HYBRIDIZATION AND ALLOPOLYPLOIDY IN CENTRAL AMERICAN *POLYSTICHUM*: CYTOLOGICAL AND ISOZYME DOCUMENTATION[1]

David S. Barrington[2]

ABSTRACT

Cytological and isozyme data for four *Polystichum* species and four hybrids between them from the Sierra Talamanca of Costa Rica document hybridity and reveal a reticulate pattern of evolutionary relationships. Tetraploid *P. talamancanum* is hybridizing with tetraploid *P. orbiculatum* and with two diploid species, *P. speciosissimum* and *P. concinnum*, which also hybridize with each other. Cytological and isozyme data confirm that *P. concinnum* is one of two diploid progenitors of the allopolyploid *P. talamancanum*. *Polystichum talamancanum* and *P. orbiculatum*, also an allopolyploid, share an unidentified diploid progenitor. Homoeologous pairing demonstrated earlier in north temperate *Polystichum* hybrids was documented for these montane tropical hybrids. The demonstrated pattern of reticulation is similar to such patterns in ferns of north temperate regions.

The origin of fern species from sterile hybrids via chromosomal nondisjunction and polyploidization is supported almost entirely by work in north temperate regions (Manton, 1950; Lovis, 1977; Barrington et al., 1989). Studies in north temperate *Polystichum* (Dryopteridaceae) have played a critical role in demonstrating the contribution of hybridization and polyploidy to fern evolution (e.g., Daigobo, 1972; W. Wagner, 1973; Vida & Reichstein, 1975; D. Wagner, 1979; Barrington, 1986; Soltis et al., 1987). Recently, a similar pattern of reticulate relationships among fern species has been proposed for *Polystichum* in the Sierra Talamanca of Costa Rica and Panama (Barrington, 1985a, b, c; Barrington et al., 1986; Barrington, 1989; Barrington et al., 1989). In this paper, I use chromosome and isozyme data to document the proposed relationships and provide insights into hybridization and hybrid speciation of *Polystichum* in montane tropical regions.

Four *Polystichum* species are involved in hybridization in the Sierra Talamanca (Fig. 1). They comprise two broad-leafed diploid species, *P. concinnum* Lell. ex Barr. and *P. speciosissimum* (Kunze) R. Tryon & A. Tryon, and two narrow-leafed tetraploid species, *P. talamancanum* Barr. and *P. orbiculatum* (Desv.) Gay (Barrington, 1985a, b; Barrington, 1989). The forest-dwelling *P. concinnum* is endemic to the Sierra Talamanca, as is the low-alpine *P. talamancanum*. In contrast, the high-alpine *P. speciosissimum* is known from Mexico and Guatemala as well as Costa Rica, and *P. orbiculatum* is common in the high-alpine from Mexico to Bolivia.

Four hybrids between these species have been proposed (Fig. 1). Hypotheses for hybridity and hybrid parentage as well as definitions of species were based on morphological characters and degree of spore abortion (Barrington, 1989). Morphologically, the hybrids are noteworthy in that they include combinations of both morphologically disparate species such as *Polystichum concinnum* and *P. speciosissimum* and morphologically similar species such as *P. orbiculatum* and *P. talaman-*

[1] Rolla Tryon originally suggested that *Polystichum* in the American tropics was in need of systematic study. I thank Luis Diego Gómez P. for his support of my fieldwork in the Sierra Talamanca. Steven R. Hill, Peter T. Hope, Bruce Howlett, and Peter Zika all provided assistance in the field. Cathy Paris provided valuable insights in the laboratory and careful readings of the manuscript. David S. Conant also provided a critical review. An anonymous reviewer provided extensive, valuable suggestions that substantially improved this work. Electrophoretic and cytological work reported here was begun at the University of Kansas during the spring of 1985, when I spent a sabbatical leave in C. H. Haufler's lab. I thank Dr. Haufler for training in isozyme electrophoresis. The University of Vermont provided support for laboratory and fieldwork through its Institutional Grants BRSG-79 and BSCI85-1; this contribution was the specific goal of the latter grant.

[2] Pringle Herbarium, Department of Botany, University of Vermont, Burlington, Vermont 05405-0086, U.S.A.

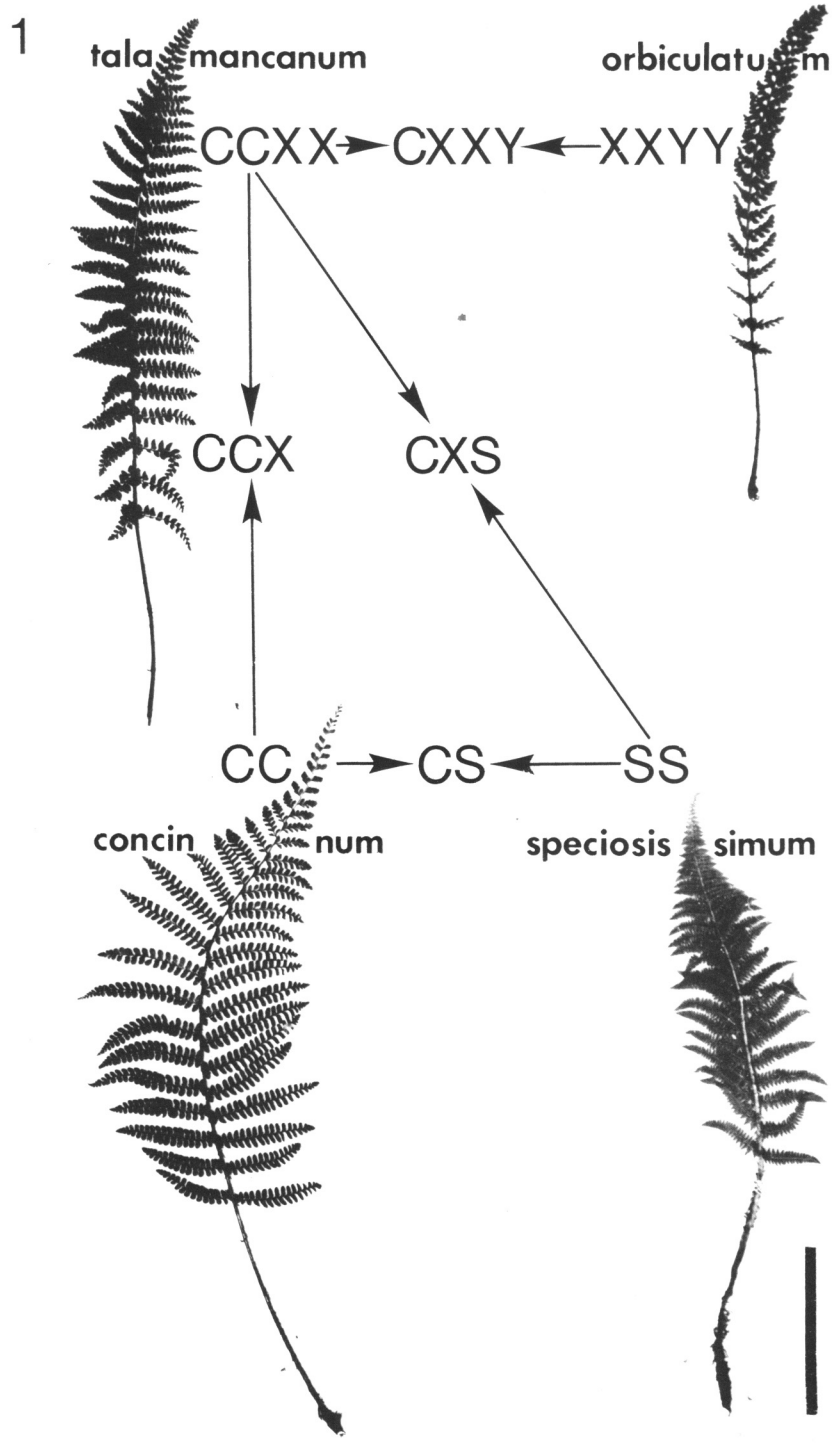
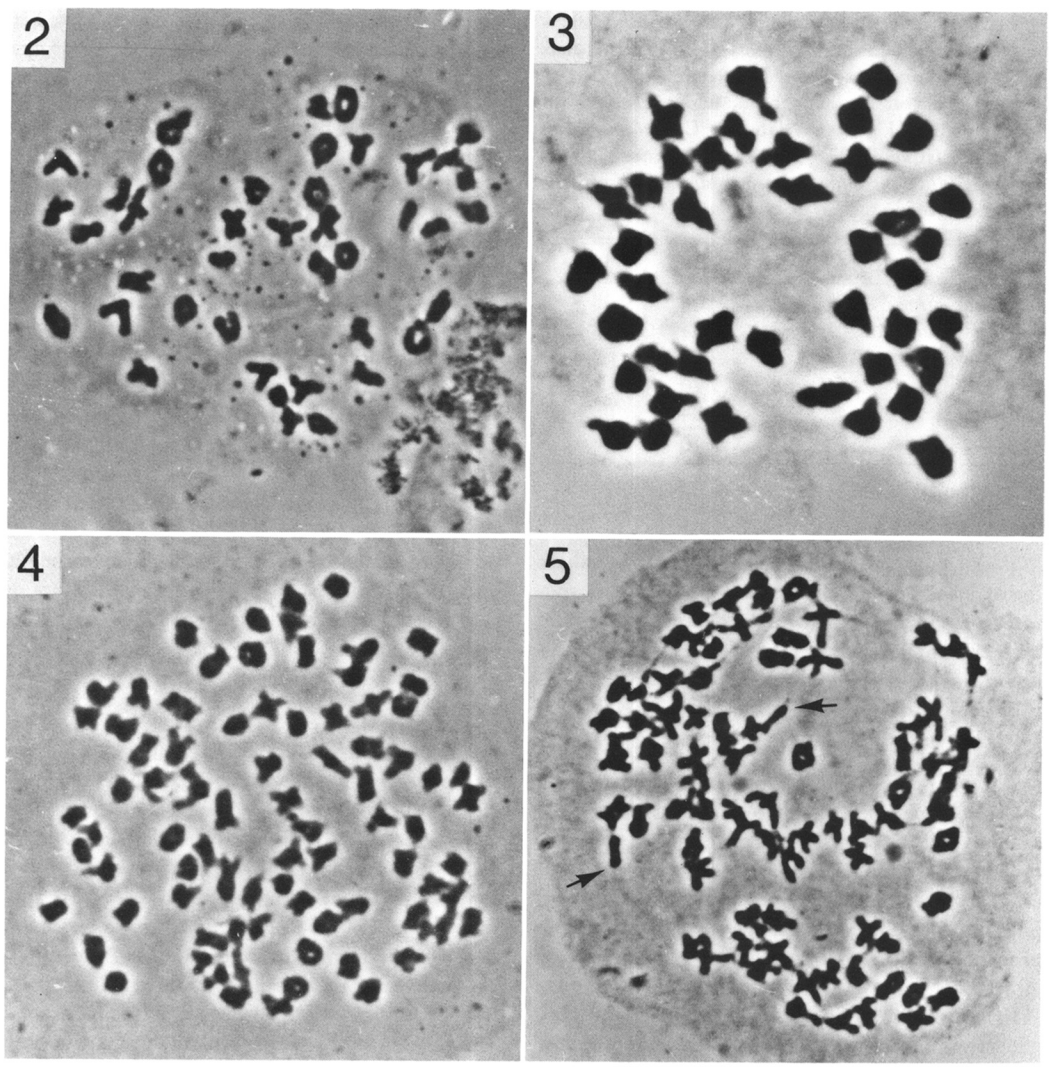


FIGURE 1. Proposed relationships between species and hybrids of *Polystichum* at Cerro de la Muerte, Sierra Talamanca, Costa Rica. Genomes are as follows: C: *P. concinnum*; S: *P. speciosissimum*; X: genome of undetermined derivation shared only by *P. orbiculatum* and *P. talamancanum*; Y: genome of undetermined derivation unique to *P. orbiculatum*. Collection numbers for greenhouse-grown leaves of species (all Barrington collections deposited at VT): *P. concinnum, 816*; *P. orbiculatum, 1273*; *P. speciosissimum, 1700*; *P. talamancanum, 1252*. Scale bar, lower right, is 15 cm.



FIGURES 2–5. Meiosis I of *Polystichum* species from Costa Rica.—2. *P. concinnum*, 41 pairs (*Barrington 822* VT).—3. *P. speciosissimum*, 41 pairs (*Barrington 976* VT).—4. *P. talamancanum*, 82 pairs (*Barrington 1261* VT).—5. *P. orbiculatum*, 81 pairs and 2 univalents indicated with arrows (*Barrington 1273* VT).

canum. The hybrids between closely allied species of *Polystichum* are especially difficult to distinguish morphologically because of overlap in ontogenetic and environmentally induced variation (Barrington, 1985a). A combination of morphological, cytological, and isozyme data is necessary to establish evolutionary relationships among these *Polystichum* taxa.

METHODS

FIELDWORK

Species and hybrids of *Polystichum* were collected during several trips to the Cerro de la Muerte, San José and Cartago provinces, Costa Rica, between 1980 and 1989. The study set comes from the montane rainforest and subalpine rain páramo at altitudes between 2,800 and 3,400 m; details of habitat and altitude for species and hybrids have been reported elsewhere (Barrington, 1985a, b, 1989).

CYTOLOGY

Field-collected species and hybrids for cytological work were cultivated at the University of Vermont greenhouses. Young sporangia for study of meiotic chromosome number and pairing were fixed in freshly mixed Farmer's solution (3 : 1 absolute ethanol : glacial acetic acid) for 18–24 hours, rinsed in 70% ethanol, and stored in 70% ethanol at 0°C.

TABLE 1. Fixed and common allozyme bands characteristic of Costa Rican *Polystichum* species. Both bands of fixed heterozygotes reported. Bands are reported as percent of fastest migrating band. Bands diagnostic of proposed diploid genomes labeled: C = *concinnum*, S = *speciosissimum*, X = genome of unknown origin shared by *talamancanum* and *orbiculatum*, Y = genome of unknown origin unique to *orbiculatum*.

	IDH		PGI-2		SkDH		TPI-1	
concinnum	82		100	C	100	C	68	
speciosissimum	100	S	83	S	84		68	
talamancanum	82		100	C	100	C	68	
			63	X	70	X		
orbiculatum	82		74	Y	84		86	Y
			63	X	70	X	68	

Material thus treated remained useful for analysis for at least three years. Staining of crushed sporangia was in 2% ferric acetocarmine at room temperature for about five minutes; Hoyer's solution was thoroughly mixed into the staining mixture before squashing.

ISOZYME ELECTROPHORESIS

To document the heritage of hybrids, fresh leaf samples from greenhouse-grown species and hybrids were ground in the phosphate extraction buffer of Haufler (1985) in preparation for electrophoresis on 12% starch gels. Electrophoretic procedures and staining schedules are as reported in Soltis et al. (1983). Triosephosphate isomerase (TPI), phosphoglucomutase (PGM), and phosphoglucose isomerase (PGI) were resolved on buffer system 6 of Soltis et al. (1983). Leucine aminopeptidase (LAP), aspartate aminotransferase (AAT), and hexokinase (HK) were assayed using buffer system 8 of Haufler (1985). System 11 of Haufler (1985) was used to resolve fructose 1-6 diphosphatase (F16DP), shikimate dehydrogenase (SkDH), isocitrate dehydrogenase (IDH), and malate dehydrogenase (MDH). All isozymes migrated anodally; isozymes were numbered from most anodal to nearest the origin. Allozymes are reported as percent of fastest-migrating band.

RESULTS

CHROMOSOME NUMBERS OF SPECIES

Here I provide chromosome numbers for each of the four species as a basis for inferences about parentage of the hybrids encountered. The counts are all first records for the species. On the basis of counts for nine individuals, *P. concinnum* is diploid with 41 pairs of chromosomes at meiosis I (Fig. 2). *Polystichum speciosissimum* is also diploid, with 41 pairs in meiosis I, based on several counts of a single individual (Fig. 3). *Polystichum talamancanum* is tetraploid, since it showed 82 pairs of chromosomes in meiosis I, based on a sample of five individuals (Fig. 4). Material from two sporophytes of *P. orbiculatum* collected on Cerro de la Muerte yielded counts of 82 pairs or 81 pairs and two univalents (Fig. 5), documenting it as tetraploid.

ISOZYME PATTERNS OF THE SPECIES

Unique combinations of fixed (or in one case very common) bands characterize the four *Polystichum* species from the Cerro de la Muerte (Table 1, Figs. 12–15). Two bands are diagnostic of *P. concinnum*: SkDH^{100} (present in all 14 sporophytes sampled) and PGI-2^{100} (present in 13 of 14 sporophytes sampled). *Polystichum speciosissimum* also has two diagnostic bands: IDH-2^{100} and PGI-2^{83}, both fixed in a sample of nine sporophytes. PGI-2^{100} and SkDH^{100}, shared with *P. concinnum*, were present in 25 sampled plants of *P. talamancanum*; the other alleles at each of these two loci (PGI-2^{63} and SkDH^{70}) have so far not been seen in diploid *Polystichum* species from Costa Rica. PGI-2^{63} and SkDH^{70}, fixed in *P. talamancanum* but absent in *P. concinnum*, were also present in all seven sampled sporophytes of *P. orbiculatum*. Two bands at fixed heterozygous loci, PGI-2^{74} and TPI-1^{86}, were unique to *P. orbiculatum* among Costa Rican species.

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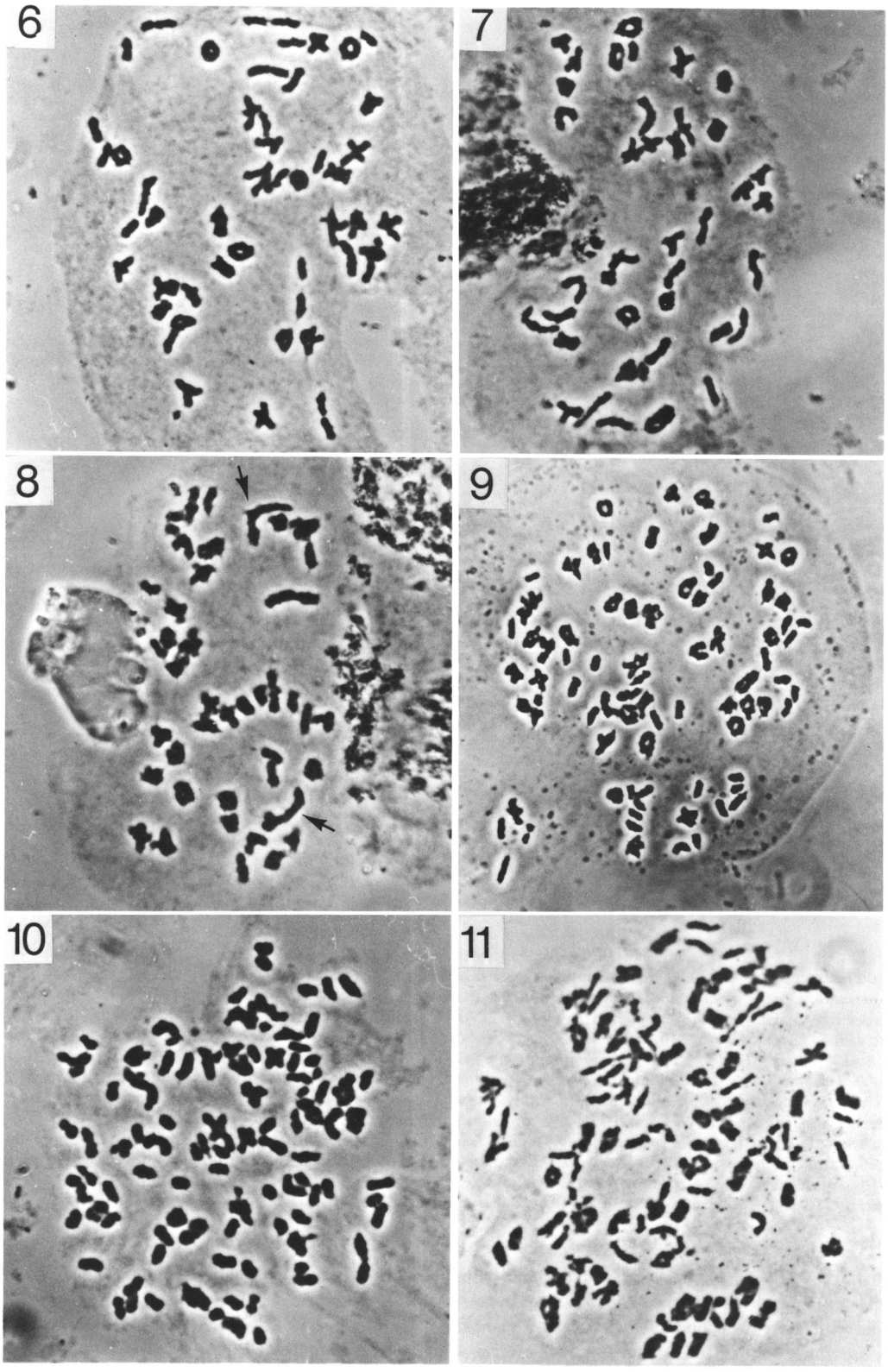
FIGURES 6–11. Meiosis I of *Polystichum* hybrids from Costa Rica.—6. *P. concinnum* × *P. speciosissimum*, 32 pairs and 18 univalents (*Barrington 1274* VT).—7. *P. concinnum* × *P. speciosissimum*, 40 pairs and 2 univalents (*Barrington 1274* VT).—8. *P. concinnum* × *P. speciosissimum*, 2 trivalents (indicated with arrows), 34 pairs, and 8 univalents (*Barrington 1274* VT).—9. *Polystichum concinnum* × *P. talamancanum*, 41 pairs and 41 univalents (*Barrington 1278* VT).—10. *Polystichum speciosissimum* × *P. talamancanum*, 31 pairs and 60 univalents (*Barrington 973* VT).—11. *Polystichum orbiculatum* × *P. talamancanum*, 51 pairs and 62 univalents (*Barrington 1283* GH, VT).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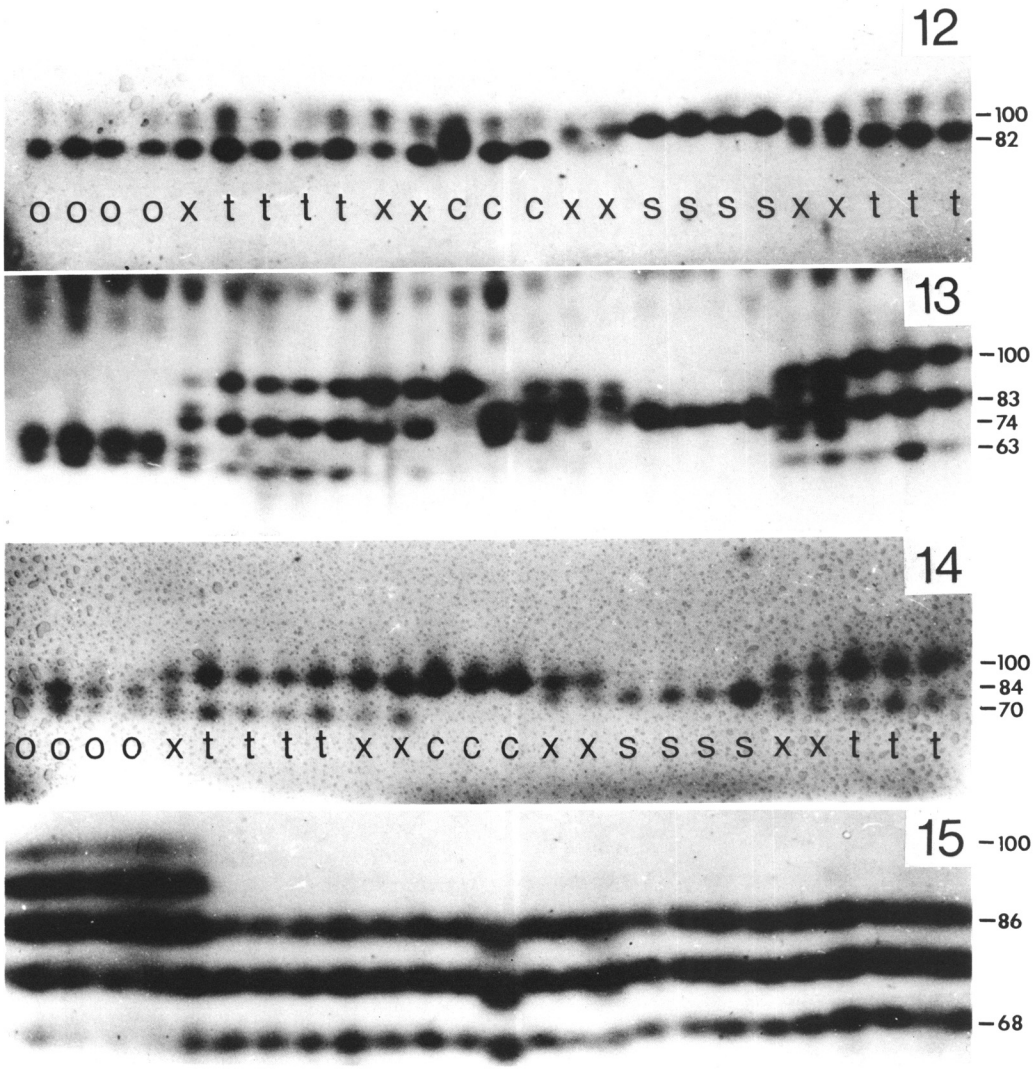

FIGURES 12–15. Isozymes of Costa Rican polystichums: c = *P. concinnum*, o = *P. orbiculatum*, s = *P. speciosissimum*, t = *P. talamancanum*, x = hybrid between adjacent species. Mobilities indicated to right of gels are average percents of fastest band at each locus. Barrington collection numbers for source plants for isozymes in order left to right (vouchers deposited at VT): o, *1273*, *1504*, *1509*, *978*; x, *1283*; t, *1263*, *1155*, *1270*, *1261*; x, *1244*, *1260*; c, *727*, *816*, *822*; x, *1274*, *1516*; s, *1513*, *1708*, *1704*, *1700*; x, *1500*, *973*; t, *1250*, *1280*, *1282*.—12. IDH.—13. PGI-2: *P. concinnum* shows three genotypes, two with the *P. concinnum* marker PGI-2^{100}; in *P. orbiculatum* × *P. talamancanum* note heterodimer combining PGI-2^{74} (*P. orbiculatum* marker) with PGI-2^{100} (*P. talamancanum* marker inherited from *P. concinnum*); in *P. speciosissimum* × *P. talamancanum* note heterodimers combining both PGI-2^{63} and PGI-2^{100} (*P. talamancanum* marker inherited from *P. concinnum*) with PGI-2^{83} (marker inherited from *P. speciosissimum*).—14. SkDH.—15. TPI-1: *P. orbiculatum* band interpretation—fixed heterozygote combines bands of unmodified allozymes 68 and 86 and their respective modified versions at 86 and 100 with all possible heterodimers. TPI-1^{86} co-migrates with the modified variant of TPI-1^{68}. Other taxa have only the slower triplet. Alternatively 86 and 100 are the unmodified products.

Each of the two tetraploid species consistently showed a single heterozygous banding pattern at loci with more than one allele. *Polystichum talamancanum* showed fixed heterozygous patterns at HK, LAP, MDH, PGI-1, PGI-2, and SkDH in all sampled plants. All sampled plants of *P. orbiculatum* showed fixed heterozygous expression at AAT, HK, LAP, PGI-1, PGI-2, PGM, SkDH, and TPI-1. Complexity of the fixed heterozygous pattern at TPI-1 is hypothesized to be increased by

post-translational modification, as discussed in Gastony (1988); see caption to Figure 15 for interpretation.

CHROMOSOME NUMBERS AND PAIRING BEHAVIOR OF HYBRIDS

The four putative hybrids from the Cerro de la Muerte were documented with information on chromosome number and pairing behavior. The hybrid between the two diploid species, *P. concinnum* and *P. speciosissimum*, was documented to be diploid based on counts of two hybrid individuals (*Barrington 705* and *1274*). Pairing was notably variable within individuals, ranging from 32 pairs and 18 univalents to 40 pairs and 2 univalents (Figs. 6, 7). Trivalents were also observed (Fig. 8). The hybrid between *P. concinnum* and *P. talamancanum*, based on chromosome counts of six individuals, is triploid with 41 pairs and 41 univalents at meiosis I (Fig. 9). The hybrid between *P. speciosissimum* and *P. talamancanum* is represented in this study by two individuals (*Barrington 973* and *1500*), the first of which yielded a triploid count of 31 pairs and 61 univalents in meiosis I (Fig. 10). The fourth hybrid, *P. orbiculatum* × *P. talamancanum*, is represented by a single individual, *Barrington 1283*. This hybrid yielded a tetraploid count of 51 pairs and 62 univalents in meiosis I (Fig. 11).

ISOZYME PATTERNS OF HYBRIDS

Hybrids have isozyme profiles consistent with their parentage as proposed from morphological criteria (Figs. 12–15). Based on two hybrid plants, *Barrington 1274* and *1516*, *Polystichum concinnum* × *P. speciosissimum* includes the marker bands of *P. concinnum* (PGI-2^{100} and SkDH^{100}) and *P. speciosissimum* (IDH-2^{100} and PGI-2^{83}). The hybrid between *P. concinnum* and *P. talamancanum* has the same diagnostic bands and fixed heterozygous loci as *P. talamancanum*, based on electrophoretic analysis of three individuals. Both individuals of *P. speciosissimum* × *P. talamancanum* include the marker bands reported for the proposed parents (IDH-2^{100} and PGI-2^{83} for *P. speciosissimum*; PGI-2^{100} and SkDH^{100} for *P. talamancanum*). *Polystichum orbiculatum* × *P. talamancanum* includes the diagnostic bands for *P. talamancanum* and *P. orbiculatum* (that is, PGI-2^{100} and SkDH^{100} shared with *P. talamancanum*, PGI-2^{63} and SkDH^{70} shared with both *P. talamancanum* and *P. orbiculatum*, and PGI-2^{74} and TPI-1^{86} shared only with *P. orbiculatum*).

DISCUSSION

INFERENCES ABOUT EVOLUTIONARY RELATIONSHIPS

Consideration of the cytological and electrophoretic data presented here leads to hypotheses for evolutionary relationships between *Polystichum* species on the Cerro de la Muerte (Fig. 1, Table 1). *Polystichum talamancanum* is an allotetraploid, since it has 82 pairs of chromosomes in meiosis I and its heterozygous banding patterns are all fixed. Corroborating evidence is available: the triploid *P. speciosissimum* × *P. talamancanum* consistently shows fewer than 41 pairs of chromosomes, suggesting that the hybrid combines three nonhomologous sets of chromosomes. Hence the two sets of chromosomes contributed by *P. talamancanum* are not homologous, so *P. talamancanum* must be an allotetraploid. Furthermore, because all marker allozymes for *P. concinnum* are included in the fixed heterozygous banding patterns of *P. talamancanum*, one of the progenitors of *P. talamancanum* is almost certainly *P. concinnum*. This interpretation is supported by the pairing pattern (equal number of univalents and pairs) in *P. concinnum* × *P. talamancanum*, typical of backcrosses between allotetraploids and their diploid progenitors. *Polystichum talamancanum* is almost certainly recent in origin, since its geographic range is highly restricted and coterminous with one of its progenitors (Stebbins, 1971).

Polystichum orbiculatum, a common alpine species from Bolivia to Mexico, is allotetraploid at Cerro de la Muerte; it shows the fixed heterozygous pattern typical of allopolyploids that combine different alleles from each of their diploid progenitors. *Polystichum orbiculatum*, as broadly circumscribed by recent taxonomists (Smith, 1981; Stolze, 1981), may also include one or both of its diploid progenitor species, since small-spored plants have been encountered in Venezuela (Barrington et al., 1986). A similar species perhaps involved in the ancestry of *P. orbiculatum* is *P. sodiroi* Christ of Ecuador, for which a diploid count of $2n = 82$ has been reported (Sorsa in Fabbri, 1965).

Polystichum orbiculatum evidently shares one parent with *P. talamancanum*, since a subset of marker allozymes is consistently shared by the two allotetraploid species. Corroborating evidence for this conclusion is the pairing pattern in *P. orbiculatum* × *P. talamancanum*. Its 51 pairs and 62 univalents are interpreted as representing two homologous sets of chromosomes, one contributed by each of the two tetraploids reported here, and two nonhomologous sets (some members of which

pair), representing a *concinnum* genome contributed by *P. talamancanum* and a genome of unknown origin contributed by *P. orbiculatum*. The two homologous sets, which undergo allosyndetic pairing in this hybrid, are presumably derived from a single diploid progenitor, so far not documented from the Cerro. This implication of a single diploid progenitor in the origin of more than one allotetraploid is a pattern already documented in the north temperate zone (e.g., *Dryopteris cristata* and *D. carthusiana*, Werth, 1989).

GENERAL SIGNIFICANCE

There is no evidence that the species on Cerro de la Muerte constitute a monophyletic group; on the contrary, there is ample morphological evidence that the diploid species present or implicated in the origin of hybrid species on the Cerro are members of different species groups with geographically disparate affinities. For instance, *Polystichum concinnum* is allied to the diverse complex of species from southern Mexico (Barrington, 1989), but *P. orbiculatum* is presumably Andean in origin (Barrington et al., 1986). Although *P. speciosissimum* is morphologically distinct enough to have been recognized as a monotypic species of the genus *Plecosorus*, its chromosome behavior in meiosis I suggests that it is not strongly differentiated from the remainder of the taxa here. Even in a large and morphologically diverse genus like *Polystichum*, hybridization and polyploidy depend on geographic and ecological proximity. The scope of *Polystichum* species involved in secondary interactions is not limited by phylogenetic proximity (as inferred from morphology).

A high frequency of homoeologous pairing is documented here for hybrids between tropical-montane *Polystichum* species, just as it was for north temperate hybrids (Wagner, 1973). There is also notable variation in pairing in these hybrids. Furthermore, degree of homoeologous pairing in hybrids is independent of morphological similarity in these species. Especially telling is the hybrid between *Polystichum concinnum* and *P. speciosissimum*, which shows virtually complete pair formation in some cells although the progenitor species are strongly differentiated morphologically (Barrington, 1985b). This emerging picture of unusual residual homology (homoeology), typical in *Polystichum* but rare elsewhere in the Polypodiales, may reflect one of two cytological phenomena. It is possible that divergence of whole genomes is not so great in *Polystichum* species as in other genera of ferns, in which case the genus has a noteworthy place in studies of fern evolution. Alternatively, pairing may be under less stringent genetic control in *Polystichum* than in other fern groups, thus permitting the relatively high frequency of homoeologous pairing observed in *Polystichum* hybrids (Wagner, 1973; Barrington, 1986).

The pattern of interactions among *Polystichum* species in high-montane Costa Rica emerging from this work and from earlier papers is notable for its similarity to patterns of hybridization and speciation in north temperate ferns in the Dryopteridaceae, Aspleniaceae, and other groups (Lovis, 1977; Barrington et al., 1989). The ecology of interaction among species (Barrington, 1985b), the morphology of the hybrids and hybrid species (Barrington, 1985a, b, 1989), the basic features of chromosome behavior at meiosis I, and isozyme patterns are all similar to those seen in north temperate polyploid complexes. Since Pleistocene climatological change was significant in tropical America (van der Hammen, 1974), vegetational change and ecological disturbance in the Sierra Talamanca may have been qualitatively like that in the north temperate zone. Hence, similar Pleistocene climatological history may be the underlying determinant of speciation phenomena in the genus *Polystichum* in both regions.

LITERATURE CITED

BARRINGTON, D. S. 1985a. The morphology and origin of a new *Polystichum* hybrid from Costa Rica. Syst. Bot. 10: 199–204.

———. 1985b. Hybridisation in Costa Rican *Polystichum*. Proc. Royal Soc. Edinburgh 86B: 335–340.

———. 1985c. The present evolutionary and taxonomic status of the fern genus *Polystichum*: the 1984 Botanical Society of America Pteridophyte Section symposium. Amer. Fern J. 75: 22–28.

———. 1986. The morphology and cytology of *Polystichum* ×*potteri* hybr. nov. (= *P. acrostichoides* × *P. braunii*). Rhodora 88: 297–313.

———. 1989. New species and combinations in tropical American *Polystichum* (Dryopteridaceae). Ann. Missouri Bot. Gard. 76: 365–373.

———, C. H. HAUFLER & C. R. WERTH. 1989. Hybridization, reticulation, and species concepts in ferns. Amer. Fern J. 79: 55–64.

———, C. A. PARIS & T. A. RANKER. 1986. Systematic inferences from spore and stomate size in the ferns. Amer. Fern J. 76: 149–159.

DAIGOBO, S. 1972. Taxonomical studies on the fern genus *Polystichum* in Japan, Ryukyu, and Taiwan. Sci. Rep. Tokyo Bunrika Daigaku. Sect. B. 15: 57–80.

FABBRI, F. 1965. Secondo supplemento alle Tavole cromosomiche delle Pteridophyta di Alberto Chiarugi. Caryologia 16: 675–731.

GASTONY, G. J. 1988. The *Pellaea glabella* complex: electrophoretic evidence for the derivations of aga-

mosporous taxa and a revised taxonomy. Amer. Fern J. 78: 44–67.

HAUFLER, C. H. 1985. Enzyme variability and modes of evolution in *Bommeria* (Pteridaceae). Syst. Bot. 10: 92–104.

LOVIS, J. D. 1977. Evolutionary patterns and processes in ferns. Adv. Bot. Res. 4: 229–415.

MANTON, I. 1950. Problems of Cytology and Evolution in the Pteridophyta. Cambridge Univ. Press, Cambridge, England.

SMITH, A. R. 1981. Part 2. Pteridophytes. *In:* D. E. Breedlove (editor), Flora of Chiapas. California Academy of Sciences, San Francisco.

SOLTIS, D. E., C. H. HAUFLER, D. C. DARROW & G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73: 9–27.

SOLTIS, P. S., D. E. SOLTIS & E. R. ALVERSON. 1987. Electrophoretic and morphological confirmation of interspecific hybridization between *Polystichum kruckebergii* and *P. munitum*. Amer. Fern J. 77: 42–49.

STEBBINS, G. L. 1971. Chromosomal Evolution and Higher Plants. Addison-Wesley, Reading, Massachusetts.

STOLZE, R. G. 1981. Ferns and fern allies of Guatemala. Part 2. Polypodiaceae. Fieldiana, Bot. n.s. 6: 1–522.

VAN DER HAMMEN, T. 1974. The Pleistocene changes in vegetation and climate in tropical South America. J. Biogeogr. 1: 3–26.

VIDA, G. & T. REICHSTEIN. 1975. Taxonomic problems in the fern genus *Polystichum* caused by hybridization. Pp. 126–135 *in* S. M. Walters (editor), European Floristic and Taxonomic Studies. E. W. Classey, Faringdon, England.

WAGNER, D. H. 1979. Systematics of *Polystichum* in western North America and north of Mexico. Pteridologia 1: 1–64.

WAGNER, W. H., JR. 1973. Reticulation in holly ferns (*Polystichum*) in the western United States and adjacent Canada. Amer. Fern J. 63: 99–115.

WERTH, C. R. 1989. Isozyme evidence on the origin of *Dryopteris cristata* and *D. carthusiana*. Amer. J. Bot. 76(6, supplement): 208.