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# THE STOMATAL COMPLEX IN *AGAVE*: GROUPS DESERTICOLAE, CAMPANIFLORAE, UMBELLIFLORAE



By

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ABSTRACT: The stomatal cell complexes of 23 taxa in the genus *Agave* are described, measured, and illustrated. The taxa belong to three taxonomic groups preponderant in Baja California and other parts of the Sonoran Desert region: Deserticolae, 17 taxa; Campaniflorae, 3 taxa; Umbelliflorae, 3 taxa.

The stomatal characteristics are compared with the contemporary systematic revision of Gentry (1978) at both the sectional and species levels. Stomatal differences are significant and helpful to *Agave* taxonomy at the sectional level, but are found to be irregular as species criteria. One species, *Agave fortiflora*, on the basis of stomatal anatomy, was found misaligned with the Umbelliflorae and was removed to another section.

The apparent xeromorphic structure of agave stomates are compared with rainfall regimes of respective species. No correlations are found between apparent xeromorphic stomatal structures and the most arid habitats of agave species.

## INTRODUCTION

During the course of making a taxonomic revision of the genus *Agave* of the California Gulf region, Gentry (1978) made a classification based on the gross morphology of the leaf, the inflorescence, the flower at anthesis, and the growth and reproductive habits. It became clear that levels of taxa and their perimeters were subtle and hard to define. Therefore, a critical study of the epidermis was made to see if such characters could be of correlative value in the proposed system.

Characters of the epidermis and stomata have been found to be of considerable value in identification and classification in various flowering-plant groups by Stace (1966), Tomlinson (1974), and others. Various accounts of the anatomy of *Agave* leaves include descriptions of epidermis and stomata. Probably the most extensive is that of Müller (1909) in which about 95 species of *Agave* are described and a key devised for their separation and identification. Since no study exists for *Agave* species inhabiting the California Gulf region, we examined the twenty-three taxa which occur in that area. The taxa fall into three sections or groups of the genus *Agave*: the Deserticolae, the Campaniflorae, and the Umbelliflorae. A Sonoran Desert species, *A. fortiflora*, is also examined as to its relationship with the Umbelliflorae with which it was placed in an earlier paper by Gentry (1972).

More recently, attempts have been made to classify and characterize *Agave* stomata for phylogenetic purposes (Stebbins and Khush 1961; Shah and Gopal 1969), but as yet only one other study successfully utilized leaf anatomy, including the study of epidermal characters, to distinguish genera in the Agavaceae (see Blunden, Yi, and Jewers 1973).

The Deserticolae includes 17 taxa which are found in the Sonoran Desert in southeastern California, Arizona, Baja California, and Sono-

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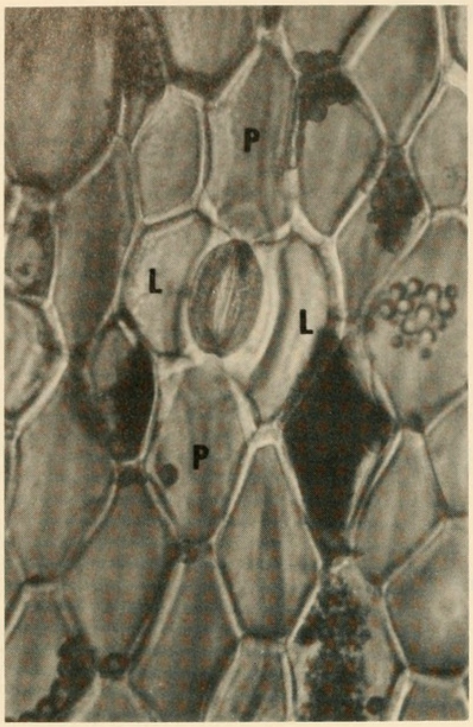



FIGURE 1. Surface view of the stomatal complex of *Agave sobria* ssp. *roseana* (×400). *P*, polar subsidiary cells; *L*, lateral subsidiary cells.

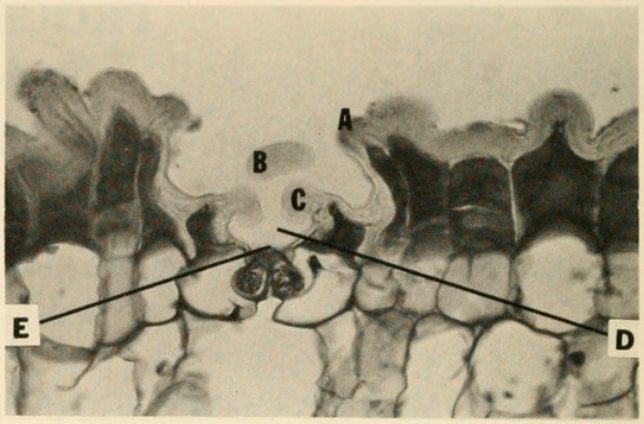



FIGURE 2. Cross-section of epidermis of *Agave deserti*. *A*, margin; *B*, cross-section of the polar lip; *C*, longisection of the lateral lip; *D*, chamber below the lateral lips (inner aperture); *E*, stoma.

ra. Most species have a slender inflorescence bearing small, compact umbels and are either suckering or simple multiannuals. The short, open flower tube is characteristic. Nearly all are xerophytic. The Campaniflorae, native to the southern half of peninsular Baja California, consists of three species with broad campanulate flowers borne in panicles with smaller bracts. The Umbelliflorae consists of three taxa found in Baja California. They are distinguished by flowers borne in large umbellate branches subtended by conspicuous sheathing bracts, and they are mainly large perennials branching from the leaf axils.

## MATERIAL AND METHODS

Materials were obtained primarily from herbarium specimens, though a few specimens were obtained from living material at the Desert Botanical Garden, from Gentry's personal collection in Murrieta, California, and one from Santa Barbara Botanic Garden, California. Fresh material was preserved in Craf's III or formalin-acetic-ethanol. Although one slide was prepared for each species, numerous herbarium specimens were examined under the dissecting microscope to insure that a representative sample was taken. In addition, leaf peelings of over 40 species representing most other sections of the genus were prepared for general comparative purposes.

Peelings of leaf epidermis were obtained readily from either preserved or dried material. Dried material was boiled 20 minutes in a detergent solution to soften it and to facilitate the removal of underlying tissues. Such material was rinsed in distilled water prior to dehydration and staining. Permanent mounts were made from peelings dehydrated in ethanol and stained in a 1% solution of Safranin O and 0.5% solution of Fast Green and mounted in Harleco Synthetic Resin.

An attempt was made to prepare plastic imprints according to the method of Sinclair and Dunn (1961). Due to the relatively deep suprastomatal chamber of most species examined, the method proved to be unsatisfactory, as has been noted for *Agave* by Shah and Gopal (1969). Though the imprint method is not suitable for detailed morphological studies, sufficient detail was obtained to count stomata.

After examining the leaves from several species, a uniformity was noted with regard to the structure of the stomata. Therefore, a single leaf was used. Five stomata were measured for each leaf. These measurements were taken about one-half way up from the base of the leaf.

Leaf sections were required in order to interpret surface features of the epidermal peelings. Such sections were prepared from material preserved in Craf's III or FAA and embedded in paraffin, and dehydrated and stained in 1% Safranin O and 0.5% Fast Green according to the method of Johansen (1940). Sectioning was im-

proved in hardened material by soaking the cut block in cold water for one hour to several days.

## General Morphology of the Stomatal Complex

In *Agave*, four epidermal cells surround the guard cells. These cells appear different from other epidermal cells in that two of them lie polar and two lie lateral to the guard cells. Often they stain differently from other epidermal cells, and they may possess a distinctive bulbous lip which protrudes into a cavity above the stoma called the suprastomatal chamber. Morphologically, the stomatal complex appears to be tetracytic, but as noted by Blunden et al. (1973), the term is purely descriptive as developmental studies have not been carried out in this paper. We will refer to these cells as subsidiary cells following Tomlinson (1974), who retains the term for structurally specialized cells distinct from other epidermal cells associated with the guard cells of the mature stomata (Fig. 1). The guard cells and associated subsidiary cells are oriented parallel to the long axis of the leaf.

The stomatal-complex proper lies at the bottom of a cavity, the suprastomatal chamber, created by the various elaborations of supra-epidermal structures, which consist of a dark-staining waxy or cellulosic layer capped by a red-staining cuticular layer (Fig. 2). That the stomata are truly sunken is questionable; the stomata are actually depressed below the cuticular and subcuticular layers only and just slightly depressed below the level of the epidermis itself.

The aforementioned lips may be termed polar lips if they arise from the polar subsidiary cells or lateral lips if they arise from the lateral subsidiary cells. The polar lips are elongated parallel to the long axis of the leaf and lie in a plane above the lateral lips which are elongated at right angles to the polar lips. This configuration of the lips causes great variation in the shape of the suprastomatal cavity in different planes of surface view, as noted by McClendon (1908). In our study five levels of change were seen for many species (Fig. 3): (A) the surface pore; (B) the level of the polar lips; (C) the level of the lateral lips; (D) the level of the chamber below the lateral lips or the inner aperture; (E) the level of the stomatal aperture. In some species fewer than five levels were observed, as in *A. shawii* ssp. *goldmaniana* where the polar lips are absent.

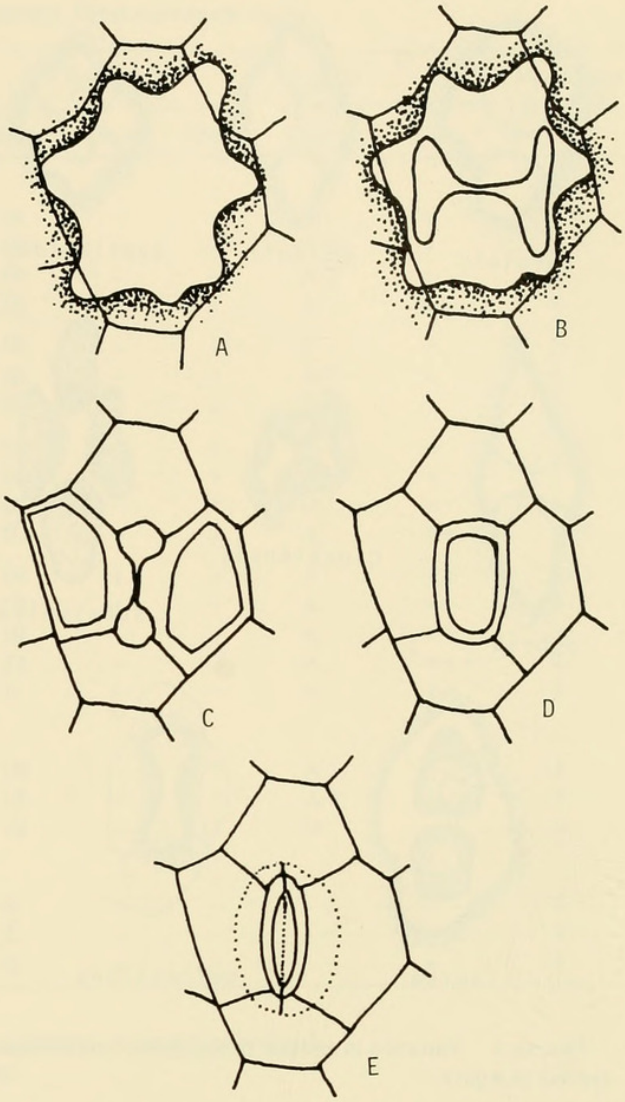



FIGURE 3. Levels of change in surface view of the *Agave* stomatal complex. *A*, surface pore; *B*, view of the polar lips; *C*, the lateral lips; *D*, the inner aperture; *E*, the stoma.

The epidermal cells which surround the stomatal complex may also possess lips or papillae, but these are less definite in form and number than those associated with the subsidiary cells; 7–11 such cells surround the stomatal complex. Variations in the nature and development of the epidermal papillae or cuticle account for the variation in the texture of leaf surfaces in *Agave*. The higher the papillae, the more asperous the leaf surface. Epidermal cells in general are either isodiametric (most species) or elongate (*A. sobria* ssp. *sobria*, *A. sobria* ssp. *roseana*, *A. sobria* ssp. *frailensis*, *A. vizcainoensis*, and *A. subsimplex*), with end walls either transverse or oblique.

The margin of the surface pore is of particular interest as it is formed in two different ways. In

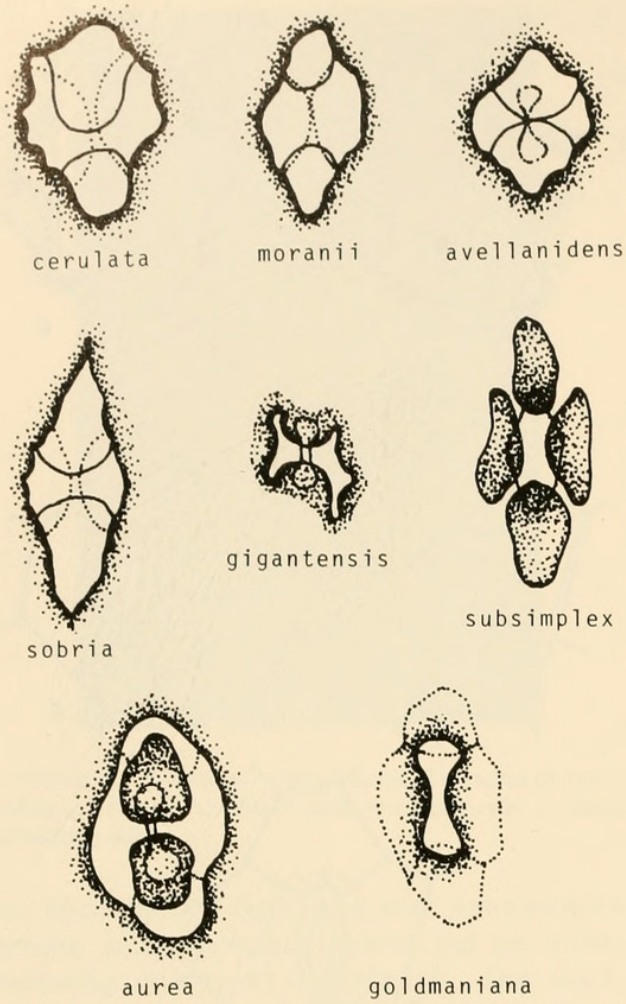



FIGURE 4. Variation in margins of the surface pore in eight species of *Agave*.

most Deserticolae and all Campaniflorae, the surface pore is formed by the confluent cuticles of the cells surrounding the stomatal complex (in Fig. 4 see *A. cerulata*, *A. moranii*, *A. avellanidens*, and *A. aurea*). In some species the margin may appear irregular due to the presence of epidermal papillae (*A. deserti*) or, as in *A. moranii* where papillae are absent, the margin may appear entire. In the Umbelliflorae and in some Deserticolae, the surface pore is formed by the polar and lateral lips themselves, and the margin thus formed is entire (in Fig. 4 see *A. subsimplex* and *A. shawii* ssp. *goldmaniana*).

In several species (Table 1) a peculiar and distinctive ovoid structure can be seen at the level of the inner aperture. This appears to be due to a greater thickening of the cell wall of the polar and lateral-subsidiary cells adjacent to the inner aperture. We refer to this collarlike structure as a "rim."

Stomata are so numerous in *A. deserti* and *A. cerulata* that the suprastomatal chambers tend to run together laterally forming a compound suprastomatal chamber or channel. A different situation is found in *A. sobria* where the margins of the surface pores tend to run together, aligned with the leaf axis rather than transversely. When "channelling" is present, it is here referred to as transverse or axial channelling, respectively.

For the most part stomata are evenly distributed on both surfaces of the leaf, but they tend to become more sparse near the very base of the leaf. Consistent with the findings of other workers (Weisner and Baar 1914), there are more stomata on the upper surface of the leaf than on the lower (Table 2).

The following characteristics of the stomatal complexes are described for each species:

1. Number of levels of change in different planes of surface view
2. Shape and nature of surface pore
3. Length and width of the stomatal complex
4. Width of the polar and lateral lips
5. Position of the polar and lateral lips
6. Length and width of the inner aperture
7. Length and width of the stoma
8. Presence of channelling
9. Presence of rim

The following descriptions are given in the form of brief diagnoses. The species are arranged alphabetically under the sections. Summaries of epidermal characters are given in Tables 1 and 2. Figures 5 to 50 show surface views of the *Agave* stomatal complex (photos all $\pm \times 200$), stomatal ideographs, and rainfall silhouettes of species habitats. The ideographs represent the relative sizes and shapes of the four subsidiary cells (white rectangles) and the sizes and positions of the polar lips (black rectangles). Sources of materials used are cited by collection numbers at the end of each paragraph.

## Description of Stomatal Complexes

### Deserticolae

*Agave avellanidens* Trelease (Figs. 5 & 14)

Five planes in surface view, margin scalloped, extending out over the lateral subsidiary cells. Stomatal complex 105–130$\mu$ long, 75–90$\mu$ wide, oval to roundish in outline; polar lips 2, broad, extending towards one another but not touching, 42–50$\mu$ wide; lateral lips 2, apart or touching,

TABLE 1. COMPARISONS OF EPIDERMAL CHARACTERS OF *Agave*.

| | Cuticle[1] | | Stomatal[2] | | | Position of polar lips[3] | Epid. cell elong.[4] | No. planes in surface view |
|---|---|---|---|---|---|---|---|---|
| | Roughness | Thickness | Marg. | Rim | Chan. | | | |
| DESERTICOLAE | | | | | | | | |
| *avellanidens* | + | ++ | IR | – | + | A | + | 5 |
| *c. cerulata* | +++ | +++ | IR | – | + | A-T | + | 5 |
| *c. nelsonii* | +++ | +++ | IR | – | + | A-T | + | 5 |
| *c. subcerulata* | +++ | +++ | IR | – | + | A | + | 5 |
| *d. deserti* | +++ | +++ | IR | – | + | T | + | 5 |
| *d. pringlei* | +++ | +++ | IR | – | + | A-T | + | 5 |
| *d. simplex* | +++ | +++ | IR | – | + | T-O | + | 5 |
| *gigantensis* | + | ++ | ID | – | + | A-T | + | 5 |
| *margaritae* | ++ | +++ | IR | – | + | A | + | 5 |
| *mckelveyana* | + | ++ | IR | – | * | O | + | 5 |
| *moranii* | + | ++ | ID | + | + | A | ++ | 4–5 |
| *s. frailensis* | + | + | IR | + | + | A | ++ | 4–5 |
| *s. roseana* | + | + | IR | + | + | A | ++ | 5 |
| *s. sobria* | +++ | +++ | IR | – | + | A | ++ | 5 |
| *subsimplex* | ++ | ++ | IR | + | – | A | +++ | 4 |
| *vizcainoensis* | + | + | E | + | – | A | ++ | 5 |
| CAMPANIFLORAE | | | | | | | | |
| *aurea* | ++ | ++ | IR | – | * | A | + | 5 |
| *capensis* | ++ | ++ | IR | – | * | A-T | + | 5 |
| *promontorii* | ++ | +++ | IR | – | * | O | + | 5 |
| UMBELLIFLORAE | | | | | | | | |
| *s. goldmaniana* | + | ++ | E | + | – | – | + | 3 |
| *sebastiana* | + | ++ | E | + | – | – | ++ | 3 |
| *s. shawii* | + | ++ | E | + | – | – | + | 3 |

[1] + = smooth or thin, +++ = rough or thick.
[2] IR = irregular, ID = indefinite, E = entire, – = absent, + = present, * = very occasional.
[3] A = polar lips apart, T = lips touching, O = lips overlapping.
[4] + = relative elongation.

35–45$\mu$ wide; inner aperture blunt oval, 46–60$\mu$ long, 28–35$\mu$ wide; stoma 30–40$\mu$ long, 15–20$\mu$ wide; channelling absent or occasional, transverse; surface smooth with small ridges of cutin, appearing slightly striate. Rim absent. *(Gentry 11929)*

*Agave cerulata* ssp. *cerulata* Trelease (Figs. 6 & 15)

Five planes in surface view, the margin of the surface pore irregular and extending out beyond the lateral cells. Stomatal complex 115–155$\mu$ long, 85–100$\mu$ wide; polar lips 2, 50–60$\mu$ wide, separated to touching, broadest at base, lateral lips 2, 40–63$\mu$ wide, separated to overlapping; inner aperture rectangular 30–35$\mu$ long and 31–40$\mu$ wide. Stoma 33–40$\mu$ long, 18–20$\mu$ wide. Surface papillate and rough to touch; channelling present, transverse. Rim absent. (Gentry Garden, Murrieta, California)

*Agave cerulata* ssp. *nelsoni* (Trelease) Gentry (Figs. 7 & 16)

Similar to the above taxon, five planes in surface view, margin irregular and extending out beyond the lateral subsidiary cells. Stomatal complex 128–142$\mu$ long, 90–100$\mu$ wide, slightly longer than wide; polar lips 2, prominent, broadest at base, apart or touching, 55–75$\mu$ wide; lateral lips 2, touching to overlapping, 45–55$\mu$ wide; inner aperture elongate, 70–80$\mu$ long, 23–29$\mu$ wide. Stoma 34–38$\mu$ long, 19–22$\mu$ wide. Channelling occasional, transverse, surface papillate. Rim absent. (*Gentry 10373*)

*Agave cerulata* ssp. *subcerulata* Gentry (Figs. 8 & 17)

Five planes in surface view, margin irregular and extending out beyond the lateral subsidiary cells. Stomatal complex 140–180$\mu$ long, 90–100$\mu$ wide, more or less roundish in outline; polar lips

TABLE 2. STOMATAL MEASUREMENTS (MICRONS) OF *Agave*.

| Taxa | Av. L&W stomate complex | Width polar lips | Width lat. lips | Av. L&W inner aperture | Av. L&W lateral cells | Av. L&W polar cells | Av. L&W stoma | Stomata/mm² U | Stomata/mm² L |
|---|---|---|---|---|---|---|---|---|---|
| DESERTICOLAE | | | | | | | | | |
| *avellanidens* | 120 × 80 | 45 | 39 | — | 66 × 21 | 37 × 42 | — | 48 | |
| *c. cerulata* | 140 × 95 | 55 | 48 | 33 × 35 | 66 × 27 | 51 × 45 | 36 × 19 | 52 | 33 |
| *c. nelsonii* | 132 × 96 | 67 | 53 | 75 × 26 | 58 × 28 | 35 × 50 | 36 × 19 | 37 | 31 |
| *c. subcerulata* | 150 × 88 | 45 | 42 | 59 × 27 | 56 × 22 | 40 × 51 | 27 × 11 | 45 | |
| *d. deserti* | 128 × 82 | 46 | 44 | — | 49 × 18 | 30 × 38 | — | 52 | 34 |
| *d. pringlei* | 110 × 105 | 45 | 38 | 47 × 18 | 48 × 22 | 32 × 42 | 28 × 10 | 61 | |
| *d. simplex* | 131 × 112 | 47 | 31 | 35 × 19 | 65 × 24 | 32 × 55 | — | | |
| *gigantensis* | 142 × 84 | 39 | 36 | 41 × 21 | 59 × 20 | 38 × 41 | — | 52 | |
| *margaritae* | 135 × 87 | 42 | 37 | 54 × 20 | 63 × 35 | 40 × 39 | — | 36 | |
| *mckelveyana* | 114 × 80 | 35 | 23 | 37 × 20 | 47 × 22 | 33 × 51 | — | 23 | |
| *moranii* | 134 × 76 | 28 | 42 | 58 × 27 | 67 × 17 | 32 × 40 | 36 × 17 | 41 | |
| *s. frailensis* | 197 × 107 | 40 | 53 | 72 × 38 | 70 × 36 | 71 × 46 | 35 × 14 | 30 | |
| *s. roseana* | 197 × 78 | 45 | 49 | 63 × 25 | 85 × 28 | 73 × 36 | 33 × 14 | 34 | 30 |
| *s. sobria* | 153 × 91 | 48 | 37 | — | 71 × 35 | 48 × 49 | — | 36 | |
| *subsimplex* | 171 × 64 | 32 | 44 | 68 × 21 | 59 × 15 | 66 × 35 | 31 × 8 | 42 | |
| *vizcainoensis* | 163 × 70 | 29 | 56 | 68 × 26 | 66 × 15 | 54 × 33 | 31 × 11 | 38 | |
| CAMPANIFLORAE | | | | | | | | | |
| *aurea* | 152 × 85 | 47 | 36 | 54 × 26 | 64 × 22 | 42 × 44 | 38 × 18 | 18 | 12 |
| *capensis* | 125 × 80 | 33 | 38 | 47 × 23 | 41 × 21 | 38 × 34 | 34 × 13 | 31 | 19 |
| *promontorii* | 126 × 84 | 67 | 40 | 43 × 29 | 65 × 25 | 38 × 47 | — | 21 | 18 |
| UMBELLIFLORAE | | | | | | | | | |
| *s. goldmaniana* | 150 × 84 | — | 67 | — | 69 × 15 | 40 × 43 | 41 × 18 | 42 | |
| *sebastiana* | 135 × 76 | — | 52 | 59 × 28 | 64 × 20 | 48 × 39 | 36 × 21 | 40 | |
| *s. shawii* | 142 × 78 | — | 53 | 43 × 41 | 72 × 20 | 48 × 43 | 39 × 22 | 47 | 36 |

2, prominent, broadest at base, apart, 40–50$\mu$ wide; lateral lips 2, touching, 30–50$\mu$ wide; inner aperture somewhat irregular, generally oblong, 54–65$\mu$ long, 19–30$\mu$ wide. Stoma 25–30$\mu$ long, 10–15$\mu$ wide. Channelling present, transverse; surface papillate. Rim absent. (Based on *Gentry 23175, 11892*)

*Agave deserti* Engelman ssp. *deserti* (Figs. 9 & 18)

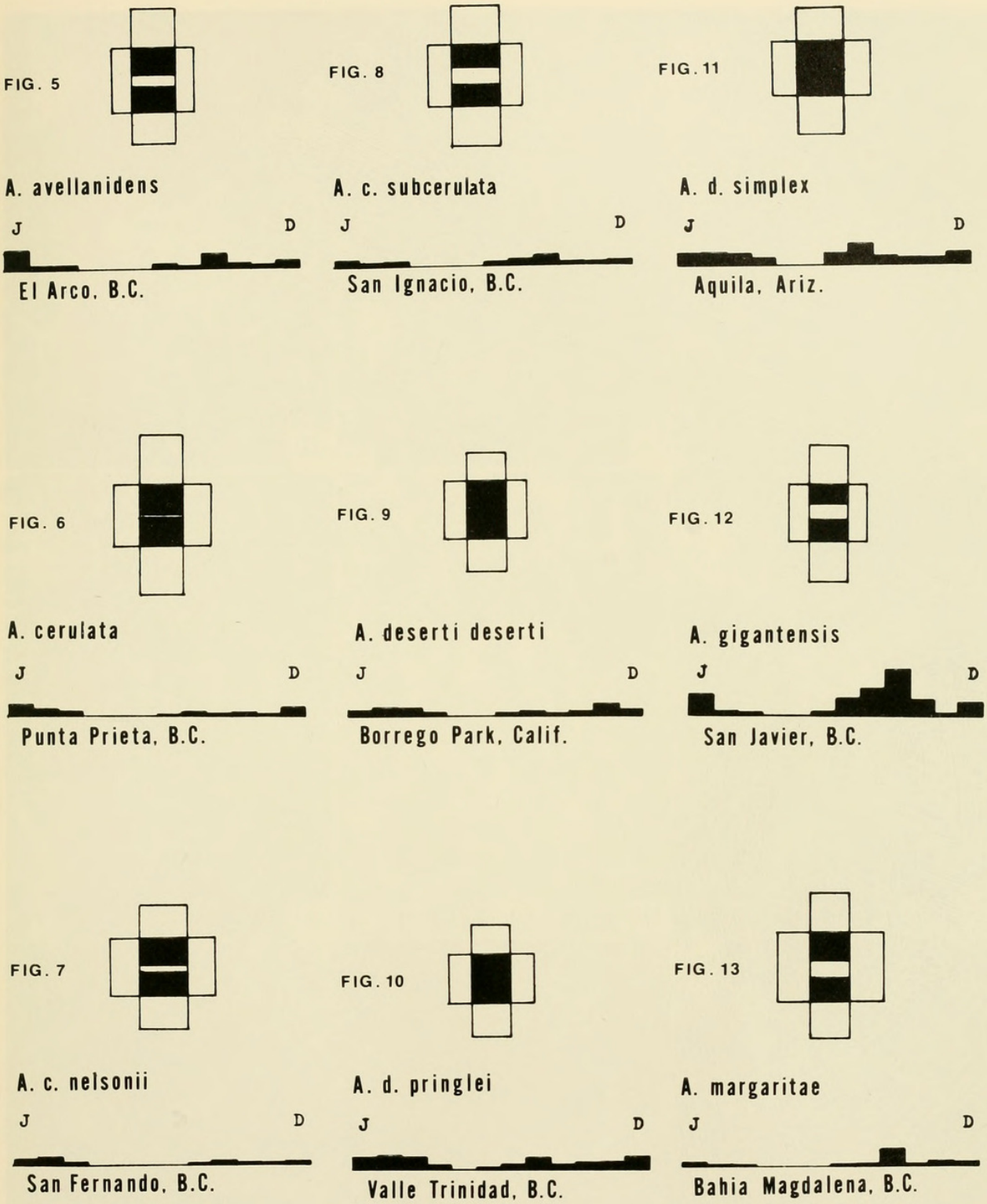
Five planes in surface view, margin irregular and extending out over the lateral subsidiary cells. Stomatal complex 110–130$\mu$ long, 80–90$\mu$ wide, somewhat smaller than the *cerulata* type but similar to it. Polar lips 2, prominent, broadest at base, touching or overlapping, 35–50$\mu$ wide; lateral lips 2, 45–50$\mu$ broad, touching or overlapping; inner aperture 31–35$\mu$ long, 17–20$\mu$ wide rectangular. Channelling present, transverse; surface rough the papillae somewhat more acute than *cerulata*. Rim absent. (*Gentry 19741*)

*Agave deserti* ssp. *pringlei* (Engelman ex Trelease) Gentry (Figs. 10 & 19)

Five planes in surface view, margin irregular as in subspecies *deserti*; stomatal complex 100–135$\mu$ long, 85–115$\mu$ wide, whole complex roundish; polar lips 2, prominent, broadest at base, touching, apart, or overlapping, 38–50$\mu$ wide; lateral lips 28–40$\mu$ wide, touching; inner aperture 40–54$\mu$ long, 15–22$\mu$ wide, somewhat constricted toward the center, stoma 22–30$\mu$ long, 10–11$\mu$ wide; channelling present, transverse (as many as 6 stomata in a chain were observed); surface rough. Rim absent. (*Gentry 19959, 16723*)

*Agave deserti* ssp. *simplex* (Trelease) Gentry (Figs. 11 & 20)

Five planes in surface view, margin highly irregular, much as in *A. deserti*; stomatal complex 131$\mu$ long, 112$\mu$ wide, polar lips 47$\mu$ wide, closed, lateral lips 2, 31$\mu$ wide touching, inner aperture and stoma obscure, the cuticle very

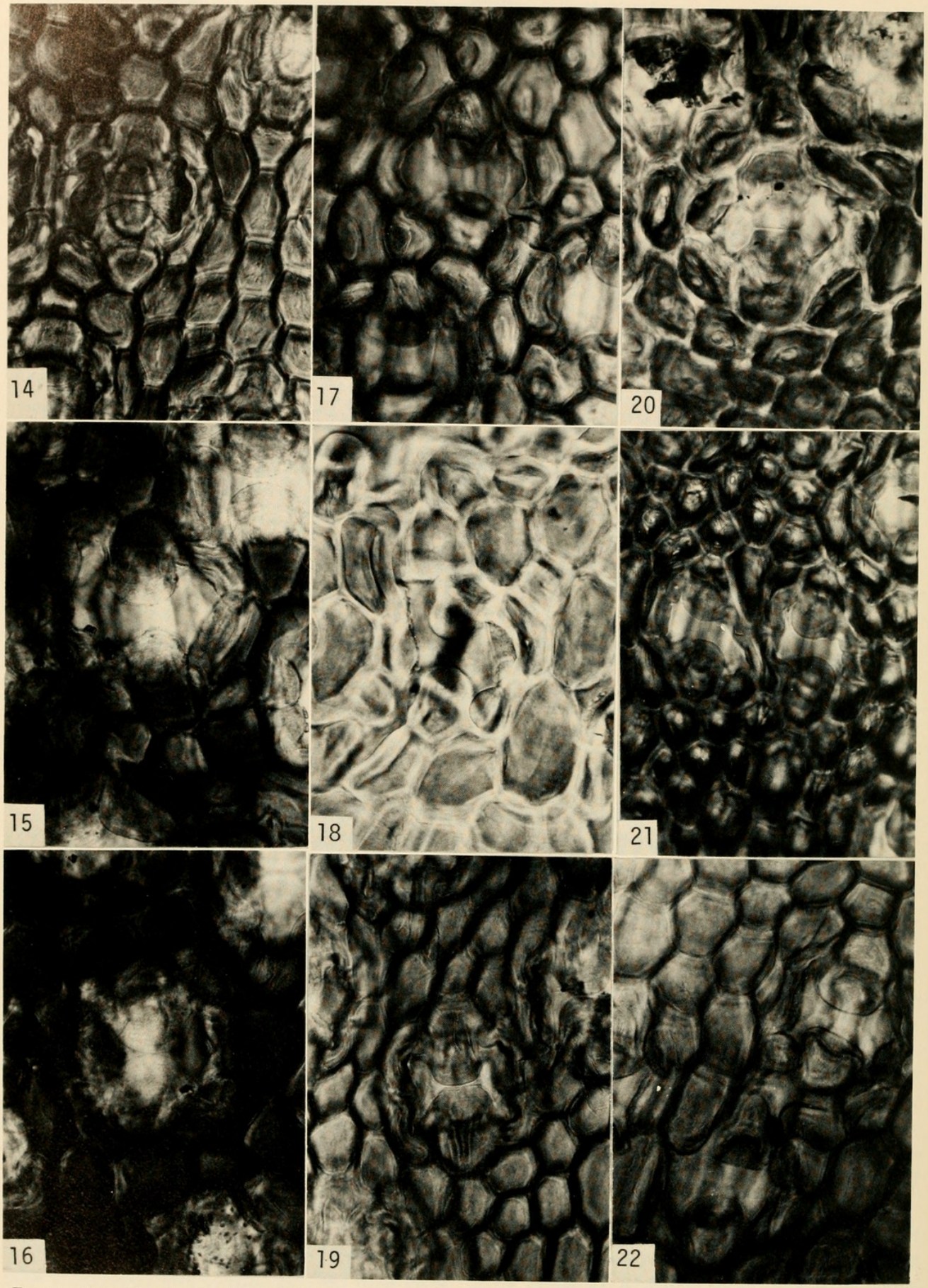



FIGURES 5 TO 13. Ideographs of the *Agave* stomatal complexes with rainfall silhouettes of *Agave* habitats. Fig. 5. *A. avellanidens*. Fig. 6. *A. cerulata cerulata*. Fig. 7. *A. cerulata nelsonii*. Fig. 8. *A. cerulata subcerulata*. Fig. 9. *A. deserti deserti*. Fig. 10. *A. deserti pringlei*. Fig. 11. *A. deserti simplex*. Fig. 12. *A. gigantensis*. Fig. 13. *A. margaritae*.

thick, surface papillate, channelling present, transverse. Rim absent. (*Gentry 23404*)

*Agave gigantensis* Gentry (Figs. 12 & 21)

Four levels in surface view, the margin of the surface pore obscure; stomatal complex 116–160$\mu$ long, 80–90$\mu$ wide; polar lips 2, bulbous, 32–46$\mu$ wide, mostly slightly apart or touching, level with the leaf surface and continuous with the surface cutin, aperture formed at this level perpendicular to the long axis of the leaf, lateral




FIGURES 14 TO 22. Surface views of the *Agave* stomatal complexes. Fig. 14. *A. avellanidens*. Fig. 15. *A. cerulata cerulata*. Fig. 16. *A. cerulata nelsonii*. Fig. 17. *A. cerulata subcerulata*. Fig. 18. *A. deserti deserti*. Fig. 19. *A. deserti pringlei*. Fig. 20. *A. deserti simplex*. Fig. 21. *A. gigantensis*. Fig. 22. *A. margaritae*.

lips below, 32–40μ wide, overlapping; inner aperture 40–50μ long, 20–26μ wide; stoma obscure; channelling occasional, transverse, surface smooth. Rim absent. (*Gentry 10237, 23320, Barclay & Argueles 1990*)

*Agave margaritae* Brandegee (Figs. 13 & 22)

Five planes in surface view; margin of surface pore undulate and extending out beyond the lateral subsidiary cells; stomatal complex 115–160μ long, 82–100μ wide, somewhat elongate; polar lips 2, 39–45μ wide, slightly constricted toward base, apart; lateral lips 2, slightly overlapping, 35–40μ wide; inner aperture 50–60μ long, 18–22μ wide; stoma obscure; channelling occasional, transverse, general surface somewhat rough. Rim absent. (*Gentry et al. 11903*)

*Agave mckelveyana* Gentry (Figs. 23 & 32)

Five planes in surface view; the margin of the surface pore irregular with 4–6 marginal papillae, extending over the lateral subsidiary cells; stomatal complex 105–125μ long, 75–85μ wide; polar lips 2, overlapping, 32–39μ broad, lateral lips 2, overlapping, 21–23μ broad, polar lips distinctly broader than the lateral lips; inner aperture 32–40μ long, 19–22μ wide; channelling very occasional, transverse; surface papillate. Rim absent. (*Gentry & Ogden 9961*)

*Agave moranii* Gentry (Figs. 24 & 33)

Four to five levels of change in surface view, the margin of the surface pore obscure or weakly developed, elongate; stomatal complex 122–150μ long, 72–80μ wide; polar lips 2, narrower than the lateral lips, far apart, 24–30μ wide; lateral lips 2, touching or overlapping, more conspicuous than the polar lips, 40–43μ wide; inner aperture 55–60μ long, 25–30μ wide, oblong to oval; stoma 32–40μ long, 15–18μ wide; channelling occasional, transverse; surface relatively smooth, cutin in small ridges. Rim present. (*Gentry 23287*)

*Agave sobria* ssp. *frailensis* Gentry (Figs. 25 & 34)

Four to five planes in surface view; margin of the surface pore somewhat obscure, elongate, tending to connect with margins of adjacent stomates; stomatal complex elongate, nearly two times as long as broad, 185–203μ long, 98–115μ wide; polar lips 2, not well-developed, far apart, 30–45μ broad; lateral lips broad, prominent, touching or overlapping, 50–64μ wide, configuration of polar and lateral lips resembling that of *A. moranii*; inner aperture oblong oval, 65–70μ long, 15–20μ wide; stoma 25–30μ long, 13–16μ wide; surface essentially smooth, slightly striate; channelling present, axial. Rim present. (*Gentry 11257, 11264*)

*Agave sobria* ssp. *roseana* (Trelease) Gentry (Figs. 26 & 35)

Five planes in surface view; margin elongate, undulate, tending to connect with margins of adjacent stomates; stomatal complex 140–175μ long, 70–79μ wide; polar lips 2, far apart, 42–47μ wide; lateral lips 2, touching or overlapping slightly; 30–45μ wide; inner aperture 62–73μ long, 20–22μ wide; stoma 30–31μ long, 18–20μ wide; channelling present, axial; surface smooth, papillae visible but short. Rim present. (*Gentry 11277*)

*Agave sobria* ssp. *sobria* Brandegee (Figs. 27 & 36)

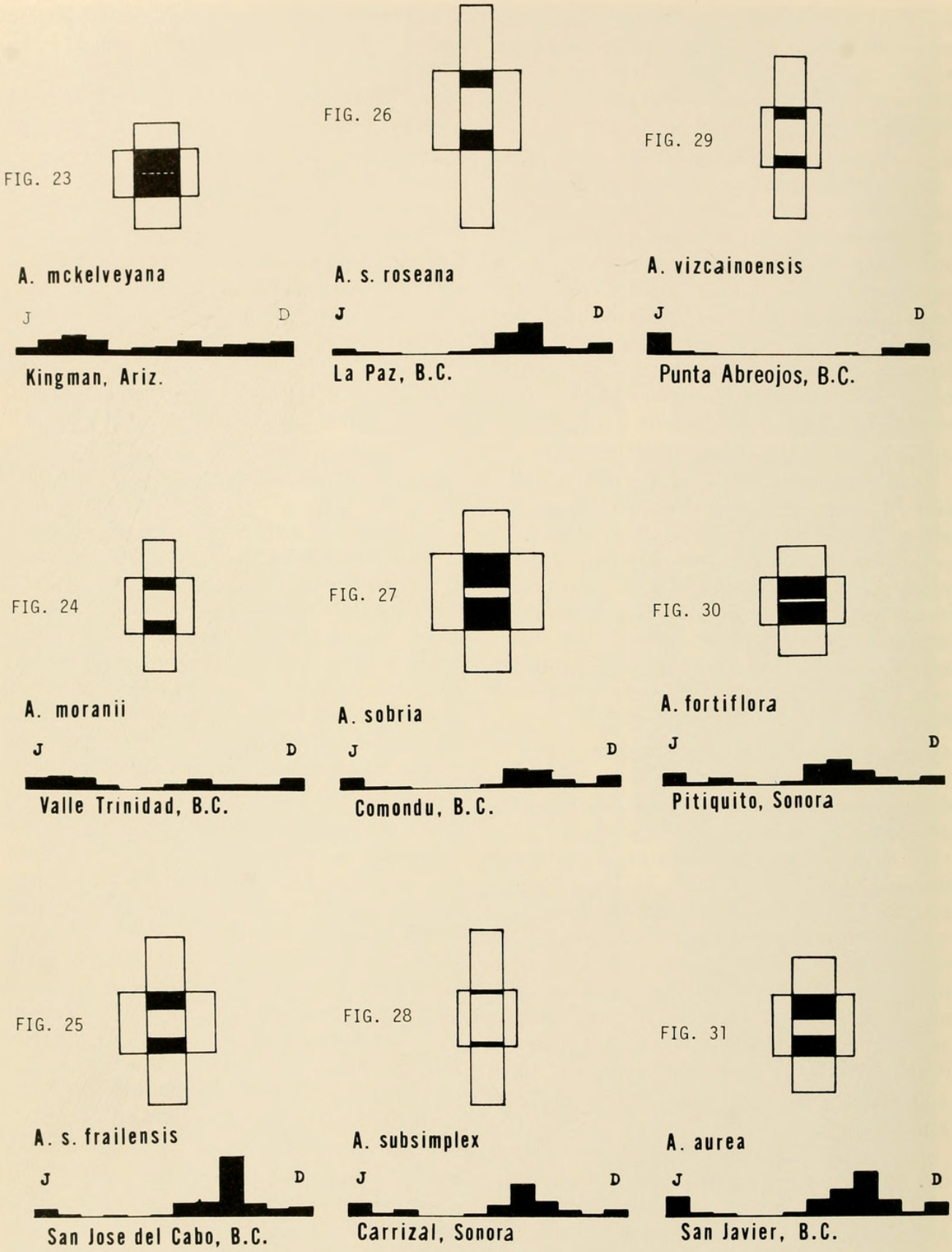
Five planes in surface view; margin of the surface pore irregular, elongate, and with some tendency to pass over the lateral subsidiary cells, this frequently not as marked as in *A. cerulata* and *A. deserti*; stomatal complex 137–175μ long, 90–107μ wide; polar lips 2, prominent, generally apart, sometimes touching, 40–55μ wide; lateral lips 2, touching or overlapping slightly, 30–44μ wide; inner aperture and stoma obscure; surface lumpy, channelling axial and transverse. Rim absent. (*Gentry 12387, 11811*)

*Agave subsimplex* Trelease (Figs. 28 & 37)

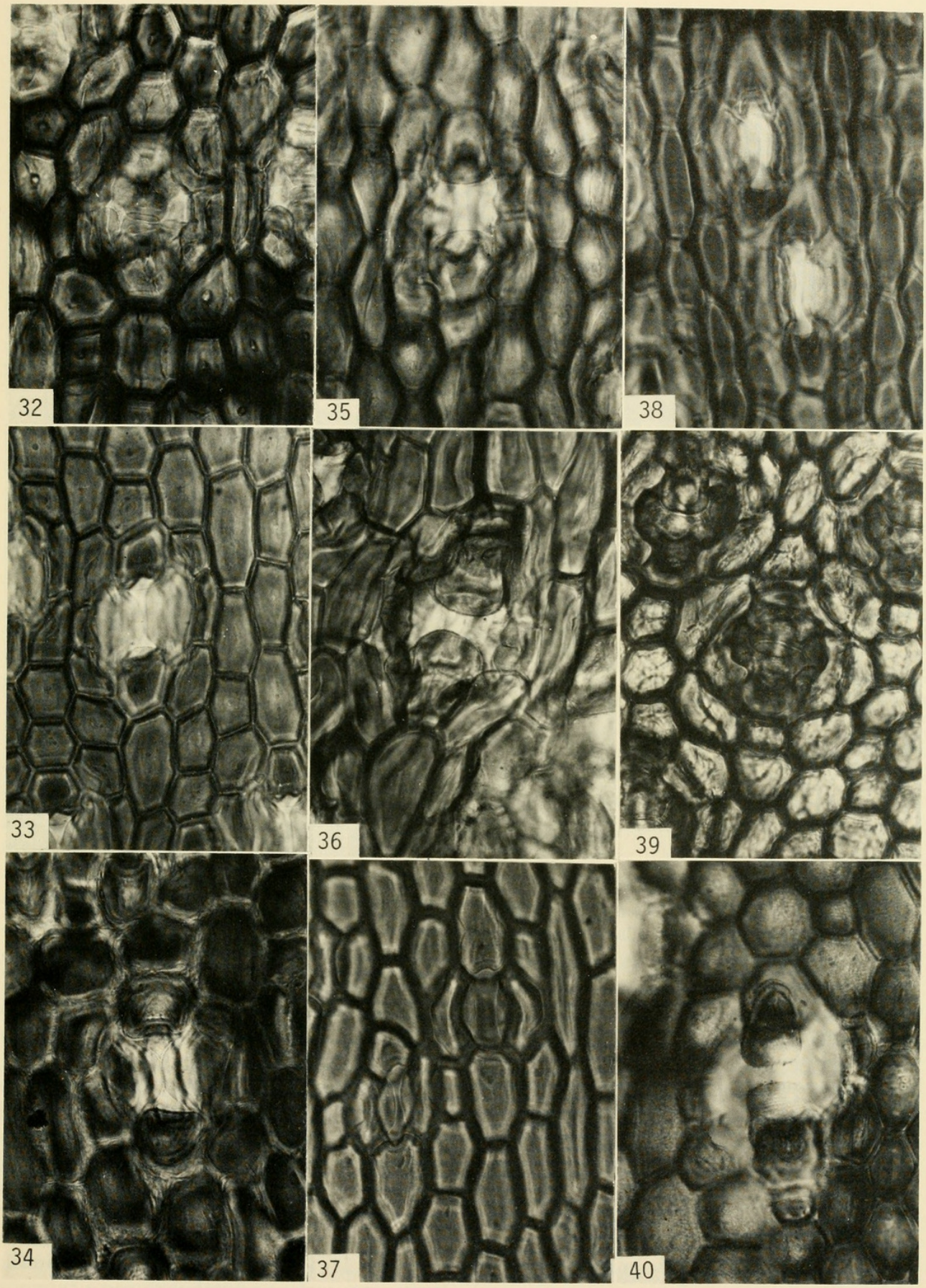
Four levels in surface view; the polar and lateral lips forming a keyhole-shaped surface pore with an entire margin; stomatal complex distinctly elongate, 150–190μ long, 58–72μ wide; polar lips 2, obscure, far apart, 28–34μ wide; lateral lips 2, prominent, 38–50μ wide, apart; inner aperture 60–80μ long, 15–25μ wide, elongate, sometimes attenuated at the ends; stoma 26–34μ long, 7–9μ wide; surface generally smooth, but with small ridges of cuticle running parallel with the long axis of the leaf; rim present. (*Gentry 10217*)

*Agave vizcainoensis* Gentry (Figs. 29 & 38)

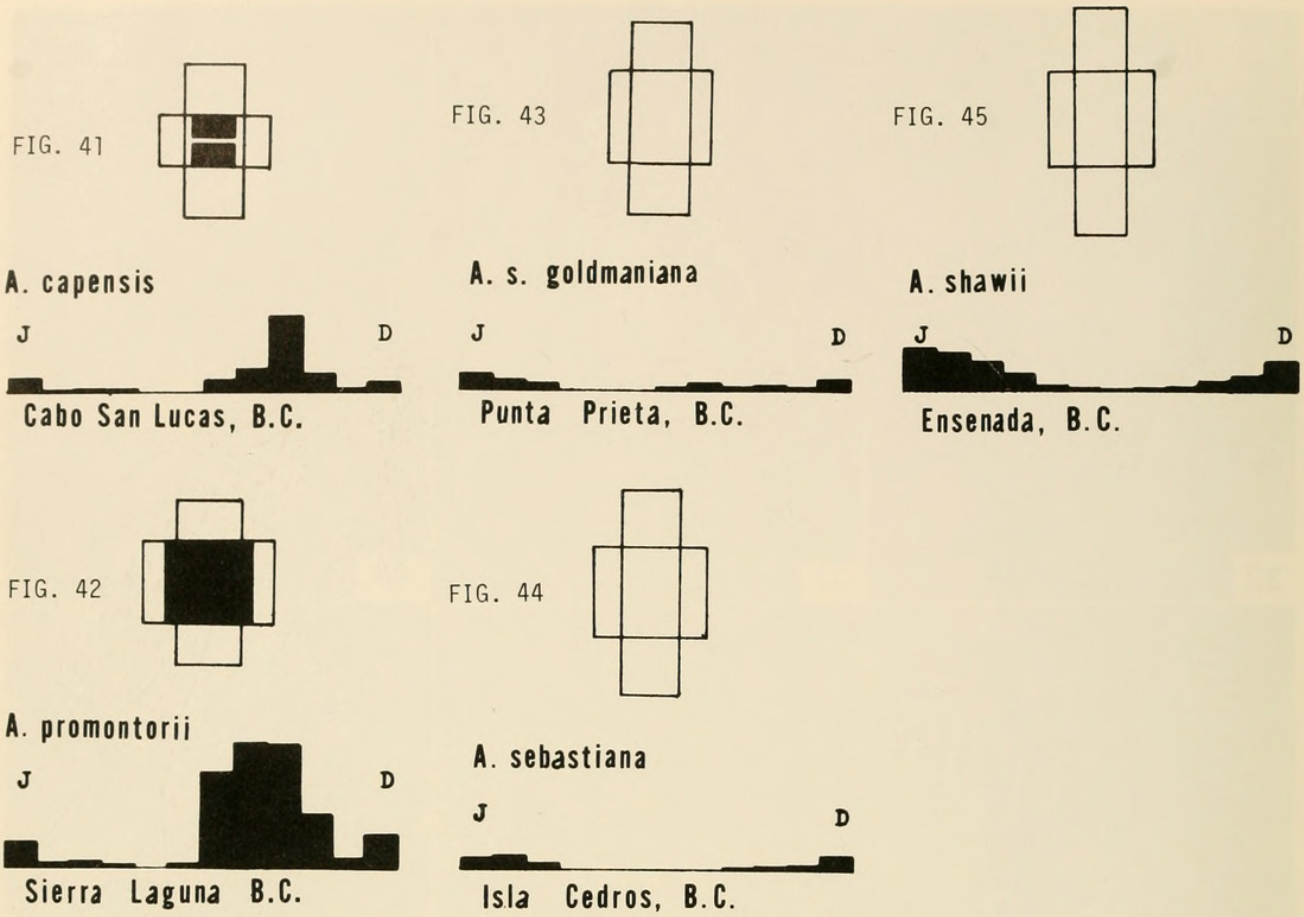
Five planes in surface view, the margin of the surface pore elongate, nearly entire, slightly longer and broader than the stomatal complex; stomatal complex 160–172μ long, 65–73μ wide; polar lips 2, narrower than the lateral lips, 26–31μ wide, constricted toward base, far apart, never touching; lateral lips 2, broad, 54–60μ wide,




FIGURES 23 TO 31. Ideographs of the *Agave* stomatal complexes with rainfall silhouettes of *Agave* habitats. Fig. 23. *A. mckelveyana*. Fig. 24. *A. moranii*. Fig. 25. *A. sobria frailensis*. Fig. 26. *A. sobria roseana*. Fig. 27. *A. sobria sobria*. Fig. 28. *A. subsimplex*. Fig. 29. *A. vizcainoensis*. Fig. 30. *A. fortiflora*. Fig. 31. *A. aurea*.




FIGURES 32 TO 40. Surface views of the *Agave* stomatal complexes. Fig. 32. *A. mckelveyana*. Fig. 33. *A. moranii*. Fig. 34. *A. sobria frailensis*. Fig. 35. *A. sobria roseana*. Fig. 36. *A sobria sobria*. Fig. 37. *A. subsimplex*. Fig. 38. *A. vizcainoensis*. Fig. 39. *A. fortiflora*. Fig. 40. *A. aurea*.




FIGURES 41 TO 45. Ideographs of the *Agave* stomatal complexes with rainfall silhouettes of *Agave* habitats. Fig. 41. *A. capensis*. Fig. 42. *A promontorii*. Fig. 43. *A. shawii goldmaniana*. Fig. 44. *A. sebastiana*. Fig. 45. *A. shawii shawii*.

close together but not touching; inner aperture 65–72$\mu$ long, 22–30$\mu$ wide, stoma 35–39$\mu$ long, 10–15$\mu$ wide; channelling absent, surface smooth. Rim present, distinct. (*Gentry 10339, 7469*)

**Agave fortiflora* Gentry (Figs. 30 & 39)

Five layers in surface view; margin irregular due to cuticular papillae, similar to many Deserticolae in general aspect. Stomatal complex 105–134$\mu$ long, 83–99$\mu$ wide; polar lips prominent, larger than the lateral lips, open or touching, 35–45$\mu$ wide, lateral lips 23–30$\mu$ wide touching or overlapping; inner aperture 43–45$\mu$ long, 14–20$\mu$ wide; stoma 25–30$\mu$ long, 10–15$\mu$ wide; channelling occasional, some polar connections; surface smooth but papillae visible. Rim absent. (*Gentry 11630, 19808*)

Campaniflorae

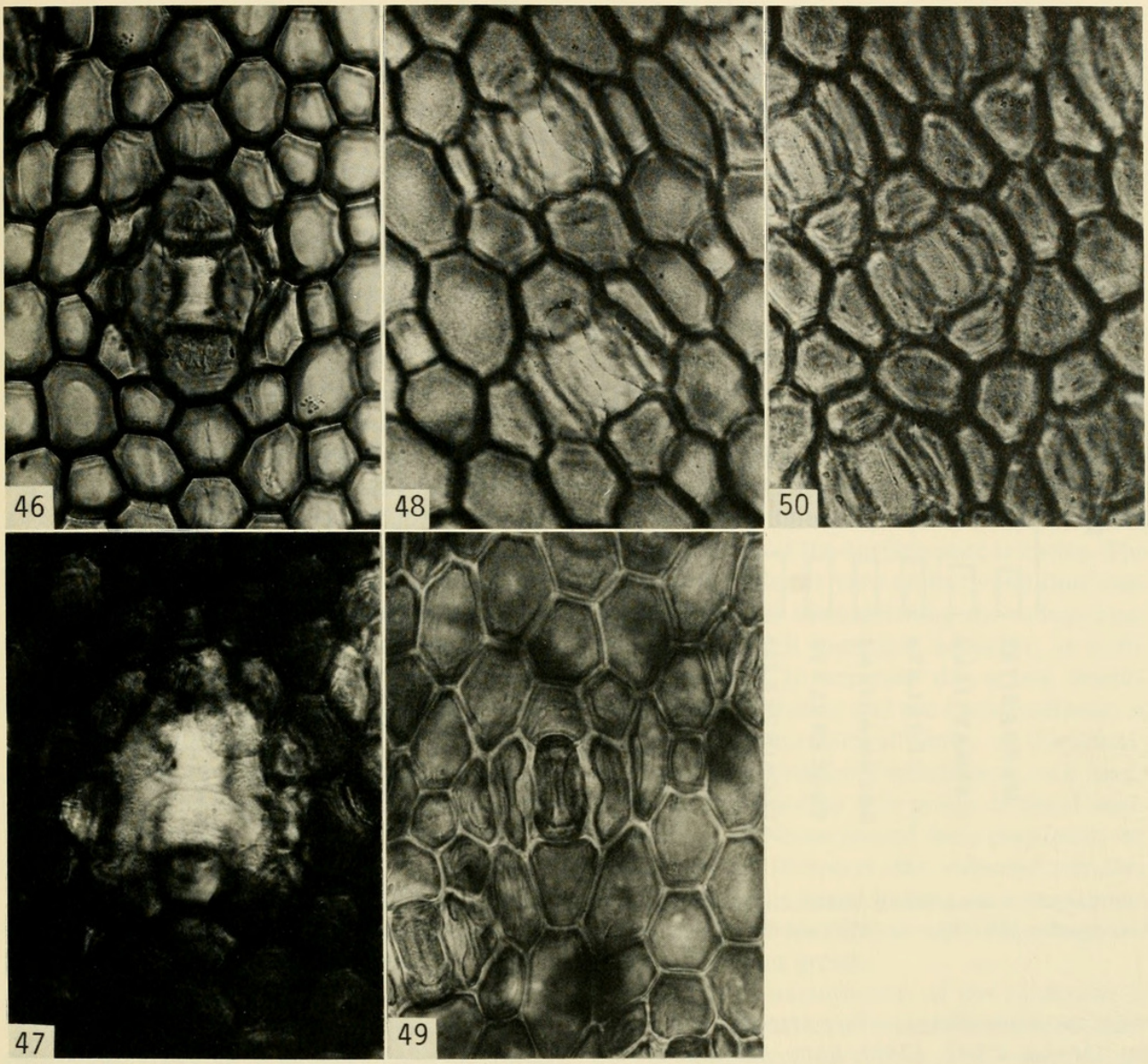
*Agave aurea* Brandegee (Figs. 31 & 40)

Five planes in surface view; margin somewhat irregular and extending out beyond the lateral subsidiary cells, not continuous with the polar lips; stomatal complex 145–160$\mu$ long, 80–92$\mu$ wide, oval in outline; polar lips 2, conspicuous, constricted at base, apart, 40–50$\mu$ wide; lateral lips 2, touching, 35–37$\mu$ wide; inner aperture 47–60$\mu$ long, 20–30$\mu$ wide, oval; stoma 34–40$\mu$ long, 15–18$\mu$ wide; channelling occasional, transverse; surface smooth to slightly wrinkled, papillae when visible, small; cuticle distinctly yellow after staining. Rim absent. (*Gentry 12338*)

*Agave capensis* Gentry (Figs. 41 & 46)

Five planes in surface view; margin as in *A. aurea*, not continuous with the polar lips; stomatal complex 110–135$\mu$ long, 75–85$\mu$ wide, oval; polar lips 2, apart, overlap occasionally, 30–35$\mu$ wide; lateral lips 2, mostly overlapping, larger than the polar lips, 35–40$\mu$ wide; inner aperture 42–50$\mu$ long, 20–28$\mu$ wide; stoma 32–36$\mu$ long, 11–15$\mu$ wide; channelling occasional, transverse; surface smooth with some cutinous ridges; rim absent. (Santa Barbara Botanical Garden)

* Not aligned as to group.




FIGURES 46 TO 50. Surface views of the *Agave* stomatal complexes. Fig. 46. *A. capensis*. Fig. 47. *A. promontorii*. Fig. 48. *A. shawii goldmaniana*. Fig. 49. *A. sebastiana*. Fig. 50. *A. shawii shawii*.

*Agave promontorii* Trelease (Figs. 42 & 47)

Five planes in surface view; margin very irregular with as many as 8 cuticular papillae obscuring the lips below; stomatal complex 110–145$\mu$ long, 80–87$\mu$ wide; polar lips 2, broad, overlapping, 60–72$\mu$ wide (nearly 2× those of *capensis*); lateral lips 2, touching, 35–45$\mu$ wide, narrower than the polar lips; inner aperture 40–54$\mu$ long, 28–33$\mu$ wide, square to rectangular, stoma obscure, channelling occasional, transverse, surface more or less smooth; rim absent. (*Gentry 11218*)

Umbelliflorae

*Agave shawii* ssp. *goldmaniana* (Trelease) Gentry (Figs. 43 & 48)

Three levels of change in surface view, the surface pore formed by the lateral lips, entire, keyhole-shaped; stomatal complex elongate, 140–190$\mu$ long, 68–88$\mu$ wide, polar lips absent, lateral lips prominent, apart or touching, 46–75$\mu$ wide; inner aperture 65–82$\mu$ long, 37–42$\mu$ wide, stoma 40–45$\mu$ long, 16–20$\mu$ wide; channelling absent, surface smooth, papillae absent. Rim present. (Desert Botanical Garden)

*Agave sebastiana* Greene (Figs. 44 & 49)

Not distinguishable from ssp. *goldmaniana*. Three levels of change in surface view; stomatal complex 110–160$\mu$ long, 72–82$\mu$ wide, polar lips essentially absent, lateral lips prominent, 40–55$\mu$ wide, open; inner aperture oval, 56–63$\mu$ long, 27–30$\mu$ wide; stoma 35–36$\mu$ long, 18–25$\mu$

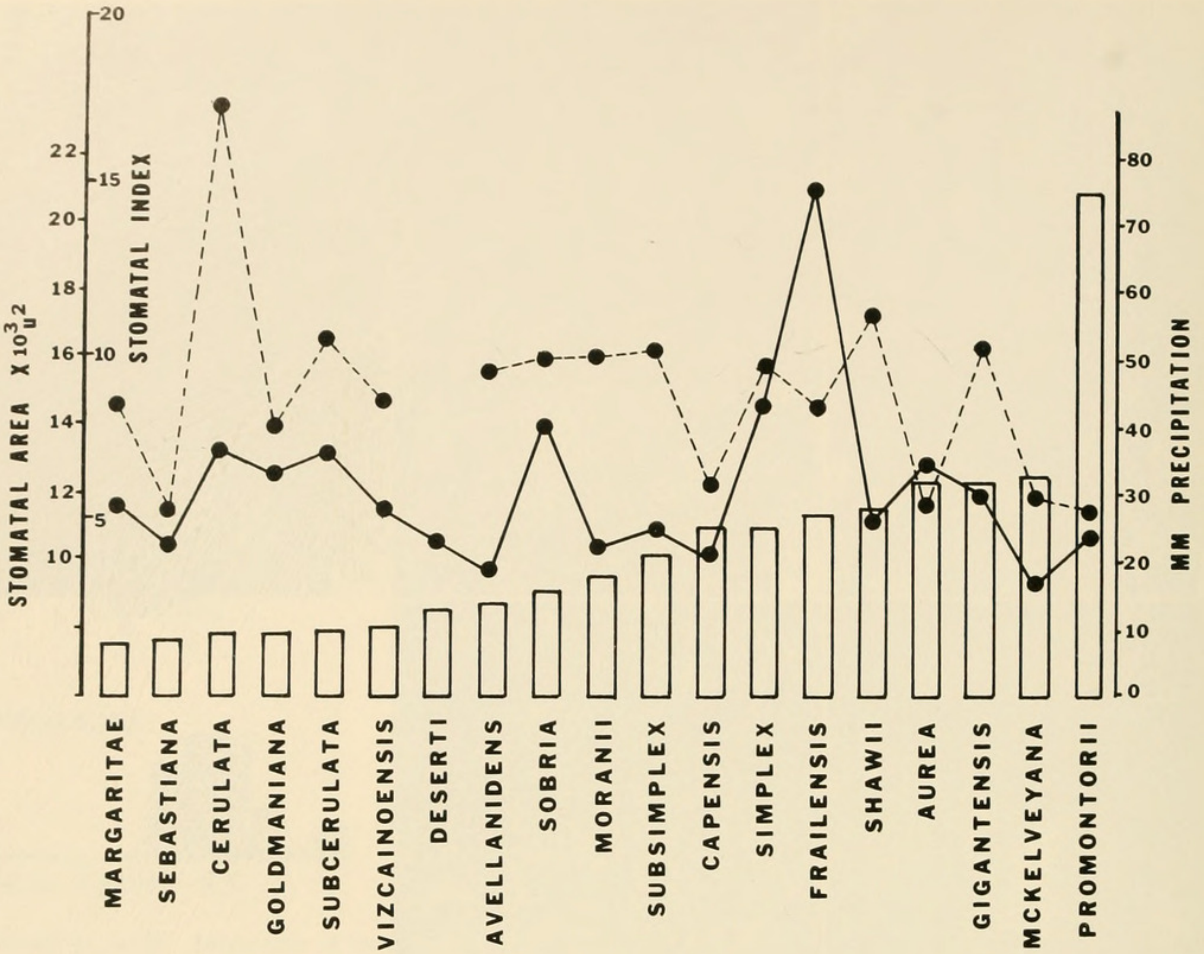



FIGURE 51. Average annual rainfall of *Agave* habitats compared with stomatal surface areas (data from Table 2; for example, *A. margaritae* 135 × 87 = 11,745$\mu^2$) and the stomatal index (I = S/(E + S) × 100), after Wicks (1935).

wide; no channelling, surface smooth. Rim present. (*Moran 15142, 17430; Beauchamp 2095*)

*Agave shawii* Engelman ssp. *shawii* (Figs. 45 & 50)

Very similar to the above subspecies. Stomatal complex elongate, 137–150$\mu$ long, 67–83$\mu$ wide; polar lips absent or rudimentary, lateral lips prominent, 45–60$\mu$ broad; inner aperture 42–60$\mu$ long, 30–35$\mu$ wide, stoma 34–39$\mu$ long, 20–21$\mu$ wide; channelling absent, surface smooth. Rim present. (*Gentry 10397*)

## Discussion and Conclusions

Certain conclusions can be drawn regarding the value of the stomatal cell complex for systematic studies. We found, as did McClendon (1908), that in many taxa the surface aperture (level of the polar lips) of the suprastomatal passage is usually elongated at right angles to the long axis of the leaf, and farther down (level of the lateral lips) it is elongated parallel to the long axis of the leaf. Blunden et al. (1973) observed the reverse in *A. ellemeetiana*. We have no material of this species, but we have observed in others that the surface pore may appear elongated parallel to the long axis of the leaf if the polar lips are absent or rudimentary—as in all the taxa of the Umbelliflorae and in *A. subsimplex*.

The presence of four cells surrounding the guard cells appears to be a constant character in over 80 species of *Agave*, if we include our data with those of Blunden et al. (1973). That the stomatal complex is only morphologically tetracytic must be emphasized, as our observations refer to the appearance of the stomata when fully differentiated.

A puzzling feature described by Blunden et al. (1973) for several agavaceous genera and by Blunden and Binns (1970) for *Yucca glauca* is

the presence of beaded cell walls. We have observed no such structure in our work with *Agave*. It is possible that the epidermal portion containing the beaded cell walls is not obtained in leaf peelings.

Taxonomic use of the epidermal characters can be considered on the sectional, specific, and subspecific levels. The stomata of the Umbelliflorae, Campaniflorae, and Deserticolae are all distinct from one another, as represented by their respective ideographs and by their surface views (Figs. 5–50).

Most of the stomatal elements or characters are common to all three groups, but a few are lacking in one or another group. While stomatal rims are lacking in the Campaniflorae, they are present in the Umbelliflorae, and absent or present in the Deserticolae. While the lack of a single element may not be sufficient to support group status, it becomes notably significant when supported by other characters. Variations in the lips, margins, and number of changes in levels of surface view of the stomata are especially useful in distinguishing groups. The Umbelliflorae and Campaniflorae are small distinct groups, but the Deserticolae is large, widespread, and complex in the variability displayed by its seventeen taxa.

In the species of the Deserticolae—a fairly coherent group macromorphologically—we have found a surprising amount of diversity in stomatal structure. Major features of the stomatal complex were found to be constant for a species, while variation between species was often apparent. Species such as *A. cerulata* and *A. deserti* possess thick cuticles with elaborate surfaces, deeply depressed stomata, and conspicuous polar and lateral lips which tend to obscure the suprastomatal chamber. In contrast, *A. sobria frailensis*, *A. sobria roseana*, *A. subsimplex*, and *A. vizcainoensis* possess relatively smooth surfaces, with stomata not deeply depressed, and poorly developed polar lips leaving the suprastomatal chamber below "unprotected."

In order to distinguish species of the Deserticolae, it was found necessary in most cases to use a combination of leaf-surface characters with the variations in the lips, margins, and number of changes in the levels in surface view of the stomata. For instance, *A. cerulata* and *A. deserti* can be distinguished from *A. sobria roseana* and *A. sobria frailensis* by the presence of a rim in the latter two subspecies. *A. subsimplex* can be distinguished from all other Deserticolae in having four planes in surface view, a rim, and the margin of the surface pore formed by the lateral lips. Other species were not found separable by stomatal characters, as the two complexes represented by *A. deserti* and *A. cerulata*, both of which have highly variable overlapping subspecific forms.

In the Campaniflorae, *A. aurea* can be distinguished from *A. capensis* in that the polar lips of *aurea* are 40–50$\mu$ wide, while those of *capensis* are 30–35$\mu$ wide. *A. promontorii* is distinguished from the first two not only by its wider polar lips (60–72$\mu$), but also by its thicker cuticle and strongly overlapping polar lips.

In the Umbelliflorae the polar lips are absent and the margin of the surface pore is entire. The stomatal complex is very uniform and thus cannot be used for distinguishing the subspecies. The stomatal-cell complex, however, is sufficiently distinct to separate this group readily from the Deserticolae and the Campaniflorae. A species with uncertain affinities, *A. fortiflora*, was compared with the Umbelliflorae, as it bears some resemblance to this group in floral morphology. It had been placed there previously by Gentry (1972). However, the stomatal complex of *A. fortiflora* is found to bear no resemblance to that of the Umbelliflorae and it has been removed from that group.

Microscopic examination of the epidermis is frequently of particular value in the identification of species. This is true with isolated leaf specimens, which in form or armature may be atypical of a given species. For instance, *Howell 10660*, a leaf specimen from San Bartoleme Bay, resembles in size and armature *A. margaritae*, but examination of the stomata shows it to be conspecific with *A. vizcainoensis*. As another example, the latter taxon, on gross morphological appearance and smooth cuticle, was first judged to be a part of *A. gigantensis*, but when stomatal structure was examinaed, *A. vizcainoensis* was set aside as a separate species.

It would be possible to classify *Agave* on epidermal criteria alone (cf. Müller 1909), but the relationships would appear very different from those obtained on the criteria of leaf and inflorescence morphology. A sound taxonomic system must be built on a broad range of criteria. Epidermal criteria are a valuable supplement to

classical morphology and to the newer guides of growth and breeding habits.

The thick cuticles with elaborate surfaces, deeply depressed stomata, and conspicuous polar and lateral lips, which tend to obscure or cover the suprastomatal chamber, appear to be xeromorphic adaptations for desert survival. This observation is discussed below.

*Cuticular Structure and the Environment*

The cuticle of *Agave* is a remarkable structure forming a translucent envelope over the functional cells within the leaf. It appears to diffuse sunlight, insolate against extreme temperatures, protect against winds and mechanical abrasion, reduce transpiration, and support the succulent, heavy leaf as a whole. The unusual thickness and other characteristics of the cuticle suggest that it has evolved in response to the aridity of the environments in which most of the species occur. McClendon (1908) paid particular attention to this theme and observed that the more xerophytic species of *Yucca*, *Nolina*, *Dasylirion*, and *Agave* had specialized stomatal structures. He states that the suprastomatal passages are guarded by four lips, which with the associated subsidiary cells, narrow or close the passage in several species, e.g., *Yucca elata*, *Y. rostrata*, *Agave victoriae-reginae*, *A. schottii*. *Nolina*, as well as some *Agave* species, have stomata aligned in deep grooves. These structures, he noted, served by "protecting the stomata from the dry air" (McClendon 1908:316).

In Figures 5 to 50 we have provided rainfall silhouettes along with stomatal ideographs and surface views of the stomata. Rainfall data were extracted from the National Climatic Center (1973) for Arizona, and Hastings (1964a and 1964b) for Mexico. With these we hope to call attention graphically to possible correlation between stomatal morphology and rainfall patterns, the most critical environmental factor for desert plants. Detailed conmparisons between environmental factors and epidermal structure is beyond the scope of this study, but a few observations can be made.

The rainfall profiles show that all stations, except for one or two, and their nearby *Agave* populations are strictly desertic. One certain exception is the Sierra Laguna station in the Cape District, with 747 mm of annual rainfall; it is the montane habitat of *Agave promontorii*, a species associated with forests. For several Baja California stations the available records are short term, 5–7 years, and therefore of limited reliability. The one for *A. cerulata* ssp. *nelsonii*, San Fernando on Sierra San Miguel, is obviously rejectable because of its low figure of only 66 mm for average annual rainfall. Generally, we can see no correlations between rainfall patterns and variations in stomatal structure. This is the case for annual rainfall, length of dry season, and for both winter-rainfall season and summer-rainfall season. The measurements of stomatal structures are also noncorrelative with rainfall patterns, as for instance, the average surface area of the stomatal complex ($120\mu \times 80\mu = 9{,}600\mu$ for *A. avellanidens*, and so on for the rest) (Fig. 28).

On the basis of rainfall data we do not find, as McClendon (1908) observed, that closure of the suprastomatal passage is a xeromorphic adaptation. *A. promontorii*, for instance, has the surface opening completely closed by the polar lips (Fig. 47), but its habitat is by far the most watered of all our subject species. Taxa of the *A. shawii* group are all characterized by lipless open stomatal surface openings (Figs. 48–50) in a smooth cuticle of moderate thickness, yet both *sebastiana* and *goldmaniana* occupy the drier habitats having less than 100 mm of annual rainfall. However, these three taxa occupy the heavy fog belt along the outer peninsular coast. Their distinctive, elongate, lipless stomata may well reflect structurally the sustaining dew of the fog-desert. This apparent correlation is also supported by the relatively open lips and shallowly depressed stomata in smooth cuticular surfaces observed in *A. sobria* ssp. *frailensis*, *A. sobria* ssp. *roseana*, and *A. vizcainoensis* (group Deserticolae), all of which are maritime dwellers where fog or high humidity are relatively frequent. However, *A. moranii*, with similar stomatal structure, is an inland species on the very arid eastern slopes of the Sierra San Pedro Mártir. There are no fog or humidity data available to verify these observations.

At this point we cannot argue for or against the concept of epidermal structure having developed in response to desert aridity. It is apparent that we need to know much more about both structure and function as well as climatological and other factors distinguishing habitats. For instance, the habit of day closure–night

opening of stomates may be more significant in desertic evolution than the structures discussed above. This habit reduces transpiration, conserves moisture, and contributes towards survival in arid climates. Many succulents, including agaves, have been found with this physiologic habit, known as CAM (for Crassulacean Acid Metabolism). There is a rapidly growing literature on this subject (see Mooney et al. 1974; Szarek and Troughton 1976).

The agave cuticle appears to function like an air-conditioned greenhouse protecting the tender, living leaf tissue from desertic extremes. The general characteristics of this structure are common to all agaves and some other related genera. However, the stomates of the two narrow endemic groups, Umbelliflorae and Campaniflorae, have distinctive features. Just how the structure and function of their stomates are correlated with their narrow environments is not clear at this writing. Nor is it clear how or if these structures have any survival value for the species. Some ideas need testing, others appear as self-evident truths, and still others are transitional to other ideas. We hope that the idea about protective xeromorphic stomates will stimulate further research.

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