

# Pollen Morphology of Some *Vaccinium* Species and Their Hybrids

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**Abstract.** Pollen morphology of 10 *Vaccinium* species, 10 interspecific hybrids, and 3 colchicine-induced polyploids were studied using scanning electron microscopy. Size, exine patterning, furrow and pore size and shape, and general appearance were investigated. Although differing morphological features were generally lacking, some interspecific differences and variation within hybrid populations were noted. Furrow and pore sizes, and the associating opericuli were the most valuable characters. Some species had large furrows with large rounded pores and others had small furrows with insignificant pores. Exine patterning was minimal and, when present, generally occurred in the intercolporate regions. F<sub>1</sub> populations of *V. corymbosum* × *V. elliotii* had the greatest exine variation and the degree of exine patterning revealed a possible dosage effect. Exine patterns suggest that the ancestors of *V. myrsinites* may be *V. darrowi* and *V. elliotii* rather than *V. darrowi* and *V. tenellum* as previously proposed by Camp.

Scanning electron microscopy (SEM) studies on pollen morphology have proven to be a useful plant identification tool for taxonomists and horticulturists. They can provide a means of distinguishing between closely related species and clones (4, 5, 13) and of determining phylogenetic relationships at both specific and generic levels (12). Size and shape of the grain, furrow and pore number and appearance, and exine patterning are some characteristics commonly used (11).

The genus *Vaccinium* is comprised of a large number of species and 3 ploidy levels. A previous study (12) revealed differences in size and exine patterning among 3 interspecific crosses, but no taxonomic relationships were concluded. The present study was conducted to characterize the pollen of the various *Vaccinium* species, interspecific hybrids, and colchicine-induced polyploids for purposes of determining inter- and intraspecific differences, comparing hybrids and parental species, studying the effects of polyploid induction, and providing information about inheritance and taxonomic relationships.

## Materials and Methods

Plant material was obtained from the germplasm collection of the Univ. of Florida and included 10 species, 10 interspecific crosses, and 3 colchicine-induced polyploids. The plants ranged in chromosome number from  $2n = 2x = 24$  to  $2n = 12x = 144$  (Table 1). In most cases 3 clones from each group were sampled to determine intraspecific variation. Pollen was collected at anthesis from field- or greenhouse-grown plants in Spring 1983. Pollen was removed from the anthers and placed on polished aluminum stubs covered with double-faced transparent tape. Samples were air-dried and stored in desiccators prior to and after coating. Samples were sputter-coated with gold to a thickness of about 400 nm using a Giko Engineering IB-2 Ion Coater. Pollen was examined with an Hitachi S-450 SEM at an operating voltage of 20 kV (7). Photomicrographs using Polaroid Type 55 film were taken of groups of pollen at ×420

to assess pollen fertility; of individual pollen grains at ×1200, ×1500, or ×2000 to study general morphology of the grains; and at ×15,000 to determine exine patterning.

## Results and Discussion

**Species.** Pollen analysis of the 10 species studied revealed few intra- or interspecific differences. The 4 end products of meiosis remained together in a tetrad, as is characteristic of *Vaccinium*, and individual pollen grains were tricolporate for all species.

The greatest intraspecific differences were observed in the fertility of the different clones. This was most pronounced in *V. atrococcum* (Fig. 1A and B) but also was observed in *V. elliotii*, *V. stamineum*, and *V. ashei*. Of the 3 *V. ashei* clones observed, the one cultivar ('Bonita') appeared to be more fertile than the 2 wild clones which had many poorly filled pollen grains, suggesting a possible correlation between general fertility and breeder selection.

Many of the wild plants examined appeared to have a surprisingly low fertility level as shown by poorly filled tetrads, especially in *V. darrowi* (Fig. 1C), *V. fuscatum* (Fig. 1D), and *V. ashei* (Fig. 1E). Fertility of wild plants would be expected to be higher due to the effects of natural selection. The low fertility observed in our plants may have various causes. Low fertility of polyploids (*V. fuscatum* and *V. ashei*) could indicate their recent amphidiploid evolution from other species. The diploids (*V. darrowi* and *V. elliotii*) previously have proven to be lower in fertility than other ploidies (3) and may reflect a high rate of gene flow between diploid species. Alternatively, the low fertility could be explained if the diploids are actually tetraploids of extinct  $2x = 12$  species, as some research has indicated (1, 8, 14). Sterility problems have been noted in other *Vaccinium* species by other workers (9, 10).

Intraspecific differences were slight, except for differences in apparent pollen fertility. For the 3 clones examined of each species, the pollen usually had the same general shape, size, exine patterning, and furrow and pore type. An exception was *V. stamineum*; one of the *V. stamineum* clones had tetrads that were distinctly triangular (Fig. 2A) compared to the rounded tetrads of the other 2 (Fig. 2B). Pollen from the triangular tetrads was also slightly concave in the intercolporate region; however,

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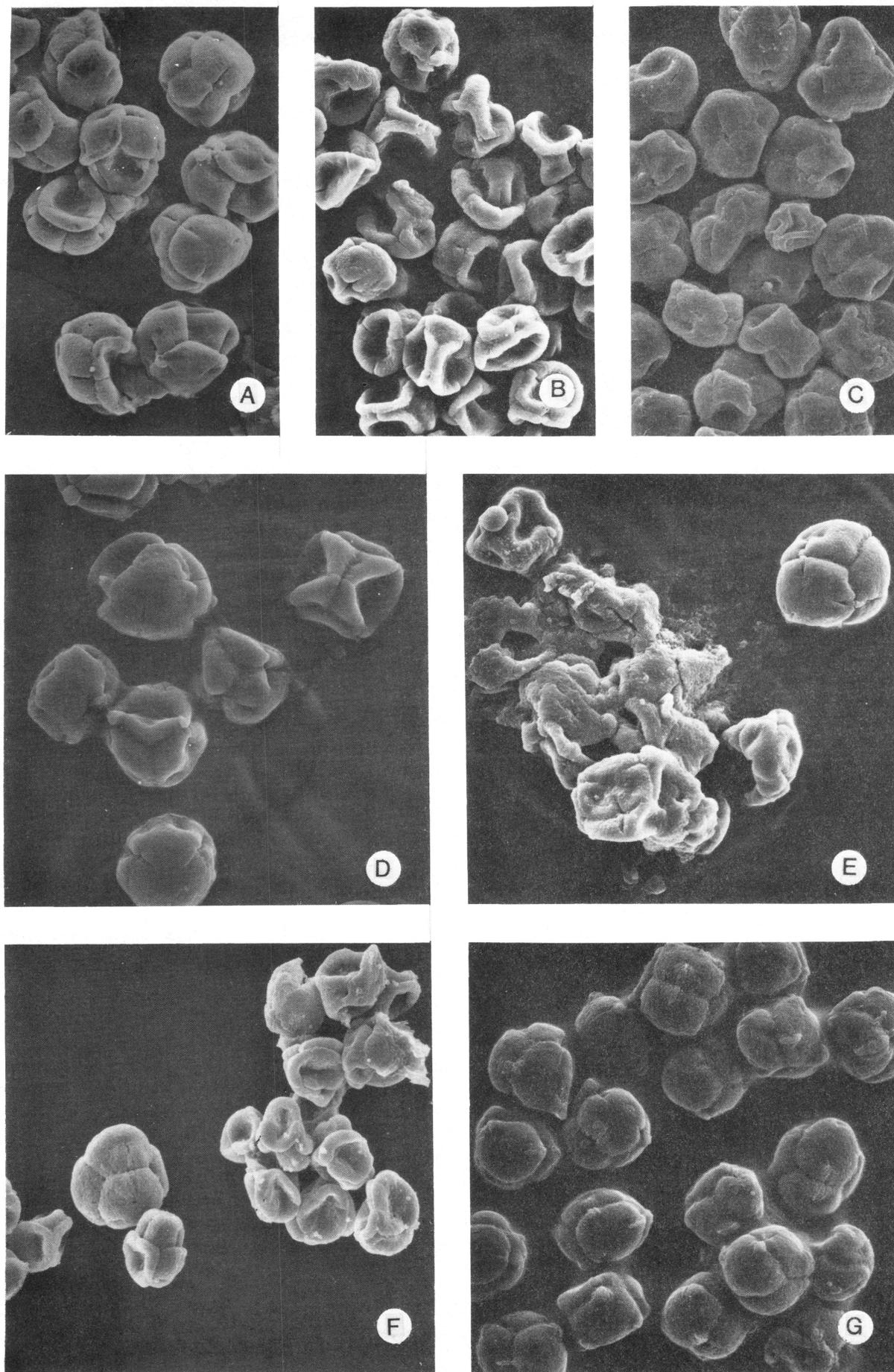


Fig. 1. Pollen tetrads of *Vaccinium* species and intraspecific hybrids magnified  $\times 412$ . (A) *V. atrococcum*. (B) *V. atrococcum*. (C) *V. darrowii*. (D) *V. fuscatum*. (E) *V. ashei*. (F) *V. corymbosum*  $\times$  *V. elliottii*. (G) *V. corymbosum*  $\times$  *V. elliottii*.

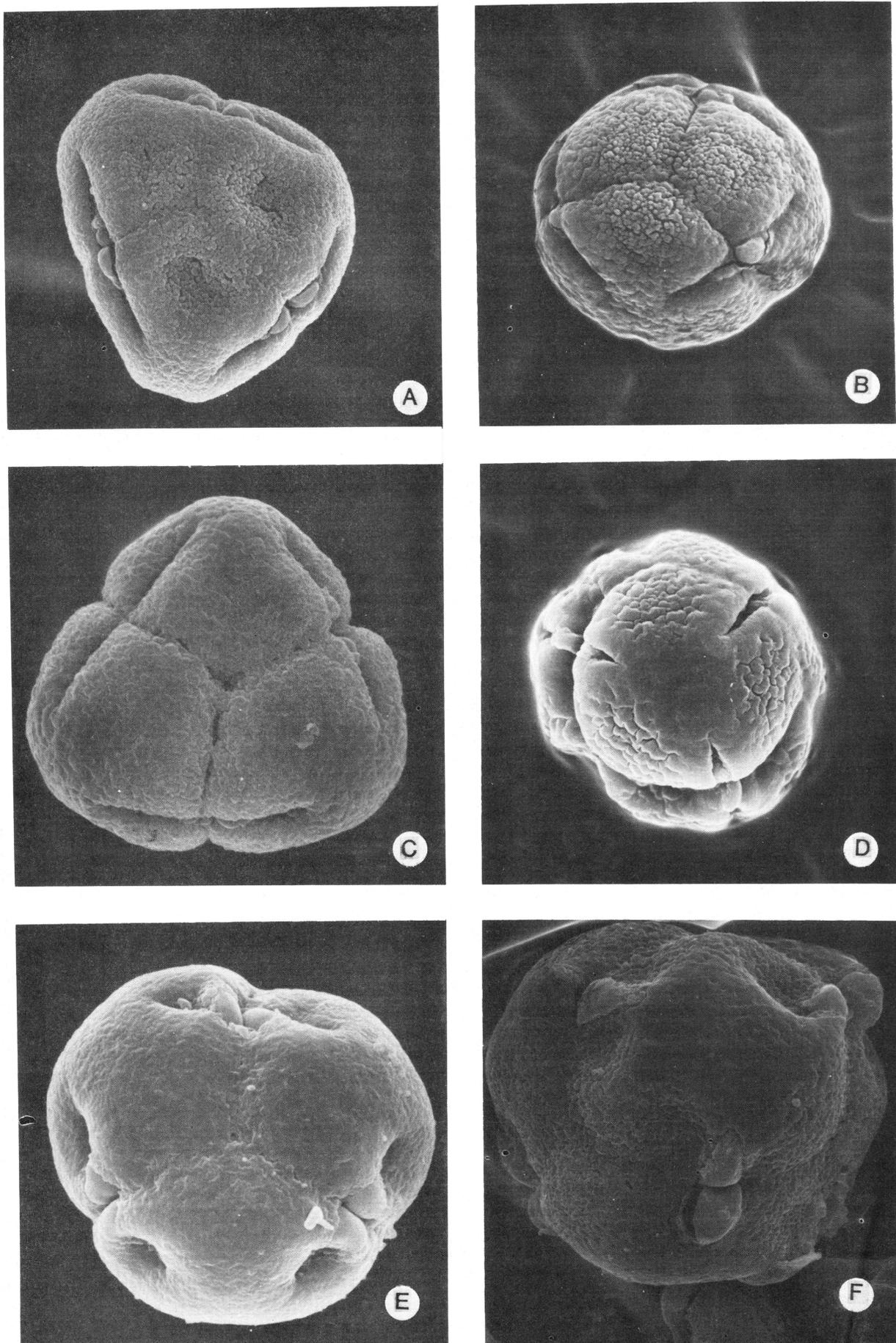


Fig. 2. Individual pollen tetrads of *Vaccinium* species and hybrids magnified  $\times 1470$ . (A) *V. stamineum*. (B) *V. stamineum*. (C) *V. ashei*. (D) *V. atrococcum*. (E) *V. corymbosum*. (F) *V. corymbosum* x *V. elliotii* hybrid.

Table 1. Plant material and their chromosome numbers.

Species <sup>z</sup>	Chromosome number (2n)	Interspecific Hybrid	Chromosome number (2n)
<i>V. arboreum</i> Marsh	24	<i>V. atrococcum</i> × <i>V. elliotii</i>	24
<i>V. atrococcum</i> Heller	24	<i>V. darrowi</i> × <i>V. elliotii</i>	24
<i>V. darrowi</i> Camp	24	<i>V. darrowi</i> × <i>V. tenellum</i>	24
<i>V. elliotii</i> Chap.	24	<i>V. elliotii</i> × <i>V. stamineum</i>	24
<i>V. stamineum</i> L.	24	<i>V. corymbosum</i> × <i>V. elliotii</i>	36
<i>V. tenellum</i> Aiton	24	<i>V. corymbosum</i> × <i>V. elliotii</i>	48
<i>V. corymbosum</i> L.	48	<i>V. corymbosum</i> × <i>V. darrowi</i>	48
<i>V. fuscatum</i> Aiton	48	<i>V. corymbosum</i> × <i>V. myrsinites</i>	48
<i>V. myrsinites</i> Lam.	48	<i>V. ashei</i> × <i>V. darrowi</i>	60
<i>V. ashei</i> Reade	72	<i>V. corymbosum</i> × <i>V. ashei</i>	60
Fla 83-59 <sup>y</sup>	144	Fla 80-46 <sup>x</sup>	96
		US 40 <sup>w</sup>	120

<sup>z</sup>According to Camp (2).

<sup>x</sup>Doubled *V. ashei* 'Bluebelle'.

<sup>y</sup>Doubled *V. corymbosum* × *V. fuscatum* (may simply be the southern form of *V. corymbosum*).

<sup>w</sup>Doubled *V. ashei* × *V. corymbosum*.

furrow size and exine patterning did not appear to vary greatly among the 3 *V. stamineum* seedlings.

The largest interspecific differences were noted in exine patterning and furrow and pore size. Differences in pollen size also were noted among species, with species of higher ploidy generally having larger sized pollen. Cockerham and Galletta (3) found a similar relationship, but in our case it was not a consistent character and could not be used for reliable species separation.

Exine patterning of *Vaccinium* pollen is not elaborate and ranges from the psilate (smooth) surface of *V. corymbosum* (Fig. 3A) and *V. fuscatum*, to the fossulate (deeply fissured) surface of *V. atrococcum* (Fig. 3B). Grains of *V. ashei* (Fig. 2C), and to a lesser extent, *V. elliotii*, tended to be patterned over the entire surface. The other species that exhibited patterns did so primarily in the intercolporate regions of the pollen grain with the area surrounding the pores and the "pore" itself being relatively smooth, as in *V. atrococcum* (Fig. 2D).

The outermost exine layer (tectum) in some cases showed distinctive patterning, as on *V. elliotii* and *V. arboreum*. The tectum of *V. arboreum* was distinctly different from that of the other species and was characterized by a foveolate surface with secondary striations (Fig. 3G). This may indicate that *V. arboreum* is more distantly related to the other species.

Furrow and pore size ranged from the large, concave type observed on *V. corymbosum* (Fig. 2E) to the smaller furrows and pores of *V. atrococcum* (Fig. 2D). The operculum, however, was observed easily in most of the species, regardless of pore type.

*Interspecific hybrids.* Interspecific hybrids showed much within-cross variation, as would be expected from a hybrid population between highly heterozygous plants. The population which

showed the greatest amount of variation was *V. corymbosum* × *V. elliotii*. Part of this variation may be attributed to differences in ploidy level among the hybrids. Progeny from this cross included both triploids and tetraploids, the 2 classes arising from fertilization of 2x *V. corymbosum* gametes by normal (x) or unreduced (2x) gametes of *V. elliotii*. The most obvious within-population difference was in fertility, which depended largely on ploidy. Triploids usually had pollen that was shrunken and poorly filled (Fig. 1F) compared to pollen from tetraploids (Fig. 1E). The well-filled tetrad present in Fig. 1F may contain 4 unreduced gametes as a result in a premeiotic doubling of a microsporocyte, in which case each grain would be 3x. If this is true, then the plant could cross with *V. ashei* and act as a bridge between *V. ashei*, *V. corymbosum*, and *V. elliotii*.

Exine patterning for the *V. corymbosum* × *V. elliotii* population also appeared to vary with ploidy level. Triploids generally had a smooth exine while the exine of tetraploids was more deeply fissured. Since patterning of pollen of the tetraploids resembled *V. elliotii* patterning, and since these tetraploid hybrids contain 2 *V. elliotii* genomes, there may be a dosage effect in the inheritance of exine patterning in *Vaccinium*.

Furrow and pore type varied both among and within ploidy levels. Some pollen grains from triploids had furrows with no obvious pores, while others had pores (Fig. 4E). Tetraploids generally had larger furrows with prominent pores which varied in shape from the smooth, rounded type in Fig. 2F to the flatter, striated type of Fig. 4A.

The hybrids from the other crosses were generally characterized by large amounts of infertile pollen. Degree of infertility did not seem to depend on whether the hybrid was from a homoploid or heteroploid cross. The cross that appeared to be most fertile was *V. darrowi* × *V. elliotii* (Fig. 5A) and the least fertile was *V. elliotii* × *V. stamineum* (Fig. 5B). The latter was not surprising since *V. stamineum* is in the section *Polycodium* of *Vaccinium*, whereas *V. elliotii* and all of the other species in this study except *V. arboreum* are in section *Cyanococcus*.

Progeny from homoploid crosses involving *V. elliotii* generally resembled *V. elliotii* in exine patterning. The exception was *V. elliotii* × *V. atrococcum*, where the hybrid resembled neither parent, even though both are well-fissured. The best example of where exine patterning suggested an unexpected relationship was in *V. elliotii* (Fig. 3H), *V. darrowi* (Fig. 3C), and their hybrid (Fig. 3D). Exine patterning of the hybrid resembled that of *V. myrsinites* (Fig. 3E); however, the proposed ancestors of *V. myrsinites* are *V. darrowi* and *V. tenellum* (6). Exine patterning on *V. darrowi* × *V. tenellum* hybrids (Fig. 3F) does not resemble that of *V. myrsinites* as closely as that from *V. darrowi* × *V. elliotii* hybrids. *V. darrowi* × *V. elliotii* hybrids also have a more prominent operculum (Fig. 5A) than either parent and resemble that of *V. myrsinites* (Fig. 5C). An effort should be made to double the chromosome number of *V. darrowi* × *V. elliotii* hybrids to see if their pollen resembles *V. myrsinites* pollen.

It was difficult to get reliable observations for furrow and pore sizes of the hybrids because the area was often misshapen as a result of poorly filled pollen grains. Exine patterning of the tetraploid hybrids always appeared intermediate between the parents. It was never as smooth as *V. corymbosum* but it also was never as fissured as the other parent. Exine patterning of pentaploid hybrids was intermediate for *V. corymbosum* × *V. ashei*. *Vaccinium ashei* × *V. darrowi* hybrids (Fig. 4B) resembled *V. ashei* in that they had similar patterning, which was present over the entire surface of the grain.

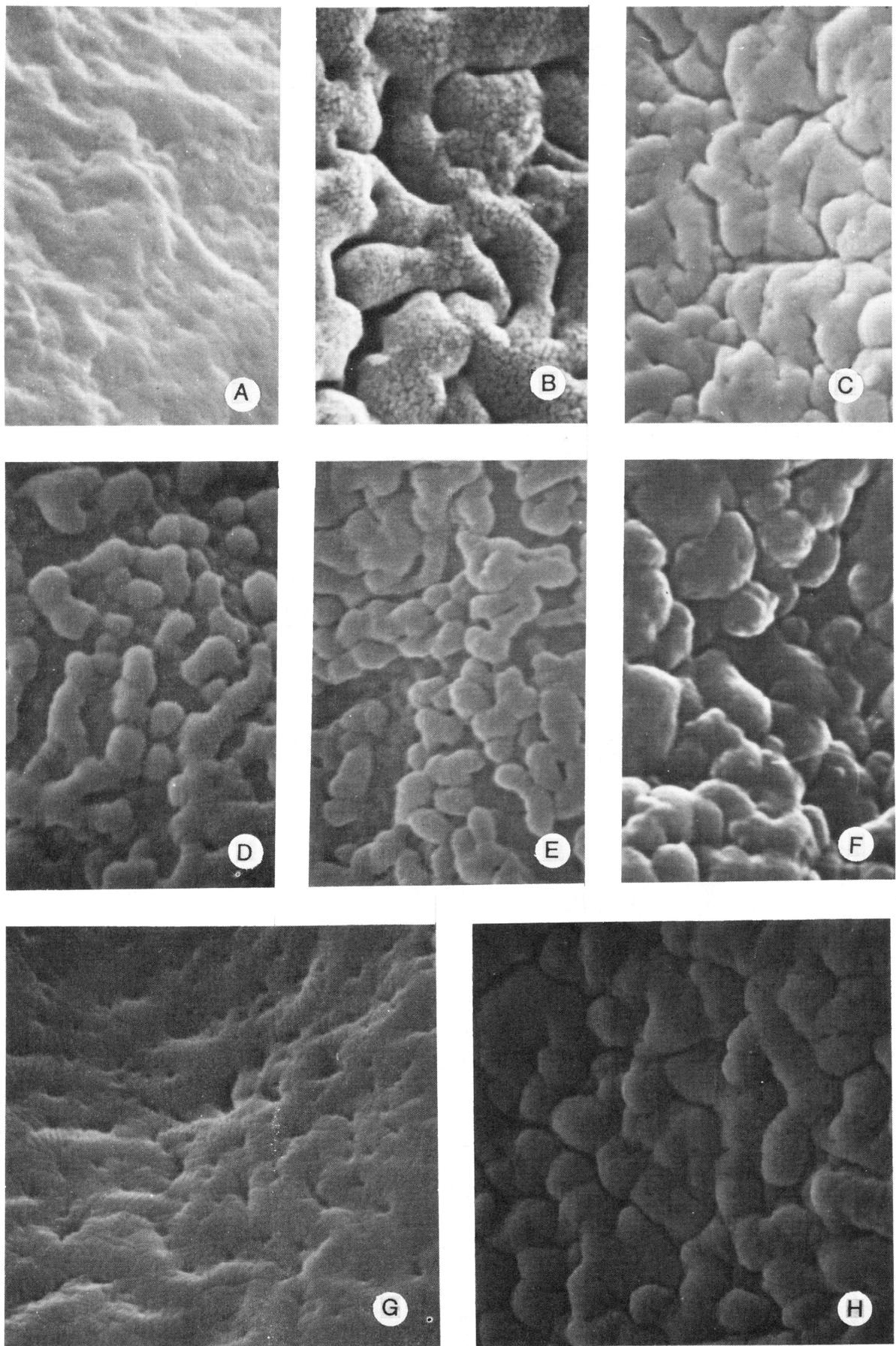


Fig. 3. Surface structures of *Vaccinium* species and interspecific hybrids magnified  $\times 14,400$ . (A) *V. corymbosum*. (B) *V. atrococcum*. (C) *V. darrowi*. (D) *V. darrowi*  $\times$  *V. elliotii* hybrid. (E) *V. myrsinites*. (F) *V. darrowi*  $\times$  *V. tenellum* hybrid. (G) *V. arboreum*. (H) *V. elliotii*.

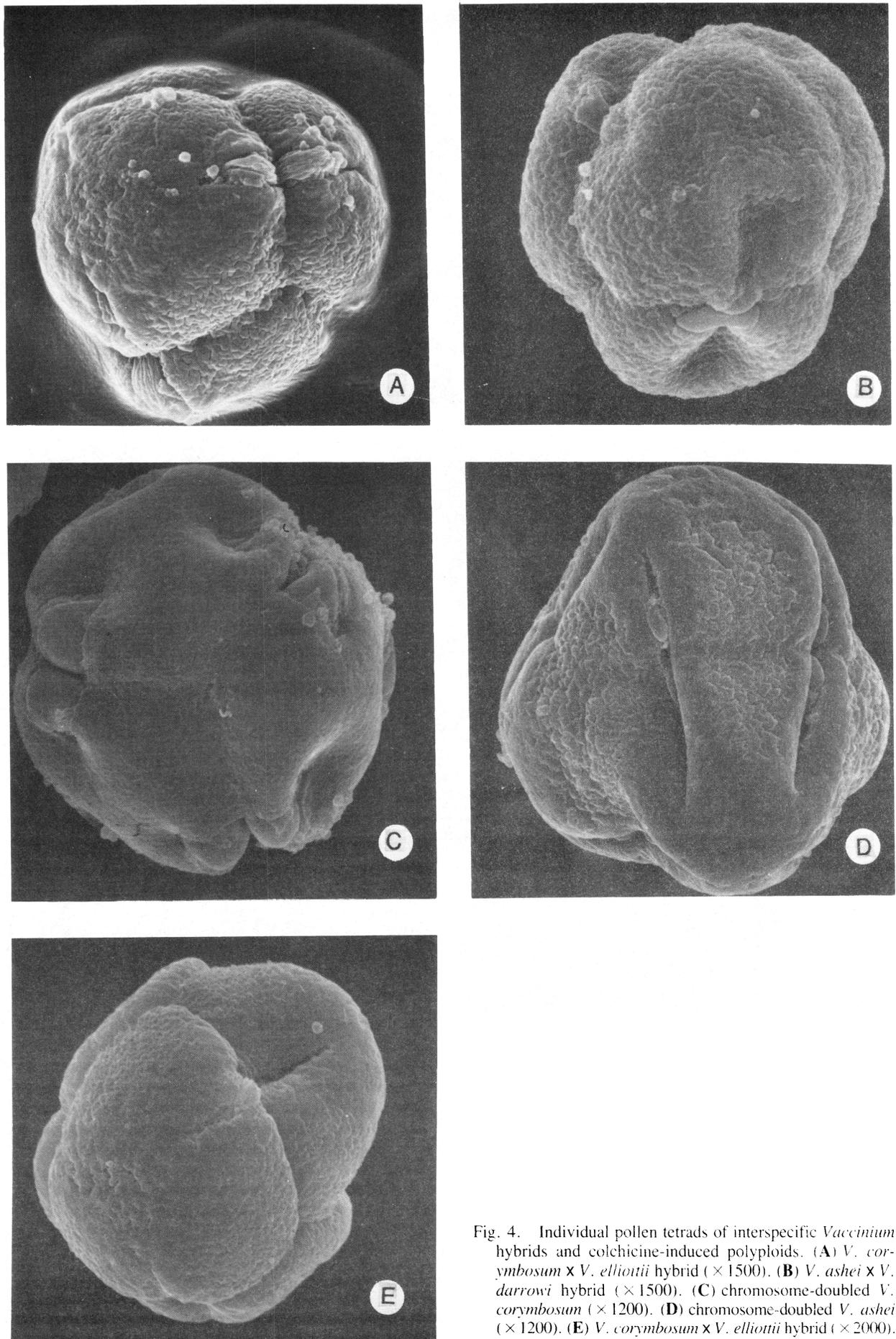


Fig. 4. Individual pollen tetrads of interspecific *Vaccinium* hybrids and colchicine-induced polyploids. (A) *V. corymbosum* x *V. elliotii* hybrid ( $\times 1500$ ). (B) *V. ashei* x *V. darrowi* hybrid ( $\times 1500$ ). (C) chromosome-doubled *V. corymbosum* ( $\times 1200$ ). (D) chromosome-doubled *V. ashei* ( $\times 1200$ ). (E) *V. corymbosum* x *V. elliotii* hybrid ( $\times 2000$ ).

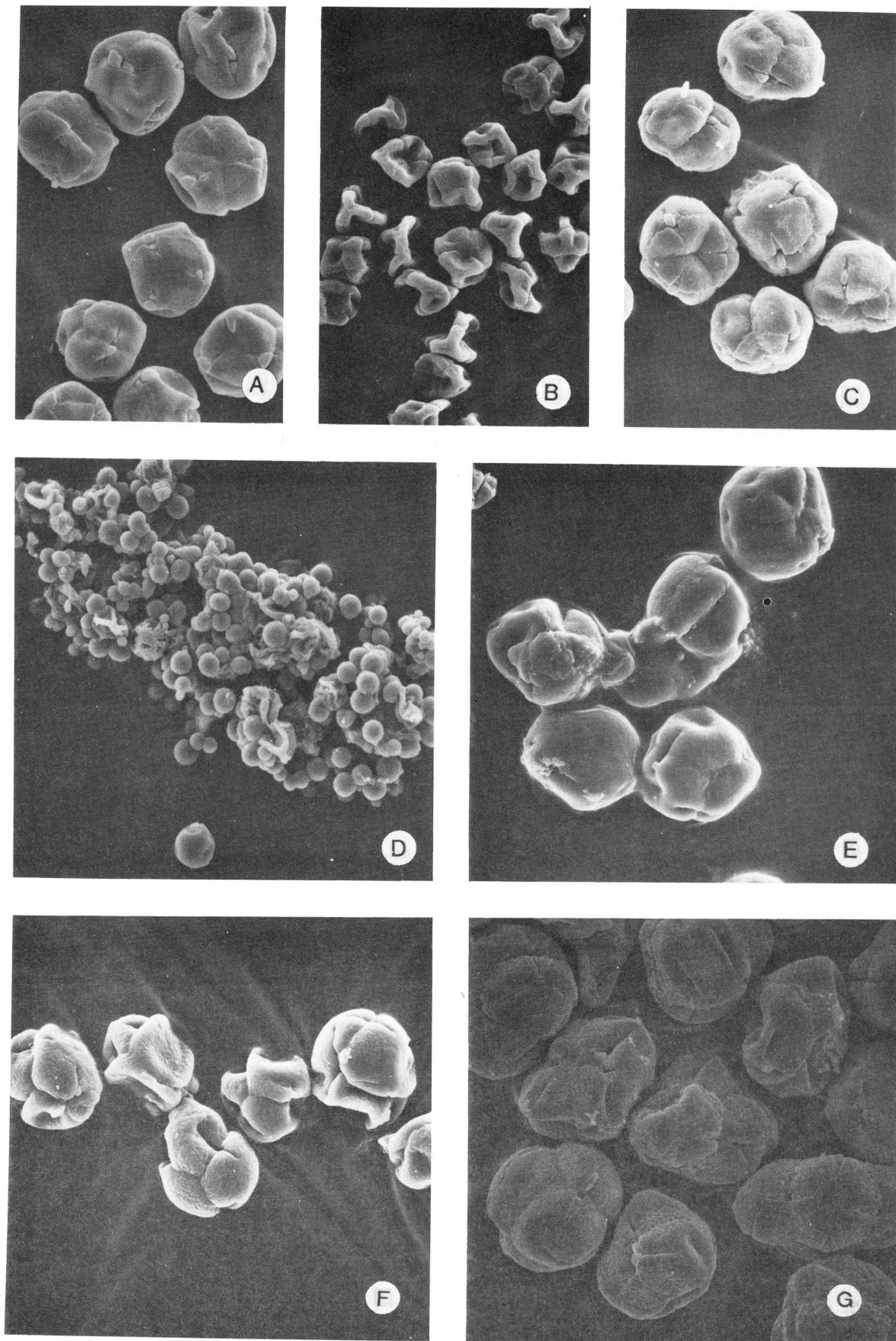


Fig. 5. Pollen tetrads of *Vaccinium* species, interspecific hybrids and colchiploids magnified  $\times 395$ . (A) *V. darrowi* x *V. elliottii*. (B) *V. elliottii* x *V. stamineum*. (C) *V. myrsinites*. (D) *V. ashei* x *V. darrowi*. (E) chromosome-doubled *V. corymbosum* x *V. ashei* hybrid. (F) *V. ashei* x *V. corymbosum*. (G) chromosome-doubled *V. ashei*.

The spherical bodies observed in Fig. 5D were more evident in the *V. ashei* × *V. darrowi* hybrids than in any of the other interspecific crosses. They were also observed in smaller numbers in the plants shown in Fig. 1F, 4A,C,E, and 5G. Their presence in many of the plants seemed to be associated with underdeveloped tetrads. Underdeveloped tetrads are generally released in groups which stick together while normal tetrads are solitary and usually not associated with these bodies. The origin and nature of these bodies is unknown; they may be the "globular substance" observed by Stushnoff and Hough (15) on irregularly developed pollen of 'Coville'.

*Colchicine-induced polyploids.* In general, tetrads from colchicine-induced polyploids resembled those of nondoubled plants in their respective species, except for an increase in pollen size. Fertility of pentaploid *V. corymbosum* × *V. ashei* hybrids apparently was increased by chromosome doubling. Decaploid 'US 40' (Fig. 5E) had more well-filled grains than the pentaploid shown in Fig. 5F. Tetrads from doubled *V. ashei* 'Bluebelle' plants (Fig. 4D) were not only larger than those of normal *V. ashei* (Fig. 2C), but exine patterning also was different. However, this may be due to intraspecific variation and not chromosome number. Some of the 12x *V. ashei* pollen had 4 pores instead of 3 (Fig. 5G).

Tetrads of octoploid *V. corymbosum* × *V. fuscatum* hybrids were surprisingly similar to tetrads of tetraploid *V. corymbosum* in all respects except size (Fig. 2E and 4C). Tetrad size did not appear to be much different among 8x, 10x, and 12x plants. The spherical bodies present in interspecific crosses were also observed on the colchicine-induced polyploids (Fig. 5F and 5G).

The questions that lead to this investigation were difficult to answer because of the lack of easily identifiable intraspecific differences and because it was hard to obtain good observations from the poorly developed tetrads of interspecific hybrids. Larger sample sizes, which would enable better determination of intra- and interspecific variation, might lead to more definite conclusions. The study, however, did suggest that exine patterning may provide useful information on taxonomic relationships and inheritance in *Vaccinium*.

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