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VARIATION IN THE ORGANIZATION OF THE STOMATAL COMPLEX IN THE LEAF EPIDERMIS OF MONOCOTYLEDONS AND ITS BEARING ON THEIR PHYLOGENY[1]

G. LEDYARD STEBBINS AND GURDEV S. KHUSH

ABSTRACT

STEBBINS, G. L., and G. S. KHUSH. (U. California, Davis.) Variation in the organization of the stomatal complex in the leaf epidermis of monocotyledons and its bearing on their phylogeny. Amer. Jour. Bot. 48(1): 51–59. Illus. 1961.—Using macerated pieces of epidermal tissue from living plants and herbarium specimens, stomatal complexes of 192 species belonging to 49 families of monocotyledons were studied. Four categories are recognized, 2 with 4 or more subsidiary cells surrounding the guard cells, 1 with 2 subsidiaries, and 1 with none. Development of the 2-subsidiary type, studied in acetocarmine preparations of *Juncus effusus* and *Sagittaria montevidensis*, resembles that in Gramineae previously described. No correlation was found between type of stomatal complex and either leaf shape or type of xylem vessel, but some correlation exists between this character and type of seed germination, vascular anatomy of seedling, growth habit of mature plant, and geographic distribution. Types with 4 or more subsidiaries are most commonly phanerophytes with tropical distribution, many vascular bundles in the cotyledon, and hypogeal germination. Complexes with 2 subsidiaries occur in many families of a diverse nature, but the most primitive plants with this type are hydrophytes or helophytes with tropical or temperate distribution, 1 vascular bundle in the cotyledon, and epigeal germination. Stomatal complexes without subsidiaries are almost confined to the Liliales and their more specialized derivatives. These plants are predominantly geophytes with temperate or tropical distribution, 2 bundles in the cotyledon, and epigeal germination. Reasons are advanced for suggesting that the type with many subsidiaries is the most primitive and the other 2 types have been derived from it independently by reduction of the number of subsidiary cells.

ALTHOUGH most systems of classification of angiosperms emphasize chiefly floral morphology, information from other botanical disciplines is becoming increasingly useful. Thus, pioneer works of Bailey and his associates on the vascular anatomy of dicotyledons and of Cheadle and his students on the vascular anatomy of monocotyledons have made significant contributions to our understanding of the phylogeny of angiosperms. The review by Constance (1955) of these and other studies has justly emphasized the importance of basing hypotheses about phylogeny on evidence derived from as many characteristics as possible.

The stomatal cell complex in angiosperms was first studied by Strasburger (1866) and particularly by Vesque (1889) who recognized 4 broad categories based on the presence and arrangement of accessory cells as well as their mode of development. Vesque, however, studied only dicotyledons. More recently Florin (1931) has made a detailed study of stomatal complexes in gymnosperms, in which he presented some data on development, while Dehnel (1957) has amplified our knowledge about the dicotyledons, presenting a full review of the literature to date. There exists, however, no comparable study of stomatal complexes in monocotyledons.

The use of information about stomatal complexes

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in studying phylogeny should be particularly enlightening in view of our detailed knowledge regarding their development. Our observations are at variance with those of Tognini (1897) who states that several modes of development of stomata may occur in different organs of the same plant. In all the material studied by us, the developmental sequences are precisely the same for a particular complex. Thus in as different genera as *Juncus* and *Sagittaria,* which fall into the same category as regards their stomatal complex, the differentiation of the stomatal complex takes place in similar sequences. Similarly, developmental modes are constant, even as to minute details, from organ to organ within the same plant. Thus, in barley the present authors followed the same developmental sequences in as different organs as coleoptile, leaf and awn of the lemma.

Another point which makes the study of the stomatal complexes a desirable tool in taxonomic research is that genera and even families show great constancy for their possession of a particular complex, yet there is considerable variability from one higher taxon to another. Thus at the level where classical methods of cytology and genetics cannot be applied, this study of stomatal complexes should help us to understand true evolutionary relationships of monocotyledons.

MATERIALS AND METHODS.—Stomatal complexes were examined in 192 species belonging to 49 of the 68 families of monocotyledons recognized by Hutchinson. In addition, more than 50 genera of Gramineae have been examined by the senior author, either directly or through published figures. The arrangement of families is according to Hutchinson (1934), and his system is followed strictly throughout the descriptive section. Representatives of the other 18 families are either aquatic and thus lack stomata or are aphyllous and hence unsuitable for investigation. Investigations were made on either dried material from herbarium specimens or preserved material from living plants. Herbarium specimens were obtained from the herbarium of the University of California, Berkeley, and from the Botany Department herbarium, University of California, Davis. Leaves from living plants were fixed in 3:1 alcohol–acetic acid and stored in 70% alcohol. All of the numerous specimens taken from Botanical Garden, U. C., Berkeley, and Strybing Arboretum, Golden Gate Park, San Francisco, were already labelled and the rest were determined with the help of appropriate manuals. Bailey's (1949) manual was used to determine the identity of the cultivated plants. The generic identity of all specimens was verified by the senior author. A table has been prepared listing the names and source of material of all of the 192 species studied, but it proved to be too long to include in the present paper. Mimeographed copies of it will be sent by the senior author upon request.

There is no difficulty in peeling off the epidermis in green material. Dried material was boiled in water for 10–15 min., and, in most cases, with a little manipulation the epidermis can be easily peeled off. In some species like *Astelia* and palms, treatment with 5% NaOH at oven temperature of 60°F. for 2 hr. was very helpful. Small pieces of epidermis separated from the rest of the mesophyll tissue were mounted and stained with acetocarmine. Observations can be made immediately after mounting, but after 10–12 hr. the remains of the nuclei stain more clearly. Measurements of guard cells and epidermal cells were made with an ocular micrometer. In each case, 10 cells were measured at random and the mean length calculated. In some species a few stomatal rows are interspersed with rows of epidermal cells which lack stomata. Length of epidermal cells in such rows is longer than the length of epidermal cells found between stomatal rows. In that case 5 cells from each region were measured. In species where leaves are absent as in some species of *Scirpus, Juncus,* and *Leptocarpus,* preparations were made from peels of stems. Camera lucida drawings were made 10–12 hr. after mounting and are reproduced at a magnification of ×240.

For studying development, the meristematic bases of developing leaves were fixed in a mixture of 3 parts absolute alcohol–1 part glacial acetic acid, mounted whole in a solution of acetocarmine diluted to ½ strength with 50% acetic acid, and heated to boiling for a short time. The mounts, sealed in a paraffin-beeswax mixture and kept in a refrigerator, lasted for several weeks, and the details of the epidermal cells could be seen clearly enough by focussing on the upper epidermis with the oil immersion lens. Camera lucida drawings are reproduced in fig. 1–4 at a magnification of ×535.

DESCRIPTION OF RESULTS.—The various types of stomatal complexes in monocotyledons can be divided into 4 broad categories. (1) Two guard cells and 4–6 subsidiary cells around the guard cells in all 4 directions (fig. 13, 21, 22); (2) Two guard cells and 4–6 subsidiary cells 2 of which are roundish and smaller than the rest and at the ends of guard cells, the rest being lateral to guard cells (fig. 10, 12, 16, 24); (3) Two guard cells and 2 subsidiary cells lateral to the guard cells. This, the most common type of stomatal complex, is distributed over many families (fig. 5–9, 11, 17, 23); (4) Two guard cells without any subsidiary cells; the second most common type of stomatal complex (fig. 18, 19, 20).

The first type is found in the families Commelinaceae, Araceae, Bromeliaceae, Musaceae, Strelitziaceae, Zingiberaceae and Cannaceae (table 3). *Tradescantia, Rhoeo* and *Zebrina* of Commelinaceae have 4 subsidiary cells, 1 on each of the 4 sides of paired guard cells and forming a beautiful square (Stebbins and Jain, 1960). *Commelina,* of the same family, has 6, 2 additional being present on the sides (fig. 21). In Araceae, 2 genera, *Scindapsis*

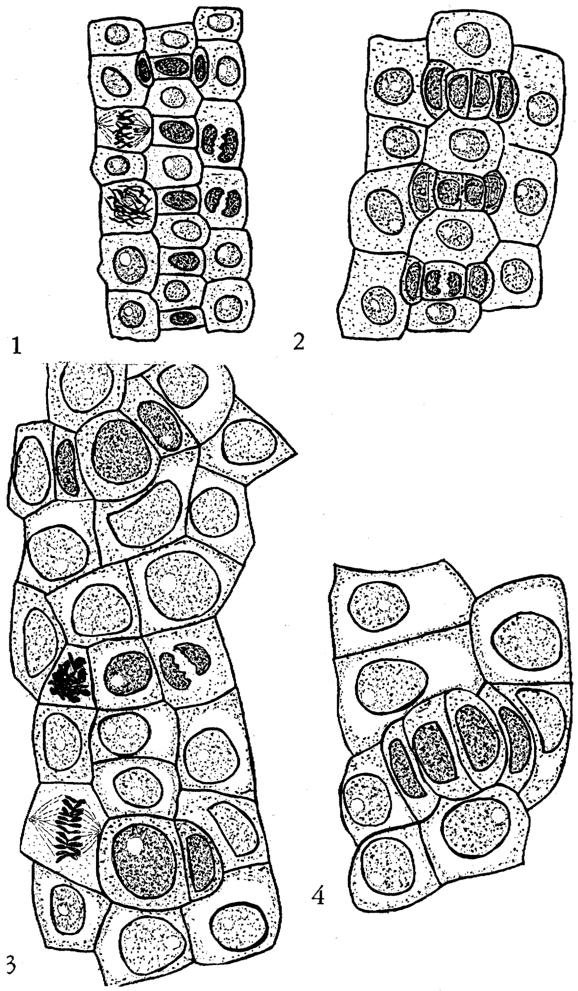

Fig. 1–4. Stomatal development.—Fig. 1. *Juncus effusus* (Juncaceae): starting from the bottom, one can see that guard mother cell with darkly staining nucleus has been formed as the result of unequal division of ordinary epidermal cell. In the center 2 guard mother cells are acquiring subsidiaries through divisions in the adjoining epidermal cells. At the top 2 small subsidiary cells with darker staining nuclei have been formed on both sides of guard mother cell, as the result of asymmetrical divisions.—Fig. 2. Later stages of development in *Juncus effusus.* At the bottom, the guard mother cell is in the process of division. At the top, the guard mother cell has completed division.—Fig. 3. Developmental stages in *Sagittaria montevidensis* (Alismataceae). At the bottom, a subsidiary cell has already formed on one side of guard mother cell, and the epidermal cell on the other side is in the process of division. In the center, epidermal cells on both sides of the guard mother cell are going through divisions which will eventually produce subsidiary cells. At the top, subsidiary cells have been produced on both sides of the guard mother cell.—Fig. 4. *Sagittaria montevidensis:* guard mother cell has divided into 2 paired guard cells.

and *Dieffenbachia,* have 6–7 subsidiary cells grouped around the paired guard cells in a haphazard manner (fig. 22), while other genera examined have 2–4. In Bromeliaceae, all the 5 genera examined, *Bilbergia, Puya, Dyckia, Pitcairnia* and *Vriesia,* have more than 4 subsidiary cells, but it is very difficult to determine the exact number because of extreme thickening of cell walls. Mention may be made of the various members of the order Zingiberales, where the subsidiary cells are not very well differentiated from the rest of the epidermal cells. For example *Musa* (fig. 14) of the family Musaceae and *Tapeinochilus* (fig. 15) of the Zingiberaceae belong to this category. *Strelitzia nicolei* of Strelitziaceae possesses many but not very well-defined subsidiary cells (fig. 13). The second type is found in the families Palmae, Pandanaceae and Cyclanthaceae. The appearance of the complex in these 3 families is very similar (fig. 10, 12, 16, 24–26) and supports Hutchinson's viewpoint of placing these 3 orders near to each other in the evolutionary scale. Within the Palmae there is some variation in the number of subsidiary cells present. Thus *Caryota* and *Calamus* have 2 subsidiary cells (fig. 17; table 3), and *Phytelephas* aff. *microcarpa* has 6 very well-defined subsidiary cells (fig. 16). Other genera examined, *Phoenix, Washingtonia, Cocos* and *Chamaedorea* all have 4 subsidiary cells, 2 lateral and 2 small roundish ones at the ends (fig. 26). In the seedling leaf of *Phoenix* the number is 6 (fig. 25). Of 3 genera of Pandanaceae, *Pandanus* and *Freycinetia* have 4 subsidiary cells each like palms (fig. 24), and *Sararanga* has only 2. Both

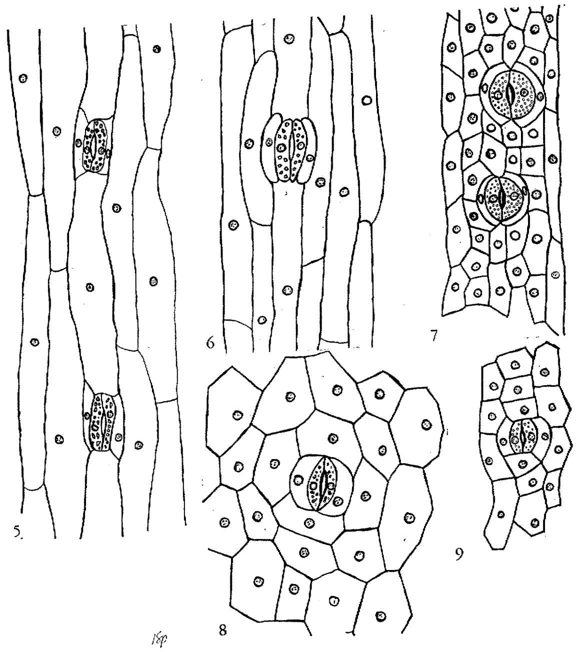

Fig. 5–9. Mature stomatal complexes with 2 subsidiary cells.—Fig. 5. *Juncus effusus* (Juncaceae).—Fig. 6. *Anigosanthus flavidus* (Haemodoraceae).—Fig. 7. *Xanthorrhoea preissii* (Xanthorrhoeaceae).—Fig. 8. *Pontederia cordata* (Pontederiaceae).—Fig. 9. *Astelia pumila* (Liliaceae).

TABLE 1. *Families with no subsidiary cells as the predominant condition. Genera deviating from the modal condition are listed separately under their family, the number of subsidiaries is given in parentheses, followed by the number of species studied. Orders which appear in 2 tables are marked with an asterisk (*).*

		NUMBER STUDIED	
ORDER	FAMILY	Genera	Species
LILIALES*	Liliaceae	34	40
	Astelia (2)-5 spp.		
	Tecophilaceae	3	3
	Trilliaceae	1	1
	Smilacaceae	1	1
ALSTROEMERIALES	Alstroemeriaceae	1	1
	Philesiaceae	1	1
ARALES*	Lemnaceae	1	1
AMARYLLIDALES	Amaryllidaceae	10	10
	Iridaceae	4	4
DIOSCOREALES	Dioscoreaeceae	2	2
AGAVALES	Xanthorrhoeaceae	6	8
	Xanthorrhoea (2)-2 spp.		
	Agavaceae	11	15
	Doryanthes (2+)-1 sp.		
HAEMODORALES*	Apostasiaceae	1	1
	Taccaceae	1	2
BURMANNIALES	Burmanniaceae	1	1
ORCHIDALES	Orchidaceae	4	4

genera of Cyclanthaceae studied have 4 subsidiary cells.

Worth mentioning is *Scheuchzeria palustris* (fig. 10), belonging to the unrelated family Scheuchzeriaceae, which has a similar pattern, with 4 subsidiary cells but the end cells are not very well defined, as in Palmae and Pandanaceae.

The third type is spread consistently over 24 families out of 50 examined (table 2) and is present in occasional members of other families, for example, the genus *Astelia* (fig. 9) of Liliaceae, *Xanthorrhoea* (fig. 7) of Xanthorrhoeaceae, *Doryanthes* (fig. 23) of Agavaceae and *Sararanga* of Pandanaceae. It was found in all species studied of the orders Butomales, Alismatales (except *Scheuchzeria*), Juncales, Graminales, Cyperales, Typhales, Haemodorales (except families Apostasiaceae and Taccaceae), Juncaginales, Potamogetonales, Aponagetonales, Eriocaulales, Xyridales, familes Flagellariaceae and Mayacaceae of Commelinales, Marantaceae and Lowiaceae of Zingiberales, and Pontedericeae of Liliales.

The fourth type is limited only to the orders closely related to Liliales (table 1, 2) and seems to have arisen only once in evolutionary history. It occurs in Liliales (except family Pontederiaceae and genus *Astelia* of Liliaceae), Dioscoreales, Alstroemeriales, Amaryllidales, Iridales, Agavales (except genera *Doryanthes* and *Xanthorrhoea* of families Agavaceae and Xanthorrhoeaceae respectively). Burmanniales and Orchidales are characterized by this complex. In addition it is present in the families Apostasiaceae and Taccaceae of Haemodorales and Lemnaceae of Arales.

It would be inappropriate to discuss the results without giving a brief description of the developmental patterns of the complexes discussed above. Bünning and Biegert (1953) followed by Stebbins and Jain (1960) have investigated thoroughly the developmental sequences in *Allium*. This genus is a good representative of type 4, which lacks subsidiary cells. The differentiation of the guard mother cell from an ordinary epidermal cell occurs by an asymmetrical division. Before division, the nucleus migrates to the distal end of the meristematic cell. The cytoplasm at the distal end is much denser than at the proximal end. As the result of asymmetrical division, the smaller cell, which has a higher density of cytoplasm, divides once again to give rise to 2 paired guard cells. Bünning and Biegert (1953) found that by centrifuging, that is, by changing the position of nuclei in the cells, differentiation could be inhibited. Thus differentiation is strictly epigenetic depending upon the organization of the cytoplasm and the position of the nucleus prior to division.

Developmental sequences of the third type, with 2 subsidiary cells, were studied by Campbell (1881) in corn, Porterfield (1937) in bamboo, Flint and Moreland (1946) in sugar cane, by Kaufman (1957) in rice, and recently verified in other tribes of Gramineae by Stebbins and Shah (1961).

The present authors investigated the development of this type of complex in *Juncus* and *Sagittaria.* Initial differentiation of the guard mother cell is as in *Allium*, except that it occurs at an earlier stage, when all of cells are in the meristematic con-

TABLE 2. *Families with 2 subsidiary cells as the predominant condition*

		NUMBER STUDIED	
ORDER	FAMILY	Genera	Species
BUTOMALES	Butomaceae	2	2
ALISMATALES*	Alismataceae	2	2
JUNCAGINALES	Juncaginaceae	1	1
	Liliaceae	1	1
POTAMOGETONALES	Potamogetonaceae	1	1
COMMELINALES*	Flagellariaceae	2	2
	Mayacaceae	1	1
XYRIDALES	Xyridaceae	1	1
	Rapataeaceae	1	1
ERIOCAULALES	Eriocaulaceae	3	3
ZINGIBERALES*	Lowiaceae	1	1
	Marantaceae	2	2
LILIALES*	Pontederiaceae	2	2
TYPHALES	Sparganiaceae	1	3
	Typhaceae	1	1
HAEMODORALES*	Haemodoraceae	10	10
	Hypoxidaceae	2	2
	Velloziaceae	1	1
	Philydraceae	1	1
JUNCALES	Juncaceae	2	3
	Centrolepidaceae	2	2
	Restionaceae	3	3
CYPERALES	Cyperaceae	4	9
GRAMINALES	Gramineae	50	50

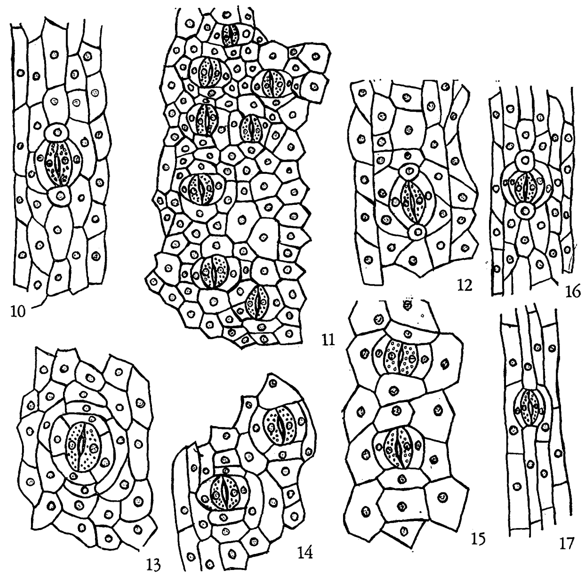

Fig. 10–17. Mature complexes with variable numbers of subsidiary cells.—Fig. 10. *Scheuchzeria palustris* (Scheuchzeriaceae). Mature complex with 4 subsidiary cells. Cells at the ends are smaller and roundish.—Fig. 11. *Typha domingensis* (Typhaceae): showing mature stomatal complexes. In this family, along with related family Sparaniaceae, the number of subsidiary cells is variable ranging from 0 to 4, with 2 being the most common.—Fig. 12. *Pandanus haerbachii* (Pandanaceae): mature stomatal complex with 4 subsidiary cells.—Fig. 13. *Strelitzia nicolei* (Strelitziaceae): mature complex with many subsidiary cells; exact number is difficult to tell unless developmental stages are studied.—Fig. 14. *Musa paradisiaca* (Musaceae).—Fig. 15. *Tapeinochilus queenslandiae* (Zingiberaceae). In fig. 14, 15 the number of subsidiary cells is difficult to tell without developmental stages, but it is always more than 2.—Fig. 16. *Phytelephas* aff. *microcarpa* (Palmae): mature complex with 6 subsidiary cells.—Fig. 17. *Calamus oxleyanus* (Palmae): mature complex with 2 subsidiary cells.

dition and cells are much smaller as compared to those of differentiated cells of *Allium.* As the result of asymmetrical division, 2 cells are produced, 1 with denser cytoplasm and darkly staining nucleus and smaller in size than the other (fig. 1). This forms the guard mother cell. The other sister cell forms an undifferentiated epidermal cell. Later stages, however, are different from those of *Allium.* Instead of dividing itself, the guard mother cell induces asymmetrical divisions in epidermal cells, situated adjacent to it (fig. 1), with the result that a small cell with densely staining nucleus is formed on each of the proximal sides of the guard mother cell. These form the subsidiary cells of the mature complex. After this triad has formed, the guard mother cell divides to give rise to 2 paired guard cells (fig. 2). In *Sagittaria,* developmental sequences are exactly the same except that cells in various stages are distributed in an irregular manner (fig. 3, 4) as compared to *Juncus,* in which cells in various stages of development are arranged in rows, the youngest near the base and oldest towards the top.

In *Tradescantia,* studied by Strasburger (1866) and Campbell (1881), and in *Zebrina* and *Commelina,* studied by Stebbins and Jain (1960), which belong to the first type, the processes are similar to the second type except that the guard mother cell induces 4–6 mitoses in neighboring epidermal cells before it divides. Although development has not been studied in palms, Pandanales and Cyclanthales, it is probably similar to that of *Tradescantia.* In the later paragraphs, therefore, types 1 and 2 will be considered together, as possessing many subsidiaries. Several lines of evidence which indicate that the guard mother cell actually does exert an inductive influence leading to subsidiary cell formation are presented by Stebbins and Shah (1961).

Early in this study, the various genera were found to differ widely in the relative size of the guard cells compared to the surrounding epidermal cells. In some types, such as most grasses, sedges, and *Allium,* the epidermal cells are much larger than the guard cells; in others, they are of about the same size or even smaller. Since there appeared to be a relationship between these differences and the type of stomatal complex, the ratio in length of ordinary epidermal cells to guard cells (E/GC) was computed for all of the species studied. From these data the association of many subsidiary cells with a low epidermal/guard cell ratio is clearly evident, while species with 2, and especially those with no subsidiaries include an increasingly high proportion of examples with a high E/GC ratio.

DISCUSSION:—*Relation between stomatal characteristics and other vegetative characteristics of the plant.*—Before considering the significance of these results with respect to the phylogeny and classification of the monocotyledons, the grouping accord-

TABLE 3. *Families with more than 2 subsidiary cells as the predominant condition*

		NUMBER STUDIED	
ORDER	FAMILY	Genera	Species
ALISMATALES*	Scheuchzeriaceae	1	1
COMMELINALES*	Commelinaceae	4	4
BROMELIALES	Bromeliaceae	5	5
ZINGIBERALES*	Musaceae	1	1
	Strelitziaceae	1	1
	Zingiberaceae	5	5
	Amomum (2)–1 sp.		
	Zingiber (2)–1 sp.		
	Cannaceae	1	1
ARALES*	Araceae	7	7
	Arisaema (2)–1 sp.		
PALMAES	Palmae	7	7
	Calamus (2)–1 sp.		
	Caryota (2)–1 sp.		
PANDANALES	Pandanaceae	3	5
	Sararanga (2)–1 sp.		
CYCLANTHALES	Cyclanthaceae	2	2

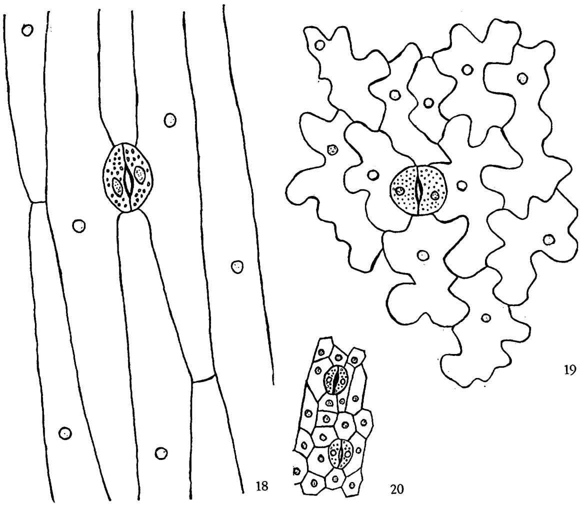

Fig. 18–20. Mature complexes with no subsidiary cells.—Fig. 18. *Nothoscordum inodorum* (Liliaceae).—Fig. 19. *Lapageria rosea* (Philesiaceae).—Fig. 20. *Phormium colensoi* (Agavaceae).

ing to stomatal type which has been presented must be compared with that resulting from emphasis upon various other vegetative and reproductive characteristics regarded as significant indicators of relationships in the subclass. The first point to be emphasized is that the nature of the stomatal complex bears no relationship to the shape of the leaf and is much more stable. The type with 2 subsidiaries occurs in the sagittate leaves of *Sagittaria,* the elliptic leaves of *Maranta,* the linear ones of grasses and sedges, and in the stems of nearly leafless plants like some species of *Juncus* and *Scirpus.* Four or more subsidiary cells occur in the linear leaves of Bromeliaceae, the elliptic leaves of Commelinaceae, many of the variously shaped and petioled types found in Araceae, as well as in the compound leaves of palms. Stomata without subsidiary cells are found in the linear leaves of many Liliaceae, the ensiform leaves of Iridaceae, the elliptic, netted veined leaves of *Smilax* and *Dioscorea,* and the elaborately lobed ones of *Tacca.* In general, most of the leaves with petioles or sheaths also possess subsidiary cells in their stomata, but *Allium, Smilax,* and the Dioscoreales are exceptions, while many leaves with subsidiary cells, such as those of the Mayacaceae, Xyridaceae, Eriocaulaceae, and *Luzula* are simple linear types without petioles or sheaths.

Equally difficult to detect is any association between stomatal types and the character of the xylem vessels, as discussed by Cheadle (1953, 1955). The families which he places in his group (a), lacking vessels (Cheadle 1953, p. 37), are all reduced aquatics, most of which lack stomata altogether. Among those with vessels only in the roots are Agavaceae, Alstroemeriaceae, Amaryllidaceae, and Trilliaceae, which lack subsidiary cells; Alismataceae, Butomaceae, and 6 other families with 2 subsidiaries; and Araceae, Cannaceae, and Strelitziaceae with more than 2. His groups (c) and (d) likewise contain all 3 types of families as regards the stomatal complex. One must comment here, however, that these 4 groups of Cheadle are equally heterogeneous in regard to floral structure, just as are the groupings based upon the stomatal complex.

Another vegetative characteristic which has received much attention in monocotyledons is the mode of germination and vascular anatomy of the seedling (Sargant, 1903; Boyd, 1932). After a lengthy review of this characteristic, Boyd points out that phylogenetic advancement in respect to it may or may not be correlated with specialization in floral characteristics. This character is, however, much better correlated with the type of stomatal complex than is vessel anatomy. When table 3 of Boyd's paper is compared with the 3 summary tables included in the present work, a striking homogeneity is found in the seedling type of those groups with 2 subsidiary cells. Except for a few species of Haemodoraceae and the Lowiaceae, all of them have but a single vascular bundle in the cotyledon, and all except the Gramineae and Cyperaceae have type A or epigeal germination with an elongating, liguleless cotyledon and lateral emergence of the first leaf through a slit. The families

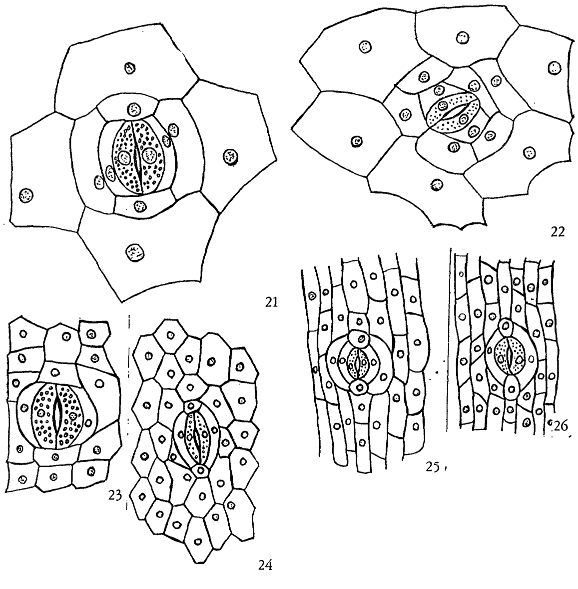

Fig. 21–26. Mature complexes with 2–8 subsidiary cells. —Fig. 21. *Commelina communis* (Commelinaceae): mature complex with 6 subsidiary cells.—Fig. 22. *Scindapsis aureus* (Araceae): mature complex with 8 subsidiary cells.—Fig. 23. *Doryanthes palmeri* (Agavaceae): mature complex with 2 subsidiary cells.—Fig. 24. *Freycinetia angustifolia* (Pandanaceae): mature complex with 4 subsidiary cells.—Fig. 25. Mature complex in the seedling leaf of *Phoenix canariensis* (Palmae) with 6 subsidiary cells.—Fig. 26. Mature complex in the leaf of adult plant of *Phoenix canariensis* (Palmae) showing 4 subsidiary cells.

with no subsidiaries, on the other hand, have predominantly 2 vascular bundles in the cotyledon, and although type A germination is the most common, types B and C are not infrequently found. Finally, the group with many subsidiaries contains mostly families with many vascular bundles in the cotyledon, the only deviating families being the Commelinaceae with 2 and the Bromeliaceae with 1 bundle. Furthermore, hypogeal germination of type C is the most common, with type B next in frequency, while type A is found in this group only in some of the Bromeliaceae and Zingiberaceae.

An equally good or even better correlation exists between stomatal type and habit of growth. When the plants in tables 1–3 are classified according to life form, as recognized by Raunkiaer (1937), those with 2 subsidiary cells are found to consist almost entirely of hemicryptophytes, helophytes, and aquatic types. Geophytes are completely lacking, and phanerophytes are found only in the grasses (bamboos, *Arundo*, etc.), the Velloziaceae, and Flagellariaceae. Among the species having no subsidiaries, on the other hand, geophytes are in the majority, with phanerophytes next in frequency. Hemicryptophytes are found only in some Liliaceae and Orchidaceae, while helophytes and hydrophytes are completely lacking from this group, except for the much reduced Lemnaceae. Finally, the group with more than 2 subsidiaries contains a majority of phanerophytes, a few hemicryptophytes in Commelinaceae, Zingiberaceae, Cannaceae, and Araceae, geophytes in Zingiberaceae and Araceae, and a single helophyte, *Scheuchzeria*. This correlation with growth habit must be seriously considered in any discussion of the phylogenetic progression of stomatal types.

CONNECTIONS BETWEEN STOMATAL TYPE AND CERTAIN REPRODUCTIVE CHARACTERISTICS.—In discussing the association between type of stomatal complex and characteristics of the flower and inflorescence, 4 characters should be considered, since they have figured prominently in various phylogenetic schemes proposed for the monocotyledons. These are separate carpels vs. syncarpous ovary, hypogyny vs. epigyny, uniseriate vs. biseriate perianth, and the development of a large spathe or bract subtending the inflorescence.

The first point to note is that all monocotyledons having apocarpous gynoecia have stomatal complexes with subsidiary cells. In most of these groups, such as the Alismatales and Butomales, their number is 2, but *Scheuchzeria* has usually 4, and some of the palms, perhaps including those with nearly free carpels, have the same number. Nearly free carpels are found also in primitive Liliaceae (*Tofieldia*), which lack subsidiaries.

In respect to hypogyny vs. epigyny, which served as a major diagnostic characteristic in the system of Bessey (1915), the evidence from stomatal complexes supports the conclusion of Hutchinson (1934) that epigyny has arisen repeatedly during the evolution of the monocotyledons, and should be regarded as a character of minor importance in delimiting families. Nearly all of the family realignments which Hutchinson made on the basis of this assumption are supported by the stomatal evidence.

The evidence from stomatal types also supports in general the conclusion of Hutchinson that the development of a biseriate perianth occurred relatively few times in the phylogeny of the group, and therefore should be given greater importance. All of the families placed by Hutchinson in his Division Calyciferae have stomatal complexes with 2 subsidiary cells except for the Bromeliales and many Zingiberales, which have more than 2. Thus, the hypothesis that the Zingiberales are parallel to and not related or ancestral to the Orchidales is fully supported by the present evidence.

Finally, the present evidence supports the concept of Engler and Gilg (1924) and particularly of Hallier (1912) regarding the series Spadiciflorae, containing the Araceae, Lemnaceae, Palmaceae, Pandanaceae, and Cyclanthaceae. All of these families, except for the Lemnaceae, agree in having stomatal complexes with many subsidiaries. Furthermore they agree with each other in the small size of their epidermal cells, except for certain probably derived genera of Araceae with large, non-evergreen leaves, such as *Lysichiton* and *Arisaema*. In this latter respect they differ from the Commelinaceae and to a lesser extent the Bromeliacae, the 2 other families with many subsidiaries, but do resemble the anomalous, apparently primitive genus *Scheuchzeria*. The present authors, therefore, favor the retention of the Spadiciflorae as an interrelated group of orders, rather than scattering its components over various branches of the Division Corolliferae, as is done by Hutchinson. If this change is made, then the remaining members of the Corolliferae, except for some groups which will be discussed below, agree in possessing stomatal complexes without subsidiary cells.

GEOGRAPHIC DISTRIBUTION OF THE STOMATAL TYPES.—The geographic distribution of plants with the 3 types of stomatal complex shows a regular progression. The orders with no subsidiaries are predominantly temperate, except for Amaryllidales, Dioscoreales, Burmanniales, and Orchidales. These are obviously the most specialized families in the group. Furthermore, the Amaryllidaceae are well represented in temperate as well as in tropical regions, while the bulk of tropical orchids, being epiphytic, are in growth habit the most specialized of their family. In this group, therefore, the trend of specialization among modern forms appears to go from temperate to tropical types.

The orders with 2 subsidiaries are of 3 types, helophytes or temperate hydrophytes, such as the Butomales, Alismatales, Juncaginales, and Potamogetonales; tropical or southern hemisphere families like Flagellariaceae, Xyridales, Eriocaulales, Zingiberales, and Haemodorales; and 2 widespread families, Cyperaceae and Gramineae. The Gramineae are almost certainly tropical in origin (Bews, 1929), and the same may be true of the Cyperaceae. The marsh and water plants, although world wide, are somewhat better represented in the tropics and the southern hemisphere than in the north, so that this group shows a recognizable progression of specialization from tropical to temperate types.

Finally, the group with many subsidiaries consists almost entirely of tropical or predominantly tropical families, the only exception being *Scheuchzeria.* This group, therefore, almost certainly originated in the tropics, and only a few members of the Commelinaceae and Araceae have been successful in temperate regions.

The information now presented can afford a basis for deciding which of the types of stomatal development should be considered the most primitive, and what have been the principal lines of phylogenetic progression with respect to this character. The first point to emphasize is that, with a few exceptions, the orders belonging to the group which lacks subsidiary cells are all clearly related to each other and, in respect to floral characteristics, can be regarded as one or a few related and parallel lines of increasing specialization starting with the more primitive Liliales. The exceptions are as follows. The Lemnaceae are probably a much reduced derivative of the Arales, which have lost their subsidiary cells along with most of their other types of specialized cells. The Pontederiaceae, the only family possessing subsidiaries which Hutchinson places in the Liliales, probably belong more correctly next to the Commelinaceae, where they are placed by Engler and Gilg (1924) and perhaps should be included in the order Commelinales. Erdtman (1953) states that their pollen is most similar to that of the Xyridales. Perhaps, therefore, the Pontederiaceae should be placed in an order by themselves, between the Commelinales and Xyridales. At any rate, they deserve further study. Three genera of Liliales and Agavales, *Astelia* of the Liliaceae, *Xanthorrhoea* of the Xanthorrhoeaceae, and *Doryanthes* of the Agavaceae, differ from other genera of their families in possessing subsidiary cells. When this fact was discovered, a particular effort was made to examine genera placed near to them in Hutchinson's system, but all those examined turned out to have stomata of the Lilialean type, without subsidiaries. The systematic position of these 3 genera, one of widespread antarctic distribution and the 2 others endemic to Australia, deserves further investigation. Finally, the Apostasiaceae and Taccaceae, placed by Hutchinson in the Haemodorales, are without subsidiaries, while the remainder of the Haemodorales possess them. This suggests that the arrangement of Engler and Gilg, which places the Apostasiaceae as an aberrant offshoot of Orchidaceae and the Taccaceae next to the Dioscoreaceae, may be more nearly correct. None of these exceptions, therefore, is an insuperable obstacle to the hypothesis that the group with no subsidiaries is monophyletic, and had its origin in primitive Liliales in the general affinity of the Heloniadeae and Narthecieae.

On the other hand, the types with 2 subsidiaries and with more than 2 are much more closely interconnected. In the Zingiberaceae, Araceae, Palmae, and Pandanaceae both types are found, and with the relatively small sampling which has been made of these families their relative abundance cannot yet be estimated. The data available, however, do suggest that the types with 2 subsidiaries are relatively advanced within their family. *Amomum* and *Zingiber* both belong to the tribe Zingibereae, which Hutchinson places last in his synopsis of the Zingiberaceae; *Arisaema* is in the Areae, the last of the tribes of Araceae; while in the Palmae *Calamus* is a specialized climber and *Caryota* occupies a relatively advanced position in the family. This indicates that types with 2 subsidiaries are in general derived by reduction from those with several. Further evidence in favor of such a trend is the high epidermal/guard cell ratio of many of the types with 2 subsidiaries. This indicates a greater amount of cellular differentiation in the epidermis.

The types with more than 2 subsidiaries appear likewise to be more primitive than those with none. The reasons for this assumption are as follows. First, they occur in a number of distantly related groups, some of which are obviously of a relictual character (*Scheuchzeria*) and have primitive floral structures. Second, they are associated with a low epidermal/guard cell ratio, and hence with a smaller amount of differentiation in the epidermis as a whole. Third, the plants having several subsidiaries are mostly evergreen phanerophytes, which are generally regarded as representing the most primitive type of life form (Raunkiaer, 1937). Fourth, they are chiefly tropical in distribution. Recent discussions of angiosperm phylogeny have emphasized that available evidence strongly supports a tropical origin for most families of dicotyledons (Axelrod, 1952), and a similar origin for the monocotyledons might be expected. Finally, the monocotyledonous stomatal type with several subsidiaries and a low epidermal/guard cell ratio is closely similar to the haplocheilic type of stomatal complex described by Florin (1931, 1933) in a variety of gymnosperms, including *Ephedra, Ginkgo,* and Cycadales as well as conifers. He considers this to be the most primitive type of stomatal complex in the class. The writers believe, therefore, that the haplocheilic type in primitive monocotyledons is directly descended from the same type in some as yet unidentified pre-angiosperm ancestor, rather than from any form similar to modern dicotyledons.

The present evidence suggests also that the types with 2 and with no subsidiaries are independently derived from the primitive type with several. The principal evidence for this hypothesis is their association with quite different life forms. One might suggest that the type with 2 well-defined subsidiaries, as found in Alismataceae, Eriocaulaceae, Xyridaceae, Juncaceae, Cyperaceae, and Gramineae, became first established in association with the marsh or aquatic habit, in which it predominates. The function of the subsidiary cells is not known, but their consistent presence in these families suggests that they must serve some function, particularly in view of the fact that stomata without subsidiary cells are rather easily induced in these types through abnormal environments during development (Stebbins et al., unpubl.), and might be expected to occur naturally through mutation unless such mutants had a strongly negative selective value. On the other hand, the frequent association of no subsidiaries with geophytes and xeric rosette plants like the Agavaceae and Aloinae suggests that this type of stomatal complex may have originated under relatively dry conditions. In both instances, the specialized stomatal complex, once developed, probably acquired a selective value which made it difficult to modify by mutation and selection, so that it persisted after the evolutionary line bearing it had changed its adaptation radically from the habitat in which the initial evolution of the stomatal type took place.

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