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Modes and mechanisms of speciation in pteridophytes: Implications of contrasting patterns in ferns representing temperate and tropical habitats

CHRISTOPHER H. HAUFLER,* ELISABETH A. HOOPER,† and JAMES P. THERRIEN*
*Department of Ecology and Evolutionary Biology, Haworth Hall, University of Kansas, Lawrence 66045, Kansas and †Department of Biology, Truman State University, Kirksville, Missouri, USA

Abstract

Discovering how biological diversification results in species is one of the primary challenges facing evolutionary biologists. In the ferns, evidence indicates that dissimilar speciation modes and mechanisms may differentiate some temperate and tropical groups. The *Polypodium sibiricum* group contains three related diploid species that all inhabit rock outcrops in temperate forests. Although differing lettle in gross leaf morphology and joined by the distinctive morphological synapomorphy of sporangiasters, these three species have an average interspecific genetic identity developed from isozymic comparisons of only 0.460. A likely mode of speciation is that periodic glaciation pushed *Po. sibiricum* populations south and, with the retreat of the glaciers, southern populations persisted, evolved diagnostic traits, and ultimately erected postzygotic barriers to interbreeding. This hypothesis follows a classic allopatric speciation model and interspecific distinctions may have been reinforced through contact mediated by subsequent ice ages. In contrast, a monophyletic group of four diploid, epiphytic *Pleopeltis* species centered in Mexico has an isozymically-determined average interspecific genetic identity value of 0.849. In spite of this high value, these species show greater morphological discrimination than do the *Polypodium* species. Although the species ranges overlap, they appear to occupy ecologically discrete habitats. These *Pleopeltis* species may have originated through adaptation to different ecological zones and developed individual morphologies in the process. The high interspecific genetic identity values among the *Pleopeltis* species suggest a relatively recent and/or rapid process. These hypotheses should be tested by further biosystematic investigations and the discovery of additional monophyletic assemblages with similar patterns of speciation.

Keywords: DNA sequencing, ferns, isozymes, *Pleopeltis*, *Polypodium*, speciation

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Introduction

Although evolutionary biologists have yet to achieve the elusive goal of formulating a single, universally accepted concept or definition of the word, species, most scientists agree that such entities constitute real biological systems. At any one point in the history of life on earth, it is possible to discern discrete units whose boundaries can be defined and that are isolated from other such units. Across time, species as individuals are born (originate) and die (become extinct). While extant, they may be successful (persist for an extended period of time, develop an extensive geographic range) or unsuccessful (exist briefly, achieve only a narrow range) and they may leave progeny in the form of descendent species. Species have been recognized morphologically, behaviorally, genetically and ecologically. Their reality can be demonstrated experimentally and testable hypotheses recounting their ancestry can be generated. To date, in excess of 1.7 million species have been formally recognized, although many more than that are yet to be discovered and delimited. Species descriptions constitute scientific hypotheses that

Correspondence: Christopher H. Haufler
(Email: vulgare@ukans.edu).

can be tested and falsified, and such hypotheses are the fundamental units in systematic biology.

The boundaries that maintain species as separate individuals may be external forces that isolate lineages (e.g. geographic barriers, incompatibility systems, habitat specialization) or internal cohesion that maintains species as integrated systems (e.g. gene flow, developmental constraints). Identifying how these boundaries originate and are maintained requires analysis at the interface of population biology and systematics. In the present paper, we approach this interface as we consider some of the ways that fern lineages may originate. To develop perspectives on fern species and speciation we discuss examples from two well-studied groups in order to illustrate contrasting modes and mechanisms. These examples demonstrate that even in a group of plants lacking the sophisticated and dynamic mechanisms found in flowering plants, speciation is a complex, fascinating, and challenging issue for evolutionary biologists to consider.

Species and ferns

Just as has been the case for the rest of biology, fern systematists have applied a variety of definitions to species. Of necessity, early workers employed morphologically based evidence, but even today morphology continues to be the primary criterion in recognizing species and formulating initial hypotheses about new lineages. Modern floristic surveys are based primarily on morphology and provide the first clues to identify certain individuals or populations as potentially unique, and deserving of further scrutiny. Once a species has been diagnosed, it represents a working hypothesis that can be tested through the application of new evidence or analytical methods. In the ferns, the first auxiliary tool that had a major impact on species concepts was the development of techniques for determining chromosome numbers and studying meiotic behavior (Manton 1950). The new perspectives that were obtained through chromosomal studies led to a revolution in the recognition of species boundaries in many groups. By discovering polyploids in taxa that had been considered simply 'polymorphic assemblages', Manton demonstrated that evolutionary mechanisms in ferns were more complex than previously appreciated and that hybridization between distinct species was an important component of the history of fern lineages. Manton (1950) and her students effectively built a new perception of fern species. They analyzed meiosis in artificial hybrids to characterize the limits of fern lineages as reproductively isolated units, and to identify genetic similarities between genomes. In this way, fern biologists were among the early proponents of the 'biological species' concept (Mayr 1942; 1969). These groundbreaking studies showed that interactions among 'primary' species involving hybridization and polyploidy generated 'secondary' species (terminology *sensu*, Grant 1981), and resulted in reticulate species complexes whose intricacies challenged subsequent systematists. As new techniques became available, the chromo-somally defined species of Manton (1950) and her students constituted the working hypotheses for further analysis.

With the advent of electrophoretic analyses of isozymes, it became possible to generate more precise species signatures that could be traced through intricate and convoluted paths of hybridization and polyploidy (Haufler 1985; 1987; Werth 1989). Species complexes that were characterized through morphological and chromosomal analyses (e.g. *Asplenium*,Wagner 1954; *Polypodium*, Shivas 1961) were further refined by isozyme characters (Werth *et al*. 1985; Haufler *et al*. 1995a, respectively). In some cases, all of the working hypotheses concerning allopolyploid origins were confirmed (Werth *et al*. 1985), whereas in others, new hypotheses were required and new species were recognized in the process (Haufler & Windham 1991; Haufler *et al*. 1995a). However, even in such 'modern' studies, the species definition employed by the authors focused primarily on morphological evidence and reproductive biology, and did not formally incorporate elements of their evolutionary history.

Among the more curious aspects of fern systematics is the application of phylogenetic principles in defining species and developing hypotheses about their ancestry. For decades, Wagner (1961) and his students promoted an explicitly phylogenetic approach to studying fern lineages. Ultimately, Wagner's 'groundplan-divergence' methods became the basis of modern algorithms used in reconstructing evolutionary history ('Wagner trees'). Given the extensive application of these fern-originated methods to other groups of organisms, it has been unfortunate that inherent difficulties of working with ferns have constrained the formulation of explicit phylogenetic hypotheses among pteridophytes. Successful application of 'evolutionary species' definitions in pteridophyte systematics has been limited because fern lineages have (i) very long histories, (ii) extensive gaps in information through extinction, and (iii) a restricted suite of informative morphological traits that can be polarized and used to establish transformation series.

As a corollary to the difficulties encountered in recognizing 'evolutionary species' in ferns, it has also been difficult to develop well-supported hypotheses about the evolutionary histories and interrelationships among lineages. Without confidence in the phylogeny of the species, the proposal of modes and mechanisms of speciation has been quite limited; unless sister species are compared, erroneous conclusions about their origins will

result. With the advent of methods for analyzing the structure of DNA molecules, opportunities for formulating new perceptions of phylogenetic relationships and assessing sister taxon status have been enhanced (Wolf 1995). Combining data from DNA sequences with those from other lines of evidence can yield greater clarity in establishing the ancestor/descendant relationships that are critical in formulating hypotheses about modes and mechanisms of speciation.

Speciation and ferns

Evolutionary biologists have amassed considerable evidence to document the pattern or mode of organismal diversification, but we know much less about the processes or mechanisms that initiate the splitting of lineages to generate greater diversity. We appreciate that these processes have occured and that they are continuing to lead to even more diversification because we can see the results, and we can develop reasonable hypotheses about the ancestry and interrelationships of current species. At the same time, we have embarrassingly little information on the origins of species and the mechanisms by which species are initiated.

Recent studies have helped to elucidate some of the dynamics that have generated species diversity in the ferns. Unveiling the ancestry of allopolyploid species has benefited greatly from isozymic approaches (e.g. Werth *et al*. 1985; Werth 1989; Haufler *et al*. 1995a). In some cases, seemingly radical and unlikely hypotheses for species origins have been supported by new and revealing data. For example, hypotheses that past episodes of secondary speciation in *Dryopteris* involved now extinct species (Wagner 1971) have been robustly supported by isozymic studies (Werth 1991); the seemingly bizarre hypothesis of species origins via unreduced spores (Morzenti 1967) appears to be an accurate depiction of past events (Gastony 1986); and the much debated 'tertiary speciation' (Haufler 1989) mediated by gene silencing (Werth & Windham 1991) has gained support from isozymic studies (Gastony 1991).

Unfortunately, just as with chromosomal data, it became apparent in many cases that isozymes were not up to the task of testing hypotheses concerned with the phylogenies of primary species. Whereas the origins of secondary species appear to have been relatively recent events, and represent instantaneous and radical modifications of genomes [because most involve interspecific hybridization (allopolyploidy)], the proximate mechanisms of primary speciation probably seldom involve immediate or obvious changes in observable features, and it is difficult to discern whether such processes occured quite recently or in the distant past. Further, the 'spark' that launched new primary lineages may be incredibly subtle, involving a small number of individuals and a short time period. In most cases, primary speciation remains a tightly locked black box, the contents of which are difficult to study or to reconstruct with certainty.

There are further impediments to developing a clear understanding of the modes and mechanisms of primary speciation in ferns. First, although the monophyly of modern leptosporangiate ferns appears solid (Pryer *et al*. 1995), this group has a long history, and extinction has undoubtedly resulted in a loss of information about the interrelationships of groups. Second, chromosome numbers in the ferns are very high (Klekowski & Baker 1966) and this feature of their history may have arisen through a high frequency of allopolyploidy (Wagner & Wagner 1980; Haufler 1987), a process that would scramble discrete lineages. Third, although high chromosome numbers provide evidence of a polyploid genetic condition, isozymic analyses indicate that the high basal chromosome numbers in fern genera nonetheless have typical diploid genetic expression. This seeming paradox of polyploid chromosome numbers and diploid isozymic expression could have resulted from episodes of allopolyploidy followed by silencing of duplicated genes (Haufler 1987). If that is the case, potentially significant information about the history of lineages may be lost as gene expression is turned off and mutation of DNA sequences is no longer selectively constrained. Finally, adding to the already complex problems associated with proposing hypotheses about primary divergence events, unexpectedly low genetic identities were discovered between congeneric fern species (Haufler 1987; Soltis & Soltis 1989). Even species that were nearly identical morphologically appeared highly divergent genetically and shared only a few allozymes. With limited genetic overlap between sister species, accurately assessing allozymic homologies became a challenging task because it was difficult to be certain that bands of stained protein migrating to the same position on starch gels were actually the same molecule. Given the low genetic identities, there were very few synapomorphic isozymes that could be coded and therefore (except for closely related congeners), isozymes proved to be an ineffective molecular tool for testing phylogenetic hypotheses or tracing the lineages of extant taxa.

To test speciation hypotheses in any group, it is important to be certain of comparing most closely related lineages. Because of the inherent problems in studying the evolutionary history of ferns, confidence in predictions concerning the monophyly of any set of species has been low. This situation was not ameliorated by evidence from isozymes. Fortunately, however, recent studies of fern DNA sequences have demonstrated that this molecular

tool can and should be used to assess relationships among fern lineages (Hasebe *et al*. 1995; Wolf 1995). Once a more robust hypothesis of interspecific relationships is obtained, combining evidence from DNA with genetic identity measures derived from isozymes can provide new perspectives on species and speciation. Uniparentally inherited (Gastony & Yatskievych 1992) and more conservative regions of chloroplast DNA may yield greater clarity of species divergence than other measures, and should be less prone to the problems of allopolyploid mixing of lineages (Haufler 1995). Once sister species relationships have been verified, isozymes can be used to estimate the level of divergence between lineages.

Until recently, nearly all studies of fern population dynamics were based on temperate species. However, isozymic analyses of species in Hawaii (Ranker 1992a,b; 1994; Ranker *et al*. 1996) and tropical America (Hooper & Haufler 1997) showed that some morphologically distinct species have very high interspecific genetic identity values (Nei's I). These studies have provided new perspectives on evolutionary patterns and processes in the ferns, and have suggested that more than one model of how fern lineages diversify should be entertained. The discovery that interspecific genetic identities are not always high indicated that either:

1. Studies restricted to temperate groups failed to include all of the members of the clades and the genetic identities were artificially reduced because the closest relatives were missing from the data set. Following this argument, temperate species groups under investigation were not monophyletic because their phylogenetic 'roots' were related to tropical species and these tropical representatives were not included in the data set. Alternatively,
2. The mechanisms giving rise to temperate species were different from those involved with at least some of the tropical species.

In this paper, using a combination of data from morphology, biogeography, isozymes and DNA, we provide evidence that the second hypothesis is most likely correct, and we develop syntheses of information that may be helpful in understanding the different pressures and possibilities associated with fern speciation.

Materials and methods

Whole genomic DNA was isolated following the protocol of Doyle and Doyle (1987). Concentrations of each DNA sample were determined with a spectrophotometer, and adjusted to 100 µg/mL for use in PCR reactions. The noncoding chloroplast DNA region between the *trnL* and *trnF* genes was PCR amplified using the primers TRNLE (5′-GGTTCAAGTCCCTCTATCCC-3′) and TRNFF (5′-ATTTGAACTGGTGACACGAG-3′) designed by Taberlet *et al*. (1991). The PCR thermal cycling profile for amplification included an initial 2 min at 94 °C, followed by 35 cycles of 1.5 min at 94 °C, 1.5 min at 50 °C, and 2 min at 72 °C, with a final extension period of 72 °C for 7 min. Amplified DNA was cleaned using QIAquick PCR purification tubes (Qiagen, Chatsworth, CA, USA), and sequenced using Thermo Sequenase dye terminator cycle sequencing premix kits, (ver. 2.0; Amersham Pharmacia, Pistcataway, NJ, USA), following the manufacturer's protocols. The same primers used for amplification were used for sequencing, and sequence reactions were electrophoresed using an Applied Biosystems (Foster City, CA, USA) model 310 genetic analyzer.

Complete sequences for the cpDNA *trnL–trnF* noncoding region were obtained for 32 taxa and deposited in Genbank (Table 1). The length of the sequences obtained, excluding the primer annealing sites, ranged from 159 bp to 310 bp. DNA sequences were aligned with CLUSTAL W 1.5 (Thompson *et al*. 1994), using the default settings (opening gap cost, 10; extending gap cost, 5), resulting in a total alignment length of 390 characters.

The sequence data were analyzed using PAUP 4.0.064 (Swofford 2000). The *g1* statistic for tree length distribution was computed for 100 000 random trees to provide an estimate of the phylogenetic information content of the sequence data (Huelsenbeck 1991). The distribution of tree lengths was skewed ($g1 = -0.9587$), suggesting that the data were potentially phylogenetically informative.

Heuristic searches were made using the maximum parsimony optimality criterion, with stepwise random addition of taxa, TBR branch swapping with the MulTrees option in effect and collapsing of zero length branches. Gaps (hypothesized insertions/deletions) were treated as missing data.

The heuristic search resulted in 180 most parsimonious trees of 618 steps. Topologies obtained were rooted using *Pecluma ptilodon*, *Pecluma ptilodon* var. *robusta* and *Pecluma divaricata* as a monophyletic outgroup. A strict consensus tree with decay indices and fast bootstrap values is shown in Fig. 1.

Results and Discussion

Polypodium sibiricum *group*

Three diploid species allied to the widespread tetraploid *Polypodium vulgare* constitute the *Polypodium sibiricum* group. Originally considered to be a circum-temperate, polymorphic single species, *Po. vulgare* is currently recognized as a monophyletic complex of seven diploid and seven polyploid species (Haufler *et al.* 1995a). Data from diverse sources were used in revising the systematics of

Table 1 Collection information for vouchers of specimens used in obtaining chloroplast DNA sequences of the *trnL–trnF* intergenic spacer region

Taxon	Location	Genbank Accession No.
Pecluma divaricata (E. Fourn.) Mickel & Beitel	Reserva Maquipucuna, Canton Quito, Ecuador. *K. Wilson 2703* (DAV)	AF159192
Pecluma ptilodon (Kunze) M. G. Price	Mexico. *Lefebre 3085* (UC)	AF159193
Pecluma ptilodon (Kunze) M. G. Price var. *robusta* Fée	Paraguay. No voucher	AF159191
Pleopeltis angusta Hb. et Bp. ex Willd.	Guatemala, Guatemala City. *W. Wagner s.n.* (UC)	AF159199
Pleopeltis astrolepis (Leibmann) E. Fourn.	Costa Rica *Andrews s.n.* (NEMO)	AF159202
Pleopeltis complanata (Weatherby) E.A. Hooper	Alajuela, Costa Rica. *Andrews 456* (NEMO)	AF159200
Pleopeltis conzattii (Weatherby) R. Tryon & A. Tryon	Oaxaca, Mexico. *Andrews 555* (NEMO)	AF159203
Pleopeltis crassinervata (Fée) T. Moore	San Luis Potosi, Mexico. *Andrews, MS22* (NEMO)	AF159206
Pleopeltis fructuosa (Maxon & Weatherby in Weatherby) Lellinger	Costa Rica. *Andrews s.n.* (NEMO)	AF159201
Pleopeltis mexicana (Fée) Mickel & Beitel	Oaxaca, Mexico. *Andrews 616* (NEMO)	AF159205
Pleopeltis polylepis (Roemer ex Kunze) T. Moore	Chihuahua, Mexico. *Andrews CP21* (NEMO)	AF159204
Pleopeltis polypodioides (L.) E. G. Andrews & Windham var. *michauxiana* (Weatherby) E.G. Andrews & Windham	Tyler Co., Texas, USA. *Kelly Irwin s.n.* (NEMO)	AF159196
Pleopeltis thyssanolepis (A. Braun ex Klotzsch) E.G. Andrews & Windham	Oaxaca, Mexico. *Andrews 1000* (KANU)	AF159198
Polypodium amorphum Suksdorf	Skamia Co. Washington, USA. *Haufler and Soltis s.n.* (KANU)	AF159182
Polypodium appalachianum Haufler & Windham	Sullivan Co., New Hampshire, USA. *Haufler s.n.* (KANU)	AF159181
Polypodium australe Fée	Cliffs at Anstey Cove, Torquay Coast; England. *C. Haufler, A.C. Jermy, & E. Rabe s.n.* (KANU)	AF159183
Polypodium azoricum (Carv. Vasc.) Ros. Fernandes	Azores Islands, E. Hennipman, No voucher	AF159184
Polypodium californicum Kaulf.	San Diego Co., California, USA. *Smith 2558* (UC)	AF159189
Polypodium fauriei H. Christ	Nikko, Japan. *Yahara* s.n. (TI)	AF159186
Polypodium fuscopetiolatum A. R. Smith	Yaracuy, Venezuela. *A. Smith 1345* (UC)	AF159178
Polypodium glaberulum Mickel & Beitel	Oaxaca, Mexico. *Barrington and Haufler 922* (KANU)	AF159180
Polypodium glycyrrhiza D. C. Eaton	Humboldt Co., California, USA. *Therrien s.n.* (KANU)	AF159188
Polypodium guttatum Maxon	Original source unknown, native to Mexico planted UC Bot. Garden (UC)	AF159195
Polypodium macaronesicum A. Bobrov	Tenerife, Canary Islands. *E. Hennipman FS082*. (Hennipman's personal herbarium)	AF159185
Polypodium pellucidum Kaulf.	Volcano National Park, Hawaii, USA. *Li, Haufler, and Werth s.n.* (KANU)	AF159190
Polypodium plesiosorum Kunze	Chiapas, Mexico. *Barrington and Haufler 924* (KANU)	AF159179
Polypodium ptilorhizon H. Christ	La Palma, Costa Rica. *C. Horich s.n.* (UC)	AF159194
Polypodium rhodopleuron Kunze	Vera Cruz, Mexico. *Barrington, Haufler, and Palacios-Rios 925* (KANU)	AF159177
Polypodium rosei Maxon	Sinaloa, Mexico. *M. Kimnach 107* (UC)	AF159197
Polypodium scouleri Hook. & Grev.	Marin Co., California, USA. *Therrien s.n.* (KANU)	AF159187
Polypodium sibiricum Siplivinskij	Saskatchewan, Canada. *Haufler and Rabe s.n.* (KANU)	AF159176
Polypodium sibiricum Siplivinskij	Tokachi Prefecture, Japan. *Haufler, Barrington and Paris s.n.* (KANU)	AF159175

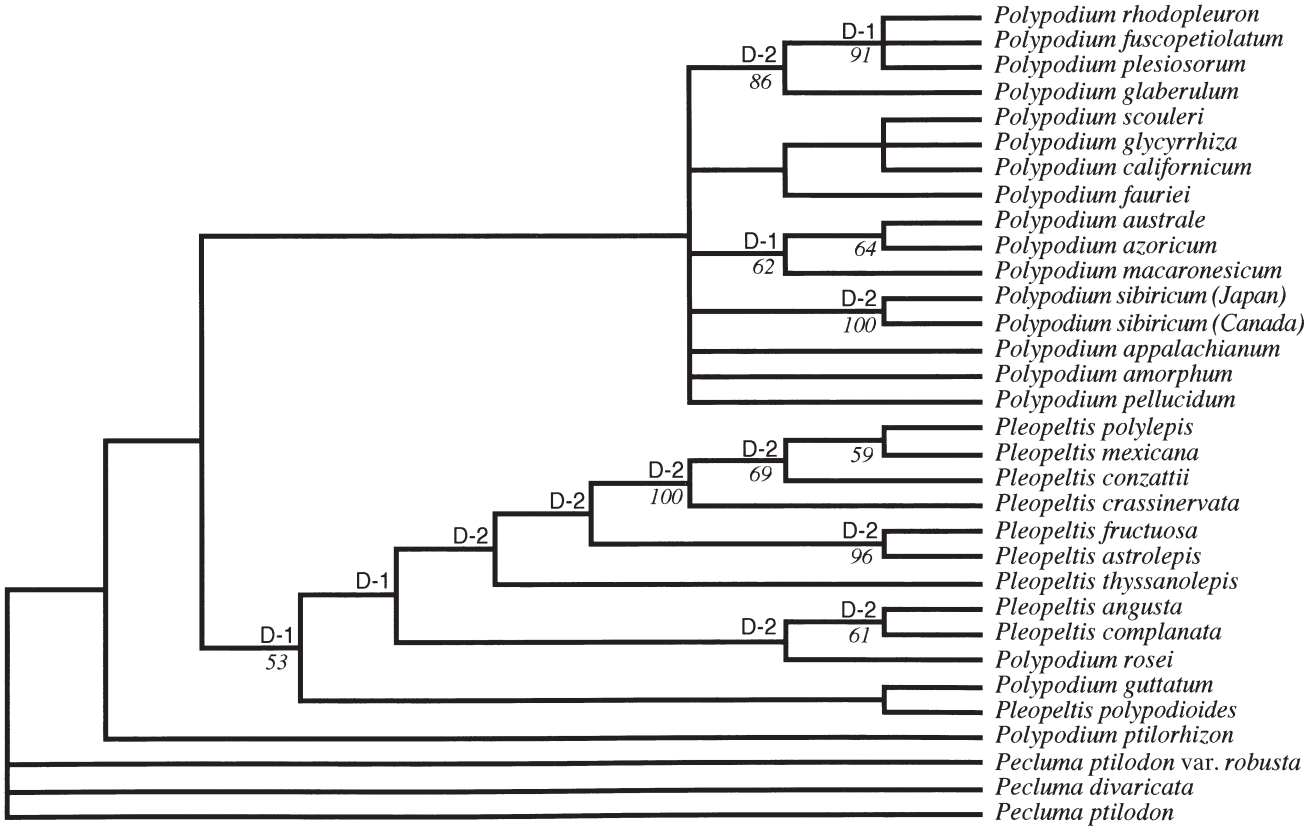

Fig. 1 Strict consensus tree of 180 most parsimonious trees based on PAUP analyses of chloroplast DNA sequences of the *trnL–trnF* spacer region. The shortest tree contained 618 steps and had a CI of 0.712 and RI of 0.716. Nodes that were maintained at one and two steps longer than the most parsimonious are labeled with D-1 or D-2. Fast bootstrap values are in italics below the appropriate branches.

the *Po. vulgare* complex. The first advances were provided by Manton (1950) and Shivas (1961) who used evidence from chromosome number and meiotic pairing behavior in hybrids to delimit diploid species, identify polyploids and propose hypotheses about their origins from diploids. Lang (1971) and Lloyd & Lang (1964) applied similar techniques in revising and summarizing relationships among North American representatives of the *Po. vulgare* complex. Haufler *et al.* (1995a) employed isozymes to test established patterns of species relationships and discovered that (i) some of the available hypotheses regarding the origins of polyploid species were consistent with results from the isozyme analyses, but other hypotheses were falsified; (ii) to revise the polyploid origin hypotheses required the circumscription of additional diploid species; (iii) although peculiar morphological features of the sori (sporangial derivatives known as 'sporangiasters') had been reported by previous workers (e.g. Martens 1943; Manton 1950; Shivas 1961; Lang 1971), the systematic significance of these structures only emerged from the revised hypotheses; (iv) isozymes provided clear fingerprints for identifying the diploid progenitors of polyploid species; however, (v) these protein variants were not useful in testing hypotheses about relationships among the diploid species. Subsequent DNA analyses (Haufler & 1995b) generated additional details about the polyploids, and further established the value of sporangiasters as informative morphological cues in understanding the relationships among the diploids.

In the context of developing perspectives and testing hypotheses of the modes and mechanisms of speciation, two aspects of the *Po. vulgare* complex are significant. First, all data indicate that this circum-temperate complex is monophyletic with phylogenetic roots in the New World tropics (Haufler & Ranker 1995), and second, the three diploid species that feature sori with sporangiasters (*Po. amorphum*, *Po. appalachianum* and *Po. sibiricum*, hereinafter referred to as 'the *Polypodium sibiricum* group') are sister species and therefore represent appropriate taxa for considering mechanisms that may have promoted diversification and lineage splitting within a coherent clade. The subtleness of the morphological distinctions of these

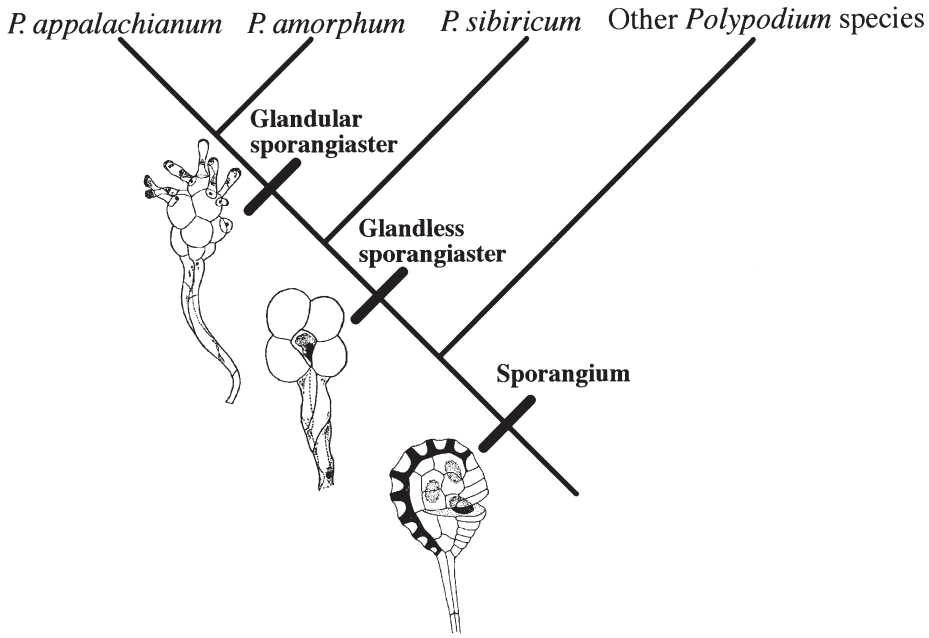

Fig. 2 Hypothesis for reconstructing the phylogeny of the *Polypodium sibiricum* species group supported by sporangiaster morphology and development, adapted from Haufler *et al*., 1995. The ancestral condition of having only sporangia in sori is transformed into the condition of having sporangia plus sporangiasters, both of which have common developmental origins. In *P. appalachianum* and *P. amorphum*, the sporangiasters become more complex by having glandular hairs.

three species is emphasized if it is noted that although specimens of them are present in prominent herbaria around the world, each was described only recently as a distinct taxa: *Po. amorphum* in 1969 (Lang 1969), *Po. sibiricum* in 1974 (Siplivinsky 1974), and *Po. appalachianum* in 1991 (Haufler & Windham 1991). Given the subtle nature of the morphological differentiation of these three species, the genetic (isozymic) distinctness of the lineages is surprising. Genetic identities among the *Polypodium sibiricum* group averages only 0.460 (Haufler *et al*. 1995a), suggesting that the three species have been distinct for a relatively long period of time. Nonetheless, the possession of the synapomorphic sporangiasters and the retention of plesiomorphic rhizome and leaf traits that are common to other members of the *Po. vulgare* complex provides a morphological basis for hypothesizing that the *Polypodium sibiricum* group represents a monophyletic assemblage.

Because they have been studied developmentally (Kott & Britton 1982), a transformation series for sporangiasters can be proposed and used as part of the evidence for reconstructing the phylogeny of the three species in the *Polypodium sibiricum* group (Haufler *et al*. 1995a). Sporangiasters appear to have originated from sporangial initials, and therefore the ancestral condition of lacking sporangiasters is consistent with the developmental data. Although all three taxa have sporangiasters, the morphology of these distinctive soral components varies among the species. Those in *Po. sibiricum* appear as a stalk topped by a cluster of large, clear cells whereas the ones in both *Po. amorphum* and *Po. appalachianum* look like maces and bear an additional set of glandular trichomes attached to the cluster of cells (Fig. 2). It may be hypothesized that the transformation series progressed from having only sporangia to bearing simple sporangiasters to having the more structurally complex glandular sporangiasters. This transformation series provides strong support for the phylogenetic hypothesis shown in Fig. 2. This cladistic topography was consistent with analyses of chloroplast DNA restriction sites (Haufler *et al*. 1995b) and also with the tree obtained by analyzing sequences from the *trnL–trnF* spacer region of the chloroplast (Fig. 1).

The distribution of the three species (Fig. 3) provides important clues to their relationships. *Polypodium sibiricum*, which masqueraded under the guise of *Po. virginianum* in most floras, is a particularly widespread diploid taxon that is circum-boreal from Siberia across northern Japan to northern North America (from Alaska to northern Ontario, Canada). *Polypodium amorphum* is more narrowly distributed in western North America from Oregon north into southern British Columbia, and *Po. appalachianum* is confined to rock outcrops in the mountains of eastern North America. By coordinating this biogeographic information with that from morphology and molecules, a scenario for the diversification of this monophyletic group can be developed. *Polypodium sibiricum* is currently found in regions that in the past were covered by ice. Evidence indicates that the ranges of species with northern distributions became compressed and migrated

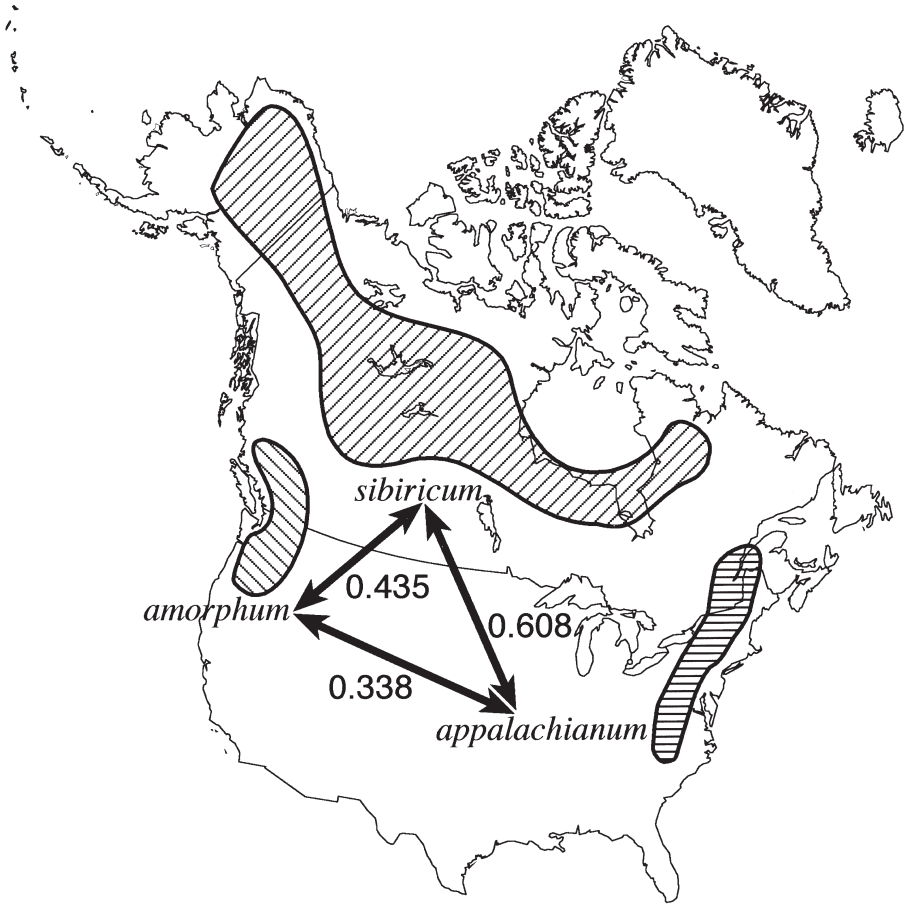

Fig. 3 Approximate ranges in North America of members of the *Polypodium sibiricum* species group (▨, *P. sibiricum*; ▧, *P. amorphum*; ▤, *P. appalachianum*) and a summary of genetic identity results (from Haufler *et al*., 1995).

south during ice ages (Davis 1983). Currently, the southern edge of the range of *Po. sibiricum* in North America is around 55°N latitude. It is likely that the range of this boreal taxon was driven south by the advancing ice sheet and that it settled around 40°N latitude. As the ice retreated, the majority of *Po. sibiricum* populations returned to their current boreal locale, but some isolated remnants may have persisted in the mountains of eastern and western North America. A classic allopatric speciation model can be invoked to explain the subsequent changes in the three lineages. Because they were initially isolated by distance, and because the original populations may have been relatively small, morphological and genetic changes may have accumulated in each of the geographically separated sets of populations. The species that we define today therefore would have been initiated by past climatic events and the resulting migration of populations.

The ice age event that provided the impetus for the origin of the three diploid species was probably not the most recent ice incursion. Evidence to support this hypothesis is seen in two aspects of the biology of these species. First, interspecific genetic identity values are low, indicating that the diploids have been isolated from each other for a considerable period of time. Second, there is solid evidence to support the hypothesis that allotetraploid species originated because the ranges of the diploids overlapped subsequent to their genetic isolation from each other (Haufler *et al*. 1995a) Tetraploid *Po. virginianum* originated through allopolyploid speciation following hybridization between *Po. sibiricum* and *Po. appalachianum*, whereas tetraploid *Po. saximontanum* originated because of hybridization between *Po. sibiricum* and *Po. amorphum*. The most likely scenario to explain these allopolyploid events is that subsequent ice ages drove *Po. sibiricum* into the range of the more southern diploids and provided the opportunity for interspecific hybrids to form. Because substantial genetic differentiation had accumulated to separate the diploids, these hybrids were undoubtedly sterile and could be perpetuated as fertile species only through polyploidization.

In the *Po. sibiricum* group, therefore, primary speciation leading to the origins of *Po. amorphum* and *Po. appalachianum* probably took place through isolation by distance

(prezygotic isolation) and subsequent genetic differentiation of the geographically separated populations (postzygotic isolation). The speciation vector was probably the long-term climatic disturbance and the resulting shifts in species ranges. Interspecific differentiation occured after the isolation, and the event or events that sparked the diversification had little or nothing to do with adaptation or ecological specialization. A markedly different scenario must be developed to explain the origin of species in the *Pleopeltis polylepis* group.

Pleopeltis polylepis *group*

Pleopeltis is a neotropical genus that traditionally has included species having simple, entire leaves with peltate and centrally clathrate laminar and soral scales (paraphyses). The taxonomic circumscription of the genus is currently undergoing modification, however, because it appears that many or all of the pinnatifid, scaly-leafed species traditionally placed in *Polypodium* (subgenus *Marginaria*) may actually belong in *Pleopeltis* (Windham 1993). Although the generic boundaries of *Pleopeltis* are still unsettled, allozymes studies of simple-leafed members of the genus (Hooper 1994) and DNA sequence data (Fig. 2) have identified a few clearly defined monophyletic groups. One such group, the *Pleopeltis polylepis* group, includes four diploid species: *Pl. polylepis*, *Pl. conzattii*, *Pl. crassinervata*, and *Pl. mexicana*. These four species occur in Mexico and all but *Pl. conzattii* extend into northern Central America as far south as Nicaragua (Fig. 4). The species are predominantly epiphytic and are found at mid- to high elevations (1000–3000 m) in the mountainous regions of Mexico and Central America (Table 2).

Although members of the *Pl. polylepis* group are superficially similar in appearance, closer examination reveals numerous morphological features, both qualitative and quantitative, that differentiate them. For example, *Pl. crassinervata* is easily recognized by its conspicuous blackened veins in the basal portion of the leaf and its concolorous scales at the rhizome apex; *Pl. conzattii* has unique leaf and soral scales that are lanceolate and long-attenuate at the tip and has black-centered scales along the midrib; *Pl. mexicana* has slightly dimorphic leaves and concolorous soral scales; and *Pl. polylepis* has very densely scaly leaves and large, usually overlapping scales on the lower leaf surface. Thus, all four *Pleopeltis* species can be readily identified by a competent botanist and they have been formally recognized as distinct entities since the mid-nineteenth century (*Pl. polylepis*, *Pl.*

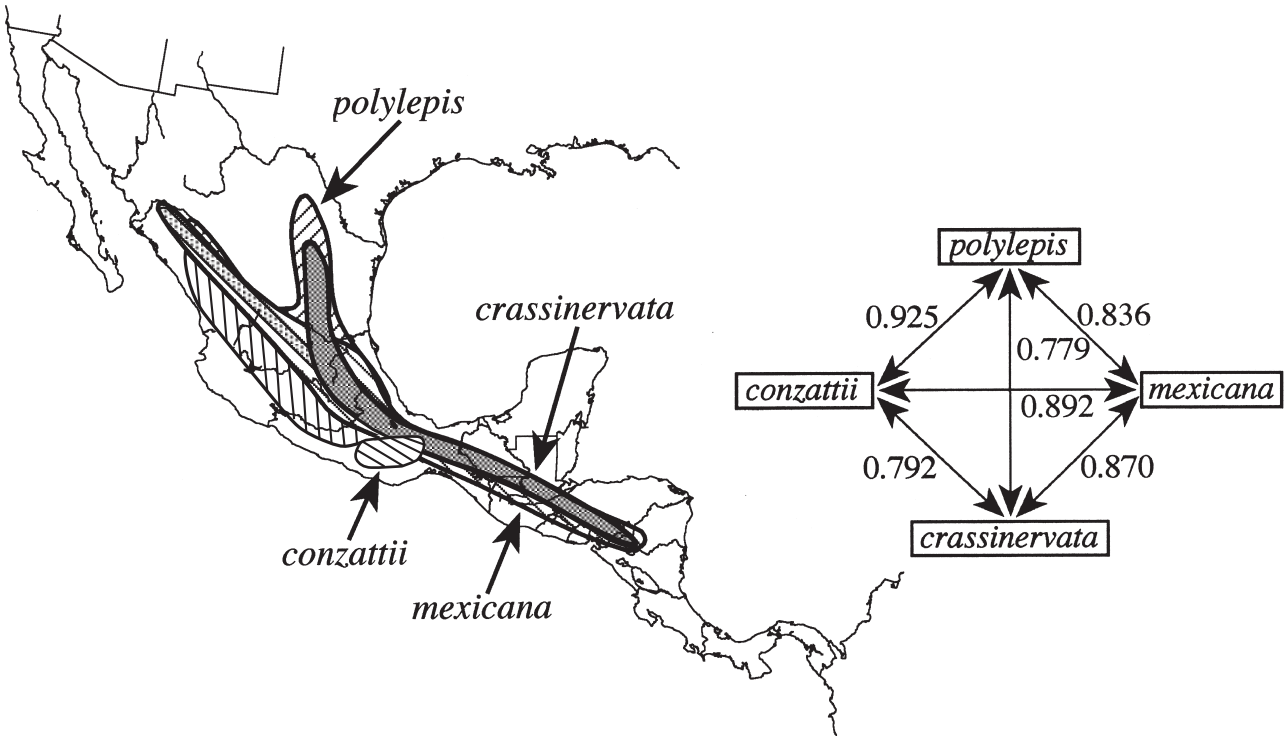

Fig. 4 Approximate ranges in Mexico and Central America of members of the *Pleopeltis polylepis* species group (▨, *P. polylepis*; ⬚, *P. mexicana*; ▩, *P. crassinervata*; ▧, *P. conzattii*) and a summary of genetic identity results (from Hooper, 1994).

Table 2 Comparison of distributions and broad ecological preferences among members of the *Pleopeltis polylepis* group

	Pleopeltis polylepis	*Pleopeltis conzattii*	*Pleopeltis mexicana*	*Pleopeltis crassinervata*
Elevation	1500–3400 m	1800–3000 m	900–2800 m	500–1800 m
Distribution	Northern, broad	Central, narrow	Southern, broad	Eastern and southern, broad
Habitat	Widespread	Drier slopes	Wetter slopes	Wetter slopes

crassinervata and *Pl. mexicana*) or early twentieth century (*Pl. conzattii*).

Providing a marked contrast to the readily discernible morphological features that distinguish these four species from each other, surveys of isozyme variation in simple-leafed *Pleopeltis* species (Hooper 1994) revealed remarkably little genetic divergence among the members of the *Pleopeltis polylepis* group (Fig. 1). Interspecific genetic identity values (Nei's I) averaged 0.849, which is much higher than found among most temperate diploid fern species (Soltis & Soltis 1989). Thus, it appears that in the *Pleopeltis polylepis* group, morphological divergence exceeds genetic divergence. This is directly opposite to the pattern described above for the temperate *Polypodium sibiricum* group, where genetic divergence seems to have outpaced morphological divergence.

Understanding the historical factors that have shaped the current distribution of plants in the tropics and influenced their genetic constitution is very difficult. In the case of *Pleopeltis*, the ranges of the four species in the *Pleopeltis polylepis* group overlap for a large part, but where they overlap, the species are generally restricted to different ecological zones (Table 2). For example, in Mexico, *Pl. crassinervata* is restricted to the wetter Atlantic slope of the Sierra Madre Oriental and occurs at lower elevations (usually below 1500 m) than the other three species. *Pleopeltis conzattii* is restricted to the states of Oaxaca and Guerrero in Mexico and occurs at high elevations (1800–3000 m) and in drier microhabitats than the other high elevation species. *Pleopeltis mexicana* occurs throughout southern Mexico (mostly south of the Tropic of Cancer) usually between 1000 and 2800 meters in elevation and, finally, *Pl. polylepis*, which is the most widespread species in Mexico, occurs throughout the mountainous regions (both the Eastern and Western Cordillera) and generally at high elevations (1500–3800 m). These latter two species are the most similar with respect to range and altitudinal distribution (although *Pl. polylepis* tends to occur at higher elevations) and yet they rarely grow together.

It is proposed that the diversity of habitat types in upland tropical regions has led to the diversification evident in *Pleopeltis*. Once adapted and locked into their ecological zones, new species are isolated from their congeners. Although it is convenient to argue that these spore-producing plants should have few limitations to long distance wind dispersal, evidence indicates that members of the *Pleopeltis polylepis* group are outcrossers and single spore dispersal is unlikely (Hooper & Haufler 1997). Based on isozyme evidence indicating that species in the *Pleopeltis polylepis* group are genetically very similar to each other, it appears that speciation is either a rapid process or that it has occured relatively recently. During this process, considerable morphological modification is observed relative to the changes that accumulate in basic genetic composition. The speciation model that appears to be most consistent with the results we obtained from comparisons of morphological, biogeographic, ecological and molecular data can be best described as a microallopatric or perhaps even approaching a sympatric model involving peripheral isolation and rapid adaptation to differing habitats.

Summary and synthesis

The world view regarding ferns and other spore-producing vascular land plants (vascular cryptogams) is that they speciate and diversify through a relatively simple set of mechanisms. Because it is likely that these ancient lineages lack the complex behaviors, chromosomal gimmicks and coevolutionary interactions that are found in the seed plants, it is generally thought that the processes associated with lineage splitting and isolation of species in the vascular cryptogams are limited to postzygotic genetic barriers and perhaps modest ecological specialization. However, the evidence presented here indicates that although such mechanisms are certainly components of speciation in the ferns, even in these relatively simple plants, lineage splitting may incorporate a wider range of possibilities than has been appreciated in the past. Although such a range of options in fern speciation may have been suspected, the coordination of data from isozymes and DNA, with those from morphology, ecology and biogeography, represent a solid foundation of evidence for postulating some generalities that may help to guide future investigations of the patterns and processes of evolution among vascular cryptogams.

In temperate zones, fern habitats are primarily terrestrial or epilithic. Thus, while tied to the features of the substrate, the temperate ferns do not appear to have par-

ticularly diverse adaptations to complex ecologies. Some species are associated with more basic or more acidic habitats, but there does not seem to be evidence of complex biotic interactions or associations that could participate in limiting species boundaries. Supporting this interpretation is the observation that nearly all temperate species appear to be isolated by postzygotic genetic barriers. When sperms and eggs from such species meet, either an inviable zygote is produced or a sterile hybrid individual results. In many cases, such hybrids are quite vigorous, but they are almost always incapable of producing viable spores. The strength of the genetic barriers separating closely related species may be associated directly with the weakness of the ecological or other prezygotic barriers to gene exchange. Indeed, speciation may be initiated through isolation by distance (a prezygotic barrier), but establishment of species boundaries ultimately relies on accumulating major genetic changes that result in postzygotic barriers as well. Unless these postzygotic barriers are in place, changes in ranges may lead to homogenization of lineages and loss of species distinction. As a result, temperate fern species may be some of the best examples of 'good biological species' (*sensu* Mayr 1969) that exist. Reproductive barriers isolating even nearest relatives in almost all temperate pteridophyte lineages appear very strong and absolute (but see Mayer & Mesler 1993).

Lineage splitting in temperate pteridophytes appears to follow classic models of allopatric speciation (Fig. 5). First, new habitats open at the periphery of current ranges through some type of disturbance or other habitat modification. In the case of the *Polypodium sibiricum* group, this disturbance was most likely linked to climate change and glaciation. Although more studies are necessary, research on gametophyte ecology in temperate ferns indicates that these minute plants require open soil and therefore disturbance of some kind to colonize new habitats (Cousens *et al*. 1985). As long as gene flow is maintained between the peripheral, founder population and the larger, established population, little or no change will occur in the lineage. In *Po. sibiricum*, isozymic evidence indicates that populations from Siberia, northern Japan, and Saskatchewan, Canada differ little in allozymic constitution (Haufler *et al.* 1995a). Once separated by sufficient distance, however, the founders may diverge in isolation, possibly through genetic drift and/or accumulation of novel mutations. If the incipient lineage splitting is not carried to completion, and the populations come back into contact, F_1 hybrids may be fertile and hybrid swarms may result [as has been documented in *Polystichum* (Mayer & Mesler 1993)], potentially erasing any accumulated changes. On the other hand, if they are isolated long enough to establish postzygotic genetic barriers to gene exchange, the lineages will persist as separate and distinct

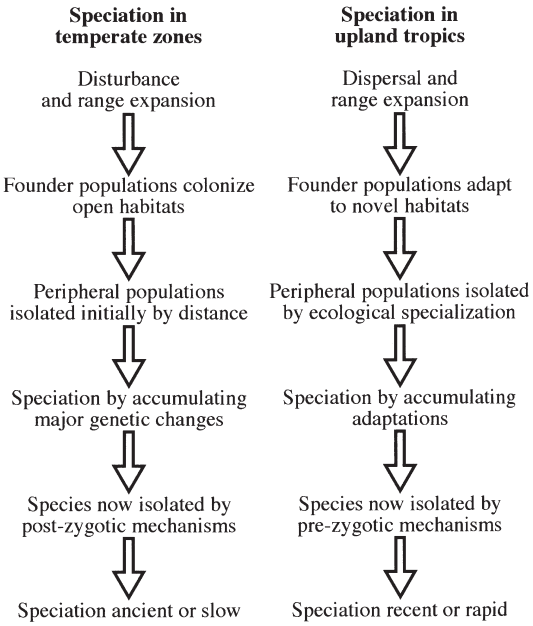

Fig. 5 Flow chart contrasting hypothesized pathways of speciation in temperate and upland tropical habitats. See text for further elaboration and discussion.

species. In the *Polypodium sibiricum* group, it appears that each of the three species is well isolated genetically and that one or more ice ages have occured since their origin. As the glaciers pushed *Po. sibiricum* southward, populations of this species have come back into contact with their more southern derivatives. As a result, secondary allotetraploid species (*Po. virginianum* and *Po. saximontanum*) have resulted from the sterile F_1 hybrids between the primary diploids (Haufler *et al.* 1995a). Primary speciation among such temperate species appears to require a long period of time to occur because it requires both prezygotic isolation by distance and subsequent postzygotic accumulation of genetic barriers to gene exchange.

In contrast, at least among some closely related species in tropical habitats, speciation may result from quite a different set of parameters and mechanisms (Fig. 5). The evidence presented here for the *Pleopeltis polylepis* group demonstrates that readily recognized morphological gaps can evolve without accumulating isozymically detectable genetic differentiation. Instead of being separated by physical distance (as seen among members of the *Polypodium sibiricum* group discussed above), the ranges of *Pl. polylepis* and its relatives overlap each other. However, the species appear to prefer different altitudes and perhaps also different ecologies (Table 2). Speciation in this set of *Pleopeltis* species may have occurred as follows: First, an ancestral species expands its range and colonizes new areas. Disturbance may play a role in opening opportu-

nities for colonization by providing open habitats for spore germination and gametophyte development. Alternatively, gametophytes of tropical species may have growth requirements and abilities that could narrow or widen the possible sites for successful establishment and growth. Second, range expansion could result in two outcomes that are significant in speciation. The new peripheral populations may become separated by distance from the main distribution of the species or the peripheral populations may encounter novel habitats that provide selective pressure for change to occur. In either of these cases, the peripheral population could become genetically differentiated from the main range of the ancestral species. Given the results reported here, it seems likely that peripheral populations are adapting to new environmental pressures. These changes may be reflected in a modified morphology for the residents of the peripheral populations and the changes may lead directly to effective isolation of the two lineages. Because the peripheral populations are adapted to the new ecology, they may develop prezygotic isolation from their ancestor populations (i.e. residents of the two ecologically differentiated populations may not tolerate the conditions of their neighbors even though they may be within spore-dispersal range). Speciation following this scenario would require only a single step and may therefore occur more rapidly than that described for temperate species.

The complexity of habitat structuring in upland tropical zones contrasts markedly with the simplicity of temperate forest biomes (and perhaps also lowland tropical regions). It is probable that speciation in many temperate situations requires large-scale shifts in ranges, significant disturbance and the possibility of forming isolated remnant populations that can diverge through genetic drift. In tropical biomes, dramatic shifts in temperature and rainfall patterns can accompany elevational and latitudinal gradients and may provide opportunities for single-step speciation through adaptation.

Conclusions and future directions

The constellation of data sets synthesized here helps to reveal some of the mysteries of the speciation black box for *Polypodium* and *Pleopeltis*. Not only is it clear that different modes and mechanisms are involved in the process of speciation in each of these cases, but it is also apparent that species isolation differs in the two species groups discussed. Whereas postzygotic genetic isolation is necessary to separate the *Polypodium* species, prezygotic ecological isolation may be the only mechanism necessary in the *Pleopeltis* group.

Our results also demonstrate that future studies of speciation in pteridophytes should begin with a clear hypothesis about the monophyly of the group being investigated. Speciation models should only be developed when sister taxa are being compared. This study also points out the value of coordinating as many different lines of evidence as possible in considering both the phylogeny and the evolutionary mechanisms involved with biological diversification. Each of the approaches provided valuable insights that helped to resolve some component of the species biology. Morphology and biogeography were used to craft testable hypotheses about the historical relationships among the *Polypodium* and *Pleopeltis* species groups. Without knowledge of the chromosomal constitution of the species, we could not have been sure we were comparing diploid to diploid. The DNA sequences provided an independent test of the hypothesized ancestry of the species. Isozymes yielded insights concerning the genetic distinctness of the lineages and a yardstick for estimating the relative time since the origin of the species. Together, these data address many of the open questions about the biology of fern species, but they also show that there are still more unknown aspects remaining to be explored. A particularly poorly understood element involves the biology of the gametophyte generation. Obviously, it is the gametophyte that directs the initial establishment of populations and controls sexual reproduction and genetic exchange among individuals. Especially among the tropical species, we need more studies of the ecophysiological aspects of their biology before we can state clearly that selection has led to adaptations to different ecological features or that species are isolated by differences in their habitats. Particularly in tropical pteridophytes, the dearth of biosystematic investigations limits our capacity to understand and appreciate the breadth of evolutionary possibilities exercised by these lineages.

Without additional studies, broad comparisons of tropical and temperate speciation mechanisms is not possible. We need more broad-based biosystematic studies of tropical fern groups to discover whether the *Pleopeltis polylepis* group model holds for other groups. It is likely that the complexity of tropical systems includes an equally complex variety of speciation models. Nonetheless, the striking contrast that emerges from this study demonstrates that speciation in ferns may be a remarkably flexible and dynamic assemblage of modes and mechanisms. Hopefully, biosystematic analyses can become more commonplace among tropical groups and such synthetic approaches can provide a more solid foundation for erecting models of evolutionary diversification.

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