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Pollination-Induced Changes in the Morphology and Physiology of *Dendrobium* Orchid Flowers Prior to Fertilization: The Roles of Ethylene and Auxin

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ABSTRACT

Pollination in *Dendrobium*, as in several other orchids, induces rapid growth in the width of both the ovary and the column (the organ containing the pollinia and the stigma). The visible effects of that growth do not occur when non-pollinated flowers are exposed to ethylene or after application of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) to the stigma of non-pollinated flowers. However, growth of the ovary and column of pollinated flowers is inhibited by the ethylene antagonist 1-methylcyclopropene (1-MCP) and the ethylene synthesis inhibitor aminooxyacetic acid (AOA). The effects on growth, including column and ovary growth, were similar following the

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application of an auxin such as 1-naphthylacetic acid (NAA) to the stigma, while studies with ethylene inhibitors showed that NAA acted through ethylene. The known presence in the pollinia of ACC and an auxin-like compound apparently explains the initial growth of the column and ovary in response to pollination.

KEYWORDS: 1-MCP, ACC, auxin, antiauxin, ethylene, ethylene antagonist, ovary growth, column growth, pollen tube, pollinia

I. INTRODUCTION

Pollination can induce rapid changes in flower form and color, as well as early flower senescence. Most of these changes also occur, although later, in unpollinated flowers. These early effects of pollination have been reported in many plant families, but are quite common in the Orchidaceae, where they usually occur before fertilization. With many orchids, therefore, it is possible to distinguish between the effects of fertilization and those of pollination. Hildebrand (1863a,b,c) and Fitting (1909) noted that the post-pollination effects in orchids depended on the species.

In the first detailed study on *Dendrobium*, Hildebrand (1863a) observed that fertilization in *D. nobile* took place several months after pollination, while early post-pollination effects were visible within 2–3 weeks of pollination. The same has been found in commercial *Dendrobium*

cultivars, which are crosses of *Dendrobium* species (Luangsuwalai et al. 2008). The visible post-pollination phenomena in *Dendrobium* are, therefore, not due to fertilization but to pollination per se.

The purpose of this review is to present the data that are now available on post-pollination phenomena in *Dendrobium* orchids, more than 150 years after the publications of Hildebrand. After a short introduction to orchid flower morphology, the early post-pollination phenomena in some orchids are discussed to show the range of effects. Some examples of early work on hormones will also be reviewed in orchids other than *Dendrobium*. This is followed by an analysis of *Dendrobium* where three groups of early visible pollination effects can be distinguished: a) color changes, b) growth reactions, and c) senescence. The purpose of this review is to summarize the role of hormones, mainly ethylene and auxin, in the initial growth of the ovary and column of *Dendrobium* orchids after pollination.

II. ORCHID FLOWER STRUCTURE

The Asteraceae are likely the largest family of flowering plants, while the Orchidaceae appear to be second largest. Although new discoveries in the field are relatively rare, new orchid species currently are described at a rate of about 500 per year, mainly based on taxonomic work. New genera have been described recently at a rate of about 10–13 per year (Chase et al. 2015).

Orchids generally have a bilaterally symmetric flower made up of three sepals and three petals, forming two whorls of colored leaf-like appendages (Figure 1.1). These orchid flower appendages are also called tepals, but here we will use the terms "petal" and "sepal." The median petal is normally bigger, more colored, and dotted and/or ornamented. This floral leaf is known as a lip or labellum and serves as a landing platform for insects. The ovary is inferior. The androecium (male parts; consisting of 1–3 fertile anthers) and gynoecium (female parts) are usually fused into a single structure called a column, whereby the male parts (pollen) are situated above the stigma and style (Singer et al. 2004). It has been suggested that orchids derive from species with six anthers, and that during evolution three, four, and five functional anthers were lost, producing the extant orchid subfamilies Apostasioideae, Cypripedioideae, and the monandrous orchids, respectively (Johnson and Edwards 2000).

In many species the pollen grains are packaged into large conglomerates (pollinia) that are removed as a single unit from the flower. The

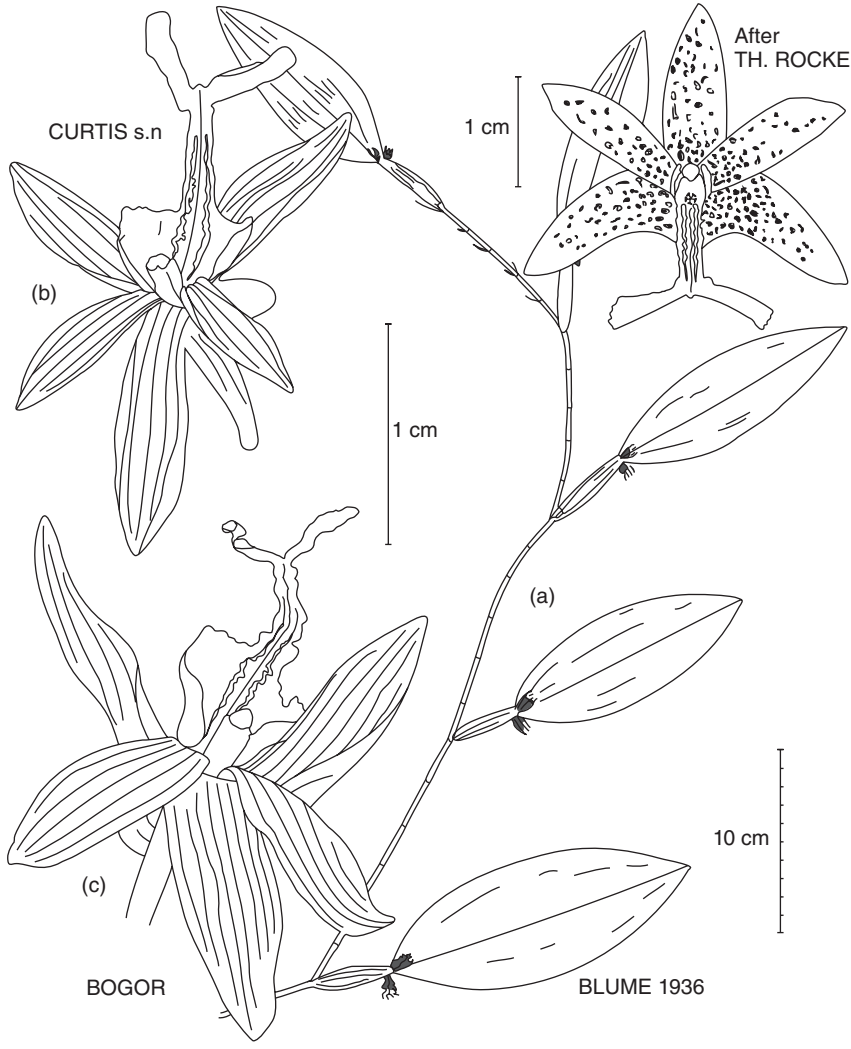


Figure 1.1 *Dendrobium appendiculatum* [syn. *Flickingeria appendiculata* (Blume)] flowers. (*Source:* © Seidenfaden (1980); reproduced with permission.)

pollinia, in most species four per flower, can have stalks as well as secretions that stick the pollinia to the pollinator's body. If pollinia have such accessory structures for attachment to the pollinator, they are called pollinaria. Pollinaria can consist of more than one pollinium (Pacini and Hesse 2002; Singer et al. 2004). In many orchid species

each pollinium often contains several thousands to tens of thousands of pollen grains. In one species a pollinium reportedly contained about four million pollen grains (Darwin 1877; Schill et al. 1992). In orchid flowers with pollinia/pollinaria the stigmatic surface is a cavity that fits one or a few pollinia/pollinaria.

III. POST-POLLINATION EFFECTS IN ORCHIDS OTHER THAN *DENDROBIUM*

Hildebrand (1863a,b,c) studied about 30 orchid species. The degree of ovary development, by the time of pollination, depended on the species: some (for example, *Listera ovata*, now called *Neottia ovata*, and *Neottia nidus-avis*) had ovules that showed relatively advanced development, although an embryo-sac was not yet present. After pollination, the ovary enlarged and the ovules started to grow and develop further, before the pollen tubes reached the ovary. In the absence of pollination, the ovary withered. The sepals and petals in most species soon senesced after pollination but did not show early abscission. Sepals and petals were usually found in a dry state at the top of ripe fruit. In some species these dry sepals/petals eventually fell off. In *Neottia ovata*, by contrast, little effect of pollination was found on sepals and petals. They remained attached to the dehiscent fruit while still fresh and succulent (Hildebrand 1863a).

Similar observations were reported by Fitting (1909). Pollination induced early flower senescence in species of the genera *Aerides*, *Bletia*, *Calanthe*, *Coelogyne*, *Dendrobium*, *Oncidium*, *Orchis*, *Phalaenopsis*, *Platanthera*, *Rhynchostylis*, *Stanhopea*, *Trichoglottis*, and *Vanda*. Fitting also showed this in *Rhenanthera* × *maingayi*, now *Arachnis* × *maingayi* (the updated name from Yam et al. 2009). Exceptions were the absence of early senescence in *Liparis latifolia* (now *Stichorkis latifolia*) and in *Cymbidium sanguinolentum* (now *C. chloranthum*) (updated names from Yam et al. 2009).

Phalaenopsis violacea petals and sepals are white, sometimes greenish white, with some violet. Within 2–3 days of pollination, the white parts became yellowish and the violet parts turned red. The flower closed almost fully and started to wilt. The petals and sepals became turgid again 2–3 days later and then started to become green. They subsequently stayed fresh for several months (Fitting 1909). In *Phalaenopsis lueddemanniana* the petals and sepals did not wilt after pollination, but within one week of pollination they became fleshy, turned green, and lost nonchlorophyllous pigments (red spots and stripes). Similar results were

obtained with *P. amboinensis*, *P. fasciata*, *P. hieroglyphica*, and *P. pallens*. Such regreening has also been reported in *P. mannii* and *P. mariae*, in the orchid *Menadenium labiosum*, and in *Miltonia* species (Tran et al. 1995).

In many species, Fitting (1909) observed pollination-induced swelling of the column (the organ containing the male and female parts) as well as of the subtending ovary. Additionally, pollination resulted in stigmatic closure in species from the genera *Coelogyne*, *Cymbidium*, *Stanhopea*, and *Phalaenopsis*.

Later work generally confirmed these early observations on post-pollination effects. Experiments included genera such as *Angraecum* and *Cattleya* (Strauss and Arditti 1982), *Calypso* (Proctor and Harder 1995), *Lemnoglossum* and *Odontoglossum* (Clifford and Owens 1988), *Cleistes* (Gregg 1991), *Mystacidium* (Luit and Johnson 2001), *Cochniella* (Abdala-Roberts et al. 2007), *Acampe* and *Bletilla* (Huda and Wilcock 2012), and *Epidendrum* (Vega and Marques 2014). Mathur and Mohan Ram (1978) reported an increase in anthocyanin in flowers of *Lantana camara* L. during thrips-pollinated senescence. In *Ophrys fusca*, analysis of the labellum transcriptome showed downregulation of transcripts involved in the synthesis of scents and pigments (associated with color fading of the lip), and upregulation of transcripts indicating senescence (Monteiro et al. 2012).

IV. ROLE OF HORMONES IN ORCHIDS OTHER THAN *DENDROBIUM*

Results of Burg and Dijkman (1967) suggested that at least ethylene and auxin had roles in the pollination-induced early senescence of *Vanda* orchids. This was confirmed by Arditti et al. (1970) in *Cymbidium*. Application of abscisic acid (ABA) to the stigma of *Cymbidium* increased the anthocyanin levels, which also takes place after pollination. However, ABA treatment did not induce the typical column swelling, loss of column curvature, or stigmatic closure (Arditti et al. 1970). Application of the auxin 1-naphthyleneacetic acid (NAA) to the stigma, by contrast, produced all the symptoms brought about by pollination, including increased anthocyanin production, early petal and sepal wilting, stigmatic closure, and the swelling and loss of curvature of the column. Application of gibberellic acid (GA_3) also induced these pollination effects, although only at high concentrations. Kinetin, a cytokinin, had almost no effect (Arditti et al. 1971). Ethylene treatment induced an increase in anthocyanin levels and petal and sepal wilting, but did not result in straightening of the column or in stigmatic closure (Arditti et al. 1973).

Application of labeled IAA (indoleacetic acid) to the stigmas of *Angraecum* and *Cattleya* orchids resulted in virtual immobilization at the point of application. Some of the auxin was conjugated into IAA-aspartate, but this also did not move. As the pollination signal spreads quickly to all floral segments, it was suggested that additional substances, either from the pollinia or produced by pollinated flowers, participated in the production of the early pollination phenomena (Strauss and Arditti 1982).

Working with *Cymbidium*, Woltering (1990) and Woltering et al. (1995) showed that 1-aminocyclopropane-1-carboxylic acid (ACC) is not mobile. Therefore, ACC is not the signal responsible for inter-organ communication during senescence. The data suggested that pollination-induced ethylene, which is the cause of the increase in ACC concentration in various flower parts, rather than ACC is transported from the column to the sepals where it induces early senescence.

O'Neill et al. (1993) found that substances from pollinia do not need to be transported out of the stigma, as pollination in *Phalaenopsis* rapidly induced transcription of genes required for ethylene production, first in the stigma and then in other parts of the flowers. The data suggested that an autocatalytic rise in ethylene production was induced first in the stigma, then in other parts of the flower. Porat et al. (1998) found that an aqueous extract of pollinia contained two high-performance liquid chromatography (HPLC) peaks, one of ACC, the direct precursor of ethylene, and the other having auxin activity while not being free auxin. This is consistent with a role of both ethylene and auxin-type compounds, but does not rule out the possibility that there are other active substances in the pollinia that help mediate the various post-pollination effects. Porat et al. (1995) concluded that an increase in ethylene sensitivity following pollination was the initial event that triggered the increase in ethylene production in *Phalaenopsis*. This increase in sensitivity has not yet been explained.

Novak et al. (2014) pointed out that the required role of auxin in the maturation of the ovaries, and of the ovules, seems unique to orchids. They concluded that the data from *Phalaenopsis* species (Zhang and O'Neill 1993; Tsai et al. 2008) indicate that an auxin-like compound from pollinia is involved in a) the initiation of elongation of ovary epidermal cells, thereby forming trichomes; b) the increase in ovary diameter; and c) depending on the species, the initiation of ovule development or the maturation of partially developed ovules. They also concluded that treatment with ethylene did not induce these effects. Nonetheless, auxin will stimulate ethylene production, and a combination of auxin and ethylene is required for optimal ovary/ovule

development. Novak et al. (2014) also concluded that auxin was a cause of stigmatic closure in *Phalaenopsis*. Closure was also partially due to auxin-induced ethylene production, but treatment with ethylene alone had no effect. They additionally suggested that flower senescence was induced by an auxin-like compound in the pollinia, whereby auxin at least in part would act by increasing ethylene production. It is indeed possible that an auxin-like compound in pollinia has this effect, but a role of ACC in pollinia, which can also lead to a rise in ethylene production through increased gene expression, should not be ignored. Furthermore, in *Dendrobium*, ethylene is adequate to explain the effect of pollination on epinasty, downward movement of the petals and sepals, venation, and senescence (see Sections VI and VII).

The idea that the effects of auxin and ethylene during pollination-induced early senescence in *Phalaenopsis* are coordinated also follows from gene expression data. Auxin upregulated the ethylene biosynthetic genes in *Phalaenopsis*, *Phal-ACS2* and *Phal-ACS3*, and auxin-induced ethylene production was secondarily enhanced through ethylene-stimulated *Phal-ACS1* expression (Bui and O'Neill 1998). Independent of ethylene, auxin lowered transcript levels of *Phalaenopsis* MADS6, which counteracts petal and sepal senescence and inhibits the completion of ovary and ovule maturation after pollination. Nonetheless, this gene has both auxin and ethylene response elements in the promoter region, which suggests that ethylene can also affect its expression (Tsai et al. 2008).

V. POLLINATION IN *DENDROBIUM*

The genus *Dendrobium* has been classified to the tribe Dendrobieae (together with *Bulbophyllum*), in the large subfamily Epidendroideae of the Orchidaceae (Pridgeon et al. 2014). The genus contains about 1200 species, found in South, East, and Southeast Asia, including India, China, Japan, and Australia. Plants are usually epiphytic (as the genus name indicates), sometimes growing on rocks, and rarely are terrestrial. Species are found in climates as diverse as alpine and desert-like, but many are from forests. Flower morphology differs widely. Examples of rather outstanding flower shapes are shown in Figure 1.2a–c (for the more common flower shape, see Figures 1.5 and 1.6).

The genus seems to contain the shortest-lived flower – flowers of *D. appendiculatum* open for five minutes only (van der Cingel 2001). The genus apparently also contains a species that produces one of the longest-lasting flowers. *D. cuthbertsonii* (Figure 1.3) plants grow on rocks and in trees at high altitudes (700 to 3500 m) and bloom in the

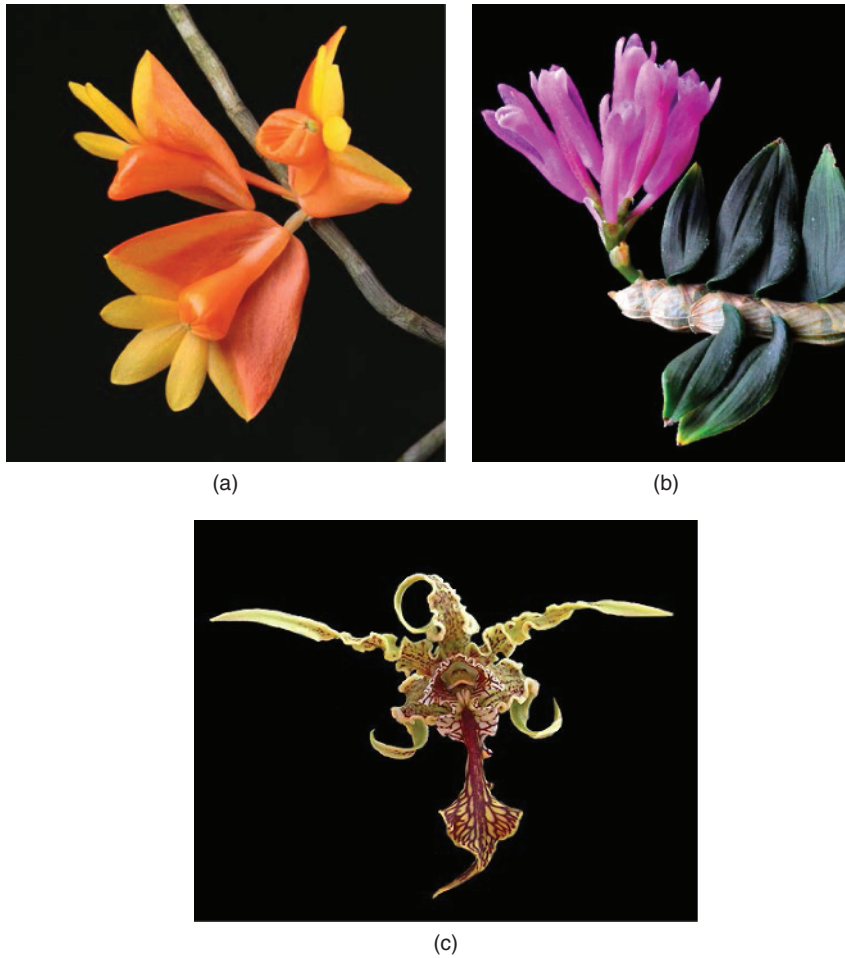


Figure 1.2 Atypical flower morphology in the genus *Dendrobium*. *Dendrobium chrysopterum* (a), *D. limpidum* (b), and *D. spectabile* (c). *D. chrysopterum* and *D. limpidum* from Papua New Guinea described by Schuiteman and de Vogel (2001 and 2003, respectively). For the common flower form of *Dendrobium*, see Figures 1.5 and 1.6. (*Sources:* (a) Photo credit: *Dendrobium chrysopterum* Schuit. & de Vogel. © Andre Schuiteman. (b) Photo credit: WWF/Bob Bowser/B2 Photography, Creative Commons. (c) Photo credit: © Don Dennis with permission.)

cold season. When grown in a mild climate (about 25°C), flowers are said to last up to nine months (Schordje 2013).

Many *Dendrobium* species have fragrant but nectarless flowers, which seem to be predominantly pollinated by bees, but pollination

(a) (b)

Figure 1.3 *Dendrobium cuthbertsonii*, showing its variability in flower color and shape. (*Source*: Photo credit: © Simon Pugh-Jones. https://wsbeorchids.org/2017/365-days-of-orchids-day-221-dendrobium-cuthbertsonii/.)

by flies, wasps, bumblebees, and birds has also been recorded. Bird-pollinated flowers are usually brightly colored and are found generally only at higher altitudes. *D. antennatum* is an exceptional case as it seems to be pollinated by the struggle between a spider that resides in the flower and a visiting insect (van der Cingel 2001).

Most *Dendrobium* species seem to bear pollinia (almost always four) without attachments and with no viscous material. Two pollinia are often connected, which is usually called a pollinarium (Telepova-Texier 2005). Different species have been shown to have different shapes of pollinaria ranging from fusiform to slightly curved or comma shaped, with the inside surface slightly flattened, having two almost same-sized parts of each pair clinging together either fully along the entire length or only partially (Figure 1.4) (Chaudhary et al. 2012). Each pollinium of *Dendrobium* 'Kenny' contained about 38,000 pollen grains, while pollinia of 'Pompadour' contained about 49,000 (Luangsuwalai et al. 2008).

Upon pollination in *D. speciosum*, the pollinia become submerged into the viscous liquid of the stigmatic cup. This liquid contains detached stigmatic cells and mucilage. The mucilage is considered essential for the hydration and germination of the pollen. The stigmatic fluid and stigmatic cells apparently have a considerable osmotic potential, but water still flows into the dehydrated pollen grains (Slater 1991).

The pollen grains are hydrated progressively from the outside of the pollinium to the inside. After four days the pollen tetrads in the

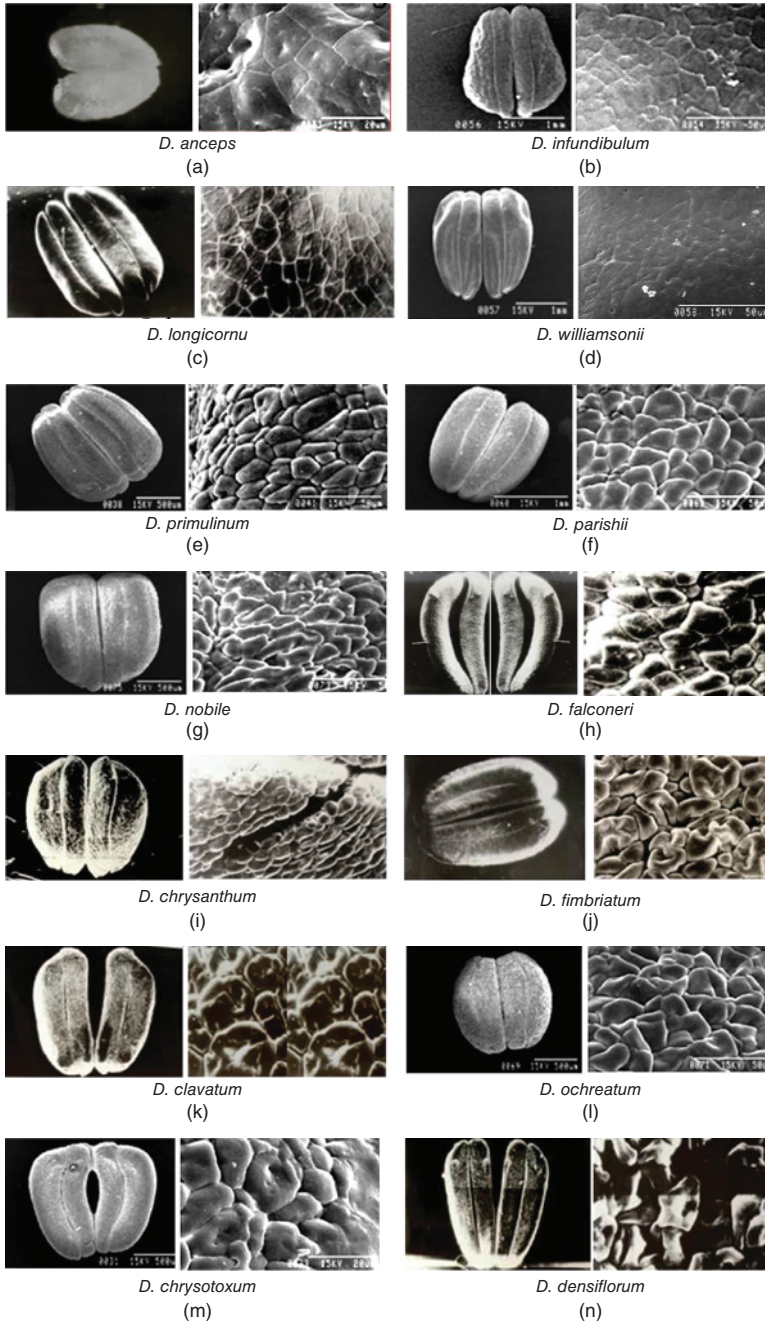


Figure 1.4 Morphology of pollinia (left) and magnified surface of pollen showing distinct types of exine morphology (right) in (a) *D. anceps*, (b) *D. infundibulum*, (c) *D. longicornu*, (d) *D. williamsonii*, (e) *D. primulinum*, (f) *D. parishii*, (g) *D. nobile*, (h) *D. falconeri*, (i) *D. chrysanthum*, (j) *D. fimbriatum*, (k) *D. ocreatum*, (l) *D. acreatum*, (m) *D. chrysotoxum*, and (n) *D. densiflorum*. (*Source*: Modified from Chaudhary et al. 2012).

center of the pollinium were clumped together, with individual grains non-hydrated, while the tetrads on the periphery had broken apart, the grains had hydrated, and pollen tubes were initiated. This continued until the grains in the center of the pollinium had hydrated, germinated, and produced pollen tubes, by day 7 after pollination. An area of the pollinium that was not in contact with the mucilage did not show pollen hydration and germination (Slater 1991).

Non-hydrated pollen grains had a dense cytoplasm, and were compacted together. The grains swelled as they hydrated and the cytoplasm became vacuolate. After germination the cytoplasm transferred to the growing pollen tube tip. The pollen tubes grew through the maze of mucilage, detached stigmatic cells, and pollen tubes, towards the wall of the stigmatic cup. Upon contacting this wall, they veered towards the stylar canal and then proceeded towards the ovary. The detached cells of the stigma, insofar as located near the entrance to the stylar canal, had lost starch from the amyloplasts by the time the pollen tubes had passed. It was suggested that this might nourish the growing pollen tubes, as the detached stigma cells located in the remainder of the stigmatic fluid did not lose starch (Slater 1991).

Slater and Calder (1988, 1990) showed that the stigma of *D. speciosum* provided the material that adhered the pollinia to the pollinator. Many orchids from other genera have a modified median stigmatic lobe (called a rostellum) which helps adherence of the pollinia to the pollinator. Such a structure is not present in *D. speciosa* (and in most, if not all, other *Dendrobium* species). Instead the pollinia are naked, compact, and not sticky. Pollinators get a smear of stigmatic fluid when they pass the stigma, either depositing a pollinium or not. When they advance further, pollinia will adhere to the stigmatic fluid on their body. The viscous material in the stigma of *D. speciosum* thus also functions as "glue" for the transfer of pollinia. The stigma of *D. speciosum* is rather unique as it has glandular cells that are detached and separated from each other by their highly viscous secretion (Slater 1991).

In *D. nobile*, Hildebrand (1863a) found that the pollen tubes formed a cord when passing through a channel of the column. On reaching the ovary cavity this cord divided into three parts, with each of these parts subsequently dividing into two. By day 11 of pollination, the ovary had become more developed but no ovules were yet observed. Two months after pollination ovules had formed, showing different degrees of development. At that time the cords of the pollen tubes had apparently not changed. Three months after pollination all ovules had fully developed. Their embryo-sacs were distinct, but no pollen tube had yet reached them. Four months after pollination the first two or three cells of the embryo had

formed, showing fertilization, and pollen tubes were observed to die. It took, therefore, four months for the ovary, which contained no ovules at the time of pollination, to form ovules and then for these to be fertilized.

VI. VISIBLE POST-POLLINATION EFFECTS IN *DENDROBIUM*

If pollinia were applied to the viscous fluid of the stigma of *D. nobile* flowers, the lip folded up round the column within two days of pollination. The other petals and sepals subsequently inclined over it, thus the flower closed (Hildebrand 1863a). The column and the ovary swelled after pollination. The flowers withered about nine days after pollination. The petals and sepals did not abscise, but were found, eventually, on the top of the ovary. If no pollen was applied to the stigma, the flower remained unchanged for about 20–30 days. It then started to wither, and the whole flower abscised. The ovary had not grown in the non-pollinated flowers (Hildebrand 1863a).

In *D. speciosum*, pollination induced flower closure and a more intense perianth color. This was followed by swelling of the column and ovary and, after four days, cell division in the ovary and development of the ovules. The pedicel and the ovary surface became green (Slater 1991). Huda and Wilcock (2012) recorded the first floral change in pollinated and non-pollinated flowers. In *D. aphyllum* the non-pollinated control flowers did not change for 13 days. After pollination, pink dotting on the sepals and petals was found by day 6, as well as upward curling of the lip. Intact flowers on non-pollinated controls of *D. fimbriatum* did not change for five days, whereas pollinated flowers began to close within three days. Flowers of *D. lindleyi* began to close by day 6 after pollination, but some changes were also found in controls by this time. *D. palpebrae* flowers faded and began to close by day 4 after pollination, whilst in non-pollinated controls the first change took place after 14 days.

Similar changes have been observed in commercial *Dendrobium* cultivars. A list of the observed visible effects in ‘Kenny’ (also called ‘Miss Teen’) is presented in Table 1.1. The sepals of ‘Kenny’ are whitish with light purple lateral parts, and petals (including the lip) are whitish with light purple mainly at the distal ends (Figure 1.5a, b). The upper surface of the lip in many *Dendrobium* cultivars shows five parallel hairy ridges which guide the pollinating insect to the reproductive parts. One ridge is found at the median axis, and two on either side of this axis. It has been suggested that these ridges reflect incident light and thereby provide visual cues that attract potential pollinators and help to orientate them on the lip surface (Davies et al. 2006). The ridges of

Table 1.1 Time (in days after pollination taking place, just after full flower opening) to the visible post-pollination effects in *Dendrobium* 'Kenny' (= 'Miss Teen') pollinated with pollinia of 'Burana Jade', compared to flowers at the same stage that were not pollinated. Cut inflorescences were bearing 5–7 floral buds and 4–5 open flowers, of which the two lowermost were pollinated. Cut inflorescences were placed in water, at 25°C. Data for flowers on intact plants were gathered at the same developmental stage as those on cut inflorescences. Data on pollinated flowers on cut inflorescences placed in water were very similar to those in cut inflorescences, except for abscission of flowers which did not occur in pollinated flowers on intact plants, but took place on day 11.2 ± 2.3 in pollinated flowers on cut inflorescences placed in water (Luangsuwalai 2007).

Post-pollination symptom	Time to symptom appearance (days)		
	Intact plant		Cut inflorescence
	Non-pollinated	Pollinated	Non-pollinated
Epinasty	17.8 ± 2.8	2.8 ± 0.4	12.1 ± 2.1
Venation	–	4.0 ± 0.0	–
Lip yellowing	20.9 ± 2.0	4.2 ± 0.4	14.4 ± 2.0
Petals/sepals close	20.9 ± 2.2	4.4 ± 0.5	13.1 ± 2.3
Petals/sepals yellow adaxially	–	5.0 ± 0.1	–
Petals/sepals fold laterally	20.8 ± 1.9	6.2 ± 0.4	13.8 ± 2.4
Ovary thickens	–	7.0 ± 0.0	–
Petals/sepals desiccate	24.3 ± 2.5	8.2 ± 0.4	15.0 ± 2.4[1]
Flowers abscise	26.1 ± 2.1	–	16.6 ± 2.3[2]

Data are means ± SD (n = 10).
[1] Observed in 50% of the flowers.
[2] Observed in 100% of the flowers.
–: Symptom not observed.

'Kenny' are deeper purple than the remainder of the lip. Additionally, the flower peduncle and the ovary in 'Kenny' are light purple on the dorsal part (Luangsuwalai 2007).

The *Dendrobium* cultivar 'Pompadour' has uniformly dark pink petals/sepals and peduncles (Figure 1.5g, h), while 'Karen' has uniformly medium purple petals/sepals and peduncles (Figure 1.5c). In both cultivars, the lower (abaxial) sides of the petals/sepals have the same color as the upper (adaxial) sides. The crossing background of many *Dendrobium* cultivars can be found in Lavarack et al. (2000).

About 4–7 days after pollination, the surface of the petals and sepals showed slight wrinkling, and an increase was noted in the color of the vascular bundles compared to the regions between these bundles (Figure 1.5h, i). This relative increase in the coloration of the vascular bundles seems mainly due to the loss of color in the regions between the vascular bundles, but it cannot be excluded that an increase in net anthocyanin synthesis occurred in the bundles at

this time. In 'Kenny' the purple color of the upper sides of the petals and sepals, other than the lip, first faded (Figure 1.5i), then became slightly reddish in color. By day 10, symptoms of inward movement of the two lateral sides of the petals (Figure 1.5k) and water soaking were found (Figure 1.5l). In 'Pomdadour' this water soaking after pollination was visible later. Water soaking is the infiltration of cell sap into the cell walls and intercellular air spaces, resulting in a glassy appearance, and is apparently due to massive programmed cell death (PCD) in the mesophyll cells (van Doorn et al. 2011). This water-soaking symptom induced by pollination is similar to chilling injury (Phetsirikoon et al. 2012; Sirikesorn et al. 2013) and to changes following ethylene treatment (Sirikesorn et al. 2015). The petals and sepals of pollinated 'Kenny' flowers subsequently wilted, became brownish in color, and finally desiccated (Figure 1.5k, m). This took place by days 10–12 following pollination. The time to each visible symptom in the pollinated *Dendrobium* flowers also depended on the source of pollinia (Table 1.2).

A. Color Changes

One of the initial physical changes following pollination is often to the color of specific parts of the individual flower. For example, the first color change, on day 1–2 after pollination, was observed in the ridges of the labellum (Figure 1.6). In 'Karen' the proximal part of the lip subsequently became less purple and turned yellow (Figure 1.6a, right), while, in contrast, with 'Pompadour' the dark pink lip did not obviously change color (Figure 1.6b, right). Yellowing of the proximal lip part was clear by about days 4–5 after pollination. In 'Karen' the color change to yellow of the lip was quite conspicuous, while at the same time the distal part of the lip turned towards red in this cultivar (Figure 1.6a). By day 5 the back (adaxial side) of the petals and sepals showed yellowing in 'Karen' (Figures 1.5f, left and 1.6a, right), 'Kenny' (Figure 1.6c), and 'Ekapol Anna' (Figure 1.6d, right). The color of the ovary and the subtending pedicel of pollinated flowers became green, starting five days after pollination (Figure 1.5f, left).

In 'Pompadour' the dark pink became very dark pink (reddish) by day 4 following pollination (Figure 1.6b, right). Subsequently the color of all petals and sepals of 'Kenny' and 'Ekapol Anna' faded, and further changes in color were similar to those in 'Karen'. In these three cultivars the color of the ovary and the subtending pedicel of pollinated flowers also became green (Figure 1.5f, left). In 'Pompadour', having a dark pink ovary and pedicel, the greening was visible relatively late

'Kenny'
(a)

'Kenny'
(b)

'Karen'
(c)

'Pompadour'
(d)

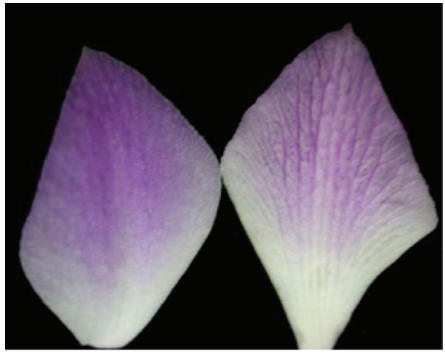
'Pompadour
(e)

'Karen'
(f)

Figure 1.5 Post-pollination symptoms in *Dendrobium* flowers. Flowers of 'Kenny' (a, b), 'Karen' (c, left), and 'Pompadour' (d, left) before pollination. Flowers of 'Kenny' pollinated with pollinia of 'Burana Jade' (c, right), and 'Pompadour' pollinated with pollinia of 'Pompadour' (d, right). Epinasty of pollinated 'Karen' and 'Pompadour' flowers is clearly visible; all pictures taken on day 3 following pollination. Column swelling of pollinated (e, right) compared with no swelling on non-pollinated (e, left) flowers of 'Pompadour'; picture taken on day 4 following pollination. Note the anther cap on top of the column, hiding the pollinia, and the stigma just below the anther cap. Also note the shiny stigma fluid. The column on the right side (e) has just been pollinated using four pollinia. Column (ovary) swelling of non-pollinated (f, right) compared with pollinated (f, left) flowers of 'Karen'; picture taken on day 3 after pollination. The lack

‘Pompadour’
(g)

‘Pompadour’
(h)



‘Kenny’
(i)

‘Sonia Bom #17’
(j)

‘Kenny’
(k)

‘Kenny’
(l)

‘Kenny’
(m)

of wilting of non-pollinated (g, left) compared with wilting of pollinated ‘Pompadour’ flowers (g, right). Venation in petals of pollinated flowers of ‘Pompadour’ (h), and on non-pollinated (i, left) and pollinated (i, right) flowers of ‘Kenny’; picture taken on day 3 after pollination. Inward movement of the lateral sides of petals on pollinated flowers of ‘Sonia Bom #17’ (j, right), and fading (k) and water soaking (l) of pollinated ‘Kenny’ flowers; pictures taken on day 5 after pollination. Inflorescence of ‘Kenny’ with three flowers that remained non-pollinated (m, left) and three pollinated flowers (m, right), on day 10 following pollination. Note epinasty, flower closure, browning, lack of turgor, and the onset of desiccation of petals and sepals. (Photo credit: ©Saichol Ketsa.)

Table 1.2 Time to visible post-pollination symptoms in *Dendrobium* 'Kenny' flowers, after pollination with pollinia from either 'Sakura', 'Pompadour', or 'Willie'. Flowers were attached to cut inflorescences placed in water at 25°C (Luangsuwalai 2007).

Symptom	Time to symptom appearance (days)[1]		
	'Sakura'	'Pompadour'	'Willie'
Epinasty	1.1	1.1	1.1n.s.
Flower closure	2.7	3.1	3.7n.s.
Venation	4.0b	4.4b	6.0a
Lip yellowing	4.2c	6.1b	7.1a
Petals and sepals fade	6.0b	8.2a	8.0a
Petals and sepals brown	8.6c	9.3a	8.9b

[1] Means in the same row showing a different letter are statistically different at $P \leq 0.01$.
n.s.: Not significant.

(i.e. seven days after pollination) (Figure 1.5e, right). In 'Ekapol Anna' the ridges and proximal lip, petal, and sepal turned to darker yellow, while the lip became light purple after pollination (Figure 1.6d, right). Rebecca et al. (2008) reported that pollinated flowers of both *Dendrobium* 'Sonia' and *Dendrobium* 'Savin White' showed earlier senescence and signs of wilting, including loss of anthocyanins, causing the flower to look dull and weak, compared to non-pollinated flowers which exhibited a longer vase life.

B. Growth Reactions

Visible growth reactions in *Dendrobium* also occur soon after pollination and include epinasty, flower closure, petal lateral inward rolling, column growth, and ovary growth. Epinasty of the flower stem (pedicel) resulted in a change in flower position on the inflorescence (Figure 1.5c, right; 1.5d, right). The surface of non-pollinated flowers as observed by a pollinator is exposed outward, while after epinasty it is oriented downward and is therefore more difficult to distinguish. Flower epinasty was visible within about one day when 'Kenny' flowers were pollinated with pollinia of 'Pompadour', 'Sakura', or 'Willie'. Detailed measurements showed that the effect was detectable 2–24 h after pollination, depending on the cultivar. In 'Pompadour' pollinated with 'Pompadour', the effect was detectable within 3 h after pollination (Rugkong 1997; Figure 1.5d, right). In Sonia Bom #17 pollinated with pollinia of 'Pompadour', the effect was detectable one day after pollination (Luangsuwalai 2007; Figure 1.5j, right). In 'Karen' pollinated with pollinia of 'White 5N', epinasty was detectable within 1.5 days

'Karen'
(a)

'Pompadour'
(b)

'Kenny'
(c)

'Ekapol Anna'
(d)

Figure 1.6 Color changes after pollination of *Dendrobium* 'Karen' flowers, non-pollinated (a, left) and pollinated (a, right) with pollinia of 'Walter Oumae 5N'; of 'Pompadour' flowers, non-pollinated (b, left) and pollinated (b, right) with pollinia of 'Pompadour'; of 'Kenny' flowers pollinated with pollinia of 'Sakura' (c); and of 'Ekapol Anna' flowers, non-pollinated (d, left) and pollinated (d, right) with pollinia of 'Pompadour'. No color change is shown in the pollinated flower of 'Pompadour' (b, right), and color change of the labellum of 'Karen' (a, right), 'Kenny' (c), and 'Ekapol Anna' (d, right) is visible following pollination. Pictures taken on day 4 following pollination for 'Pompadour', and on day 3 for 'Karen', 'Kenny', and 'Ekapol Anna'. (Photo credit: ©Saichol Ketsa.)

(Sukhotu 2006; Figure 1.5c, right), and in 'Karen' pollinated with pollinia of 'Wanna' within 2 h (Promyou et al. 2014).

Flower closure (Figure 1.5d, right), also called drooping, might be due to a change at the petal or sepal base. It took place by about day 7 following pollination. Similarly, petal and sepal lateral inward rolling might be due to a growth reaction or to a loss of turgor in the tissue. It was observed by about days 2–4 after pollination (Tables 1.1 and 1.2).

The column width increased (Figure 1.5e, right), but column length did not change following pollination – the growth in column width was detectable by day 3 (Ketsa and Rugkong 1999). The ovary, situated just below the column, also grew in width (Figure 1.5f, left), with the onset of ovary growth being found within three days of pollination (Ketsa et al. 2006).

Removal of the petals and sepals at the time of pollination resulted in 50% reduction in ovary growth, indicating that metabolites from the senescing petals/sepals served to enable the ovary to grow. However, a difference in growth was found within days 2–3 after pollination (i.e. before visible senescence symptoms had developed) (Ketsa and Rugkong 1999).

Pollination affected the distance between the lip and the peduncle, which rapidly decreased within 12 h following pollination, leading to flower epinasty symptoms (Figure 1.5c, right; 1.5d, right; 1.5j, right). In contrast, the distance between lip and peduncle in non-pollinated flowers remained unchanged (Figure 1.5c, left; 1.5d, left; 1.5j, left). Application of ethylene inhibitors, 1-methylcyclopropene (1-MCP) and aminooxyacetic acid (AOA), to the stigma surface prior to pollination resulted in a delay or prevention of epinasty (Sukhotu 2006; Luangsuwalai 2007). This indicates that ethylene synthesis induced by pollination causes epinasty of pollinated *Dendrobium* flowers similar to that occurring in poinsettia (Staby et al. 1978), tomato (Ursin and Bradford 1989), and mulberry (Suzuki et al. 1990).

It should be noted that the *Dendrobium* cultivars studied did not show closure of the stigma following pollination. This was also true for several other *Dendrobium* cultivars that had been investigated earlier (such as 'Sonia Bom Jo' and 'Burana Jade').

C. Abscission

In flowers of *Dendrobium* cultivars studied on the plant, pollination did not induce abscission of the petals or of the sepals. Also, abscission of pollinated flowers was not observed in intact plants (Table 1.1). In cut inflorescences placed in water, petal and sepal abscission was not observed. In these inflorescences, flowers abscised after pollination, earlier than

in non-pollinated flowers (Table 1.1). These responses are in contrast to many other species where pollination does induce flower or petal abscission (Stead and Moore 1979; Halevy et al. 1984; van Doorn 2002).

VII. ROLE OF HORMONES IN THE VISIBLE POST-POLLINATION PHENOMENA IN *DENDROBIUM*

A. Hormones and Their Precursors in *Dendrobium* Pollinia

Pollinia of *Dendrobium* cultivars contain considerable amounts of ACC and one or more compounds with auxin activity (Promyou et al. 2014). The compounds that induced the post-pollination phenomena moved into agar blocks, indicating that they were water-soluble. When agar blocks that had been in contact with pollinia were placed in the stigma, all the normal post-pollination phenomena were observed, showing that the hormones involved all transferred to the agar (Promyou et al. 2014). Agar blocks that had been in contact with pollinia for 3 h at 25°C had no effect, but after 6 and 12 h of contact the post-pollination effects were observed and were earlier with longer contact. Conversely, pollinia that had been in contact with agar blocks became less able subsequently to induce the post-pollination phenomena (Promyou et al. 2014). The post-pollination effects of the agar blocks were correlated with the ACC concentrations in the agar blocks that had been in contact with pollinia. The effects were also correlated with the ACC concentration in the pollinia that had been in contact with agar blocks. The effects of the agar blocks were also correlated with their auxin concentration following contact with the pollinia (Promyou et al. 2014).

Ketsa and Luangsuwalai (1996) pollinated 'Pompadour' with pollinia of 'Pompadour' and five other cultivars ('Caesar 2N', 'Jew Yuay Tew', 'Sabin', 'Sonia Bom #28', and 'Walter Oumae Taba 4N'). The ACC concentration in the pollinia, expressed per gram FW (fresh weight), was the same in all cultivars, but the ACC content per pollinium varied from 0.05 nmol to 0.14 nmol. The time to flower senescence varied from one to five days after pollination, depending on the cultivars from which the pollinium was taken. This time was highly and inversely correlated (r^2 = 0.96) with the ACC concentration of the pollinia used for pollination. Ethylene production by the flowers was also higher after pollination with pollinia having a higher ACC concentration. These data suggest that ACC in pollinia is an important factor in the induction of an increase in ethylene synthesis and of the time to pollination-induced flower senescence (Ketsa and Luangsuwalai 1996).

B. Ethylene Production after Pollination

Ethylene production by individual flowers of 'Kenny' after pollination was detectable within 3–6 h of pollination (Ketsa and Rugkong 1999). The first maximum peak of ethylene production was observed after about 12 h. A second maximum peak was observed by days 4–5 after pollination (Figure 1.7a), which coincided with the onset of petal and sepal senescence (Luangsuwalai et al. 2011). In other cultivar–pollinia combinations, the ethylene production stayed high from day 1 until after visible flower senescence (Ketsa and Luangsuwalai 1996). Pollination stimulated ethylene production, increased ACC concentration, and increased both ACC synthase (ACS) and ACC oxidase (ACO) activities (Ketsa and Luangsuwalai 1996; Sukhotu 2006; Luangsuwalai et al. 2011). These results support the conclusion that pollination induced ethylene production in pollinated flowers of *Dendrobium* via increases in ACS and ACO activity (O'Neill et al. 1993). An inhibitor of ethylene action, 1-MCP (Blankenship and Dole 2003), reduced ethylene production, ACC concentration, and ACS and ACO activities in pollinated flowers. These results suggest that ethylene production of pollinated flowers of *Dendrobium* is an autocatalytic system (Satoh et al. 2005).

The high ethylene production after pollination, up to senescence, was solely due to the column and ovary. No increase was observed in petals or sepals (Figure 1.7b). It is, therefore, likely that the post-pollination phenomena were due to ethylene produced in the column and diffusing into the petals and sepals. In related studies, ACC applied to the stigma of *Cymbidium* flowers was not mobile (Woltering 1990; Woltering et al. 1995), and auxin from pollinia apparently also produced ethylene only in the column. In *Cymbidium* orchids, Woltering et al. (1995) also concluded that ethylene produced in the stigmatic region following pollination is the mobile factor that is responsible for the ethylene-related post-pollination effects.

Post-pollination effects due to ethylene seem to be due to three sources: a) ethylene produced from pollinia-borne ACC, b) ethylene produced by pollinia-borne auxin-like compounds, and c) ethylene produced by an autocatalytic mechanism based on an initial increase in ethylene concentration.

The ethylene production of 'Kenny' flowers pollinated with pollinia from 'Kenny' and four other cultivars ('Karen', 'Pompadour', 'Sakura', and 'Willie') was evaluated by Luangsuwalai et al. (2008). A sustained increase in ethylene production was only found from the interaction with pollinia from 'Pompadour', 'Sakura', and 'Willie' (Figure 1.8). Pollination with pollinia of these cultivars also resulted in the normal

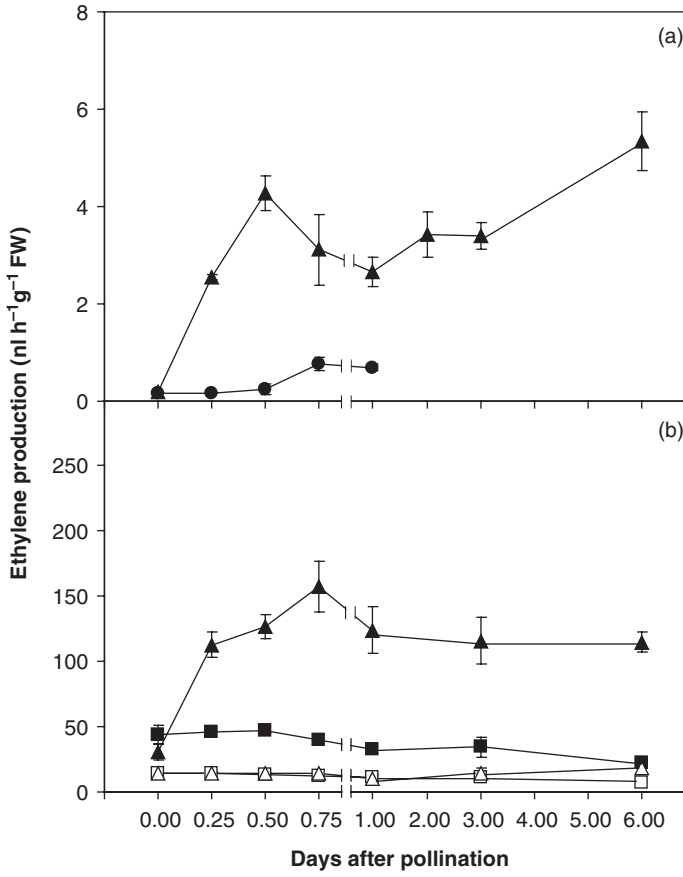


Figure 1.7 Ethylene production in non-pollinated and pollinated *Dendrobium* 'Kenny' flowers. Ethylene production in whole flowers (a). Flowers not pollinated (●), and flowers pollinated with pollinia of *Dendrobium* 'Sakura' (▲). Results are means of 10 replicates ± SD. Ethylene production of the column and ovary, and the perianth (sepals and petals, including the lip) (b). Column and ovary of non-pollinated flowers (■), column and ovary of flowers pollinated with pollinia of *Dendrobium* 'Sakura' (▲), perianth of non-pollinated flowers (□), and perianth of flowers pollinated with pollinia of 'Sakura' (△). Results are means of 5 replicates ± SD. (*Source*: Luangsuwalai et al. 2011.)

post-pollination phenomena. Pollination with the pollinia of 'Kenny' and 'Karen' did not produce any post-pollination phenomena, and growth of the pollen tubes did not occur (Luangsuwalai et al. 2008). Lack of post-pollination effects of these pollinia was also found after pollinating cultivars other than 'Kenny', showing that the effect was due

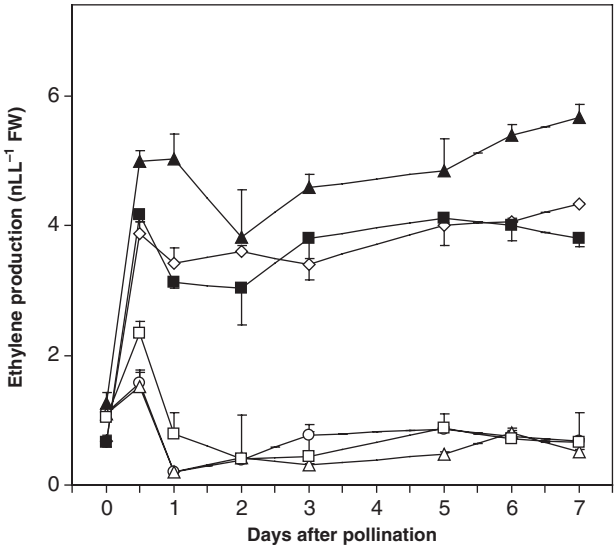


Figure 1.8 Ethylene production by non-pollinated *Dendrobium* 'Kenny' flowers (○), and by flowers pollinated with pollinia from 'Kenny' (△), 'Karen' (□), 'Pompadour' (◇), 'Sakura' (▲), and 'Willie' (■). Results are means of 10 replications (20 inflorescences, two per vial, each inflorescence bearing only five open flowers) ± SE. (*Source*: Luangsuwalai et al. 2008.)

to the pollinia and not to the mother plant. The pollinia that showed a lack of a post-pollination effect had a lower ACC concentration compared with the other cultivars (Table 1.3). The ACC concentration in the pollinia was correlated with the presence or absence of post-pollination effects (Luangsuwalai et al. 2008). The lack of post-pollination

Table 1.3 Concentrations of ACC and unconjugated IAA in pollinia of various *Dendrobium* cultivars. Data are the means of three replications each consisting of 30 mg pollinia for ACC assessment and 300 mg for IAA measurement (Luangsuwalai 2007).

Cultivar	ACC concentration (nmol ACC/g FW)[1]	Unconjugated IAA concentration (μg IAA/g FW)[1]
'Kenny'	492 c	43 c
'Karen'	410 d	49 c
'Pompadour'	548 c	140 b
'Sakura'	1246 a	352 a
'Willie'	996 b	310 a

[1] Means within columns not sharing the same letter are significantly different by DMRT (Duncan's multiple range test) at $P = 0.05$. Significant at $P = 0.05$ and $n = 100$.

effects was also correlated with a lower concentration (expressed per mg DW (dry weight)) and a lower content of auxin (Luangsuwalai and Ketsa 2013). The lower ACC concentration explains, at least in part, the lack of post-pollination effects by these pollinia. The concentrations of the auxin-like compounds in the pollinia need further investigation.

Ethylene produced in the ovary of zucchini squash (*Cucurbita pepo* L.) in the days immediately after anthesis plays an important role as a negative regulator of fruit set and early fruit development. Pollination/fertilization induces fruit set and development by preventing the production and action of ethylene immediately after anthesis. In the absence of pollination/fertilization, ethylene is induced in the ovary, aborting its normal development. Auxins can mimic pollination/fertilization by preventing the induction of ethylene and, therefore, the abortion of fruit development. The effect of pollination/fertilization on ethylene production and signaling in the ovary, therefore, could be mediated by auxins (Martínez et al. 2013). This suggests that ethylene can either upregulate or downregulate early ovary growth depending on plant species.

C. ACC, ACC Synthase, and ACC Oxidase

Pollination increased ACC concentration and ACO activity in the columns more rapidly and to higher levels than in the perianth, while pollination increased ACS activity in the perianth more rapidly and to higher levels than in the column. ACC concentration in the perianth was, therefore, lower than in the column, while ACS activity in the perianth was higher than that in the column+ovary. These results may be due to ACC in the perianth having a more rapid turnover than in the column+ovary or to ACC in the perianth being transported into the column+ovary and converted to ethylene and, in turn, regulating perianth senescence (Sukhotu 2006). The higher ACO activity in the column+ovary supports this suggestion but needs confirmation.

The transcript abundance of the isolated *Dendrobium* ACO gene was highly upregulated in the perianth and column+ovary within 6–12 h of pollination. This increase might be partially responsible for the increase in perianth and column ACO activity. The expression of the *Dendrobium* ACO gene after pollination (Sukhotu 2006) is similar to that of an ACO gene in *Phalaenopsis*, where a rapid and large upregulation was observed in the column of pollinated flowers (Nadeau et al. 1993), similar to that in *Doritaenopsis* (Nadeau and O'Neill 1995), plum (Fernandez-Otero et al. 2006), and tomato (Llop-Tous et al. 2000).

Together, this can explain why the column and the ovary combined is the main source of ethylene in *Dendrobium* flowers, which is similar to the results obtained in *Phalaenopsis* flowers.

D. Treatment with Ethylene or ACC

Exposure of non-pollinated inflorescences to ethylene induced the following changes: a) epinasty of the peduncles, b) color change in the petals and sepals, c) downward movement of the petals and sepals, d) venation and water soaking, and e) wilting of the petals and sepals. It did not promote growth of either the column or the ovary (Ketsa and Rugkong 2000a,b; Luangsuwalai et al. 2008; Sirikesorn et al. 2015).

Ketsa and Rugkong (2000a) found that pollination increased the sensitivity of flowers to exogenous ethylene. Previously, Porat et al. (1994) had exposed pollinated and unpollinated *Dendrobium* 'Jacquelin Thomas' to ethylene, and also observed an increase in ethylene sensitivity after pollination. They excluded a role of endogenous ethylene, as the flower pedicels were placed in an aqueous solution containing 0.5 mM aminooxyacetic (AOA), which prevented the pollination-induced increase in ethylene production. Their data, therefore, showed that ethylene sensitivity per se was increased by pollination. The effect was found very rapidly after pollination. One possible interpretation is that pollinia contain a factor or factors that increase ethylene sensitivity. Additionally, pollination decreased the transcript abundance of an ethylene receptor gene in *Dendrobium* petals (Thongkum et al. 2015), which might lead to increased ethylene sensitivity as the number of ethylene receptor proteins is inversely correlated with ethylene sensitivity (Klee 2004; Liu and Wen 2012).

Application of 0.02 nmol ACC to non-pollinated stigma of 'Pompadour' resulted in all post-pollination phenomena except column and ovary growth. However, the effects observed were smaller in magnitude than those from pollination. For example, senescence in the controls was found on days 13–19, whilst after pollination with 'Pompadour' pollinia it occurred on day 2. Placing 0.02 nmol ACC in the stigma of non-pollinated flowers resulted in senescence on day 7 (Ketsa et al. 2001). The ACC treatment resulted in an increase in ethylene production by the flowers, which was already detectable 2 h after treatment. After pollination with 'Pompadour' pollinia, ethylene production also increased within 2 h (Ketsa et al. 2001).

E. Endogenous Auxin and Treatment with Auxin

Ovary growth or ovary development is one of the major post-pollination developmental processes in plants. It has long been known that ovary development after pollination depends on a supply of auxin derived from the pollen (Ketsa et al. 2001; Mól et al. 2004), and from the developing ovules and seeds (Gustafson 1939). Pollen of *Ruella tuberosa* L. (Acanthaceae) induced a burst of ethylene production and premature senescence of *Dendrobium* 'Pompadour' flowers after pollination, similar to that occurring with pollinia of *Dendrobium* 'Pompadour', but pollen of *R. tuberosa* did not increase ovary growth in the same way that occurred with pollinia of *Dendrobium* 'Pompadour' (Table 1.4). This result indicates that factors other than those related to ethylene production seem to be involved in ovary growth of *Dendrobium* orchid flowers. For example, NAA increased ovary growth of *Dendrobium* 'Pompadour' flowers (Ketsa and Rugkong 2000b), indicating that a factor associated with pollination may be auxin in the pollinia. In fact, pollinia of *Dendrobium* cultivars have been shown to contain auxin (Luangsuwalai and Ketsa 2013; Promyou et al. 2014). A lack of post-pollination effects has been correlated with a lower concentration (expressed per mg DW) and a lower content of auxin (Luangsuwalai and Ketsa 2013).

Application of the stable synthetic auxin NAA to the stigma of virgin flowers on 'Pompadour' inflorescences resulted in all of the post-pollination phenomena, including growth of the column and the ovary.

Table 1.4 Ovary diameter of *Dendrobium* 'Pompadour' flowers with and without 0.02 nmol ACC treatment (Ketsa et al. 2001).

Treatment	Ovary diameter (cm)[1]	
	Day 0	Day 7
Dendrobium 'Pompadour' without pollination	0.28	0.27 b
Dendrobium 'Pompadour' × *Ruellia tuberosa*	0.28	0.28 b
Dendrobium 'Pompadour' × *Dendrobium* 'Pompadour'	0.27	0.39 a
Dendrobium 'Pompadour' × *Dendrobium* 'Pompadour' and ACC	0.28	0.37 a
Dendrobium 'Pompadour' × *Ruellia tuberosa* and ACC	0.27	0.27 b
Dendrobium 'Pompadour' and ACC	0.28	0.26 b
F-test	n.s.	**

[1] Means within columns not sharing the same letter are significantly different at $P = 0.01$ by DMRT (Duncan's multiple range test).
** Significant at $P = 0.01$ and $n = 25$.
n.s.: Not significant.

NAA also increased the rate of ethylene production similar to that occurring with pollination (Ketsa and Rugkong 2000b). The time course of the effects depended on the NAA concentration. When applied at relatively low concentrations (5 μg), NAA induced changes such as senescence at a later point in time than occurred with pollination. At relatively high concentrations (20 μg), NAA induced the post-pollination symptoms faster than pollination, and promoted more ovary growth than that resulting from pollination (Ketsa and Rugkong 2000b).

Auxin-like compounds induced all post-pollination phenomena, but were required for growth of the column and ovary. Pollinia contain at least one auxin-like compound. The chemical nature of the auxin-like compound is not known in *Dendrobium*, or in any other orchid. The effectivity of this compound in pollinia was quantified using agar blocks and the standard *Avena* curvature test according to the standard Went method (Went and Thimann 1937; Barkawi et al. 2010). Pollinia were brought in contact with the agar blocks, and the agar blocks were then placed in the stigma. The minimum amount of the auxin-like compound that induced the post-pollination effects induced a curvature of about 25–30° in the *Avena* test (Promyou et al. 2014).

F. Antagonists of Ethylene

Two antagonists of ethylene synthesis have been applied to the stigma of *Dendrobium* flowers, prior to pollination. AOA inhibits the activity of ACS (Bradford et al. 1982), while cobalt chloride inhibits the activity of ACO (Yang and Hoffmann 1984). Whole inflorescences bearing only open flowers were either not pollinated, pollinated, or the stigma received a volume of either distilled water as a control or AOA (0.15 or 0.30 μmol per flower in aqueous solution) for 24 h prior to pollination. Cobalt chloride was similarly applied to the stigma at 0.1, 0.2, and 0.4 nmol (Ketsa et al. 2006).

Both the AOA and cobalt chloride applications to the stigma prevented or delayed the effects of pollination on epinasty, flower color, venation, petal and sepal senescence, and the growth of the column and the ovary (Ketsa and Rugkong 2000b; Wisutiamonkul 2001; Ketsa et al. 2001, 2005, 2006; Luangsuwalai et al. 2008). The effects of AOA application and pollination on epinasty, lip yellowing, and venation and water soaking in the petals and sepals were the same at 0.15 and 0.30 nmol AOA (Luangsuwalai et al. 2008). The inhibiting effect of cobalt chloride on ovary growth was larger at 0.4 than at 0.1 nmol (Ketsa et al. 2006).

The AOA-induced inhibition of the pollination effect on ovary growth was partially alleviated by ACC, indicating that the effect of pollination

on ovary growth was due, at least in part, to an effect of ethylene (Ketsa and Rugkong 2000b). The stigma AOA treatment also prevented the increase in ethylene production and the increase in ACC concentration in whole flowers after pollination (Ketsa et al. 2001).

Interestingly, placement of AOA on the stigma also inhibited the effect of simultaneously applied NAA on ovary growth, showing that the effect of NAA is at least partially increased through production of ethylene. This was corroborated by ethylene measurements: AOA prevented the increase in ethylene production induced by NAA (Ketsa and Rugkong 2000b).

The compound 1-MCP inhibits the ethylene receptor (Blankenship and Dole 2003). Treatment with 1-MCP prevented or delayed most of the post-pollination phenomena in *Dendrobium* (Ketsa et al. 2006; Sukhotu 2006; Luangsuwalai 2007), indicating that ethylene perception was involved. Ethylene perception is required for the autocatalytic rise in ethylene production, whereby a small concentration of ethylene is perceived by the receptors, which results in increased expression of the genes involved in ethylene production, such as those encoding ACS and ACO. This increases the activity of the respective enzymes, and thus ethylene production (Klee 2004; Liu and Wen 2012). An application of 1-MCP also largely prevented the effect on ovary growth of placing 0.2 μmol NAA on the stigma, suggesting that auxin was required to act through ethylene in order to promote ovary growth (Ketsa et al. 2006).

Pollen tube growth has also been shown to be regulated by ethylene (Dhawan and Malik 1981; Zhang and O'Neill 1993; Song et al. 1998; Latha and Jayasree 2002), as it was inhibited after treatment of the flowers with 500 nL L^{-1} 1-MCP, and after placing 0.15 or 0.30 μmol AOA, or 0.2 or 0.4 nmol cobalt chloride, on the stigma (Table 1.5). The 1-MCP treatment was the most effective (Luangsuwalai et al. 2008). These data are consistent with experiments with other plant species where pollen germination and pollen tube growth were promoted by ethylene (Dhawan and Malik 1981; Zhang and O'Neill 1993; Song et al. 1998; Latha and Jayasree 2002).

Silver ions also block the ethylene receptors. Silver ions hardly move in plants as they strongly attach to cell walls (including the inner xylem walls). Silver has, therefore, often been applied in the much more mobile thiosulphate complex (Veen and van der Geijn 1978). When 0.3 μmol silver nitrate was applied to the stigma, prior to pollination, the effect of pollination on ovary growth was completely prevented (Ketsa et al. 2006). The data suggest that the pollination-induced increase in ethylene production in the column takes place, at least in its initial steps, not far from the bottom of the stigma cavity.

G. Antagonists of Auxin

An auxin antagonist, α-(*p*-chlorophenoxy)-isobutyric acid (PCIB), was applied to the stigma at 0.025, 0.05, and 0.10 μmol, before pollination, or together with NAA in non-pollinated flowers. PCIB inhibits the effects of auxin but does not affect auxin transport (Zhao and Hasenstein 2010). At 0.05 μmol, PCIB completely prevented the effects of pollination on ovary growth. At 0.025 and 0.10 μmol, it partially prevented the effect on ovary growth of placing 0.2 μmol NAA on the stigma (Ketsa et al. 2006), but in another test it completely prevented it at 0.02 μmol (Wisutiamonkul 2001). Similarly, the PCIB concentrations of 0.025 and 0.10 μmol prevented column growth and other post-pollination phenomena in pollinated flowers of *Dendrobium* (Wisutiamonkul 2001; Ketsa et al. 2006). Treatment of the stigma with the auxin antagonist PCIB, prior to pollination, reduced both pollen germination and pollen tube growth similar to ethylene inhibitors (Table 1.5).

Table 1.5 Pollen germination and pollen tube growth, determined 7 days after pollination, in *Dendrobium* 'Kenny' pollinated with 'Pompadour' pollinia. One day prior to pollination, the stigma was treated for 24 h with indicated chemicals. In a separate experiment, the inflorescences were exposed, prior to pollination, for 4 h to 1-MCP (Luangsuwalai et al. 2008).

	Pollen germination (%)[1]	Pollen tube length (mm)[1]
Ethylene and auxin antagonist treatments		
Pollinated with 'Pompadour' pollinia	96 a	0.42 a
'Pompadour' pollinia, after placement of 0.15 μmol AOA on the stigma	78 b	0.09 cd
'Pompadour' pollinia, after placement of 0.30 μmol AOA on the stigma	82 b	0.07 d
'Pompadour' pollinia, after placement of 0.2 nmol $CoCl_2$ on the stigma	82 b	0.14 bcd
'Pompadour' pollinia, after placement of 0.4 nmol $CoCl_2$ on the stigma	80 b	0.14 bcd
'Pompadour' pollinia, after 5 μg PCIB on the stigma	80 b	0.17 bc
'Pompadour' pollinia, after 10 μg PCIB on the stigma	82 b	0.22 b
1-MCP treatment		
Pollinated with 'Pompadour' pollinia	81 a	0.53 a
'Pompadour' pollinia, after treatment of the flowers with 500 nl l^{-1} 1-MCP	57 b	0.26 b

[1] Means within a column, per treatment group, not sharing the same letter are significantly different for $P < 0.01$.
PCIB: α-(*p*-chlorophenoxy)-isobutyric acid.

Additionally, the anti-auxin 2,3,5-triiodobenzoic acid (TIBA) was applied to the stigma at 0.010, 0.015, and 0.020 μmol, before pollination, or together with NAA in non-pollinated flowers. TIBA inhibits polar auxin transport. This transport requires the activity of specific auxin influx and efflux carriers that are located on the plasma membrane of transporting cells. TIBA apparently inhibits auxin efflux carrier activity (Al-Hammadi et al. 2003). These three TIBA concentrations reduced, to about half, the effect of pollination on ovary growth, without differences between the concentrations (Ketsa et al. 2006). TIBA application to the stigma also prevented column growth and other post-pollination phenomena in pollinated flowers of *Dendrobium* (Luangsuwalai 2007). These results overall appear to indicate that both ethylene and auxin are required for pollen germination and for pollen tube growth. In contrast, application of TIBA to the ovary of zucchini squash after anthesis can induce fruit set and early fruit growth (Martínez et al. 2013). This suggests that auxin can either upregulate or downregulate early ovary growth depending on the plant species.

VIII. CONCLUSIONS

Pollination of *Dendrobium* flowers induces a rapid growth of the column and ovary. Pollinia contain ACC, the direct precursor of ethylene, and at least one auxin-like compound. Auxins induced ethylene production and can have an effect on initial growth of the column and ovary mediated through ethylene action. Ethylene was required for initial rapid growth of the column and ovary. The pollinia-borne chemicals accounted for growth of the column and ovary after pollination.

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