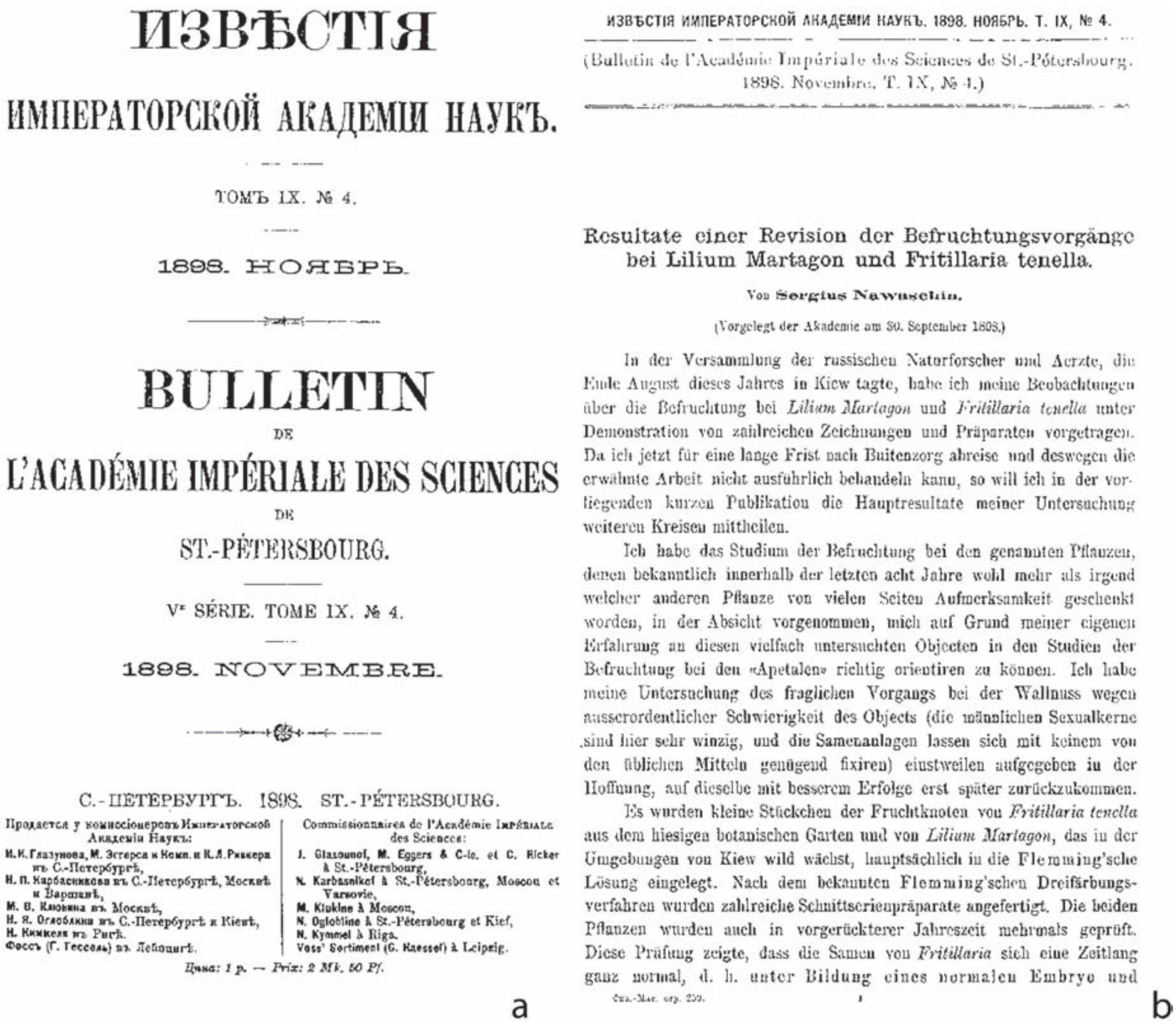



**Fig. 1.1** Diagram of a 'Polygonum' type of embryo sac showing the disposition of cells

The process of fertilization in flowering plants, including the encounter of the male and female gametes and the actual fusion of gametic nuclei, presents a degree of complexity not found in other groups of plants. Pollination, resulting in the transfer of pollen grains from the anther to the stigmatic surface of the appropriate flower type, is the beginning of a cascade of events that delivers the male gamete to the vicinity of the egg. Following germination of pollen grains on the stigma, the resulting pollen tubes carrying the two male gametes (produced by a mitotic division of the generative cell of the pollen grain) navigate through the carbohydrate-rich matrix of the stigma, style, and the ovular tissues, and reach the vicinity of the embryo sac. Fusion of the male and female gametes takes place when the pollen tube enters the embryo sac and releases the sperm. Hitherto partially or totally uncharacterized extracellular matrix components of the stigma and style spring into action to sustain pollen tube growth, and the ever-present signaling molecules generated by the diploid cells of the ovule or the haploid cells of the embryo sac for pollen tube attraction contribute to successful fertilization (Johnson and Preuss 2002). Following fertilization, the ovule develops into the seed enclosed in the ovary, which becomes the fruit. Although these facts – the bare bones of the reproductive biology of flowering plants – have long been known, perspectives on the molecular genetics of the individual phases involved have come from recent cell biological studies and analyses of female gametophytic mutants of *Arabidopsis thaliana* (Brassicaceae; hereafter referred to by genus name only). The purpose of this chapter is to present an overview of the peripheral and central events of fertilization in flowering plants with a focus on both old and new literature.

## 1.1 Discovery of Double Fertilization

Unambiguous proof of the actual fusion of the male and female gametes embodied in fertilization in flowering plants can be traced to a monographic publication of Strasburger (1884). This work was devoted mostly to the nuclear cytology of pollen grains and pollen tubes of plants belonging to a wide range of families, and to the fate of male gametes delivered by pollen tubes in the embryo sacs



ИЗВѢСТІЯ

ИМПЕРАТОРСКОЙ АКАДЕМІИ НАУКЪ.

ТОМЪ IX. № 4.

1898. НОЯБРЬ.

BULLETIN

DE

L'ACADÉMIE IMPÉRIALE DES SCIENCES

DE

ST.-PÉTERSBOURG.

V$^{e}$ SÉRIE. TOME IX. № 4.

1898. NOVEMBRE.

С.-ПЕТЕРБУРГЪ. 1898. ST.-PÉTERSBOURG.

Продается у комиссіонеровъ Императорской Академіи Наукъ:
И. И. Глазунова, М. Эггерса и Комп. и К. Л. Риккера въ С.-Петербургѣ,
Н. П. Карбасникова въ С.-Петербургѣ, Москвѣ и Варшавѣ,
М. В. Клюкина въ Москвѣ,
Н. Я. Оглоблина въ С.-Петербургѣ и Кіевѣ,
Н. Киммеля въ Ригѣ,
Фоссъ (Г. Гессель) въ Лейпцигѣ.

Commissionnaires de l'Académie Impériale des Sciences:
J. Glasounof, M. Eggers & C-ie. et C. Ricker à St.-Pétersbourg,
N. Karbasnikof à St.-Pétersbourg, Moscou et Varsovie,
M. Klukine à Moscou,
N. Oglobline à St.-Pétersbourg et Kief,
N. Kymmel à Riga,
Voss' Sortiment (G. Haessel) à Leipzig.

*Цѣна: 1 р. — Prix: 2 Mk. 50 Pf.*

a

ИЗВѢСТІЯ ИМПЕРАТОРСКОЙ АКАДЕМИИ НАУКЪ. 1898. НОЯБРЬ. Т. IX, № 4.

(Bulletin de l'Académie Impériale des Sciences de St.-Pétersbourg. 1898. Novembre. T. IX, № 4.)

Resultate einer Revision der Befruchtungsvorgänge bei Lilium Martagon und Fritillaria tenella.

Von Sergius Nawaschin.

(Vorgelegt der Akademie am 30. September 1898.)

In der Versammlung der russischen Naturforscher und Aerzte, die Ende August dieses Jahres in Kiew tagte, habe ich meine Beobachtungen über die Befruchtung bei *Lilium Martagon* und *Fritillaria tenella* unter Demonstration von zahlreichen Zeichnungen und Präparaten vorgetragen. Da ich jetzt für eine lange Frist nach Buitenzorg abreise und deswegen die erwähnte Arbeit nicht ausführlich behandeln kann, so will ich in der vorliegenden kurzen Publikation die Hauptresultate meiner Untersuchung weiteren Kreisen mittheilen.

Ich habe das Studium der Befruchtung bei den genannten Pflanzen, denen bekanntlich innerhalb der letzten acht Jahre wohl mehr als irgend welcher anderen Pflanze von vielen Seiten Aufmerksamkeit geschenkt worden, in der Absicht vorgenommen, mich auf Grund meiner eigenen Erfahrung an diesen vielfach untersuchten Objecten in den Studien der Befruchtung bei den «Apetalen» richtig orientiren zu können. Ich habe meine Untersuchung des fraglichen Vorgangs bei der Wallnuss wegen ausserordentlicher Schwierigkeit des Objects (die männlichen Sexualkerne sind hier sehr winzig, und die Samenanlagen lassen sich mit keinem von den üblichen Mitteln genügend fixiren) einstweilen aufgegeben in der Hoffnung, auf dieselbe mit besserem Erfolge erst später zurückzukommen.

Es wurden kleine Stückchen der Fruchtknoten von *Fritillaria tenella* aus dem hiesigen botanischen Garten und von *Lilium Martagon*, das in der Umgebungen von Kiew wild wächst, hauptsächlich in die Flemming'sche Lösung eingelegt. Nach dem bekannten Flemming'schen Dreifärbungsverfahren wurden zahlreiche Schnittserienpräparate angefertigt. Die beiden Pflanzen wurden auch in vorgerückterer Jahreszeit mehrmals geprüft. Diese Prüfung zeigte, dass die Samen von *Fritillaria* sich eine Zeitlang ganz normal, d. h. unter Bildung eines normalen Embryo und

Изв.-Мат. стр. 259. 1

b

**Fig. 1.2a,b** Discovery of double fertilization. **a** Cover page of the journal in which Nawaschin's discovery of double fertilization was first published. **b** First page of the article describing double fertilization

of *Gloxinia hybrida* (Gesneriaceae), *Himantoglossum hircinum*, *Orchis latifolia* (Orchidaceae), and *Monotropa hypopitys* (Pyrolaceae). The most complete, illustrated details were provided on *M. hypopitys*, in which it was shown that one of the two male gametes conveyed by the pollen tube fused with the nucleus of the egg. At that time the male gametes were known as generative nuclei and it was uncertain whether these gametes were true cells or naked nuclei. However, the observation that a male gamete fused with the egg in the act of fertilization was contrary to a previous puzzling finding that this event was orchestrated by the diffusion of the cytoplasmic contents of the pollen tube (see Maheshwari 1950). Although Strasburger's work identified the embryo as the resulting product of fertilization, understanding of the fate of the second male gamete discharged by the pollen tube, and the source of origin of the endosperm (albumen), remained major hurdles in gaining a complete insight into the dynamics of fertilization in angiosperms.

### 1.1.1 Who Discovered Double Fertilization?

The breakthrough in the discovery of double fertilization occurred when S. Nawaschin in Russia showed that, in ovules of *Lilium martagon* and *Fritillaria tenella* (Liliaceae), both male gametes from the pollen tube penetrated the embryo sac; whereas

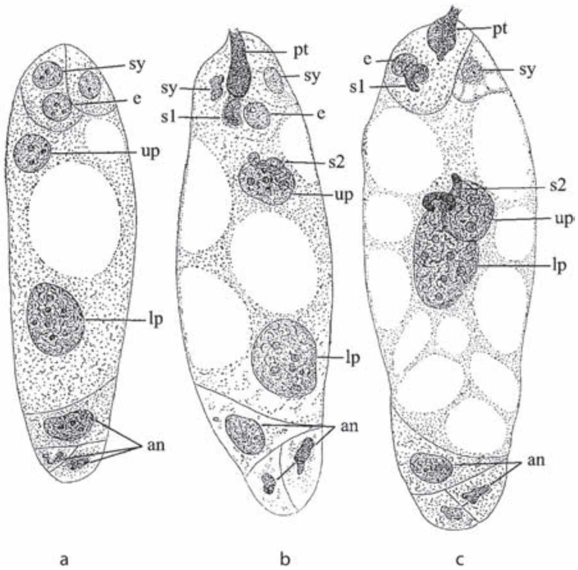



**Fig. 1.3a–c** Double fertilization in *Lilium martagon*. **a** Mature embryo sac showing the egg apparatus, consisting of the egg and synergids, antipodals, upper polar nucleus, and lower polar nucleus. **b** Mature embryo sac after discharge of male gametes from the pollen tube. The nucleus of one sperm has entered the egg and that of the second sperm is in contact with the upper polar nucleus. The nucleus of one of the synergids is disintegrating. **c** Union of one sperm with the egg nucleus and of the second sperm with the two polar nuclei. *an* Antipodals, *e* egg cell, *lp* lower polar nucleus, *pt* pollen tube, *s1* sperm that fuses with the egg, *s2* sperm that fuses with the polar nucleus, *sy* synergid, *up* upper polar nucleus. (Reprinted from Guignard 1899a)

one of them fused with the nucleus of the egg cell, the other fused with the polar fusion nucleus (at that time known as the definitive nucleus) floating in the central cell, initiating a second fertilization event (Nawaschin 1898, 1899). The results of this work were presented orally on 24 August 1898 to the botanical section of the "Naturforscherversammlung" held in Kiew, Russia (20–30 August 1898) and published as an abstract in the following year (Nawaschin 1899); the full paper appeared a few months after the meeting (Nawaschin 1898). Thus, reverent credit is due to Nawaschin for this legendary discovery of the two fusion events during fertilization in flowering plants (Fig. 1.2a,b). The phenomenon observed by Nawaschin was also independently confirmed in *L. martagon* and *Lilium pyrenaicum* by L. Guignard (1899a, 1899b) in France. The account of this investigation was communicated to the Academy of Sciences in Paris on 4 April 1899 and was published soon afterwards in its Report ("Comptes Rendus") (Guignard 1899a). Exactly the same paper, with a footnoted reference to the earlier paper with volume number and a middle page number, was also published in another journal in the same year (Guignard 1899b). The work described in these two papers, which included a reference to Nawaschin's 1899 abstract, was accompanied by a series of illustrations in the form of line drawings showing the two fusion events (Fig. 1.3a–c). Guignard's description and figures portrayed a precise two-step sequence of events involving the fusion of the second sperm with the upper polar nucleus, followed by integration of this fusion product into the lower polar nucleus. Within a few months of the publication of Guignard's papers, full confirmation of the startling discovery of fusion of the second sperm with the polar fusion nucleus came from a reexamination of previously prepared slides of fertilized ovules of *L. martagon* by E. Sargant in England (Sargant 1899). The coincident choice of ovules of species of *Lilium* and *Fritillaria* by investigators working in three European countries as the classic experimental system in these pioneering studies is not surprising because of the relatively large size of the embryo sac and its equally conspicuous nuclei as seen in microscopic preparations of ovules of these two genera. Indeed, because of this and other advantages, slides demonstrating embryo sac development in various species of *Lilium* and *Fritillaria* have been popular in the teaching of general plant biology; species of these genera have also been favored systems of subsequent investigators because embryo sac development in them appeared to be a simplified version of a complex series of nuclear fusions and divisions that did not have parallels in other plants studied (Maheshwari 1950). To designate the two fertilization events that occur at the inception of the sporophytic phase in flowering plants, Guignard (1899a, 1899b), in a seemingly visionary act, used the term 'double copulation' in the title of the first two papers and 'double fécondation' in later publications. Strasburger (1900) referred to the two fertilization events as 'doppelten Befruchtung' in the title of a paper, and nearly the same term ['die doppelte Befruchtung' and 'двойное оплодотворенiе' (in Russian)] appeared in the text of two papers by Nawaschin (1900a, 1900b). The term 'double fertilization' now in universal use was first employed in the title of a paper by Thomas (1900) and in the text of a paper by Sargant (1900). Putting to rest the prevalent assumption that the endosperm was generated by fusion of the two polar nuclei, the abovementioned investigators also concluded correctly that the product of fusion of the second sperm with the polar fusion nucleus gives rise to the endosperm, typically constituted of cells with chromosomes of biparental origin from the coalescence of three nuclei. The discovery of double fertilization in the liliaceous species, and the confirmation of its occurrence in many other angiosperms, including both monocotyledons (monocots) and dicotyledons (eudicots), within a period of just over a year – for example, additional species within the Liliaceae such as *Fritillaria meleagris*, *Scilla bifolia*, *Lilium candidum*, *Tulipa celsiana*, *Tulipa gesneriana*, and *Tulipa sylvestris* (Guignard 1899c, 1900a, 1900b), *Narcissus poeticus* of the Amaryllidaceae (Guignard 1900a), and *Himantoglossum hircinum*, *Orchis latifolia*, *Orchis maculata*, and *Orchis mascula* of the Orchidaceae (Strasburger 1900) (all monocots), *Erigeron philadelphicus*, *Erigeron strigosa*, *Guizotia oleiflora*, *Helianthus annuus* (sunflower), *Heliopsis patula*, *Rudbeckia grandiflora*, *Rudbeckia laciniata*, *Rudbeckia speciosa*, *Silphium integrifolium*, *Silphium laciniatum*, *Silphium terebinthinaceum*, and *Spilanthes oleracea* of the Asteraceae (Guignard 1900a; Land 1900; Nawaschin 1900a, 1900b), *Hibiscus trionum* of the Malvaceae (Guignard 1900a), *Anemone nemorosa*, *Caltha palustris*, *Clematis viticella*, *Delphinium elatum*, *Helleborus foetidus*, *Nigella sativa*, and *Ranunculus flammula* of the Ranunculaceae (Guignard 1900a; Nawaschin 1900a, 1900b; Thomas 1900), *Reseda lutea* of the Resedaceae (Guignard 1900a), *Juglans* sp. of the Juglandaceae (Nawaschin 1900a, 1900b), and *Monotropa hypopitys* of the Pyrolaceae (Strasburger 1900) (all eudicots) – may be said to have ushered in twentieth century plant embryology, paving the way for what will surely go down as the golden age in the study of reproductive biology of flowering plants. Appropriately, the centennial of this discovery has been marked by the publication of several reviews on this topic (Jensen 1998; Erdelská and Dubová 2000; Faure 2001; Koul 2001; Friedman 2001b; Raghavan 2003b). Besides paying tribute to Nawaschin and Guignard, these articles show how their discovery has driven the field of plant embryology for more than a century, including most current research in this field.

### 1.1.2 Universality of Double Fertilization in Flowering Plants

The momentum created in the waning years of the nineteenth century to establish double fertilization as a ubiquitous feature in the reproductive biology of flowering plants was followed by a sustained effort in the first 2 years of the twentieth century leading to the discovery of this phenomenon in additional members of the Ranunculaceae (Guignard 1901c), Liliaceae (Ikeda 1902), Juglandaceae (Karsten 1902), and Pyrolaceae (Shibata 1902), as well as in plants belonging to Poaceae (Guignard 1901a), Najadaceae (Guignard 1901b), Solanaceae, Gentianaceae (Guignard 1901d), Asclepiadaceae (Frye 1902), Brassicaceae (Guignard 1902), and Ceratophyllaceae (Strasburger 1902). Guérin (1904), in a monograph devoted entirely to the topic of fertilization in seed-bearing plants, and Coulter and Chamberlain (1912) in their classic book on the *Morphology of Angiosperms*, refer to 16 families of angiosperms, encompassing about 40 genera and over 60 species definitely known to have a second fertilization event; these two publications surveyed the literature up to the end of 1902. From that time onwards, along with the presence of a reduced female gametophyte and embryo-nourishing endosperm, the occurrence of double fertilization was accepted as a general feature of the reproductive biology of angiosperms. Indeed, under this assumption, there were only occasional references to double fertilization in the numerous publications dating from the early 1900s to the present dealing with the variability and diversity of reproductive processes in flowering plants with special reference to their embryogenesis and endosperm development (Johansen 1950; Maheshwari 1950; Davis 1966; Johri et al. 1992). However, this period was notable for providing the first glimpses of electron microscopic details of double fertilization in several plants, including cotton (*Gossypium hirsutum*; Malvaceae; Jensen and Fisher 1967), maize ( *Zea mays*; Poaceae; Diboll 1968; van Lammeren 1986), barley (*Hordeum vulgare*; Poaceae; Cass and Jensen 1970; Mogensen 1982, 1988), *Linum catharticum* (Linaceae; d'Alascio Deschamps 1974), spinach (*Spinacia oleracea*; Chenopodiaceae; Wilms 1981), *Plumbago zeylanica* (Plumbaginaceae; Russell 1982, 1983),

http://www.springer.com/978-3-540-27791-0

Double Fertilization
Embryo and Endosperm Development in Flowering Plants
Raghavan, V.
2006, XX, 237 p. 75 illus., 27 illus. in color., Hardcover
ISBN: 978-3-540-27791-0