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Systematic Inferences from Spore and Stomate Size in the Ferns

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Size of equivalent cells has traditionally been assumed to be constant within species and variable between species. Systematists have commonly measured cells with constant form, such as spores and stomates, as a means of distinguishing species and hybrids in polyploid complexes, since the best known factor determining cell size is ploidy level (Stebbins, 1950). Preliminary hypotheses about polyploid evolution can be generated directly from herbarium specimens based on spore and stomate measurement data.

The first evidence of a relation between cell size and polyploidy in the ferns was Lawton's 1932 demonstration that prothallial cells, lower epidermal cells, and stomates in induced apogamous races of *Dryopteris marginalis* and *Woodwardia virginica* vary directly with ploidy level. Butters and Tryon's 1948 work on *Woodsia × abbeae* included epidermal cell and annulus cell measurements that corroborated data from spore germination experiments to document an instance of somatic autopolyploidization.

More recently a considerable body of evidence has been assembled supporting the contention that spore size is related to ploidy level in the ferns. Manton (1950) noted that Vancouver Island *Cystopteris fragilis* ($n = 84$) has smaller spores than Swiss *Cystopteris alpina* ($n = 126$). Hagenah (1961) postulated a polyploid series in species of *Cystopteris* based on spore-size measurements. In a monograph of *Cystopteris*, Blasdell (1963) concluded that it was possible to infer ploidy level from spore size in that genus. Blasdell assigned ploidy levels to cytologically unknown components of polyploid complexes based only on spore size (see Lovis, 1977 for a critique). Wagner (1966) demonstrated that there were two varieties of *Gymnocarpium dryopteris*: a larger-spored typical variety, which is tetraploid, and a smaller-spored variety *disjunctum*, which is diploid. Schneller (1974) recently provided an analysis of ploidy level and spore size in the *Dryopteris filix-mas* group of Europe. He concluded that ploidy and spore size are positively correlated for diploid to pentaploid cytotypes in a closely knit phylogenetic group.

In an exhaustive analysis of spore features of northeastern North American *Isoetes*, Kott and Britton (1983) provided new insight into spore-size variability. Whereas megaspore variation within sporangia of a single plant, between sporangia of a single plant, and between plants of a population was found to be more or less equivalent and negligible, variation between populations of a species was demonstrated to be twice that between plants of a population. Microspores were found to be slightly less variable. Kott and Britton (1983) suggested that

spore size can be used to characterize species so long as a sample of at least 20 spores be measured from single plants of a representative set of populations. They demonstrated that, between species, both microspore and megaspore sizes varied directly with ploidy level.

Some spore-measurement analyses have yielded confusing results. Brown (1964) found that spores of the tetraploid *Woodsia oregana* var. *cathcartiana* were the same size or smaller than those of var. *oregana*, which is diploid. His results, however, are apparently a consequence of having mixed diploid and tetraploid cytotypes of variety *oregana* (see Windham & Haufler, 1986). More intriguing are the apparently inconsistent results for *Dryopteris*. Manton (1950) found it odd that spores of diploid *Dryopteris abbreviata* and tetraploid *D. filix-mas* were almost identical in size. Britton (1968) concluded from an analysis of eastern North American *Dryopteris* species that spore size and ploidy level are not closely correlated in the genus, based on documentation of relatively large spore sizes of the diploid species *D. fragrans* and *D. dilatata* (= *D. expansa*). He found that mean spore size of *D. dilatata* (= *D. expansa*) was indistinguishable from that of the tetraploids *D. campyloptera* and *D. spinulosa*. Wagner (1971) provided spore-length data for an expanded set of *Dryopteris* species in his survey of Appalachian wood ferns. His data corroborate Britton's observation that there is no simple relationship between spore length and ploidy level in *Dryopteris*.

The observations on *Dryopteris* by Manton, Britton, and Wagner suggest that sizes of diploid spores within a genus can be quite different. This variation in spore size among diploid species depends on at least three factors. 1) Size varies with adaptation for dispersal. Small size increases the likelihood of dispersal outside the region of the parent, but spore size increases on islands so that propagules can remain on the island rather than disperse into terrain that cannot host gametophyte development and function (Carlquist, 1966). 2) Spore size is also thought to increase for nutritional reasons (as in the evolution of megaspores). 3) Cox and Hickey (1984) provided evidence that environmental parameters strongly affect spore size in *Isoetes storkii* of Costa Rica. Their investigation of three populations whose habitats differed in mean temperature, mean daily solar radiation, and altitude demonstrated that plants in colder, shadier, higher habitats had smaller spores. This paper suggests that analyses of cell size variation among species should, when possible, include a representative sample of populations from a set of characteristic habitats for each species. Hence, spore size is determined not only by ploidy level but by the reproductive biology and environmental regime of a taxon.

Stomate size (usually as length of the guard cells) has also been correlated with ploidy level. Wagner (1954) noted that tetraploid members of the Appalachian *Asplenium* complex had larger stomates than the diploids. Lovis and Reichstein (1968) provided data on stomate length for two sterile diploid hybrids and each of their derived fertile tetraploids. In each case the tetraploid stomates are longer than those of the corresponding diploid, and there is little overlap of diploid and tetraploid stomate samples. Schneller (1974) reported stomate lengths for members of the *D. filix-mas* complex in Europe. He demonstrated a correlation between ploidy level and stomate size for diploid to pentaploid cytological

ances. Stomate measurements are particularly useful in assessing sterile hybrids, since irregular spores preclude spore comparisons.

Counterintuitive results of stomate-size analysis have also been reported. Barrington (1986) reported stomate lengths of diploid *Polystichum acrostichoides*, tetraploid *P. braunii*, and their hybrid, which is known as both triploid and tetraploid. The order of these taxa based on stomate length (smallest to largest) was: tetraploid progenitor, triploid hybrid, diploid progenitor, tetraploid hybrid. Barrington suggested that *P. braunii* was derived from diploid Asian species with much smaller stomates than those of *P. acrostichoides*, but could not explain the anomalous guard-cell sizes of the hybrids. We provide an analysis of the anomalous stomate sizes in this hybrid below.

Experimental error in measurement has been a problem in the work with spore and stomate size. Factors affecting the measurement of cells include: 1) calibration of microscope; 2) characteristics of mounting medium; 3) definition of cell boundary; and 4) choice of cells for measurement. Lovis (1964) encountered a 30% discrepancy between his data sets and those of Meyer in spore-size measurements of the European *Asplenium trichomanes* complex. Difference in mounting medium accounted for 6% of the difference: Lovis found that his spores, in gum chloral, yielded measurements 6% higher than Meyer's spores of the same taxon, which were measured dry. Lovis also determined that Meyer's measuring all spores versus his measuring only those whose long axis was perpendicular to the line of sight accounted for a further 1.5–3.5% discrepancy. Nevertheless, he was unable to account for a residual discrepancy of over 20%, which is equivalent to the difference between a diploid and a tetraploid taxon. Lovis's efforts are evidence that caution must be used in comparing spore measurements made by different investigators or those made with different equipment.

Analysis of data on spore and stomate size has not usually included statistical analysis. Selling (1944) applied statistical techniques to problems of spore size, but his excellent example was not followed until recently. Analyses have seldom included simple descriptive statistics such as means and standard deviations, let alone tests of the significance of spore-size differences among related taxa.

We provide new spore and stomate measurement data for three groups of ferns in three different families in order to assess the value of cell measurements in making systematic and evolutionary inferences from herbarium specimens. The methods are not equivalent, nor are the analyses: these data sets are independently brought to bear on the general problem of cell size and fern systematics. In *Adiantum*, we document herbarium specimens of a new tetraploid taxon from eastern North America. In *Polypodium*, we explore the problem of allopolyploid versus autopolyploid origin of tetraploid *P. californicum*. In *Polystichum*, we suggest a possible candidate for a missing diploid progenitor in a new tropical montane reticulate complex.

ADIANTUM IN NORTHEASTERN NORTH AMERICA

Recent systematic work on the *Adiantum pedatum* complex in eastern North America has demonstrated that there are three divergent entities there: *A. pe-*

TABLE 1. Spore Size (μm) in *Adiantum pedatum*.

Taxon	Mean length (s.d.)	Observed limits	N	No. sporophytes
Woodland 2n	37.03 (2.494)	31.8–42.0	200	8
Serpentine 2n	42.96 (4.416)	32.0–52.8	385	16
Tetraploid	51.36 (5.162)	35.2–68.8	549	22

datum ssp. *pedatum*, a diploid of rich, deciduous woodlands; ssp. *calderi*, a diploid of serpentine substrates (formerly var. *aleuticum*; see Cody, 1983); and an unnamed tetraploid taxon of serpentine outcrops in north-central Vermont (Paris, 1986; Paris & Windham, in prep.). Although these entities have several diagnostic electrophoretic markers, they are not easily distinguished by their structural characters, most of which overlap considerably between taxa. Phenotypic plasticity further obscures species boundaries. Especially difficult to discriminate are the diploid and the tetraploid plants on serpentine, in part because the tetraploids, when growing in exposed areas, resemble their serpentine diploid progenitors quite closely. Because tetraploid plants have consistently larger spores than diploids, spore size can be used effectively to differentiate the two serpentine taxa. The predictable relationship between spore size and ploidy level in *A. pedatum* has made it possible to survey herbarium collections from serpentine areas within the Appalachian Mountain ultramafic belt in order to define the range of the new tetraploid taxon.

Materials and methods.—Spores were mounted in Hoyer's medium on glass slides and measured at $400\times$ using an ocular micrometer. Twenty-five spores per sporophyte were measured, each in its longest dimension as it was oriented on the slide. The perispore, thin and unornamented in this group, was included in the measurement. An analysis of variance was performed on the data using the BMDP7D program (Dixon, 1983). Independence of spore size and ploidy level was tested using the G-test of Sokal and Rohlf (1981). To test whether *A. pedatum* spores swell in Hoyer's medium over time, a sample of 25 spores from each of two sporophytes was mounted in Hoyer's medium and measured after two days and again after 85 days. Significance of the difference in sample means was tested using Student's *t*-test.

Results.—Mean spore sizes for the three taxa were $37.03\ \mu\text{m}$ for the woodland diploid, $42.96\ \mu\text{m}$ for the serpentine diploid, and $51.36\ \mu\text{m}$ for the new tetraploid (Table 1). A G-test of independence of spore size and ploidy level, using spores from cytologically or electrophoretically documented sporophytes, yielded evidence that spore size and ploidy level are not independent in this group (Table 2). Thus spore size may be used as a reliable indicator of ploidy in the *A. pedatum* complex. An analysis of variance (Table 3A) demonstrated that the means among taxa are significantly different ($P < 0.001$). Not only are both diploids different from the tetraploid, but the diploids differ significantly from one another ($P < 0.01$), as demonstrated by a GT-2 test for multiple comparisons among pairs of means (Sokal and Rohlf, 1981; Table 3B). Non-parametric tests

TABLE 2. Test of Independence of Spore Size and Ploidy for *Adiantum* Taxa on Serpentine ($N = 6$ sporophytes/taxon, 25 spores/sporophyte).

	Spores $\leq 47 \mu\text{m}$	Spores $> 47 \mu\text{m}$	Row totals
Diploid	125	25	150
Tetraploid	50	100	150
Column totals	175	125	300
G Statistic (with Williams' correction for a 2×2 table) = 80.97***			

*** = $P < 0.001$.

that do not assume homoscedasticity show equally significant differences. A t -test of the difference in the mean size of spore samples mounted in Hoyer's medium for 2 days and for 85 days was insignificant.

POLYSTICHUM IN COSTA RICA AND ANDEAN SOUTH AMERICA

Evidence of a tropical montane polyploid complex reminiscent of well-known North Temperate complexes in the genus *Polystichum* now exists (Barrington, 1985a). The complex includes two unnamed species endemic to Costa Rica, an allotetraploid and one of its diploid progenitors. Morphologically, the best candidate for the second progenitor is *P. polyphyllum*, a common polymorphic species of the Andes, Costa Rica, and Mexico. However, *P. polyphyllum* is tetraploid based on counts from Costa Rica, Ecuador (Barrington, unpublished data), and Mexico (Smith in Barrington, 1985b). Spore length and width measurements were made to establish the relationship between spore length and ploidy level in *Polystichum* and search for a diploid cytotype of *P. polyphyllum* in northern Latin America. Included in the study set were four entities: 1) the endemic diploid from Costa Rica (ten sporophytes, documented to be diploid); 2) the

TABLE 3. Tests Comparing Mean Spore Length for *Adiantum pedatum* Taxa.

A. Analysis of variance			
Source of variation	Degrees of freedom	MS	F
Among taxa	2	17,694.1541	857.83***
Within taxa	1131	20.6267	
B. GT-2 test for differences between means (pairwise comparisons)			
Pair	Difference between means (in μm)		
Woodland 2n vs. serpentine 2n	5.931**		
Serpentine 2n vs. tetraploid	8.394**		
Woodland 2n vs. tetraploid	14.325**		

** = $P < 0.01$.*** = $P < 0.001$.

TABLE 4. Spore Measurements (in μm) for *Polystichum* Species (30 spores/sporophyte. Standard deviation is for sample means from grand mean.)

Taxon	Spore length (s.d.)	Spore width (s.d.)	Spore volume (s.d.)	No. plants
Endemic diploid	38.52 (1.41)	28.75 (1.02)	16,940 (1590)	10
Endemic tetraploid	46.20 (3.05)	33.27 (2.50)	27,620 (5430)	10
Costa Rica <i>polyphyllum</i>	40.14 (0.59)	29.20 (0.85)	18,110 (1270)	5
Andean <i>polyphyllum</i>	41.67 (5.18)	30.25 (3.36)	20,870 (6730)	8

endemic tetraploid from Costa Rica (ten sporophytes, documented to be tetraploid); 3) *P. polyphyllum* from Costa Rica (four sporophytes, documented to be tetraploid); and 4) *P. polyphyllum* from the northern Andes from Venezuela to Ecuador (ploidy unknown).

Materials and methods.—Spore measurements of *Polystichum* species were made using a phase contrast microscope with a calibrated ocular micrometer at $400\times$. Measurements comprised length and width of exospore wall of those spores whose long axis was parallel to the slide surface. Damaged spores and irregular spores were excluded from measurement.

To assess the differential effects of mounting media on *Polystichum* spores over time, 20 spores from a single sporophyte (Barrington 1275, VT) of the endemic tetraploid were mounted in Hoyer's medium, Permout, and lactic acid on glass slides and were measured seven times over a two-week period. Spores in Hoyer's medium swelled 15% in two weeks, but spores in the other two media remained unchanged. To characterize spore size for each of the taxa in the study group, 30 spores from each sporophyte mounted in Permout were measured. Volume of each spore was computed using the formula for an ellipsoid, $V = \pi LW^2/6$ (where L is the longest dimension of the exospore and W is width measured at right angles to L). Descriptive statistics were developed using the Minitab Statistical Package (Ryan et al., 1976). To test the hypothesis that spore length could serve as a predictor of ploidy level, independence of spore size and ploidy level was tested using the G-test of Sokal and Rohlf (1981).

Results.—The mean spore lengths for the four sets of plants were $38.52\ \mu\text{m}$ for the endemic diploid, $46.20\ \mu\text{m}$ for the endemic tetraploid, $40.14\ \mu\text{m}$ for *P. polyphyllum* in Costa Rica, and $41.67\ \mu\text{m}$ for Andean *P. polyphyllum* (Table 4). The mean volumes for these same taxa were $16,940\ \mu\text{m}^3$, $22,630\ \mu\text{m}^3$, $18,110\ \mu\text{m}^3$,

TABLE 5. Test of Independence of Spore Size and Ploidy for *Polystichum* Species ($N = 10$ sporophytes/taxon, 30 spores/sporophyte).

	Spores $\leq 42.36\ \mu\text{m}$	Spores $> 42.36\ \mu\text{m}$	Row totals
Costa Rica diploid	277	23	300
Costa Rica tetraploid	50	250	300
Column totals	327	273	600
G Statistic (with Williams' correction for a 2×2 table) = 393.24843***			

*** = $P < 0.001$.

and $20,870 \mu\text{m}^3$, respectively. Spore length was a valid predictor of ploidy level for the pair of endemic Costa Rican species, since Sokal and Rohlf's G-statistic allowed the rejection of the null hypothesis that spore size and ploidy level were independent of one another (Table 5). Mean spore size of the two samples of *P. polyphyllum* plants was similar to that for the Costa Rican diploid. However, standard deviation of mean spore length by sporophyte was much more variable in Andean *P. polyphyllum* than in the first three samples, because small-spored sporophytes were encountered in Venezuela.

POLYPODIUM IN CALIFORNIA

Polypodium glycyrrhiza is a diploid species ($n = 37$) that occurs along the west coast of North America from Monterey County, California, north to the Aleutian Islands. *Polypodium californicum* consists of two cytological races that tend to occupy different habitats. The diploid race ($n = 37$) extends from the San Francisco region south along the coast to Baja California, and the tetraploid race ($n = 74$) is found from Monterey County north to Del Norte County, California and in the foothills of the Sierra Nevada. Although there is evidence to suggest that allopolyploidy is a common mode of speciation among other *Polypodium* species in North America and Europe (Manton, 1950, 1957; Shivas, 1961; Manton & Shivas, 1953; Lloyd & Lang, 1964; Lang, 1971), the origin of the tetraploid *P. californicum* is still problematical.

Lloyd and Lang (1964) suggested that the tetraploid race arose through allopolyploidy from *P. glycyrrhiza* and diploid *P. californicum*. However, Ranker (1982) and Ranker and Mesler (1982) presented evidence based on morphology and guard-cell size that supported both allopolyploid and autopolyploid origins of the tetraploid. To test their assumption that guard cells could be used as indicators of ploidy level, guard cell data were collected from plants of known ploidy of all three taxa for this study.

Materials and methods.—Pinnae from dried specimens of known chromosome number were placed in 95% ethanol until most of the chlorophyll was extracted. The pinnae were then rehydrated in near-boiling water for about one minute and placed on a glass microscope slide with a few drops of water. Twenty-five guard cells per specimen were measured with an ocular micrometer at a magnification of $400\times$.

Results.—Inspection of the data from diploid *P. californicum* revealed a strongly bimodal distribution of guard-cell size: a cluster of four individuals with smaller guard cells from drier, more southern sites and a cluster of three individuals with larger guard cells from more mesic, northern sites (Table 6). These clusters were treated as distinct groups in subsequent analyses. The mean guard-cell sizes were $50.4 \mu\text{m}$ for *P. glycyrrhiza*, $54.3 \mu\text{m}$ for diploid *P. californicum* from southern California, $65.2 \mu\text{m}$ for diploid *P. californicum* from northern California, and $60.4 \mu\text{m}$ for tetraploid *P. californicum* (Table 6). A nested analysis of variance showed that there were significant amounts of variation both among plants within taxa and among taxa (Table 6). Pairwise comparisons of means using the GT-2 method indicated that all means differed significantly ($P < 0.05$).

TABLE 6. Comparison of Mean Guard-Cell Length for *Polypodium* Taxa.

A. Guard-cell measurements (<i>N</i> = 25 guard cells/plant)				
Taxon	Mean length, μm	s.d.	No. of plants	Range of plant means
<i>P. glycyrrhiza</i> , 2 <i>n</i>	50.4	3.293	22	42.8–57.8
<i>P. californicum</i> , 2 <i>n</i>				
Southern cluster	54.3	1.352	4	52.7–55.6
Northern cluster	65.2	3.007	3	62.1–68.1
<i>P. californicum</i> , 4 <i>n</i>	60.4	3.564	12	54.5–66.9

B. Nested analysis of variance for guard-cell data			
Source of variation	Degrees of freedom	MS	<i>F</i>
Among taxa	3	1468.7033	36.45***
Among plants within taxa	37	40.2949	32.53***
Within plants	984	1.2389	

*** = *P* < .001.

ANALYSIS AND DISCUSSION

Measurements of spores and stomates serve as useful probes for establishing hypotheses of evolutionary relationships within polyploid complexes. Spore and stomate size of polyploids is dependent on two factors: size of cells in diploid progenitors and ploidy level. Taken together, these two factors may be used to predict cell size of missing members of polyploid complexes from cell-size means of the known members, so long as environmental variation does not compromise the analysis.

In this paper we have provided new evidence that cell size is correlated with ploidy level in ferns from investigations of three reticulate complexes in three different families. That each study involves a tetraploid and at least one of its progenitors is suggested by results of other studies, cytological or electrophoretic.

Little information exists about the variation in size of spores or stomates in the diploid members of a polyploid complex. The two eastern North American diploid taxa in the *Adiantum pedatum* complex differ significantly with respect to spore size. In this case, the observed differences in size are consistent with the theory that species in insular habitats have larger spores (Carlquist, 1966). The serpentine taxon of *A. pedatum* has larger spores than its ally, which is widespread in woodlands. We suggest that serpentine outcrops are analogous to islands for serpentine-adapted plant species, so that spores of serpentine *Adiantum pedatum* have responded to selection for limited dispersability.

We contend that the spore size of an allotetraploid can be predicted from the known size of its diploid progenitors by multiplying a constant reflecting the increase (in volume or linear dimension) due to polyploidy by the mean of the two diploid spore volumes. Increase in cell size due to polyploidy is not necessarily consistent among species or even among tissues of a single organism (Sin-

nott, 1960). However, the assumption that doubling of chromosome number yields a doubling of cell volume gave meaningful results in this study. Using a sphere as a model, doubling volume would give a concomitant increase of 1.26 diameters. We multiplied the average of the mean lengths of the tetrahedral-globose spores of two diploid adiantums by 1.26 to predict the mean spore length for the tetraploid *Adiantum* from Vermont to be 50.39 μm , which is close to the observed mean spore length of 51.36 μm .

We predicted the mean spore volume of a second progenitor for the tetraploid *Polystichum* endemic to Costa Rica to be 10,670 μm^3 by solving for a missing diploid rather than for the tetraploid. Although *P. polyphyllum* is tetraploid in Costa Rica and thus could not serve as a progenitor of the endemic tetraploid, a diploid progenitor of *P. polyphyllum* could be an ancestor. The sole diploid progenitor of an autotetraploid *P. polyphyllum* would be predicted to have a spore volume of 9050 μm^3 . Although spores of most Andean *P. polyphyllum* measured for this study were within the range for a tetraploid, a few collections from Venezuela and Colombia had smaller spores that approached the predicted size for diploids. Hence, the data suggest that a diploid progenitor of *P. polyphyllum* and the endemic Costa Rican tetraploid may be found in the northernmost Andes.

Guard-cell size can also be used as a valid indicator of ploidy. The results from *Polypodium* show that in northern California, where the diploid *P. glycyrrhiza* is sympatric with tetraploid *P. californicum*, the two can be distinguished using guard-cell size. South of this region, the relatively small guard cells of diploid *P. californicum* make this character a less useful predictor. These data also can be used to address the question of the origin of the tetraploid cytotype of *P. californicum*. They indicate that this taxon could have arisen through allopolyploidy from two progenitors, one similar to the southern cluster of diploid *P. californicum* and the other *P. glycyrrhiza*. Both the southern and northern clusters of diploid *P. californicum* have guard cells that are too large to accommodate an autopolyploid origin of the tetraploid. Environmentally induced variability similar to that encountered by Cox and Hickey (1984) may complicate the interpretation of stomate-size data in this group.

Calculation of expected guard-cell lengths for the triploid and tetraploid cytotypes of the hybrid between *P. acrostichoides* and *P. braunii* in Vermont provides another test of the contention that cell sizes of unknown members of polyploid complexes can be predicted from cell sizes of known members. Data from Barrington (1986) for guard-cell lengths of the four taxa involved, along with values predicted from guard-cell lengths of the progenitors and from ploidy level, are reported in Table 7 (see Appendix). The predicted values for the hybrid guard cells are similar to the observed values. These calculations demonstrate that the unexpected values for hybrid guard-cell sizes are predicted from a model including two factors, one reflecting ploidy level and the other the size of the progenitor guard cells. The close match of the observed value to the predicted value for the triploid suggests that a two-fold increase in the volume of polyploidized fern cells may be common enough to use as a preliminary indicator of ploidy level in ferns.

TABLE 7. Analysis of Guard-Cell Pair Measurements in μm for *Polystichum* $\times$ *potteri* and its progenitors ($N = 30$ guard cell pairs per sporophyte).

Taxon	Ploidy level	Genomic constitution	Observed mean guard-cell length (s.d.)	Predicted mean guard-cell length
<i>acrostichoides</i>	2n	aa	53.6 (0.97)	—
<i>braunii</i>	4n	bbbb	47.8 (1.14)	—
$\times$ <i>potteri</i>	3n	abb	50.0 (1.83)	49.7
$\times$ <i>potteri</i>	4n	aabb	59.8, 64.0*	58.5

* Two counts of same sporophyte, collections from GH and MICH, respectively.

In summary, using data from three genera we have provided additional support for the hypothesis that ploidy level and cell size are correlated in ferns. We have also provided some tests of the hypothesis that cell sizes of diploids and their tetraploid derivatives can serve as a basis for predicting the size of missing members of their reticulate complex. We suggest that more exhaustive data sets from better-known reticulate complexes would be very enlightening tests of this simple hypothesis. Especially important would be a critical analysis of complexes in which polyploids fail to conform to predictions, such as Appalachian *Dryopteris* (Wagner, 1971).

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APPENDIX

To calculate the predicted length values for the hybrids, haploid length values for the progenitor genomes were derived by multiplying the observed guard-cell lengths by $1/1.26$ (for the diploid species) and $1/1.26^2$ (for the tetraploid species). $1/1.26$ is the ratio of diameters of two spheres, the first having half the volume of the second. Then the predicted value was computed by multiplying the average of the haploid values for each component genome— $(a + a + b + b)/4$ for the tetraploid cytotype and $(a + b + b)/3$ for the triploid cytotype—by a coefficient reflecting increase in diameter expected for a given ploidy level of the hybrid ($1.26^{3/2}$ for the triploid, 1.26^2 for the tetraploid).