Pseudopollen in Eria Lindl. Section Mycaranthes Rchb.f. (Orchidaceae)

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INTRODUCTION

The flowers of many epidendroid orchid species are visited by insects searching for nectar, oils or droplets of fragrance (van der Pijl and Dodson, 1969; Arditti, 1992; Dressler, 1990, 1993) and it has been shown that orchid species that reward pollinators in this way often double their chances of fruiting (Neiland and Wilcock, 1998). A significant number of epidendroid orchids, however, reward potential pollinators with food-laden pseudopollen and these species tend not to produce nectar (van der Pijl and Dodson, 1969).

Pseudopollen is a mealy substance and is usually formed by the fragmentation of uniseriate, multicellular, labellar trichomes into individual component cells or short chains of cells. Some food-hairs, however, simply become detached from the labellar surface. Species that produce pseudopollen are to be found in the genera Maxillaria Ruiz & Pav. (Janse, 1886; Porsch, 1905; van der Pijl and Janse, 1886; Porsch, 1905; van der Pijl and Pav. (Janse, 1886; Porsch, 1905; van der Pijl and Pseudopollen is a whitish, mealy material produced upon the labella of a number of orchid species as labellar hairs either become detached or fragment. Since individual hair cells are rich in protein and starch, it has been speculated that pseudopollen functions as a reward for visiting insects. Although some 90 years have passed since Beck first described pseudopollen for a small number of Eria spp, currently assigned to section Mycaranthes Rchb.f., we still know little about the character of pseudopollen in this taxon. The use of SEM and histochemistry would re-address this deficit in our knowledge whereas comparison of pseudopollen in Eria (southern Asia), Maxillaria (tropical and sub-tropical America), Polystachya (largely tropical Africa and Madagascar) and Dendrobium unicum (Thailand and Laos) would perhaps help us to understand better how this feature may have arisen and evolved on a number of different continents.

Methods Pseudopollen morphology is described using light microscopy and scanning electron microscopy. Hairs were tested for starch, lipid and protein using IKI, Sudan III, and the xanthoproteic test, respectively.

Key words: Evolution, food-hairs, histochemistry, labellum, light microscopy, papillae, pollinators, pseudopollen, scanning electron microscopy, wax.
devoid of food material can still attract insects solely by deceit. Meliponini (stingless bees) and halictid bees are the main pollinators of *Maxillaria* and *Polystachya* spp., respectively (Goss, 1977; Petterson and Nilsson, 1993; Singer and Cocucci, 1999; Roubik, 2000) but, unfortunately, records of insects actually gathering pseudopollen are rare. Nevertheless, old records report that Euglossine bees have been observed collecting pseudopollen from *Maxillaria* flowers (Dodson and Frymire, 1961; Dodson, 1962). More recently, *Trigona* spp. (meliponini) have been observed collecting hairs, presumably pseudopollen, from the label of *M. ochroleuca* Lodd. ex Lindl. and *M. brasiliensis* Brieger & Bicalho (Singer, 2003; Singer and Koehler, 2004). Moreover, Davies and Turner (2004b) have suggested that the pseudopollen of *D. unicum* is gathered by small eusocial bees since the main food here is starch and starch alone is unlikely to satisfy the nutritional requirements of solitary bees. Furthermore, as pollen is not used by wasps, these insects are unlikely to visit pseudopollen-producing flowers. Our knowledge of the pollination biology of *Eria* is even more vague and it has been speculated that beetles (Beck, 1914) or small bees (Dressler, 1990) may pollinate these flowers.

Beck (1914) was the first to study the pseudopollen of *Eria* in any detail. He examined *E. monostachya* Lindl. var. *pleiostachya* Beck & Lerchen and *E. paniculata* Lindl., both of which are currently placed in section *Mycaranthes* Rchb.f. This section, which contains about 20 species (Seidenfaden, 1982), is well represented in Sumatra and Borneo with a few species reaching Thailand, Indochina, New Guinea, the Philippines and Malaya. Species assigned to this section tend to be non-pseudobulbous mountain epiphytes but some are lowland plants. They have fairly long, thick but not fleshy, leafy stems with narrow, lanceolate leaves. Usually three or four terminal or sub-terminal inflorescences arise together and these are covered with short woolly hairs. Each inflorescence bears numerous, small flowers with widely spreading tepals, tomentose on the outer surface, and a labellum with well-developed side lobes. The labellum has a farinaceous median ridge connecting a large callus to a large mealy callus on the mid-lobe and the column-foot is relatively long (Seidenfaden, 1982; Seidenfaden and Wood, 1992).

Beck (1914), in describing *E. monostachya* var. *pleiostachya*, claimed that the part of the labellum that produces pseudopollen lacks a cuticle and that epidermal cells swell early in the development of pseudopollen. The papillae thus formed are vacuolate with a little peripheral cytoplasm, contain nuclei and become ellipsoid, pyriform or clavate. The base of each papilla has a short ‘stalk’. Soon, starch grains develop within the papillae but these may be absent. The papillae now measure some 30–92 μm × 30–40 μm although most are 50–60 μm in length and have a very characteristic cuticle of fine, wavy ‘lines’. Beck also reported that the ‘stalk’ is very fragile and that the papillae are detached by passing insects. His histochemical tests showed that whereas the swollen part of the papilla stained for cellulose with chlorzinc iodide (Schulze’s solution), the ‘stalk’ did not and therefore must have had a different chemical composition. Histochemical tests did not reveal the presence of sugars within the pseudopollen. Furthermore, Beck proposed that during the course of evolution, pseudopollen gradually replaced nectar in these flowers and that only relatively large insects, probably beetles, attracted by scent, could possibly pollinate the flower since the reproductive organs occur some 2.5 mm above the pseudopollen. As only herbarium (presumably dried and pressed) material of *E. paniculata* was available to Beck, he could not be certain that the subject of his study was actually this species. However, the structure and detachment of pseudopollen here was similar to *E. monostachya*. The papillae measured 55–88 μm in length and contained much starch, as indeed did the pseudopollen of *E. stricta* Lindl.

The aim of the present paper is to examine the range of pseudopollen morphology found within *Eria* section *Mycaranthes* based on a greater number of taxa and to compare the results with those obtained by Beck (1914). Scanning electron microscopy (SEM) studies, coupled with histochemical analyses at light microscopy level, would perhaps better allow us to speculate as to what the pollinators could be. Finally, since *Eria* spp. and *D. unicum* occur exclusively in Asia, whereas *Maxillaria* spp. grow solely in the American tropics and subtropics and *Polystachya* spp. occur largely in tropical Africa and Madagascar with some representatives in southern Africa, Asia, Australia and central South America, comparison of their pseudopollen could perhaps yield useful information about the way this feature may have arisen and evolved in a number of unrelated genera on different continents.

**MATERIALS AND METHODS**

Twenty-eight samples of spirit-preserved flowers representing eight species of *Eria* currently assigned to section *Mycaranthes* (Table 1) and collected from a number of localities in south-east Asia (Fig. 1) were examined. Those specimens whose accession numbers are prefixed ‘K’ were obtained from the herbarium of the Royal Botanic Gardens, Kew, UK, whereas those prefixed ‘SBG’ were obtained from the Singapore Botanic Gardens. The former were kept in ‘Copenhagen mix’ (70 cm³ industrial methylated spirit : 2 cm³ glycerol : 28 cm³ water) but as some of these specimens were collected as long ago as 1929, many had formerly been stored in a range of preservatives that contained formalin. The specimens obtained from the Singapore Botanic Gardens, however, were collected much more recently, sent to us in and subsequently stored in 5 % formalin. The authorities for plant names follow Brummitt and Powell (1992).

**Scanning electron microscopy**

Labellae were transferred to and stored in tubes of 70 % (v/v) ethanol. They were dehydrated in 90 % (v/v) ethanol (15 min at room temperature) followed by two changes of 100 % ethanol (30 min each at room temperature) and subjected to critical-point drying (Balzers 030 CPD) using liquid CO₂. The specimens were then mounted on stubs by means of double-sided carbon adhesive tabs, coated
with gold (Edwards S150B sputter coater) and examined using a JSM 5200 LV-SEM at an accelerating voltage of 20 kV.

**Histochemistry**

Pseudopollen was tested for starch using a dilute iodine/potassium iodide (IKI) solution, for lipids using a saturated ethanolic solution of Sudan III and for protein by means of the xanthoproteic test as outlined in our previous papers (Davies et al., 2000, 2003a, b).

**RESULTS**

Light microscopy revealed that pseudopollen of all species of *Eria* section *Mycaranthes* examined is very similar in form in that the labellar trichomes are unicellular and clavate with ‘stalks’ of varying lengths. Moreover, these hairs easily become detached from the surface of the labellum (Fig. 2A). A number of small spherical cytoplasmic organelles are visible within the swollen tip of each hair, even in the unstained state (Fig. 2B). In common with *D. unicum* and pseudopollen-forming species of *Maxillaria* and *Polystachya*, those species that have been observed in *vivo* by earlier authors appear not to produce nectar.

Examination by SEM further revealed that the labellar trichomes, regardless of species, are striated (Figs 3A–C and 4A–E) and that these raised cuticular ridges tend to be shorter and more densely arranged towards the apex of the hair (Fig. 4C and E). Furthermore, the apices often have a flaky appearance, possibly due to wax deposits (Fig. 4A and B) and both apices and ridges stained intensely with Sudan III indicating high lipid content.

Histochemistry also showed that the labellar hairs are rich in aromatic amino acids and starch grains (Table 2). The former are distributed throughout the cytoplasm, whereas the latter are confined to amyloplasts that correspond in shape, size and position to the small spherical organelles visible in unstained preparations. Lipid droplets were not detected in the labellar hairs of any species, regardless of whether the flowers were preserved in solutions containing alcohol or formalin.

**DISCUSSION**

**Comparative morphology and histochemistry**

Despite their wide geographical distribution, the pseudopollen of all eight taxa showed considerable uniformity, in as much as it was equally difficult to distinguish between the
pseudopollen of well-defined species (Figs 3A–C and 4C–E) as it was to distinguish between that of taxa such as *E. oblitterata* (Blume) Rchb.f. (Fig. 4A) and *E. ridleyi* Rolfe (Fig. 4B) which are considered by some authors to be conspecific (Seidenfaden and Wood, 1992). Consequently, pseudopollen in *Eria* section *Mycaranthes* is a highly conservative character and is thus of little value in taxonomy at species level. Indeed, data relating to pseudopollen alone would support the concept that these taxa form a species complex. Seidenfaden (Seidenfaden, 1982; Seidenfaden and Wood, 1992) states that *Mycaranthes* is well separated on morphological grounds from all other sections of *Eria* and that several of its species are morphologically very variable. It would appear that in the specimens examined here, unicellular, clavate, pseudopollen-forming hairs develop from papillae as described by Beck (1914). This is supported by the presence of cuticular ridges and flaky deposits upon both trichomes and papillae. The multicellular, moniliform, pseudopollen-forming hairs of *M. sanderiana*
Rchb.f. also arise in like manner (Davies et al., 2000), although it is speculated that such hairs may have evolved in the *M. discolor* (Lodd. ex Lindl.) Rchb.f. alliance from simple uniseriate, multicellular trichomes (Davies et al., 2003a).

In *Eria*, pseudopollen is formed as clavate hairs become detached from the labellar surface. These pseudopollen-forming hairs are very different from those found in *Maxillaria*, *Polystachya* and *Dendrobium* in that they are unicellular, not multicellular. Moreover, unlike the pseudopollen-forming hairs of *Maxillaria* and *Dendrobium*, they do not fragment, rather, the complete hair becomes detached from the surface of the labellum as occurs in certain species of *Polystachya*. Indeed, these hairs are very similar in shape to those of *P. campyloglossa* Rolfe. Also, the hairs are rich in aromatic amino acids, and protein forms the main food reserve here as in *Maxillaria* and *Polystachya* although, unlike the former genus, the protein in *Eria* does not occur in a well-defined protein-body, but is distributed throughout the cytoplasm as in *Polystachya* spp. and *D. unicum*. Starch is also present within the pseudopollen of *Eria* and occurs in numerous small amyloplasts, as in *Maxillaria* and *Polystachya* spp., unlike *D. unicum* where each component cell contains a single, relatively large

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![Fig. 3. SEM studies of clavate, unicellular labellar hairs of (A) *E. paniculata* K20109-000 showing (B) cuticular ridges and pronounced flaky deposits. Trichome of *E. paniculata* K18323-000 (C) becoming detached from labellar surface. Note the associated filamentous structure at the base of the trichome. An abundance of such structures (see Fig. 3A) may account for earlier reports that, on dispersal of pseudopollen, the labellum becomes ‘hairy’ (Beck, 1914). Scale bars: A = 50 μm; B and C = 10 μm.](https://academic.oup.com/aob/article-abstract/94/5/707/152055)
starch grain (Kjellsson and Rasmussen, 1987; Davies and Winters, 1998; Davies et al., 2000, 2002, 2003; Davies and Turner, 2004a, b).

The pseudopollen-forming hairs of the *Eria* spp. examined are significantly different from those hitherto described for other orchid genera in that they bear pronounced cuticular ridges and possibly wax deposits. These ridges closely resemble the waxy striations found upon the labellar papillae of *M. cerifera* Barb. Rodr. (Senghas, 1993). Labellar wax in *Maxillaria* is generally thought to be gathered by meliponini (Flach et al., 2004) and may perhaps be used for nest-building (van der Pijl and Dodson, 1969). D. W. Roubik (pers. comm.) remarks that if this is true, it would be ironic since meliponini, unlike euglossine and halictid bees, are
capable of making their own wax. Lipoidal material also occurs upon the labella of other Maxillaria spp. (Davies et al., 2003a; Davies and Turner, 2000, 2002, 2003). In short, lipid-rich surface deposits, protein and starch had been preserved in all specimens of Eria tested, indicating that conventional preservation methods were adequate and that material kept in this manner could still be used successfully for histochemical analyses even after some 60 years of preservation in fluid!

However, the presence of food materials alone is not sufficient evidence that labellar hairs function as pseudopollen. For example, E. pilifera Ridl. has unicellular, clavate hairs not unlike those observed for Eria spp. assigned to section Mycaranthes, yet, although the cytoplasm of this species is rich in protein and numerous small amyloplasts containing starch, it appears that the hairs do not become detached and therefore cannot function as pseudopollen (K. L. Davies, unpubl. res.). Even so, this does not preclude the possibility that insect pollinators may nibble at the hairs for the food they contain.

### Pollination biology

Current knowledge of the pollination biology of Eria spp. is poor. Beck (1914), basing his argument on the relative dimensions and position of floral parts, speculated that Eria flowers may be pollinated by beetles. However, Dressler (1990) suggested that the pollinators are small bees; a view in keeping with the fact that most pseudopollen-forming flowers examined to date are either pollinated by meliponini or halictid bees (Goss, 1977; Pettersson and Nilsson, 1993; Singer and Cocucci, 1999; Roubik, 2000; Singer, 2003; Singer and Koehler, 2004). This may well be the case since the labellar hairs of Eria spp. and certain species of
Polystachya, a genus known to be pollinated by halictid bees, are very similar and stain identically when tested for protein and starch. Moreover, the presence of presumed wax deposits upon the labellar hairs would also tend to argue strongly in favour of bee pollination, although, at present, there is no unequivocal evidence to support this. Wasps, however, probably do not pollinate Eria spp. since these insects do not utilize pollen and are thus unlikely to be attracted by pseudopollen. Nevertheless, it is possible that the branched multicellular hairs that occur on the reverse of tepals and upon the pedicellate ovary may also be involved in attracting/rewarding insects. These hairs easily become detached and in the case of fluid-preserved flowers, form a dense layer at the base of the specimen tube. In life, it is possible that such hairs may be gathered by insects and used for nest-building but again, evidence for this is lacking.

Ecological considerations

Representatives of Eria section Mycaranthes are restricted to south-east Asia and pseudopollen is invariably formed in these species as unicellular, clavate trichomes become detached from the labellum. These hairs contain protein that is distributed throughout the cytoplasm and starch that occurs within amyloplasts. The most remarkable feature, however, is that the pseudopollen bears flaky, wax-like deposits upon its surface. Thus, the pseudopollen of Eria spp. is very different from that observed to date for other orchids such as Maxillaria (American tropics and subtropics) (Davies et al., 2000, 2003a; Davies and Turner, 2004a), Polystachya (tropical Africa and Madagascar with some species in southern Africa, Australia and central South America) (Porsch, 1906; Beck, 1914; Davies et al., 2002) and D. unicum (Laos and Northern Thailand) (Kjellson and Rasmussen, 1987; Davies and Turner, 2004b).

Pseudopollen of diverse morphology in unrelated taxa occurring on different continents would indicate that this character is not homologous and arose independently in response to similar pollinator pressures. Moreover, differences in pseudopollen structure and the foods it contains, as well as the possible presence of wax, may confer greater pollinator selection or even allow pollination by a larger number of insect species—a matter that can only be resolved by intensive field work. However, the occurrence of moniliform, pseudopollen-forming hairs in Maxillaria and in species assigned to section Polystachya such as P. concreta (Jacq.) Garay & H.R. Sweet which occurs both in Africa and tropical America would indicate a degree of convergence. Consequently, it would be useful to establish whether the same insect species pollinate P. concreta on both continents.

In the past, orchidologists generally viewed all insect visitors as potential pollinators, regardless of whether or not they were actually observed transferring pollinia. Similarly, although great strides have already been made with respect to identifying pseudopollen-foraging insects and relating them to named orchid taxa, large gaps in our knowledge still remain. For example, although meliponini have been seen gathering pseudopollen from a small number of species, it is still not known for certain whether it is actually ingested or indeed how it is used. Nor have the energy requirements of producing pseudopollen yet been considered in terms of the reproductive success of orchids or whether the energy source it contains is sufficient for the needs of the insect or the colony. Meliponini seemingly also gather wax from the labella of Maxillaria spp. (Flach et al., 2004), but whether insects gather the pseudopollen of Eria spp. for the presumed waxy deposits it bears is not known. It may simply be that the latter protect the pseudopollen from desiccation or, alternatively, by reducing the wettability of the pseudopollen, aid its dispersal. If eventually it can be proven unequivocally that insects gather wax from the labella of orchids then this, in turn, would pose yet other problems since it is known that meliponini and honey bees can make their own wax, whereas euglossine and halictid bees neither make wax nor use it for nest building (D. W. Roubik, pers. comm.). Thus, much work remains to be done, and until it is possible to relate the micromorphology and nutritional value of labellar hairs to the behaviour of potential pollinators it will not be possible to understand fully the significance of pseudopollen and the evolutionary advantage it confers.

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LITERATURE CITED


