Plant Syst. Evol. 222: 243–269 (2000)

Plant Systematics and Evolution

The structure and function of orchid pollinaria

S. D. Johnson and **T. J. Edwards**

School of Botany and Zoology, University of Natal, Scottsville, South Africa

Received July 24, 1999
Accepted December 31, 1999

Abstract. Cohesive masses of pollen known as pollinia have evolved independently in two plant families – Orchidaceae and Asclepiadaceae. Yet, the bilateral symmetry of orchids has allowed a greater degree of specialization in pollination systems and a much greater diversity in the morphology of pollinaria – units comprising the pollinia(um) together with accessory structures for attachment to the pollinator. Pollinaria differ in the degree of cohesion of pollen in the pollinium, which may be soft, sectile (comprised of sub-units known as massulae) or hard. A single hard pollinium may contain more than a million pollen grains, yet pollen:ovule ratios in orchids are several orders of magnitude lower than in plants with powdery pollen due to the lack of wastage during transport to the stigma. Attachment of pollinia to the pollinator is usually achieved by means of a viscidium that adheres most effectively to smooth surfaces, such as the eyes and mouthparts of insects and beaks of birds. The stalk connecting a pollinium to the viscidium may be comprised of a caudicle (sporogenous in origin) and/or a stipe (derived from vegetative tissue), or be lacking altogether. Caudicles and stipes may undergo a gradual bending movement 20 s to several hours after withdrawal from the flower, the main function of which appears to be to reduce the possibility of geitonogamous pollination. Other mechanisms that promote outcrossing and pollen export in orchids include pollen carryover (achieved by sectile or soft pollinia), temporary retention of the anther cap, protandry and self-incompatibility (rare among orchids). Pollinaria ensure that large pollen loads are deposited on the stigma, thus enabling the fertilization of the large numbers of ovules in the flowers of Orchidaceae. Pollinaria also ensure efficient removal of pollen from the anther, minimal pollen wastage during transit, and a high probability of deposition on conspecific stigmas.

Key words: Orchidaceae, geitonogamy, gene flow, pollen, pollen discounting, pollen:ovule ratio, pollinaria, pollination, pollinium, seed size, self-incompatibility.

The packaging of pollen into a compact unit known as the *pollinium*, which together with accessory structures for attachment to pollinators comprises a *pollinarium*, was undoubtedly a key innovation in the evolutionary history of the Orchidaceae, and may have played a role in promoting the tremendous radiation of the group, which numbers at least 19 500 species (Dressler 1993). The purpose of this review is to provide a synthesis of published information on structural and functional aspects of orchid pollinaria. In particular, we attempted to combine two different, yet complementary, traditions in the study of orchids, namely the comparative morphological approach of systematic botany (Dressler 1993) and the ecological approach adopted in experimental pollination biology (Nilsson 1992).

The structure and function of orchid pollinaria cannot be understood in isolation from other profound morphological modifications that occurred during the evolution of the Orchidaceae. Among these were the reduction in the number of fertile stamens to just one, varying degrees of fusion of stamen and pistil into a structure know as the column (or gynostemium), and the dust-like seeds, most of which lack endosperm (Dressler 1981).

The adnation of the gynoecial and androecial whorls of the Orchidaceae and Asclepiadaceae sets these families apart in the Monocotyledonae and Dicotyledonae, respectively. In both families, pollen has been consolidated into pollinia. Both families show precursory evidence of grouped pollen grains in the form of tetrads, almost as if there has been repeated consolidation of the pollen loads during their evolution; however, the retention of actinomorphic flowers in the Asclepiadaceae has precluded the reduction in anther number that is so evident in the orchids.

The orchid anther

Hypothetical ancestral patterns of the orchids are based on a hexastaminate liliod template (Dahlgren et al. 1985, Rasmussen 1986b, Cameron et al. 1999) and require losses of 3, 4 and 5 functional anthers to produce the subfamilies Apostasioideae, Cypripedioideae and the monandrous orchids respectively (Table 1).

The Apostasioideae, with their primitively triandrous flowers and fairly poor fusion between the androecium and gynoecium, clearly represent the pleisiomorphic condition among orchids (Dressler 1993). However the specialization of their vessels (Judd et al. 1993) indicates vegetative specialization off the main phyletic lineage. The monophyly of the subfamily is well established by recent studies (Judd et al. 1993, Stern et al. 1993, Freudenstein and Rasmussen 1999, Cameron et al. 1999). Diandrous species of *Apostasia* are linked to the triandrous pattern through members of the typical section which sport a median staminode, the loss of which is considered specialized (Dressler 1990). Anther dehiscence is introse via longitudinal slits. The anthers are dorso-basally attached and, while considerable connation of staminal filaments occurs, the style is largely free. Endothecial thickenings are intimately involved in anther dehiscence (Dahlgren et al. 1985) and in the Apostasioideae are tightly packed channels

Table 1. Some androecial characters of the Orchidaceae (*o* outer, *i* inner, *l* lateral, *m* median)

Taxon	Stamens	Staminodes	Pollinia	Viscidia
Liliaceae s.l.	6(3 o, 3 i)	0	absent	absent
Apostasioideae			absent	absent
Neuwiedia	3 (1 om, 2 il)	0		
Apostasia	2 (i)	0–1 (om)		
Cypripedioideae	2 (il)	1 (om)	smear pollen, rarely soft pollinia	absent
Spiranthoideae	1 (om)	2 (il)	soft pollinia, sometimes sectile	apical when present
Orchidoideae	1 (om)	2 (il)	soft pollinia or sectile with hard massulae	basal when present
Epidendroideae	1 (om)	2 (il)	hard or, rarely, soft pollinia	basal when present

of loops or helices. Similar patterns occur in putatively basal genera in the remaining subfamilies (Freudenstein 1991). The reduced thickenings associated with the more "advanced" tribes may be due to the decrease in conventional anther dehiscence associated with the evolution of pollinaria and detachable anther caps.

The Cypripedioideae are diandrous and are further characterized by their prominent median staminodes, saccate lips and synsepalum. The group is monophyletic and is well supported in recent cladistic studies (Albert 1994, Cameron et al. 1999, Freudenstein and Rasmussen 1999). Their androecial derivation is from the loss of three adaxial stamens and retention of the lateral anthers as fertile units. The fertile anthers are subsessile and are positioned so that pollinators escaping from the labellum pass the convex stigma before coming into contact with them. The endothecial thickenings of this group are a circular arrangement of anticlinal bars which are convergent with patterns in the Vanillinae and Cranichidae (Freudenstein 1991). The median staminode is highly variable and is probably intimately involved in pollination syndromes. The organ has considerable systematic importance due to its divergence at the species level (Cribb 1987).

Within the monandrous Orchidaceae only the median abaxial anther is fertile and this is frequently flanked by the sterile remnants of the lateral abaxial stamens. With the reduction of fertile anthers comes a trend of increased pollen aggregation from primitive powdery or mealy pollen masses to complex pollinaria (Burns-Balogh and Funk 1986, Dressler 1986). In a few anomalous instances this typical pattern of androecial reduction is contradicted, thus *Encyclia cochleata* (L.) Lemee var. *triandra* has two supernumerary fertile anthers which are involved in autogamy (Catling 1990) and this situation is also recorded from *Epidendrum nocturnum* Jacq. (Ackerman 1995). Unusual androecial patterns are also present in *Diplandrochis* [= *Neottia*] which is unique in the occurrence of two fertile anthers (the medial members of the inner and outer whorls of the androecium) and the Chinese genus *Tangtsinia* [= *Cephalanthera*] which has five staminodes and a single outer median stamen (Chen 1982).

Pollen

Possibly no other family of Monocotyledonae exhibits the range of pollen wall diversity apparent in the Orchidaceae. Pollen surface sculpturing is most complex in *Diseae* and Orchidoideae, which have sectile pollinia, and least complex in orchids with hard pollinia (Schill and Pfeifer 1977). Burns-Balogh (1983) suggested phylogenetic pathways for the evolution of the exine in orchids and a more extensive structural account is available in Schill and Pfeiffer (1977). Pollen grains within the Apostasioideae and Cypripedioideae, are loosely aggregated monads, while in the monandrous orchids, monads are largely replaced by tetrads and these may be loosely packaged or tightly fused into pollinia.

The pollen of the Apostasioideae is produced in monads and grains are monosulcate, operculate and have reticulate sculpturing (Newton and Williams 1978, Schill 1978). Examination of the pollen walls reveals a semi-tectate arrangement with a tectum, columellae and a foot layer. The operculum appears to be synapomorphic for this group (Judd et al. 1993, Freudenstein and Rasmussen 1999), although Burns-Balogh and Funk (1986) argued that the condition was derived separately in *Apostasia* and *Neuwiedia.* The progressive aggregation of pollen in the remaining orchids obviates the advantages of operculate pollen. Pollen sculpturing in the Apostasiaceae is reticulate and similar to that of the *Neottieae* (Williams and Broome 1976).

Throughout the Cypripedioideae pollen is produced as adherent monads, which smear insects escaping from the trap flowers. Pollinia occur in *Phragmipedium* and *Selenipedium* but these remain soft masses representing the contents of the four pollen sacs. TEM shows that the wall structure of cypripedioid pollen is very distinctive. The foot layer has been lost and the imperforate tectum is accompanied by incipient columellae, a condition which is unique to this subfamily (Burns-Balogh 1983). In addition the sculpturing of the grains is smooth (Williams and Broome 1976, Newton and Williams 1978) which has been interpreted as a derived condition (Freudenstein and Rasmussen 1999).

Dressler (1981, 1993) subdivides the monandrous orchids into three subfamilies: Spiranthoideae, Orchidoideae and Epidendroideae. Basic pollen subunits are tetrads in all of these subfamilies; however, a number of taxa produce monads. Within the vanilloid orchids monad pollen grains are ubiquitous in the subtribes Vanillinae and Lecanorchidinae, and in the subtribe Pogoniinae, *Pogonia* and *Cleistes* have monads. *Palmorchis* (Palmorchideae) also has monads and was included in the Vanilleae by Dressler (1981) and considered vanilloid by Freudenstein and Rasmussen (1999). Recent molecular studies (Cameron et al. 1999) place *Palmorchis* as a sister group to the *Neottieae* where monads occur in *Cephalanthera* (subtribe Limodorinae). Recent evidence indicates that the vanilloid orchids are a fairly basal lineage in the Orchidaceae (Cameron et al. 1999), therefore monads in this group are not necessarily derived from tetrads.

Within the Orchidoideae both monads and tetrads have been recorded from the Diurideae in *Thelymitra* (subtribe Thelymitrinae), *Caladenia*, *Spiculaea* and *Chiloglottis* (subtribe Caladeniinae), *Codonorchis* (subtribe Chloraeinae), *Corybas* (subtribe Acianthinae), *Epiblema* and *Calochilus* (subtribe Diuridinae) and *Pterostylis* (subtribe Pterostylidinae). In *Caladenia patersoni* R. Br. both monads and tetrads were reported from the same sample. Thus some variability occurs in the pollen units of the Diurideae and Ackerman and Williams (1981) suggest that production of monads or tetrads may differ between populations or be influenced by environmental conditions. Careful consideration needs to be given to this question as it points to the secondary derivation of monads for some of these species.

The taxonomic position of some of the tribes with monads is fairly unstable (Ackerman and Williams 1980, 1981; Dressler 1981, 1993; Kores et al. 1997; Cameron et al. 1999; Freudenstein and Rasmussen 1999). Dressler (1986) cautions that slight ontogenetic changes may be responsible for the production of monads or tetrads and that monandous orchids with monads are not necessarily primitive.

The exine development in orchid pollen grains depends, to some extent, on characteristics of the pollinium. In species with mealy pollinia all the grains usually have an exine (Fitzgerald et al. 1994) and cohesion is achieved by autolysis of the tapetum which releases a lipid glue. This substance eventually penetrates the entire pollinium and consolidates all the microspores (Fitzgerald et al. 1993). The early release of the consolidating lipid may be necessary to achieve complete coverage of all grains. By contrast in *Dendrobium* where grains are tightly consolidated the inner grains often lack exine layers (Zavada 1990). Similarly in *Epidendrum ibaguense* H.B.K. the mature pollen grains reveal layering. The exine is clearly separable into an amorphous nexine, which covers all grains, and the sexine which is concentrated on the periphery of the pollinium (Blackman and Yeung 1983). Consolidation of the pollinia is achieved by a secretion fairly late in pollinium development. Again, the adhesive material is produced in tapetal cells, but penetration is limited to the spaces between adjacent peripheral tetrads. On exposure to light and drying this layer polymerizes into a hard coat (Fitzgerald et al. 1994).

In sectile pollinia, such as those of *Loroglossum hircinum* (L.) C. Rich., pollen grains are grouped in tetrads which coalesce into massulae and again the exine is limited to peripheral grains. The intine of these species often comprises two layers; the outer layer maintains the integrity of the tetrad and the inner layer surrounds each grain (Pandolfi and Pacini 1995). In the Disinae pollen tetrads are calymmate, but the massulae and pollinia are acalymmate (Chesselet and Linder 1993).

The cohesion of pollen into pollinia must be constrained, to some extent, by the interference caused by pollen walls during germination. The reduction in exine surrounding inner grains may be part of the evolutionary compromise in reducing this interference. In addition agglutination of pollinia with substances which degenerate upon contact with the stigma alleviates constraints placed upon innermost tetrads. Pandolfi and Pacini (1995) indicated that intercellular spaces between microspores are dynamic in massulae and that they increase from 15–20% to 50–60% during germination. In addition changes may occur in the male and female gametophytes of orchids which lead to delayed and asynchronous emission of pollen tubes (Pacini and Franchi 1996). Pollinium morphology is, presumably, also mediated to facilitate germination (Pacini and Franchi 1998) and this may have favoured flattened pollinia and the preservation of longitudinal furrows between fused pollinia (Figs. 11, 12).

Pollinarium

In the majority of orchids, the pollinia are joined to an adhesive viscidium (Figs. 2, 4, 8, 9, 11, 12), forming a structure known as a pollinarium. The pollinarium is thus the entire structure, including one or more pollinia, that is removed by a pollinator. In the Orchidoideae and some Vandeae where each of two pollinia are joined to separate viscidia (Figs. 9, 13), Dressler (1981) sought to introduce the term "hemipollinarium" on the technical ground that one anther can produce only one pollinarium. However, this term has not really been widely adopted, and in accordance with Dressler (1990), we use the term pollinarium to refer to the package comprising pollinia plus viscidium, regardless of whether there are one or two such packages in a flower.

The tremendous diversity of pollinaria, reflects the pivotal role of this structure in the complex pollination mechanisms of orchids, and has been found to be useful as a source of characters for taxonomy (Burns-Balogh 1982, Ackerman and Williams 1981, Chase 1987).

Pollinia

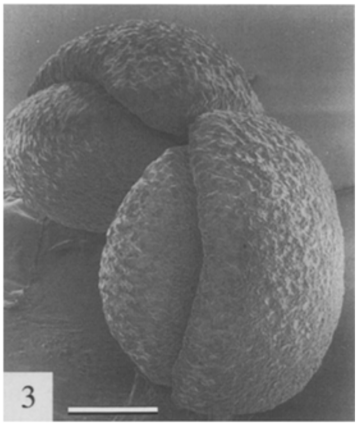
The term pollinium refers to a more or less compact and coherent mass of pollen, a condition found in the great majority of orchids (Dressler 1993). The subdivision of the pollen within an anther is based on a pattern of four locules, each producing pollen masses. The production of four pollinia is thus plesiomorphic for many groups, though Dressler (1986) indicates that eight pollinia are produced in primitive members of the Arethuseae, Epidendreae and Dendrobieae. The Epidendreae show a reduction series of six, four (Fig. 4) and finally two pollinia while four pollinia occur in most derived genera of *Dendrobieae*. Rasmussen (1986a) argued that the plesiomorphic condition in the *Dendrobiinae* and *Bulbophylinae* is more likely to be four.

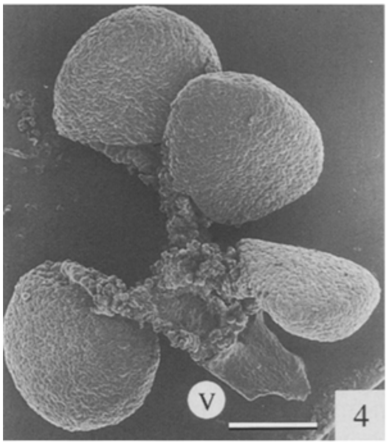
Freudenstein and Rasmussen (1996) provided an ontogenetic model explaining differences in the numbers of pollinia among orchids. Sporogenous tissue is produced by a single meristematic region in each pollen sac and the number, shape and type of pollinia are produced by septation or fusion of these meristematic regions. Within the Epidendroideae septation of the meristem results in four or eight pollinia per anther while lack of septation gives two pollinia, common in many vandoid species (Dressler 1986). In contrast, the two bipartite pollinia found in many Spiranthoideae and Orchidoideae are produced by adherence of the contents of two locules at a late ontogenetic stage, and should be recognized as distinct. Within the latter subfamilies eight pollinia result either from partitioning of the meristem by two longitudinal septa or a longitudinal and a transverse septum (Freudenstein and Rasmussen 1996).

The clear elastic material that forms a matrix which binds pollen together within the pollinium, is known as elastoviscin (Dressler 1981). Elastoviscin in *Epidendrum* was reported to be a lipid polymer by Blackman and

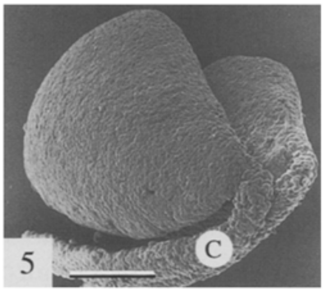

1

2

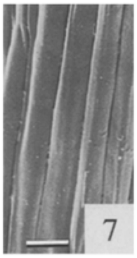

3

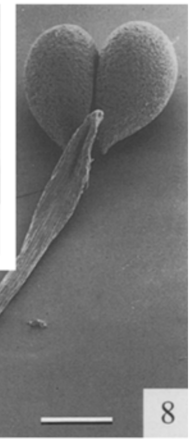


4

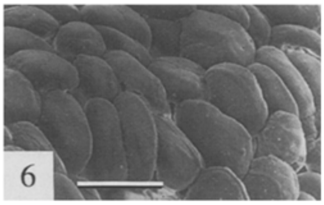


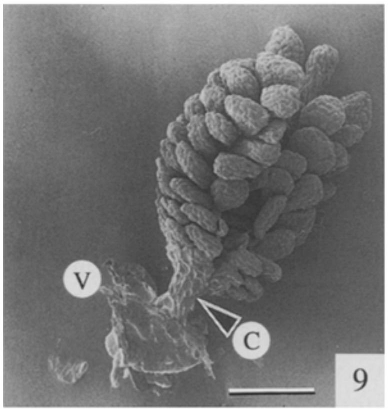
5


7

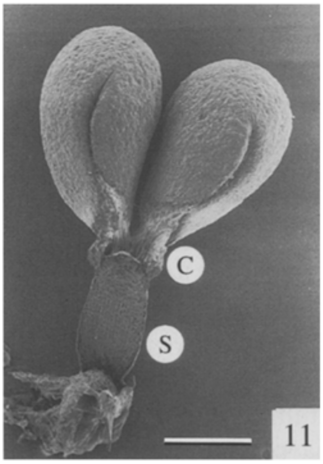


8

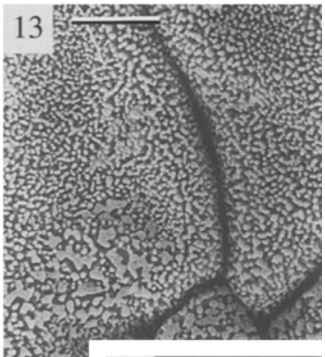

6

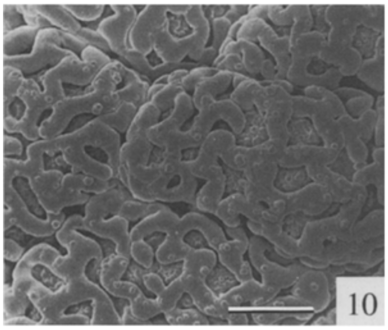


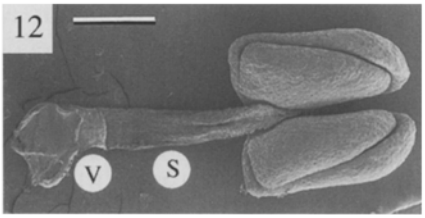
9



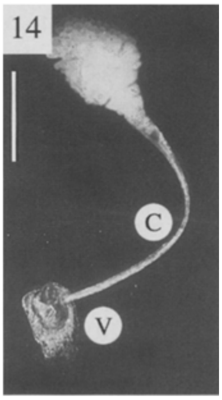
11


13


10



12



14

Yeung (1983). Schill and Wolter (1986) consider elastoviscin to be homologous with pollenkit. A different material, termed cohesion strands, was found to bind the loose pollinia of some Spiranthinae by Burns-Balogh (1982). In the Disinae, additional pollen cohesion is established by means of tectal bridges (Chesselet and Linder 1993), while Ackerman and Williams (1981) reported unique, strap-like bands connecting grains within tetrads of the Diurideae. Schill and Wolter (1986) outline the ontogeny of elastoviscin in the family.

The cohesion of pollen grains is highly variable within the Orchidaceae. Loose, powdery or sticky pollen is confined to the Apostasioideae and some Cypripedioideae, the remaining orchids having soft, sectile or hard pollinia, in order of increasing cohesion (Dressler 1986). Soft pollinia occur in *Selenipedium* and *Phragmipedium*, as well as in some of the monandrous orchids, such as *Vanilla*. The distinction between orchids having loose pollen masses and soft pollinia is not always clear, and we suggest that the term pollinia be used for any pollen masses that are removed in their entirety from the anther during a single pollinator visit.

Sectile pollinia are those that are subdivided into discrete units or massulae (Figs. 9, 13). The functional consequence, elaborated on in the second half of this review, is that several flowers may be pollinated from a single pollinium. Sectile pollinia have originated several times in the Orchidaceae, but can be grouped into two basic types. The first is restricted primarily to the Spiranthoideae and Orchidoideae and comprises fairly homogenous wedge-shaped massulae attached, in a single layer, around an elongate core of elastoviscin (Freudenstein and Rasmussen 1997). The second type occurs within the Epidendroideae and comprises irregular massulae which are interconnected by weak strands of elastoviscin (Freudenstein and Rasmussen 1997). It is clear that the evolution of pollinia is closely linked with the evolution of the stigmatic surfaces upon which they are deposited. In the taxa with sectile pollinia the stigmatic surfaces are often flat or slightly convex and have a fairly shallow adhesive covering. This facilitates the gradual erosion and enhances pollen carryover (Nilsson 1983).

Hard pollinia, the kind most common among orchids, represent the greatest degree of cohesion of pollen, and are deposited as an entire unit on the stigma, in which respect they are similar to the pollinia of Asclepiadaceae. Pollinia which require deposition in a single event correspond to concave stigmatic surfaces with deep stigmatic fluid and sometimes modifications to the rostellum (Burns-Balogh and Bernhardt 1985). Some hard pollinia are waxy in composition, while Dressler (1993) describes

Figs. 1–14. Anther and pollinarium morphology. **Fig. 1.** Anther of *Paphiopedilum callosum* Pfitz. (Cypripedioideae). Bar: 400 µm. – **Fig. 2.** Pollinarium of *Coelogyne barbata* Lindl. *ex* Griff. (Epidendroideae). Bar:1 mm. – **Fig. 3.** Sessile pollinarium of *Maxillaria variabilis* Batem. *ex* Lindl. (Epidendroideae). Bar: 200 µm. – **Fig. 4.** Pollinarium of *Epidendrum porpax* Reichb. *f.* (Epidendroideae) with four pollinia and well developed caudicles. Bar: 250 µm. – **Fig. 5.** Pollinia of *Cattleya deckeri* Klotzsch (Epidendroideae) with caudicles derived from modified pollinia. Bar: 200 µm. – **Fig. 6.** Cellular detail of caudicle tissue of *C. deckeri* showing tetrads. Bar: 20 µm. – **Fig. 7.** Cellular detail of the stipe of *Oncidium ornithorynchum* H. B. and K. (Epidendroideae). Bar: 20 µm. – **Fig. 8.** Pollinarium of *O. ornithorynchum* showing the well developed stipe and two terminal pollinia. Bar: 430 µm. – **Fig. 9.** Sectile pollinarium of *Stenoglottis longifolium* Hook. f. (Orchidoideae) with a discrete viscidium. Bar: 500 µm. – **Fig. 10.** Tectal sculpturing of pollen grains of *S. longifolium*. Bar: 5 µm. – **Fig. 11.** Pollinarium of *Gomesa recurva* Lodd. (Epidendroideae) with well developed stipe, reduced caudicles and two terminal pollinia each with a longitudinal cleft. Bar: 300 µm. – **Fig. 12.** Pollinarium of *Lycaste lasioglossa* Reichb.f. (Epidendroideae) with a well developed stipe and two lateral pollinia derived from 4 partially separated meristems. Bar: 600 µm. – **Fig. 13.** Tectal sculpturing of *Bonatea cassidea* Sond. (Orchidoideae). Bar: 9 µm. – **Fig. 14.** Pollinarium of *B. cassidea* showing the discrete viscidium and elongated sterile caudicle. Bar: 4 mm. Abbreviations: *C* caudicle; *S* stipe; *V* viscidium the very hard pollinia of "advanced" Epidendriodeae as "bony".

According to Dressler (1993), 80% of orchids have hard pollinia, 11% have sectile pollinia (mainly Orchideae and Diseae), 6.6% have soft pollinia (mainly Cranchideae and most Diuridae) and less than 3% have various types of powdery pollen/loose pollinia (exemplified by Apostasioideae, Cypripedioideae and Vanilleae).

Individual pollinia can contain between 5000 and 4 000 000 pollen grains (Schill et al. 1992). The fewest pollen grains recorded per pollinium are found in neottioid orchids, such as *Cephalanthera longifolia* Fritsch with 5547 grains (Nazarov and Gerlach 1997), intermediate numbers are found in sectile pollinia, such as those of *Orchis* species which vary between 36 000 and 62 000 grains (Darwin 1877, Nazarov and Gerlach 1997), and the highest numbers in orchids with solid pollinia, such as *Coryanthes senghasiana* G. Gerlach with 400 000 grains (Nazarov and Gerlach 1997).

Caudicles

In the monandrous orchids the adhesive compounds which attach pollinia to pollinators are derived from stigmatic tissues and this dictates a close proximity of the sexual parts. The evolution of such sexual integration carries with it the possibility of autogamy and a phylogenetic 'dead end'. Thus the sterility of the structures which connect the viscidia (stigmatic) with the pollinia must have been of cardinal importance in the family's divergence. In the Dendrobiinae, Bulbophylinae, Triphoreae (*Triphora*), Arethuseae (*Bletilla*) and Malaxideae (*Liparis*) there are no physical structures connecting the viscidial glue to the untailed pollinia but in most other groups pollinia abound with unusual artifices, derived from female tissues, which are instrumental in pollination success. In view of the proximity of sexual parts, which allow this integration, it is not surprising that autogamy occurs in 5–20% of orchid species (Catling 1990).

Caudicles are produced within the anthers and can be considered an extension of the pollinium. Their anatomical structure is consistent with derivation from sporogenous tissues (Van der Pijl and Dodson 1966, Blackman and Yeung 1983) making them a unique example of haploid tissues which have a nonsexual mechanistic function. Caudicles are a mixture of tetrads, aborted pollen mother cells and tapetal remnants in a matrix of elastoviscin. Occasionally they are devoid of pollen tetrads and comprise almost pure elastoviscin, e.g. *Lockhartia* and *Cryptarrhena* (Dressler 1986). In other instances whole pollinia serve as caudicles and these may be hard and hyaline, e.g. Podochilinae (Dressler 1981) or poorly differentiated in genera such as *Cattleya* (Fig. 5), *Epidendrum* and *Encyclia* (Fig. 4). The latter examples substantiate Dressler's (1981) assertion that 'the primitive pollinium number in the Epidendreae is eight'. The retention of eight pollinia in genera such as *Sophronitis*, *Ceratostylis*, *Brassovola* and *Laelia* would thus represent an unspecialized condition. This argument conforms with the notion that specialization of pollination syndromes often correlates with increased levels of pollen cohesion.

In the Spiranthoideae and Epidendroideae caudicles are connected to the apices of pollinia (acrotonic attachment) but in the Orchidoideae and a few other exceptions attachment is basal (basitonic attachment) (Freudenstein and Rasmussen 1999). Sterile caudicles in the sectile pollinia of the Orchidoideae are considered to have been derived from homogenous pollinia (Dressler 1993) (Fig. 9).

The remarkable elasticity of the caudicle appears to be due to the same viscin material that binds pollen tetrads in sectile pollinia, but we are not aware of any elucidation of the structure of this material. Caudicles may be up to 20 mm in length in some Habenariinae, such as *Cynorkis uniflora* Lindl. (Nilsson et al. 1992a) and *Bonatea speciosa* Willd. (Johnson and Liltved 1997) (Fig. 13). On the other hand, caudicles are reduced and hidden in a slit in some vandoid orchids

(Dressler 1993). In *Dendrobium*, hard pollinia are attached directly to the pollinator by means of a smear of glue from the rostellum (Dressler 1981).

After removal from the anther, many pollinaria undergo a bending movement localized at some point along the caudicle. Darwin (1877) showed that these movements are hygrometric and that after bending the pollinarium can be restored to its original position by exposure to water. Darwin (1877) showed that bending of pollinaria of Orchideae, including *Gymnadenia conopsea* and *Orchis mascula*, is localized at the point where the caudicle is joined to the viscidium. He suggested that it was the contraction of cells forming part of the viscidium that caused the bending movement to take place.

The caudicle usually serves as the breakage point between pollinia and viscidia during pollination. However, in orchids with sectile pollinia, such as the Orchideae and Diseae, the caudicle does not break and individual massulae, rather than whole pollinia, are deposited onto the stigmas.

Stipe

Caudicles are sometimes augmented by stalks, derived from non-sporogenous tissues, which are collectively termed stipes (Rasmussen 1986a). According to Rasmussen (1986a), the stipe is attached to the caudicle by means of viscid material from the anther and this often serves as a break point during deposition of the pollinium.

Where stipes are produced from the abaxial epidermis of the column and form vitreous plates they are referred to as tegulae (sing. tegula) (Rasmussen 1986a). Dressler's (1993) definition is broader, including all stipes derived from column tissue. Rasmussen (1986b) cautioned that the stipes present in the *Stanhopea* (Stanhopeinae) may not be homologous structures due to their multilayered nature; however, later developmental studies in *Gongora* and *Cirrhaea* show that the layers are produced by periclinal divisions within the epidermis and thus represent an elaboration of the tegula (Freudenstein and Rasmussen 1996). Rasmussen (1986a) records tegulae from Vandeae (*Vanda* and *Cleisostoma*), Oncidiinae (*Oncidium*) and Goodyerinae (*Hetaeria* and *Zeuxine*). Freudenstein (1994) augmented this list with Calypsoeae (*Calypso* and *Yoania*) and Cymbidieae (*Govenia* and *Eulophia*).

In a few isolated cases stipes are produced by sharply recurved rostellar tips which are detachable; these are termed hamulae (sing. hamulus). These structures occur in Epidendroideae: Bulbophylinae (*Bulbophyllum*, *Monomeria*), Orchidoideae: Prasophyllinae (*Prasophyllum* and *Microtis*) and Spiranthoideae: Tropideae (*Tropidia*, *Corymborkis*) (Rasmussen 1982a, 1986a). In these instances the structure is clearly convergent. Further examples of hamulae are listed from *Amplectrum*, *Corallorhiza*, *Cremastra* and *Oreorchis* (Corallorhizinae) where Freudenstein (1994) reports a strong correlation between the development of the hamulus and outcrossing. In these instances the structure is probably synapomorphic.

Perhaps the most bizarre function of the stipe among orchids is its role in forcibly ejecting the pollinaria out of *Catasetum* and *Mormodes* flowers. This mechanism, first elucidated in painstaking detail by Darwin (1877), relies on the natural elasticity of the stipe which is "springloaded", until explosively released when a bee touches a sensitive trigger. The pollinarium may be thrown more than a metre from the flower (Darwin 1877), and normally strikes a bee with sufficient force to cause subsequent aversive behaviour toward male *Catasetum* flowers, according to Romero and Nelson (1986).

It is clear from this brief synopsis that selection has driven considerable convergence and divergence in the evolution of stipes. Within the Oncidiinae, for example, stipes are highly divergent at the generic level (Williams 1972, Chase 1987). Pollinia stalks have also proved useful in a recent reassessment of the Corallorrhizinae (Freudenstein 1994).

Viscidium

In supposedly primitive orchids, such as the Cypripedioideae, an insect emerging from the flower receives a coating of stigmatic fluid on its back, to which pollen subsequently adheres as the insect brushes the anther. The role played by the stigma in pollen transfer has been refined in most orchids with the development of the viscidium, a sticky detachable structure derived from one of the stigmatic lobes that serves to attach the pollinia(ium) to the pollinator (Dresser 1993). The viscidium is connected to the pollinium by means of a caudicle, as well as a stipe in many orchids.

The sticky glue of the viscidium results from the breakdown of stigmatic cells and its composition remains a mystery, but it would be expected to be similar in structure to stigmatic mucilage. What is particularly interesting about the viscidium is the way the glue dries soon (usually within minutes) after coming into contact with the body of the pollinators. In some Orchidoideae the viscidium is covered with a macroscopic sheath (the bursicle) that is dislodged by the pollinator, triggering the drying process (Darwin 1877). An unusual mechanism of pollen deposition is found in the Listerinae which have a rostellum that squirts glue when touched; pollinia are released directly onto this fresh layer of glue (Ackerman and Mesler 1979). Viscidia of *Dendrobium* species burst on contact, leaving a smear of glue on the surface of the retreating pollinator, onto which the hard pollinia adhere (Rasmussen 1986b).

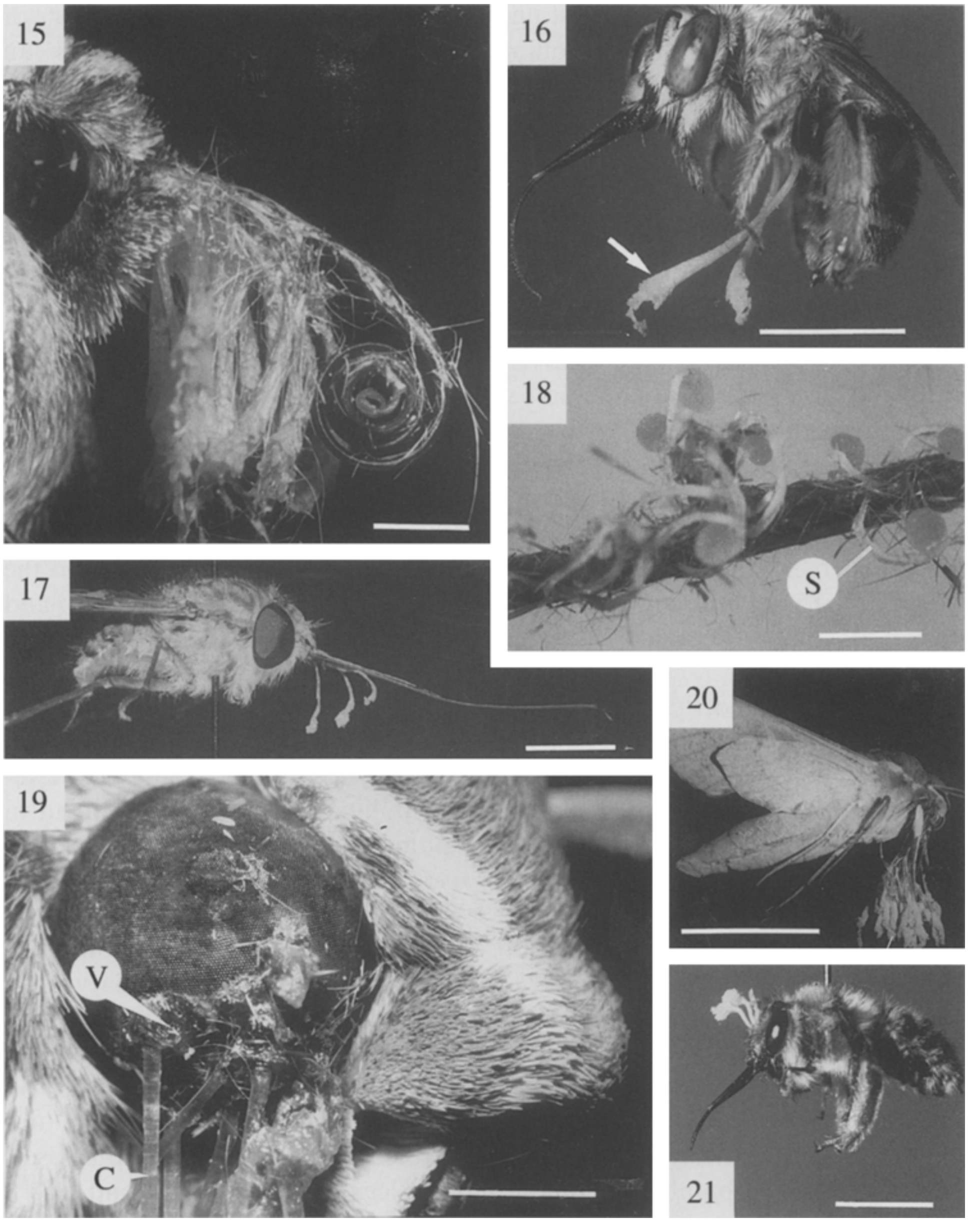
A single viscidium is found in most orchids, although many Orchideae, such as *Disa*, and some Vandeae, have two viscidia associated with two pollinaria. These are usually removed at the same time, but in the case of twin-spurred *Satyrium* flowers and the functionally subdivided flowers of many *Habenaria* and *Bontaea* species, the viscidia are removed independently (Johnson 1997, Johnson and Liltved 1997). Within *Diseae*, a single viscidium has been secondarily derived at least twice; in *Disa* section *Herschelia* by fusion of two viscidia from the lateral rostellum lobes, and in *Disa* section *Monadenia* by the innovation of a single viscidium derived from an unlobed rostellum (Linder and Kurzweil 1994, Johnson et al. 1998a).

Pollen longevity in orchids

Orchids often occur at low densities and their pollinators seldom confine their visits to orchid flowers alone, thus it is likely that several days may sometimes elapse from when a pollinarium is removed to when it is eventually deposited on a stigma. Orchid pollen within intact pollinia would thus be expected to remain viable for relatively long periods of time. Sectile pollinia of *Dactylorhiza purpurella* (T. & T. P. Steph.) Soo retained their germinability for up to 51 days after removal from the flower and storage under laboratory conditions (Neiland and Wilcock 1995), while ageing of pollen for up to eight days in a laboratory had little effect on fruit formation in *Calopogon tuberosus*, *Pogonia ophioglossoides* and *Cypripedium reginae* Walt. (Proctor 1997). Field studies by Alexandersson (1999) showed that a labelled pollinium of *Calypso bulbosa* Reichb.f. was still capable of producing a normal fruit 10 days after being removed from a flower by a bumblebee. These studies indicate a great capacity for long-distance dispersal and infrequent pollination events among orchids.

Sites of pollinarium attachment

The powdery pollen typical of the majority of angiosperms adheres most effectively to the hairy parts of insects and mammals, and feathers of birds. By contrast, the viscidium of orchid pollinaria adheres most effectively to smooth surfaces that are generally unsuitable for the placement of powdery pollen (Figs. 15–21). This simple fact about pollen attachment helps to explain many of the morphological differences between the flowers of orchids and those of other families. Bird-pollinated orchids, for instance, tend to place pollinaria on



Figs. 15–21. Attachment of orchid pollinaria to insect pollinators. – **Fig. 15.** Pollinaria of *Disa ophrydea* Lindl. (Diseae) attached to the tongue of a moth, *Cucullia minuta* (Noctuidae). Bar: 1 mm. – **Fig. 16.** Sectile pollinaria of the orchid *Disa racemosa* L.f. (Diseae) adhere to the middle legs of the bee *Amegilla niveata* (Anthophoridae). Chunks of massulae have broken away from the arrowed pollinium. Bar: 5 mm. – **Fig. 17.** Long tongued fly *Prosoeca ganglbauri* (Nemestrinidae) with pollinaria of *Brownleea macroceras* Sond. (Diseae) attached to the proboscis. Bar: 5 mm. – **Fig. 18.** Close-up view of the proboscis of a hawkmoth showing attachment of hard-type pollinaria of *Mystacidium venosum* Harv. ex Rolfe (Aerangidinae). Bar: 5 mm. – **Fig. 19.** Eye of a hawkmoth, *Theretra capensis* (Sphingidae), showing attachment of pollinaria of *Bonatea speciosa* L.f. (Willd. (*Habenariinae*). Bar: 2 mm. – **Fig. 20.** Hawkmoth, *T. capensis*, with a large load of *B. speciosa* pollinaria attached to its eyes. Bar: 20 mm. – **Fig. 21.** Pollinaria of *Satyrium erectum* Sw. (*Satyriinae*) attched to the frons of a bee, *Anthophora diversipes* (Anthophoridae). Bar: 10 mm. Abbreviations: *C* caudicle; *V* viscidium; *S* stipe

the bird's beak and therefore have short floral tubes, as it is necessary for a bird to insert its beak only a short distance into the flower, in contrast to the long floral tubes of flowers which deposit pollen on the feathers of a bird's head (Johnson 1996). Dressler (1981) suggested that the absence of bat pollination in orchids was due to the lack of suitable surfaces for pollinarium attachment on the bodies of these hairy mammals.

The precise placement of pollinaria on the bodies of pollinators, for which orchids are renowned, is achieved, firstly by filtering floral visitors by specific visual and olfactory cues, and secondly by modifications of the column and perianth parts that ensure that the pollinator is manipulated into the correct position for contact to be made between the viscidium and the exact target area on its body (Dressler 1981).

Pollen deposition in the vast majority of orchids is nototrobic (on the dorsal surface of the pollinator), but sternotrobic pollen deposition (on the ventral surface) does occur in the Diseae, and some Habenariinae and Diuridinae (Johnson et al. 1998b, Burns-Balogh and Bernhardt 1988). Commonly used sites for pollinarium attachment to insects include their mouthparts, frons, eyes, thorax, and legs (Figs. 15–21). Dressler (1981) identified 13 discrete sites for pollinarium placement on the bodies of euglossine bees. Rasmussen (1986b) describes how pollinia of *Dendrobium infundibulum* Lindley are attached precisely to a bald spot on the metanotum of the bumblebee pollinator.

A factor which undoubtedly influences the site of pollinarium placement is the probability that pollinators will remove the pollinaria. Bees, for example, frequently attempt to remove pollinaria, even when they are placed in such relatively inaccessible sites as the dorsal surface of the thorax (van der Pijl and Dodson 1966). Roubik (this volume) suggests that social meliponine bees may co-operate in the removal of pollinia from their bodies while in the nest, thus reducing the effectiveness of pollen export. Dressler (1971) made the interesting suggestion that the dull colour of pollinaria of bird-pollinated species is an adaptation to render the pollinaria cryptic and therefore less likely to be scraped off the bill.

The size and shape of the viscidium is of tremendous importance for the success of pollen export in orchids. Viscidia range in size from the tiny viscidium of *Liparis capensis* Lindl. to the very large platelike viscidia of *Satyrium carneum* R. Br. that adhere firmly to the beaks of sunbirds (Johnson 1996). Viscidium shape varies from the long flat viscidium of *Spiranthes* which adheres to the flat and rigid galea of the proboscis of bee pollinators (Catling 1983) to the chunky viscidium of *Catasetum* which is forcibly propelled onto the thorax of visiting euglossine bees (Romero and Nelson 1986). Viscidia that attach to the narrow proboscides of Lepidoptera tend to encircle the tongue, as in *Anacamptis pyramidalis* Rich. (Darwin 1877) and *Satyrium stenopetalum* Lindl. (Johnson 1997). Eye-placement of pollinaria is quite widespread in the moth-pollinated Habenariinae, such as *Bonatea speciosa* (Figs. 19–20) (Johnson and Liltved 1997), *Platanthera chlorantha* Cust. ex Reichb. and *Cynorkis uniflora* (Nilsson et al. 1992a), and is also found in some Satyriinae with elongated rostellum arms and globose viscidia, such as the fly-pollinated *Satyrium bracteatum* Lindl. (Johnson 1997). Pollinaria of orchids pollinated by Lepidoptera are invariably attached to the eyes or proboscis (and rarely the legs – Johnson and Bond 1994), as these are the only sites that are not covered with scales (Figs. 15, 18–20).

Depending on the precision of pollinarium placement and the available space on the body of the pollinator, there may be restrictions in the number of pollinaria that can be attached to a single insect. For example, only one pollinarium of *Disa racemosa* Linn.f. can be attached at a time to a site on each of the middle legs of the anthophorid bees that pollinate this species (Fig. 16) (Johnson et al. 1998b). Only one pollinarium of *Catasetum pileatum* is usually found attached to the euglossine bees that pollinate this orchid, but this has been attributed to aversion behaviour by the bees after experiencing the explosive mechanism of pollinarium attachment (Romero and Nelson 1986).

Pollen loads may become so large that insects are unable to fly properly, as observed for butterflies carrying *Disa uniflora* Berg. pollinaria (Johnson and Bond 1994) and hawkmoths carrying the formidable pollinaria of *Bonatea speciosa* (Fig. 20) (Johnson and Liltved 1997). Bernhardt (1995) suggested that *Pterostylis* releases the contents of one anther sac at a time, producing four discrete pollinia, to avoid overburdening the tiny mycetophilid gnats that pollinate these orchids. The impact of large orchid pollinarium loads on the proboscis and eyes of pollinators has not been investigated, but studies of Asclepiadaceae have shown that large numbers of pollinia attached to the feet and mouthparts of bumblebees lead to a significant decline in foraging efficiency (Morse 1981).

Several sympatric orchids may share a pollinator without competitive interference in pollen deposition due to the precision of pollinarium placement on different parts of the body of the pollinator (Dressler 1968, Nilsson 1983b, Steiner 1989). Switches from tongue to eye-placement appear to have been important for speciation in *Platanthera;* at least three species pairs in the genus are differentiated primarily by placement of the pollinaria (Hapeman and Inoue 1997). The present-day distributions of these species pairs are at least partly overlapping, but it is difficult to establish whether selection for isolating mechanisms played any role in the shifts in pollinarium placement.

Mechanisms of pollen deposition on stigmas

Pollen is deposited onto orchid stigmas either when whole pollinia become trapped in a rostellum notch and detach from the viscidium, or when the adhesion between pollen and stigmatic mucilage is powerful enough to cause whole pollinia, individual massulae or parts of soft pollen masses to break away from the remainder of the pollinarium attached to the insect.

The success of pollen deposition in orchids is dependent on a sophisticated system of breakage points. In orchids with hard pollinia, it is imperative that the adhesion between the stigma and pollinium, as well as between the viscidium and pollinator, is adequate to ensure that the pollinarium breaks at the correct point, which is usually between caudicle and stipe. The retention of the anther cap for up to

Table 2. Pollen:ovule ratios reported for orchids

Type of pollinum	Method of deposition	Genera studied	Mean pollen: ovule ratio	SD	range	n	Reference
Soft	Scraping	*Isotria*, *Cephalanthera*	3.3	0.4	2.9–3.9	6	Mehrhoff (1983), Nazarov and Gerlach (1997)
Soft	Contact	*Listera*	30.1	0	–	1	Nazarov and Gerlach (1997)
Sectile	Contact	*Orchis*, *Dactylorhiza*, *Ophrys*, *Platanthera*	19.1	7.3	10–36	19	Neiland and Wilcock (1995), Nazarov and Gerlach (1997)
Solid	Scraping	*Coryanthes*	1.2	0	–	1	Nazarov and Gerlach (1997)

40 minutes in *Tipularia discolor* was shown to prevent deposition of pollinia since the force of adhesion between the anther cap and stigma was not sufficient to break the pollinium away from the caudicle (Catling and Catling 1991).

In most orchids with soft or sectile pollinia, pollination occurs when chunks of pollen or massulae adhere to the stigma and break away from the rest of the the pollinarium (Fig. 16). Here it is essential that the weak link is between the massulae and the elastoviscin core of the pollinarium, as this type of pollinarium can serve to pollinate a sequence of flowers. The stretchiness of the elastoviscin ensures that an entire pollinarium in contact with the stigma is not jolted off the insect when it departs from the flower. The degree of pollen carryover in orchids with sectile pollinia would be in part determined by the degree of cohesion of the individual massulae by elastoviscin threads.

Pollen: ovule ratios

Cruden (1977 and this volume) presented compelling evidence that the ratio of pollen:ovule number in angiosperms was a reflection of the efficiency of the pollination process in terms of the probability of pollen grains reaching a stigma. Low pollen:ovule ratios are found in autogamous and cleistogamous species, while the highest ratios (mean of 5900:1) are found in xenogamous species. However, outbreeding plants in which pollen is clumped, either being held together by viscin threads (as in Onagraceae and Caesalpinioideae) or aggregated into pollinia (as in Asclepiadaceae and Orchidaceae) do not conform to this trend and have pollen:ovule ratios many times lower than would be expected (Cruden and Jensen 1979, Proctor and Harder 1994, Nazarov and Gerlach 1997).

Pollen:ovule ratios for orchids appear to vary according to the degree of pollen clumping and the method of pollen deposition, with the lowest ratios (1:2) being found in orchids with hard pollinia and scraping deposition, and the highest ratios (19:1) in orchids with sectile pollinia with contact deposition. Orchids with soft pollinia vary from a ratio of 3:1 in species with scraping deposition and 30:1 in a *Listera* species with contact deposition (Table 3).

Variation in pollen:ovule ratios in orchids is likely to reflect pollen carryover and its influence on typical stigmatic pollen loads. The relatively high pollen:ovule ratios in orchids with sectile pollinia, for example, can be explained in terms of their extensive pollen carryover. A single pollinarium of *Orchis mascula* L. can pollinate at least eight flowers (Johnson and Nilsson 1999), and typical stigmatic loads of *Orchis* species are only about a quarter of the amount of pollen found in a pollinium (Neiland and Wilcock 1995). In orchids in which an entire solid pollinium is deposited on a stigma, it would be expected that the pollen:ovule ratio would be about one, as borne out Nazarov and Gerlach's (1997) study of *Coryanthes senghasiana* (Table 2). Orchids with soft pollinia have either a scraping type rostellum which presumably removes much of the pollen load and may explain the low pollen:ovule ratios in species of *Cephalanthera* and *Isotria* (Nazarov and Gerlach 1997, Mehrhoff 1983), or a contact deposition of the *Orchis* type which may allow more extensive pollen carryover and explain the high pollen:ovule ratio of 31:1 in *Listera ovata* R. Br. (Nazarov and Gerlach 1997).

The number of pollen grains per pollinium in orchids varies from as few as 5500 in *Cephalanthera longifolia* to as many as 400 000 in *Coryanthes senghasiana* (Nazarov and Gerlach 1997). The number of ovules in orchids flowers is correlated with the number of pollen grains typically deposited onto the stigma after a pollinator visit (Proctor and Harder 1994, Neiland and Wilcock 1995, Nazarov and Gerlach 1997).

Spatial dispersal of pollinaria

Gene flow via pollen and seeds is important for determining the spatial scale of genetic divergence within and among plant populations

Table 3. Published data on dispersal of pollinia within orchid populations

Species	Pollen vector	Pollinium type	Method	Geitonogamy (% of pollen transfers)	Mean pollen transfer distance (m)	Maximum pollen transfer distance	Reference
Prasophyllum fimbria	Bees and wasps	Sectile	Histochemical stain	22	8	27	Peakall (1989a)
Caladenia tentactulata#	Wasps	Sectile	Histochemical stain	<10	17	58	Peakall and Beattie (1996)
Microtis parviflora	Ants	Sectile	Histochemical stain	70	0.22	0.45	Peakall and Beattie (1991)
Cypripedium calceolus	Bees	Ssoft	Histochemical stain	0	5.2	23	Tremblay (1994)
Comparettia falcata	Humming bird	Solid	Histochemical stain	85	–	–	Salguero-Faria and Ackerman (1999)
Aerangis ellisii	Hawkmoth	Solid	Microtags	30	c. 5	76	Nilsson et al. (1992b)
Calypso bulbosa#	Bumblebee	Solid	Microtags	18–37	15	200	Alexandersson (1999)

single-flowered inflorescences

(Levin and Kerster 1974). Factors influencing pollen dispersal include behaviour of the pollinators, degree of pollen carryover from flower to flower and the density of the population (Levin 1981).

Studies of plants with loose pollen have shown that pollen dispersal curves are invariably leptokurtic with most pollen being dispersed among neighbouring plants a few meters away, and occasional dispersal to plants several hundred meters away (Table 4). Methods such as the use of dye analogues for loose pollen are generally unsuitable for measurement of dispersal of orchid pollinaria, necessitating the use of novel methods. These include the "microtags" developed by Nilsson et al. (1992b). Uniquely numbered tags with a breadth of only 0.5 mm are glued to individual pollinia while still in the anther, and subsequently traced to their deposition sites on stigmas, enabling the very accurate measurement of intra and inter-plant dispersal of pollinia. Results for the hawkmoth-pollinated Malagasy orchid *Aerangis ellisii* Schlechter show that most transfer occurred within 5 meters from the parent plant, with occasional dispersal as far as 76 m (Table 4). The frequency of geitonogamous pollen transfer was 30%. The same technique was used by Alexandersson (1999) to measure dispersal of pollinia of the boreal orchid *Calypso bulbosa* (Table 3).

Microtags are suitable only for orchids with solid-type pollinia. For studies of orchids with massulate pollinia, Peakall (1989a) developed a technique whereby pollen is coloured by histochemical stains applied in drop form to pollinia while still in the anther. Up to six colours can be used simultaneously within one population. Results for *Prasophyllum fimbria* Reichb.f. using this technique showed that 22% of pollen transfers were geitonogamous and that the mean pollen flow distance for allogamous pollen transfer was 8 m (Peakall 1989a). By contrast 51% of the pollen flow in the ant-pollinated orchid *Microtis parviflora* R.

Table 4. Recorded bending times for orchid pollinaria

Species	Location of bending	Bending time	n	Comments	Reference
Dactylorhiza sambucina	Caudicle	20 s	?	Generalized food-deception, bee-pollinated	Nilsson (1980)
Orchis spitzelii	Caudicle	26 s	20	Generalized food deception, bee-pollinated	Fritz (1990)
Orchis morio	Caudicle	30 s	25	Generalized food deception, bee-pollinated	Johnson and Nilsson (1999)
Orchis mascula	Caudicle	40 s	27	Generalized food deception, bee-pollinated	Johnson and Nilsson (1999)
		30 s	?		Darwin (1877)
Himantoglossum hircinum	caudicle	30 s	?	Deceptive, bee-pollinated	Darwin (1877)
Orchis militaris	Caudicle	42 s	30	Generalized food deception, bee-pollinated	Johnson and Nilsson (unpublished)
Anacamptis pyramidalis	Caudicle	30 s	10	Deceptive, lepidopteran -pollinated	Johnson and Nilsson (unpublished)
		32 s	3		Darwin (1877)
Gymnadenia conopsea	Caudicle	2 s	30	Nectariferous, lepidopteran -pollinated	Johnson and Nilsson (unpublished)
		30–60 s			Darwin (1877)
Platanthera chlorantha	Caudicle	80 s	21	Nectariferous, moth-pollinated	Johnson and Nilsson (1999)
Platanthera blephariglottis		60 s	?	Nectariferous, lepidopteran -pollinated	Cole and Firmage (1984)
Coeloglossum viride	Caudicle	6 min	3	Nectariferous, wasp- and beetle-pollinated	Johnson and Nilsson (unpublished)
		20–30 min			Darwin (1877)
Ophrys insectifera	Caudicle	6 min	?	Sexual-deception, wasp-pollinated	Darwin (1877)
Cycnoches ventricosum	Stipe	15 min	?	Pollinated by male euglossine bees	Darwin (1877)
Cycnoches lehmannii	Stipe	40 min	?	Pollinated by male euglossine bees, anther cap falls off after 2–3hrs	van der Pijl and Dodson (1966)
Catasetum eburneum	Stipe	"Several minutes"	?	Pollinated by male euglossine bees, hermaphrodite flowers	van der Pijl and Dodson (1966)

Table 4 (continued)

Species	Location of bending	Bending time	n	Comments	Reference
Oncidium sp.	Stipe	"Several hours"	?		Darwin (1877)
Mormodes ignea	Stipe	12–15 min	15	Pollinated by male euglossine bees	Darwin (1877)
Mormodes luxata	Stipe	15 min	?	Pollinated by male euglossine bees	Darwin (1877)
Mormodes aff. buccinator	Stipe	30 min	?	Pollinated by male euglossine bees	van der Pijl and Dodson (1966)

Br. were within the same inflorescence and the mean pollen flow distance was only 22.2 cm (Table 3). Observations of pollinator behaviour on orchids suggest that pollen dispersal distances may be greater in deceptive species due to the tendency of pollinators to depart from unrewarding patches, and conversely to remain in rewarding patches (Nilsson 1980, Peakall and Beattie 1996, Alexandersson 1999).

Mechanisms that promote outcrossing and efficiency of pollen transfer

Geitonogamy (transfer of pollen among flowers of the same plant), can reduce plant fitness in several ways: it can lead to inbreeding depression in self-compatible species and clog the stigma, and can also diminish the male component of reproductive success through pollen discounting (reduction in the efficiency of pollen export). For these reasons, it is not surprising that most orchids show sophisticated floral adaptations to maximize cross-mating opportunities.

1. Pollen carryover. Pollen carryover has not been well studied in orchids, partly due to the formidable difficulties in carrying out suitable experiments. It would be expected that pollen carryover would be limited in orchids with solid pollinaria, and be more extensive in orchids with sectile or mealy pollinaria.

Johnson and Nilsson (1999) allowed insects carrying freshly withdrawn pollinaria to visit a sequence of emasculated virgin flowers of *Orchis mascula* and *Platanthera chlorantha* in a greenhouse. The average number of flowers receiving pollen from a single pollinium was 6.6 for *O. mascula* and 13.8 for *P. chlorantha.* The fraction of pollen carried over from flower to flower was 0.67 for *O. mascula* and 0.87 for *P. chlorantha.* This difference is probably due to the greater number of massulae within individual pollinia of *P. chlorantha* (366, versus 70 in *O. mascula*) and the gentler action of the moth pollinators of *P. chlorantha* compared to the bumblebees that pollinate *O. mascula* (Johnson and Nilsson 1999). Interestingly, these pollen carryover figures are within the range of 0.5–0.99 reported for plants with loose pollen (Robertson 1992 and refs therein), suggesting that at least for orchids with sectile pollinia, restricted pollen carryover may not pose serious problems for geitonogamy and pollen discounting.

While solid pollinaria would be expected to have no carryover, i.e. be deposited in the next flower that is visited, this is not the case in practice. The capture of insects carrying large loads of solid-type pollinaria indicates that pollen carryover may be extensive due to imperfections in the pollen-transfer process. In the case of solid-type pollinaria that first undergo a bending movement before insertion in the stigma, insects may visit a long sequence of flowers before pollination occurs.

In general pollen carryover is beneficial to the male component of plant fitness as it reduces the possibility of geitonogamy and increases the number of potential mates. One way in which pollen carryover could be achieved in orchids with solid-type pollinia, is to have several pollinia in one pollinarium. Orchids which have up to eight solid-type pollinia in one pollinarium are particularly interesting as up to eight flowers in a sequence could theoretically be pollinated by a single pollinarium, although no evidence exists to validate this possibility.

2. Bending movements of pollinaria. After withdrawal from a flower, the caudicle or stipe of many orchids undergoes a bending movement such that the pollen masses become correctly orientated to strike the stigma. The movement may take anything from a few seconds to several hours (Table 4). The fact that the bending movement occurs slowly was interpreted by Darwin (1877) as a mechanism to prevent self-pollination among flowers on the same plant (geitonogamy). In some cases bending movements are involved in autogamy, as shown for example in the bee orchid *Ophrys apifera* Huds. (Darwin 1877, Catling 1990).

The degree to which gradual pollinarium bending will act to reduce or eliminate geitonogamy will depend on the time taken for a pollinator to leave an inflorescence. Observation of deceptive *Orchis* species in Europe indicate that pollinators spend less time on an inflorescence than the time taken for a pollinarium to undergo a bending movement, thus outcrossing is virtually assured (Nilsson 1980, Johnson and Nilsson 1999). In *Orchis mascula* L., for instance, pollinaria take an average of 30–40 s to undergo bending after withdrawal, yet bumblebee pollinators spend less than 10 s per inflorescence (Nilsson 1983a). Likewise with *Dactylorhiza sambucina* (L.) Soo, Nilsson (1980) found that bumblebees never spend more than 20 s on an inflorescence which is the time that it takes for pollinarium bending to occur in this species (Table 3).

Pollinators visit more flowers and forage longer on the inflorescences of nectariferous orchids. This is borne out by the observations of Dafni and Ivri (1979) who found that bees spend just 2–5 s on the deceptive flowers of *Orchis collina* Sol., as opposed to 10–60 s on the nectar-producing inflorescences of *Orchis coriophora* L., and Johnson and Nilsson (1999) who found that addition of artificial nectar to flowers of *Orchis mascula* and *Orchis morio* L. increased the mean amount of time spent by bumblebees on an inflorescence from less than 10 s to about 60 s. As a consequence, it would be expected that natural selection would favour relatively delayed pollinaria bending times in nectariferous orchids. Data are sketchy, but indicate that pollinarium bending is effective in preventing geitonogamy even in nectariferous orchids. Pollinaria bending time for the rewarding orchid *Platanthera blephariglottis* Lindl. is about 60 s, longer than the c. 34 s that lepidopteran pollinators spend on average on an inflorescence (Cole and Firmage 1984). Pollinaria bending for *Platanthera chlorantha* takes 80 s, which is longer than the time taken by moths to visit an inflorescence (Johnson and Nilsson 1999).

Nierenberg (1972) suggested that bending of the stipe in *Oncidium* species was related to the amount of time spent by the pollinators, but unfortunately actual times for pollinator visits and bending of the stipe were not given. The longest pollinaria bending times (up to several hours) are found in orchids with stipes and solid type pollinaria, such as *Oncidium* (Table 4). This may partly be due to the fact that orchids with solid-type pollinaria lack pollen carryover, making them particularly vulnerable to geitonogamy when pollinators visit several flowers on an inflorescence. Euglossine bees are known to linger for long periods at fragrance-producing orchid flowers as they focus on fragrance collection. Dressler (1981) notes that euglossine bees will often re-enter the same flowers several times, thus posing a risk of self-pollination. For this reason, long pollinaria bending times would be expected in euglossine bee-pollinated orchids as a mechanism to prevent geitonogamy. Indeed, some of the longest pollinarium bending times are found in genera such as *Cycnoches* and *Mormodes* (Table 4). Pollinaria of *Mormodes* curl up after removal from the anther and gradually straighten out. However, the elaborate pollinarium bending (technically straightening) found in *Mormodes* and *Cycnoches* is difficult to explain in terms of the outcrossing/reduction of pollen discounting hypothesis as these orchids largely have unisexual flowers.

3. Shrinking of the pollinium. It was recently reported that pollinia of *Bulbophyllum involutum* Borba, Semir and F. Barros and *B. ipanemense* Hoehne shrink in width by as much as 50% after removal from the anther, apparently as a result of dehydration (Borba and Semir 1999). Only after 105–135 minutes do the pollinia shrink enough to be inserted in the narrow entrance to the stigmatic cavity. Since the milichiid fly pollinators of these species spend up to about 15 minutes in a flower after removing the pollinaria, the mechanism appears to prevent self-pollination (Borba and Semir 1999).

4. Retention of the anther cap. Dressler (1981) suggested that retention of the anther cap for a few minutes to a few hours after a pollinarium is withdrawn from a flower would have much the same effect as pollinarium bending, namely to reduce the incidence of geitonogamous self-pollination. According to van der Pijl and Dodson (1966) anther caps of *Catasetum* species are retained for at least 20 minutes after ejection from the anther, and in *Cycnoches lehmanni* Nichols. for 2–3 hours after removal from the column. In *Tipularia discolor* the anther cap is retained for 8–40 minutes after pollinarium removal, the exact time being dependent on the ambient humidity (Catling and Catling 1991).

5. Protandry. In the majority of orchid flowers, removal and deposition of pollen happen simultaneously with little risk of self-pollination because pollinaria are removed only as the insect backs out of the flower, or, as discussed above, pollinaria have first to undergo a bending movement before they are in a position to contact the stigma. Nevertheless, protandry (male followed by a female phase in flowers) does occur in orchids (van der Pijl and Dodson 1966, Ackerman and Mesler 1979). The nectariferous flowers in *Spiranthes* are protandrous (accomplished by changing the position of the column with respect to the lip) and open sequentially from the base of the tall upright inflorescence (Darwin 1877, Catling 1983). Foraging bumblebees land at the bottom of an inflorescence and forage upwards, thus ensuring that pollen is deposited on the older and lower flowers in the female stage and removed from the upper and younger flowers in the male stage – thus cross-pollination and pollen export is maximized. A similar strategy occurs in the related genus *Goodyera* (Ackerman 1975).

Protandry may also be effective in orchids which have a tendency to be revisited repeatedly by the same pollinator. Warford (1992) observed euglossine bees lingering for as long as 45 minutes at *Notylia* inflorescences. *Notylia* flowers are strongly protandrous, the stigma remaining tightly closed for two days after anthesis, and then opening to reveal a narrow slit just wide enough to accommodate one of the wafer-like pollinia (Warford 1992). Euglossine bee-pollinated orchids in the Catasetinae are either protandrous, as in some species of *Mormodes*, *Clowesia* and *Dressleria*, or unisexual, as in *Catasetum* and *Cycnoches* (Romero and Nelson 1990). These traits, together with the pollinarium bending and anther cap retention found in the group, may represent a suite of adaptations which ensure outcrossing and efficient pollen export despite the tendency of euglossine bees to linger and repeatedly enter flowers. Protandry also occurs in the Stanhopeinae, a subtribe in which pollination by euglossine bees is ubiquitous.

6. Self-incompatibility. Most orchids appear to be self-compatible, yet most of these appear to be predominately outcrossing as a result of mechanisms such as floral longevity, pollinarium bending and deception that reduce within-plant pollen transfer (geitonogamy). Even in self-compatible orchids, reduction in seed set is generally in evidence following self-pollination (cf Nilsson 1983a, Johnson 1994). This may be a consequence of inbreeding depression due to expression of deleterious alleles previously masked by heterozygosity.

Self-incompatibility appears to be rare among orchids but does occur in *Coelogyne*, *Lycaste*, *Chondrorhyncha* (Edwards unpublished data), *Notylia* (Warford 1992), *Oncidium* (Scott 1865, Sanford 1964) and *Dendrobium* (Johansen 1990). Self-incompatibility in orchids appears to be gametophytic and is usually expressed in flower abscission and not inhibition of germination or inhibition of pollen tube growth (Johansen 1990).

It is notable that in many of the orchid genera known to be self-incompatible, individuals are characterized by mass flowering which increases the possibility of geitonogamy. Self-pollinated flowers of many Epidendroideae fall off the plant, but in most *Orchidae* and *Diseae* self-pollinated flowers develop into fruits, albeit with fewer seeds than outcrossed flowers. In some orchids self-pollination results in fruits that are indistinguishable from outcrossed fruits in terms of seed number (Peakall 1989b) or seed fertility (Ackerman and Montalvo 1990, Salguero-Faría and Ackerman 1999).

Mechanisms of self-pollination

Although orchids are widely considered to exemplify sophisticated floral adaptations for outcrossing, species capable of autogamy (automatic self-pollination) occur in virtually every tribe and subtribe (Catling 1990). Autogamy in orchids is facilitated by the close proximity of anther and stigma and many of the mechanisms of autogamy involve modification of the pollinarium. In orchids with sectile pollinia, the massulae are often friable and fall onto the stigmatic surface, sometimes even in the bud stage (Kurzweil and Johnson 1993). In some cases whole pollinia fall or slide onto the stigmatic surface (Mehrhoff 1983). Caudicles in autogamous species are often either weak, allowing pollinia to flip across onto the stigma when the flower is jarred, or undergo a bending movement which brings the pollinium into contact with the stigma of the same flower. A similar effect is achieved by bending of the stipe in, for example, *Oncidium glossomystax* Reichb.f. (van der Pijl and Dodson 1966).

There are differing degrees of autogamy among orchids. Most retain the potential for outcrossing by having functional pollinaria that will self-pollinate the stigma only if it is not removed by insects. Only a few orchids are truly cleistogamous or apomictic (Catling 1990). Autogamy is most frequent among orchids with a weedy habit or those that occur in habitats that are marginal for pollinator activity (Hagerup 1952, Johnson et al. 1994, Williamson 1984). Catling and Catling (1991) showed that autogamy in North American orchids is more common at higher latitudes.

Pollinaria: a key innovation in the radiation of orchids?

In terms of numbers of species, the Orchidaceae are rivalled only by the Asteraceae. The Orchidaceae today numbers between 18 000 and 25 000 species (Atwood 1986). The most recent estimate is that of Dressler (1993) who gives 19 500 species for the family.

Pollinia are not unique to the Orchidaceae. An analogous structure has evolved in the Asclepiadaceae, and it is instructive to ask whether these families share common evolutionary outcomes as a result of their independent evolution of pollinia. The answer is a disappointing "no". The flowers of Asclepiadaceae are invariably radially symmetrical, as opposed to the bilateral symmetry of orchids. Certainly asclepiads have not shown the same rampant speciation that has been evident in the orchid family, although the richness of Asclepiadaceae exceeds that of orchids in semi-arid regions. Perhaps the comparison is meaningless, since the two families differ in so many other intrinsic characteristics, such as growth form and floral symmetry. It is nevertheless interesting that many Asclepiadaceae are quite promiscuous in their pollination systems, suggesting that the association between pollinia and specialized pollination systems is not an obligate one.

The evolution of pollinaria

Although monads and pollinia represent the two extremes on either end of the pollen clumping scale, there are many intermediate conditions among the angiosperms. Pollen occurs in tetrads in several families and dispersal of pollen in clumps is achieved by viscin threads in Onagraceae and Ericaceae, filamentous threads in Strelitziaceae and polyads in mimosoid legumes. One of the implications of pollen clumping is that the chances of multiple paternity are reduced. Studies of orchids with solid-type pollinia show that the vast majority of flowers are pollinated by just one pollinium (Nilsson et al. 1992b, Alexandersson 1999). Kress (1981) argued that single paternity may confer advantages to plants with pollen dispersed in clumps because competition is reduced among seeds which are full siblings. He pointed out that the trend for wind-pollinated plants to be uni-ovulate may be a consequence of selection to avoid competition among seeds. The converse argument is that multiple paternity encourages gametophytic competition among pollen grains for access to ovules and therefore ensures fitter offspring, particularly when resources are limiting and ovule abortion is a necessity (Ellstrand 1984).

Genetic implications aside, it is clear that pollen dispersal in clumps is associated with greater ovule numbers in families such as Orchidaceae, Asclepiadaceae, Onagraceae and Ericacaeae (Cruden 1977, Kress 1981). There are two ways of interpreting this trend: either selection has favoured many small seeds, in which case pollen clumping is a secondary adaptation to ensure fertilization of large numbers of ovules or, alternatively, selection has favoured pollen clumping and the evolution of large number of ovules is a secondary adaptation to ensure fertilization by large numbers of pollen grains. We will explore the merits of both of these hypotheses.

1. The pollen export hypothesis. Successful export of pollen to conspecific stigmas is important for the male component of reproductive success, and selection should thus favour any modifications that enhance pollen export.

Studies of species with loose pollen have revealed substantial losses through pollen falling off the bodies of insects, or deposited on foreign stigmas. There is now ample evidence that a very high percentage (typically more than 99%) of the pollen of xenogamous angiosperms is wasted during transport. For example, Rademaker et al. (1997) found that only 0.15% of pollen of *Echium vulgare* was deposited onto stigmas, less than 0.5% of pollen removed from the flowers of *Rhexia virginica* L. (Melastomataceae) was deposited on stigmas (Larson and Barret 1999) and only 0.52% of the pollen removed from flowers of *Erythronium grandiflorum* Benth. was deposited on stigmas (Thomson and Thomson 1989). Such wastage could lead to the evolution of higher pollen:ovule ratios or mechanisms such as pollen clumping that minimize pollen wastage. Estimates of the probability of pollen reaching a stigma range from 20% in *Cymbidiella flabellata* Rolfe. (Nilsson et al. 1986) to 51% in some populations of *Cypripedium acuale* Ait. (O'Connell and Johnston 1998).

The efficiency of pollen export in orchids with solid pollinaria that are deposited in their entirety can be estimated by the ratio of pollinaria removed and deposited. Estimates of the efficiency of pollinia export in orchids with sectile pollinaria are made more difficult by the fact that a single pollinarium can pollinate several flowers.

Pollinaria may also promote pollen export when pollinator visits are infrequent, as the entire pollen complement of a flower can be removed in a single visit. Studies of plants with loose pollen show that only 7–65% of pollen in an anther is removed in a typical visit, thus leading to low removal efficiency when a flower is visited just once (de Jong et al. 1992). Indeed, it has been argued that attraction of repeated pollinator visits to ensure efficient pollen export has been an important factor in the evolution of floral traits in plants with loose pollen (Bell 1985). Data for orchids show that pollinator visits are often infrequent

(Johnson and Bond 1997, Neiland and Wilcock 1998), perhaps as a consequence of low population densities and non-rewarding pollination strategies, and this may have promoted the cohesion of pollen into pollinaria to ensure efficient pollen removal. Conversely, when pollinator visits are frequent it may pay to dispense pollen in small amounts and thereby spread the risk among several vectors (Thomson et al. 1989).

Although, pollen clumped into pollinia is unlikely to end up on foreign stigmas, especially of non-orchids, there is always the risk that a generalist vector will not visit another flower of the same species and therefore squander all of the pollen from a flower. Ensuring that the pollinium reaches its target destination is obviously important to male fitness of orchid flowers and may have driven selection for the specialized pollination systems that are characteristic of the Orchidaceae. The trend noted by Tremblay (1991) for pollination systems to be more specialized in orchids with solid pollinaria than in orchids with mealy pollen, such as the Cypripedioideae, is consistent with this idea.

One of the ways in which pollen is lost from typical angiosperm flowers is through the activities of pollen-collecting insects. Pollen packaged in pollinia is generally unavailable to pollen-collecting insects, although recent studies show that bees may actively collect the pollen of some orchids with loose pollen masses, such as *Cleistes divaricata* (L.) Ames and *C. bifaria* (Fernald) Catling and Gregg (Gregg 1991). Other orchids, such as some *Maxillaria*, *Polystachya* and *Cephalanthera* species, attract pollen-collecting insects by falsely advertizing the presence of pollen through modifications of the labellum (van der Pijl and Dodson 1966, Dafni and Ivri 1981).

In their hypothetical sequence of the evolution of orchids in which great importance is placed on pollinator driving forces, van der Pijl and Dodson (1966) consider small seeds to have evolved subsequent to pollinaria. They cite the Asclepiadaceae as an example of a family in which pollinia have evolved despite having large non-mycotrophic seeds.

In summary, the pollen export hypothesis is supported by data which show that pollinaria can improve male reproductive success through efficient removal from the anther, minimal wastage due to pollen falling or being groomed off the bodies of pollinators, and a high probability of being deposited on a conspecific stigma.

2. The small seeds hypothesis. A pivotal aspect in the evolution of the Orchidaceae has been the reduction of the seeds and their contents to an absolute minimum. In all but a few species, endosperm and a recognizable embryo are absent from the seed. This loss of reserves must have occurred in conjunction with increasing reliance on fungal symbionts. The reduced energy investment per ovule meant that more ovules could be produced. For such a system to be successful, fertilization capacity must be enhanced through the deposition of much larger amounts of pollen on the stigma, as can be achieved through the evolution of pollinaria (cf. Neiland and Wilcock 1995, Nazarov and Gerlach 1997).

Dressler (1981) argued that small seeds (associated with the mycotrophic habit) would have preceded (and provided a powerful stimulus for) the evolution of pollen clumping that is ultimately expressed in pollinia. Similar views were expressed by Darwin (1877) who considered pollinia to have evolved subsequent to small seeds in orchids. Recent evidence indicates that the small-seeded Apostasioideae are indeed the sister group to the remainder of the Orchidaceae (Cameron et al. 1999), and there are thus good phylogenetic grounds for believing that small mycotrophic seeds were the initial evolutionary impetus for the evolution of pollinaria.

The evolution of the epiphytic habit in orchids would have required very light dustlike seeds to effectively colonize new growth sites in forest canopies (Dressler 1981). Numerous small seeds are also found in other largely epiphytic families, such as the Bromeliaceae and Gesneriaceae. While pollen is not clumped in these families, many of the species are pollinated by vertebrates which would be expected to deposit large enough stigmatic pollen loads to ensure fertilization of the large number of ovules.

The reduction of fertile anthers from six to one, and the close alliance of male and female whorls in families such as Cannaceae, Marantaceae, Costaceae and Zingiberaceae is remarkably similar to Orchidaceae. Within these lineages there is an overwhelming reduction of tepals and their replacement by petaloid staminodes which are frequently modified into a labellum remarkably similar to those of Orchidaceae. Yet the pollen of these families does not cohere into pollinia. The key functional difference may be the mycotrophic habit of Orchidaceae which is pivotal in the evolution of dust seed in this slow growing group.

Conclusions

The most likely evolutionary scenario for orchids is that both small seeds and the need for efficient pollen export were complementary selective pressures for the evolution of pollinaria. Ancestral orchids probably had small seeds which would have promoted the cohesion of pollen as it leads to greater stigmatic pollen loads. At the same time, infrequent visitation by insects would have favoured a pollen packaging strategy that allowed removal of all of the pollen from the anther in one visit with minimal wastage in transit and minimal probability of it being deposited on foreign stigmas.

The innovation of pollinaria undoubtedly led to greater floral specialization in the orchids (Tremblay 1991), and thereby contributed indirectly to pollinator-driven speciation in the family (cf. Johnson et al. 1998a). In addition, the phenomenally high number of recombinants possible after each successful pollination event in orchids would have supplied the variation on which natural selection can act.

On the other hand, pollinaria impose certain constraints on the evolutionary options within the family. Pollination by pollen-collecting bees is rare among orchids, with the exception of a few species with powdery pollen, such as *Apostasia* (Dressler 1986) or loose pollen masses such as *Cleistes* (Gregg 1991). Likewise, wind-pollination is not viable for orchids due to the aggregation of pollen into weighty units. The ability of orchids to utilize promiscuous pollinators is limited because of the risk of losing the entire pollen complement of a flower to an unreliable vector. Orchids have followed an evolutionary pathway of specialized pollen transfer and have flourished in terms of numbers of species, but at the same time have failed to dominate ecosystems in the way that wind-pollinated plants, such as the Poaceae, or those with generalist pollination systems, such as the Asteraceae, have been able to do.

References

Ackerman J. D. (1975) Reproductive biology of *Goodyera oblongifolia* (Orchidaceae). Madrona 23: 191–198.

Ackerman J. D. (1995) An orchid flora of Puerto Rico and the Virgin Islands. Mem. N.Y. Bot. Gdn 73: 1–203.

Ackerman J. D., Mesler M. R. (1979) Pollination biology of *Listera cordata* (Orchidaceae). Amer. J. Bot. 6: 820–824.

Ackerman J. D., Williams N. H. (1980) Pollen morphology of the tribe *Neottieae* and its impact on the classification of the Orchidaceae. Grana 19: 7–18.

Ackerman J. D., Williams N. H. (1981) Pollen morphology of the *Chloraeinae* (Orchidaceae: Diurideae) and related subtribes. Amer. J. Bot. 68: 1392–1402.

Ackerman J. D., Montalvo A. M. (1990) Short- and long-term limitations to fruit production in a tropical orchid. Ecology 71: 263–272.

Albert V. A. (1994) Cladistic relationships of the slipper orchids (Cypripedioideae: Orchidaceae) from congruent morphological and molecular data. Lindleyana 9: 115–132.

Alexandersson R. (1999) Reproductive biology of the deceptive orchid *Calypso bulbosa*. PhD thesis, University of Umea.

Atwood J. T. (1986) The size of the Orchidaceae and the systematic distribution of epiphytic orchids. Selbyana 9: 171–186.

Bell G. (1985) On the function of flowers. Proc. Roy. Soc. Lond. B. 224: 233–265.

Bernhardt P. (1995) Observations on the floral biology of *Pterostylis curta* (Orchidaceae). Cunninghamia 5: 1–8.

Blackman S. J., Yeung E. C. (1983) Comparative anatomy of pollinia and caudicles of an orchid (*Epidendrum*). Bot. Gaz. 144: 331–337.

Borba E. L., Semir J. (1999) Temporal variation in pollinarium size after its removal in species of *Bulbophyllum*: a different mechanism preventing self-pollination in Orchidaceae. Plant Syst. Evol. 217: 197–204.

Burns-Balogh P. (1982) Generic redefinition in the subtribe *Spiranthinae* (Orchidaceae). Amer. J. Bot. 69: 1119–1132.

Burns-Balogh P. (1983) A theory on the Evolution of the exine in Orchidaceae. Amer. J. Bot. 69: 1119–1132.

Burns-Balogh P., Funk V. A. (1986) A phylogenetic analysis of the Orchidaceae. Smithsonian Contrib. Bot. 61: 1–79.

Burns-Balogh P., Bernhardt P. (1985) Evolutionary trends in the androecium of the Orchidaceae. Plant Syst. Evol. 149: 119–134.

Burns-Balogh P., Bernhardt P. (1988) Floral evolution and phylogeny in the tribe Thelymitreae (Orchidaceae: Neottioideae). Plant Syst. Evol. 159: 19–47.

Cameron K. M., Chase M. W., Whitten W. M., Kores P. J., Jarrell D. C., Albert V. A., Yukawa T., Hills G., Goldman D. H. (1999) A phylogenetic analysis of the Orchidaceae: evidence from *rbc*L nucleotide sequences. Amer. J. Bot. 86: 208–224.

Catling P. M. (1983) Pollination of northeastern North American *Spiranthes* (Orchidaceae). Can. J. Bot. 61: 1080–1093.

Catling P. M. (1990) Auto-pollination in the Orchidaceae. In: Arditti J. (ed.) Orchid biology, reviews and perspectives V. Timber Press, Portland, Oregon, pp. 121–158.

Catling P. M., Catling V. R. (1991) A synopsis of breeding systems and pollination in North American orchids. Lindleyana 6: 187–210.

Chase M. W. (1987) Systematic implications of pollinarium morphology in *Oncidium* Sw., *Odontoglossum* Kunth, and allied genera (Orchidaceae). Lindleyana 2: 8–28.

Chen S. C. (1982) The origin and early differentiation of the Orchidaceae. Acta Phytotaxon. Sin. 20: 1–22.

Chesselet P., Linder H. P. (1993) Pollen morphology of the *Diseae* (Orchidoideae: Orchidaceae). Grana 32: 101–110.

Cole F. R., Firmage D. H. (1984) The floral ecology of *Platanthera blephariglottis*. Am. J. Bot. 71: 700–710.

Cribb P. J. (1987) The Genus *Paphiopedilum*. Kew: Royal Botanic Gardens; Portland, U.K.

Cruden R. W. (1977) Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.

Cruden R. W., Jensen K. G. (1979) Viscin threads, pollination efficiency and low pollen-ovule ratios. Amer. J. Bot. 66: 875–879.

Dafni A., Ivri Y. (1979) Pollination ecology of, and hybridization between, *Orchis coriophora* L. and *O. collina* Sol. ex. Russ. (Orchidaceae) in Israel. New Phytol. 83: 181–187.

Dafni A., Ivri Y. (1981) The flower biology of *Cephalanthera longifolia* (Orchidaceae) – pollen imitation and facultative floral mimicry. Plant Syst. Evol. 137: 229–240.

Dahlgren R. M. T., Clifford H. T., Yeo P. F. (1985) The families of the monocotyledons: structure, evolution, and taxonomy. Springer, Berlin.

Darwin C. (1877) The various contrivances by which orchids are fertlized by insects. 2nd edn. John Murray, London.

De Jong T. J., Klinkhamer P. G. L., Staalduinen M. J. (1992) The consequences of pollination biology for selection of mass or extended blooming. Funct. Ecol. 6: 606–615.

Dressler R. L. (1971) Dark pollinia in hummingbird-pollinated orchids, or do hummingirds suffer from strabismus? Am. Nat. 105: 80–83.

Dressler R. L. (1968) Pollination by euglossine bees. Evolution 22: 202–210.

Dressler R. L. (1981) The Orchids: Natural History and classification. Harvard University Press, Cambridge, Mass.

Dressler R. L. (1986) Features of pollinaria and orchid classification. Lindleyana 1: 125–130.

Dressler R. L. (1990) The Neottieae in orchid classification. Lindleyana 5: 2, 102–109.

Dressler R. L. (1993) Phylogeny and classification of the orchid family. Timber Press, Portland.

Ellstrand N. C. (1984) Multiple paternity within the fruits of the wild radish, *Raphanus sativus*. Evolution 123: 819–828.

Fitzgerald M. A., Calder D. M., Knox R. B. (1993) Secretory events in the freeze-substituted tapetum of the orchid *Pterostylis concinna* FitzG. Plant Syst. Evol. [Suppl.] 7: 53–62.

Fitzgerald M. A., Barnes S. H., Blackmore S., Calder D. M., Knox R. B. (1994) Pollen development and cohesion in a mealy and a hard type of orchid pollinium. J. Plant Sci. 155: 481–491.

Freudenstein J. V. (1994) Gynostemium structure and relationhips of the *Corallorhizinae* (Orchidaceae: Epidendroideae). Plant Syst. Evol. 193: 1–19.

Freudenstein J. V. (1991) A systematic study of endothecial thickenings in the Orchidaceae. Amer. J. Bot. 78: 766–781.

Freudenstein J. V., Rasmussen F. N. (1996) Pollinium development and number in the Orchidaceae. Am. J. Bot. 83: 7, 813–824.

Freudenstein J. V., Rasmussen F. N. (1997) Sectile pollinia and relationships in the Orchidaceae. Plant Syst. Evol. 205: 125–146.

Freudenstein J. V., Rasmussen F. N. (1999) What does morphology tell us about orchid relationships? – A cladistic analysis. Amer. J. Bot. 86: 225–248.

Fritz A. L. (1990) Deceit pollination of *Orchis spitzelii* (Orchidaceae) on the island of Gotland in the Baltic: a suboptimal system. Nord. J. Bot. 9: 577–587.

Gregg K. B. (1991) Defrauding the deceitful orchid: pollen collection by pollinators of *Cleistes divaricata* and *C. bifaria*. Lindleyana 6: 214–220.

Hagerup O. (1952) Bud autogamy in some southern orchids. Phytomorphology 2: 51–60.

Hapeman J. R., Inoue K. (1997) Plant-pollinator interactions and floral radiation in *Platanthera* (Orchidaceae). In: Givnish T. J., Sytsma K. J. (eds.) Molecular evolution and adaptive radiation. Cambridge University Press, Cambridge, pp. 433–454.

Harder L. D., Wilson W. G. (1998) A clarification of pollen discounting and its joint effects with inbreeding depression on mating system evolution. Am. Nat. 152: 684–695.

Johansen B. (1990) Incompatibility in *Dendrobium* (Orchidaceae). Bot. J. Linn. Soc. 103: 165–196.

Johnson S. D. (1994) Evidence for Batesian mimicry in a butterfly-pollinated orchid. Biol. J. Linn. Soc. 53: 91–104.

Johnson S. D. (1996) Bird pollination in South African species of *Satyrium* (Orchidaceae). Plant Syst. Evol. 204: 91–98.

Johnson S. D. (1997) Insect pollination and floral mechanisms in South African species of *Satyrium* (Orchidaceae). Plant Syst. Evol. 204: 195–206.

Johnson S. D., Bond W. J. (1994) Red flowers and butterfly pollination in the fynbos of South Africa. In: Arianoutsou M., Groves R. H. (eds.) Plant-animal interactions in Mediterranean-type ecosystems. Kluwer Academic Publishers, Dordrecht, pp. 137–148.

Johnson S. D., Bond W. J. (1997) Evidence for widespread pollen-limitation of fruiting success in Cape wildflowers. Oecologia 109: 530–534.

Johnson S. D., Liltved W. R. (1997) Hawkmoth pollination of *Bonatea speciosa* (Orchidaceae) in a South African coastal forest. Nord. J. Bot. 17: 5–10.

Johnson S. D., Nilsson L. A. (1999) Pollen carryover, geitonogamy and the evolution of deception in orchids. Ecology 80: 2607–2619.

Johnson S. D., Steiner K. E., Kurzweil H. (1994) Auto-pollination in *Disa* species (Orchidcaeae) of the Cape mountain cloud zone. Lindleyana 9: 3–6.

Johnson S. D., Linder H. P., Steiner K. E. (1998a) Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). Amer. J. Bot. 85: 402–411.

Johnson S. D., Steiner K. E., Whitehead V. B., Vogelpoel L. (1998b) Pollination ecology and maintenance of species integrity in co-occurring *Disa racemosa* L.f. and *Disa venosa* Sw. (Orchidaceae) in South Africa. Ann. Missouri Bot. Gard. 85: 231–241.

Judd W. S., Stern W. L., Cheadle V. I. (1993) Phylogenetic position of *Apostasia* and *Neuwiedia* (Orchidaceae). Bot. J. Linn. Soc. 113: 87–94.

Kores P. J., Cameron K. M., Molvray M., Chase M. (1997) The phylogenetic relationships of Orchidoideae and Spiranthoideae (Orchidaceae) as inferred from *rbc*L plastid sequences. Lindleyana 12: 1–11.

Kress W. J. (1981) Sibling competition and evolution of pollen unit, ovule number, and pollen vector in angiosperms. Syst. Bot. 6: 101–112.

Kurzweil H., Johnson S. D. (1993) Auto-pollination of *Monadenia bracteata*. S. Afr. Orchid. J. 24: 21–22.

Larson B. M. H., Barrett S. C. H. (1999) The ecology of pollen limitation in buzz-pollinated

Rhexia virginica (Melastomataceae). J. Ecol. 87: 371–381.

Levin D. A. (1981) Dispersal versus gene flow in plants. Ann. Missouri Bot. Gard. 68: 233–253.

Levin D. A., Kerster H. W. (1974) Gene flow in seed plants. Evol. Biol. 7: 139–220.

Linder H. P., Kurzweil H. (1994) The phylogeny and classification of the Diseae (Orchidoideae: Orchidaceae). Ann. Missouri Bot. Gard. 81: 687–713.

Mehroff L. A. (1983) Pollination in the genus *Isotria* (Orchidaceae). Amer. J. Bot. 70: 1444–1453.

Morse D. H. (1981) Modification of bumblebee foraging: the effect of milkweed pollinia. Ecology 62: 89–97.

Nazarov V. V., Gerlach G. (1997) The potential seed productivity of orchid flowers and peculiarities of their pollination systems. Lindleyana 12: 188–204.

Neiland M. R. M., Wilcock C. C. (1995) Maximization of reproductive success by European Orchidaceae under conditions of infrequent pollination. Protoplasma 187: 39–48.

Newton G. D., Williams N. H. (1978) Pollen morphology of the Cypripedioideae and the Apostasioideae (Orchidaceae). Selbyana 2: 169–182.

Nierenberg L. (1972) The mechanism for the maintenance of species integrity in sympatrically occurring Oncidiums in the Caribbean. Amer. Orch. Soc. Bull. 41: 873–882.

Nilsson L. A. (1980) The pollination ecology of *Dactylorhiza sambucina* (Orchidaceae). Bot. Notiser 133: 367–385.

Nilsson L. A. (1983a) Anthecology of *Orchis mascula* (Orchidaceae). Nord. J. Bot. 3: 157–179.

Nilsson L. A. (1983b) Processes of isolation and introgressive interplay between *Platanthera bifolia* (L.) Rich. and *P. chlorantha* (Custer) Reichb. (Orchidaceae). Bot. J. Linn. Soc. 87: 325–350.

Nilsson L. A. (1992) Orchid pollination biology. Trends Ecol. Evol. 7: 255–259.

Nilsson L. A., Jonsson L., Rason L., Randrianjohany E. (1985) The pollination of *Cymbidiella flabellata* (Orchidaceae) in Madagascar: a system pollinated by sphecid wasps. Nord. J. Bot. 6: 411–422.

Nilsson L. A., Rabakonandrianina E., Razananaivo R., Randriamanindry J. (1992a) Long pollinia on eyes: hawk-moth pollination of *Cynorkis uniflora* Lindley (Orchidaceae) in Madagascar. Bot. J. Linn. Soc. 109: 145–160.

Nilsson L. A., Rabakonandrianina E., Pettersson B. (1992b) Exact tracking of pollen transfer and mating in plants. Nature 360: 666–667.

O'Connell L. M., Johnston M. O. (1998) Male and female pollination success in a deceptive orchid, a selection study. Ecology 79: 1246–1260.

Pacini E., Franchi G. G. (1996) Some cytological, ecological and evolutionary aspects of Pollination. Acta Societatis Botanicorum Poloniae 65: 11–16.

Pacini E., Franchi G. G. (1998) Pollen dispersal units, gynoecium and pollination. In: Owens S. J., Rudall P. J. (eds.) Reproductive biology. Royal Botanic Gardens, Kew, pp. 183–195.

Pandolfi T., Pacini E. (1995) The pollinium of *Loroglossum hircinum* (Orchidaceae) between pollination and pollen tube emission. Plant Syst. Evol. 196: 141–151.

Peakall R. (1989a) A new technique for monitoring pollen flow in orchids. Oecologia 79: 361–365.

Peakall R. (1989b) The unique pollination of *Leporella fimbriata* (Orchidaceae): pollination by pseudocopulating male ants (*Myrmecia urens, Formicidae*). Plant Syst. Evol. 167: 137–148.

Peakall R., Beattie A. J. (1991) The genetic consequences of worker ant pollination in a self-compatible, clonal orchid. Evolution 45: 1837–1848.

Peakall R., Beattie A. J. (1996) Ecological and genetic consequence of pollination by sexual deception in the orchid *Caladenia tentactulata*. Evolution 50: 2207–2220.

Proctor H. C. (1997) Effect of pollen age on fruit set, fruit weight, and seed set of three orchid species. Can. J. Bot. 76: 420–427.

Proctor H. C., Harder L. D. (1994) Pollen load, capsule weight, and seed production in three orchid species. Can. J. Bot. 72: 249–255.

Rademaker M. C. J., De Jong T. J., Klinkhamer P. G. L. (1997) Pollen dynamics of bumble-bee visitation on *Echium vulgare*. Funct. Ecol. 11: 554–563.

Rasmussen F. N. (1982a) The gynostemium of neottioid orchids. Opera Bot. 69: 1–96.

Rasmussen F. N. (1982b) The gynostemium of *Bulbophyllum ecornutum*. Bot. J. Linn. Soc. 91: 447–456.

Rasmussen F. N. (1986a) On the contrivances by which pollinia are attached to viscidia. Lindleyana 1: 21–32.

Rasmussen F. N. (1986b) Ontogeny and phylogeny in Orchidaceae. Lindleyana 1: 114–124.

Robertson A. W. (1992) The relationship between floral display size and geitonogamy in *Mysotis colensoi* (Kirk) MacBride (Boraginaceae). Biol. J. Linn. Soc. 46: 333–349.

Romero G. A. (1990) Phylogenetic relationships in subtribe Catasetinae (Orchidaceae, Cymbidieae). Lindleyana 5: 160–181.

Romero G. A., Nelson C. E. (1986) Sexual dimorphism in *Catasetum* orchids: forcible pollen emplacement and male flower competition. Science 232: 1538–1540.

Roubik D. W. (2000) Deceptive orchids with Meliponini as pollinators. Plant Syst. Evol. 222: 271–279.

Salguero-Faría J., Ackerman J. D. (1999) A nectar reward: is more better. Biotropica 31: 303–311.

Sanford W. W. (1964) Sexual compatibility relationships in *Oncidium* and related genera. Amer. Orchid Soc. Bull. 33: 1035–1048.

Schill R. (1978) Palynologische Untersuchungen zur systematischen Stellung der Apostasiaceae. Botanische Jahrbucher fur Systematik, Pflanzengeschichte und Pflanzengeographie 99: 353–362.

Schill R., Dannenbaum C., Neyer P. (1992) Quantitative Untersuchungen an Orchideen-pollinien. Bot. Jahrb. Syst. 114: 153–171.

Schill R., Pfeiffer W. (1977) Untersuchungen an Orchideenpollinien unter besonderen Beruecksichtigung ihrer Feinskulpturen. Pollen et Spores 19: 5–118.

Schill R., Wolter M. (1986) Ontogeny of elastoviscin in the Orchidaceae. Nord. J. Bot. 5: 575–580.

Scott J. (1865) On the individual sterility and cross-impregnation of certain species of *Oncidium*. Bot. J. Linn. Soc. 8: 162–167.

Steiner K. E. (1989) The pollination of *Disperis* (Orchidaceae) by oil-collecting bees in southern Africa. Lindleyana 4: 164–183.

Thomson J. D., Thomson B. A. (1989) Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. Evolution 43: 657–661.

Tremblay R. L. (1991) Trends in the pollination ecology of the Orchidaceae: evolution and systematics. Can. J. Bot. 70: 642–650.

Tremblay R. L. (1994) Frequency and consequences of multi-parental pollinations in a population of *Cypripedium calceolus* var. *pubescens* (Orchidaceae). Lindleyana 9: 161–167.

Stern W. L., Cheadle V. I., Thorsch J. (1993) Apostasiads, systematic anatomy, and the origins of Orchidaceae. Bot. J. Linn. Soc. 111: 411–455.

Thomson J. D., McKenna M. A., Cruzn M. B. (1989) Temporal patterns of nectar and pollen production in *Aralia hispida*: implications for reproductive success. Ecology 70: 1061–1068.

Van der Pijl L., Dodson C. H. (1966) Orchid flowers: their pollination and evolution. University of Miami Press.

Warford N. (1992) Pollination biology: the reciprocal agreement between *Notylia* and *Euglossa viridissima*. Amer. Orchid. Soc. Bull. 61: 884–889.

Williams N. H. (1972) A reconsideration of *Ada* and the glumaceous brassias. Brittonia 24: 93–110.

Williams N. H., Broome C. R. (1976) Scanning electron microscope studies of orchid pollen. Amer. Orchid. Soc. Bull. 45: 699–707.

Williamson G. (1984) Observations of a mechanism by which self-pollination may occur in *Eulophia* (Orchidaceae). J. S. Afr. Bot. 50: 417–423.

Zavada M. S. (1990) A contribution to the study of pollen wall ultrastructure of orchid pollinia. Ann. Missouri Bot. Gard. 77: 785–801.

Address of the authors: S. D. Johnson and T. J. Edwards, School of Botany and Zoology, University of Natal, P. Bag X01, Scottsville, 3209, South Africa.