

CARPOLOGY OF ORCHIDS

FINN N. RASMUSSEN* AND BO JOHANSEN

Institute of Biology, University of Copenhagen, Gothersgade 140, DK-1123 Copenhagen K, Denmark. Email: FinnR@bot.ku.dk

ABSTRACT. Orchid fruits have been largely neglected in orchid research. More variable than generally imagined, orchid fruits have a basic structure that remains inadequately described and understood. Yet structure and function of orchid fruits are important for many aspects of orchid conservation and may contribute significantly to the understanding of orchid phylogeny. Carpological studies of orchids are part of the ongoing research of the monocot phylogeny research group at the University of Copenhagen. A model of the orchidaceous ovary explaining the pattern of six valves is presented here, and the “subplacental cushions” in the ovary are shown to be cords of pollen tubes.

Key words: Orchidaceae, carpels, carpology, endocarpic trichomes, fruits, intra-ovarian trichomes

INTRODUCTION

It is because of the flowers that we find orchids interesting in the first place. The immense variation seen in orchid flowers and pollination mechanisms captivates the mind of those interested in natural history and is hence the ultimate reason for the wish to conserve orchids.

The next stage, the fruit, has been much neglected in orchid research. Orchid fruits, however, are much more variable than generally imagined, and their basic structure is still not completely clarified, despite the obvious fact that structure and function of orchid fruits are no less important than flowers.

The primary reason for this state of affairs is, of course, that we recognize species by the flowers. Most orchids collected in nature have been flowering specimens, and non-flowering specimens collected for cultivation rarely produce fruits in captivity. Orchid taxonomy and identification has become a study of flowers. Even very striking fruit features are ignored, because they are neither present in the herbarium nor in the greenhouse; and when seen in nature, they cannot be identified.

MATERIALS AND METHODS

Ovaries of a large number of orchids flowering or fruiting after artificial pollination in the Botanical Garden, University of Copenhagen, were hand sectioned and inspected in stereomicroscope and with transmitted light microscopy at low magnification. Ovaries of ca. 50 orchid species collected at anthesis in the greenhouses or in nature were fixed in FAA and embedded in Paraplast®. Then 10 µm thick sections were deparafinized and stained in Safranin/Fast Green

for examination in transmitted light microscopy. Fruits of *Dendrobium thyrsiflorum* Rchb.f. ex André (live plants collected by G. Seidenfaden, GT 8338 × GT 8500, Thailand; see Johansen 1990) were cross-pollinated and collected 2, 4, 6, and 8 weeks after pollination. The developing fruits were fixed, embedded, and stained as described above. Fruits of cross-pollinated flowers of *D. secundum* Lindl. (Live plants collected by G. Seidenfaden, GT 9454 × GT 9248, Thailand; see Johansen 1990) were collected 2, 4, 6, 8, and 12 weeks after pollination, fixed in a mixture of PFA/GA (2 % paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer), dehydrated, and critical point dried for SEM according to standard procedures.

Transmitted light microscopy images were digitized directly from the Leica DM RXA 2 microscope using a Hamamatsu C-2400 (ORCA II) camera. SEM observations were made using a JEOL JSM P15 Scanning Electron Microscope.

RESULTS: STRUCTURE OF ORCHID FRUITS

Orchids belong to the lily-like monocots in the broad sense and may constitute the sister group to the remainder of the clade that has become known as Asparagales (The designation “Orchidales” has priority, but the principle of priority is not mandatory for ordinal names). The flowers in this group are generally 3-merous. In Orchidaceae, the flowers are epigynous, and the ovary is thus inferior to perianth-segments and stamens. Epigyny in lily-like plants usually is regarded as being a result of congenital fusion of the basal parts of the perianth and the stamens, but also may be imagined as a result of a cup-shaped receptacle that envelops the ovary—a view advanced by Pfitzer (1886) and accepted by Dressler (1981, 1993).

* Corresponding author.

One would expect orchid fruits to be constructed like the fruits seen in Amaryllidaceae, Hypoxidaceae, and other families with inferior capsules made of three carpels, but they are really much different and have puzzled botanists since Robert Brown and Lindley.

How Many Carpels?

FIGURE 1 shows a cross section of a typical orchid fruit as viewed by Francis Bauer in 1801. This and other beautiful drawings of orchid flower details were published in Bauer & Lindley (1830–1838). The ovary is composed of six distinct parts, unlike any other fruits we know from the monocotyledons; and at maturity, the capsule of most species will open along dehiscence lines separating these six elements. Lindley assumed that the ovary was made up of six carpels, three fertile ones carrying luminal V-shaped placentae and three narrower, sterile carpels, carrying the stigmata (Bauer & Lindley 1830–1838; Lindley 1847). Robert Brown (1831) preferred a less complicated interpretation: orchids have only three carpels that alternate with the inner whorl of stamens. This interpretation places the midline of each carpel directly below a stigma lobe, and the placentae are marginal, as in comparable families. This view was accepted by later authorities such as Payer (1857), van Tieghem (1875), and Pfitzer (1886), but others regarded the question as unresolved (Prillieux 1857, Eichler 1875, and Arber 1925 who ascribed Lindley's 6-carpel model to Saunders 1923; see FIGURE 2).

Garay (1960) suggested that uni-locular orchid ovaries comprise just one carpel, a view apparently adopted by Arditti (1992). Barbosa Rodrigues is cited by Hallé (1980) for having advanced a model with no less than 12 carpels in 1883. Österberg (1883) and Swamy (1948), unaware of Österberg's work, tried to elucidate the problem by studies of vascularization (FIGURE 4), but inferences from this approach are limited by the fact that vascular bundles are very plastic. Anastomoses and new side branches may arise as a function of distance to other bundles and depth of the tissue (Schmid 1972).

Although prominent orchidologists (Schlechter 1927, Dressler 1981) accepted Brown's model, dissent continues to be expressed. A very different view was published by Vermeulen (1966), who suggested that the three placentiferous elements might not be carpels at all but ovule-bearing stem-like organs. He even introduced a special term, "stachyospermy," for this way of producing a fruit. Veyret (1981, 1988) argued for the 6-carpel view after analysing fruits of *Sobralia* and *Elleanthus*. Odoux et al. (2003) de-

scribed a cross section of a fruit of *Vanilla planifolia* as showing three carpels with laminal placentation along their midnerves, which would place the carpels opposite the petals and the inner stamen whorl and bring them out of alignment with the stigma lobes.

Hallé (1977, 1980, 1981, 1986), the most important recent contributor to orchid carpology, regarded the carpel number and ovary structure as still open for interpretation. He pointed out that the distinctive pattern with six "valves" or "elements" is still in need of an explanation. We offer one here.

Split-Carpel Model

The orchidaceous ovary can be described within the framework of the standard liliiflorous gynoecium. No extranumerary carpels, receptacular cups, shifted whorls, or unusual ovuliferous structures are needed, if this ovary is recognized as inferior and the tepal bases as involved in the structure. After studying a great number of orchid ovaries, we suggest that the hexamerous pattern seen in most orchid fruits is produced by the bases of the sepals and petals, which are less amalgamated into the inferior ovary than in, for example, Amaryllidaceae (Rasmussen et al. in press). The "sterile valve" ("jugum" in Venverloo et al. 2003) corresponds to a sepal base and may in many cases appear to split the carpel opposite to it. In some cases, however, it is evidently an element adnate to the carpel, sometimes even with a separate vascular strand (FIGURES 3, 4). The broader "fertile valve" is then made up of a petal base and two carpel-halves, carrying one marginal placenta from each (FIGURE 5). In most species, the carpel walls thus will split along the midline at maturity, which is indeed the most common mode of dehiscence in capsule-fruited liliiflorous monocots; but the sepal base will most often remain as a separate "sterile valve." All six "valves" will usually remain fused near the apex of the capsule, rendering the characteristic lantern-like impression of a dehiscent orchid capsule. The stamen bases are integrated to the extent that no trace is left in the inferior ovary, which is to be expected considering their strong integration in the gynostemium.

This interpretation of the orchidaceous ovary, in accordance with general monocot floral morphology, is compatible with all phenomena we have seen so far in orchid ovaries and fruits.

Observations of Variation

Despite this simple ground plan, orchid fruits are extremely variable in size, shape, texture, or-

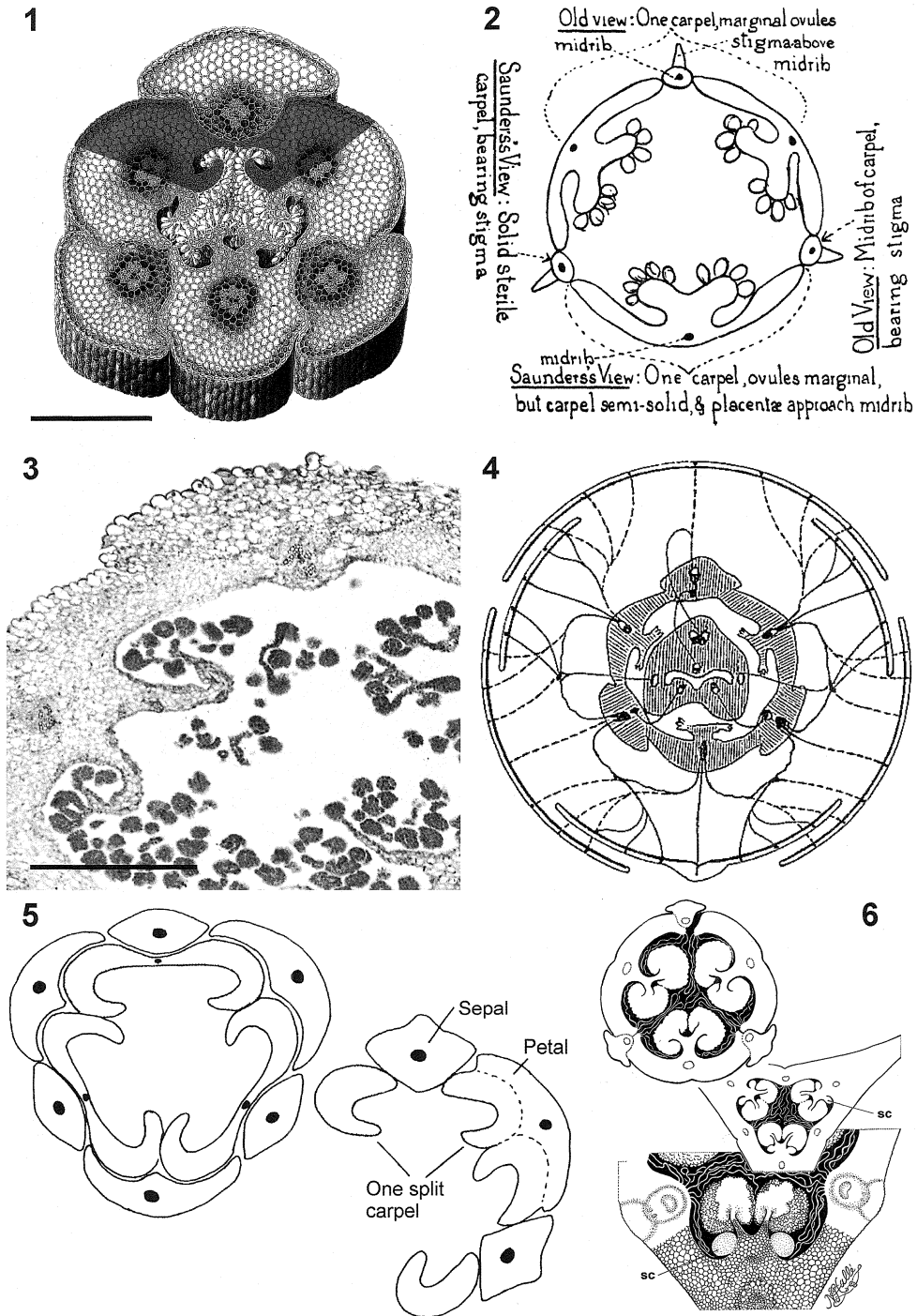


PLATE A. Cross sections and interpretations of orchid ovaries. Flowers are shown as resupinated with labellum lowermost in all sections. FIGURE 1. *Bletia purpurea* (Lam.) DC, three sterile valves alternate with three placentiferous valves (Bauer 1801 in Bauer & Lindley 1830: pl. IX as *B. verecunda*). Tissue corresponding to the median carpel and bases of adjoining lateral petals is shadowed here. Scale bar = 1 mm. FIGURE 2. Two interpretations of the orchidaceous ovary rendered by Arber (1925: fig. *cli*): "Old view" (Brown 1831) and "Saunders's view" (Lindley 1830). FIGURE 3. *Gavilea glandulifera* (Poepp.) M.N. Correa. The sterile valve (top) is seen on the outer side of the carpel, the vascular strands are not fused. Scale bar = 500 μ . (Collected by

namentation, way of dehiscence, number of seeds, and seed dispersal mechanisms. Prillieux (1857) recognized seven different dehiscence types and observed that particular modes of dehiscence may characterize genera. The criteria were number of valves (3 + 3, 3, 2, or 1) and apically fused vs. free and recurving valves. Prillieux did not distinguish the case where the three placentiferous valves recurve, as the sterile ones remain apically conjoined; and he was not aware that some orchid fruits do not dehisce at all.

The most beautiful contribution to orchid carpology was published by Beer in 1863. The folio work "Beiträge zur Morphologie und Biologie der Familie der Orchideen" contains 12 lithographic color plates of orchid seeds and fruits, depicting 238 different fruits of mainly neotropical orchids along with descriptions of fruit development observed in culture. Beer even suggested a classification of the orchid family into six main groups characterized by a combination of floral and fruit characters. This scheme, very much at variance with established classifications, was completely ignored by later authors.

Le Maout and Decaisne (1868) illustrated the dehiscence modes described by Prillieux (1857). Their drawings were included in Pfitzer (1882) and in his treatment of Orchidaceae in *Die Natürlichen Pflanzenfamilien* (Pfitzer 1889).

Horowitz (1901) undertook a detailed anatomical study (supervised by Pfitzer) of 38 orchid fruits. He concluded that the main difference between the sterile and the fertile valves is that the cells of the former do not get much larger during maturation, while the cells of the fertile valves undergo considerable lateral growth. He also suggested a classification of orchid fruits based on number and distribution of vascular bundles, and recognized six different ways of dehiscence.

Fruits of a number of Colombian orchids are present on several of the more than 200 beautiful orchid plates painted from 1783–1816 for a grand folio report in the *Real Expedición Botánica*. Publication of the report was much delayed

and began in the last part of the 20th century. The orchid paintings are treated in Schweinfurth et al. (1963, 1969) and in Fernandez Perez (1985); three more volumes are planned. The fruits, however, are not as detailed as those of Beer (1863), and the text provides little discussion of fruit morphology.

Orchid fruit observations have been rare and scattered for a long time; only the works on New Caledonian orchids by Hallé (1977, 1980, 1981, 1986) contain numerous illustrations of orchid fruit details and penetrating discussions of their structure and biology. Silhouettes of fruit cross sections ("carpograms") were used in Hallé (1981) together with formula-like data lines for presenting a large amount of fruit data. Reeve and Woods (1989) included cross sections of the often spectacularly winged fruits in most of their drawings of species of *Dendrobium* sect. *Oxyglossum*. Ververloo et al. (2003) showed cross sections of several species in a paper on post-pollination phenomena in *Phalaenopsis* spp.

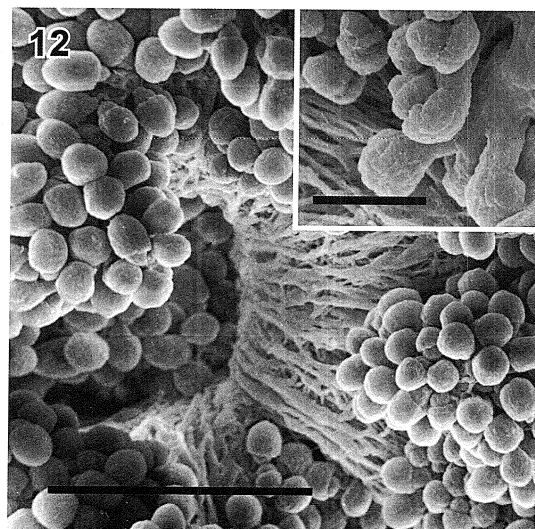
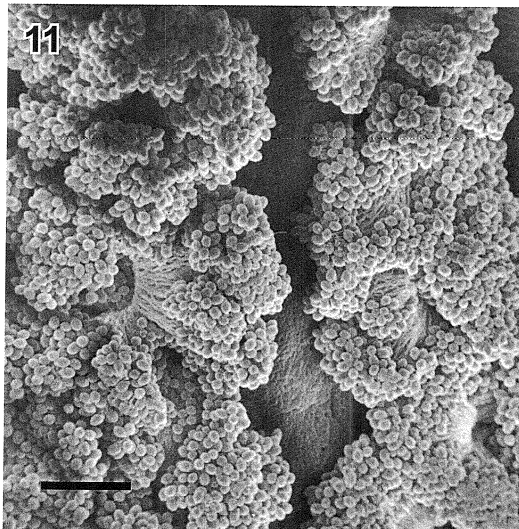
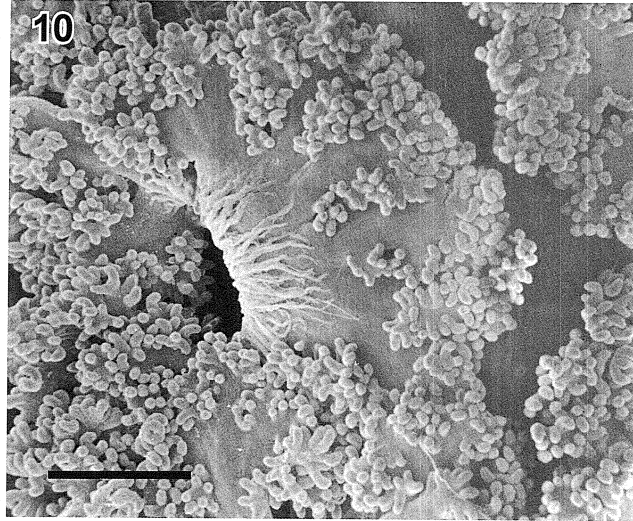
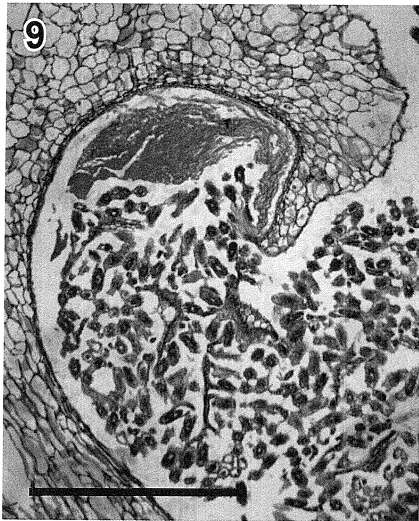
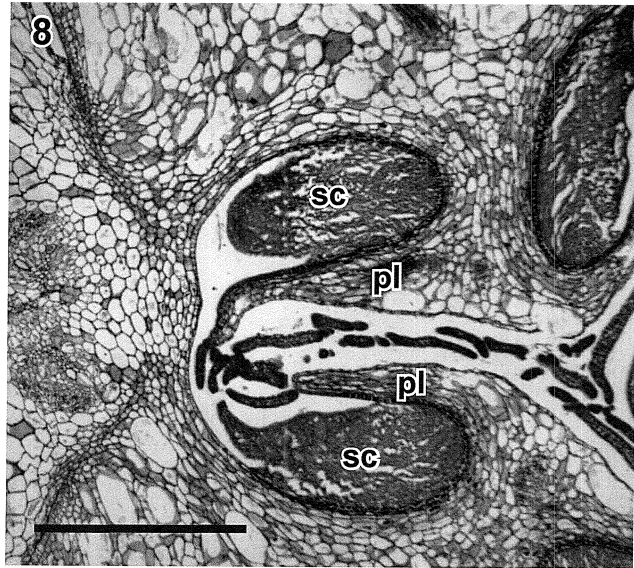
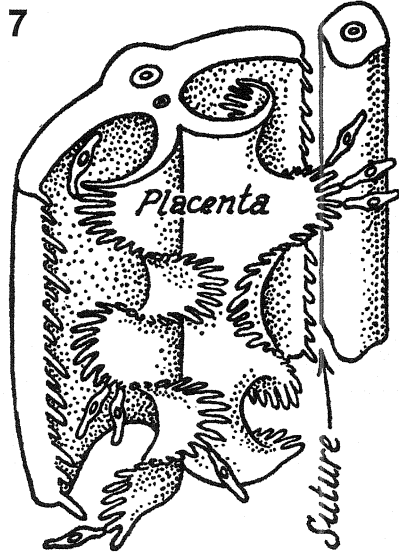
Often overlooked are the non-dehiscent, more or less fleshy fruits of some orchids. Examples are found in *Apostasia*, *Selenipedium*, *Cyrtosia*, *Vanilla*, and *Palmorchis* (Dressler 1981, 1993; Kocyan & Endress 2001). In *Neuwiedia*, some species typically have 6-valvate capsules with caducous fertile valves, but others have genuine, gooseberry-like berries (K.A. Kristiansen pers. comm., Kocyan & Endress 2001, Rasmussen et al. in press).

Endocarpic Trichomes

Hairline structures inside the ovary of orchids seem to have been first recognized by du Petit-Thouars (1822). They have been observed and discussed in some detail by Blume (1848), Beer (1857), Prillieux (1857), Horowitz (1901), Malguth (1901), and Hallé (1986), but often with incomplete references to other treatments. Hallé, for example, was unfamiliar with the comprehensive work of Malguth. In a recent survey of intra-ovarian trichomes in monocotyledons (Ru-

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N. Jacobsen 1979–3085, Argentina, Prov. Rio Negro). FIGURE 4. *Phaius tankervilleae* (Aiton) Blume, "Vasco-gram" by Österberg (1883: fig. 34 as *P. grandifolius*). Median strands from the carpels continue into the style; strands of the sterile valves become midnerves of the sepals, with the one from the median sterile valve branching and also supplying the anther. FIGURE 5. The "split carpel model" of the orchidaceous ovary. Left: Ovary with incomplete splitting of the carpel, as in FIGURE 3. Right: Typical orchid ovary with three sterile valves (sepal bases) and three fertile valves, each consisting of two half-carpels and a petal base. FIGURE 6. Endocarpic trichomes emanate from the midline (eventually sutures) of the carpels of *Micropera fasciculata* (Lindley) Garay (top) and *Trachoma subluteum* (Rupp) Garay (middle, bottom). "Subplacental cushions" (coussinet sous-placentaires) are seen next to the placenta (sc) in *T. subluteum* (details from Hallé 1986: pl. 9, © Publications Scientifiques du Muséum national d'Histoire naturelle, Paris).



dall et al. 1998), orchids were not mentioned at all.

Beer (1857) had already noted that endocarpic trichomes seemed to be found only in epiphytic genera. Hallé (1986) found that they are a characteristic of the subtribe Sarcanthinae (=Aeridinae). Freudenstein and Rasmussen (1999) scored them as present in 41 genera, including all "Aeridinae," in most of the genera formerly classified as "vandoids" (Vandoideae in Dressler 1981), and more surprisingly in the terrestrial orchidoid genus *Prasophyllum*.

Considerable variation has been observed in appearance and texture of the endocarpic trichomes. They may be up to 4 cm long, curled, and hose-like, sometimes cylindrical (Hallé 1986), or laterally conjoined forming mat-like structures (Malguth in Horowitz 1901). In some fruits, they are developed as stiff brushes that remain on the margins of one set of valves after dehiscence (Malguth 1901, Hallé 1989), perhaps acting as sieves that bring a bryophyte peristome to mind rather than elaters. We suspect that the resiniferous papillae seen in the fruits of *Vanilla* (Berg 1865, Roux 1954, Odoux et al. 2003) may be homologous structures.

Knowledge remains incomplete regarding the distribution, homologies, range of variation, and structural details of endocarpic trichomes; and we continue to observe and collect data for a more comprehensive survey. To date, no observational data have elucidated their ecological significance. Prillieux (1857) and particularly Hallé (1986) emphasized the resemblance to the elaters seen in the sporangia of liverworts and some pteridophytes, and concluded that their function is most probably also similar. This seems plausible in most cases, where they are strongly hygroscopic one-celled trichomes emerging from the lines of dehiscence in the capsules (near the median nerve of the carpels), eventually becoming detached, and sometimes taking up more volume than the seeds. Malguth (1901) speculated that they also might function as regulators of the environment inside the de-

veloping ovary or even for transport of nutrition, as they in some cases have numerous dots that he interpreted as pores. He also suggested they might provide the mature seeds with glutinous material enabling them to stick on branches, which would be an interesting alternative to the seed-stickiness described by Barthlott and Ziegler (1980).

Hallé (1986) also observed and depicted peculiar, seemingly inexplicable intraovarian structures that he called "coussinets sous-placentaire," subplacental cushions, in the corners of the placenta (FIGURE 6). Viewed in three dimensions over an interval of time they are, as we shall see, natural transient phenomena in connection with fertilization.

DISCUSSION: WHAT GOES ON IN AN ORCHID OVARY?

The awesome number of ovules and later seeds in orchid ovaries has long fascinated biologists. Darwin (1862) estimated the number of seeds in *Cephalanthera damasonium* (as *C. grandiflora*) and *Orchis maculata* at 6020 and 6200, respectively, describing in detail how he arrived at his number. He also related a count of 371,250 seeds in a *Gongora* sp. (as *Acropera*). Arditti and Ghani (2000) cited counts of 4 million seeds in *Cycnoches*, but such extremes are in fact rare. The 1.5–2 cm long fruit of *Dendrobium secundum* contains ca. 15,000 seeds. Even small capsules will often hold a few thousand seeds, which is still far more than in non-orchidaceous plants. As for many other unique orchid characters, this is viewed as a consequence of the mycotrophic germination (Benzing 1987, Rasmussen 1995). The chance of germination for any one seed is very low, so a huge surplus of very mobile seeds is necessary simply for population maintenance. On the other hand, mycotrophy dispenses largely with the need for stored nutrition, allowing seeds to become very small but still capable of germination. An extremely high number of ovules per fruit neces-

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PLATE B. Stages in the development of *Dendrobium* placentae, pollen tubes, and ovules. FIGURE 7. Schematic placentiferous and sterile valve in an orchid ovary. Note the wavy margin (placenta) of the carpel and the sutural papillae where the carpel will split at dehiscence of the fruit. (Hallé 1977: pl. 2, fig. 6, © Publications Scientifiques du Muséum national d'Histoire naturelle, Paris). FIGURE 8. *Dendrobium thyrsiflorum* Rchb.f. ex André 2 weeks after pollination. Two sets of placental lobes (pl) almost fill the lumen of the ovary; "subplacental cushions" (sc) are dense masses of pollen tubes very distinct at the bases of the lobes. Scale bar = 500 μ m. FIGURE 9. *Dendrobium thyrsiflorum* 8 weeks after fertilization. The young ovules fill most of the lumen of the ovary. Scale bar = 0.5 mm. FIGURE 10. *Dendrobium secundum* Lindl. SEM of placenta 8 weeks after pollination. The pollen tubes have nearly reached the developing ovules. Scale bar = 100 μ m. FIGURES 11, 12. *Dendrobium secundum* 12 weeks after pollination, with fertilization imminent. Scale bar = 200 μ m. Insert: detail of ovules with two integuments clearly seen. Scale bar = 50 μ m.

sitates an equally high number of pollen being transferred to the stigma, leading to all the well known specializations of the orchid flower.

As in all liliiflorous monocots, the ovules will develop along the margins of the carpels, where the placentae, which in orchids usually are undulate, optimize the usage of available space in the fruit (FIGURE 7). Development of the ovary into a fruit and formation of ovules, however, will only start if the fruit is pollinated (Brongniart 1831; Hildebrand 1863; Swamy 1949a, 1949b; Veyret 1965). Pollination events and hence fruit set is often low in orchids, especially in epiphytic orchids (e.g., Ackerman & Montalvo 1990, Aragón & Ackerman 2004), but the effect of this seems counterbalanced by the high number of ovules per fruit. For every seed, a pollen grain must germinate on the stigma and produce a pollen tube that will grow all the way down the stylar canal into the fruit lumen, cross the placenta, and enter the micropyle of an ovule. Observations on several species of *Dendrobium* show that in cross section single tubes may be 2–10 μm in diam. depending on species. The tubes collapse and flatten behind the sperm cells, but they nevertheless form a conspicuous cord because of their sheer number. Orchid pollen contains little or no reserve nutrients, but pollen tube growth is promoted by mucilage secreted by the stigma and stylar canal (Johansen 1990). After passing through the stylar canal they will spread and continue along the corners of the placentae. Viewed in cross section, they form the “subplacental cushions” (FIGURE 8). When the tube apices with the sperm cells fan out over the placentae, the cushions become less prominent and the ovules, having developed in the meantime, begin to appear in cross sections of the fruit (FIGURES 9, 10). Observations on fruit development in *Dendrobium farmeri* indicate that when the pollen tubes reach their targets, the ovary has increased in size by a factor of ten (Johansen 1990) and is stuffed with ovules ready for fertilization (FIGURES 11, 12). The size of the fruit also depends on the amount of pollen applied to the stigma. When 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ pollinarium were used for pollination in *Dendrobium secundum*, the fruit size decreased with decreasing amounts of pollen applied, indicating that the number of pollen cells in the pollinarium roughly corresponds to the number of ovules developed, and hence little or no pollen competition takes place in this species.

Time from pollination to fertilization in orchid fruits has been measured from 4 days to 10 months (Wirth & Withner 1959, Veyret 1965). In *Dendrobium chrysotoxum*, development of fruit stops shortly after fertilization (ca. 80 days after pollination). The fruit will eventually ma-

ture and dehisce in the next dry season a year after pollination (Johansen 1990).

The time required for the fruit to ripen is known from a number of cultivated orchid species (45 days to 20 months; see surveys in Wirth & Withner 1959, Lucke 1971, Arditti 1992). In addition, more complete event tables worked out for a limited number of species record the following events: fruit age in days after pollination, physical fruit size, and such stages as the beginning of cell divisions in the ovary wall, differentiation of archesporial cell, megasporogenesis, megagametogenesis, fertilization, seed coat formation, seed disconnection from placenta, and fruit dehiscence. See comparable surveys in Veyret (1965), Arditti (1992), and a detailed table for *Epidendrum ibaguense* in Taylor et al. 1982. Much of the development is initiated and regulated by the primary pollination event at the stigmatic surface (O'Neill 1997). The sequence of these events appears consistent, but the timing differs and is probably adapted to the ecological conditions at the preferred habitat. Structural and developmental constraints are likely to be involved as well, but very little is known yet. Not surprisingly, almost all information on the ontogeny of orchid fruits is concerned with ovules and seeds; little data concerns the ovary walls and placentae; and hardly any data are available on papillae and trichomes.

CONCLUSIONS: THE CARPOLOGY CHALLENGE

The ability to set fruit and successfully disperse seeds is critical for maintaining a viable population and indispensable for spreading and forming new populations and hence for the long-term survival of a species. Much research has focused on pollination; also ecological factors such as pollen load, availability of resources, fruit predation, and controlling fruit set and reproduction efficacy have been investigated (e.g., Montalvo & Ackerman 1987, Zimmerman & Aide 1989, Snow & Whigham 1989, Ackerman & Montalvo 1990, Ackerman & Zimmerman 1994, Ackerman 1998, Melendez-Ackerman et al. 2000). The structure, physiology, and mechanical function of the fruit, however, are still very little known. Apparently this part of the plant is just as specialized and uniquely adapted as other orchidaceous structures that attract much more attention. A detailed study of orchid fruits might offer some genuine surprises.

The bottleneck for this research is availability of study material. Orchid fruits should be studied in-situ at accessible sites to investigate not only incompatibility, fruit set, and timing but also dehiscence and release of seeds. For this

research, fruits should be collected and fixed for structural studies. In collections of cultivated orchids, fruits can be produced and fixed at intervals to study timing of developmental processes and function of endocarpic structures. As pollination triggers the development of ovules in most orchids, and as many thousands of ovules are developed simultaneously, orchids are ideal plants for studying the molecular background of ovule and gametophyte development in angiosperms. For these reasons, we currently are using various molecular techniques to study MADS-box gene expression during ovule development in orchids. Fruit character data (Skipper et al. 2006) mapped on phylogenies may help clarify where and how these attributes evolved. The aerodynamics of seed shedding and dispersal may be studied experimentally.

An understanding of the structure and biology of orchid fruits may offer crucial insights, applicable when addressing conservation questions.

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IN-VITRO GERMINATION AND CLONAL PROPAGATION OF ENDEMIC *TILLANDSIA CALIFANII* RAUH (BROMELIACEAE) FROM MEXICO

M.D. GARCIA-SUAREZ*

Departamento de Biología, Universidad Autónoma Metropolitana Iztapalapa. Apartado Postal 55-535, Mexico City, DF, 09340, México. Email: loli@xanum.uam.mx

V. RICO-GRAY

Instituto de Ecología, Apartado Postal 63, Xalapa, 91000, VER, México.

N. MOLINA-ACEVES

Departamento de Biología, Universidad Autónoma Metropolitana Iztapalapa. Apartado Postal 55-535, México City, DF, 09340, México.

H. SERRANO

Departamento de Ciencias de la Salud, Universidad Autónoma Metropolitana Iztapalapa. Apartado Postal 55-535, México City, DF, 09340, México.

ABSTRACT. *Tillandsia califanii* is an endemic epiphytic bromeliad from the Tehuacán-Zapotitlán-Cuicatlán region, Puebla, México. Its basic reproductive patterns and distribution are poorly documented. The authors studied its distribution in two natural populations in the Zapotitlán Valley and performed a series of laboratory experiments to determine the basic culture conditions to determine a propagation strategy. In nature, *T. califanii* commonly propagates asexually producing up to four plantlets per mother plant. Most *T. califanii* individuals (90%) use *Beaucarnea gracilis* as phorophyte, and only 2% live on the columnar cacti *Cephalocereus columna-trajani*. Almost 50% of the *T. califanii* populations include individuals measuring 20–40 cm in height, considered as adult individuals in reproductive stage. Flowering occurs August–October. Inflorescence, formed by a spike, reaches 60 cm in height and produces an average of 20 capsules and up to 2000 seeds per spike. Seeds disperse late February–May. Viability tests show that most seeds contained a highly damaged embryo, which contributed to low survival. The low germination and survival observed in the field may be associated to the endemism of *T. califanii*, thus resulting in a genetically vulnerable species.

Key words: micropropagation, in-vitro seed germination, *Tillandsia*, epiphyte

INTRODUCTION

Tillandsia califanii Rauh (Bromeliaceae) is an endemic species of the semiarid Tehuacán-Zapotitlán-Cuicatlán region of Puebla, México. This epiphytic monocot mainly grows in the crowns of the succulent endemic tree *Beaucarnea gracilis* Lem. (Nolinaceae), which is the main component of the Izotal plant community. At the Cardonal plant community, smaller populations are established on the columnar cacti *Cephalocereus columna-trajani* (Karw.) K.Schum. and *Neobuxbaumia tetetzo* (J.M.Coult.) Backeb. (Cactaceae). Thus their abundance and distribution depend on the spatial arrangement and distribution pattern of their hosts as well as germination within the sites on their hosts (Bennett 1986, 1987; Benzing 1990; García-Suárez et al. 2003).

Anatomical features of *Tillandsia califanii* al-

low its growth and development in this arid environment as a drought-resistant tank bromeliad. Xerophytic adaptations include water-absorbing scaly trichomes on the epidermis of its leaves and sclerified roots that work mainly as holdfasts. Rauh described *T. califanii* in 1971 (Smith & Downs 1977), and Gardner (1984) demonstrated the viability of its pollen.

Tillandsia califanii is the only endemic bromeliad among the 600 endemic plant species growing within the Zapotitlán Valley (Dávila et al. 1995). The distribution and abundance of these endemics has been described by García-Suárez et al. (2003).

The physiology of epiphyte seeds has not been studied intensively, except for some well known orchids and bromeliads (Benzing 1980). In-vitro germination can be used for rapid propagation, not only of epiphytic species with commercial value, but also as an alternative method for conserving species that are part of the rich

* Corresponding author.