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CHARACTER ANALYSIS OF THE SEED COAT IN SPIRANTHOIDEAE AND ORCHIDOIDEAE, WITH SPECIAL REFERENCE TO THE DIURIDEAE (ORCHIDACEAE)[1]

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Previous work on seed types within Orchidaceae has demonstrated that characters associated with the seed coat may have considerable phylogenetic utility. Application of these characters has been complicated in practice by the absence of quantitative descriptors and in some instances by their apparent lack of congruity with the taxa under consideration. Using quantitative descriptors of size and shape, we have demonstrated that some of the existing seed classes do not represent well delimited, discrete entities, and we have proposed new seed classes to meet these criteria. In the spiranthoid-orchidoid complex, the characters yielding the most clearly delimited shape classes are cell number and variability and degree and stochasticity of medial cell elongation. Of lesser, but still appreciable, significance are the presence of varying types and degrees of intercellular gaps, and some, but not all, features of cell walls. Four seed classes are evident on the basis of these characters in Spiranthoideae and Orchidoideae. These seed types are briefly described, and their distribution among the taxa examined for this study is reported. It is hoped that these more strictly delimited seed classes will facilitate phylogenetic analysis in the family.

Phylogenetic relationships within the Orchidaceae have been discussed extensively in a series of recent publications by Garay (1960, 1972), Dressler (1981, 1986, 1990a, b, c, 1993), Rasmussen (1982, 1986), Burns-Balogh and Funk (1986), and Chase et al. (in press). These treatments have greatly increased our knowledge of Orchidaceae, but they are marked by controversy surrounding the delimitation of major lineages within the family, and a lack of consensus on phylogenetic relationships. This lack of resolution may be due partly to the heavy reliance these analyses placed on floral morphology, a suite of characters possibly more labile than previously thought and therefore potentially subject to high levels of homoplasy (Dodson, 1962; Chase and Pippen, 1990; Chase and Palmer, 1992). To compensate for the emphasis placed on floral morphology, there has been a renewed effort in recent years to examine other aspects such as vegetative anatomy (Stern et al., 1993), floral ontogeny (Kurzweil, 1987, 1988), cpDNA restriction sites (Chase and Palmer, 1992; Yukawa et al., 1993), DNA sequences (Cameron et al., unpublished data), and seed coat morphology (Barthlott, 1976; Barthlott and Ziegler, 1981; Ziegler, 1981; Chase and Pippen, 1988, 1990; Chase and Hills, 1992). The current study, which focuses on the delimitation of the seed coat characters within the two putatively most primitive subfamilies of monandrous orchids and evaluates the utility of these characters for the purpose of phylogenetic inference, extends this avenue of investigation.

Characters associated with the orchid seed coat have been noted by Clifford and Smith (1969), Arditti, Michaud, and Healey (1979, 1980), Healey, Michaud, and Arditti (1980), Barthlott and Ziegler (1981), Ziegler (1981), Tohda (1983, 1985, 1986), and Chase and Pippen (1988, 1990). The majority of these studies have been purely descriptive, but Arditti, Michaud, and Healey (1979, 1980), Healey, Michaud, and Arditti (1980), Stoutamire (1983), and Tohda (1985, 1986) attempted, with only limited success, to utilize simple quantitative data to distinguish between taxa of orchids (e.g., cell size, cell number per seed, cell number per seed length, and cell number per seed diameter). In addition to quantitative characters, numerous qualitative differences in the form of the testa have been described, but there has been little consistency between, and in some instances, within treatments; the descriptive terminology used to characterize these differences has often been imprecise; and no rigorous examination of specific characters occurred to see if they display discrete variation. The general applicability of seed coat characters would be enhanced if a more rigorous definition ensured that only discontinuous characters were used and that suites of characters form well-delimited entities. However, to gain a better understanding of the difficulties of achieving this goal, it may be helpful to discuss first the structure and form of the orchid seed coat in general, and then proceed to the more specific problem of character delimitation within subfamilies Spiranthoideae and Orchidoideae.

Description of orchid seed coats—Orchid seeds vary in size from ≈150 to 6,000 μm, with the majority of taxa in the 300 to 800 μm range (Figs. 1–5). Seeds can vary in shape from filiform to fusiform, clavate to ellipsoidal,

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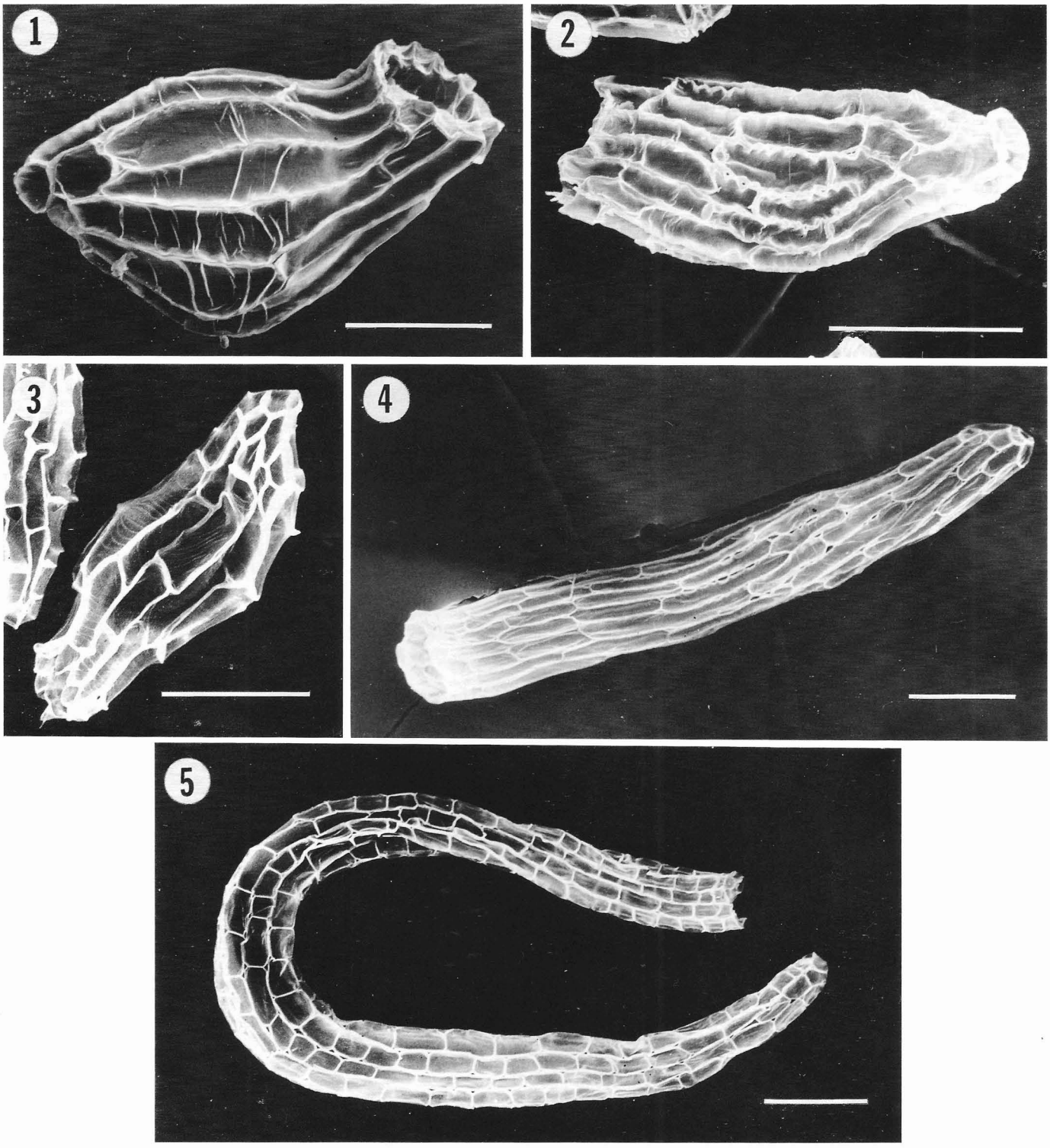


Figs. 1–5. Seeds found in Orchidoideae and Spiranthoideae. Scale bars = 100 μm. **1.** *Disa rungweensis.* **2.** *Diuris corymbosa.* **3.** *Spiranthes vernalis.* **4.** *Acianthus huegelii.* **5.** *Zeuxine elongata.*

and are sometimes prominently winged. In some orchids, seeds are covered by a hard coat (e.g., *Apostasia, Cyrtosia, Epistephium, Galeola, Neuwiedia, Palmorchis, Selenipedium,* and *Vanilla*), but the testa in most species only loosely surrounds the embryo, and is generally rather papery in texture. This seed coat is derived solely from the outer integument and is uniseriate in all but a few taxa. The number of cells that forms the testa varies greatly among the different seed classes, ranging from <20 cells in some species of *Disa,* to ≈ 600 in some

species of *Hetaeria.* Individual cells may be subquadrate, oblong, subelliptical, or irregular in outline. Their size may be uniform, or some cells may be noticeably larger or smaller within areas such as the medial region or the chalazal end. Anticlinal walls frequently appear strongly raised, whereas the outer periclinal surface is generally sunken or concave, giving the outer surface of the seed a tesselate appearance.

Numerous characters are associated with testa cell walls (Figs. 6–11). Anticlinal walls may be high (Figs. 9, 10) or low (Fig. 7), and the wall tissue tough or membranaceous. Adhesion zones between cells may show a distinct lamella, or the zone may be covered by a membranaceous film (Fig. 11). Anticlinal walls may be forced up into an arch (Fig. 8). There may be distinct gaps between the walls (Fig. 6), referred to as "intercellularen" by Ziegler (1981) when they occur at the vertices (anglicized here to "intercellular gaps"), or beading (Clifford and Smith, 1969) when repeated gaps occur along longitudinal anticlinal walls (Fig. 7). In addition, some or all of the walls may be sculptured. This sculpturing can take the form of ridges (Fig. 8), reticulations, perforations (Fig. 10), or scattered verrucosities, and can be fairly consistent within genera and subtribes. In some instances the color of the mature seed coat has also been used as a distinguishing character (Barthlott and Ziegler, 1981; Ziegler, 1981). However, seed color has rarely been mentioned by other authors and is difficult to evaluate when working with archival specimens and SEM micrographs.

At present the most comprehensive work on seed coat morphology within Orchidaceae is a dissertation completed in 1981 by Ziegler, aspects of which were published in a short article by Barthlott and Ziegler (1981). Barthlott and Ziegler's survey of the family recognizes 20 different seed types based on overall seed shape, relative elongation of some cells in the seed coat, height and sculpturing of walls, and features of wall adhesion zones between adjacent cells, including the presence of intercellular gaps and beading. Three of these seed types are subdivided further into subtypes, and those of the *Orchis*-type are noted in the Discussion. In Orchidoideae and Spiranthoideae, only filiform and fusiform seeds are present. Within these two subfamilies (excluding Neottieae, which is now regarded by Dressler [1993] as a primitive member of the subfamily Epidendroideae), Ziegler (1981) characterized three seed types: *Orchis* type with elongate medial cells, thin and high anticlinal cell walls, no intercellular gaps and occasional beading or ridge-like sculpturing (Fig. 3); *Disa-Diuris* type with elongate medial cells, thick and high anticlinal cell walls, relatively few intercellular gaps, some beading, and an absence of ridge-like sculpturing (Figs. 1, 2); and *Goodyera* type with medial cells only slightly or not longer than the apical or chalazal cells, thin and low anticlinal walls, numerous intercellular gaps, occasional beading between adjacent cells, and cell walls without ridge-like thickenings or other forms of sculpturing (Figs. 4, 5).

Ziegler's (1981) delimitation of the three seed types in Spiranthoideae and Orchidoideae is problematic for two reasons. First, many of the characters that define a particular seed type are not unique to it. Thus, elongated medial cells characterize both *Orchis* and *Disa-Diuris* types. Intercellular gaps and beading are considered diagnostic of the *Goodyera* type, yet they are also found in the *Disa-Diuris* type and even sporadically in the *Orchis* type (e.g., Fig. 3). Likewise, high anticlinal walls are characteristic of both the *Orchis* and *Disa-Diuris* types. Second, many of the differences Barthlott and Ziegler use are in fact quantitative, not qualitative, and they have not provided the reader with a metric by which these can be evaluated. As an example, seeds of *Orchis spectabilis* (*Orchis* type) and *Orthoceras strictum* (*Disa-Diuris* type) both have *Orchis* type features as defined by Ziegler (1981). According to Ziegler, there is a difference in wall hardness between these two seed types, but it was not evident in our samples, nor is hardness quantified or otherwise objectively defined. Similarly, though a given feature may not explicitly characterize more than one group, the feature may in fact be more or less evident in other groups. For example, Ziegler's *Orchis*-type *Spiranthes sinensis* (Fig. 3) has minor medial cell elongation more characteristic of his *Goodyera* type. Conversely, the *Goodyera*-type *Acianthus halleanus* shows about as much elongation as the *Disa-Diuris*-type seeds of *Microtis atrata.*

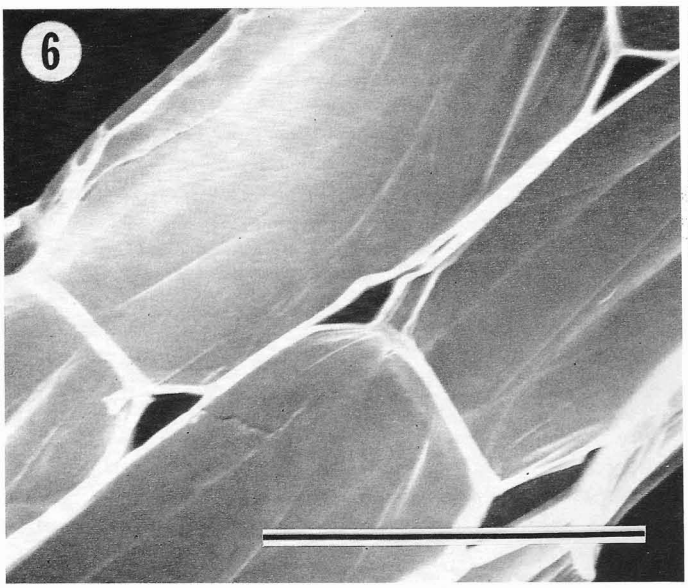
More recently, Dressler (1993) noted that Barthlott has recognized numerous intermediates between the *Diuris* and *Goodyera* type, which underscores the difficulty of characterizing seed types as they are currently defined by Barthlott and Ziegler (1981).

However, despite the need for additional work, there is some correspondence between Barthlott and Ziegler's seed types and phylogenetic entities in Orchidaceae, and it was this fact that drew our attention to the problem of character delimitation. It was our hope that by applying quantitative methods to what is essentially a quantitative problem of shape analysis, we could build on previous work with orchid seed coats. A more consistent interpretation of seed characters should assist in elucidating phylogenetic relationships of orchidoid and spiranthoid genera.

METHODS

Seeds both from freshly collected, naturally dehiscing capsules and herbarium specimens were used to determine whether dried and fresh specimens were comparable. Seeds were placed on SEM (scanning electron microscope) stubs and coated to a thickness of 50–120 nm with gold-palladium in a Hummer X sputter coater. The specimens were examined and photographed on an Amray 1700 scanning electron microscope, with a filament voltage of 30 kv, though on occasion 15 kv was also used. No differences due to voltage were observed. Magnifications of the entire seed varied from 30 to 250×, and details of the wall adhesion zones were examined at 500–1,500×.

Photographs were digitized at 200 dots per inch with a hand scanner, and measurements were taken on the digitized images using one or more of the following software packages: MeasurementTV (by Garr Updegraff), Image (by Wayne Rasband), and ImageFractal (Wayne Rasband's original program with additional programming by Bill Sheriff). Details about these programs can be found in Molvray, Kores, and Darwin (1993).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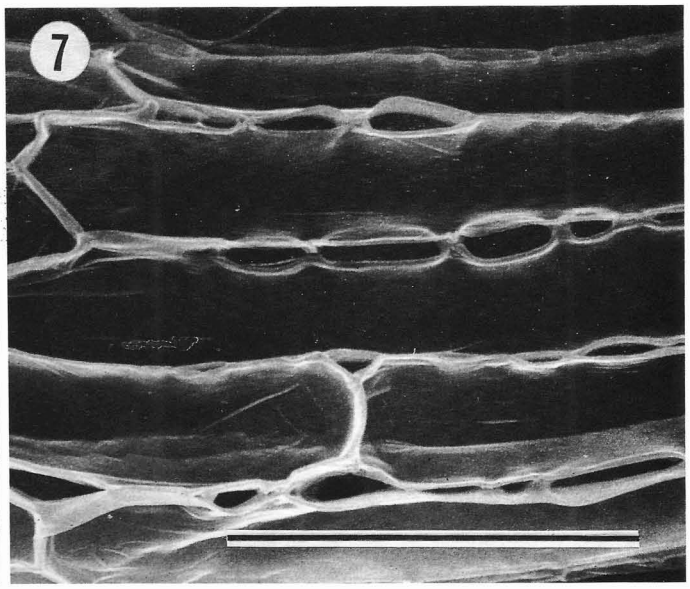
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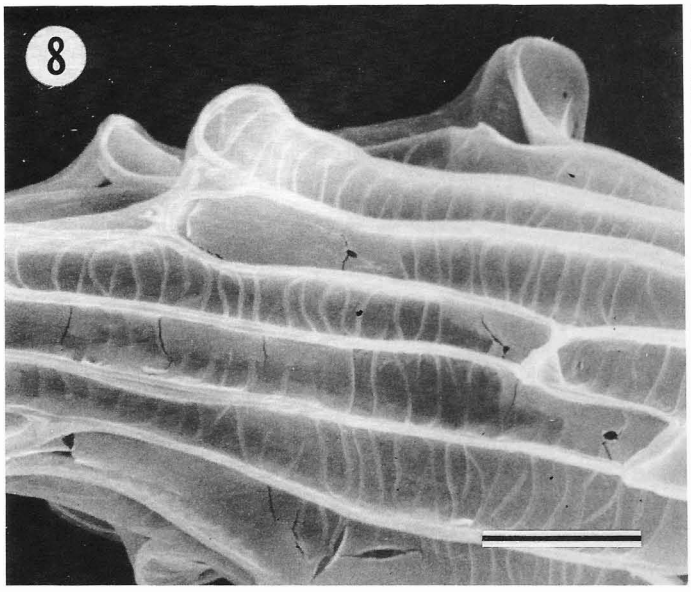
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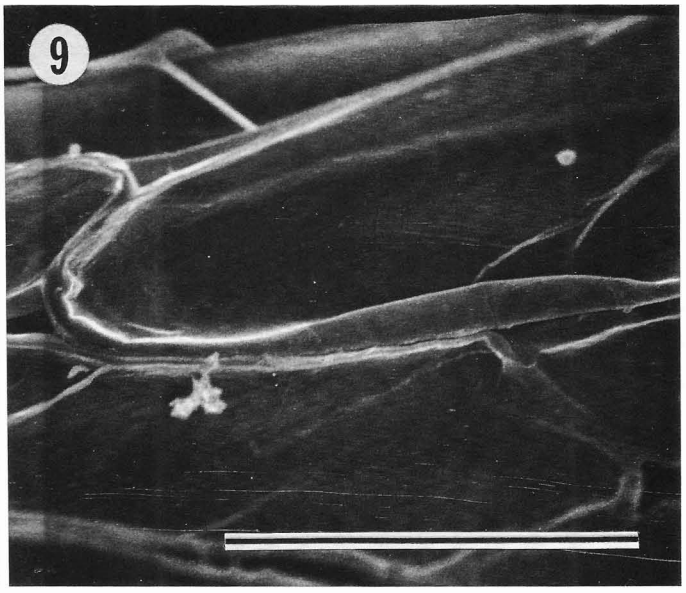
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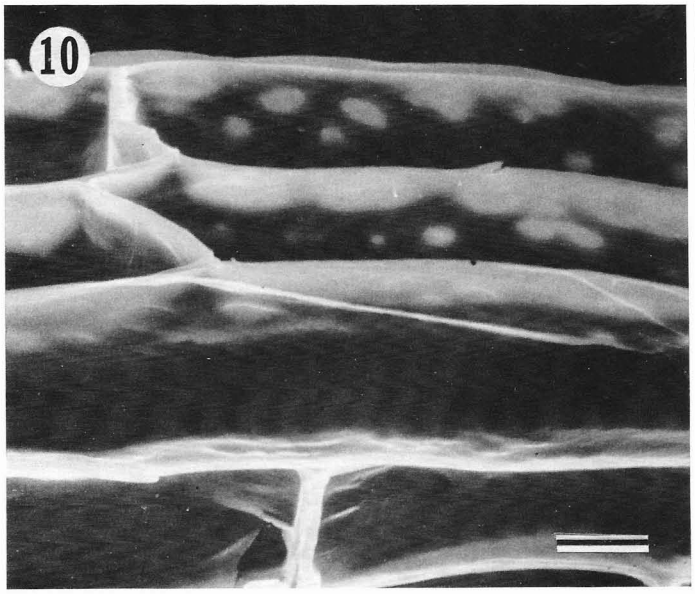
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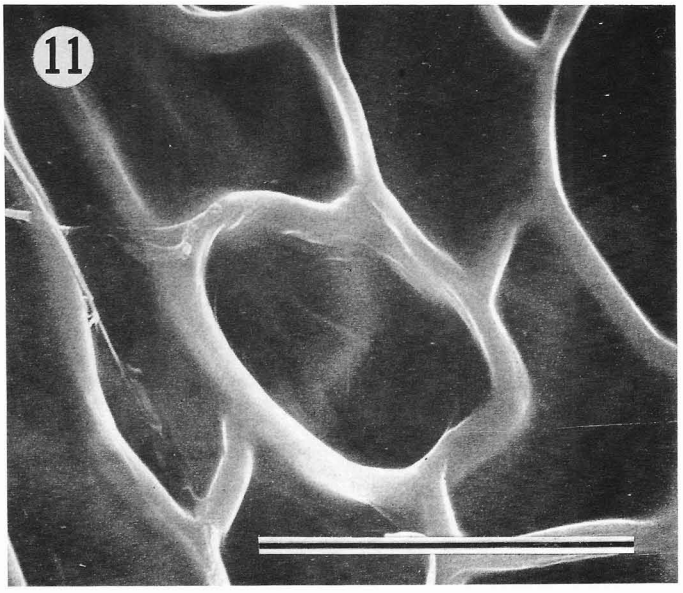
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TABLE 1. Table of characters used in delimiting orchid seed types. (See Methods for details.)

Character	Description
cellno	number lengthwise multiplied times number widthwise
5celldiff	suddenness of transition between medial and other cells
avmed/se	average medial cell length in relation to seed length
avmedcel	average medial cell length
stdevlen	standard deviation of cell length measurements
intercel	proportion of gaps at cell vertices in medial section
medianob	median of obtuse angle measures
stdevob	standard deviation of obtuse angle measures
maxminpar	measure of parallelism between opposing cell walls (see text)
stdevpar	standard deviation of cell width measurements

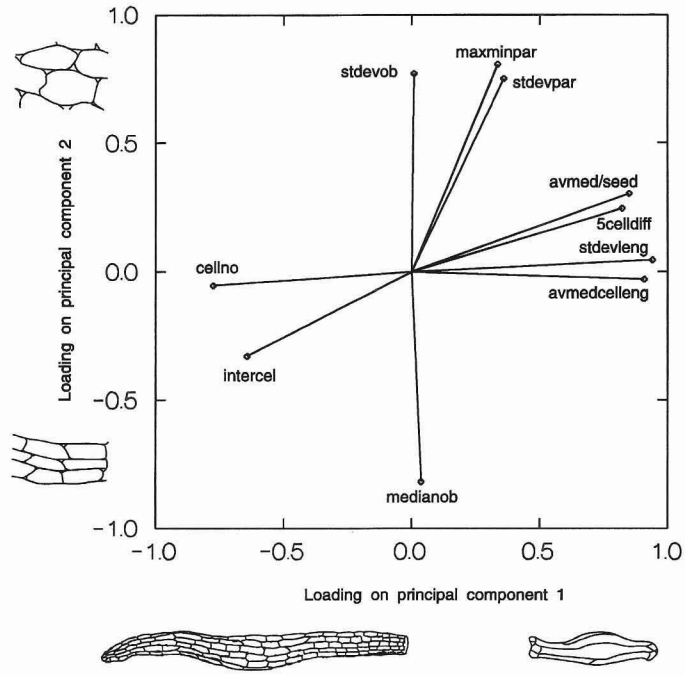


Fig. 12. Loadings of ten variables on the first two principal components.

To ensure that seeds obtained from herbarium specimens were mature and to observe the ontogeny of certain characters, seed coat development was followed in *Zeuxine strateumatica, Calopogon tuberosa, Pogonia ophioglossoides, Spiranthes vernalis,* and *Ludisia discolor.* Immature capsules from these taxa were collected at 3-d intervals, fixed in formalin-alcohol-acetic acid (FAA), dehydrated, embedded in Paraplast, sectioned, and the sections made into permanent slides. Seed coat development was followed until no further changes were evident on these slides.

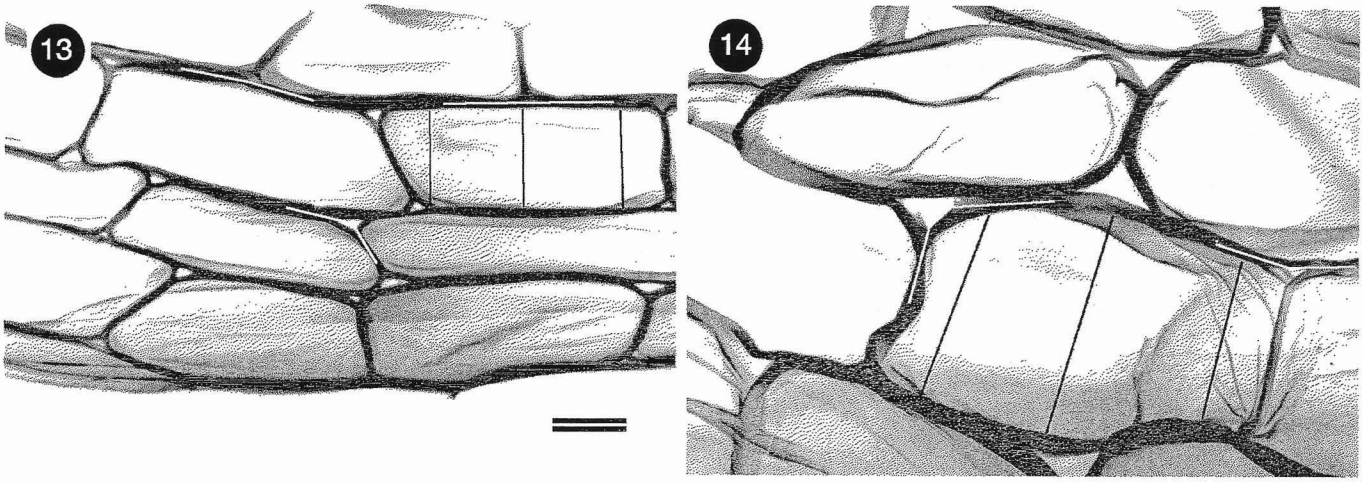
Shape differences were quantified and evaluated using both classical and contour-oriented approaches. Contour data were subjected to eigenshape analysis using EIGENS and related software written in 1981 by Schweitzer and Lohmann. This technique is described in more detail by Lohmann and Schweitzer (1990), Ray (1992), and Kores and Molvray (1993). It should be noted that eigenshape analysis requires at least one landmark to place the starting point, which then controls the orientation of the final shape. Orchid seed testa cells have no biological landmarks. To overcome this problem, a starting point was chosen for each cell to ensure that the long axis of every cell would be uniformly oriented in the final analysis. According to Schweitzer and Lohmann (1981), distribution of shapes of original specimens along eigenvectors can be studied, and the eigenshapes themselves can be used as variables in further analyses. We employed both approaches, using the sample centroid (i.e., the first eigenshape in this software) of all medial cell shapes from one seed in order to smooth out individual cell variation, and then in higher level analyses projecting these first eigenshapes onto the principal vectors of variation within subtribes or larger subsets of the data.

In addition to *x,y* coordinates of cell outlines, other data collected included seed lengths, cell lengths, cell number, and angle measures at cell vertices (Table 1). Medial cell elongation and the number of testa cells are related characters described by a group of metrics (variables 1–5 in Table 1). Cell number is an estimate obtained by multiplying the number of cells counted in a lengthwise file times the number counted in the width. It is, therefore, actually close to half the true number of testa cells. To gauge the suddenness of transition in length between medial and end cells, length measures were arranged by size, and the sum of the four largest differences between five measurements was used. Average medial cell length was used both directly and as a proportion of total seed length. A maximum of 30 cells was measured per seed if possible, but the sample size had to be reduced for some specimens (such as very small seeds or those in poor condition). Figure 12 shows the loadings on the first and second principal components of these characters. Several variables have essentially the same explanatory power, but they are used together despite their similarity to weight medial cell characters, which contribute significantly to the resolution of seed types.

Another set of characters, which measured the degree of regularity in seed coat cell organization, was used to differentiate among seeds with cells arranged in orderly, parallel files (Fig. 13) and those with more disorderly patterns (Fig. 14). The greater the maximum difference between repeated cell width measurements, the less parallel opposing walls are. Average difference between the two largest and smallest measurements was used to smooth aberrations in cell width. Angles between adjoining walls at cell vertices were also measured. Differences

←

Figs. 6–11. Details of wall structure and sculpturing. Scale bars = 50 μm except in Fig. 10. **6.** Intercellular gaps. *Zeuxine elongata.* **7.** Beading along longitudinal anticlinal walls and intercellular gaps at vertices. *Acianthus huegelii.* **8.** Ridging and high arched end walls. *Spiranthes vernalis.* **9.** High out-rolled anticlinal walls. *Porphyrostachys parviflora.* **10.** Perforations. *Megastylis montana.* Scale bar = 10 μm. **11.** Epidendroid wall structure with membranous covering. *Epistephium laxiflorum.*



Figs. 13-14. Examples of angle and cell width measurements. Scale bar = 10 μm. **13.** Seed with squarish cells. *Hetaeria yakusimensis.* **14.** Seed with rounded cells. *Gavilea lutea.*

in the degree of acuteness at cell vertices appeared to have no taxonomic significance. However, obtuse angles provided another measure of cell orderliness. Seeds with regular files of more or less rectangular cells have vertex angles close to either 90° or 180°, but those with irregular files of rounded cells have vertex angles of all possible degrees, including 180°. Measuring simply the most obtuse angle obscures this difference in cell organization. Therefore the most obtuse angle >100° but <170° was chosen, unless 180° was the only choice. Ten measurements per seed were taken for widths and angles. Figures 13, 14 show how the measurements of cell width and vertex angles were made.

In addition to raw measurement and count data, several transformations and manipulations were tried. Because the character of interest often seemed to be degree of uniformity or variation in cells, rather than an absolute quantity, standard deviations of measures and angles were calculated, and proved particularly useful in cell length and width measures.

Multivariate analyses, including principal components and canonical variates analyses, were performed on the measurements using Ntsys-pc (Rohlf, 1992), SYSTAT, and SAS.

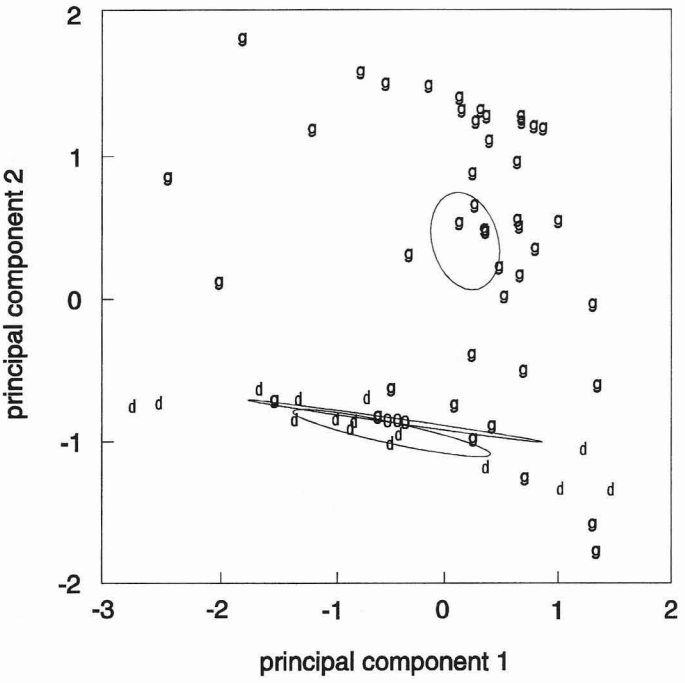


Fig. 15. Principal components analysis labelled with Ziegler's (1981) and Barthlott and Ziegler's (1981) seed classes. o = *Orchis*-type, d = *Diuris*-type, g = *Goodyera*-type. See Results for explanation. Ellipses are 95% confidence intervals on the location of the sample centroid.

RESULTS

Quantitative characters—Ten quantitative and two qualitative characters proved sufficient to differentiate four seed types (Table 1 and below). These seed classes differ from those of Ziegler (1981) and Barthlott and Ziegler (1981). It was not possible to reconstruct their groupings using either our characters or limiting the variables to the quantifiable ones Ziegler notes in his thesis (1981): seed size, medial cell length, cell number per seed, and the presence of intercellular gaps. Figure 15 is a graph of Barthlott and Ziegler's seed types distributed along the first and second principal components of variation based on those four characters. Their *Goodyera* type is partially distinct from the *Disa-Diuris* and *Orchis* types, but also overlaps entirely with the *Disa-Diuris* and *Orchis* types. Few seeds in our study included *Orchis*-type representatives, so our sample of this group is too small to be significant. However, even with that limitation, statistical analysis confirms the impression that the *Disa-Diuris* and *Orchis* types are indistinguishable in some cases. Differentiation is somewhat improved using all ten of our characters, but overlap is still great. Extensive overlap is evident even in canonical variates analysis. Barthlott and Ziegler measured seed size but did not quantify the other variables. They also use color and hardness, but do not define these qualities with the precision that would allow other workers to use them consistently. Hardness specif-

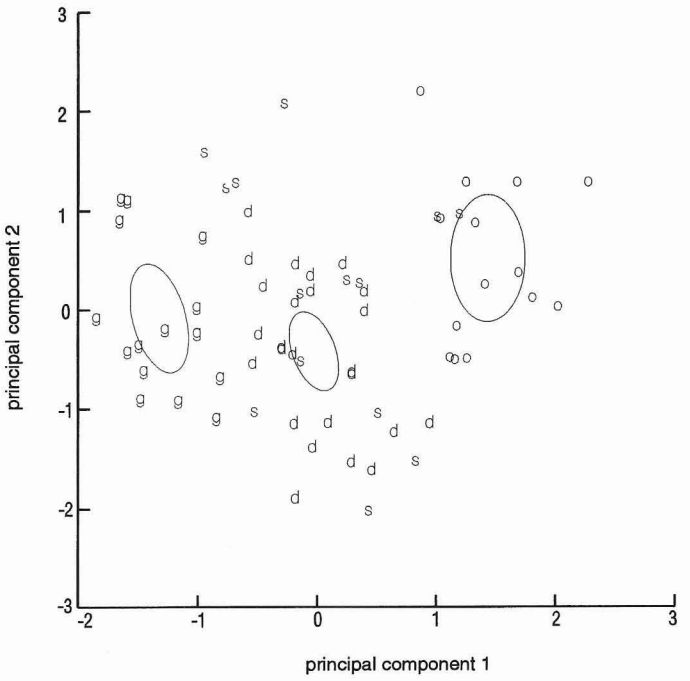


Fig. 16. Principal components analysis of orchid seed measurement data. Ellipses are 95% confidence intervals on the location of the sample centroid.

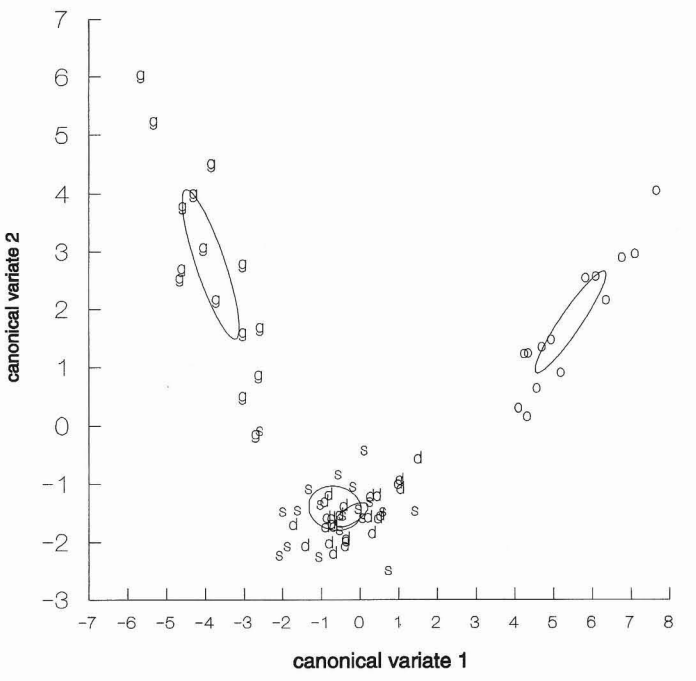


Fig. 17. Canonical variates analysis of orchid seed measurement data. Ellipses are 95% confidence intervals on the location of the sample centroid.

ically is supposed to distinguish between the *Orchis* and *Disa-Diuris* types, but no intuitively obvious difference was evident in our samples.

In addition to number of cells per seed, proportion of cells adjoining intercellular gaps, and average medial cell length, we had two other characters that measured the relationship between medial and other cells, two characters that measured whether the cells were disorderly or arranged in files, and three characters measuring the degree of variability present in major parameters. Principal components analysis of the ten measures was applied to detect unbiased groups. Figure 16 shows the three major groups found using morphometric data. The three groups are generally contiguous but overlap is minimal. Coupled with two characters that we did not quantify, ridging and high arched end walls, four seed classes are delimited: orchidoid, diuroid, spiranthoid, and goodyeroid. The spiranthoid class is not present in Barthlott and Ziegler's (1981) system, but the other classes share elements with theirs, yet are somewhat differently circumscribed. We have, therefore, retained similar but not identical names.

Orchidoid seeds have few testa cells (20 cells or fewer) and highly elongate medial cells with an abrupt transition between small, isodiametric end cells and long medial cells (Fig. 1). Except that wall hardness does not enter into this concept, the circumscription is very similar to Barthlott and Ziegler's *Orchis* type.

Diuroid seeds have somewhat elongate medial cells but no abrupt transition zone, and an intermediate number of cells (over 20 and up to 60) (Figs. 2, 4). Intercellular gaps and beading occur in this type (Figs. 6, 7) but may not be present in every seed. Some diuroid seeds approach orchidoid ones in their low cell number and the elongation of medial cells, but there are two differences that were consistent in our samples that can be used to distinguish the two classes in this case. First, chalazal cells in the diuroid type tend to be elongated rectangles, and are almost never isodiametric (Fig. 2), whereas in orchidoid seeds basal cells are commonly isodiametric, or, if there are none, the highly elongated cells of the medial section reach right to the base (Fig. 1). Second, careful averages of all medial cells yield smaller measures in diuroid seeds because they almost always have a few shorter cells present somewhere in the medial portion of the seed. The diuroid group contains elements of Barthlott and Ziegler's *Disa-Diuris* and *Goodyera* types. It also appears to be similar to the *Dactylorhiza* and some of the *Habenaria* variants of the *Orchis* type. These variants are beyond the scope of our study, but there is no reason to postulate a special relationship between these genera and those with diuroid seeds. We suspect that close scrutiny of the variants would discover fundamental divergence from the diuroid type.

Spiranthoid and diuroid seeds are morphometrically similar, though the former has greater range of variation in many characters. The spiranthoid type is primarily distinguished by qualitative characters: high anticlinal walls that often roll outward or, especially in the case of transverse anticlinals, that are forced up into a characteristic arch (Figs. 3, 8, 9). The high arched end walls are visible early in ovule development. A specific type of ridging may also occur in spiranthoid seeds and is characteristic of Spiranthinae. These ridges are all similar in appearance and position, implying that they share a similar ontogeny. Ideally, ridge and wall characters should be quantified, but the methodology is problematic and yet to be resolved. Both characters are consistent and visually strik-

TABLE 2. Genera and species examined, seed type, and specimen examined (suprageneric taxa sensu Dressler, 1993). Seed types: d = diuroid, dt = diuroid with unusually long medial cells, g = goodyeroid, o = orchidoid, s = spiranthoid.

Taxon	Seed type	Specimen examined
Orchidoideae		
Diurideae		
Acianthinae		
Acianthus aegridantennatus	d	*Franc 1801 p. p.* (P)
Acianthus amplexicaulis	d	*Blaxell 1366* (AD)
Acianthus confusus	d	*Vieillard 1317 p. p.* (P)
Acianthus cymbalarifolius	d	*Hennecart s. n.* (P)
Acianthus exsertus	d	*Kores & Molvray s. n.* (NO)
Acianthus fornicatus	d	*Kores & Molvray s. n.* (NO)
Acianthus halleanus	d	*McKee 15093* (P)
Acianthus macroglossus	d	*Schlechter 14862* (P)
Acianthus uvarius	d	*Veillon 5461*
Corybas despectans	d	*Clarke s. n.* (AD)
Corybas unguiculatus	d	*Bates 464* (AD)
Cyrtostylis reniformis	d	*Max Koeh s. n.* (NSW)
Stigmatodactylus croftianus	g	*Carr 16911* (MO)
Townsonia viridis	g	*Dunse 51718* (MO), *Rogers 5180c* (AD)
Caladeniinae		
Caladenia alata	g	*Hatch s. n.* (unvouchered)
Caladenia arenaria	d	*Bates 301* (AD)
Caladenia denticulata	d	*Went 235* (MO)
Caladenia patersonii	dt	*leg. ign.* AD #966051282
Leporella fimbriata	d	*Bates 23340* (AD)
Chloraeinae		
Bipinnula polysyka	d	*Perez 61853* (MO)
Chloraea densipapillosa	g	*Vargas 6373* (MO)
Gavilea lutea	g	*Werdermann 1231* (MO)
Megastylis montana	d-aberrant	*McPherson 5658* (MO)
Cryptostylidinae		
Cryptostylis leptochila	d	*Curnow & Dockrill 315* (AD)
Diuridinae		
Diuris brevifolia	d	*Bates s. n.* (AD)
Diuris corymbosa	d	*Went B4* (MO)
Orthoceras strictum	dt	*Gardner 2889* (MO)
Drakaeinae		
Chiloglottis cornuta	d	*Bates 3598* (AD)
Chiloglottis trapeziformis	dt	*Pescott s. n.* (AD)
Paracaleana minor	d	*Bates s. n.* (AD)
Prasophyllinae		
Microtis atrata	d	*Warnock 120* (MO)
Microtis media	d	*Bates 13276* (AD)
Microtis orbicularis	dt	*Copley et al. (NPKI 20405)* (AD)
Microtis unifolia	d	*Hatch s. n.* (unvouchered) *Sugimoto s. n.* (MO)
Prasophyllum brevilabre	d	*Bates 6615* (AD)
Prasophyllum colensoi	d	*Thompson 362* (MO)
Prasophyllum nigricans	d	*Alcock 1293* (AD)
Prasophyllum odoratum	d	*Leppitt s. n.* (AD)
Prasophyllum patens	d	*Bates 10601* (AD)
Pterostylidinae		
Pterostylis banksiae	g	*Hatch s. n.* (unvouchered)
Thelymitrinae		
Calochilus campestris	dt	*Blaylock 2392* (AD)
Calochilus paludosus	d?	*Hunt 2561* (AD)
Thelymitra longifolia	dt	*Hatch s. n.* (unvouchered)
Thelymitra pauciflora	dt	*Hatch s. n.* (unvouchered)
Diseae		
Corycinae		
Ceratandra grandiflora	o	(From Kurzweil, 1991)
Ceratandra venosa	o	(From Kurzweil, 1991)
Corycium flanagani	o	(From Kurzweil, 1991)
Corycium magnum	o	*Kunhardt s. n.* (unvouchered)
Corycium orobanchoides	o	(From Kurzweil, 1991)
Disperis fanninae	o	*Kunhardt s. n.* (unvouchered)
Pterygodium caffrum	o	(From Kurzweil, 1991)
Pteryodium leucanthum	o	(From Kurzweil, 1991)
Disinae		
Brownleea caerulea	o	*Medley Wood 5379* (MO)
Disa cornuta	o	*Dahlstrand 190* (MO)

TABLE 2. Continued.

Taxon	Seed type	Specimen examined
Disa crassicornis	o	*Kunhardt s. n.* (unvouchered)
Disa rungweensis	o	*Pawek 14274* (MO)
Satyriinae		
Satyrium longicauda	o	*Kunhardt s. n.* (unvouchered)
Orchideae		
Orchidinae		
Orchis spectabilis	o	*Sidney s. n.* (MO)
Spiranthoideae		
Cranichidaeae		
Cranichidinae		
Cranichis lehmannii	s	*Hirtz 1021* (MO)
Goodyerinae		
Anoectochilus sandwichensis	g	*Degener & Shear 3258* (MO)
Cheirostylis lepida	g	*Christiansen 2439* (MO)
Cheirostylis yunnanensis	g	*Henery 10423* (MO)
Erythrodes killipii	g	*Correa et al. 2933* (MO)
Erythrodes vescifera	g	*D'Arcy & Sytsma* (MO)
Gonatostylis bougainvillei	g	*McPherson 5706* (MO)
Goodyera procera	g	*Levine 2138* (MO)
Goodyera pubescens	g	*Ewan s. n.* (TUL)
Goodyera repens		*Ohba s. n.* (unvouchered)
Goodyera striata	g	*Ostlund 3839* (MO)
Hetaeria yakusimensis	g	*Nakauma s. n.* (TI)
Myrmechis japonica	g	*Segawa s. n.* (TI)
Platythelys vaginata	g?	(unknown)
Zeuxine boninensis	g	*Toyoshima s. n.* (TI)
Zeuxine elongata	g	*Hall et al. 42041* (MO)
Zeuxine strateumatica	d	*Satomi 21405* (TI)
Pachyplectroninae		
Pachyplectron arifolium	d-aberrant	*McPherson 1812* (MO)
Prescottinae		
Aa maderoi	s	*Hirtz 1341* (MO)
Altensteinia fimbriata	s	*Hirtz 2579* (MO)
Altensteinia virescens	s	*Hirtz 1225* (MO)
Gomphichis valida	s	Hirtz 1589 (MO)
Porphyrostachys parviflora	s	*Lopez 8060* (MO)
Prescottia stachyodes	s	*Davidse et al. 23458* (MO)
Spiranthinae		
Brachystele unilateralis	s	*Morrison 17545* (MO)
Cyclopogon elatus	s	*Proctor 17486* (MO)
Cyclopogon elliptica	s	*Hirtz 1384* (MO)
Deiregyne rhombilabia	s	*"Juan G." 2163* (MO)
Pelexia hystrantha	s	*Vavrek & Gonzalez 640* (MO)
Sarcoglottis schaffneri	s-d	*Lyonnet 618* (MO)
Spiranthes chinensis	s	*Hatch ex cult.* (unvouchered) *Togashi 1539* (TI)
Spiranthes vernalis	s	*Ewan s. n.* (TUL)
Tropideae		
Corymborkis coymbosa	epidendroid	*Chapman 6650* (MO)
Tropidia polystachya	epidendroid	*Rugel 177* (MO)
Epidendroideae		
Triphoreae		
Monophyllorchis maculata	g-aberrant	*Dressler 3409* (MO)
Psilochilus macrophyllus	g-aberrant	*Valeur 723* (MO)
Triphora trianthophora	s-aberrant	*Palmer 16436* (MO)

ing, making recognition of the seed type unambiguous within Cranichideae.

The goodyeroid type includes highly elongate, filiform seeds with numerous (more than 60, and commonly in excess of 90) small cells that show only slight elongation in the medial segment (Figs. 4, 5). Intercellular gaps are common and may be present at almost all cell vertices (Fig. 6). Beading may also occur (Fig. 7). Goodyeroid seeds are a morphological subset of Barthlott and Ziegler's (1981) *Goodyera*-type seed.

We tested how well the three morphometrically discernible groups could be identified by employing canonical variates analysis in which seeds were assigned to one of the three groups. The three groups are quite distinct (Fig. 17), and confirm the intuitive impression that these three classes are simple to identify. Characters that most

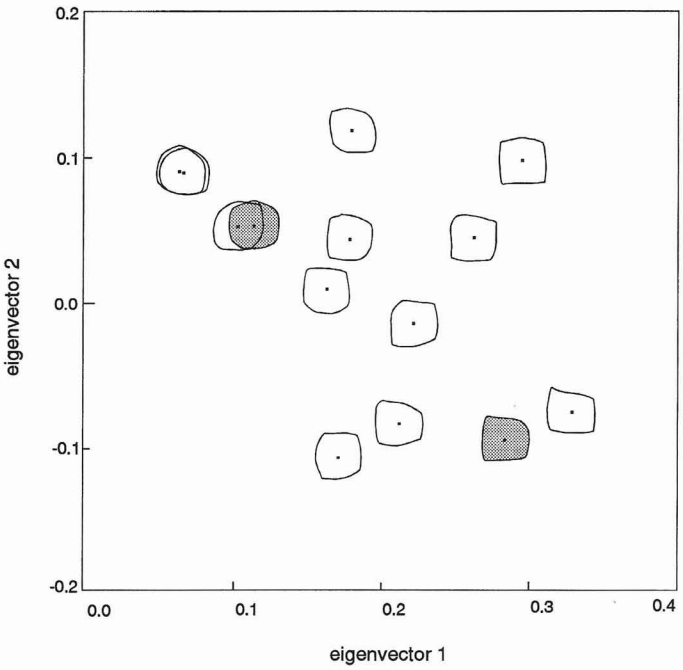


Fig. 18. Projection of "squared" eigenshapes on first two eigenvectors. Shaded shapes are members of subtribe Goodyerinae; z = seed of *Zeuxine,* g = seed of *Goodyera.*

strongly differentiate the groups are the proportion of average medial cell length to seed length (i.e., degree of medial cell elongation) and degree of parallelism between opposing walls, as measured by the uniformity of cell widths and vertex angles (i.e., the degree to which cells are arranged in relatively orderly longitudinal files).

Table 2 lists the species and specimens examined and the seed type to which they belong.

Goodyeroid seeds appeared to have two subtypes that were not well distinguished by our measurements because the differences involve degree of cell roundedness, whereas overall cell size and length–width proportions are similar. The difference is sufficiently subtle that it might be based on a false perception of pattern where there is none, a possibility we tested by quantifying this character. Eigenshape analysis was performed on cells that had been distorted to fit within a square, thus removing length and width differences, but preserving relative shapes at the corners. A clear gradient from round to square cells is evident (Fig. 18), although the variation appears to be continuous. Another interesting point is the lack of correlation between these cell shapes and phylogeny. Both *Zeuxine* and *Goodyera,* for example, have species with rounded cells as well as more rectangular cells, implying that this character may arise relatively easily and be subject to convergence at higher taxonomic levels, although it does appear to be consistent within species. Cell shape differences in goodyeroid seeds therefore have not been used in the present analysis, despite the fact that they are visually striking in some cases.

Qualitative characters—Characters associated with anticlinal wall morphology, such as whether adjacent walls are covered with a membrane (Fig. 11) or whether the middle lamella is sunken (Figs. 6–10), seem to be both conservative and informative at tribal and higher taxonomic levels. It was, for instance, the dissimilarity in wall structure between Neottieae and other Orchidoideae that first alerted us to the fact that it was misplaced and should not be part of our analysis. Neottieae have membrane-covered walls similar to Vanilleae, and the tribe's placement in Epidendroideae has been confirmed by *rbcL* data (Chase et al., in press; Cameron et al., unpublished data).

Morphology associated with anticlinal walls was uniform in the three groups of interest to us, Spiranthoideae, Orchideae, and Diurideae. All have longitudinal anticlinal walls merely appressed with no visible middle lamella and therefore with a sunken line between the walls. Transverse anticlinal walls are generally covered with a slight membrane (Figs. 6–10). (See also Ziegler, 1981). The wall-to-wall adhesion zone was used to differentiate these taxa from others.

Wall height is another character that may prove useful if it can be quantified. Differences in height are evident early in development and appear to be consistent within some groups. However, without quantitative data, it is difficult to discern whether variation is discrete. It was beyond the scope of our work to explore techniques, such as plastic embedding of seeds and TEM (transmission electron microscope) cross sections, that would allow wall height to be measured, hence we have not used this character despite its potential value.

Characters associated with wall sculpturing, as opposed to the above characters of the wall itself, must be applied with caution. In specific groups these characters appear to be homologous and supportive of relationship. Characters such as transverse ridges, which are not uncommon in Orchidaceae, may not be reliable at subtribal and higher levels. Ridges appear in all Spiranthinae we studied.

Only one other instance of notable wall sculpturing occurred in our sample: round translucent areas visible at high magnification in the anticlinal walls of *Megastylis montana,* subtribe Chloraeinae (Fig. 10). The subtribe as a whole is anomalous, and this particular character is otherwise reported only from two species of Cymbideae and one of Gastrodieae, both in the Epidendroideae (Ziegler, 1981). Morphometrically, *Megastylis* is most similar to *Pachyplectron*: both have similar, unusually large, cells in an otherwise diuroid seed such that the size of the whole seed approaches goodyeroid dimensions, but the number of cells does not.

One character mentioned both by Ziegler (1981) and Barthlott and Ziegler (1981) that we have avoided is wall hardness. Except for extremely hard seeds, this character is not only difficult to identify, it is difficult to apply consistently. Noticeably hard seeds are found in several orchid genera, including for instance *Selenipedium, Vanilla,* and *Disa,* that apparently have no direct relationship to each other. Evidence suggests that this character has arisen repeatedly in response to selective pressures such as the need to survive a digestive tract (*Vanilla*) or dry periods (*Disa*). Further study may find an efficient way of quantifying hardness, and the character's value can then be studied more rigorously.

Color of the mature, dry seed was also used by Barth-

lott and Ziegler, but we feel that the influence of undefined factors, including storage, is too great to allow us to use color with confidence.

DISCUSSION

The recognition of four seed types within Orchidoideae and Spiranthoideae, agrees with the core concept set forth by Ziegler (1981) and Barthlott and Ziegler (1981) in their work on seed coat morphology, but there is considerable difference in the delimitation of our orchidoid, goodyeroid, spiranthoid and diuroid seed types, and the *Orchis, Goodyera,* and *Disa-Diuris* seed types suggested in previous publications. Some genera (e.g., *Orchis, Goodyera,* or *Diuris*) are treated similarly in both systems, but we feel that there is considerably less overlap among classes in the delimitation presented here.

Under our classification the orchidoid type is found in all members of Orchideae and Diseae examined. This distribution contrasts with Barthlott and Ziegler's treatment, where *Orchis*-type seed is restricted to the tribe Orchideae and the subtribe Huttonaeinae within Diseae, while the other taxa within Diseae are characterized as having *Disa-Diuris*-type seeds.

Our concept of the goodyeroid seed also differs substantially from Ziegler's (1981) *Goodyera* type. Under their system all members of the subfamily Spiranthoideae (sensu Dressler, 1993) and some members of Diurideae have the *Goodyera* type. In our classification there is a distinction between goodyeroid and spiranthoid seeds. The goodyeroid type is found in all members of the subtribe Goodyerinae examined, and in some Diurideae [*Stigmatodactylus* and *Townsonia* (Acianthinae), *Chloraea* and *Gavilea* (Chloraeinae), *Pterostylis banksiae,* (Pterostylidinae), and *Caladenia alata* (Caladeninae)]. Other members of Diurideae we examined had diuroid seeds.

The spiranthoid type, with cell number, arrangement, and size similar to, though more variable than, the diuroid type, appears in Prescottiinae and Spiranthinae. Of Cranichidinae, we sampled only *Cranichis,* which also has a spiranthoid seed.

Also of interest is the genus *Triphora* (subfamily Epidendroideae: tribe Triphoreae). This genus has seeds with ridge-like sculpturing and slightly arched end walls, typical spiranthoid attributes, but its seed size and cell number are more representative of goodyeroid seeds. Other characteristics of this genus, such as the structure of the anther and pollen seems consistent with the subfamily Epidendroideae, and its atypical seed type may be convergent rather than indicative of a close relationship between Triphoreae and representatives of Spiranthoideae.

Our diuroid type includes many of the same genera Ziegler (1981) and Barthlott and Ziegler (1981) originally assigned to their *Disa-Diuris* type, exclusive of the tribe Diseae, which we regard as a separate seed type. This circumscription is similar to Barthlott's more recently established *Disa-* and *Diuris*-type seed classes, first reported by Dressler (1993). Unlike other seed types that show some congruence with previously recognized lineages within the family, the distribution of the diuroid seed class is somewhat problematic. Diuroid seeds are only found within Diurideae, but their occurrence is sporadic. All genera from Drakaeinae and Prasophyllinae have diuroid seeds, as well as some members of Acianthinae, Chloraeinae, Caladeniinae, and Diuridinae, but taxa with goodyeroid seeds are also present within many of these subtribes. In *Caladenia* there are even goodyeroid and diuroid seeds in different species. If one assumes the diuroid seed is homologous throughout its distribution, the clear implication of this patchy distribution within Diurideae is that the tribe is polyphyletic, and that affinities must be determined on a genus-by-genus basis. Our seed characters suggest a closer relationship between Diurideae and Spiranthoideae than the other members of Orchidoideae as suggested by Dressler (1993) or Epidendroideae as suggested by Burns-Balogh and Funk (1986).

Another critical difference between this study and the previous accounts by Barthlott (1976), Barthlott and Ziegler (1981), and Dressler's work (1993) based on those accounts is the lack of congruence with Dressler's proposed phylogeny for the basal clades of monandrous orchids. Under our system of classification at least three distinct types are present within Spiranthoideae (sensu Dressler) whereas Barthlott and Ziegler, and Dressler's work based on theirs, recognize one type. Similarly, our two seed types within Diurideae differ in circumscription from Barthlott and Ziegler's, and therefore also from Dressler's. Based on our work, seed coat morphology would appear to provide little or no support for either a monophyletic Orchidoideae or Spiranthoideae (sensu Dressler, 1993). Molvray and Kores (unpublished data) will explore the phylogenetic implications of orchidoid and spiranthoid seed characters.

As previous workers have also shown, there are a number of conservative and phylogenetically informative characters in the orchid seed coat. What we have attempted to show here is that quantitative methods can assist the process of character choice by confirming the degree of character state consistency. Characters, as the determinant of subsequent taxon delimitation, are the foundation of all phylogenetic work, and phylogenies can only be as good as the characters upon which they are based.

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