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History of orchid propagation: a mirror of the history of biotechnology

Tim Wing Yam · Joseph Arditti

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Part I: Seed germination

Abstract Orchid seeds are nearly microscopic in size. Because of that, many fanciful theories were proposed for the origin of orchids. Almost 400 years separate the time when orchid seeds were seen for the first time and the development of a practical asymbiotic method for their germination. The seeds were first observed and drawn during the sixteenth century. Seedlings were first described and illustrated in 1804. The association between orchid and fungi was observed as early as 1824, while the requirement for mycorrhiza for seed germination was established in 1899. An asymbiotic method for orchid seed germination was developed in 1921. After Knudson's media B and C were formulated, orchids growing and hybridization became widespread. Hybrids which early growers may not have even imagined became possible.

Keywords Clonal propagation · In vitro propagation · Mycorrhiza · Orchid seeds · Propagation · Seed germination

Introduction

A convincing argument can be made that research into orchid propagation (and the procedures themselves) were always in the forefront of biotechnology (or at least propagation methods) of their time. The first method for horticultural orchid seed germination (Moore 1849; for reviews, see Arditti 1984; Yam et al. 2002) was a major and radical departure from the manner in which other seeds were germinated 160 years ago. David Moore's (1807–1879) approach was an innovative major horticultural and biological advance (Moore 1849). Half a century after Moore's discovery, Noël Bernard (1874–1911) made another quantum jump when he formulated a method for symbiotic germination of orchid seeds in vitro (Bernard 1899, 1909a; Bernard 1990; for reviews, see Boullard 1985; Arditti 1990; Rasmussen 1995; Yam et al. 2002). His is probably the first method for in vitro propagation of any plant. It utilizes what were at the time modern and advanced microbiological procedures. Bernard also predicted that a day would come when orchid growers would have laboratories as part of their establishments. This is the case at present not only for orchids, but also for other plants.

Lewis Knudson's (1884–1958) method for the asymbiotic germination of orchid seeds (Knudson 1921, 1922a; for reviews, see Arditti 1984, 1990; Yam et al. 2002) was the first practical procedure for in vitro propagation of any plant in pure (i.e., axenic) culture. His method was a significant conceptual and technological innovation which foreshadowed modern biotechnology.

David Moore may have based his work (Moore 1849) on reports that orchid seeds can germinate if scattered at the base of a mature plant. However, Bernard's discovery and method were not based on any previous procedures and/or

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research by others. They were solely a result of his brilliance (Bernard 1899, 1909a; Boullard 1985; Arditti 1990; Bernard 1990; Yam et al. 2002). Knudson developed the asymbiotic method as a result of a sharp mind, incisive reasoning, and on the basis of his own pioneering research with other plants (Knudson 1921, 1922a, b; for a review, see Arditti 1990). The micropropagation of orchids by means of tissue culture has a more complex history which is not free of controversy and includes unusual episodes (Arditti 1977b, 1985, 2001; Arditti and Arditti 1985; Torrey 1985a, b; Arditti and Krikorian 1996; Easton 2001).

Seed germination accidentally or in nature

Orchid seeds (Fig. 1) are dust-like and nearly impossible to observe individually with unaided eyes. It is very probable that they remained unnoticed for much of history. If the ancient Greeks noticed these seeds, their scientists and philosophers did not write about them, not even Theophrastus (370–285 B.C.), who is often called the Father of Botany and who was also the first Western naturalist to describe an orchid, or Dioscorides (ca. 20–70 A.D.) who wrote about orchids later (Lashley and Arditti 1982; Arditti 1992). The Roman naturalist Caius Plinius Secundus (Pliny The Elder; A.D. 23 or 24–79) wrote about orchids in his *Treatise on Natural History* without even alluding to orchid seeds (Lawler 1984; Arditti 1992; Jacquet 1994). Orchids and their seeds were not mentioned in the Ebers papyri (ca. 1500 B.C.).

Despite the fact that the use of orchids to stimulate lactation originated in ancient Mesopotamia (Lawler 1984; Jacquet 1994), there is no mention of these plants and their seeds in Assyrian writing of the Ashurbanipal period (668–627 B.C.). Many plants are mentioned in the bible, but orchids and their seeds are not (Dunn and Arditti 2009). There are no published reports on whether seeds are mentioned in the rich Islamic-Arabic literature on natural history, botany, and even orchids (Jacquet 1994). The Turkish *Tercüme-i Cedide Fil-Havasil Müfredi* by Mehmet Ali which dates back to 1691–1692 describes salep, an orchid product (Sezik 1967, 1984; Arditti 1992), but seeds are not mentioned. And none of these sources mention orchid seedlings. If seeds are mentioned in the ancient Chinese, Indian, Japanese, and Korean literature, no one seems to have discovered the writings.

Plinius and Plinius: first descriptions of orchid seeds

There are three known early descriptions of orchid seeds in the west. All were published many years after they were written with the first description being the second to be

published. The first to be published, *Herbarium Amboinense*, is a six-volume work written between 1654 and 1702 by Georgius Everhadus Rumphius (1627–1702; Fig. 2), ‘Plinius Indicus’, in Ambon, Indonesia (de Wit 1977; Beekman 2003) and published by Professor Joannes Burman (1706– or 1707–1779) in Holland half a century (1741–1750) after Rumphius’s death (Wehner et al. 2002).

Conrad Gesner (1516–1565; Fig. 3), ‘Plinius Germanicus’, a Swiss scientist, was actually the first to describe and draw orchid seeds (Arditti 1992; Jacquet 1994; Wehner et al. 2002). However, his book, *Opera Botanica* (Gesner 1751), was published between 1751 and 1771 by Christopher Jacob Trew (1695–1769). This does not really matter because no one seems to have paid much if any attention to orchid seeds for a long time even after *Herbarium Amboinense* and *Opera Botanica* were published.

The artists of a Spanish scientific expedition to New Grenada (now Colombia) also drew orchid seeds, which they did between 1783 and 1816. These, the second drawings after Gesner’s, are the first to indicate size. It is not clear at present if the artists who drew them had access to or were familiar with *Herbarium Amboinense* and *Opera Botanica*. Publication of these illustrations (Perez-Arbelaez et al. 1954; Schweinfurth et al. 1963, 1969; Fernandez Perez 1985) was delayed 150 years (Arditti and Ghani 2000).

Bock or Tragus: fancy, not facts

Since orchid seeds were neither seen nor known, it is not surprising that several fanciful ideas were proposed to explain the origin of orchids. A number of authors associated several birds and four-legged animals with orchids. One of the more interesting associations is between goats and *Himantoglossum hircinum* (L.) Spreng. [*Satyrion hircinum* L., *Loroglossum hircinum* (L.) Rich.]. This orchid produces caproic acid, a substance which smells like goats. This explains the supposition that it is derived from goat semen which dropped to the ground during copulation by goats and “fermented” into orchids (Arditti 1972).

Jeremy (Jerome) Bock, who is also known as Hieronymus Tragus (1498–1554), suggested that orchids came about from semen of birds and beasts which fell to the ground when they copulated. He wrote: “As soon as the flowers abscise little pods arise in which no more is found than pure dust or flour. These plants arise miraculously from the seeds or sperm of junipers, blackbirds and thrushes, in Latin they are named turi and nerubae; these Satyrions are found nowhere else except in the meadows where these birds search for food”. Had Bock (Tragus)

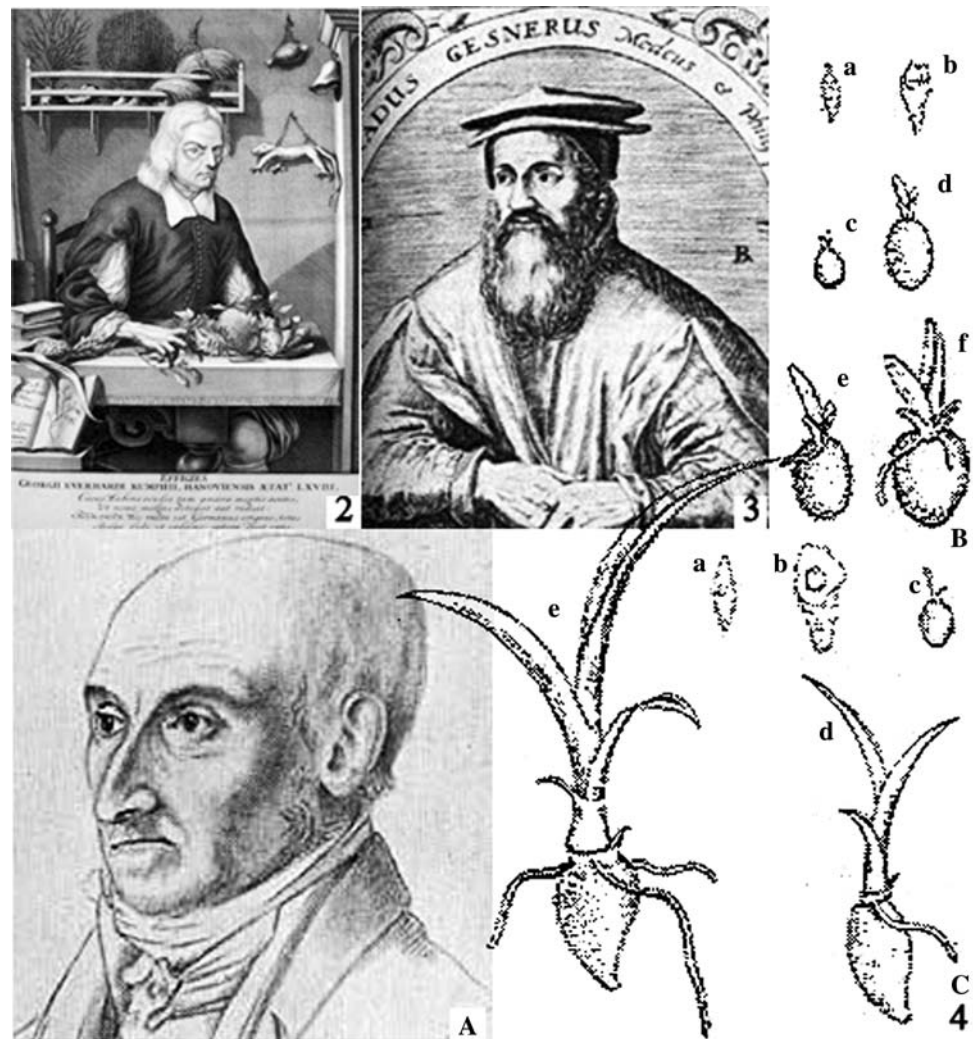


Fig. 1 Orchid seeds (Beer 1863)

recognized the true nature of the “dust or flour” and appreciated its nature he would have preceded Rumphius in describing orchid seeds (for reviews, see Arditti 1992; Yam et al. 2002).

Athanasius Kircher (1601–1680), a German Jesuit, expounded on Bock’s ideas in his *Mundus Subterraneus* (published 1664–1665 in Amsterdam) and wrote that “these plants arise from the latent survival force in the cadavers of

Figs. 2–4 First observers of orchid seeds and seedlings.
2 Georgius Everhardus Rumphius, ca. 1627–1702.
3 Conrad Gesner, 1516–1565.
4 Richard Anthony Salisbury, 1761–1829 (A); seedlings of *Orchis morio* (B), and *Limodorum verecundum* (C)



certain animals [and] animal semen [that] falls to the ground in mountains and meadows.” As proof, Kircher drew images of flowers (these and other illustration which are relevant to this section can be seen in Arditti 1992; Yam et al. 2002) which resemble animals (birds, goats, humanoid, sheep) whose cadavers and semen gave rise to orchids.

Naumburg and Wächter: eighteenth century observations and reports

Samuel Johann Naumburg (1768–1799), Professor at Erfurt (Thuringia, Germany), wrote a paper which includes drawings of orchid ovaries. He stated in the paper that: “Das Saamenbehältniß ist eine Kapßel [in free translation: “the seed container is a capsule”]... Die Kapßeln enthalten eine grosse Menge ganz kleiner brauner Saamen [“the capsules contain many very small brown seeds”]” and in a footnote: “Semina plurima, minima, brunnea [“seeds many, small, brown”]” (Naumburg 1794).

A forester named Johann Karl Augustin Wächter (1773–1846) became intrigued by Naumburg’s paper and hand-pollinated orchids after reading it (Wächter 1799–1801). He drew what appears to be a swollen ovary of “*Ophrys Nidus*” (probably *Neottia nidus-avis*) and wrote that the ovary of *Orchis militaris* became swollen after hand pollination and produced “eine grosse Menge Samen” (a great many seeds).

If additional reports were published after that they are either buried deep in seldom seen and little known book(s) and/or journal(s) or lost because subsequent authors did not cite additional reports. Page by page searches through some of the old literature in several libraries have also not uncovered any relevant publications.

Observations and reports in the nineteenth century

As the nineteenth century started, the general belief among botanists was that orchids rarely produced seeds which, even if present, never germinated. However, early in this

century, an eminent British botanist described germinating orchid seeds and developing seedlings for the very first time. They happened to be those of European species (Salisbury 1804; for reviews, see Arditti 1967, 1979, 1984, 1990, 1992). Many other important observations and discoveries were made later in the nineteenth century.

Salisbury: seeds and germination of temperate climate orchids

Richard Anthony Salisbury (1761–1829; Fig. 4A) was eccentric, hard to get along with, mired in scandal during part of his life and apparently disdainful of at least some Victorian moral constraints. He was also an excellent botanist (for more details and illustrations, see Yam et al. 2002) and a contemporary of (1) Robert Brown (1773–1858), the noted British botanist who studied orchid pollination and fertilization and discovered cell nuclei while doing it, (2) John Lindley (1799–1865), who is often referred to as the father of orchidology, and (3) Charles Darwin (1809–1882) who is probably the most influential thinker of all time. At that time, orchid seedlings were not known or believed to occur in nature. This changed on 5 January 1802 when Salisbury read a paper to the Linnean Society describing germinating orchid seeds and seedlings. The talk was published 2 years later (Salisbury 1804). It was enhanced by illustrations of seeds and seedlings of *Orchis morio* Linn. and *Limodorum verecundum* Prodr. (Fig. 4Ba–f, Ca–e). Despite being the first modern description of seeds and seedlings it did not seem to have drawn much if any attention and/or to have stimulated additional studies and/or reports at the time regarding British or other European orchid seeds and/or seedlings.

Link: a tropical orchid seedling and a missed opportunity

Salisbury's drawings depicted the external appearance and morphology of orchid seedlings and seeds. They did not show structural features of either or the mycorrhizal association of the seedlings. However, the German botanist Heinrich Friedrich Link (1767–1851; Fig. 5A) illustrated these characteristics very well (Link 1824, 1839–1842, 1840, 1849a, b). Link's drawings (Fig. 5Ba–k) may not have been the first but they are excellent even by current standards. It is also interesting to note that Link drew seeds and seedlings of a tropical orchid, *Oeceoclades maculata*, before anyone else. It is obvious that Link saw mycorrhizae in cells of seedlings, but he did not appreciate their importance.

Cameron: seedlings in a garden

Sometime between 1835 and 1838, David Cameron (ca. 1787–1848), Curator of the Birminham Botanical Garden

at the time (and before that, gardener for Robert Barclay of Barclay's Bank fame), saw “self-sown seedlings in several of the pots” which contained “British Orchidaceae [which were] cultivated [with] alpine plants.” Some of the seedlings were “very small, and evidently seedlings of that year, others were much stronger. Of plants so obtained several... *Gymnadenia conopsea*, *Orchis maculata*, and *O. latifolia*...” flowered (Cameron 1844, 1848).

Herbert: foreshadowing Darwin

Among British clergymen, the Rev. Stephen Hales (1677–1761) studied water uptake by plants; the Rev. John Henslow (1796–1861) was Professor of Botany at Cambridge, wrote about flower structure of orchids and other plants (Henslow 1888) and befriended young Charles Darwin (1809–1882); and the less well known but more eccentric Rev. James Neil used plants as the subjects of his religious parables (Neil 1880). The Honorable and Very Reverend William Herbert (1778–1847; Fig. 6), Dean of Manchester, followed in this tradition (Herbert 1846, 1847). He seems to have been an enlightened clergyman having “asserted that it was preposterous to suppose all the existing form of vegetables... to have been so specially created by the Almighty, and... I suspected that the various forms... to have also branched out from smaller number of original types...” (Herbert 1847). These views, published in 1847, 12 years before publication of Darwin's *Origin of Species* in 1859, lead to attacks on him “as a person who was minishing from the power and wisdom of God” (Herbert 1847). Dean Herbert countered these attacks by suggesting that “immense operations of ages before the creation of man... were not compressed within a diurnal week of our terrestrial life, but filled a gigantic page in the great volume of antecedent time” (Herbert 1847).

Herbert's arguments regarding plants was even more explicit: “I am... unwilling to assent to the assertion, that every plant... or even a distinct species, or... genus, had a special creation” (Herbert 1847). After laying this theological foundation, Herbert stated: “If I can show that in one genus of plants cross breeding is not only easy, but more easily obtained than fertility by the plant's own pollen, and that in others, so closely allied to it as to make it a question whether they are not sections of one genus, cross-breeding cannot be affected generally, and in no case easily; that in some genera of plants many or all the cross-bred varieties are fertile, and in other nearly allied thereto all, or almost are sterile... [he proceeds with examples from animals]... the assertion that the races... must have had separate origin because their crossed product is sterile... must fall to the ground” (Herbert 1847).

Herbert tested his hypothesis with the intent to prove his point by experimenting with the hybridization of many

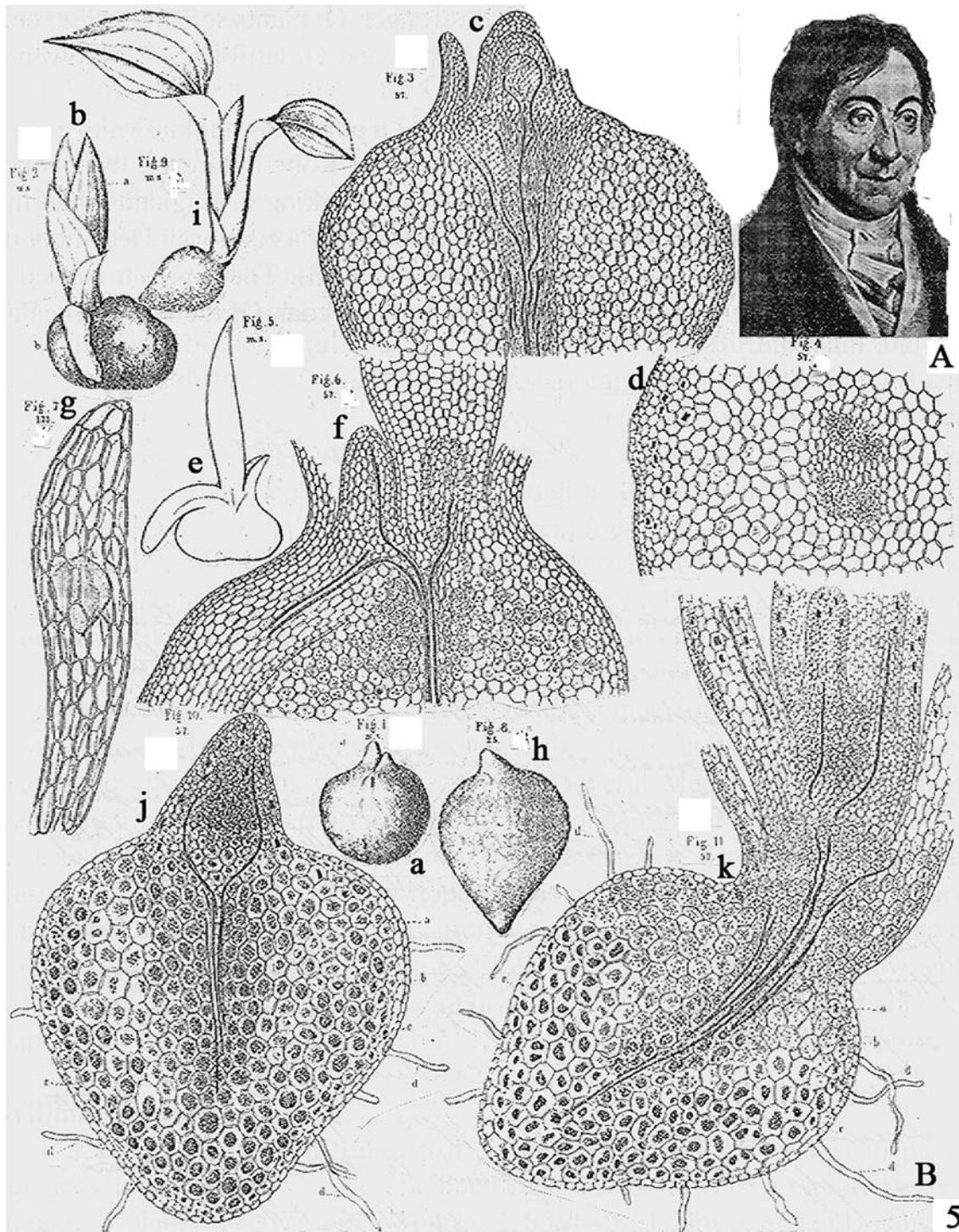
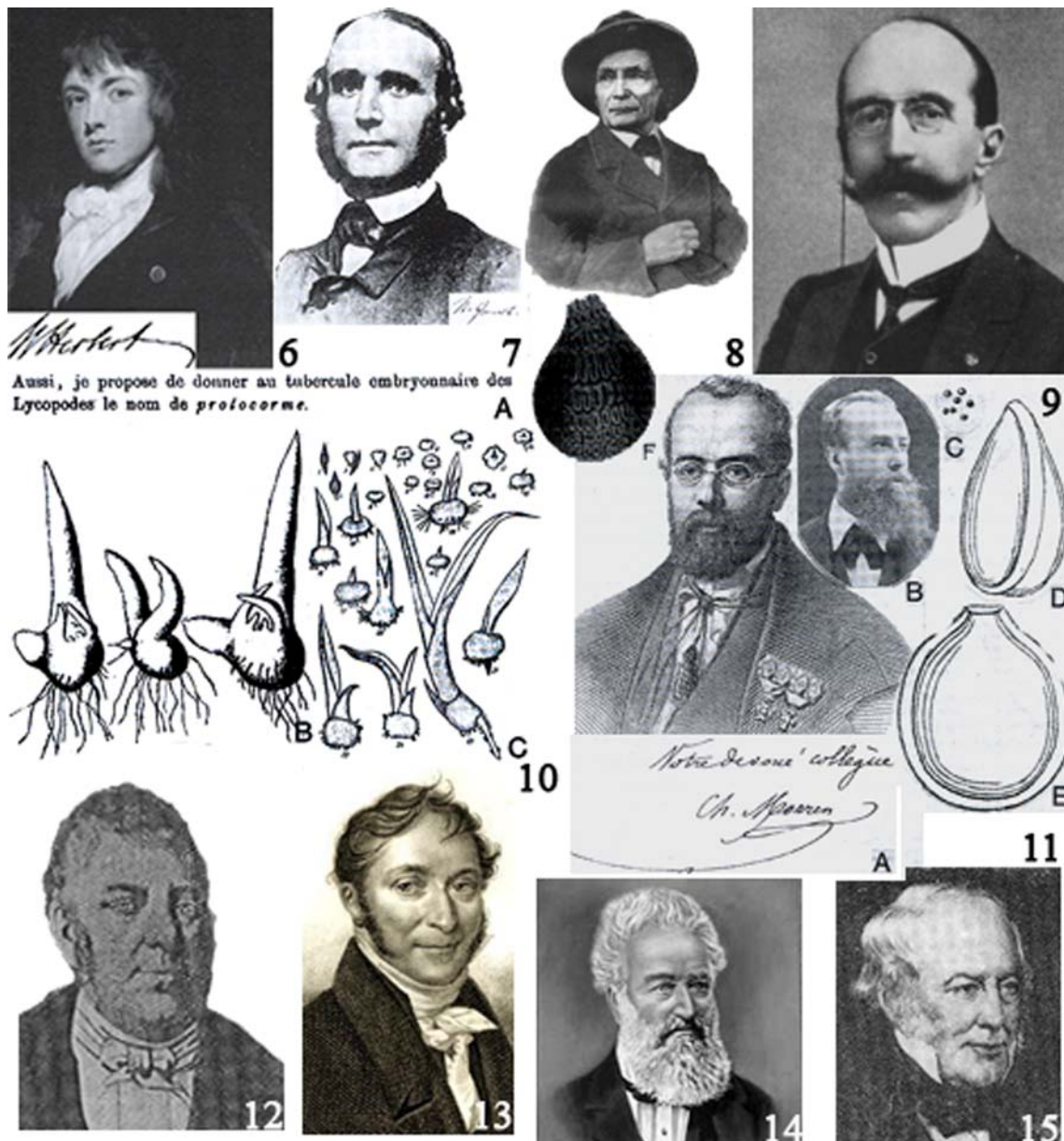


Fig. 5 Early drawings of orchid mycorrhiza. **A** Heinrich Friedrich Link, 1767–1851. **B** Germinating seed and seedlings of *Oeceoclades maculata* (the sequence is *g*, *a* and *c*, *h* and *j*, *b*, *e* and *f*, *i* and *k*; *d* is a cross-section of a root)

plants including orchids, his view being that “cross breeding amongst Orchidaceous plants would perhaps lead to very startling results; but unfortunately they are not easily raised by seed” (Herbert 1847, communicated in

October 1846). He reported that he produced orchid seeds and raised seedlings of *Bletia*, *Cattleya*, *Ophrys aranifera* and *Orchis monorchis* (now *Herminium monorchis*). Unfortunately, he did not describe his method of seed



Figs. 6–15 Students of seed germination. **6** The Honorable and Very Reverend William Herbert Dean of Manchester, 1778–1847. **7** Johann Friedrich Thilo Irmisch, 1816–1879. **8** Jean-Henri-Fabre, 1823–1915. **9** Melchior Treub, 1851–1910. **10** Lycopod (**B**) and orchid (**C**) seedlings (there is no **A** in this figure). **11** Charles Francois Antoine

Morren, 1807–1858 (**A**); Édouard Morren, 1833–1886 (**B**); slightly magnified *Vanilla* seeds (**C**); side (**D**) and front (**E**) view of a *Vanilla* seed, $\times 166$; *Vanilla* seed showing detail of testa, $\times 142$. **12** Joseph Henri Francois Neumann, 1850–1858. **13** Louis Claude Noisette, 1772–1849. **14** David Moore, 1807–1879. **15** John Harris, 1782–1855

germination. This is an important weakness because knowledge of how to germinate orchid seeds, if it existed at the time, was not widespread. Herbert also failed to describe his seeds and seedlings. It is possible that he used Cameron's method or one similar to it, but the lack of information reduces the value and importance of his report. However, it must be remembered that Dean Herbert hybridized plants and germinated seeds to prove a theological point. Orchid seed germination was not his main interest.

Irmisch: anatomical and morphological observations

In Germany, the first reports of orchid seed and seedlings were by Johann Friedrich Thilo Irmisch, 1816–1879; Fig. 7), a major figure in plant morphology during the nineteenth century (Müllerott 1980). Orchids were not his primary interest, but he published several papers on German species (Irmisch 1842, 1854a, b, 1863, 1877). They contained morphological and/or anatomical line drawings. The excellent drawings in his major work on orchids

(Irmish 1853) may well be the first detailed anatomical and morphological illustrations of seed germination and seedling development, especially as they pertain to European terrestrial species.

Fabre: Orchid germination and filaments

Jean-Henri Fabre (1823–1915; Fig. 8), is mainly associated with studies of insect behavior (Fabre 1856). He became interested in orchids due to “the asymmetry of their blossoms, the unusual structure of their pollen, and their innumerable seeds” (Legros 1971), but was primarily concerned with the structure of orchid tubers (Fabre 1855, 1856). This led him to a study entitled, “Inquiries Respecting the Tubercules of *Himantoglossum hircinum*,” which was published as a thesis in 1855 (Legros 1971).

On studying *Ophrys apifera* subsequently, Fabre observed many bulbiform bodies. He also saw the seeds of this species and described them as being microscopic, covered with a fusiform seed coat and containing a spheroid embryo measuring 0.25 mm in diameter (Fabre 1856). Fabre described the seeds as germinating after extended “incubation” in humus. He wrote that on swelling they change in shape only at the apex and are covered with long delicate filaments. The filaments could have been trichomes which are often produced by protocorms or mycorrhizal hyphae. If they were the latter, Fabre failed to appreciate their importance. He also wrote that the seedlings become spheroids (i.e., protocorms) following continued growth (Fabre 1856).

Treub: from lycopods to orchids

Melchior Treub (1851 Holland–1910 France; Fig. 9) studied at the University of Leiden when the Chair of Botany was occupied by Willem Frederik Reinier Suringar (1832–1898) who was interested in lichens. Probably because of this Treub’s dissertation dealt with the nature of lichens (Schröter 1912; Went 1911).

Lichens did not hold Treub’s interest for long and he moved on to studies of other plants including orchids. His work was excellent and he had a promising future in Holland. But as fate would have it, Herman Christian Carl Scheffer (Holland 1855–Indonesia 1880), director of the Botanical Garden in Buitenzorg (now Bogor), died and Treub became his successor (Went 1911). He served in that capacity until 1909.

Treub made a number of major contributions to orchid studies in general and the understanding of their embryology, seeds, and seedlings in particular. While still in Holland, he studied the embryology and seed development of several species (Treub 1879). The line drawings (by Treub himself) are excellent. However, his most important

contribution to orchid science was unintentional. He made it in a paper on the embryology of club mosses (Treub 1890) by proposing the term *protocorme* for an early stage in the germination of lycopods (Fig. 10). Noël Bernard (see below) must have read Treub’s paper and 10 years later applied the term to orchid seedlings (Arditti 1989, 1990, 1992).

Prescottia: first seedlings of a tropical orchid outside the tropics

The first reported germination of the seeds of a tropical orchid, *Prescottia plantaginea* Lindl. outside the tropics, was observed in a horticultural establishment in the UK. However, the date is in question and the seed source is not certain (for a review, see Arditti 1984). Two dates are listed for the production of these seedlings. One is 1822 and the other is 1832. It is possible that the seeds, and therefore seedlings, may have been produced before 1832, but the available information does not point to 1822 with certainty (for a discussion, see Arditti 1992, pp. 40–42).

Prescottia plantaginea could have been introduced into cultivation shortly after John Forbes (1798–1823), a British collector in Brazil, sent plants to the garden of the Horticultural Society of London in the autumn of 1822. The source of the seeds which produced the seedlings in the UK is not clear, and there are no known reports that they were seen by anyone. *P. plantaginea* produces seeds apomictically or through self pollination. Therefore, one possibility is that the seeds were produced after the plants arrived in the UK and ripened after 1822. Another possibility is that fruits may have been present on the plants that were sent to the UK. If plants collected in their natural habitats bore fruits, the capsules continued to develop en route, ripened on arrival, and the seeds could have matured a short time after the plants arrived in Britain in 1822.

Regardless of how the seeds got to the Horticultural Society gardens, they could germinate because a suitable mycorrhizal fungus was probably present at the site. Such a fungus could have come from a British native orchid or from the roots of mature plants of *P. plantaginea*. Many seedlings were raised at the Chiswick garden of the Horticultural Society (Anonymous 1858a, b; Hoene 1949; for a review, see Arditti 1992).

The seedlings and the method by which they were produced did not draw much attention at the time and Lindley wrote about them only in 1858 (Anonymous 1858a, b). One reason for this could have been the lack of popularity of the genus. Another may have been the accidental nature of the germination. If the germination was intentional there is a good chance that it would have been published by whoever did it. In fact, it is surprising that no one took credit for it at the time.

Charles Morren: first seed production by a tropical orchid outside the tropics

Vanilla, the only orchid grown as a plantation crop, is associated with the first known intentional production of seeds of a tropical orchid in Europe. The spice vanilla may have been imported into Europe as early as 1510. Plants may have introduced for the first time in 1739, but they died. However, plants introduced into the UK in 1753 survived. (Delteil 1884, 1902; for reviews, see Lawler 1984; Arditti 1984, 1992). A Mr. Parmentier of d'Enghien introduced *Vanilla* into Belgium (Morren 1838–1839). Some plants were cultivated at the Liege Botanical Garden where a flower which opened on 16 February was pollinated by Professor Charles Morren (1807–1858; Fig. 11A). The fruit ripened a year later (Morren 1829a, b, 1837, 1838a, b, 1838–1839, 1839a, b, 1850, 1852, 1860; Poiteau 1858; MN 1845, 1849; de Visiani 1845; Anonymous 1855a, b, no date; van Gorkom 1884; Delteil 1884, 1902; Childers et al. 1959).

Charles François Antoine Morren (1807–1858) attended the Royal Atheneum in Bruxelles and graduated on 14 August 1825 *summa cum laude*. Following his graduation, Morren went to the University of Gand where he received his “*diplome de candidat*” on 1 August 1826. Two months after that Morren was given an award for his research on the anatomy of *Orchis latifolia*. Morren received his doctorate in 1829. This was followed by a period of travel, research, publications, and many honors.

On 31 August 1834, Morren passed an examination making him ‘*candidat de médecine*.’ Altogether his biography lists 235 papers (Morren 1860) but it does not include three of his contributions on *Vanilla* (Morren 1838a, b, 1839b). Most of his papers are in French, but he also published in Latin, Dutch, German, and English. He also established and edited several journals.

Morren became interested in orchids early in his life and worked on the anatomy of *Orchis latifolia* (Morren 1829b), fruits of *Leptotes* (Morren 1839b), “*Cypripedes*” (Morren 1850), and other subjects (Morren 1852). However, his major contribution was the first manual pollination of *Vanilla* anywhere (Morren 1829a, 1837, 1838a, b, 1838–1839, 1839a, 1850; Poiteau 1858; MN 1845, 1849; de Visiani 1845; Anonymous 1855a, b, van Gorkom 1884; Delteil 1884, 1902; Childers et al. 1959). In fact, it is possible to argue that, despite his wide interests, many achievements, numerous publications and a very productive life, this may prove to be his most memorable contribution to plant science, orchids, *Vanilla* cultivation, and the economy of several countries. While doing this work, Charles Morren or his son, Édouard Morren (1833–1886; Fig. 11B) also observed and drew *Vanilla* seeds (Morren 1852; Fig. 11C–F). These seem to have been the

very first seeds of a tropical orchid to be produced by hand pollination and observed outside the tropics and probably anywhere. They were certainly the first *Vanilla* seeds to be described and drawn. This was more than half a century after seeds of a European orchid, *Habenaria bifolia*, were produced through hand pollination (Wächter 1799–1801).

It is necessary to use the word “seem” above because, according to claims made by himself and repeated by others, Joseph Henri Francois Neumann (1850–1858; Fig. 12) may have been the first to pollinate *Vanilla* in France in 1830 (Neumann 1838, 1841a, b; Delteil 1884, 1902; van Gorkom 1884). If his claims are true, Neumann could have noticed seeds that may have been produced as a result of the pollination especially because several years later he claimed to have grown orchid seedlings (Neumann 1844; for a review, see Arditti 1984).

Neumann’s *Vanilla* claim is questionable because (Busse 1899):

1. This was an important discovery and one for which Neumann clearly wanted to establish priority for himself (the late Professor Ernest E. Ball used to say that the French are very concerned with *priorité*). Therefore, it stands to reason that he would have published it immediately, not almost 10 years after making it.
2. The French wanted to establish a vanilla industry in Réunion and other colonies and needed to pollinate *Vanilla*. Therefore, it is reasonable to assume that such an important discovery would have been made known and used immediately.
3. Neumann’s appears to have had a tendency to claim *priorité* for discoveries made by others by writing articles which claimed that he had made these discoveries before their actual discoverers without publishing them (for a review, see Arditti 1984).

These considerations render very unlikely the possibility that Neumann pollinated *Vanilla* and produced seeds before Morren (for more details, see Yam et al. 2002).

Horticultural seed germination

As orchid growing became popular, growers wanted to germinate their seeds in horticultural establishments.

Early attempts

At about the time *Prescottia* seedlings were reported in the UK, French orchid growers attempted to germinate *Orchis* seeds (Anonymous 1822) using a method like the one described by Louis Claude Noisette (1772–1849; Fig. 13; Noisette 1826), but failed. Noisette’s method is to

place orchid seeds on light soil and cover them with fine moss. The method can work only if mycorrhizal fungi are present in the moss or soil, but this was not known at the time.

Neumann: another questionable priority claim

Neumann, who claimed that he pollinated *Vanilla* in 1830, before anyone else (Neumann 1838, 1841a, b; Delteil 1884, 1902; van Gorkom 1884) made a second unprovable claim in 1844. As with *Vanilla*, the only “evidence” for his claim is his making it. This time, Neumann reported that he produced seeds of *Calanthe veratrifolia* R. Br. by pollinating the flowers, germinated them and grew seedlings. He also claimed that his seedlings would bloom in the “following year” (Neumann 1844). If Neumann had seedlings which flowered, even a very intensive search of the literature failed to discover any report(s) about them. Not even French authors who glorified every orchid discovery, no matter how insignificant, made by their compatriots mention Neumann’s plants (Costantin 1913a, b, 1917, 1926; Costantin and Magrou 1922a, b). This is a reasonably clear indication that Neumann’s seedlings either did not exist or died before flowering in the “following year.” There is also another *triste*, but obviously contrived, report regarding premature death of seedlings which would have established priority for a different French orchid grower (Rivière 1866a, b).

Dean Herbert: absentee germination

Dean Herbert’s claim that he “raised *Bletia*, *Cattleya*, *Orchis monorchis* (L.) R. Br. and *Ophrys aranifera* Huds. from seed” is also questionable because he reported that his plants died probably because he was absent “during the greatest part of the year... from the place where [they] were deposited” (Herbert 1847). Perhaps had he “remained on the spot” (Herbert 1847) he could have produced plants.

David Moore: germinating orchid seeds in a botanical garden

Several horticulturists in Ireland (Moore 1849) and Britain (Cole 1849; Gallier 1849) attempted to germinate orchid seeds under horticultural conditions (Naudin 1849, 1850, 1865; Anonymous 1850, 1853; Arditti 1980, 1984, 1992). The first to succeed was David Moore (Fig. 14), Director of the Glasnevin Botanical Gardens in Ireland.

David Moore (1807 Dundee, Scotland–1879, Ireland) had a special interest in insectivorous plants (Nelson and Seaward 1981), and “orchids were probably just another group of plants to” him (Dr. E. C. Nelson, National

Botanic Gardens, Glasnevin, Dublin, Ireland, personal communication). Still, he showed some interest in orchids as evidenced by an article on the importation of orchids and a description of a *Catasetum* (Moore 1834; Nelson 1981). Also, on becoming director of the Glasnevin Botanical Gardens, he added many plants to the initial collection of 65 orchid species (Nelson 1981).

Moore was also interested in fruit production through hand pollination and seems to have been the first horticulturist to produce cocoa fruits in Ireland (Anonymous 1869). This probably caused him to pollinate *Cattleya forbesii*, *Epidendrum crassifolium*, *Epidendrum elongatum*, and *Phaius albus* and produce seeds. He started to experiment with the germination of these seeds around 1844 and continued with his experiments despite the Irish potato famine (1846) and his wife’s death (1847). He published his findings in 1849 (Moore 1849; Anonymous 1850) and commented that “at the present time there are few subjects connected with plant growing on which there is less recorded information than that of growing Orchids from seeds...” and added that the reasons why orchid seeds do not germinate in large numbers like those of other plants is not clear. After that he asserted that “when Orchid seed does vegetate under favorable circumstances, a very large number of the myriads of extremely minute seeds contained in the ovaries are perfect, whether artificially impregnated or not.” After that, Moore proceeded to describe his germination method (Moore 1849):

“The manner of sowing the seeds, and treating the young seedlings, has been to allow the fine dust-like seed to fall from the ovaries as soon as they show symptoms of ripeness, which is readily known by ovaries bursting open on one side. When this takes place, they are either taken from the plant and shaken gently over the surfaces of the other Orchid-pots, on the loose material used for growing them in, or on pots prepared for the purpose, after which constant shade, a steady high temperature, with an abundance of moisture, are all requisites which are absolutely necessary to ensure success. In the course of eight or nine days after sowing, the seeds, which at first had the appearance of fine white powder, begin to assume darker colour to the naked eye, and if looked at with a Coddington, or even a simple lens, evident signs of approaching vegetation may be perceived, which increase until the protrusion of the young radicle and cotyledon takes place which varies from a fortnight to three weeks. From this period of their growth the young plants grow rapidly and the rootlets lay hold of whatever material is supplied to them. If the seeds happen either accidentally or intentionally to be made to vegetate on bare wood, as in some instances has been the case here, the young roots extend themselves in different directions, adhering closely to the

bark, and make great progress compared with the growth of the stems, thus affording beautiful examples of the manner in which epiphytal plants fix themselves so firmly... “The principal difficulty to contend with in rearing the young seedlings has been found to consist in their treatment during the first year, particularly the winter months... The second year’s growth has been one during which the plants made much progress and the only two kinds which have been brought to a flowering state have bloomed the third season. These are *Epidendrum crassifolium* and *Phaius albus*, the latter being now in flower, exactly 3 years from the sowing of the seeds.”

Moore was director of the Glasnevin Botanic Gardens for 30 years after publication of his paper on orchid seed germination. He continued to import orchids from various parts of the world but does not seem to have continued to work on the germination of orchid seeds and the cultivation of seedlings. Moore was also a member of many learned societies. In 1864, he was awarded an honorary doctorate by the University of Zurich. He was made a member of The Royal Dublin Society in 1878.

In his waning days, Moore took part in religious polemics and contributed to a collection of “lectures” by several anti-evolutionists. His claim was that he proved creation through “design in the structure and fertilization of plants” (Moore 1875). By using the word “design” Moore may have foreshadowed current pseudoscientific claims of “intelligent design.” This is surprising in view of Moore’s cordial correspondence with Darwin regarding insectivorous plants and potatoes during June and July of 1874. Darwin also wrote him a very nice letter on 3 May 1879 (i.e., about two months before his death; Nelson 1981; Nelson and Seaward 1981).

Moore’s other “contribution” to orchids rivals and perhaps exceeds seed germination in importance. It was his eldest son with his third wife Margaret Baker, Frederik Moore (1857–1950), who developed a passion for orchids and became well known as an orchid expert.

Richard Gallier and J. Cole: orchid seed germination by two gardeners

Two British horticulturists, Richard Gallier and J. Cole, also shared their experiences (Cole 1849; Gallier 1849; Anonymous 1850) after Moore published his report. Gallier was gardener for J. Tildesley, Esq., of West Bromwich, Staffordshire in Britain. Cole held a similar position for J. Willmore at Oldford near Birmingham. As he was located near Birmingham it is possible that he could have interacted with David Cameron (see above), but if he did there are no known records of such an interaction. Very little is known about Cole and Gallier and attempts to obtain information about them failed.

Cole’s note was the first to be published after Moore’s report. He wrote that his employer informed him:

“that *Bletia* [now *Phaius*] *Tankervilleæ* was some years since obtained from seeds sown in common soil; also *Epidendrum elongatum* sown on blocks of wood covered with moss. I have sown other sorts of Orchids at various times and in different ways, but without success... a few have been hybridised successfully here, so far as obtaining seed to all appearance perfect... and it has been sown, but it did not vegetate. *Cattleya labiata* was crossed with *C. guttata*, and swelled its pod (sic); *Calanthe veratrifolia* with *Bletia Tankervilleæ*; *Dendrobium moniliforme* with other *Dendrobes*; and *Stanhopea Wardii* with one of the other *Stanhopes*... I have the hybridised seed pod of *Stanhopea Wardii* by me, and shall be pleased to present some of the seeds to Mr. Moore or any other gentleman who may take an interest in raising seedlings.”

J. Cole also stated that he intended to carry out further experiments and planned to report his findings. If he did, we could not find any reports he may have published. The germination methods described by Cole can be successful and do not seem to have been published before his letter. The reputation of *The Gardeners’ Chronicle* (the most important horticultural publication in the world at the time which was nicknamed “The Times of Horticulture” in allusion to the famed *Times* of London) provides every reason to believe that Cole’s report was accurate and factual.

Gallier’s report was also published in the *The Gardeners’ Chronicle* (Gallier 1849). He crossed *Dendrobium nobile* with *Dendrobium chrysanthum*, obtained seed and:

“sowed it in three ways: some on a log, with natural moss growing on it, suspended in a shady part of the Orchid-house; some was sown on an inverted flower pot, the inside of which was stuffed with sphagnum, and placed in a pan of water... neither of these two sowings vegetated.”

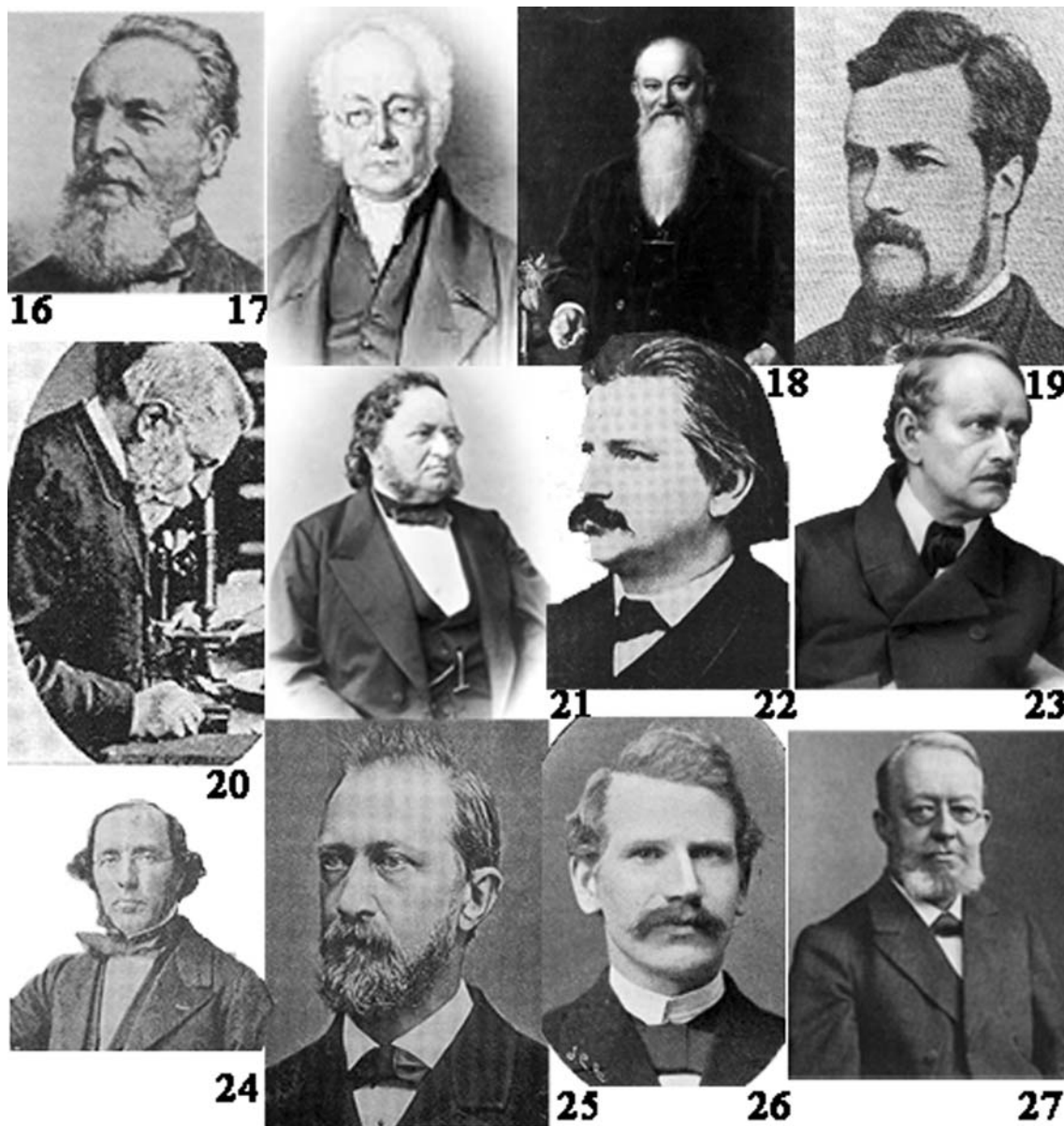
For his third method, Gallier used two pots, one filled with sand and the other with water. He spread the seeds on a floating piece of cork covered with a bell jar. Then he placed the entire contraption in a shady part of the greenhouse. Two seeds germinated after three weeks. Eventually, Gallier had five plants all of which died when he removed the cork from under the bell jar and suspended it from the roof of his greenhouse (Gallier 1849).

A Belgian journal reported Moore’s, and Cole’s experiences (Anonymous 1850) and stated that orchid propagation through seed germination in the greenhouses of Europe would open a new avenue for the culture of “these bizarre plants” (Anonymous 1850).

John Dominy, John Harris and Harry Veitch: the first orchid hybrid

Since this review is limited to orchid seeds and seedlings, the history of orchid hybridization will be mentioned only as it relates to seed germination. *Calanthe Dominyi*, the first commercial orchid hybrid, was produced in the Veitch establishment in the UK. A gregarious surgeon named John Harris (1782–1855, Fig. 15; Arditti 1980), advised an excellent horticulturist, John Dominy (1816–1891, Fig. 16) who was employed by an enlightened

nursery owner, Harry J. Veitch (1840–1924; Fig. 17), the owner of the well known orchid nursery which was established by his father, James Veitch (1792–1863; Fig. 18) to cross orchids. Dominy made the first cross in 1853, seeds were harvested in 1854 and the first plant bloomed in October 1856 (Veitch 1885, 1886; Veitch and Sons 1887–1894). This well documented chronology indicates that the first germination of orchid seeds as part of: (1) a commercial venture, and (2) the first successful attempt to produce a hybrid took place in 1854 in England.



Figs. 16–27 Orchid breeders and scientists. **16** John Dominy, 1816–1891. **17** Harry J. Veitch, 1840–1924. **18** James Veitch, 1792–1863. **19** Auguste Rivière, 1805 or 1821–1827. **20** Édouard Ernest Prillieux, 1829–1915. **21** Johann Georg Beer, 1803–1873. **22** Hermann Schacht,

1814–1864. **23** Mathias Jacob Schleiden, 1804–1881. **24** Gaspard Adolphe Chatin, 1813–1901. **25** Hubert Leitgeb, 1835–1888. **26** Oscar Drude, 1852–1933. **27** Albert Bernhard Frank, 1839–1900

Even making the first cross was not simple. To fully appreciate history as it unfolded it is best to quote a person who was not only present as it happened but also made it happen, Harry J. Veitch (1886):

“... very few [horticulturists and gardeners] possessed even an elementary knowledge of botany. They could... distinguish... the stamens and pistils of many flowers... and they were aware of the functions of those organs, but the confluence of those organs into the solid column of an Orchid flower was to them a profound mystery.

It was Mr. [actually Dr.] John Harris, a surgeon, of Exeter, who suggested to Dominy the possibility of muling Orchids, and who pointed out to him the reproductive organs seated in the column, and showed that the application of the pollinia to the stigmatic surface was analogous to the dusting of the stigma of other flowers with pollen. This simple fact being once fairly grasped, the work of hybridization proceeded apace... Capsules were produced in abundance... dehiscent... and seed was at length in hand. Then arose a great difficulty... which still exists... to discover the most suitable method of raising seedlings. The seeds of Orchids are... so minute... that an ordinary pocket lens is powerless to enable one to know whether the seeds are likely to contain a germ or are mere lifeless dust. Following, or at least believing that we were following Nature... every method or available means that could be thought of was brought into request to secure the germination of the seed. It was sown upon locks of wood, pieces of tree fern stems, strips of cork, upon the moss that surfaced the pots of the growing plants, in fact in any situation that seemed to promise favorable results. But... we seem far off as ever from hitting upon a method by which at least a moderate amount of success may be calculated upon.

Seeds we get in profusion, but... little of it germinates... The seeds of hundreds of capsules have been sown without yielding a single result. In very many cases only a solitary plant had been raised from a capsule that must have contained thousands of seeds; in very few instances indeed has the number of seedlings reached a hundred. It is true that we have raised many seedlings in the aggregate, but many of them have appeared when least expected, and when we consider the myriads of seeds that have been sown, and the comparatively few plants raised, we cannot be said to have achieved great success...”

The seed germination method used at the Veitch establishment was similar to the methods used by Moore, Cole, and Gallier.

Orchid seed germination: 1850–1899

Several horticulturists germinated orchids and produced hybrids during the second half of the nineteenth century.

John and John: Orchid Hybrids

Dominy (Fig. 16), Cole, Gallier, and Veitch (Fig. 17, 18; DCGV) made their seed germination methods known by publishing them in *The Gardeners' Chronicle*. Within a year of the original articles there was also at least one report in Belgium in French about Moore's and Cole's findings (Anonymous 1850) in a magazine which was probably also read in France. Three additional reports were published a number of years after that in France and Belgium (Bergman 1879, 1881, 1882, 1889). Further, Dominy and Harris were great conversationalists who enjoyed and readily took part in extensive conversations (Arditti 1980). This was enhanced by the fact that Dominy possessed “... wide knowledge which he was always willing to communicate orally...” (Anonymous 1891a). Therefore, the news that orchid seeds can be germinated probably travelled fast among those who were producing seeds and attempting to germinate them (Anderson 1862, 1863; Gosse 1862, 1863), most without success: “... there is nothing unusual in obtaining seed-pods [sic] and seed in abundance from more than one species; but I have never yet been fortunate enough to get the seed to germinate” (Anderson 1862). And when the seeds did germinate, “... all of the [seedlings] found the means of getting out of the world by a route I never could fathom” (Beaton 1862). Thus, it is not surprising that a large number of hybrids were produced (i.e., seeds of additional orchids were germinated) shortly after the reports by Moore, Cole, and Gallier and close on the heels of Dominy's first hybrid (Veitch 1886).

The second hybrid, a *Cattleya* flowered first in 1859. The first *Paphiopedilum* flowered in 1869 (Veitch 1885, 1886; Veitch and Sons 1887–1894; for a review, see Arditti 1984). Successful germination of orchid seeds and establishment of seedlings remained newsworthy and a subject for discussion for several years (Anonymous 1869, 1891a, b; Douglas 1882a, b; Scheidweiler 1844, 1845; Godefroy-Lebeuf 1886; Ignotus 1894). The Moore–Cole–Gallier–Dominy–Veitch (MCGDV) method was also described in a book on orchid cultivation in India (Jenning 1875), but it is not clear whether it was used there or taken from British sources and inserted in the book. There are no known hybrids and instances of orchid seed germination during that period from India or any other area except the UK and Europe. This book is also found in the library of the Singapore Botanic Gardens but it is not clear when it was acquired. Even if it was acquired in the 1880s, the seeds which produced the first artificial orchid hybrid in

Singapore, *Spathoglottis* Primrose, were germinated in vitro in the 1920s on a medium formulated by Lewis Knudson (*Vanda* Miss Joaquim, discovered by Miss Agnes Joaquim in her garden in 1893 is believed to be a natural hybrid by orchid scientists and knowledgeable growers).

The Faculty of Medicine in Paris, France, maintained a botanical garden (FMPBG) which had a collection of 1,200 species and varieties (Rivière 1866a, b) Auguste Rivière (1805– or 1821–1877; Fig. 19) carried out orchid research there starting in April 1837 (for a review, see Arditti 1984), or 1840 (Rivière 1866a, b). In 1865, Rivière at that time chief gardener at the Luxemburg palace in France, claimed to have discovered how to pollinate orchids sometime between 1840 and 1857 (Anonymous 1857). Strangely, he waited for 10–25 years to report his discovery, did so after orchids were pollinated successfully in the UK, and claimed to have done it 1–2 years before the British. The first report regarding Rivière's purported discovery appeared in the official organ of the French empire (*Journal Officiel de l'Empire Français, Gazette National*) in a convoluted anonymous note regarding an oral report concerning the FMPBG orchid collection and Rivière's experiments (Anonymous 1857). In what must be described as circular referencing, Rivière used this anonymous note to buttress his report (Rivière 1866a, b). It is hard not to ask whether these reports described a real discovery or a fabricated one for the purpose of producing a coveted *priorité* for Rivière and France.

Rivière reported that his (unpublished) preliminary experiments with pollination in 1843 included *Epidendrum crassifolium* (*Encyclia crassifolia* at present; Rivière 1866a, b) which set fruit. Towards the end of June 1848, the capsules began cracking and released seeds. Rivière collected the seeds “*précieusement*” and “sowed the seeds [on the 5th of July] on two pieces of peat moss lying in two dishes in order to keep, by imbibition, a decent humidity. The pieces of peat moss were later placed on a layer of rotting manure, in the open air, and covered with two tightly fitting glass bell jars. During the day I sheltered... from the sun's rays; I was taking an almost fatherly care of them. On the 28th of the same month, imagine my joy, gentlemen, when I found out that most of my seeds were germinating” (Rivière 1866a, b). But, “*chose triste!*” despite Rivière's fathering, his plants died due to circumstances he characterized as unusual, but did not describe (Rivière 1866a, b).

It may well be that the events of 1848 (suspiciously 1 year before Moore's report) did take place as Rivière recollected them 18 years later in 1866. However, it is also necessary to inquire why Rivière published his observations so long after he made them and why did he not describe exactly what killed his seedlings. There can be no doubt that he knew that germination of tropical orchids

seeds under horticultural conditions was an important advance which should have been published immediately. It is clear that Rivière appreciated the value of publication. That is why he published his findings even if late. Or did he fabricate a story?

There are interesting and perhaps even disturbing coincidences: (1) Link's reported working with *Oeceoclades maculata* and so did Rivière's, and (2) Rivière germinated *Epidendrum crassifolium* just like Moore. Rivière was probably familiar with Moore's and Link's work (Prillieux and Rivière 1856a, b). Therefore, it is possible to suspect that his success with orchids which were known to germinate was not accidental. Hence, it is necessary to ask if Rivière's paper in 1866 is genuine or contrived for the sole purpose of creating the impression that he made the discovery before Moore. Questions can also be raised about Rivière's remark that in 1843 he observed “accidental” germination of *Epidendrum nocturnum* seeds. If he saw germinating seeds in 1843, why did not Rivière publish his observations at the time? The fact that he did not raises serious doubts about his veracity.

Rivière seems to have been industrious: “In 1854 [conveniently before the first orchid hybrid was produced in the UK] still obsessed by the thought of restarting my experiments... I [Rivière] made some new experiments but this time *in secret* [emphasis added because the need for secrecy is not obvious]. The plant I chose was *Oeceoclades maculata* or *Angraecum maculatum*, a little orchid from Brazil. It was pollinated through my efforts in February 1854; its fruits reached maturity on the 4th of July of the same year and it dropped its seeds on the table around it” (Rivière 1866a, b). Rivière spread the seeds on some pots, but had to be gone during a critical period. He returned on the following 6 August to find that the seeds germinated and some of the seedlings survived. On seeing the seedlings, Rivière wrote Édouard Ernest Prillieux (1829–1915; Fig. 20) and asked him to study their development.

Prillieux became interested in orchids early in his life. He studied the dehiscence of their fruits and other subjects (Prillieux 1856, 1857) and joined Rivière in studying seed germination and seedling development of *Oeceoclades maculata*, which was known as *Angraecum maculatum* at the time (Prillieux and Rivière 1856a, b), and of *Miltonia spectabilis* (Prillieux 1860). These were not the first detailed anatomical studies of germinating orchid seeds and young seedlings in general or those of *Oeceoclades maculata* in particular (Link 1840 preceded them), but they added new details.

Several papers were published in France after that. They were reports regarding the first British hybrids (Bergman 1879, 1881, 1882) as well as French seedlings and crosses (Bergman 1881; Bleu 1881). Publications on seedlings elsewhere (mostly the UK and Belgium) were similar.

There were no major advances in the technology, horticulture, and basic understanding of orchid seed germination and seedling culture until 1899 (for reviews, see Arditti 1967, 1979, 1984, 1990, 1992 and literature cited therein), despite the publication of numerous articles (Anonymous 1855a, b, 1887, 1896; Anderson 1862, 1863; Beaton 1862; Gosse 1862, 1863; Jenning 1875; Bergman 1879, 1881; Bleu 1881; W S 1887; L 1892, 1893, 1894; Scheidweiler 1844; Maron 1898).

Even if to some extent more artistic than purely botanical, horticultural, or biological, the most notable orchid seed publication to appear between 1850 and 1899 was *Beiträge zur Morphologie und Biologie der Familie der Orchideen*, a book by Joseph or Johann Georg Beer (1803–1873; Fig. 21) which was published in 1863. In *Beiträge*, Beer described and illustrated orchid fruits, seeds (frontispiece), and seedlings. All seeds are depicted in color and magnified 100 times. The drawings are morphologically accurate and artistically magnificent. Beer's artistic ability, patience, and botanical expertise are obvious. His are probably the first detailed color renditions of orchid seeds and seedlings to be published.

The role of mycorrhiza in orchid seed germination

Despite the fact that orchid seeds were being germinated under horticultural conditions their requirements were not known.

Many observations but no discovery

Several botanists saw orchid mycorrhiza during the last half of the nineteenth century, but only one of them appreciated its importance.

- Heinrich Friedrich Link (1767–1851; Fig. 5) may have been the first botanist to draw orchid endophytes. His drawing shows fungi inside root cells of *Goodyera procera* (*Goodyera repens* R. Br.) very clearly (Link 1824, 1839–1842, 1840, 1849a, b).
- Schleiden von Reissek suggested in 1846 that fungi may be present in the roots of several orchids, *Neottia nidus-avis* among them (von Reissek 1847).
- Johann Georg Beer (Fig. 21) drew orchid seeds, seedlings and organs in great and beautiful detail (Beer 1854, 1863).
- Hermann Schacht (1814–1864; Fig. 22) saw hyphae in roots of *Corallorhiza*, *Epipogium*, *Goodyera*, *Limodorum* and *Neottia* (Schacht 1854a, b).
- Mathias Jacob Schleiden (1804–1881 Fig. 23) observed hyphae while studying roots and tuber cells of *Neottia nidus avis* L (Schleiden 1854).
- Gaspard Adolphe Chatin (1813–1901; Fig. 24) published two papers on orchid anatomy which point to fungi in root cells (Chatin 1856, 1858).
- Édouard Ernest Prillieux (1829–1915; Fig. 20) depicted fungi in seedlings of *Angraecum maculatum* (Prillieux and Rivičre 1856a, b) and tubers of *Neottia nidus avis* (Prillieux 1856).
- Hubert Leitgeb (1835–1888; Fig. 25) studied orchid roots and their cells (Leitgeb 1864a, b, c, 1865).
- Oscar Drude (1852–1933; Fig. 26) investigated the biology of *Monotropa hypopitys* and *Neottia nidus avis* (Drude 1873).
- Albert Mollberg drew the fungi in roots of *Cephalanthera grandiflora* Babgnt. (Mollberg 1884).
- Albert Bernhard Frank (1839–1900; Fig. 27) coined the term mycorrhiza: “*Der ganze Körper ist also weder Baumwurzel noch Pilz allein, sondern ähnlich wie der Thallus der Flechten, eine Vereinigung zweier verschiedener Wesen zu einem einheitlichen morphologischen Organ, welches vielleicht passend als Pilzwurzel, Mykorrhiza* [the two words are printed with single spaces between the letters; *mykorrhiza* is spelled with a single “r”] *bezeichnet werden kann.*” [The entire body is neither tree root nor fungus alone, but like the thallus of lichens a unique morphological organ which can be referred to as fungusroot, mycorrhiza (Frank 1885)]. He redescribed and redefined the phenomenon in his textbook (Frank 1892): “... *Pilzgewebe... in... organischer Verwachsung mit... Wurzeln... und... gemeinschaftlich... wächst, das Pilz und Wurzel ein... gemeinsam arbeitendes Organ darstellen, welches ich Pilzwurzel, Mykorrhiza, genannt habe.*” (in free translation: fungal hyphae grow organically together with roots forming a common organ which I named fungusroot, mycorrhiza).
- H. Wahrlich examined many tropical orchids and some European ones while working in Moscow before Frank's new term (i.e., *mykorrhiza*) became widely accepted and concluded that the yellow clumps he saw in root cells were fungi (Wahrlich 1886).
- Pierre Augustin Clement Dangeard (1862–1947; Fig. 28) and L. Armand studied the mycorrhiza of *Ophrys aranifera* and published two very interesting articles. They contain good drawings and the suggestion that the fungus was a parasite which caused no harm to the orchid (Dangeard and Armand 1887, 1898).
- Daniel Trembly MacDougal (1865–1958; Fig. 29) investigated orchid mycorrhiza, especially that of *Aplectrum* and *Corallorhiza* and drew several correct conclusions, but did not observe seedlings (MacDougal 1898, 1899a, b, 1944; Arditti and Ernst 1993a).
- Professor Gottlieb Haberlandt (1854–1945; Fig. 30), the great German physiologist/anatomist, who reported



Figs. 28–38 Orchid scientists. **28** Pierre Augustin Dangeard, 1862–1947. **29** Daniel Trembly MacDougal, 1865–1958. **30** Gottlieb Haberlandt, 1854–1945. **31** *Neottia nidus avis*. **32** Noël Bernard, 1874–1911. **33** Julien Costantin, 1857–1936. **34** Gaston Bonnier,

1853–1922. **35** Leon Guignard, 1852–1923. **36** Marie-Louise Bernard (born Martin), 1878–1946. **37** Francis Bernard, 1908–ca. 1991. **38** Joseph Magrou, 1883–1951

the presence of fungal mycelium in root cells of *Neottia nidus-avis* (the orchids which led N. Bernard to his discovery; Fig. 31), *Corallorhiza innata*, *Epipogon gmelini*, and *Wulfschlaegelia* “but attached no significance to it” (Haberlandt 1914; Pridgeon 1990).

- Melchior Treub (1851–1910; Fig. 9), who reported seeing endophytes in seedlings and young plants of lycopods (Treub 1890). He formulated the term “protocorm” to describe seedlings of lycopods. Noël Bernard used the term to describe the early stage of orchid seed germination. With time, the initial use of “protocorm” for lycopods was forgotten and the term is

currently used almost exclusively for orchids. A reference to endophyte(s) in protocorm(s) by Treub was misinterpreted leading to the suggestion that he saw orchid mycorrhiza without appreciating its importance. This is not the case. Treub did not work with orchid seedlings and probably never saw their endophytes (for reviews which include discussions of the history of orchid mycorrhiza, see Arditti 1992; Magnus 1900; Harley 1969; Warcup 1975; Arditti 1979, 1984, 1990, 1992; Hadley 1982; Harley and Smith 1983; some reviews are part of history themselves: Burgeff 1909, 1932, 1936, 1938, 1943, 1954, 1959).

A single observations and a major discovery

Of the botanists listed above who saw orchid endophytes (Beer, Chatin, Drude, Link, Frank, Leitgeb, Mollberg, Prillieux, Reissek, Schacht, Schleiden, and Wahrlich), none drew correct conclusions about the role of the fungus. In their defence it must be said that they studied roots and rhizomes for the most part. It is not easy to draw proper conclusions about the role of the fungus in orchid seed germination from seeing it in these organs. None of the horticulturists who germinated orchid seeds on the surfaces of potting media which supported mature orchids seemed to even suspect the participation in the process of another organism, especially fungus. The reason for this is simple: They never saw the endophyte. Even had they seen the fungi it is reasonable to assume that they would have assumed them to be pathogens.

Only the “genius of Pasteur applied to orchids” and a “Mozart of Plant Biology” (Bernard 1990) could appreciate the role of the fungi in orchid seed germination. Noël Bernard (1874–1911; Fig. 32) possessed these unique characteristics. He saw *Neottia nidus-avis* seedlings which harbored the endophyte and drew correct conclusion about the nature of the fungi and their function in orchid seed germination (Le Dantec 1911; Pérez 1911, 1912; Bernard 1921; Derx 1936; Blarighem et al. 1937; Magrou 1937a, b; Moreau 1958; Boullard 1985; Arditti 1979, 1984, 1990, 1992; Bernard 1990).

Noël Bernard, his life and times

Noël Bernard, was born on 13 March 1874, the son of Francois Bernard, 46, and his second wife, Marie Marguerite Sabot, 19. According to one report, his father died in December 1879, but the late Prof. Francis Bernard, Noël's son, stated that his father was orphaned at the age of 12 (i.e., Francois died in 1886). Marie Marguerite, a young mother and widow, had to work very hard to support her son and herself, but had trouble making ends meet. Noël had to help as soon as he could and became a mathematics tutor while still a young student.

An outstanding student with a fascinating, sometimes abrasive, personality (Boullard 1985), Noël was admitted to the École Normale Supérieure and the École Polytechnique. At the age of 21, Bernard decided to become a biologist and Julien Costantin (1857–1936; Fig. 33) became his mentor. Costantin considered him to be his star pupil. And, having lost a son at war, Costantin may have found a replacement in Bernard.

Bernard earned his *Licencié* in *Sciences Naturelles* in November 1897 and decided to specialize in orchids, but was drafted before continuing with his studies. As a soldier, he was stationed at the Mulum barracks near Fontainebleau forest. There, while taking a walk on 3 May

1899, Bernard made his great discovery (Bernard 1899). Together with the very discovery of orchids (by Theophrastus in Europe and unknown individuals elsewhere) and the establishment of the Orchidaceae (by Lindley), Bernard's is one of the five most important orchid discoveries, the other two being Prof. Lewis Knudson's method for asymbiotic seed germination and Dr. Gavino Rotor's first micropropagation.

After completing his service, Bernard returned to the École Normale Supérieure where he worked with Julien Costantin (Fig. 33) and Gaston Bonnier (Fig. 34) while living on a property owned by Leon Guignard (Fig. 35). In 1901, Bernard took a position at the University of Caen.

Bernard married Marie Louise Martin (Fig. 36), a mathematics teacher, on 8 August 1907. He was 33 and she was 29. On 30 April 1908, the pregnant Marie Louise went bike riding, fell and their son Francis (Fig. 37) was born prematurely. Bernard kept the tiny (1.5-kg) baby alive by feeding him a mixture of malt, water and orange or lemon juice and placing him in an incubator (which may have been one of the incubators left over from Pasteur's days and still in service).

Later in 1908, Bernard became Professor of Botany at Poitiers. There, he made numerous major contributions to botany, orchids, potatoes and symbiosis. Sadly, he only had 3 years to live.

Early in 1910, Bernard's cousin Joseph Magrou (1883–1951; Fig. 38) and the family physician diagnosed him with tuberculosis, an incurable disease in those days (Bernard predicted that someday there would be a cure; Bernard 1911a). Bernard and his wife moved to an estate in Mauroc, not far from Poitiers. He died at 03:00 on 26 January 1911 after much suffering and was buried in a small cemetery at Saint Benoît near Mauroc. His grave was marked with a concrete plate which bears the inscription (Boullard 1985):

Noël Bernard, Professeur A La Faculté des
Sciences de l'Université de Poitiers–1874/1911

Like Noël Bernard's mother, Marie Margaret Sabot, his wife Marie Louise Martin did not remarry. She raised Francis as a single mother earning a living as an educator and a school administrator. Marie Louise died in 1946. Francis was then 3 years old. Eventually, he became a noted myrmecologist and a marine biologist. Francis Bernard died on 16 June 1990, but not before writing a memoir of his father (Bernard 1990). He was survived by his wife, Michelle, two grown sons and four grandsons (for more details, see Yam et al. 2002).

Noël Bernard: mycorrhiza and orchid seed germination

As mentioned above, a number of botanists saw, described, and drew fungi in orchid seedlings, roots, and rhizomes,

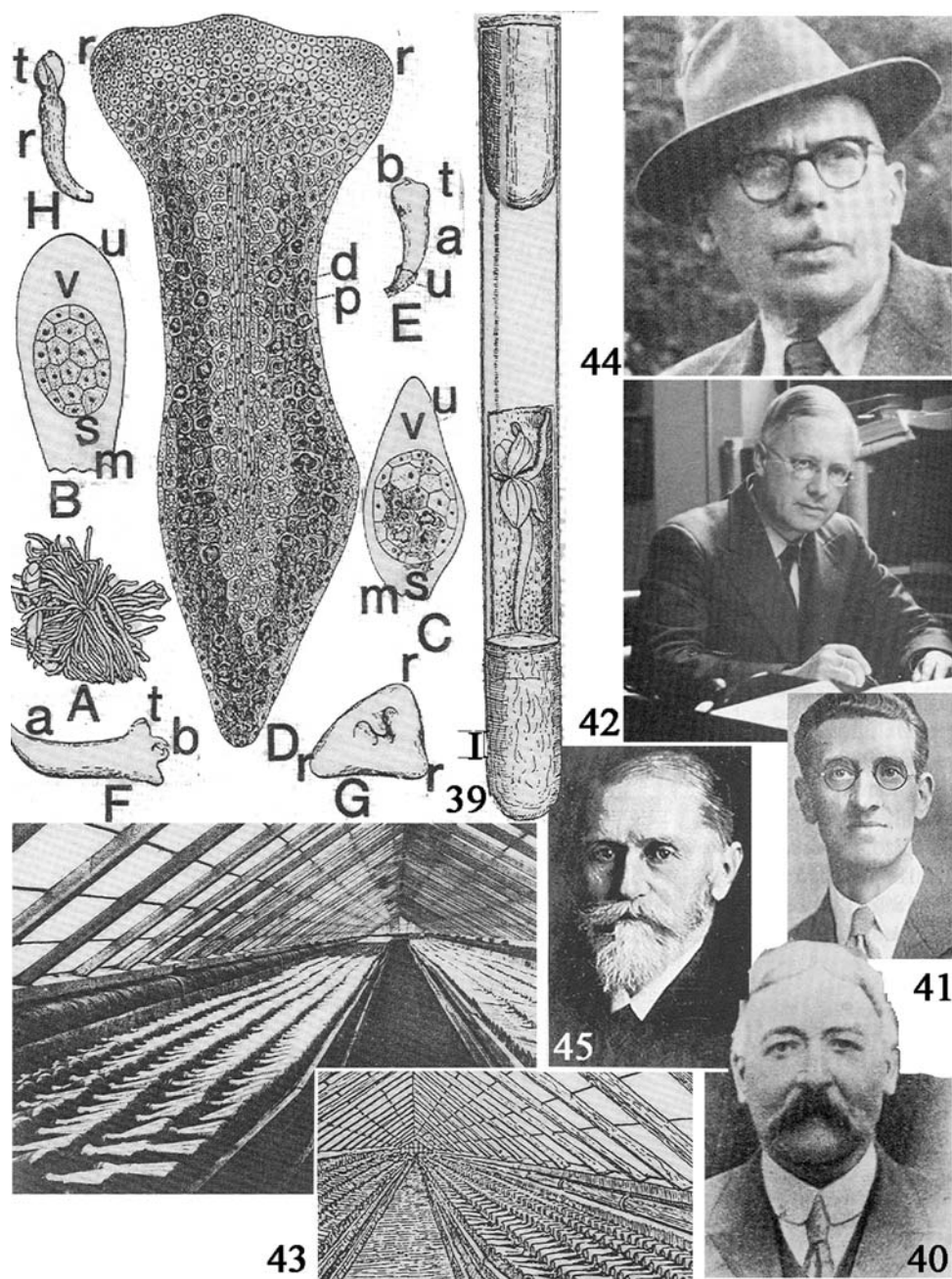
but none of them discovered the role of the endophyte. Bernard did. What he observed during his walk on 3 May 1899 were seedlings of *Neottia nidus-avis*, 3 mm (Boullard 1985) to 5 mm long (Bernard 1899) all of them colonized by fungi (Fig. 39). He also saw germinating seeds of *Neottia*. No one reported seeing them before him.

Bernard described his discovery in a paper published 15 May 1899 (Bernard 1899; Boullard 1985). He reported seeing details reported by others before him also saw and described: (1) parenchymatous cells which contained starch, (2) a network of hyphae in some cells layers, and (3) epidermal cells free of fungi and starch grains (Boullard

1985). He also noted that all germinating seeds contained fungi. His genius came into play at this point and he wrote that “mycorrhizae are indispensable for the plant [meaning the seeds of course] during the germination period [and] *Neottia nidus-avis* plants are associated with their fungi during all stages of development” (Bernard 1899).

After subsequent research, Bernard provided additional details: “Although the fungi can live apart from their host plants, the orchids themselves require the presence of their guests for their own development. I have sown the seeds of many orchids ‘aseptically’... under these conditions they have not freely germinated; they swell, and later on they

Figs. 39–45 Students of orchid mycorrhiza. **39** Roots and seedling of *Neottia nidus-avis*: **A** roots before the development of flower stalks; **B** seed showing the vegetative tip of the embryo (*v*), suspensor (*s*), seed coat (*u*) and opening at the base of the latter, $\times 58.86$; **C** Seed at the start of germination following fungal penetration (*symbols* the same as in **B**), $\times 66.49$; **D** cross-section of seedlings during the first year of development showing living (*p*) and degenerating (*d*) pelotons and points at which the first roots will develop (*r*), $\times 40.37$; **E** external view of a developing seedling showing apical bud (*b*), swelling which will eventually forma protocorm (*t*), embryonic axis (*a*) and remnant of seed coat (*u*), $\times 5.14$; **F** a more advanced seedling (*symbols* as in **E**), $\times 4.96$; **G** front view of the seedling in **F** showing areas of future root development, $\times 7.98$; **H** excised root showing swelling (*t*) and fungus infected area of root, $\times 3.17$; **I** *Phalaenopsis* seedling, 18 months old, produced through the Bernard method. **40** Joseph Charlesworth, 1851–1920. **41** Gurney Wilson. **42** John Ramsbottom, 1885–1974. **43** The Charlesworth greenhouses filled with flasks in which seeds were germinated symbiotically. **44** Hans Edmund Nicola Burgeff, 1883–1978. **45** Ernst Stahl, 1848–1919



get green, but their growth remains insignificant. On the other hand, if germs of the appropriate fungus are sown with the seeds, they commence to germinate almost immediately in a very regular manner... I have examined a large number of young orchids which had germinated in very varying conditions, and I always noticed that they were invaded by the fungus from the beginning of their life. The orchids are therefore practically dependent on their parasitic fungi, since they do not grow without them.”

It would have been easy for Bernard to simply describe what he saw like those before him. He did not, and instead studied the physiological, evolutionary, and symbiotic implications. He could have concluded that the fungi were pathogens. A third possibility was to conclude that the fungi entered the seedlings after they germinated, not before. His genius was that he did not reach these conclusions.

Bernard studied orchids, potatoes, fungi, symbiosis, and some genetics during the last 2 years of his life. His productivity was immense and excellent (Bernard 1899, 1900, 1902a, b, c, 1903, 1904a, b, c, 1905a, b, 1906a, b, c, d, 1907, 1908, 1909a, b, 1911a, b; for a translation of some of Bernard’s papers into English, see Jacquet 2007) despite having to care for an injured wife and a premature baby while being very ill.

Francis Bernard described his father as a precocious genius. He compared him to Mozart for several reasons, one of them being that his “greatest period of... creativity [was] up to around [the age of] 22–35” (Bernard 1990). According to Francis Bernard, “decline sets in after this age...” Given N. Bernard’s early death it is impossible to state with certainty if decline would have set early in his life. At the end of his life when he was 36–37, Noël Bernard discovered phytoalexins and devised the zones of inhibition (“halo”) method of studying the effects of antifungal and antimicrobial compounds. This suggests that he would have continued to be a productive scientist. One of his papers on phytoalexins was published during his lifetime (Bernard 1909b). The second (Bernard 1911b) was completed and edited by his mentor Julien Costantin (1857–1936) and his cousin Joseph Magrou (1883–1951) who continued some of Bernard’s work (Magrou 1925, 1937a, b; Magrou and Magrou 1935; Anonymous 1951; Mariat and Segretain 1952).

Noël Bernard did not devise a practical method for asexual germination of orchid seeds, but his research just before he died indicates that he might have done so had he lived long enough (Arditti 1990). He did predict that a time would come when orchid gardens will include laboratories. Bernard did not explain why orchid seeds, especially ones of temperate climate species, require fungi for germination. However, no one has done that to this day (for reviews, see Arditti et al. 1990; Rasmussen 1995; Yam

et al. 2002). Francis Bernard was correct in suggesting that his father was a genius, but he may have erred in classifying him as one who might have declined after turning 35 (for more details, see Arditti 1984; Yam et al. 2002).

The twentieth century: great strides forward

The first horticultural methods for orchid seed germination were developed in the middle of the nineteenth century. Bernard made his discovery in 1899. However, truly major discoveries in orchid seed germination, both basic and practical, were made during the twentieth century.

Applications of Bernard’s discovery

The Moore–Cole–Gallier–Dominy–Veitch (MCGDV) methods of orchid seed germination were used by British orchid growers almost immediately after their publication in *The Gardeners’ Chronicle* in 1849 (Neumann 1844; Scheidweiler 1844; Moore 1849; Cole 1849; Gallier 1849; Anonymous 1906a, b, 1921, 1925; Black 1906; Manhardt 1906; Wilson 1906; Grignan 1912, 1914a, b, 1916; Denis 1914; Bauer 1915; for reviews, see Arditti 1980, 1984, 1990, 1992). And the availability of a seed germination method made possible the start of orchid hybridization.

During that period (1849–ca. 1910), seeds were generally sown on the surface of potting mixes in pots which supported orchids. Despite appearing to be simple this method required an operator who had “an ‘eye’ for a likely surface” (Black 1906). After the surface was selected the operator had to “clip [it] over... evenly and give it a good watering” (Black 1906). Seed were distributed uniformly. The pots were placed in a well illuminated area away from direct sunlight (Black 1906). With all that, success required the sowing of “*Odontoglossum* seeds... on pots containing *Odontoglossum* plants, *Cypripedium* [*Paphiopedilum*] on *Cypripedium* [*Paphiopedilum*], etc.” (Black 1906). The only exception was *Laeliocattleya* seeds. They could germinate on any compost. This suggests that *Laeliocattleya* seeds could germinate in the presence of fungi from different orchids, but the growers did not know it at the time. These methods or similar ones were used by growers and breeders in the UK, France, and perhaps Belgium (Jancke 1907, 1915; Hefka 1914; Young 1893; Anonymous 1894; Burberry 1894; Wrigley 1895), but there is no convincing evidence that they were used anywhere else. The methods were not very effective and germination was uncertain (Hammerschmidt 1915; for reviews, see Arditti 1984, 1990). In spite of and maybe because of the problems with these methods, attempts were made by growers to enhance seedling growth through CO₂ enrichment (Witt 1913; Fischer 1914), a procedure far ahead of its time.

Commercial in vitro symbiotic germination

Joseph Charlesworth (1850– or 1851–1920; Fig. 40; Anonymous 1920) collected in the Andes sometime between 1887 and 1889. Probably this is why he decided to specialize in *Odontoglossum*, first as an importer (there was no CITES then) and subsequently as a hybridizer. His hybridization program became very extensive by 1894 and he started to sell plants in 1898 (R A R 1887). He developed his business and breeding program rapidly and by 1906 his company could offer many seedlings for sale.

Charlesworth's was not satisfied with the MCGDV germination methods and on reading Bernard's he decided to develop new methods involving mycorrhizal fungi (Anonymous 1922a, b). He encountered difficulties and turned to Gurney Wilson (Fig. 41) for help. Wilson recommended contacting mycologist John Ramsbottom (1885–1974; Fig. 42). The two met at the Royal Horticultural Society Holland House exhibition and Charlesworth prevailed upon Ramsbottom to visit the Charlesworth establishment at Haywards Heath in 1913.

Charlesworth and Ramsbottom established a good working relationship. They carried basic and applied research and developed a successful in vitro symbiotic method for orchid seed germination (Anonymous 1921; Ramsbottom 1922a, b, c, 1929). Charlesworth's died in 1920 but by 1922 his firm had many in vitro seed cultures in greenhouses (Ramsbottom 1922a; Fig. 43). The 1922 Charlesworth catalog had 2,245 entries and “an immense number of choice hybrids” and even several color plates. According to a writer (probably Gurney Wilson) in the *Orchid Review* in that year, the descriptions were “remarkably correct in nomenclature and typographical details” (Anonymous 1922c). By 1924, the Charlesworth catalog offered 2,422 items (Anonymous 1924a, b). This attests to the success of the Charlesworth–Ramsbottom symbiotic method for orchid seed germination. As a result, it gained widespread use throughout the world until Professor Lewis Knudson formulated his asymbiotic method (for reviews, see Arditti 1967, 1979, 1984, 1990, 1992; Yam et al. 2002). The development of this method may be one reason why volume 25 (1917) of the *Orchid Review* was dedicated to him.

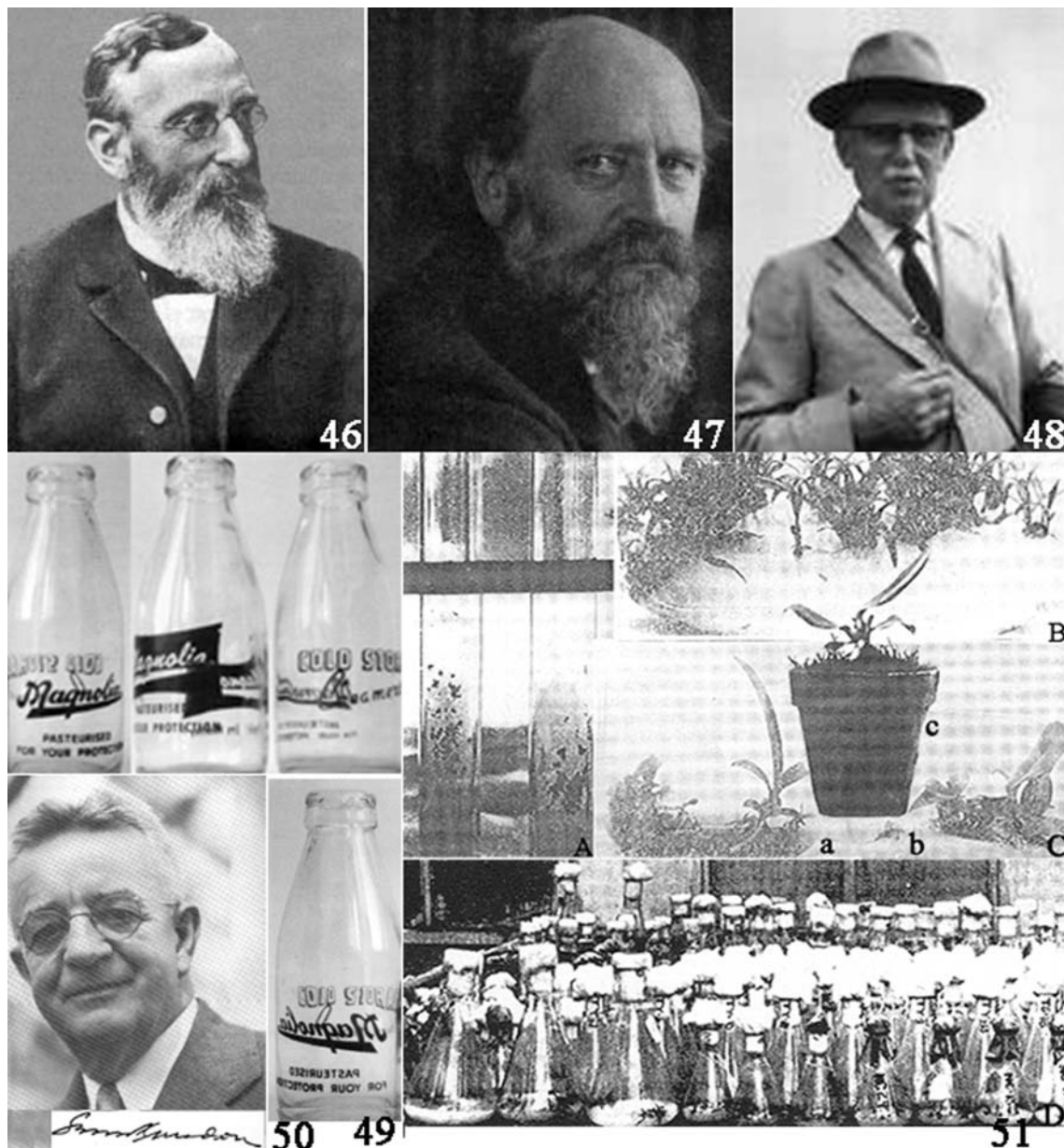
Ramsbottom joined the British Museum in 1910, became Keeper of Botany in 1930 and held this position until his retirement in 1950. He remained active 20 years after his retirement in the British Museum, the Linnean Society of London, the British Society of Mycopathology, the National Rose Society (UK), the Royal Horticultural Society, the South London Botanical Institute, and other groups. He never returned to orchids and when asked by one of us (JA) in 1968 to reminisce about his days with Charlesworth he wrote: “I am a little surprised and much

amused... at 82 I am much out of touch! However I shall be very pleased to have a chat... one day, preferably in the afternoon...” His visitor (J.A.) described the chat as “... entertaining and informative despite the fact that Ramsbottom was recovering from a stroke at the time. One point he made was that orchids were never his primary interest and that he worked on them only due to the association with Charlesworth.” He died in 1974 [for additional details about Joseph Charlesworth as well as John Ramsbottom and, the chat between him and a then young orchid scientist (J.A.), see Arditti 1990; Yam et al. 2002].

Hans Burgeff: *Orcheomyces* and *Mycelium radicans*

Two major works on orchid fungi were published in 1909. One was Noël Bernard's definitive book length review on orchid mycorrhiza, *L'évolution dans la symbiose, les orchidées et leur champignons commensaux* (Bernard 1909a). He was then 35 years old with 2 years to live. The second work was a dissertation by a 26-year-old German botanist, Hans Edmund Nikola Burgeff (1883–1976; Fig. 44), called *Die Wurzelpilze der Orchideen, ihre Kultur und ihr Leben in der Pflanze* (Burgeff 1909). He remained active for more than 50 years after that with orchids being his main interest (Haber 1963; Knapp 1978) and wrote reviews, research papers, and more books on orchids (Burgeff 1909, 1911, 1932, 1936, 1938, 1943, 1959). Before becoming a professor, Burgeff worked with a number of well-known German plant scientists of his era. They included Peter Clausen in Freiburg and Berlin who taught him fungus culture methods; Ernst Stahl (1848–1919; Fig. 45) from whom he learned synecology; Wilhelm Pfeffer (1845–1920; Fig. 46) in whose laboratory he acquired knowledge of plant physiology; and Karl von Goebel (1855–1932; Fig. 47) who also had an interest in orchids. He also worked at the Bogor Botanical Gardens in Indonesia. After leaving Goebel's laboratory he was Professor at Halle (1920–1921), Munich (1921–1923) and Göttingen (1923–1925). He left Göttingen to become Director of the Botanical Institute at the University of Würzburg where he was also a Professor and worked on orchid seed germination, mycorrhiza, and symbiosis (Burgeff 1932). Burgeff remained at Würzburg until his retirement in 1952 but continued to work on the germination of seeds of terrestrial orchids (Burgeff 1954) and conservation.

Burgeff developed methods for the isolation and culture of endophytes and symbiotic orchid seed germination (Burgeff 1911). He concluded that orchid endophytes were separate group of fungi named them *Orcheomyces* and established a ponderous and awkward nomenclatural system which used the words *Mycelium radicans* (*M. R.*) followed by the name of the orchid from which the fungus was isolated. An example is *Mycelium radicans*



Figs. 46–51 Asymbiotic germination of orchid seeds. **46** Wilhelm Pfeffer, 1845–1920. **47** Karl von Goebel, 1855–1932. **48** Richard Eric Holttum, 1895–1990. **49** Magnolia brand milk bottles which were used for orchid seed germination in Singapore. This brand was and still is owned by the Cold Storage Grocery Company. **50** Lewis Knudson, 1884–1958. **51** Knudson's asymbiotic cultures: **A** One of

Knudson's earliest experiments: test tubes with three-month-old cultures; **B** asymbiotic *Cymbidium* seedlings in Erlenmeyer flask, $\times 0.68$; **C** older seedlings: *a* *Cattleya*, *b* *Laeliocattleya*, $\times 3$, *c* potted 15-month-old *Cattleya* seedling, $\times 0.44$; **D** some of Knudson's earliest cultures: Erlenmeyer flasks containing seedlings transferred from smaller test tubes

Thrixspermum arachnites. In fact, orchid fungi are not part of a separate group. Burgeff was mistaken.

He also believed that there was strong orchid/fungus specificity. In this he was partially correct because temperate orchids (north in the Northern Hemisphere and south in the Southern Hemisphere) in general often do not germinate asymbiotically and may require specific fungi (for reviews, see Burgeff 1936; Arditti 1979, 1992; Rasmussen 1995; Yam et al. 2002). Burgeff tried to germinate orchid

seeds asymbiotically but had no success. He used Knudson's media (B and C) after their publication and attempted to improve them (Burgeff 1936). On his trip to Indonesia, Burgeff visited Singapore and introduced Prof. R. E. Holttum (1895–1990; Fig. 48), then Director of the Singapore Botanic Gardens, how to use Knudson's method (Yam 1995, 2007). This led to the production of the first intentional hybrid in Singapore, *Spathoglottis Primrose* in 1932 (all orchid scientists and most knowledgeable

orchidists believe that the National Flower of Singapore *Vanda Miss Joaquim*, discovered in 1893 by Miss Agnes Joaquim is a natural hybrid; Yam et al. 2002; Arditti and Hew 2007). Many hybrids were produced in Singapore after that by germinating the seeds in Magnolia-brand milk bottles (Fig. 49) which were sold by the Cold Storage grocery store.

Asymbiotic germination

Bernard tried to germinate *Bletilla hyacinthina* seeds on several media supplemented with salep (a decoction made of dried *Orchis* tubers; Lawler 1984) and determined that the optimal level was 2% (Bernard 1903, 1904a, b, 1909a; Burgeff 1959). *Orchis* tubers contain 16–61% mucilage (this varies with the species), 0.5–25% starch (which cannot be hydrolyzed or utilized by orchid seeds), 0.9–2.7% reducing sugars (many of which can support seed germination), 0.2–1.4% sucrose (which supports orchid seed), and low levels of nitrogenous substances (Sezik 1967, 1984; Ernst and Rodriguez 1984; Lawler 1984). Therefore, the 2% salep in Bernard's medium was too low a concentration to provide sufficient levels of appropriate sugars for germination. Bernard also attempted to germinate *Laelia* seeds on a medium with a combination of salep and sucrose. This medium may have pointed to an asymbiotic medium for orchid seed germination, but if it did, Bernard did not live long enough to carry out further experiments.

As mentioned above, Burgeff's attempts to germinate orchid seeds asymbiotically also failed. He explained his failure as being due to his culture vessels which were "made of ordinary glass, which gives off alkali [that brings] an end to the life of the seedlings by a rapidly increasing alkalinity" (Burgeff 1959). This explanation is not convincing because:

- Orchid seeds are known to germinate in a wide variety of food and drink bottles and jars of all kinds. These are usually made of ordinary glass.
- Both orchid seeds and seedlings tolerate a wide pH range during germination (Piriyakanjanakul and Vajrabhaya 1980).
- If Burgeff was aware that alkalinity which accumulated in the medium caused the problem he could have transferred his seedlings to new medium as often as needed to prevent the death of the seedlings.
- Burgeff formulated a potassium phosphate ($\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$) buffer for the Knudson B medium (Burgeff 1936, 1959) and could have used it for his excessively alkaline culture media.
- He could have used culture vessels made of non-toxic glass. A photograph in one of his books (Burgeff 1936)

is of a Jena Glass brand Erlenmeyer flask. Jena Glass is not "ordinary glass."

The only logical conclusion from these facts is that Burgeff did not formulate a good and proper medium and did not maintain his cultures under suitable conditions.

Lewis Knudson: asymbiotic seed germination

Using "data from the experiments of Bernard and Burgeff," Lewis Knudson (Fig. 50), a 38-year-old American plant physiologist at Cornell University concluded that "the fungus might... digest some of the starch, pentosans and nitrogenous substances; which are digestion products, together with secretions from or products produced on decomposition of the fungus might be the cause for germination" and that "it is conceivable that germination is induced not by any action of the fungus within the embryo, but by products produced externally on digestion or secreted by the fungus" (Knudson 1922a). On the basis of this reasoning Knudson decided that "germination of orchid seeds might be obtained by the use of certain sugars" (Knudson 1922a).

The Sea Captain's Son

Lewis (no middle name) Knudson (according to Giltner Knudson, Lewis's younger son, the family always pronounced the name with a K as in Kent and u as in urea, Knudson, not Newdson) was the son of a Norwegian sea captain (who emigrated to US as an adult, lived in Milwaukee, Wisconsin and was a commander of ships on the great lakes) and his American-born wife. He was born on 15 October 1884, attended primary, junior high and high schools in the Milwaukee Public School system graduating from the latter on 1 July 1904 with an average grade of 81% (a B or 3.0 average in present terms and certainly not a grade that would have gotten him into one of the modern American highly selective universities and not an indication of future greatness).

Knudson attended the University of Missouri where he earned a B.Sc. in agriculture and graduated on 30 January 1908. He was appointed assistant in plant physiology at Cornell University immediately after that and promoted to Instructor before the end of his first term there. Three years later (1911) he received his doctorate and was appointed Assistant Professor of Plant Physiology. A year after that he was appointed Acting Head of his Department and became Full Professor by 1921. When the Department of Plant Physiology became part of the Department of Botany, Lewis Knudson's title was changed to Professor of Botany. He was head of that department in 1941 and held the post until his retirement on 30 June 1952 ruling it "with an iron

fist in a steel glove” (Wedding 1990). Knudson remained in Ithaca as Professor Emeritus until his instant death of heart attack in his home while having a drink on Sunday evening 31 August 1958. Altogether he was a man liked by most, feared by some, on bad terms with very few and respected by all (for more details about his life, some provided by his son Giltner and others who knew him, see Arditti 1990; Wedding 1990; Yam et al. 2002; Giltner and JA knew each other).

“Lewie”: *plant physiologist*

Today, “Lewie” (as he was called behind his back by the graduate students in his department; Wedding 1990) is known for his work with orchids, but his research included fungi (Knudson 1913a, 1913b), sugar metabolism (Knudson 1915, 1916, 1917), osmotic pressure (Knudson and Ginsburg 1921), amino acids (Knudson 1933a), aseptic growth of whole plants (Knudson 1915, 1916; Knudson and Lindstrom 1919; Knudson and Smith 1919), in vitro culture of detached root-cap cells about 50 years before cell cultures became common (Knudson 1919), *Calluna vulgaris* (Knudson 1928, 1929b, 1932, 1933c), amylase production by plant roots (Knudson and Smith 1919); X-ray effects on plants (Knudson 1933b, 1934b, 1940b, 1941c), ferns (Knudson 1933b, 1934b, 1940b, 1941a, b, c), and chloroplasts (Knudson 1934a, b, c, d, 1936). His research on sugars, aseptic culture and amylase probably led Knudson to orchids.

Knudson: “*Germination of Orchid Seeds Might be obtained by the Use of Certain Sugars*”

The use of salep by Bernard and Burgeff as well as some carbohydrates by the latter (starch, sucrose, glucose) led Knudson to use a “certain” sugar in a culture medium (Knudson 1922a). He concluded correctly that salep contains nutrients which could be utilized by seeds and seedlings (Knudson 1922a, 1989; Ernst and Rodriguez 1984; Arditti 1989; Janick 1989). He also theorized that the fungus hydrolyzed large molecules and rendered their component moieties available to the seeds (Knudson 1922a).

Knudson first attempted to germinate *Cattleya schroederiae* × *Cattleya gigas* seeds in December 1918 on extracts of peat and canna tubers. Within a month (January 1919) seeds on these media formed protocorms. Those on peat failed to grow into seedlings. Protocorms on canna extract (which probably contained soluble sugars) produced 1–2 leaves in five months (April 1919). In 1919, he attempted to germinate seeds of *Cattleya labiata* × *Cattleya aurea* on carrot (*Daucus carota*) and beet (*Beta vulgaris*) concoctions. The seeds germinated

and seedlings developed on both media. These findings convinced him that orchid seeds can germinate without fungus and led him to further experiments (Arditti 1990).

The next experiment was probably obvious and inevitable. Also in 1919 he placed seeds of *Cattleya mossiae* on Pfeffer’s solution (Table 1) enriched with 1% sucrose. The seeds germinated and in seven months produced protocorms 1 mm in diameter with one leaf. Not much happened on the sugar-free medium. This was Knudson’s first solution. It was probably his undesignated solution A (Engman 1984; Arditti 1990; Yam et al. 2002).

Knudson assumed that if the fungus hydrolyzed sucrose, glucose would be one of the products, and on 18 July 1919 placed seeds of *Cattleya intermedia* × *Cattleya lawrenceana* on Pfeffer’s solution and a modification of it he labeled “Medium B” (Knudson 1921, 1922a, b, 1924, 1925) which contained sucrose or glucose. Knudson departed for Spain and France after starting his cultures and did not return until approximately 1 year later. On examining his cultures on 9 June 1920 he observed that seedlings on both glucose and sucrose were well developed (Fig. 51), but the media were dehydrated.

In subsequent experiments with seeds of *Laeliocattleya*, *Cattleya* and *Epidendrum*, Knudson found that 0.8% (8 g or 0.044 moles) glucose l⁻¹ was the most suitable sugar concentration (Knudson 1922a). The most widely used sugar concentration in Knudson’s media B and C is 2% (20 g or 0.058 moles) sucrose l⁻¹ (Table 1). On complete hydrolysis that much sucrose will produce 0.058 moles glucose and 0.058 moles fructose (i.e., ca. 10 g of each monosaccharide) for a total of 0.116 moles of sugars or 2.6 times the optimal glucose concentration. However, hydrolysis in media is not complete (Ernst et al. 1971; Ernst and Arditti 1972, 1990; for a review and more details, see Yam et al. 2002).

Knudson: “*Chance favors the prepared mind*”

Chance (the quote is by Louis Pasteur) and good fortune may have had a part in some of Knudson’s early experiments. First, his experiments would have probably failed if he had used seeds which do not germinate easily. The seeds used in his experiments came from Theodore L. Mead of Oviedo, Florida, a well-known orchid grower at the time. Luckily the seeds Mr. Mead provided were those of *Cattleya* and other orchids which germinate easily (for more details, see Arditti 1984; Yam et al. 2002).

Knudson used cane sugar (sucrose from sugar cane) in his medium B (Table 1). Beet sugar is also sucrose but several reports indicate that it does not support orchid seed germination as well as that obtained from cane (for reviews, see Arditti 1967, 1979; Arditti and Ernst 1984).

Table 1 Composition of the Pfeffer, Knudson B and C, Vacin and Went, Galambos, Schenk and Hildebrand, Hoagland and Knop media (Arditti 1990) (mg l⁻¹ water unless indicated otherwise)

Component	Pfeffer ^a	Knudson		Galambos	Vacin and Went	Schenk and Hildebrand ^f	Hoagland ^g		Knop ^h
		B	C				1	2	
Macroelements									
Monoammonium phosphate, NH ₄ H ₂ PO ₄						300		136	
Ammonium sulfate, (NH ₄) ₂ SO ₄		500	500	200	500				
Calcium chloride, CaCl ₂ · 2H						200			
Calcium nitrate, Ca(NO ₃) ₂	800	1,000	1,000	1,000			820	656	800
Calcium phosphate, Ca ₃ (PO ₄) ₂					200				
Magnesium sulfate, MgSO ₄ ^b				250			240	240	200
Magnesium sulfate, MgSO ₄ · 7H ₂ O	200	250	250		250	400			
Potassium chloride, KCl	100			120					
Potassium nitrate, KNO ₃	200				525	2,500	505	606	200
Potassium phosphate, KH ₂ PO ₄	200	250	250	250	250	250	136		200
Iron									
Ferric chloride, FeCl ₃	8								
Ferric phosphate, Fe ₂ (PO ₄) ₃ ^c		50							
Ferric phosphate, FePO ₄				50					Trace
Ferrous sulfate, FeSO ₄ · 7H ₂ O			25			27.85			
Ferric tartrate, Fe ₂ (C ₄ H ₄ O ₆) ₃ · 2H ₂ O					28		5	5	
Chelating agent									
Sodium EDTA, Na ₂ EDTA						37.25			
Microelements									
Boric acid, H ₃ BO ₃						10	2.8	2.8	
Copper sulfate, CuSO ₄ · 5H ₂ O						0.025	0.08	0.08	
Manganese chloride, MnCl ₂ · 4H ₂ O							1.81	1.81	
Manganese sulfate, MnSO ₄ · H ₂ O			7.5		7.5				
Manganese sulfate, MnSO ₄ · 4H ₂ O						25			
Molybdic acid, H ₂ MoO ₄ · H ₂ O							0.02	0.02	
Sodium molybdate, Na ₂ MoO ₄ · 2H ₂ O						0.15			
Zinc sulfate, ZnSO ₄ · 7H ₂ O						10	0.22	0.22	
Amino acid									
Asparagine ^d				500					
Sugar									
Sucrose	1%	2%	2%		2%	2%			
“Sugar” ^e				2.5%					

^a Pfeffer suggested two solutions (English translation by A. J. Ewart, 1900), in which salts are dissolved in 3 or 7 l of water. Knudson dissolved the salts in 5 l of water

^b The number of waters of hydration not given

^c This formula is given in Knudson's first paper in English (Knudson 1922a), but is doubtful that he used such a salt. See text for discussion

^d This amino acid is not known to enhance seed germination and seedling growth, but Burgeff used it in a culture medium for endophytes. At 370 mg l⁻¹ in asymbiotic media asparagine is a better nitrogen source for seedlings than leucine and cystine (for reviews see Burgeff 1936; Arditti 1967, 1979; Arditti and Ernst 1984)

^e M. Galambos is reported (Domokos 1976) to have used *Zucker* (“sugar”), probably sucrose, some of which breaks down to glucose and fructose during autoclaving (Ernst et al. 1971)

^f As used for the culture of orchid plantlets (Piriyakanjanakul and Vajrabhaya 1980)

^g Knudson used Hoagland's solution in one of his experiments, but did not indicate whether it was version 1 or 2

^h Knop's solution predates Pfeffer's by approximately 35 years. The two solutions are very similar and Knop's is listed here for comparison purposes

Had Knudson used beet rather than cane sugar his experiments may have been less successful. The reasons for the difference between cane and beet sugar are not known even now. They may be due to the presence of minute levels of impurities.

Wilhelm Pfeffer listed two mineral solutions in his *Physiology of Plants* (Pfeffer 1900), both containing the same concentrations of salts dissolved in either 7 or 3 l of water. Knudson averaged the two volumes of water $[(7 + 3)/2 = 5]$, dissolved the minerals in 5 l of water (that was probably his unlabeled solution A) and was successful. However, the seeds in his experiments would have germinated equally well on either the 3 or 7 l Pfeffer's solutions (Arditti 1990; Table 1).

Knudson: from B to C

Knudson found that his medium B “was not entirely satisfactory for seeds of” *Paphiopedilum*, *Vanilla*, and North American species of *Cypripedium*. In one instance, he encountered difficulties with *Cattleya*, *Phalaenopsis*, and *Vanda*. His conclusion was that the difficulties were brought about by the absence of microelements. Because of that, he added boron, copper, manganese, and zinc to medium B “without any improvement in the case of *Cypripedium* and *Vanilla*” (Knudson 1946a). Results were better when he added iron and manganese to what he called his solution C (Table 1) which he described as being “theoretically better than [solution] B,” and superior in the case of *Cattleya* (Knudson 1946b).

Conclusion

Almost 400 years separate the time when orchid seeds were first seen and the development of a practical asexual method for their germination. Claims, counter claims and acrimony followed Knudson's discovery, but these are beyond the scope of this review (for details, see Arditti 1984, 1990, 1992; Knudson 1927, 1929a, 1930, 1935, 1940a, 1951, 1952; Yam et al. 2002). Knudson also made other contributions to orchid seed science (Knudson 1947, 1948, 1950). The important point is that after Knudson's media B and C were formulated, orchid growing and hybridization became widespread. Hybrids which early growers may not have even imagined became possible. Examples of this are colored *Phalaenopsis* hybrids. In the early days of orchid growing and even as late as 1958–1959 (Scott and Arditti 1959), there were only white *Phalaenopsis* hybrids and relatively few intergeneric crosses. Today, multicolored *Phalaenopsis* flowers are as common as multigenics like *Aranda*, *Darwinara*, *Knudsonara*, *Lindleyara*, *Mokara*, and many others.

Part II: Micropropagation

Abstract A commonly held view is that Prof. Georges Morel is the sole discoverer of orchid micropropagation and that he was the first to culture an orchid shoot tip in 1960. In fact, the first in vitro orchid propagation was carried out by Dr. Gavino Rotor in 1949. Hans Thomale was the first to culture an orchid shoot tip in 1956. The methods used by Morel to culture his shoot tips were developed by others many years before he adapted them to orchids. This review also traces the history of several techniques, additives, and peculiarities (agitated liquid cultures, coconut water, banana pulp, a patent and what appears to be an empty claim) which are associated with orchid micropropagation. A summary of plant hormone history is also outlined because micropropagation could not have been developed without phytohormones.

Orchid micropropagation: the origins

Orchid micropropagation did not originate suddenly as a brand new idea in the mind of a single person despite an unfortunately successful self-serving attempt to create this impression (Morel 1960). The origins of orchid micropropagation are intermingled with the history of tissue culture, phytohormones and other areas of plant physiology (Arditti and Krikorian 1996; Arditti 2008).¹ Its origins are in several areas of research and arose from the work of numerous scientists (Arditti and Krikorian 1996; Arditti 2008) rather than a single individual. These research lines will be presented separately until they converged and lead to orchid micropropagation. A summary of plant hormone history will also be outlined because micropropagation could not have been developed without phytohormones (see Krikorian 1995 for a more extensive history).

Plant hormones and culture media additives of plant origin

A little more than a century ago the existence of plant hormones was only being suggested. At the present time,

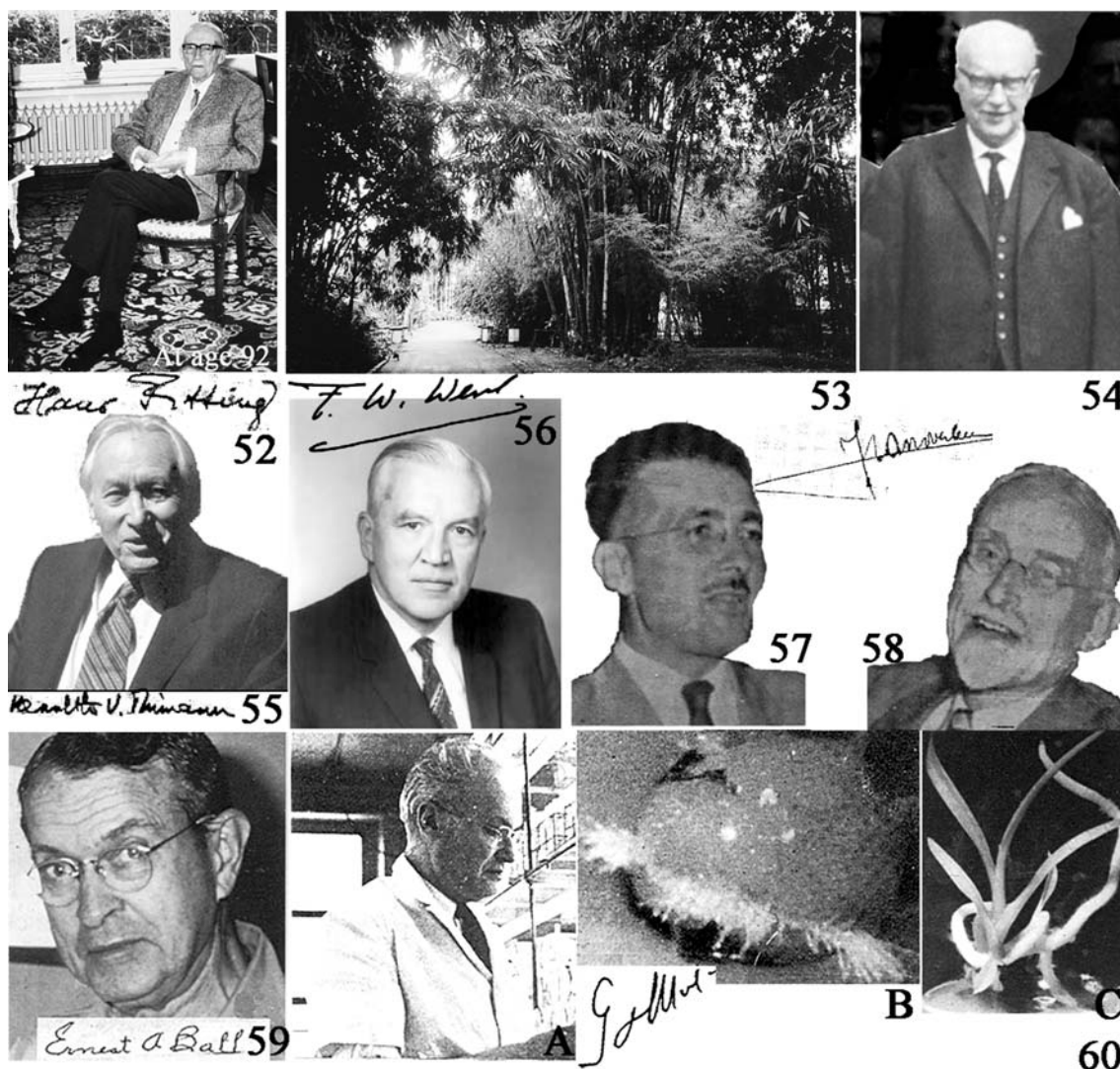
¹ Important details were removed from or “softened” in chapter 1 of *Micropropagation of Orchids*, first edition (Arditti and Ernst 1993a) under pressure in an effort not to offend or displease certain individuals and/or groups. A subsequent review (Arditti and Krikorian 1996) set the record straight by presenting the history accurately. The history chapter in the second edition of *Micropropagation of Orchids* (MO2; Arditti 2008) is also accurate. It is based on Arditti and Krikorian (1996). The review here is based on these two accurate historical presentations (Arditti and Krikorian 1996; Arditti 2008). We thank Dr. A. D. Krikorian for allowing the use of the joint paper for MO2 and by extension also here.

the use of these substances in tissue culture and micro-propagation is routine.

Auxins

The first botanist to suggest the existence of plant hormones is Gottlieb Haberlandt (1854–1945; Fig. 30), Professor of Plant Physiology in Berlin. He suggested that pollen tubes affect the growth of ovaries by releasing substances which he named Wuchsenzyme (“growth enzymes”). Haberlandt also suggested that if vegetative cells were to be cultured in the presence of pollen tubes “perhaps the latter would induce the former to divide” (Haberlandt 1902; English translation by Krikorian and Berquam 1969; Arditti and Krikorian 1996; Laimer and Ucker 2003).

Pollen tubes release a substance which induces post-pollination phenomena and ovule development in orchids. This was first shown by Professor Hans Fitting (1877–1970; Fig. 52) in his research on *Phalaenopsis* pollinia and pollination at the Bogor Botanical Gardens in Indonesia (Fig. 53) in 1909 (Fitting 1909a, b, 1910; for reviews, see Arditti 1971, 1979, 1984, 1992; Avadhani et al. 1994; Yam et al. 2009). Fitting, who was described as being “The first investigator to work with hormones and active extracts in plants” (Went and Thimann 1937), named the substance *Pollenhormon* and is therefore the first plant scientist to use the word hormone in connection with plants and to suggest that they produce hormones. He did not pursue the matter further, but if he had Fitting might have discovered auxin.



Figs. 52–60 Plant scientists. **52** Hans Fitting (1877–1970) at the age of 92. **53** Bogor Botanical Gardens, Indonesia. **54** Friedrich Laibach (1885–1967). **55** Kenneth V. Thimann (1904–1998). **56** Frits W.

Went (1904–1990). **57** Johannes van Overbeek (1908–1988). **58** Albert Blakeslee (1874–1954). **59** Ernest A. Ball (1909–1997). **60** Georges Morel (A), his *Cymbidium* protocorm (B) and a plantlet (C)

The first indication that *Pollenhormon* may be or contains auxin was provided by Friedrich Laibach (1885–1967; Fig. 54) who reported that diethyl ether can extract an active principle from it (Laibach 1930, 1932, 1933a, b; Maschmann and Laibach 1932; Laibach and Maschmann 1933). After Laibach, Kenneth V. Thimann (1904–1998, Fig. 55) showed that the ether extract contained auxin (for reviews, see Went and Thimann 1937; also see Thimann 1980; Avadhani et al. 1994; Arditti and Krikorian 1996; Yam et al. 2009).

Frits W. Went (1926, 1928; Fig. 56) discovered auxin in Utrecht, Holland, before Laibach extracted it from *Pollenhormon*. Went carried out his work (actually a Ph.D. Dissertation) after Fitting suggested the existence of *Pollenhormon* [which Fitting did not equate with auxin even in his 90s in a letter to one of us (JA)]. In letters and conversations with one of us (JA), Went indicated that he made no connection between Fitting work with orchids and his research on *Avena* coleoptiles and *Pollenhormon* and auxin (Yam et al. 2009). This is not surprising for the time (1920s). Auxin was identified as indole-3-acetic acid (IAA) in 1934 (Went and Thimann 1937; Haagen-Smit 1951) and made successful tissue culture possible (Gautheret 1935, 1937, 1983, 1985; Loo 1945a, b). Currently IAA and several of its analogs [i.e., synthetic auxins like 2,4-dichlorophenoxy acetic acid (2,4-D), naphthalene acetic acid (NAA) and indolebutyric acid (IBA), for example] are indispensable for tissue culture in general and orchid micropropagation in particular.

Coconut water and cytokinins

Haberlandt suggested in his classic paper that “one might also consider the utilization of embryo sac fluids” (Haberlandt 1902; Krikorian and Berquam 1969; Laimer and Ucker 2003). E. Hannig tested the effects of embryo sac fluids of *Raphanus* and *Cochlearia* on the growths of their own embryos (Hannig 1904; Krikorian and Berquam 1969; Laimer and Ucker 2003). It is very likely that European botanists at the time were not familiar with the liquid endosperm of coconuts. Only, those who spent time in the tropics became familiar with the colorless liquid endosperm in green coconuts. This is coconut water (CW), not coconut milk which is a white milky liquid produced by squeezing, extracting or grating the solid white endosperm (meat) of mature nuts (jelly-like and clear in green nut) that becomes copra when dried.

One Dutch botanist who spent time in the tropics [the Bogor (Buitenzorg) Botanical Gardens in Indonesia; Fig. 53] and became acquainted with CW was Johannes van Overbeek (1908–1988; Fig. 57). He and M. E. Conklin suggested its use to Albert Blakeslee (1874–1954; Fig. 58) for the culture in vitro immature embryos of *Datura*

stramonium. The embryos grew well in the presence of CW (van Overbeek et al. 1941, 1942) and an effective complex additive was introduced into plant tissue culture (van Overbeek et al. 1944; Caplin and Stewart 1948; Stewart and Shantz 1955; Pollard et al. 1961; Raghavan 1966; Tulecke et al. 1961, 1975; Krikorian 1975, 1982, 1988, 1995; Stewart and Krikorian 1975; Gautheret 1985). Ernest A. Ball (Fig. 59) was the first to use CW for the culture of apical meristems (Ball 1946; Krikorian 1975, 1982). L. Duhamet used coconut water to culture crown gall tissues (Duhamet 1950). Georges Morel (Fig. 60) cultured *Amorphophallus rivieri*, *Sauromatum guttatum*, *Gladiolus*, *Iris* and lily in CW-enriched media (Morel 1950). Nowadays, CW is used widely in tissue culture and micropropagation of many plants and there are several incorrect priority claims.

F. Mariat was first to publish on the incorporation of CW (mistakenly by referring to it as coconut milk) and copra extract as additives to orchid seed germination media (Mariat 1951; for reviews see Arditti 1967, 1977a, b, 1979, 2008; Arditti and Ernst 1984, 1993b). Today, CW is used extensively in orchid micropropagation (for reviews see Arditti 1967, 1977a, b, 1979, 2008; Arditti and Ernst 1984, 1993a). There is no agreement in the literature regarding the reasons for its effects. Research on plant tissue culture expanded between 1940 and 1965, and efforts were made to solve the problem of tissues which could not be cultured. Tobacco pith was one of these tissues (Gautheret 1985; Skoog 1994). In an effort to culture this tissue, Folke Skoog (1908–2001; Fig. 61) and his group at the University of Wisconsin, Madison formulated several media and assayed the effects of many additives (Skoog 1944; Skoog and Tsui 1948, 1951; Skoog and Miller 1957). Herring sperm DNA which was stored for a very extensive period was one of the substances they tested. The period was long enough to suggest that this DNA was used for orchid seed germination experiments by Professor John T. Curtis (1913–1961; Fig. 62; for a review see Arditti 1967). However, according to one of the co-discoverers of cytokinins, Professor Carlos O. Miller (b. 1923; Fig. 63) in correspondence with one of us (JA), Curtis and Skoog would not have shared reagents due to a very strained relationship. Regardless of its source the old DNA led to the discovery of the first cytokinin, kinetin (Strong 1958; Miller 1961, 1967; Leopold 1964; Skoog et al. 1965; Gautheret 1985; Skoog 1994).

With the requirements for auxin and several vitamins (Gautheret 1945) for plant tissue culture already well known, the discovery of cytokinins made possible the formulation of the Murashige and Skoog (MS) medium by Toshio Murashige (b. 1930; Fig. 64) and Folke Skoog (Murashige and Skoog 1962; Smith and Gould 1989; Skoog 1994). This medium is used very widely for the



Figs. 61–73 Plant researchers. **61** Folke Skoog (1908–2001). **62** John T. Curtis (1913–1961). **63** Carlos Miller. **64** Toshio Murashige (b. 1930). **65** Roger Gautheret (1910–1997). **66** H. Vöchting. **67** Julius Sachs (1832–1897). **68** William J. Robbins. **69** Walter Kötze (1893–

1970). **70** Philip R. White (1901–1968). **71** Pierre Noubécourt (1895–1961). **72** Loo Shih Wei (Western Style: Shih Wei Loo; 1907–1998). **73** Theodor Schwann (1810–1882)

culture of many plants including orchids (for reviews see Arditti and Ernst 1993a; Arditti 2008).

Banana

Powdered banana was apparently first added to a medium for orchid seed germination in Brazil (Graeflinger 1950 as cited by Withner 1959b). Incorporation of banana in culture media became widespread very quickly with several of

the subsequent users claiming to have been the originators of the practice. The most common practice at present is to add pulp of ripe bananas to media (Withner 1955; Ernst 1967; for reviews see Arditti 1967; Pages 1971, Arditti and Ernst 1984; Yam et al. 2002). The reasons for the effects of banana are not known. Attempts to find answers by fractionating banana pulp through serial extractions with a number of solvents produced inconclusive results (Arditti 1968).

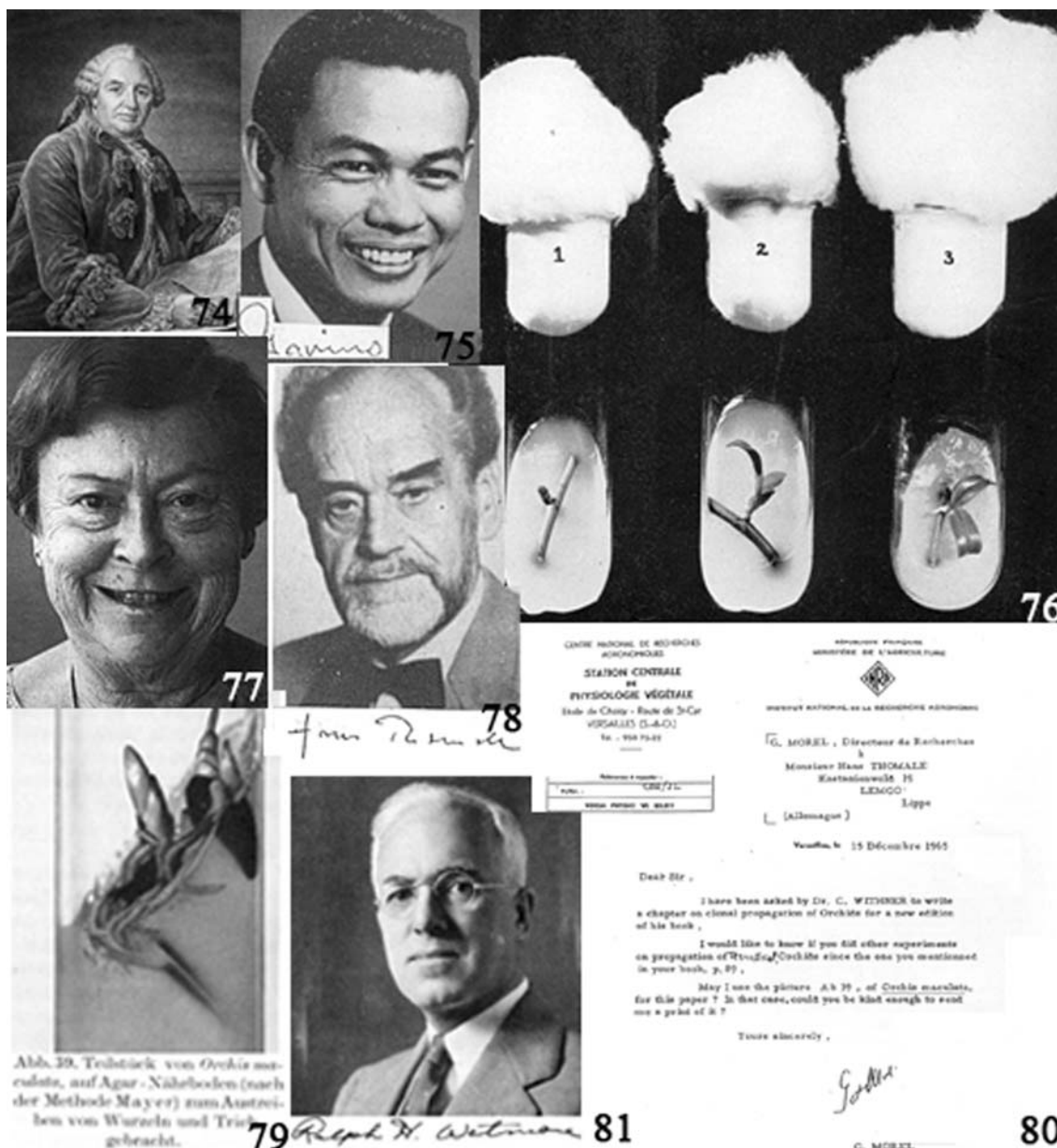
Several other plant homogenates have been evaluated for their effects on seed germination and seedling growth (Arditti 1967, 1979; Ernst 1967; Arditti and Ernst 1984). Few if any of these are used to culture orchid explants. A number of these are added to cultures of protocorm-like bodies or developing plantlets (see specific procedures).

The beginnings

Orchid micropropagation has its roots in early tissue culture research with other plants.

Tissue and organ culture of non-orchidaceous plants

Roger J. Gautheret (1910–1997; Fig. 65) was an early, well-known and influential figure in the history of plant tissue culture from his base in France. In his later years, he became a (not unbiased) historian of the field. In one of his accounts he wrote that “the progress of plant tissue culture was made possible by only a few genuine discoveries [which]... did not appear suddenly, but after a long and slow journey, unpretentiously covered by pioneers” (Gautheret 1985). According to him, a multi-talented, Frenchman, Henri-Louis Duhamel du Monceau (1700–1782; Fig. 74), a student of



Figs. 74–81 Plant tissue culture. **74** Henri-Louis Duhamel du Monceau (1700–1782). **75** Gavino Rotor Jr. (1917–2005). **76** Rotor’s cultures of *Phalaenopsis* flower stalk sections. **77** Lucie Mayer. **78**

Hans Thomale (1919–2002). **79** Thomale’s explants of *Dactylorhiza (Orchis) maculata* producing shoots and roots in vitro. **80** George Morel’s letter to Thomale. **81** Ralph W. Wetmore (1892–1989)

wound healing in trees and a writer about naval architecture (11 volumes), and science and art (18 volumes) was the first pioneer in what he describes as the “prehistory” of plant tissue culture (Gautheret 1985).

Gautheret’s view is that the description of swelling and the appearance of buds following the removal of bark and cortex from an elm tree (Gautheret 1985) in du Monceau’s book *La Physique des Arbres* (1756) was the discovery of callus formation and “a foreword for the discovery of plant tissue culture. But in 1756 the bacteriological technique was not invented, asepsis was unknown, the concept of tissue culture had not been yet expressed and finally nobody was able to appreciate Duhamel’s discovery” (Gautheret 1985). This assertion is not very convincing. Wounding-induced callus formation on mature trees bears minimal or no resemblance to tissue culture. Also, the development of grafting and budding techniques can be described as being as relevant (or irrelevant) as wound-induced callus.

A more reasonable, objective and convincing suggestion by Gautheret is that “the history of plant tissue culture begins in 1838–1839 when [M. J.] Schleiden (1838) (Fig. 22) and [T.] Schwann (1839) [Fig. 73]... stated the... cellular theory and implicitly postulated that the cell [is] totipotent” (Gautheret 1983; for an excellent review of the totipotency concept, see Krikorian 2005). Schwann even suggested that “plants may consist of cells whose capacity for independent life can be clearly demonstrated...” (translated from German by Gautheret 1985). A. Trécul in 1853, H. Vöchting (Fig. 66) in 1878, K. Goebel (Fig. 47) in 1902, J. Sachs (1832–1897; Fig. 67) between 1880 and 1882, J. Wiesner in 1884, and C. Reehinger in 1893 considered this theoretically and showed it to be the case experimentally. Reehinger proposed that isolated plant parts could be cultured in vitro by suggested that excised sections could develop in a solution (Gautheret 1983).

Early tissue culture attempts

Gottlieb Friedrich Johann Haberlandt (1854–; Fig. 30), considered by some to have originated physiological plant anatomy, was first to attempt the culture of plant cells (Haberlandt 1902; Krikorian 1975, 1982; Gautheret 1985; Laimer and Ucker 2003; for an annotated English translation accompanied by an excellent and perceptive scholarly essay, see Krikorian and Berquam 1969). In his first attempt, Haberlandt tried to culture isolated mesophyll and leaf palisade cells of *Lanium purpureum*, stinging hairs of nettle, *Urtica dioica*, glandular hairs of *Pulmonaria*, stomatal cells of *Fuchsia magellanica* Globosa, pith cells from petioles of *Eichhornia crassipes*, and three monocotyledonous species, *Tradescantia virginiana* (stamen filament hairs), *Ornithogallum umbelatum* (stomatal cells),

and *Erythronium des-canis* (stomatal cells). Haberlandt used Knop’s solution as modified by Julius Sachs and still useful today (1 g potassium nitrate, 0.5 g calcium sulfate, 0.5 g magnesium sulfate, 5 g calcium phosphate, and a trace of ferrous sulfate per liter) and added to it sucrose, glucose, glycerine, asparagine, and peptone (except for glycerine, these additives are still being used). He maintained his cultures in the light (natural daylight and photoperiods in April–June and September–November in Germany) and the dark at 18–24°C.

Haberlandt had no success and reported that: “cell division was never observed” (Krikorian and Berquam 1969; Laimer and Ucker 2003). Several reasons are probably responsible for his lack of success (Krikorian and Berquam 1969; Laimer and Ucker 2003).

- The cells he selected were mature, specialized, non-meristematic, and highly differentiated.
- His culture medium did not contain vitamins, hormones, *myo* inositol, and other additives and/or substances which at present are known to be required by tissue and cells in vitro. At the time, some of these substances were yet to be discovered and others were not known to be required.
- According to his biographers “Haberlandt could not have been less judicious in his selection...” (Krikorian and Berquam 1969; Laimer and Ucker 2003) of plants. He used three monocotyledonous species which are recalcitrant. However, it must be noted that Haberlandt had nothing to guide him in his selection of plants and explants to culture.
- “Haberlandt did not think it necessary to achieve complete sterility” (Krikorian and Berquam 1969; Laimer and Ucker 2003) and stated in fact that “the cultured plant cells were impaired only slightly in their progress by the presence of numerous bacteria in the culture solutions” (translation by Krikorian and Berquam 1969; Laimer and Ucker 2003).

Claims that the failure was due to neglect of “Duhamel’s results as well as Vöchting’s and Reehinger’s experiments... and [his] ignorance of the past” (Gautheret 1985) are baseless, spurious, without any scientific merit, unwarranted, and excessively harsh. They seem to have been driven by chauvinism more than good science. Haberlandt would have failed with most explants including ones taken from Duhamel’s species because tissues require a medium different from the one he used and do not grow in contaminated media. He was a pioneer who did not know what was required. And some media components which are known to be required at present were neither familiar nor available at the time. It is very likely that Haberlandt had no knowledge of the method used to culture *Phalaenopsis* flower stalks at the time (Anonymous

1891b). His medium could have supported growth of *Phalaenopsis* flower stalk buds, but they would have been destroyed by contamination.

Luck and the selection of different plants could have resulted in at least partial success, but not “neglecting” Duhamel’s ideas would not have led to success (Krikorian 1982). Haberlandt probably decided to ignore Duhamel’s findings because he did not consider them to be relevant (they were not!). Perhaps he might have succeeded with carrot explants, but made no attempts to culture them. He suggested the use of embryo sac fluids and used liquids from *Raphanus* and *Cochlearia* to culture embryos (Krikorian and Berquam 1969; Laimer and Ucker 2003). Therefore “it is tempting to speculate that perhaps Haberlandt... might have conceived coconut as being a source of readily available ‘embryo sac fluids’ had coconuts been generally available in Berlin” (Krikorian and Berquam 1969; Laimer and Ucker 2003); or if he had paid attention to them in Indonesia.

Others followed in Haberlandt’s footsteps with more success. S. Simon reported that poplar explants formed callus, buds and roots (Simon 1908). String bean segments were cultured by H. Winkler who did observe cell divisions (Winkler 1908; Gautheret 1985).

The idea of using buds or stem tips for mass rapid clonal propagation is more than 100 years old. As long ago as the 1890s, Carl Reehinger in Vienna tried to culture parts of roots as well as stem sections and excised buds of *Populus nigra* and *Fraxinus ornus* on sand moistened with tap water (Reehinger 1893; Krikorian 1982; Gautheret 1983). Like Haberlandt, Reehinger had no success, but concluded that sections must be thicker than 1.5 mm for successful development. By using tap water as his medium, sand for support and explants, Reehinger foreshadowed present day “tissue culture” procedures which involve

- A culture medium which incorporates organic components like a sugars, which is why aseptic technique are required
- An explant
- Agar or gellan gum (i.e., Gelrite or Phytigel) as gelling agents in solid media.

Stem- and root-tip cultures were attempted a quarter of a century after Reehinger by William J. Robbins (1890–1978; Fig. 68) at the University of Missouri (Krikorian 1982; Gautheret 1983). He excised root and stem tips from aseptic seedlings of peas, corn, and cotton, and tried to culture them in the dark on sterile glucose or fructose containing and sugar-free Pfeffer’s (Knop 1884; Pfeffer 1900; White 1943; Krikorian 1975, 1982; Murashige 1978; Arditti 1977a, b, 1992; Arditti and Krikorian 1996; see Arditti et al. 1982 for composition of this medium). The cotton explants did not grow, but those taken from corn and

peas grew normally (Robbins 1922a, b). Eventually, the cotton explants produced roots but were chlorotic and showed characteristics which were “typical of plants grown in the dark.” Robbins and his associates succeeded in maintaining their root tip cultures for almost 4.5 months (Robbins and Maneval 1923, 1924).

Walter Kotte (1893–1970; Fig. 69) cultured pea roots (independently of Robbins, but at the same time) on Knop’s salts (Knop 1884) supplemented with alanine, asparagine, glucose, glycine, a meat extract, a digest of pea seeds, peptone, and perhaps also vitamins, plant hormones, and inositol. His roots grew, but could not be subcultured (Kotte 1922a, b; White 1943).

Philip R. White (1901–1968; Fig. 70) of The Rockefeller Institute for Medical Research at Princeton New Jersey thought that apical and intercalary meristems “would be best to choose [as] materials for our first experiments” (White 1931, 1933b). While visiting the plant physiology institute at the University of Berlin (winter 1930–summer 1931) he tried to culture root tips and “some 400 stem tips” (White 1932a, b, 1933a) of *Stellaria media* in hanging drops of the U+U medium (formulated for pure cultures of *Volvox minor* and *V. globator*; Uspenski and Uspenskaja 1925). He had used this medium previously to culture root tips, embryos, and other explants (White 1933b). The tips remained alive “for periods up to three weeks... [and] during this time there... occurred active cell division... growth... differentiation... into leaves, stems and floral organs” (White 1933b). These results, disappointing by present standards, were probably due to medium composition because U+U contained no ammonium ions, vitamins, or hormones (some of them were not known or investigated at the time, new to science, or not recognized as being required).

Another substance not present in the U+U medium was *myo*-inositol which was isolated from muscles in 1850. What may be the earliest inclusion of inositol in a plant tissue culture medium was a century after its discovery (Jaquiot 1951). However *myo*-inositol was recognized as a potentially effective component of plant tissue culture media after the sugar alcohols sorbitol, meso- or *myo*-inositol and scyllo-inositol were identified as components of coconut water and isolated (Pollard et al. 1961). Its exact role in plant tissue culture media is yet to be established (Åberg 1961; Arditti and Harrison 1977), but since its addition seems harmless it is being added routinely to the Murashige Skoog and other media since it may be beneficial.

Hormones and vitamins

Thiamine (Vitamin B₁), a consistent additive to culture media at present, was isolated from rice bran in 1910–

1911, but its structure was elucidated only in 1926. Niacin (nicotinic acid), first produced by oxidizing nicotine in 1925, was added to culture media only several decades after that. Ascorbic acid (vitamin C), first isolated in 1928 and studied more extensively in 1933, is used in plant tissue culture media only rarely. The structure of riboflavin (vitamin B₂), a vitamin used in some culture media, originally isolated from eggs, was described in 1935. Biotin, discovered in egg yolks in 1936, is not in common use. Pyridoxine (vitamin B₆), used in many culture media, was isolated from rice and yeast in 1938. Pantothenic acid was isolated from liver and its structure was first elucidated ca. 1940. Folic acid was identified in 1948 after being crystallized from liver in 1943 and yeast in 1947 (for a review of vitamins and orchids, see Arditti and Harrison 1977). Of the plant hormones used in tissue culture, auxins were discovered in 1928 (Went 1926, 1928, 1990) and cytokinins in 1955 (Miller et al. 1955a, b; Miller 1961, 1967). Information that vitamins and hormones are required by explants in culture started to accumulate ca. 1936–1938 (for reviews, see White 1943; Schopfer 1949; Åberg 1961; Arditti and Ernst 1993a, b; Arditti 2008).

Culture of monocotyledonous plants

Despite lacking components which are known to be required at present, White's medium was and still is suitable for a number of tissues. Corn shoot tips cultured on it produced plants (Segelitz 1938). This is one of the earliest successful *in vitro* cultures of monocotyledonous plants. It precedes by a more than a dozen years the monocotyledonous culture which is incorrectly claimed to be the first successful one (Morel and Wetmore 1951a; for reviews of monocotyledonous cultures, see Swamy and Sivaramakrishna 1975; Hunault 1979).

Three reports that plant tissues can be cultured “for unlimited periods of time were made independently” and were published in quick succession after the first culture of a monocotyledonous explant, but not “simultaneously” as stated incorrectly (Gautheret 1985). The first one was by P. R. White (31 December 1938). Gautheret's (1939) report was second (9 January 1939) and Pierre Nobécourt's (1895–1961, Fig. 71) was the third (20 February 1939). These reports were the basis for successful stem tip cultures.

A very important and ancient crop in Hawaii and the Pacific, *Colocasia esculenta* (taro), was the second monocotyledonous plant to be propagated by what can be viewed as an early or primitive form of micropropagation. Dormant buds “borne in the axils of the leaves on the surface of the taro corm” were cultured in an effort to propagate this crop more rapidly (Kikuta and Parris 1941). Slices, 2–5 cm thick and buds “together with approximately 1 cubic

centimeter of corm tissue,” produced plants when planted in sterilized soil. The tissue explants and buds were taken from corms and the culture medium was sterile soil. Therefore, this procedure (Kikuta and Parris 1941) is similar to current micropropagation methods even if it was crude by present-day standards. This method is mentioned only in a few reviews (Arditti and Strauss 1979; Arditti and Ernst 1993a; Krikorian 1994 do mention it versus Gautheret 1982, 1983, 1985 which do not). Taro was first cultured by modern techniques 31 years later (Mapes and Cable 1972; for reviews, see Arditti and Strauss 1979; Krikorian 1994).

Rye, another monocotyledonous plant, was also cultured (de Ropp 1945) before the supposed first culture (Morel and Wetmore 1951a). Stem tips (actually the plumules) of excised embryos were cultured on White's medium containing 2% (w/v) sucrose.

Shoot tip cultures

Shih-Wei Loo (Loo Shih Wei, Chinese style; 1907–1998; Fig. 72) came to the US from China to be a graduate student at the California Institute of Technology. He received his Ph.D. in 1943, after only 2 years as a student. In 1945, he became research associate at the Botany Department of Columbia University in New York. After 1 year, he moved to the Chemistry Department and was there until 1947 when he returned to China to become Professor of Botany at Beijing University. In 1953, Loo accepted a position at the Plant Physiology Institute in Shanghai. He remained there until the end of his life. Loo suffered greatly and more than most during the Cultural Revolution, but returned to his laboratory after the turmoil. He resumed his research and mentored graduate students until his death (Loo 1978; Arditti 1999).

Loo's doctoral dissertation research involved the culturing of excised stem tips of *Asparagus officinalis* (Loo 1945a). His explants were 5–10 mm long. He cultured them on a medium utilized by James Bonner for the culture of tomato roots. Several of Loo's explants developed buds, but none formed roots probably because the medium did not contain auxin. He concluded correctly that growth of the excised stem tips was “potentially unlimited” (Loo 1945a, b). After he moved to Columbia University, Loo published a second paper on asparagus shoot tip culture (Loo 1946a) in which he showed that a semi-solid agar medium was “as good, if not better, than liquid medium.” His explants remained alive after 22 months in culture and following 35 transfers (Loo 1946a).

Prof. Loo also cultured stem tips of dodder (*Cuscuta campestris*, a parasitic flowering plant). His cultures failed to produce roots and leaves but flowered *in vitro* (Loo 1946b). This may well be the first report that “floral

organs... developed on excised stems tips in vitro” (Loo 1946b). Loo’s contemporary at the California Institute of Technology, Professor Arthur Galston of Yale University (a close friend of Loo since their days; during the last years of his life Loo corresponded and exchanged visits with J.A.) speculated that the dodder explants would have formed leaves if appropriate hormones were added to the medium (Galston 1948). Loo did not do that (auxins were not in wide use at the time and cytokinins were discovered only in 1955), but he concluded that explants require sugar for growth in vitro. Loo also cultured *Baeria chrysostoma* (Asteraceae) and obtained flowering in vitro (Loo 1946c).

There can be no doubt that Prof. Loo’s papers suggest that tissue culture of angiosperms and micropropagation would have advanced more rapidly had he remained in the USA and/or if conditions in China had been different. His important contributions to stem tip culture and ultimately to micropropagation have thus far received credit only passingly in a few reviews (Krikorian 1982; Gautheret 1983) and a few research papers (Steward and Mapes 1971a; Koda and Okazawa 1980). Loo’s work is certainly not as well known as it should be (Arditti and Krikorian 1996). It is important to note that de Ropp, Loo, and Segelitz (independently of each other) were the first to have significant success in culturing of monocotyledons in vitro, not subsequent workers despite unjustified claims to the contrary (Morel and Wetmore 1951a; Gautheret 1983, 1985).

The first successful culture of an axillary bud meristem was by Carl D. LaRue (1888–1955). He cultured water cress on White’s mineral nutrients containing 20 g (w/v) sucrose l^{-1} and “hetero-auxin... 1 part to 20 millions” (La Rue 1936). The auxin was a gift from Frits W. Went (Fig. 56), its discoverer.

Ernest ‘Ernie’ A. Ball (1909–1997; Fig. 59) was interested in shoot tips and apical meristems (Ball and Boell 1944; Ball 1946, 1972), “the capacity for growth and development of vegetative plant cells,” “polarity of the buds and subjacent cells,” “the relation between respiration and development, independence of the tip from the rest of the plant, production of subjacent tissues by the apex,” and the “totipotentiality of all living plant cells” (Ball 1946). He excised and cultured shoot apices of nasturtium, *Tropaeolum majus* L. (“55 μ high and 140 μ thick”), and lupin, *Lupinus albus* L. (“81 μ high and 250 μ thick”); the sections were 400–430 μ^3 in volume (Ball 1946).

‘Ernie’ Ball (J.A. knew him well, was his friend and they collaborated for several years) made “no provisions to achieve and maintain asepsis,” and “inoculations were performed in the laboratory, “but his cultures did not become contaminated. He cultured his explants on Robbins’ modification of ‘Pfeffer’s Solution’ plus microelements and in some cases “unautoclaved coconut milk”

(actually coconut water). Ball solidified his medium with agar which he washed in thirty 24-h changes of distilled water. The washing changed the agar color from brown to white. His explants grew very well [(Ball 1946) and also stated repeatedly by Ball in many conversations with one of us (J.A.) while he was at UCI]. It is important to make this point due to insinuations by Professor Georges Morel that this was not the case (Morel 1974). Morel’s insinuations are untrue, incorrect, entirely without foundation, self-serving, and disrespectful of a pioneering plant scientist. Loo Shih Wei and Ernest A. Ball succeed in culturing shoot tips before Georges Morel did. Perhaps this is why Morel did not cite Loo and found it necessary to malign Ball. And nursery owner Hans Thomale and Dr. Lucie Mayer, not Georges Morel were the first to culture an orchid shoot tip (Arditti and Krikorian 1996). Morel did not cite Thomale and Mayer in his initial papers on orchids. When he did cite them (Morel 1974), his comments were demeaning probably for the same reason he demeaned Ball and Loo. This pattern of maligning successful predecessors and minimizing their successes in the quest of *priorité* is neither ethical nor a behavior which generates respect for anyone who engages in it.

Prehistory of orchid micropropagation

About 120 years ago, British orchid growers placed *Phalaenopsis* flower stalk nodes in peat and produced plantlets from their buds (Anonymous 1891b; for a review, see Arditti 1984). This method of propagating *Phalaenopsis* is a “prehistoric,” simple or crude form of micropropagation because the explant (a bud or a stalk section):

- Was taken from a mature plant
- Placed in/on a “medium” (moss, albeit non-sterile), and
- “Cultured” until it produced a plantlet or died.

The method provided a means of mass rapid propagation for *Phalaenopsis* and also showed that Theodor Schwann (of cell theory fame; 1810–1882; Fig. 73) was right in suggesting in 1939 that isolated buds can “be separated from the plant and continue to grow” (in Gautheret’s translation, 1985).

This method of propagating orchids was not noticed by botanists and orchid growers at that time (and for almost a century after that) probably because of its

- Perfunctory, but not real, similarity to the rooting of cuttings (but actually very different because the *Phalaenopsis* flower stalk buds produced shoots that formed roots and grew into plants as is normally the case with plant development from buds, protocorm-like bodies and/or callus in vitro)

- Place of publication, which was one of the earliest and now obscure and rare orchid journal that is hard to find
- Language (French), because “an increasing number of scientists... read no modern languages other than English” (Krikorian and Berquam 1969).
- Age, because not many scientists take the time to read the old literature regardless of language and prominence (or lack of it) of journals.

At least one orchid grower noticed the articles. According to a short note (Anonymous 1891b), a grower named Perrenoud (no first name given) saw the reports in a so-called “*journaux anglais*” (which we have not been able to trace), modified it by placing sections of *Phalaenopsis* roots in humid enclosures and obtained a plant. No other details are available. However, it is known that *Phalaenopsis* roots can produce buds and plants (for a review, see Churchill et al. 1972). This method is reminiscent of micropropagation. Therefore, it can be viewed as being part of the pre-history of orchid (or any plant) micropropagation (Arditti and Krikorian 1996) and biotechnology.

If this method would have been noticed and given the attention it deserves, it and its discoverer (a still unknown British orchid grower) could have been an important road mark on the path to plant tissue culture and micropropagation. It is obviously more

- Relevant as a “foreword”
- Related to tissue culture
- Like micropropagation

than (what appear to be the chauvinistically) publicized and glorified observations and writings by the Seigneur du Monceau et de Vrigny, Henri-Louis Duhamel du Monceau in France (Gautheret 1985; Fig. 74).

First micropropagation of orchids

The modern history of orchid micropropagation (and in fact micropropagation in general) started when a:

1. New [tissue culture or in vitro], simple and practical method for vegetative [clonal] propagation of *Phalaenopsis* [orchids] was developed at Cornell [University]” 5 years (Rotor 1949) before the first published report regarding orchid shoot tip cultures. The medium Rotor used to culture the *Phalaenopsis* flower stalk nodes was Knudson C (KC), a solution formulated for the asymbiotic germination of orchid seeds by Lewis Knudson (1884–1958; Fig. 50), Professor of Plant Physiology at Cornell University (see Arditti 1990 for biography). Knudson’s first solution, the Knudson B medium (KB) was a modification of Pfeffer’s solution which was formulated by the

German plant physiologist Wilhelm Pfeffer (1845–1920; Fig. 46). Knudson improved KB in 1946 as his solution C (Knudson C, KC; Knudson 1946a, b) which became a useful medium for orchid seed germination. Today, KC is used extensively for orchid seed germination (Arditti et al. 1982) and the micropropagation of some orchids (Arditti and Ernst 1993a; Yam and Arditti 2007; Arditti 2008).

2. A German nurseryman suggested that shoot tip cultures can be used for micropropagation (Thomale 1956, 1957).

Gavino Rotor Jr. (1917–2005; Fig. 75) was born in Manila and received his B.S. in Agriculture from the University of the Philippines in 1937. He came to the US in 1946, received his M.S. degree at Cornell University in 1947 and Ph.D. in 1952 with a dissertation on the responses of orchids to temperature and day length. In a letter (to J. A.), Dr. Rotor wrote that he conceived the idea of propagating orchids while attending a lecture by Knudson on the role of sugars in plant growth. However, he provided no details on how a lecture on sugars led him to the idea of culturing *Phalaenopsis* flower stalk nodes. He sectioned inflorescences into segments and inserted node sections, each bearing a single bud into KC hoping that the buds would develop plants. The buds produced leaves within 14–60 days. Roots appeared after the formation of 2–3 leaves (Fig. 76). Of the 65 buds he cultured, only 7 failed to produce plants (Rotor 1949). In his letter, Rotor wrote that Knudson’s “eyes brightened when [Rotor] showed him the first successful propagation... and told him how [he] got the idea from [Knudson’s] lecture” (Arditti 1990).

Given his *Phalaenopsis* flower stalk node cultures, there can be absolutely no doubt that Dr. Gavino Rotor is the inventor not only of orchid micropropagation but also of mass rapid clonal propagation of plants in vitro (i.e., micropropagation). His method utilized:

- A defined culture medium
- Aseptic technique
- Explants.

And he drew attention to the propagation potential of his method. It can be argued that Rotor’s method was not micropropagation as the term is understood at present because:

- It produced only a single shoot per explant
- Explants contained pre-existing buds
- Callus formation or proliferation was not involved.

However, such an argument would be spurious and contrived because production of multiple plantlets from a single explant, absence of pre-existing buds and production and proliferation of callus are neither parts of the definition

of microporpagation nor a requirement for mass rapid clonal propagation in vitro. Roger Gautheret discounted the historical relevance of Rotor's discovery when one of us (J.A.) informed him of it. He probably did it in an effort to glorify his friend, colleague, and compatriot, Georges Morel (since the word chauvinism is attributed to Nicolas Chauvin, a soldier in Napoleon's *Grande Armee*, this is not surprising).

Rotor's microporpagation method was barely noticed, used, or appreciated at the time. This could be due to the fact that it was published in a magazine for hobby growers. *American Orchid Society Bulletin* (AOSB, now called *Orchids*), which is not a scientific journal. Orchid growers who read the AOSB could have been daunted by the procedure and probably failed to appreciate its importance. Scientists would have seen the method and could have put it to good use, but they probably did not read the AOSB. The method became forgotten. When it was finally rediscovered, claims of priority by Prof. Georges Morel had become a widely accepted urban legend.

Also in the late 1940s, Professor John T. Curtis (1913–1961; Fig. 62) and his associates in the Department of Botany at the University of Wisconsin described formation of multiple growing points on proliferating seedling-derived callus of *Cymbidium* and *Vanda* (Curtis and Nichol 1948). 'Calloid' is the term they applied to protuberances which developed in asymbiotic culture following treatment with barbiturates. They also reported that these tissue masses had the capacity to grow into plants (Curtis and Nichol 1948), appreciated the potential for clonal propagation, and wrote: "the practical ability to produce clonal lines of plants of potentially unlimited numbers would be of obvious value in many types of genetic and plant production work."

It must be noted here that in their initial reports the nearly forgotten Hans Thomale (Thomale 1954, 1956, 1957; Haas-von Schmude et al. 1995; Arditti 2001; Easton 2001) and the unjustifiably widely celebrated Georges Morel (1960) also called attention to the potential of their findings, but they did it after Rotor.

First culture of an orchid shoot tip or the second aseptic culture of an orchid explant

Pelargonium zonale and cyclamen, *Cyclamen persicum*, were cultured by Lucie Mayer (Fig. 77) on a relatively simple medium (Mayer 1956) before the formulation of the Murashige Skoog medium. Ms Mayer's work intrigued Hans Thomale (1919–2002; Fig. 78), German nursery owner. Mayer and Thomale joined forces for the first culture of sections ("Teilstücken" or "Pflanzenteile"), tissues ("Gewebe") and shoot tips of orchids (Thomale 1956, pp. 89–90, fig 39; Fig. 79).

Hans Thomale was born in Herne, Westphalia, Germany, raised in Cologne and resided and grew orchids in Lemgo for many years. He began to study chemistry and medicine before World War II started, but was drafted and had to interrupt his studies. After the war, he grew potatoes in a nursery and married Dr. Liselotte Kuhlman, the daughter of the owner. When he became interested in orchid seed germination, Thomale taught himself how to do it by using Prof. Hans Burgeff's (1883–1976; Fig. 44) book, *Samenkeimung der Orchideen*. In 1946, he established a laboratory and utilized it to germinate and clonally propagate both tropical orchids and species native to Germany.

On 23 September 1956, Thomale reported to a meeting of the *Deutsche Orchideen Gesellschaft* (DOG; German Orchid Society) that explants of *Dactylorhiza* (*Orchis maculata*) (Fig. 79) and several tropical orchids produced shoots in vitro. Thomale recollected, somewhat tentatively, that Mr. Lecoufle of the French orchid firm Vacherot and Lecoufle (see below) was present at that meeting. He also included a photograph of the *Orchis maculata* culture (Fig. 79) in the second edition of his book *Orchideen* (Thomale 1957). Thomale appreciated immediately the potential of his discovery. He wrote (Arditti and Ernst 1993a, b; Haas-von Schmude et al. 1995):

"It should be noted that efforts to find a propagation method for European terrestrial orchids, based on the work by Dr. L. Mayer [Mayer 1956], through the culture of sterile explants on an agar medium were successful. It is well known that vegetative parts of orchids, for example, sterile sections of *Phalaenopsis* flower stalks [Rotor 1949], which bear at least one adventitious bud [Note in Arditti and Krikorian 1996: these buds are lateral on the flower stalk and not necessarily adventitious, at least not in the strict sense of the word], can produce shoots when cultured on an agar medium. Recently it has become possible to culture undifferentiated tissues on certain nutrient media to produce roots and shoots from them. Since sufficient details were not available by the time this book went to press [i.e., the second edition which appeared in 1957; the first edition was published in 1954], it is only possible to mention that whole plants can be produced from tissue explants one cubic centimeter in size. *This is a form of vegetative multiplication whose potential cannot be overlooked*" [emphasis added]!

Thomale's book and his prescient statement about the utilization of explant cultures in vitro as a method of mass rapid propagation were published (Thomale 1957) before the first reports of *Cymbidium* "meristem" cultures (Morel 1960; Wimber 1963), but limited attention was paid to

them. Thomale behaved professionally and ethically by referring to Rotor's work (Thomale 1956, 1957). Had he not done that Thomale could have created the false impression that he originated the concept of clonal propagation in vitro. Thomale did not describe his techniques in detail. Instead, he referred to the procedures published by Mayer. Dr. Lucie Mayer took part in Thomale's first attempts to culture orchid explants (Haas-von Schmude et al. 1995; personal communication from E. Lucke and Dr. N. Haas-von Schmude, Wettengel, Germany). After her retirement to Madeira, Portugal, Dr. Mayer recalled (in a letter to one of us, J.A.) that she and Thomale also excised and cultured *Cymbidium* stem tips. Thomale's work did not become well known for several reasons:

- His first report was published in a German language magazine for orchid hobbyists which is mostly known only in Germany (Thomale 1956)
- The second publication was also in German in a little known book (Thomale 1957, the second edition of Thomale 1954) written mostly for hobbyists and commercial orchid growers.
- Few scientists read Thomale's work. Practical growers who read the books were probably bewildered by the technique, failed to understand it and never used it (there is parallel between Rotor's and Thomale's publications).

Georges Morel (1916–1973; Fig. 60) who is undeservingly, but often given, exclusive credit for being the first to culture an orchid explant in vitro (Arditti and Arditti 1985; Haas-von Schmude et al. 1995; Arditti and Krikorian 1996; Easton 2001; Arditti 2001) knew about Thomale's report at least as early as 1965 (Fig. 80). However, he did not cite it for almost 10 years. When Morel did cite it, in a chapter written for Carl. L. Withner's *The Orchids—Scientific Studies* (Withner 1974), it was 14 years after his celebrity in the orchid world was a well-established fact (Morel 1974; Haas-von Schmude et al. 1995). At that time, Thomale was known only for his medium GD for the germination of *Paphiopedilum* seeds (Thomale 1957). Even when he cited Thomale's work and published his photograph [labeling it as “after Thomale” rather than indicating that Hans Thomale provided a copy because Morel asked for one (Fig. 80), Morel diminished it by adding the qualifying statements like:

- “Pieces from the bulb of *Orchis maculata*, aseptically cultivated on nutrient medium, soon regenerated stems and roots...” and on the photograph caption “regeneration of roots and shoots occurring on a piece of tuber of *Orchis maculata*.” The wording (“stems and roots”) minimizes Thomale's achievement by implying that what was produced were not plants. Morel should have stated “... soon produced plants.”

- “[Cases like this] are very exceptional.” They are certainly not! Regardless, Thomales deserves credit for his achievement, not denigration.

Hans Thomale was finally given the recognition which was due to him, fortunately while he was still alive, due to the efforts four individuals who believed in fairness (Haas-von Schmude et al. 1995; Arditti and Krikorian 1996; Arditti 2001). Unfortunately, by then total (but undeserved) credit for priority of discovery was given to Morel. One reason for this could be Morel's deserved international standing as a prominent plant scientist. His many friends who repeatedly spoke and wrote on his behalf were another reason. His extensive travels and frequent lectures were a third reason because he used them for self-publicity. A fourth reason were orchid scientists who did not know the correct history, idolizing hobbyists and appreciative commercial growers who placed Morel on a pedestal as being the sole inventor. Resistance to new knowledge (Gaffron 1969) and/or revision of established fiction were/are a fifth reason. Examples are:

- A note marking Thomale's 75th birthday (Lucke 1994) which does not even mention his discovery because a statement to that effect was edited out by the editors of *Orchidee* (Dr. Norbert Haas-von Schmude, Wettengel, Germany, personal communication).
- An article marking the 25th anniversary of “mericloneing” (Arditti and Arditti 1985) which was “revised” on the advice of a reviewer.
- The history chapter in a book on orchid micropropagation (Arditti and Ernst 1993a) was edited heavily in an effort not to offend those who favor the urban legend of Morel as the sole discoverer.

Today, since Hans Thomale's work and his accurate prediction about micropropagation are known, it is no longer correct to state that “... Georg[e] Morel has realized for the first time the multiplication of Orchids (*sic*) by stem tips in vitro culture. Dr. (*sic*) Thomale seems to be unaware of the tissue culture history” (R. J. Gautheret, Paris, in a letter to J.A.). Patriotism, nationalism, and dedication to a “... Late collaborator...” (Gautheret, personal communication), friend and *compatriote* are not sufficient justification for ignoring historical facts and an Orwellian (i.e., 1984 style) restating of history. The one unaware of tissue culture history was Gautheret.

Plant diseases and shoot tips

The concept that stem tips, root cuttings, and even leaves can be used to produce healthy clones of horticultural plants is more than 60 years old (see Krikorian 1982; North

1953 for literature citations). During World War II, Arthur W. Dimock (1908–1972) published a method for producing *Verticillium*-free clones of chrysanthemums through the use of tip cuttings taken from 4- to 6-inch-long (ca. 10–15 cm) shoots which were shown to be disease free (Dimock 1943a, b). This method was later improved and used to free plants of other diseases (Brierly 1952; Dimock 1951b). Such methods were also used to produce disease-free carnations (Dimock 1943a, b, 1951b; Forsberg 1950; Andreasen 1951; Guba 1952; Hellmers 1955; Thammen et al. 1956). It is even possible to argue that those who originated these methods were among the first to use the terms “cultured” (Forsberg 1950) and “culturing” (Dimock 1951a; Guba 1952) in relation to producing disease-free plants.

Observations that tips of virus-infected roots could be free of infection were made 75 years ago (White 1934a, b, 1943). Earlier, virus or “abnormalities” were not seen in stem tips of tobacco, tomato, and *Solanum nodiflorum* (Sheffield 1933, 1942; Clinch 1932). Spotted wilt virus was eliminated from *Dahlia* through the use of stem tip cuttings (Holmes 1948a, b, 1955). This method was also used for the elimination of leaf spots in sweet potato, *Ipomea batatas*, which are associated with the internal-cork disease in this crop, (Holmes 1956a) as well as aspermy virus (Holmes 1956b) and other viruses (Brierly and Olson 1956) in *Chrysanthemum*.

The elimination of spotted wilt of *Dahlia* (Holmes 1948a, b) through use of stem tip cuttings left little or no doubt that apical meristems are virus-free. Confirmation of this was obtained in work with tobacco mosaic-infected tobacco, *Nicotiana tabacum*, variety Samsun (Limasset and Cornuet 1949).

These findings are not consistent with current information which is that apical meristems are not always virus-free. This inconsistency is the reason for difficulties which have been encountered in freeing many clones and cultivars of viruses (Kassanis 1967). Also, even when apical meristems are virus-free it is not always possible to obtain pathogen-free plants. In fact “in the orchid industry... Before ‘mericlone’ orchid viruses were a minor problem... However [they] are now common, wide-spread and costly” (Langhans et al. 1977) because careless culturing spread rather than contained or eliminated viruses (Tous-saint et al. 1984).

Viral infection of certain potato and *Dahlia* cultivars was a problem facing French horticulture ca. 1950 (Lecoufle 1974a, b). The culture of stem tips provided a means of freeing these plants of viruses in view of what was known about dahlias (Holmes 1948a, b) and tobacco (Limasset and Cornuet 1949). Pierre Limasset (1911–1988; Fig. 82) and Pierre Cornuet (b. 1925; Fig. 83) “suggested to their colleagues Georges Morel and Claude Martin to



Figs. 82–83 Plant virologists. **82** Pierre Limasset. **83** Pierre Cornuet. (Note: these figures were located after the final editing of the manuscript and are included here despite their very low quality because they are the only ones of these individuals that could be found)

cultivate shoot meristems of infected plants” (Gautheret 1983, 1985). Morel and Martin followed the suggestion. Their attempts were successful and virus-free dahlias (Morel and Martin 1952) and potato (Morel and Martin 1955a, b; Morel and Muller 1964; Gautheret 1983, 1985) plants were produced from infected ones.

“George Morel was an amateur orchid grower [who] had in his greenhouse a plant of *Cymbidium* Alexaderi ‘Westonbirt’... the most famous *Cymbidium* of all time, which was sadly, totally infected by *Cymbidium* mosaic virus” (Vacherot 2000). The success with *Dahlia*, potatoes and other plants (Morel and Martin 1955b; Morel 1964a, b) led Morel “[to apply] the same technique as he was using on his potatoes to the *Cymbidium* [and] produced a protocorm [sic]” (Morel 1960; Vacherot 2000; Fig. 60B) and later a plant (Fig. 60C). As indicated above, this effort was heralded in numerous lectures and publications. One catalog stated that “a beautiful thing happened to the orchids when they operated on a sick potato [because] Dr. Georges Morel, distinguished French botanist, discovered the orchid meristem process while he was trying to figure out a way to prevent virus in potatoes” (Orchids Orlando 1968). Less lyrical, but just as inaccurate paeans and odes clutter the scientific, horticultural, and hobby literature (examples are: Bertsch 1966, 1967; Marston and Vourairai 1967; Vacherot 1966, 1977; Borriss and Hiibel 1968; Vanseveren and Freson 1969; Hahn 1970; Kukułczanka and Sarosiek 1971; Lecoufle 1971; Lucke 1974; Allenberg 1976; Champagnat 1977; Rao 1977; Loo 1978; Murashige 1978; Goh 1983; Hetherington 1992). Correct versions of history are rare (Arditti 1977a, b, 2001; Haas-von Schmude et al. 1995; Arditti and Krikorian 1996; Easton 2001; Yam and Arditti 2007; Arditti 2008). Accuracy was sacrificed in a few instances as a bow to editorial pressure (Arditti and Arditti

1985; Lucke 1994; Dr. Norbert Haas-von Schmude, Wettenberg, Germany, personal communication) or in an effort not to offend established interests and views (Arditti and Ernst 1993a).

The third aseptic culture of an orchid explant

“The secret of originality is hiding your sources”
(Attributed to Mark Twain or Albert Einstein)

Many papers on orchid micropropagation start with a citation or at least a mention of Morel’s first paper on *Cymbidium* shoot tip culture (Morel 1960). Assertions that “the first application [of micropropagation] concerned the clonal propagation of orchids (Morel 1960)” are found in historical accounts by one of the founders of plant tissue culture (Gautheret 1983, 1985). Such reviews are influential because they are read extensively and then quoted or re-stated in subsequent publications. As a result, an urban legend was elevated to universal (albeit incorrect) truth and scientific dogma. Once this occurs, forces which resist knowledge strive to preserve the status quo and thus defend the urban legend (Gaffron 1969; Arditti 2004).

Questioning the accepted views can lead to unpleasant interactions (Arditti 1985; Torrey 1985a, b). Demands to revise manuscripts became conditions for publication (Arditti and Arditti 1985; Lucke 1994). Still, the prevailing historical accounts were reexamined critically and facts were placed in an accurate perspective (Arditti and Krikorian 1996). The resulting review (Arditti and Krikorian 1996) is one of the major sources used for an history chapter in the second edition of a book on orchid micropropagation (Arditti 2008) as well as the present account and two previous ones (Arditti 2004; Yam and Arditti 2007). Sadly, presenting an accurate history may create an inaccurate impression that the intent is to sully some reputations because [to quote physicist Ernst Mach (1838–1916) as quoted by the late prominent plant physiologist Hans Gaffron in 1969: “It is hardly possible to state any truth strongly without apparent injustice to some other.”] This only seems so because inaccuracies are so prevalent in the orchid literature.

Georges Morel (1916–1973; Fig. 60), entered l’Institut de Chimie in Paris where he studied agriculture and plant pathology. After that he joined L’Institut National de la Recherche Agronomique (INRA), the French institute of Agricultural Research (Gautheret 1977), where he became very influential (Vacherot 2000) and “*chef de travaux*” in 1941. In 1943, Morel joined Gautheret’s laboratory (Lecoufle 1974a, b) and worked there towards his doctorate. Despite the difficulties caused by the Nazi occupation (Paris was liberated in 1944), Morel was successful in his research.

Morel received his doctorate in 1948, went to the USA during the same year and worked until 1951 with Professor Ralph W. Wetmore (1892–1989; Fig. 81) in the Biological Laboratories at Harvard University. They worked on tissue culture of monocotyledonous plants (Morel and Wetmore 1951a) and ferns (Morel and Wetmore 1951b). On his return to France, Morel was appointed *Maître de recherches* (in 1951 or 1952) and in 1956 *Director de recherches* of the Station Centrale de Physiologie Végétale of the Centre National des Recherches Agronomiques, Ministère de l’Agriculture (Lecoufle 1974a, b).

Despite its great fame in the orchid world, Morel’s first report shoot tip culture of *Cymbidium* (Morel 1960) was more a news item than a scientific paper. It lacked detail, was sketchy, and stated that the explants were cultured on a non-existent medium he named “Knudson III.” Morel’s conclusion was “that it is relatively easy to free a *Cymbidium* from the mosaic virus... each bud will give several plants so the stock of a rare or expensive variety can be increased... [and that] experiments of the same kind are now being conducted with... *Cattleya*, *Odontoglossum*, and *Miltonia*, contaminated with different viruses” (Morel 1960). Its major contribution was the introduction of a new term into orchid terminology, “protocorm-like body” (PLB) to describe the “small flat bulblet looking exactly like [a] protocorm” (Fig. 80B) which was produced by the cultured *Cymbidium* stem tips (for an history of the term “protocorm”, see Arditti and Ernst 1993a, b; Arditti and Krikorian 1996, Arditti 2008).

It would have been impossible for anyone to repeat Morel’s work for lack of sufficient details. To reconstruct the procedures and medium or media used by Morel it would have been necessary to carefully study much of Morel’s previous work including a rather obscure paper on potatoes (Morel and Martin 1955a) and an even less well-known one on “*parasites obligatoires et de tissus végétaux*” (Morel 1948) as well as two not very easy to find papers by Dutch authors, one on *Iris* (Baruch and Quak 1966) and the other on potatoes (Quak 1961). In addition, it would have been necessary to assume that the potato and *Iris* papers were relevant. But why would any one assume that? Even if orchid scientists could find the composition of the potato or *Iris* media there were no indications that they would be suitable for orchids. In fact, the potato medium is very different from the one subsequently used for orchids by Morel. And orchid commercial and hobby growers could not be expected to engage this type of literature search.

Interestingly, Vacherot and Lecoufle (V&L) ‘La Tuilerie’, Boissy-Saint Leger (Seine-et-Oise), a French orchid firm had enough information to start commercial micropropagation of “rare or expensive” orchids before any other establishment. They moved quickly enough to have a

clonally propagated plant of *Vuylstekeara* Rutiland ‘Colombia’ bloom in December 1965 (Vacherot 1966; Lecoufle 1967), but a recent report suggests that the first plants to be cultured were “some of [their] finest cymbidiums” (Vacherot 2000).

A report that “... at ‘La Tuilerie’ our first mericlone to flower [was]... *Vuylstekeara* Rutiland ‘Colombia’... in December, 1965... (Lecoufle 1967) suggests that “mericlone” started at V&L before or at about the time Morel’s first paper was published because “... it will take just as long to grow the plants produced from meristem tissue as it takes to grow a new hybrid from seed” (Scully 1964). It may have started as early as 1956 despite what seems to be a delayed publication of Morel’s first paper in 1960 and subsequent papers (Morel 1963, 1964a, b, 1965a, b, 1970, 1971a, b, c, 1974; for much more detailed history and discussions as well as speculations regarding the reasons for the delayed publications and lack of details, see Arditti and Krikorian 1996; Arditti 2008).

The lack of citations in Morel’s first paper must also be considered. Loo and Ball (whose techniques were used), Limasset (see Fig. 82) and Cornuet (see Fig. 83; who suggested the idea of culturing shoot tips), Rotor (the first investigator to clonally propagate an orchid in vitro), Thomale and Mayer (first to culture an orchid shoot tip), and others were not cited. This is not in line with the standard practice by scientists. Two visitors to Morel’s laboratory in the mid-1960s (one a student and the other a sabbatical year researcher) suggested in conversations with one of us (J.A.) about 25 years ago that

- Such lack of citation was not unusual for French scientists, but we have seen and/or read many papers by French scientists with extensive lists of cited publications
- Morel did not spend a lot of time in the library, but he was clearly aware of Thomales work (Fig. 80), and many papers from his laboratory contain adequate and detailed citations (for examples, see most of the papers we cite, i.e., Morel 1948, 1950, 1963, 1964a, b, 1970, 1971a, b, 1974; Morel and Wetmore 1951a, b; Morel and Martin 1952, 1955a, b; Morel and Muller 1964; Champagnat 1965, 1971, 1977; Champagnat et al. 1966; Champagnat et al. 1968; Champagnat and Morel 1969, 1972; Champagnat et al. 1970; as well as those by his associates, Champagnat 1965, 1971, 1977). He also commented on one of our papers (Churchill et al. 1971a, b) a very short time after it was published, a fact which indicates that he read the literature.

When asked by one of us (J.A.) after a lecture he gave at the World Orchid Conference in Sydney in 1969 about Ball’s contribution to his work, Morel’s reply was a testy: “Ah, Ball.” This non-reply and a suggestion by someone who knew him that Morel liked prominence suggest that he

did not cite those he should have cited in an effort to claim the discovery for himself. There is also another view. Morel was described by the late Professor John Torrey of Harvard University as (1) “... one of the pioneers in the study of shoot meristem culture as well as an early advocate for its practical use in multiplication of virus free plants... interested in the free exchange of scientific information and discoveries [who] ‘did not take any patent because I feel that a scientist does not have to do this... ’” (Torrey 1985b), and (2) a very nice, kind, and modest man.

Innovators

“Good artists copy; great artists steal”

Pablo Picasso

In the opinions of many, Morel’s orchid work was very original and highly innovative, but an impartial analysis of the historical facts leads to a different conclusion. None of Morel’s work with potatoes, dahlias, and orchids was original. Media for plant tissue culture in general and stem tips of orchids in particular were formulated (Knop 1884; Loo 1945a, b, 1946a, b, c; Knudson 1946a, b; Rotor 1949; Mayer 1956, Thomale 1956, 1957) before Morel devised his own substrate by modifying those that were already in existence. A number of explants (buds, nodes, shoot tip) from several monocotyledonous species (Robbins 1922a, b; Segelitz 1938; Kikuta and Parris 1941) and orchid in particular (Rotor 1949; Thomale 1956, 1957) were cultured before Morel accomplished it (Morel and Wetmore 1951a). Several methods were published prior to his. Plants were freed of virus infection through shoot tip culture or rooting before Morel’s work with potatoes, dahlias, and orchids. And Morel’s work on potatoes and dahlias was suggested by others, namely P. Limasset and P. Cornuet (Gautheret 1983, p. 402, 1985, p. 42).

Georges Morel’s first notable achievement was the production of protocorm-like bodies (PLBs) which could be subcultured. This made micro-(mass rapid clonal) propagation of orchids possible. Morel accomplished this by cleverly using existing methods and culture procedures and combining them into a very useful new application. His second important achievement was publicity for an idea which was needed. Morel deserves credit for thoughtfully applying existing methods and information to a new technology. However, he should not be accorded the adulation normally given to individuals who conceive new ideas, make basic discoveries, and articulate new principles (Easton 2001; Arditti 2001, 2004, 2008; Yam and Arditti 2007).

Roger Gautheret, one of the early tissue culture investigators, wrote that “Ball is really the father of the so-called

micropropagation method” (Gautheret 1985, pp. 16–17), but the same arguments can be made about LaRue and Loo (La Rue 1936; Loo 1945a, b, 1946a, b, c; Ball 1946). It is possible that Gautheret credited Ball because he demonstrated that it is possible to culture stem tips in vitro. But, LaRue and Loo did the same without being credited by Gautheret. Ball (who collaborated with us during his tenure at UCI) probably did not appreciate the practical implications of his work, or if he did, he failed to state it in print (Ball 1950). The same is true of others (La Rue 1936; Loo 1945a, b, 1946c; Krikorian 1982; Arditti and Ernst 1993a, b; Arditti and Krikorian 1996; Arditti 2008).

Credits

Ball, LaRue and Loo were interested in the basic science of meristem growth and development. Therefore, they were not as much “fathers” of micropropagation as they were “uncles”. Morel is also not a “father” because Gavino Rotor Jr. was first to clonally propagate an orchid, or any plant, in vitro. Hans Thomale was first to culture an orchid shoot tip explant and to call attention to the micropropagation potential of these cultures. And the first to publish a detailed shoot tip culture method in proper scientific format was Professor Donald Wimber (1930–1997) of the University of Oregon (Wimber 1963, 1965).

The first properly published method for shoot tip cultures or the fourth aseptic culture of an orchid explant

The Dos Pueblos Orchid Company (DP) in Goleta (near Santa Barbara), California was owned by a wealthy oilman named Samuel Mosher (1893–1970; Fig. 84). It was described at the time as “the world’s largest establishment for the breeding and growing of *Cymbidium* orchids” (Anonymous, n.d.). A modern and well-equipped laboratory was part of DP. An excellent cytogeneticist named Dr. Donald E. Wimber (1930–1997; Fig. 85) worked in it on orchid chromosomes and seed germination. Observations of seedlings and young plants led Wimber to tissue culture. His first attempt pre-dated both Thomale’s and Morel’s work, but was never published. It involved the production of PLB from young leaves and thin transverse sections of shoot axes of *Cymbidium lowianum* which were cultured on the Vacin and Went medium (letter dated 13 December 1976 to J.A.). Sections of these PLBs produced plantlets when cultured on agar medium.

Mosher and the manager of DP, Kermit Hernlund, were not impressed due to the slow growth of the tissues. Wimber appreciated his new method of plantlet

production: “I knew I had something, but was rather fearful that some sort of chromosomal change might have occurred so that a faithful reproduction of the parent might not occur.” Thus, “If the cytogeneticist in Wimber had been less persuasive than the propagator he could have been the one credited with the discovery of mass rapid clonal propagation of orchids” (Arditti 2008).

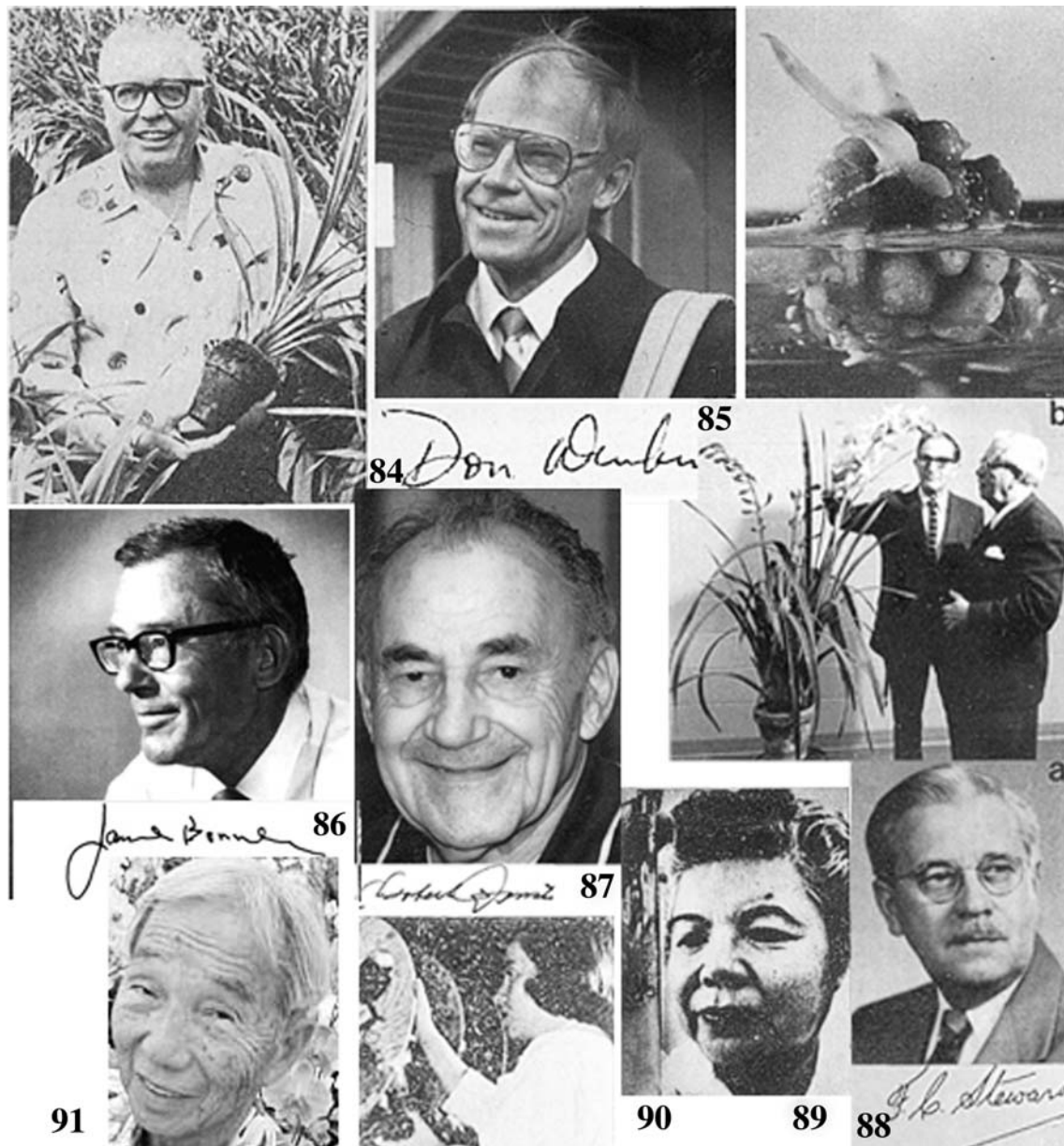
Wimber published his first paper on clonal propagation of *Cymbidium* in 1963 (Wimber 1963). Like Morel’s first paper on shoot tip culture of *Cymbidium*, Wimber’s was also published in the *American Orchid Society Bulletin*. However his paper was very different from Morel’s. Unlike Morel, Wimber wrote a real scientific paper which included all the information anyone would need to repeat his work. A subsequent paper elaborated on the initial procedures (Wimber 1965). Wimber also called attention to the propagation possibilities inherent in shoot tip cultures. All this despite the fact that he developed his method while working for a commercial concern which had every right to keep the details secret. Anyone with the requisite training could easily repeat Wimber’s work after reading his paper. Because Wimber’s was a real scientific (even if non-peer reviewed) paper (Wimber 1963), rather than a detail-free news bulletin (Morel 1960), it is reasonable to argue that Prof. Donald Wimber was the first to publish on clonal propagation of orchids through stem tip culture.

Who are the pioneers?

Dr. Gavino Rotor’s discovery in 1949 is directly traceable to Prof. Lewis Knudson through his teaching and the Knudson C culture medium. His approach was not based on any previous or similar work and therefore was the most original. On the other hand, he did not excise explants (i.e., he did not remove the buds from the flower stalks) and obtained only one plant per explant. Also, his method used the simplest medium.

Chronologically the third discoverer, Dr. Donald Wimber, originated the idea of shoot tip cultures as a result of his own research on orchid protocorms and seedlings. He is a close second to Rotor in originality because his work is based on observations he made himself. His method did involve explants (i.e., excised shoot tips) and produced multiple plantlets.

The work of Hans Thomale, (chronologically the second discoverer) and Georges Morel (the fourth and last discoverer) can be traced to Haberlandt, Loo, and Ball. A well-read, practical horticulturist, Thomale developed his method after reading Dr. L. Mayer’s paper. Morel’s procedure has several origins: (1) Ball’s and Loo’s research, (2) Knudson’s and/or Knop’s media, and (3) Limasset’s and Cornuet’s suggestion. Being based on previous work of



Figs. 84–91 Orchid growers and plant scientists. **84** Samuel Mosher (1893–1970). **85** Donald E. Wimber (1930–1997) and one of his cultures. **86** James F. Bonner (1910–1996). **87** Robert Ernst (b.1916).

88 Fredrick C. Steward alone (*a*) and with Russel C. Mott and a *Cymbidium* plant grown from a single cell (*b*). **89** Marion O. Mapes (1913–1981). **90** Kathryn Mears. **91** Yoneo Sagawa

the same nature by others with different plants as they are, Thomale's and Morel's methods are the least original.

Rotor's method was used sporadically for a while. It was not very successful or practical and was eventually abandoned and forgotten. Thomale's method was apparently never used. Wimber's and Morel's methods are the most practically useful (immediately after publication and too many years after an initial announcement, respectively).

Rotor and Thomale never received the credit they deserve for their discoveries. Their papers are mentioned in the literature seldom if ever. Wimber received much less

credit than he deserves. Morel received much more credit, publicity, fame, adulation, glory, and funding than he deserved.

Rotor indicated in private correspondence (to J.A.) that the lack of recognition did not concern him. Thomale and Mayer expressed gratitude (also in letters) when their contributions were made known. Wimber was not disturbed (in a conversation with J.A.) by the lack of recognition. Given Morel's pursuit of glory it is safe to assume that he was pleased by his fame (for additional details, see Arditti and Krikorian 1996; Arditti 2008).

The following direct quote (Arditti 2008) is one view about the allocation of credit for the discovery of micropropagation:

1. “Dr. Gavino Rotor Jr. for developing the first tissue culture (or in vitro) clonal propagation method for orchids or any other plant even if he did not use an explant as the terms is understood today. The Cornell University Department of Horticulture website reported him as deceased in its 12 April 2002 update.
2. Hans Thomale for the: (1) first clonal propagation method of orchids involving a bud or tip explant, and (2) the earliest clear suggestion that tissue culture has the potential of being used for mass rapid clonal propagation.
3. Prof. Donald E. Wimber for being the first to publish a detailed and reproducible method for the micropropagation of orchids through the culture of shoot tip explants.
4. Dr. Georges Morel for: (1) suggesting (after being alerted to the possibility by Limasset and Cornuet) that shoot tip culture can be used to free orchid plants of viruses, (2) generating considerable publicity for mass rapid clonal propagation through tissue culture, (3) calling the attention of commercial growers to the method, (4) coining the term “protocorm-like body.”
5. The firm of Vacherot and Lecoufle for the first commercial use of shoot tip cultures for mass rapid clonal propagation (on their own and/or with the advice of Dr. Georges Morel and/or Dr. Walter Bertsch).

Root cultures

Professor Lewis Knudson who worked on tannic acid (Knudson 1913a, 1913b), before becoming interested in sugars (Knudson 1915, 1916), used aseptically cultured roots to investigate enzyme secretion and carbohydrate metabolism (Knudson 1917; Krikorian and Berquam 1969; Krikorian 1975, 1982; Arditti 1990). He also studied root cap cells and demonstrated that they slough off while still alive and can be kept alive in culture for several weeks. However, they did not divide and died (Knudson 1919; Gautheret 1985) perhaps due to their nature and because auxins and cytokinins were yet to be discovered and used by Knudson. His root cultures and research on carbohydrate metabolism caused Knudson to read the literature on the germination of orchid seeds. This led him to orchid seeds and the discovery of the asymbiotic method for orchid seed germination (Knudson 1921, 1922a, b).

W. J. Robbins (1890–1979) followed a different path. His aim was to test a hypothesis suggested by Jaques Loeb in 1917 that a hormone produced by leaves had an

effect on root development in the leaf notches of *Bryophyllum* (Krikorian and Berquam 1969). To carry out the test he decided to compare growth of excised root tips in sugar-containing and sugar-free salt solutions (Loeb 1917; Krikorian and Berquam 1969). He thought that growth in a sugar containing medium “... would demonstrate that sugar was the ‘hormone’ furnished by the leaf and necessary for the growth of roots in the leaf notches” (Robbins 1957, cited by Krikorian and Berquam 1969). After that, he developed a method for long-term culture of corn roots (Robbins 1922a, b; Krikorian and Berquam 1969; Krikorian 1975, 1982; Gautheret 1983, 1985).

At the same time, W. Kotte (1893–1970), at one time director of the Pflanzenschutzamtes in Freiburg and who also worked in Haberlandt’s laboratory, was successful in culturing short root tip explants of corn and peas on several glucose, alanine, asparagine, and Justus Liebig’s meat extract containing modifications of Knop’s solution (Kotte 1922a, b; Krikorian 1975, 1982; Gautheret 1983, 1985). He wanted to study the growth of meristematic tissues because “... isolated meristematic tissues have not yet been cultured” (Kotte 1922a, translated by Krikorian and Berquam 1969).

Several additional workers tried to culture root tips, but obtained only limited growth. P. R. White was the first to have success with “indefinite” cultures of tomato root tips in 1934 (White 1934a). He was encouraged by Nobel laureate Wendell Stanley Sr. who needed a system for plant virus studies and multiplication. Three years after that, James F. Bonner (1910–1996; Fig. 86) and Robbins and White demonstrated (separately and independently) the importance of thiamine or its components thiazole and pyrimidine in root cultures (Bonner 1937; Robbins and Bartley 1937; White 1937; Gautheret 1985). An interesting sidelight to this reported by Professor Frank B. Salisbury in a biography of James Bonner is quoted here in full: “Phillip White had grown tomato roots through repeated transfers by adding yeast extract to a medium that contained the essential mineral nutrients and sucrose as an energy source. James set out to find what it was in the yeast extract that allowed the growth of the excised tomato roots. He obtained some vitamin B₁ (thiamine), which had just been synthesized, and it made the pea roots grow nicely, although growth slowed after six to eight transfers. James was ecstatic about his discovery and wrote to Phillip White to ‘tell him the joyous news’ White never answered, but he published similar experiments quickly in the *Proceedings of the National Academy of Sciences*. James’s paper was written first, but it appeared only later in *Science*, with a longer paper in the *American Journal of Botany*. James’s conclusion: ‘Be careful how you spread the joyous news’“(http://www.nap.edu/readingroom/books/biomems/jbonner.pdf).

An additional intrigue associated with this work is an allegation that Bonner's technician fabricated results and quit as soon as the papers were published. He founded a company which sold a vitamin B₁ preparation to horticulturists and gardeners using Bonner's paper as proof that vitamin B₁ enhanced plant growth. When asked about it, Bonner found a humorous reason not to answer (one of us, J.A., knew Bonner). Numerous researchers subsequently worked on root cultures: H. E. Street is the most prominent among them (Street 1973, 1977, 1979; Krikorian 1982; Gautheret 1983, 1985).

Findings similar to Bonner's with tomato roots were made in experiments with *Cymbidium* seedlings also in the California Institute of Technology (where Bonner spent his entire scientific career) by a student in the laboratory of Prof. Frits W. Went, the discoverer of auxin. The raw data languished for many years in a notebook until Went visited the University of California, Irvine, and told one of (J.A.) about the work. As a result of the conversation, he mailed the notebook to J.A. who interpreted the data and wrote a paper based on them (Hijner and Arditti 1973). Went refused to be listed as a coauthor due to his long-standing policy not to add his name to papers by his students. However, he insisted that J.A. must be listed as a coauthor.

The first printed suggestion that orchid roots can and should be cultured was in a theoretical article rather than one which reported research findings (Beechey 1970). We initiated a research project involving the culture of *Epidendrum* root tips and about the same time. Mary Ellen Farrar (later Churchill), an undergraduate student, modified a medium originally developed for the culture of wheat root tips (Ojima and Fujiwara 1962) for the purpose. The roots elongated, became thinner, remained alive for a long time, but lost their chlorophyll after 2 years (Churchill et al. 1972). *Phalaenopsis* roots, which sometimes produce plantlets spontaneously in nature (Anonymous 1885; Reichenbach Fil 1885; Fowlie 1987) proved difficult to culture initially, but were cultured eventually (Tanaka et al. 1976). Roots of *Neottia nidus-avis* (Champagnat 1971) and other orchids (for a review, see Churchill et al. 1973) which also produce buds and/or plantlets in nature seem not to have been cultured. Rhizome tips and roots of many orchids have been cultured during the last 20 years (for reviews, lists of orchids that were cultured and procedures, see Arditti and Ernst 1993a; Arditti and Krikorian 1996; Arditti 2008).

Leaf cultures

A number of the early attempts to culture plant cells and tissues by Haberlandt and others were made with leaf explants. These attempts failed because the cells were differentiated (Krikorian and Berquam 1969; Krikorian

1975, 1982; Steward and Krikorian 1975; Gautheret 1983, 1985). However, attempts to culture mature differentiated palisade parenchyma of some plants were successful (Joshi and Ball 1968a, b).

Leaf cuttings can be made of *Restrepia species* (Webb 1981). However, this did not lead to the development of tissue culture procedures for leaf explants. The tendency of juvenile leaves on protocorms to produce protocorm-like bodies led to the development of micropropagation methods through culture of leaf bases (Champagnat et al. 1970). A claim that these procedures were developed even earlier (Morel 1960, 1965a, b, 1966, 1970) is not supported by the available evidence (“Keine Angabe vorliegend”, meaning “no statements are available” in Zimmer 1978a, b).

The first unambiguous and well-documented report that leaves can produce protocorm-like bodies was made in cultures derived from *Cymbidium* shoot tips (Wimber 1965). An earlier observations in 1955 was that embryonic leaves of *Cymbidium lowianum* placed on Vacin and Went medium formed protocorm-like bodies was not published (personal communication from the late Prof. Donald E. Wimber; Arditti 1977a).

Leaf tips were first used to propagate orchids (*Epidendrum* and *Laeliocattleya*) as a result of unsuccessful attempts in our laboratory to culture foliar explants similar to those taken from peanuts (Joshi and Ball 1968a, b). After these explants failed to grow, we attempted to culture leaf tips and succeeded almost immediately.

A major advantage of leaf tip cultures is that removal of explants does not endanger the donor plant. Because of that, orchid growers and propagators were interested in these methods. To make them widely available, they were published in a number of journals and several languages (Arditti et al. 1971; Ball et al. 1971; Churchill et al. 1971a, b, 1972; Ball et al. 1971).

To succeed with these procedures, the explant must be taken before the leaf tips differentiate fully and no longer have an ability to form callus. The proper stage to take explants is while the tip is still pointed and before a notch is formed. If this is not done, the explants die rather than develop when placed in culture. Therefore, these methods require attention to detail and cannot be reproduced easily. Several failures to repeat them led to questions following their publication. The doubts were resolved following reports that the leaves of many orchids were cultured successfully (for reviews, lists of orchids that were cultured and procedures, see Arditti and Ernst 1993a, b; Arditti and Krikorian 1996; Arditti 2008).

Stems

The culture of *Arundina* stem sections was first reported at the 5th World Orchid Conference in Long Beach,

California, in 1966, but only limited information was presented at the time (Bertsch 1966; for a review, see Zimmer 1978a). Details became available in a paper which reported on the culture of seeds, shoot tips, and stem disks of this orchid (Mitra 1971). *Dendrobium* nodes were cultured in 1973 (Arditti et al. 1973; Mosich et al. 1973, 1974a, b). Stem sections of other orchids have also been cultured (for reviews, lists of orchids that were cultured and procedures, see Arditti and Ernst 1993a, b; Arditti and Krikorian 1996; Arditti 2008).

Flower buds, flowers, floral segments and reproductive organs

Excised ovaries were the first orchid floral segments to be cultured (Ito 1960, 1961, 1966, 1967). In earlier papers, Ito reported on another first: the culture of immature *Dendrobium* seeds and young seedlings (Ito 1955). This was followed by the culture of immature seeds (often and erroneously called ovules) of *Vanilla* (Withner 1955), *Phalaenopsis* (Ayers 1960), *Dendrobium* (Niimoto and Sagawa 1961), *Vanda* (Rao and Avadhani 1964) and *Paphiopedilum* (Ernst 1982; for reviews, see Withner 1959a; Arditti 1977b; Rao 1977; Zimmer 1978a, b; Czerevczenko and Kushnir 1986). Immature seeds of many additional orchids have been cultured since then (Yam et al. 2007). In some cases, this is the preferred method of sexual propagation since it saves time and facilitates the germination of several species. This is not a micropropagation method. It is a method of sexual (seed) propagation. However, since it involves the scraping of the contents of ovaries it is possible that some of resulting plantlets are produced by ovary tissue and/or cells rather than seeds.

The first young flower buds or inflorescences to be cultured have been those of *Ascofinetia*, *Neostylis* and *Vascostylis* (Intuwong and Sagawa 1973). Similar explants of *Cymbidium* (Kim and Kako 1984; Shimasaki and Uemoto 1991) *Phalaenopsis*, *Phragmipedium* (Fast 1980a, b), and other orchids were cultured subsequently (for reviews, lists of orchids that were cultured and procedures, see Arditti and Ernst 1993a; Arditti and Krikorian 1996; Arditti 2008).

Inflorescences

“In anointing ‘fathers’ and giving credits to investigators for the discovery/invention of micropropagation, a self-appointed arbiter (Gautheret 1983, 1985) did not even mention Dr. Gavino Rotor’s culture of *Phalaenopsis* flower stalk nodes” (Arditti 2008). However, there can be no doubt that Dr. Gavino Rotor’s work led the way. Others followed and cultured explants from inflorescences of

several orchids (for reviews, lists of orchids that were cultured and procedures, see Arditti and Ernst 1993a, b; Arditti and Krikorian 1996; Arditti 2008).

Darkening of culture media

The first to darken orchid seed germination media was Professor John T. Curtis (Fig. 62) at the University of Wisconsin (Curtis 1943). He used lampblack (soot produced by burning of petroleum hydrocarbons) which has only color in common with charcoal. The charcoal used in orchid media is of vegetable origin and made from organic, peat, sawdust and wood, residues obtained during production of pulp. These residues are carbonized and then activated to produce a large surface area (Weatherhead et al. 1990).

An orchid culture medium was darkened with charcoal for the first time by Prof. Peter Werkmeister in Germany (Werkmeister 1970a, b, 1971; we could not find his dates of birth and death or a portrait). Before that, charcoal was employed to darken a medium used to germinate moss spores and grow filamentous algae (Proskauer and Berman 1970; Krikorian 1988). Werkmeister darkened the medium to study the growth of roots, gravitropism, and proliferation of clonally propagated plantlets. He died not long after publishing the last of his orchid papers.

Four years after Werkmeister’s published his first paper on the darkening of orchid culture media (Werkmeister 1970a), Robert Ernst (b. 1916; Fig. 87) was the first to add charcoal to practical seedling culture media and found that *Paphiopedilum* and *Phalaenopsis* seedlings grew well on substrates darkened by this additive (Ernst 1974, 1975, 1976). His findings resulted in the formulation and widespread use of charcoal-containing media for orchid seed germination, seedling culture, and micropropagation (Ernst 1974, 1975, 1976; for a review see Weatherhead et al. 1990; Arditti and Ernst 1993a, b; Arditti 2008).

Cell and protoplast culture

Lewis Knudson’s culture of sloughed-off root cap cells of Canada field-pea and corn (Knudson 1919) was ahead of its time, but is now forgotten. As culture media he employed water and Pfeffer’s solution, which he modified by replacing dibasic potassium phosphate with the monobasic salt with or without 0.5% sucrose. Pea cells survived for 50 days when roots were also present in the culture medium. They lived for 21 days after removal of the roots despite becoming contaminated (for a review, see Arditti 1990). Knudson’s experiments suggested the release of growth substances from the roots. The cells seem to have required these substances, but this research was carried out before the discovery of auxins and cytokinins and before it

became known that vitamins are required for the culture of plant cells and explants. Still, Knudson can be viewed as a pioneer in the culture of isolated plant cells.

The first isolated cells to be cultured successfully were those of tobacco, *Nicotiana tabacum*, and marigold, *Tagetes erecta* (for reviews, see Muir et al. 1954; Steward and Krikorian 1975; Krikorian 1975, 1982; Gautheret 1983, 1985). Shortly after that, isolated mesophyll cells of *Arachis hypogea* were prompted to divide in culture and produced what can best be described as protocorm-like bodies or structures which look like them (Joshi and Ball 1968a, b).

Using an apparatus that rotates nipple culture flasks slowly (1 r.p.m.) around a horizontal axis, Professor Frederick Campion Steward (1904–1994; Fig. 88a, b), Russell C. Mott (Fig. 88b), Marion O. Mapes (1913–1981; Fig. 89) and Kathryn Mears (Fig. 90) obtained suspension cultures of carrot cells and eventually regenerated plants from them (for reviews, see Krikorian 1975, 1982, 1989; Steward and Krikorian 1975; Gautheret 1983, 1985; Arditti and Ernst 1993a; Arditti and Krikorian 1996). *Cymbidium* cell cultures were established using the same system. Plants were regenerated from these cells subsequently (Steward and Mapes 1971b). Two decades later, *Phalaenopsis* plants were regenerated from embryoids derived from a loose-celled callus (Sajise and Sagawa 1990; Sajise et al. 1990) in Professor Yoneo Sagawa's (Fig. 91) laboratory at the University of Hawaii. Other orchids cells have also been cultured (for reviews, see Arditti and Ernst 1993a, b; Arditti 2008).

The first preparation of orchid protoplasts resulted from work with leaves (i.e., mesophyll cells) of *Cymbidium* Ceres and “virus free protocorms of *Cymbidium pumilum*, *Brassia maculata*, and *Cattleya schombocattleya*” (Cape-sius and Meyer 1977). The protoplasts were used to isolate nuclei but no efforts seem to have been made to produce callus masses or regenerate plants from them. There is no “*Cattleya schombocattleya*.” Therefore what was meant could have been “*Cattleya*, *Schombocattleya*,” “*Cattleya* or *Schombocattleya*,” “*Cattleya* and *Schombocattleya*,” or “*Cattleya* X *Schombocattleya*.”

Production of orchid protoplasts and subsequent fusion between and within genera was first reported in 1978 (in an orchid magazine for hobby and commercial growers rather than a peer reviewed journal), but the ultimate fate of the fusion products has not been described in the literature (Teo and Neumann 1978a, b, c). As a result, there are still unanswered questions about these reports and they have been discounted entirely. Early isolations of orchid protoplasts have been reported from several laboratories (Chen et al. 1995; for reviews, lists of orchids that were cultured and procedures, see Arditti and Ernst 1993a; Arditti and Krikorian 1996; Arditti 2008).

Coda

The first plants to be propagated in vitro from seeds, initially symbiotically in joint culture with mycorrhizal fungi and later asymbiotically on sugar containing medium, are orchids. Their seeds are still germinated in vitro asymbiotically (see “Part I”).

Orchids are also the first plants to be propagated clonally in vitro through tissue culture methods which are now referred to as micropropagation (Yam and Arditti 1990). These techniques were first developed with flower stalk nodes of *Phalaenopsis* orchids as early as 1949 (Rotor 1949), and shoot tips of *Orchis maculata* in 1954 (Thomale 1954). The culture of *Cymbidium* shoot tips was reported later (Morel 1960; Wimber 1963). Of these, Rotor's (1949) was an original idea. The other two methods (Thomale 1954; Morel 1960; Wimber 1963) were based on the work of others. Hans Thomale and Don Wimber credited those whose work, methods and ideas he used from the outset. Georges Morel did not. Tissue culture techniques are used extensively at present to propagate orchids and many other plants and in biotechnology in the US (Zimmerman 1996) and elsewhere.

Other related firsts for orchids are the discovery of cell nuclei by Robert Brown and phytoalexins by Noël Bernard (for a review, see Arditti 1992).

Dedication

I dedicate my contribution to this historical account to Anne Westfall, Chief of Staff in the President's Office at the University of Southern California (USC), because of my son, Jonathan. Both Jonathan and I hold degrees from USC (B.A. in 2008 and Ph.D., in 1965, respectively) *Joseph Arditti*.

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