Homospory 2002: An Odyssey of Progress in Pteridophyte Genetics and Evolutionary Biology

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The homosporous vascular plants, including ferns and other graceful, primarily forest-dwelling groups such as the ground pines and horsetails, are the extant representatives of ancient lineages that once dominated the land surface. Although the very well-studied seed plants find their closest relatives (sister group) among these species (Pryer et al. 2001), called *homosporous pteridophytes*, genetic aspects of homosporous plants have not been studied extensively. In addition to the homosporous species, there are extant heterosporous pteridophytes—among them the water ferns (Marsileaceae), spike mosses (Selaginellaceae), and quillworts (Isoetaceae)—and extinct heterosporous lineages, which are well represented in the fossil record. But these "evolutionary experiments" in the heterosporous way of life, whose ancestors are homosporous, did not result in the extraordinary burst of diversification seen among the seed plants, and they are not the focus of this review. However, there are more than 10,000 homosporous pteridophyte species on Earth today, and homospory appears to be the foundation on which all extant plant groups stand. Thus valuable insights for understanding the origin and biodiversity of groups such as the flowering plants could be gained from a solid understanding of homosporous lineages.

Unfortunately, developing and testing hypotheses about the genetics and evolution of homosporous pteridophytes has been a difficult enterprise, because although homosporous plants share many structural features with their much-studied heterosporous siblings, the reproductive biology of these two groups is quite different. Nonetheless, the last quarter century has seen considerable change in our understanding of pteridophyte evolution and genetics. This review will consider how new discoveries, insights, and hypotheses have modified our perceptions of homosporous pteridophytes and assess the impact that these changes have had on understanding basic aspects of plant genetics.

Homosporous pteridophytes possess biological attributes that complicate a thorough understanding of their genetic systems and evolutionary potential. All vascular plant life cycles feature an alternation of genetically different phases: *sporophytes*, the diploid portion of the life cycle responsible for the production of spores, and *gametophytes*, the haploid generation that yields gametes. The homosporous pteridophytes produce spores that are uniform in size, whereas heterosporous species (including seed plants) produce spores of two different sizes, microspores and megaspores. This seemingly simple difference in spore size extends to the mode of sexual reproduction. Heterosporous species have unisexual gametophytes—microspores produce exclusively male gametophytes and sperm, while megaspores germinate to initiate only female gametophytes and eggs. In contrast, all spores of homosporous species can become bisexual game-

FERNS AND OTHER HOMOSPOROUS VASCULAR PLANTS HAVE HIGHLY POLYPLOID CHROMOSOME NUMBERS, BUT THEY EXPRESS TRAITS FOLLOWING DIPLOID MODELS AND, ALTHOUGH CAPABLE OF EXTREME INBREEDING, ARE PREDOMINANTLY OUTCROSSING

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tophytes, bearing both eggs and sperm on the same individual. Such distinctive genetic architecture as well as aspects of life history and ecology can influence patterns and processes of evolution. Only by fully integrating all of these components can we obtain an accurate picture of the evolutionary potential of homosporous pteridophytes. The following elements of homosporous species pertain to genetic analyses:

- Critical genetic processes (meiosis and fertilization) occur on separate plants growing in different microhabitats (figure 1). Gametophytes generally colonize moist, open soil in often disturbed microsites, whereas sporophytes are typically found in well-established, mature sites.
- Because a bisexual gametophyte is the product of a single meiotic event and produces genetically uniform eggs and sperm, biologists have predicted that homosporous pteridophytes are highly inbred and that these populations may have greatly reduced levels of genetic variation.
- All homosporous pteridophytes have high chromosome numbers, and genetic analyses and assumptions about evolutionary potential that are regularly applied to other organisms may not be appropriate.
- Gene flow in homosporous species occurs through dispersal of spores and sperm, which contrasts markedly with seed and pollen dispersal.
- In thinking about the movement of genes between individuals and populations, it is necessary to consider the high dispersal capacity of unicellular spores and to balance this against the limitations imposed by the restricted habitat requirements for growth and maturation of the gametophytes.

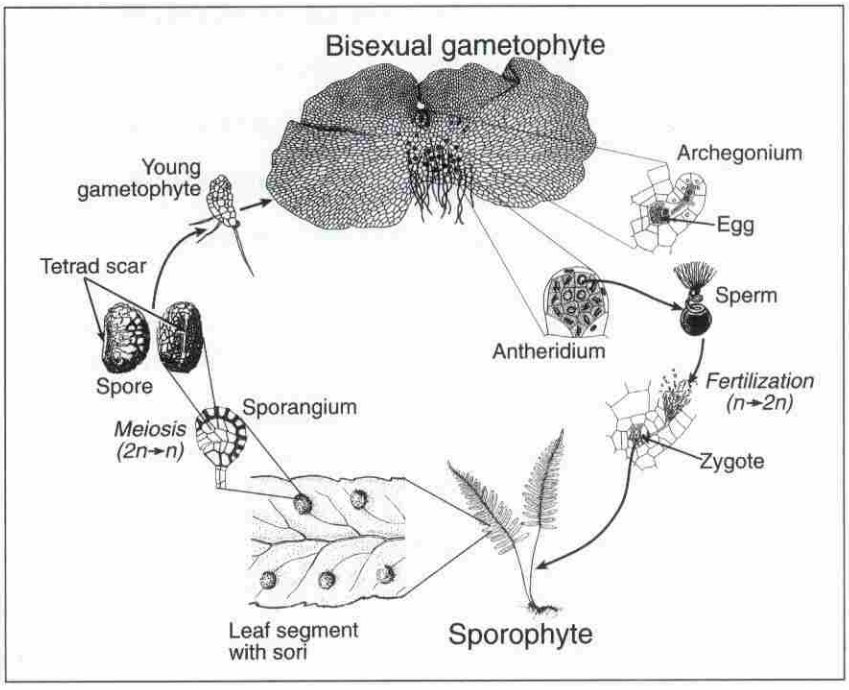

Figure 1. Homosporous pteridophytes alternate between two entirely independent generations. Bisexual gametophytes produce both egg-containing archegonia and sperm-producing antheridia. Fertilization occurs when the motile sperm swim down the necks of the archegonia and unite with the egg, forming the diploid zygote. Not all gametophytes of homosporous species become bisexual, and not all bisexual gametophytes are capable of self-fertilization. The single-celled zygote divides mitotically and matures to form the sporophyte. On the underside of sporophyte leaves are clusters of sporangia (sori). Meiosis occurs in the sporangia, producing tetrads of spores that are released into the air. Under suitable conditions, the spores germinate and grow by mitotic cell divisions to form the gametophyte.

More than 20 years ago, Edward Klekowski (1979), who can validly be called the father of modern studies on pteridophyte genetics, published a summary and synthesis of the unique features of homosporous pteridophytes. Klekowski, who had been studying these plants for more than a dozen years, argued convincingly that the features of homosporous vascular plants required a modified set of rules to develop hypotheses about their genetic composition and reproductive biology. Since 1979, Klekowski's controversial views have provided the focus for numerous studies and have led to a new set of syntheses about homosporous pteridophyte genetics.

In the following sections, a picture of homosporous plant genetic systems will be developed by proceeding in an organismal hierarchy: A description of the characteristics of individuals and their genetic makeup provides the basis for a discussion of hypotheses about how these genomic characteristics evolved, which leads to an analysis of how these features influence breeding system interactions or potential interactions between individuals, which ultimately yields a synthesis of population-level features and evolutionary processes.

In this hierarchy, the first question that must be asked is, "What constitutes an individual?" Because homosporous pteridophytes alternate between two strikingly different and fully independent generations, when one looks at a typical fern sporophyte growing in the woods, one sees only one of two components of the whole fern individual (figure 1). The sporophyte is the larger, generally perennial generation. The diminutive and generally ephemeral gametophytes are found in habitats that are usually quite dissimilar from those of the sporophyte. Both generations and the processes they contain are critical for species survival, but from a genetic perspective, the sporophyte could be considered the less important generation. Although it is true that meiosis takes place on the sporophyte, fertilization occurs when sperms unite with eggs on the gametophyte. Thus, the products of the gametophyte actually determine the genetic composition of the sporophyte. Through meiosis, the sporophyte can only release recombinations of the compo-

nent genomes that were amalgamated through fertilization on the gametophyte.

As noted above, homosporous species produce gametophytes that are potentially bisexual, having both eggs and sperms on a single, multicellular, haploid individual that grew by mitosis and differentiation from a unicellular spore. This condition actually imparts to homosporous species greater breeding system amplitude than their heterosporous relatives possess. Because gametophytic bisexuality enables fertilization to yield a variety of genetically different products, Klekowski (1979) developed a special set of terms to describe the breeding systems possible for homosporous plants (figure 2). To refer to true outcrossing (as applied to heterosporous plants), Klekowski coined the term *intergametophytic crossing*, in which a sperm from a gametophyte of one sporophyte fertilizes the egg of a gametophyte from a second sporophyte, similar to the process of the pollen from one flowering plant being transferred to the stigma of a second plant.

Corresponding to selfing in heterosporous species is *intergametophytic selfing*, in which a sperm from one gametophyte fertilizes the egg from a second gametophyte, but both gametophytes had the same sporophyte parent, similar to the pollen from the anther of one flower landing on the stigma of the same flower. Finally, to describe the type of fertilization that is possible only for bisexual gametophytes in which a sperm from a gametophyte fertilizes an egg from the same gametophyte, Klekowski suggested the term *intragametophytic selfing*. Because eggs and sperms from a single gametophyte are genetically identical, intragametophytic selfing produces sporophytes that are 100% homozygous in a single generation, a condition that takes even obligately selfing heterosporous plants many generations to approach. This capacity for absolute inbreeding has important implications when trying to understand individual and populational genetics.

The genetics of individuals

Exploring the genetics of individual homosporous plants has involved intrigue and deception. Biologists have been intrigued by homosporous plants because they are genetically distinct from seed plants. Homosporous plants have been considered stellar exemplars of polyploidy and inbreeding. Individuals are usually called polyploid if their somatic cells have more than two complete sets of chromosomes. In angiosperms, the dividing line between diploid and polyploid chromosome numbers has been set at $n = 13$ or 14 (Grant 1981). Using this base line, about 50% of flowering plants can be considered polyploid. When the same numbers are applied to the pteridophytes, over 95% may be called polyploid. Such statistics suggest that homosporous plants constitute an odd group that demands special consideration when developing ideas about patterns and processes of evolution. However, as intriguing as this unique feature of homosporous plants is, biologists were deceived when trying to extrapolate from these observations to explain genetic expression and breeding system attributes.

What if most homosporous plants are polyploid?

Klekowski (1973) advanced hypotheses that high ploidy levels actually may have been selected to maintain and release genetic variability in homosporous plants. Klekowski reasoned that if bisexual gametophytes of homosporous plants frequently fertilized themselves, lineages should rapidly become highly homozygous and lose the capacity to store and release the genetic variability necessary to remain evolutionarily active; therefore, homosporous plant lineages became polyploid as an adaptive reaction to the deleterious effects of rampant inbreeding.

Klekowski reached these conclusions by correlating direct observations and widely held assumptions about homosporous pteridophyte biology. A common evolutionary pathway in homosporous pteridophytes and other plants that results in higher chromosome numbers is a process called *allopolyploidy* (figure 3). This pathway begins when the sperm from one diploid species (A_1A_1) unites with the egg of a second diploid species (A_2A_2). In this notation, each haploid set of chromosomes is indicated by the same capital letter, A, because for hybrids to form there must be significant genetic compatibility between the component genomes (e.g., consider the similarity between the donkey and the horse that hybridize to yield vigorous but sterile mules). The subscript numbers 1 and 2, on the other hand, are used to designate the genetic distinctness of these haploid sets. When hybridization occurs between species from the same genus of homosporous plants, there appear to be few barriers to prevent the initiation of interspecific zygotes (Schneller 1981). Although many of these zygotes are not viable, in the somatic or vegetative cells of some hybrids, the two "parental" genomes are sufficiently compatible to yield cells that can replicate without problem through mitotic divisions, and robust sporophytes (A_1A_2) often result. In the reproductive cells of the sporangia, however, meiosis rather than mitosis is responsible for producing spores (figure 1). Even in the most vigorous interspecific hybrids, the reproductive cells

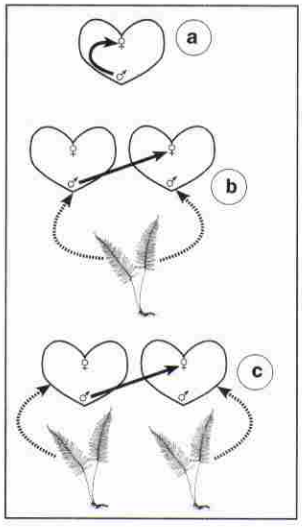

Figure 2. Breeding system options for homosporous pteridophytes. (a) Intragametophytic selfing. Sperm from a single gametophyte fertilizes the egg of that same gametophyte. (b) Intergametophytic selfing. Sperm from one gametophyte fertilizes the egg of a neighboring gametophyte, but both gametophytes arose from spores of the same sporophyte. (c) Intergametophytic crossing. Sperm from one gametophyte fertilizes the egg of a neighboring gametophyte, and the two gametophytes arose from spores of different sporophytes.

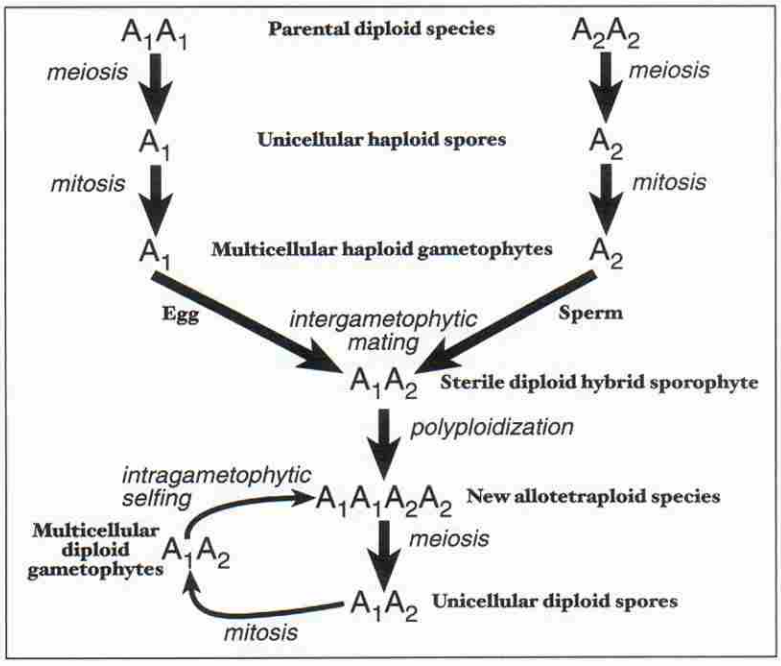

Figure 3. Allopolyploid speciation is initiated when hybridization occurs between diploid species A_1 and A_2. The resulting hybrid is reproductively incompetent (sterile) because, although homoeologous haploid genomes A_1 and A_2 are sufficiently similar to form a vigorous sporophyte (the considerable similarity of the genomes is denoted by having both share the letter A), the two genomes are sufficiently divergent to prevent bivalent formation during meiosis (critical differentiation of genomes is denoted by separate subscripts 1 and 2). Through polyploidization, that hybrid regains reproductive competency, because bivalents can form between the duplicated homologous diploid genomes A_1A_2 and A_1A_2.

poised to form spores usually fail to yield viable products, because the two haploid sets (one from each parent species) are sufficiently divergent that they will not pair properly with each other to form bivalents during meiosis (something that they do not have to accomplish during mitosis).

Sterile interspecific hybrids can regain sexual reproductive competency through genome doubling. In an allotetraploid ($A_1A_1A_2A_2$), for example, two identical genomes are present (A_1A_2 and A_1A_2) whose chromosomes can now pair perfectly and form bivalents during meiosis. Diploid spores containing one set of genes from each of the two parental species are produced. Via mitosis (having the biparental genome A_1A_2), the diploid gametophytes developing from these spores produce sperm that fertilize eggs carrying the same A_1A_2 genome, and a new $A_1A_1A_2A_2$ tetraploid sporophyte results.

In each individual of these new polyploid lineages, there are two complete genomes. Some chromosomes from each progenitor are, of course, homologous (those from set A_1 and A_1, or from set A_2 and A_2), and these pair to form bivalents during meiosis, whereas those from the different progenitors are termed *homoeologous* (from chromosome sets A_1 and A_2). Presumably the fact that the two species will form vigorous hybrids means that the bulk of their chromosomes are quite similar, even though they are not homologous. Thus, homoeologous chromosomes are those that are partially homologous. Each time a new species originates through allopolyploidy, every individual of that new lineage has both homologous and homoeologous sets of chromosomes.

Which brings us back to Klekowski's conundrum. Bisexual gametophytes of homosporous species appear likely to form new sporophytes by intragametophytic selfing (fertilizing their eggs with their own sperm). This single event would make their homologous chromosomes identical and their gene expression completely homozygous (an inbreeding nightmare). Each allopolyploid individual, however, stores additional genetic variability in its homoeologous chromosomes. Stated succinctly, because the progenitors of allopolyploids are genetically different, the polyploid amalgamation of these dissimilar sets would generate *homoeologous heterozygosity* even if the plants had become homologous homozygotes through intragametophytic selfing. Thus, each individual of an allopolyploid species should carry a certain amount of "built-in" homoeologous genetic variability, even if intragametophytic selfing eliminates homologous variability.

Is there a way to recombine the homoeologous genetic variation that is stored in each allopolyploid individual? Klekowski suggested that during meiosis it was likely for some pairing to occur between homoeologous chromosomes rather than all pairing being restricted to homologous chromosomes. Just as heterozygous diploid individuals produce variation in their progeny, pairing between homoeologues would result in variability among the offspring of even the most homologously homozygous allopolyploid. Especially given the large number of chromosomes, this seemed to be a reasonable and likely event. For this system to be relevant to organisms, however, there were three requirements:

1. The genes carried on homoeologous chromosomes must be genetically different from each other.
2. The genes must be actively translated into products used by the organism that could be acted upon by selective or other agents.
3. In homologously homozygous individuals, there must be pairing between homoeologous chromosomes to recombine this genetic variation among progeny.

Assuming these requirements were being met in naturally occurring species, how could it be detected and how could this interesting hypothesis be tested?

Genetic control of morphological and other traits.

One way to assess the genetic constitution of individuals is by considering the expression of genetically determined traits through controlled breeding programs. This is the method that Mendel used in making his important discoveries about plant

genetics. If homosporous pteridophytes were highly polyploid, each trait would be determined not by two (as in diploid organisms) but by four or more different genes. In simple diploids, crossing two suspected heterozygous individuals (Aa x Aa) yields an expected 1:2:1 ratio of homozygous dominants (AA) to heterozygotes (Aa) to homozygous recessives (aa). However, if each trait is controlled by four or more genes (or alleles of the same gene), depending on how independently the alleles segregate, the proportion of each genotype will differ substantially from the diploid 1:2:1 ratio. The segregation arrays rapidly become more complex. For example, in a tetraploid, if every diploid gamete carried a homoeologously heterozygous genotype for each trait ($A_1a_1A_2a_2$), even in the unusual situation where each allele of each duplicated gene sorted independently, only a tiny fraction of the progeny from a cross between two such individuals ($A_1a_1A_2a_2$ x $A_1a_1A_2a_2$) would express the recessive trait. Thus, if all genes and alleles were expressing active products, a very different outcome from controlled crosses among polyploids would be expected compared with that in diploids.

In the early 1900s, during the Victorian fern craze in England (Allen 1969), Andersson-Kottö (1929) conducted crossing studies. She considered the genetic control of both morphological and life cycle traits, and she discovered that most features followed diploid genetic (Mendelian) models of inheritance. The only departure from typical diploid inheritance patterns occurred among individuals having variegated leaf mutations. Klekowski (1979) drew attention to these studies of variegation as evidence for possibly polyploid genetic systems in ferns. However, variegation genetics are complicated, because such patterns result from mutations in chloroplasts and are frequently inherited through the cytoplasm rather than the nucleus (Kirk and Tilney-Bassett 1978). Andersson-Kottö (1938) herself states that "the only clear case of polyploid segregation of a mendelian character in a fern to my knowledge occurs in *Phyllitis scolopendrium*" (p. 293). This case involved a "fertile hybrid between a 'peculiar' with 50 chromosomes and a gametophyte of normal *Scolopendrium* type with 30 chromosomes, the hybrid having 80 chromosomes" (Andersson-Kottö 1938, p. 293). Andersson-Kottö's studies provided evidence that homosporous species with high chromosome numbers expressed traits consistent with diploid, not polyploid, models of inheritance, but more information was necessary before conclusions about the universality of these findings could be developed.

Cytogenetic tests for homoeologous chromosome pairing. Using inbred lines and gametophytic genetic markers from *Ceratopteris thalictroides,* Hickok (1978a, 1978b) was able to demonstrate that 1% to 3% of the progeny contained combinations of alleles that must have resulted from pairing between homoeologues. Hybrids between inbred lines showed higher percentages of aberrant progeny that most likely arose because nonhomologous chromosomes paired during meiosis, giving rise to segregants that contained more recessive alleles than they "should have" if only homologous chromosomes formed bivalents.

These cytological data elucidated a mechanism by which variability stored among homoeologous genomes could be released and demonstrated that this process was operating in *Ceratopteris.* However, the chromosome number of *C. thalictroides* is 78, double the base number of 39 found among other members of *Ceratopteris.* Thus, even relative to other *Ceratopteris* species, *C. thalictroides* is a polyploid.

Molecular tests of homoeologous gene expression. Structurally (chemically) different yet functionally similar enzymes are called isozymes and, using the technique of electrophoresis, it is possible to detect the different forms of the enzymes present in cells. The genetically pertinent aspect of these biochemical features is that each isozyme is coded by a different DNA sequence and many isozymes represent single gene products. These biochemical characters can be used to address questions about the genomic constitution of organisms.

In the seed plants, Gottlieb (1982) showed that because each allelic variant could code for a separate enzyme that may be detected electrophoretically, the higher the ploidy the greater the number of enzyme variants. For monomeric enzymes, diploids have a maximum of two variant bands for each enzyme, tetraploids have a maximum of four variants, hexaploids max out at six variants, and so forth. Gottlieb used this evidence to suggest that absolute chromosome numbers should not be the sole criterion for determining whether an organism is diploid or polyploid. Rather, he suggested, the essential attribute of polyploidy is genome multiplication and attendant increases in the number of gene loci. Assuming that different genes should produce slightly different enzyme products, Gottlieb concluded that polyploids should have more isozymes (more gene products) than diploids. In most cases, surveys of seed plants have upheld this assumption (but see Elisens and Crawford 1988), and thus have led to the application of isozyme evidence in determining ploidal levels in plants.

Using electrophoresis, it is possible to determine whether homosporous plants are isozymic diploids or polyploids. Initial work indicated that homoeologous genomes were active and that pairing between them was releasing this stored variability. Using inbred lines of the bracken fern, *Pteridium aquilinum,* Chapman and colleagues (1979) presented the results of an electrophoretic analysis of enzyme variability. This study concluded that numerous isozyme variants of each enzyme were present and that the progeny of inbred sporophytes contained enzyme combinations that would not be possible without homoeologous pairing. This work appeared to provide support for the hypothesis that (a) homosporous plants were highly polyploid and (b) the several sets of chromosomes (genomes) that were contained in each polyploid stored variability that was released through pairing between homoeologous genomes during meiosis.

Although this first attempt to test Klekowski's homoeologous heterozygosity model appeared decisive, subsequent work with other homosporous plant groups (which ultimately included a reanalysis of *P. aquilinum* by Wolf and colleagues [1987]) generated a very different picture. Plants with chromosome numbers that are decidedly high compared to those of seed plants, but are basal within homosporous plant genera (see table 1), exhibited isozyme numbers considered typical of diploid seed plants, rather than showing the anticipated multiplication of enzyme bands (Haufler and Soltis 1986). These data indicated that, rather than a single standard for differentiating diploids and polyploids, there was a series of basic chromosome numbers in the homosporous plants; moreover, only taxa having chromosome numbers that were multiples of the generically basal ones contained enzyme profiles that met Gottlieb's functional definition of polyploids (table 1). The evidence from the homosporous plants also represented a significant breakdown of the correspondence between isozyme number and chromosome number in defining polyploidy.

When reconsidered from this perspective, other lines of evidence also supported the distinction between "chromosomal" polyploidy and "genetic expression" of polyploidy. Klekowski and Davis (1977) studied the distribution of somatic mutants among gametophytic progeny of a $2n = 44$ pteridophyte and found a 1:1 segregation ratio, typical of a diploid genetic system. Schneller (1979) worked with morphological traits controlled by single genes in the lady fern (*Athyrium filix-femina*, $2n = 80$) and concluded that their pattern of inheritance could be explained by assuming a diploid genetic system. Masuyama (1986) analyzed the genetic control of breeding systems in *Phegopteris* ($2n = 60$) and indicated that a diploid model fit the data best. Finally, in controlled crossing experiments involving mutants of a "diploid" ($2n = 78$) species in the genus *Ceratopteris* (Hickok 1985), results suggested diploid expression for the single gene mutants. The accumulated evidence yielded a genetic paradox for homosporous vascular plant lineages: Although homosporous plants could be defined as polyploids based on their absolute chromosome numbers, they were diploids when it came to isozyme numbers.

A revised perspective on polyploidy in homosporous plants. On the basis of the accumulated genetic data, species having the lowest extant chromosome numbers within genera should be considered diploid, whereas polyploids are those species having multiples of such generically basal numbers. Thus, from the standpoint of genetic expression, an absolute chromosome number cutoff between diploidy and polyploidy does not exist. The hitch in this relativistic genetic definition of ploidy levels is that it relates only to the current situation and ignores the historical, evolutionary component of species. We do not know where all these seemingly superfluous chromosomes came from, nor do we understand why homosporous plant genetics should depart so markedly from well-studied angiosperm systems.

Explaining polyploid origins. Two main models have been proposed to explain the evolution of the chromosomal polyploidy–genetic diploidy paradox. Soltis and Soltis (1987a) advanced the hypothesis that the immediate ancestors of homosporous plant lineages contained high chromosome numbers and that high numbers are, therefore, a basic characteristic of the homosporous pteridophytes.

An alternative hypothesis (Haufler 1987) suggested that homosporous lineages began with low chromosome numbers and that our current homosporous "diploid" species arose through episodes of polyploidy, which were followed by genetic diploidization in these new polyploids via gene silencing and loss by extinction of progenitor taxa with lower chromosome numbers. Thus, in the group as a whole, chromosome numbers would continue to increase while genetic diploidy was maintained.

Only a few studies have focused on testing these models at the DNA level. Pichersky and colleagues (1990) found a larger-than-expected number of defective chlorophyll *a/b*-binding protein genes in *Polystichum munitum* at $2n = 82$. McGrath and colleagues (1994) discovered evidence of silenced genes in *C. richardii* at $2n = 78$, and later McGrath and Hickok (1999) showed that this species also had multiple ribosomal RNA gene loci. These studies are all consistent with models of homosporous pteridophyte evolution via cycles of polyploidy followed by gene silencing. Achieving diploidy through the silencing of superfluous genes may be selectively advantageous and, as in other organisms, diploidy may be the best compromise genomic condition for preserving and releasing the genetic variation that is required to evolve.

Breeding systems of individuals. If homosporous plant gametophytes fertilized themselves regularly (a reasonable assumption, given the bisexuality of individual gametophytes), a very different set of constraints would be imposed at the population level than if gametophytic self-fertilization were rare. Studies have indeed shown that individual gametophytes can often form sporophytes (reviewed in Klekowski 1979). However, other studies summarized by Lloyd (1974) have shown that the potential for gametophytic bisexuality does not necessarily translate to a predisposition for inbreeding.

Studies of the "isolate potential" of homosporous plants (Peck et al. 1990) provide direct evidence on the breeding system of a given species. By placing one gametophyte in each culture vessel, inducing sperm release by watering, and assaying the number of individual gametophytes that form sporophytes, the capacity of species to generate new sporophytes from single spores can be predicted. These data can then be used to generate testable hypotheses about breeding systems in nature. Species whose gametophytes have a low isolate potential (that is, they rarely form sporophytes when isolated) should be outcrossing in nature. Species that have a high isolate potential are expected to be highly inbred. Peck and colleagues (1990) demonstrated a broad range of isolate potentials among those species tested, with isolated gametophytes

in most species having far less than 100% capability to produce sporophytes. It is likely that a greater number of recessive lethal genes are present in species with a low isolate potential than in those with a high isolate potential. Because gametophytic self-fertilization would lead to total homozygosity and therefore expression of all recessive genes, gametophytes that carry only a single recessive lethal gene could not form sporophytes. In genetically diploid organisms, the joining of gametes that carry different genotypes, which must be an evolutionarily selected process, can reduce the risk of the expression of recessive lethals (however, individuals that carry recessive lethals in heterozygous combinations can still be healthy and vigorous).

Table 1. A comparison of chromosome numbers and genetic expression, as revealed via isozymes in representative pteridophyte genera.

Relative ploidal level	Isozymic expression	Actual vegetative (somatic) chromosome numbers			
		Asplenium	*Polypodium*	*Cystopteris*	*Pteridium*
6*n*	Hexaploid	216	222	252	—
4*n*	Tetraploid	144	152	168	208
2*n*	Diploid	72	74	84	104

Note: All chromosome numbers shown would be considered to be polyploid numbers in seed plants. The lowest chromosome numbers in each column are the lowest extant ones in the genera listed. In all cases, isozymic expression typical of diploid plants was observed at these generically basal chromosome numbers. These numbers show that in pteridophytes, it is not possible to use chromosome numbers to designate a single numerical dividing line between diploids and polyploids. The "diploid" number of chromosomes is different in each genus.

There also appear to be both genetical and chemical mechanisms to promote outcrossing in homosporous species. Production of a pheromone (antheridiogen) by gametophytes of some species may enhance the frequency of outcrossing. This pheromone is released from mature gametophytes (those having a defined meristem) and causes neighboring immature gametophytes to develop exclusively male gametangia (antheridia). The production of such unisexual gametophytes can increase the frequency of outcrossing (reviewed in Schneller et al. 1990). In addition to these antheridiogen mechanisms, several studies have shown that archegonia may form before antheridia, and by the time antheridia appeared, each gametophyte already had numerous archegonia (Lloyd 1974). In some cases, when gametophytes were grown individually, each produced hundreds of archegonia and never formed antheridia (Haufler and Gastony 1978).

These attributes may be coordinated and may actually reinforce each other in establishing gametophytic systems that will promote outcrossing. For example, in a given population, the first spores to germinate produce gametophytes that mature with meristems and archegonia. Neighboring spores that germinate later (or those stored in subterranean spore banks; Dyer and Lindsay 1992, Haufler and Welling 1994) fall under the influence of antheridiogen produced by the meristematic gametophytes and therefore mature as males. In the presence of water, sperms released from the male gametophytes fertilize the eggs of the female gametophytes. In addition, Klekowski (1982) has reported that some female gametophytes show evidence of a "soft selection" mechanism to ensure that the more vigorous sporophytic genotypes will be produced. Soft selection works as follows: Each female gametophyte contains numerous archegonia and, with water and actively swimming sperm present, several archegonia can become fertilized simultaneously. This establishes competition among the developing embryos. Circumstantial evidence suggests that this mechanism is applicable because few, if any, gametophytes ultimately produce more than a single sporophyte. This same mechanism can select against zygotes containing recessive lethals.

It may be that polyploidization could radically modify the breeding system of homosporous plant species. About 44% of extant homosporous plants are polyploids, even if polyploidy is defined as being above generically basal chromosome numbers (Vida 1976). Electrophoretic analyses showed that these "recent" polyploids do have more isozymes (Werth et al. 1985, Haufler and Soltis 1986, Haufler et al. 1990), and therefore more active genes per trait, than do their diploid relatives. In polyploid systems, single gene mutations, which are usually recessive, would not be immediately apparent, and the presence (because of polyploidy) of homoeologous copies of dominant genes retards the expression of any recessive mutations. In the context of the control of breeding systems, these duplicated genes would permit the accumulation of several recessive lethals, as long as at least one dominant gene was still active. With such buffering protection against lethals, polyploids could be more tolerant of intragametophytic selfing than could diploids. Masuyama and Watano (1990) summarized studies that provide evidence for the substantial differences between the breeding systems in diploid and polyploid species and concluded that the gametophytes of diploids promote outcrossing, while those of tetraploids allow inbreeding. Such a difference in breeding system may explain in part the geographic success of polyploids relative to diploids. Through inbreeding, the polyploids could establish new sporophytes from single spores, whereas the presence of recessive lethals or outcrossing mechanisms, or both, in diploids would require that at least two genetically different gametophytes mature in close proximity and that the sperm from one fertilize the egg on the other to successfully generate a new sporophyte.

Genetics of individuals: Summary and implications

Perhaps the most revolutionary discovery about pteridophyte genetics in recent years is that although homosporous plants

have many chromosomes, at a series of generically basic chromosome numbers, they are functionally diploids (table 1). The lack of correspondence between chromosome numbers and genetic expression necessitates the formulation of new hypotheses about how such a system could have evolved and what impact it has on the population biology of homosporous plants. The fact is that in vascular plants, no matter what the presumed ancestry of a species is, if it is homosporous, the species has more chromosomes than if it is heterosporous. Being homosporous may impose a different set of evolutionary constraints and predispositions on species than does being heterosporous.

What are the differences between homosporous and heterosporous organisms that may influence interpretations of their genetic systems? Homosporous plants appear more likely than heterosporous ones to become polyploid, because inherent characteristics of homosporous plants may increase the likelihood of both interspecific hybridization and chromosome doubling (figure 4).

The frequency of hybridization may be enhanced because many spores are produced, which may germinate to yield many gametophytes. If gametophytes of different species are found in the same vicinity (occupying the same "safe sites"; Cousens et al. 1985), the sharing of gametes between gametophytes (perhaps mediated by antheridiogen) is probable. Formation of hybrids is likely because homosporous plants lack the sophisticated prefertilization mechanisms that often prevent interaction between heterosporous species (e.g., insect pollination mechanisms and pollen–stigma compatibility interactions in flowering plants).

The frequency of polyploidization also may be enhanced because the transformation of sterile hybrids to fertile polyploids occurs most frequently by the production of unreduced spores during meiosis (Harlan and deWet 1975). Homosporous plants produce extraordinary quantities of spores and, because most are perennials, it is reasonable to assume that long-lived hybrid individuals will eventually produce and release unreduced spores. Finally, as pointed out by Walker (1979), once a polyploid gametophyte has been formed by a homosporous plant, it is much more likely that it will generate a polyploid sporophyte than would a heterosporous plant. In the latter case, two demonstrably rare events must occur simultaneously—both a polyploid microspore and a polyploid megaspore must be formed to produce the egg and sperm that are necessary to initiate a new sporophyte (figure 4). Because homosporous gametophytes are hermaphroditic, polyploid eggs and sperms are adjacent to each other and intragametophytic selfing (which, as discussed above, may be especially frequent in polyploids) will yield polyploid sporophytes in a single step. Furthermore, this new allopolyploid gametophyte will likely be immune to any genetic problem of inbreeding because of the fixed homoeologous heterozygosity. As Walker (1979) states succinctly, "The heterosporous plant in order to establish polyploidy must meet very rigorous conditions both in time and space, whereas the homosporous plant has to fulfill much less demanding conditions" (p. 100). It seems clear, therefore, that the basic features of homospory enhance the possibility that each of the steps critical for the evolution of higher ploidy will occur.

But what about the evolution of genetic diploidy at high chromosome numbers? If the ancestors of extant homosporous species all were diploid at high chromosome numbers, then we would not have to consider diploidization as an ongoing process that regularly proceeds to completion. However, proposing high chromosome numbers as a unique, derived feature of the homosporous pteridophytes means that species with high chromosome numbers are more closely related to each other than they are to all other species. Is such an assumption reasonable? It would follow that the homosporous genera *Lycopodium* and *Dryopteris* are more closely related than *Lycopodium* is to heterosporous *Selaginella* or *Dryopteris* is to heterosporous *Marsilea* (the heterosporous species all have low base chromosome numbers). There are strenuous arguments against such evolutionary allegiances based on paleobotanical, anatomical, morphological, and

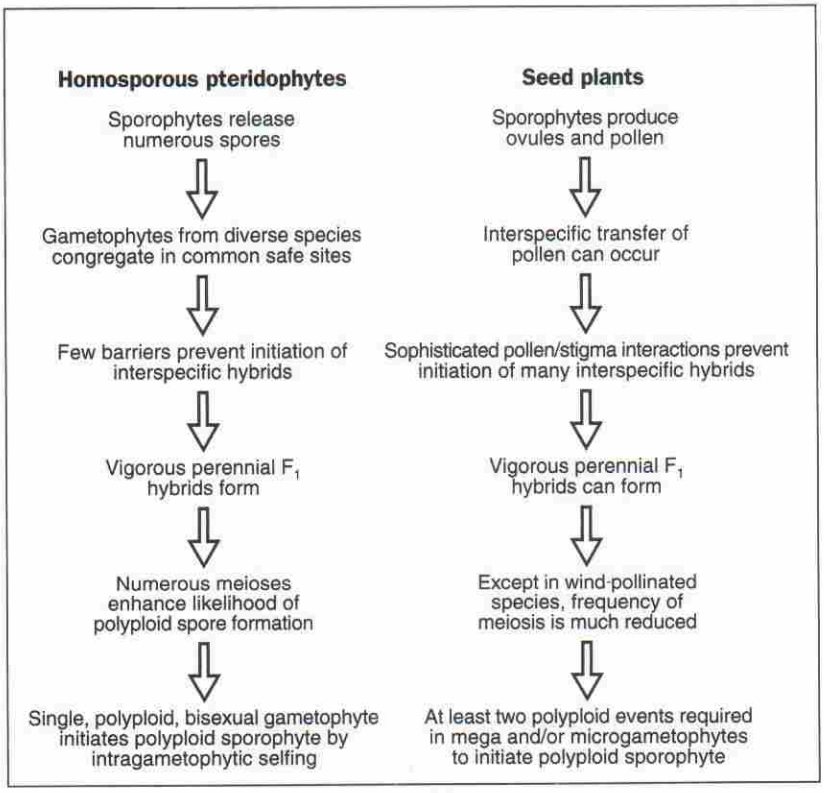

Figure 4. Comparing stages of allopolyploid formation in homosporous pteridophytes with similar aspects of seed plants. Attributes of reproductive biology promoting a high frequency of allopolyploidy in homosporous species are emphasized.

molecular evidence (Pryer et al. 2001). *Lycopodium* and *Selaginella* are sister lycopod taxa, whereas *Dryopteris* and *Marsilea* are both ferns. Alternatively, it could be that species with lower chromosome numbers evolved by extraordinary loss of chromosomes. If this were true, why would only the heterosporous pteridophytes, such as *Selaginella* and *Marsilea,* reduce their numbers of chromosomes?

An alternative path begins with all species having low chromosome numbers, with only the homosporous ones evolving high numbers. If this is true, then each homosporous lineage could have evolved the condition of chromosomal polyploidy with genetic diploidy independently. Three observations make this hypothesis likely.

1. Truly polyploid homosporous plants (which contain chromosome numbers that are multiples of extant basal sets), whose wide geographic distribution may indicate that they are "old" taxa, show a loss of duplicated isozyme loci.
2. "Younger" (that is, less widely distributed) polyploids do not show such a loss of duplicated expression. This is evidence for progressive gene silencing over time (Haufler and Werth 1986).
3. A corollary to evidence that polyploids show high rates of gametophytic self-fertilization (because any recessive lethals are "covered" by duplicated, dominant genes) is that silenced genes would be rapidly fixed into the genome. Because of this mating system, homosporous species could become diploidized more rapidly than heterosporous species, given that the latter are not capable of intragametophytic selfing.

The accumulated evidence and inferences, therefore, appear to favor the episodic origin of polyploids followed by gradual gene silencing, but more studies are necessary. More molecular studies at the genome level will help to discover whether there is solid evidence of "fossilized," silenced genes that are no longer functional.

The population genetics of homosporous plants

Although debating the origin of the current genetic composition of homosporous species should continue, it is clear that for purposes of genetic analysis, we cannot consider homosporous plant species having generically basal chromosome numbers as polyploids (table 1). This now well-documented observation has important implications at the populational level, where the evolutionary opportunities and parameters relating to diploids are quite different from those of polyploids. Models for the expression of traits based on a diploid framework will simply not produce the same results as those based on an assumption of polyploidy.

Electrophoresis and population genetics. As was the case for determining individual genetic features, electrophoretically detectable enzyme variability is very useful in examining the genetic composition of populations. In electrophoretic studies, by convention the term *isozyme* is used to refer to all of the enzyme variants coded by individual genetic loci and the term *allozyme* refers to enzymes assignable to the various alleles at a given locus.

For analyzing enzyme variation, statistics that are commonly used to compare populations and develop conclusions about types of breeding systems that operate in nature include determinations of the levels of polymorphism and heterozygosity. Polymorphism refers to the actual number of different alleles (allozymes) that are present in a population. An isozyme that has two or more allozymes at a frequency greater than 5% is said to be polymorphic. The more allozymes there are per locus, the more polymorphic the population is said to be and, presumably, the greater the likelihood that the population contains evolutionarily significant levels of genetic variability. Other aspects of the genetic composition of individuals can be important at the population level. By determining the number of heterozygous loci in individuals and populations, we can assess the type of breeding system that predominates in the population. The more heterozygotes that are present, the more outcrossing between genetically different egg and sperm that must have taken place.

Analyzing how variability is partitioned can provide insights into the evolutionary dynamics of a species. Population biologists discovered, for example, that some species have most of their total variability housed in each separate population, whereas others dispersed their variability among their populations. With outcrossing breeding systems, the movement of alleles between individuals and among populations is more likely than if inbreeding predominates. Thus, a species whose populations all contain the same alleles at about the same frequencies is likely to be primarily outcrossing. When individual populations of a species accumulate unique allelic frequencies, inbreeding is more prevalent. Common sense suggests that if species differ in the way that they compartmentalize their variability, they probably differ in other ways. Thus, analysis of the partitioning of variability can be used to compare the mating system features and gene flow characteristics (how genes are shared between individuals and populations) of species.

Population statistics. Population biologists use conformance to Hardy-Weinberg (H-W) frequencies as an indicator of the type of mating system. Populations that conform to H-W equilibrium maintain a predictable proportion of individuals as homozygotes and heterozygotes. Departures from H-W can be caused by several forces, including nonrandom breeding, selection, migration, and so on. Excesses in heterozygote frequencies are expected in outcrossing species, while excesses in homozygote frequencies are considered characteristic of inbreeding species. The fixation index *(F)* specifically examines the proportion of heterozygotes (Wright 1931). When F approaches -1, there is an excess of heterozygotes in the population, whereas when F nears $+1$, the population is deficient in heterozygotes. Translating this to the biology of the species, at $F = -1$, the populations would be highly outcrossing, at $F = 0$, the populations

would be mating randomly, and at $F = +1$, the populations would be highly inbred.

The availability of databases charting isozyme variation for pteridophytes provided another opportunity for considering the unique genetic features of homosporous plants. Population biologists had not pondered how the capacity to become totally inbred in a single generation might affect population genetic statistics. McCauley and colleagues (1985) were the first to develop special equations for working with populations of homosporous plants. Subsequently, Hedrick (1987) and Holsinger (1987) built on McCauley and colleagues' work, but Holsinger's equations are probably the most useful, because they reduce the bias of the estimates and allow the incorporation of data from more than one locus (Holsinger 1990).

Coordinating gametophytic and sporophytic mating system determinants. Prior to the development of isozyme databases, the information regarding breeding systems in homosporous plants came from laboratory studies of gametophytes. By analyzing the genetic composition of sporophyte populations, it is possible in many cases to develop accurate descriptions of the mating system of the species. Haufler and Soltis (1984) tested the laboratory-based prediction, developed by Haufler and Gastony (1978), that species of the desert fern *Bommeria* should be predominantly outcrossing. Electrophoretic studies of isozymes were used to study natural sporophyte populations and to estimate the frequencies of outcrossing and, by using conservative methods for identifying products of outcrossing events, Haufler and Soltis estimated that at least 80% of *B. hispida* sporophytes were initiated through outcrossing. Subsequent investigations (Haufler and Welling 1994) showed that *Bommeria* spores from subterranean spore banks could be stimulated to germinate and release sperm through the stimulation of antheridiogen produced by aboveground gametophytes. The sperm from genetically different, soil-bound gametophytes could fertilize eggs produced by the gametophytes on the surface. In short, studies of gametophytes and population biology revealed that complex mechanisms to promote outcrossing and preserve genetic variation have evolved, even in species with what appear to be simple breeding systems.

Analyses of isozyme studies have provided additional information about the sporophyte products of gametophyte breeding systems. If inbreeding predominated, values of F should be close to +1. However, as summarized by Soltis and Soltis (1989, 1990a, 1992), most diploid homosporous plants have F values that hover around 0, indicating that most homosporous plants probably have random mating systems rather than a predisposition for selfing. These analyses also showed that intragametophytic selfing rates in most species are near 0 (Soltis and Soltis 1990b). These findings showed, therefore, that even though cultured gametophytes could become hermaphroditic, and often could be forced to self-fertilize, such outcomes were not obtained in nature. Discoveries that cultured gametophytes failed to mimic natural conditions should not be surprising. Botanists are well aware that greenhouse experiments often have little relevance to natural populations. Some mating events that can be carried out in greenhouses would simply not occur under natural conditions. Individuals normally separated by great geographical distance can be forced to share gametes, species that cooccur in nature but never have flowers open at the same time can be crosspollinated, and in orchids even intergeneric crosses can yield fully fertile offspring, but none of these events would occur naturally. Perhaps because of the perceived simplicity of the pteridophyte systems, any sophistication that would promote outcrossing was considered unlikely.

Among diploid homosporous pteridophytes, a close correlation has been demonstrated between inferred breeding systems and the distribution of genetic variation within or between populations. Outcrossing species tend to house most of the species-wide genetic variation within individual populations (Soltis and Soltis 1987c, 1988, Wolf et al. 1991). In contrast, species with mixed mating systems (Soltis and Soltis 1987b, Murakami et al. 1997), and especially those that are inbreeding (McCauley et al. 1985, Soltis and Soltis 1986, Hauk and Haufler 1999), tend to have populations that contain only part of the species-wide genetic variation and therefore show "population substructuring." It is also interesting to note that polyploid species, which because of their gene duplication should not show evidence of genetic load and should have inbreeding systems, also show the population substructuring that is consistent with an inbreeding mating system (Suter et al. 2000). The available studies demonstrate, therefore, that homosporous pteridophyte species are not all highly inbred or evolutionarily stagnant, as had been assumed from a consideration of superficial features.

Gene flow. This term refers to the sharing of genes between individuals and populations. Most of the literature on gene flow in plants comes from the study of angiosperms where the movement of genes is often complicated by animal vectors of pollen. Presumably, gene flow in homosporous plants is accomplished by spore dispersal, and the forces acting on homosporous plants should be similar to those of wind-pollinated species such as grasses and gymnosperms.

There are two ways to measure gene flow: either directly or indirectly. Direct measurements involve tracking the release of propagules from a source plant and actually determining how far they move. Indirect measurements are based on observing the spatial distribution of particular features and then statistically determining how this distribution relates to the sharing of genes between populations.

Both of these approaches have been applied to homosporous plants. Using radioactive tracers (Conant 1978) and spaced spore traps (Peck et al. 1990), it was possible to demonstrate that the distribution of spores released from ferns was leptokcurtic, that is, most of the released spores stayed near the parent plant, and only a small proportion traveled far from the point of origin. In contrast, when studying the flora of the isolated island of Tristan de Cunha, Tryon (1966) concluded

that spores had traveled thousands of miles from South America. From these and other studies, we may deduce that although only a small percentage of spores travels great distances, because so many are released from each plant, significant range extensions can result.

Indirect procedures often use electrophoresis to analyze the distribution of isozyme variants within and between populations. Statistics have been developed that are based on the frequency of rare protein variants. When these rare proteins are confined to individual populations they are referred to as "private alleles." Slatkin (1985) showed mathematically that if gene flow is high, such "private alleles" will be at low frequencies, that is, individual populations will only rarely have unique proteins. If rates of gene flow are low, however, there will be a higher frequency of "private alleles." Using Slatkin's procedures, Soltis and Soltis (1987c) showed that rates of gene flow in *Polystichum munitum* are among the highest yet reported for plants. In a similar study of *Pteridium aquilinum*, Wolf and colleagues (1991) concluded that gene flow was so great that the neighborhood size for this prominent component of the flora of Great Britain was effectively the entire country!

Island pteridophytes provide evolutionary insights. Ever since Darwin and Wallace developed their theories about mechanisms of evolution via natural selection, islands have featured prominently as natural laboratories for testing theories and obtaining data about evolutionary patterns and processes. Island systems have also provided some valuable new information for understanding the structure of pteridophyte populations and the flow of genes among populations. Most analyses of island pteridophytes have focused on species in Hawaii. Some hypotheses about how plants with the reproductive characteristics of pteridophytes "should" function on islands have been supported, but others have not. Because pteridophytes use minute, unicellular, vagile spores for dispersal, it has been assumed that ferns and related groups will be well represented in island floras. Indeed, pteridophytes appear to be overrepresented on islands. Whereas ferns account for only about 4% of vascular plant species on Earth, they represent about 13% on the Hawaiian Islands and show similarly high proportions on other oceanic islands (Smith 1972).

Additional support for the facile dispersability of fern species comes from comparing endemism on individual islands of the Hawaiian chain. Whereas 80% of flowering plant species are endemic to single islands, only 6% of fern species are found on only one Hawaiian island (Ranker et al. 2000). Homosporous pteridophytes may disperse more readily than other plants from continents. Ranker and colleagues (1994) used allozymic evidence to show that the inbreeding polyploid *Asplenium adiantum-nigrum* had at least three and possibly as many as 17 separate introductions to Hawaii. On the other hand, not all island pteridophytes are selfing species. Evidence from populational studies demonstrates that well-established island endemics are primarily outcrossing (Ranker 1992a, Ranker et al. 1996, 2000, Li, and Haufler 1999). It may be that as species become established on islands, there is a shift in breeding systems from an inbreeding mode that facilitates single-spore colonization to an outcrossing mode. Switching to outcrossing would enable the colonists to accumulate and maintain genetic variation in populations, develop diverse responses to climate change, adapt to opening habitat opportunities, radiate, and speciate. Once outcrossing became established in a lineage, subsequent origins of island endemic offspring species would most likely start as outcrossers.

Tropical pteridophyte population dynamics. The discussions above illustrate that the genetic composition of individual pteridophyte species, the breeding capacities and interactions among gametophytes, and the migration of species via spore dispersal all have an influence on pteridophyte populations. Because most methods for determining the patterns of genetic variation require the availability of well-equipped laboratories, however, nearly all populational studies have focused on species from temperate climatic zones. When populations are locally available, fresh samples can be obtained and rapidly processed. Unfortunately, the highest diversity of pteridophytes is located in tropical regions having restricted access to laboratory facilities. The small number of studies that have involved tropical populations (Ranker 1992a, 1992b, Chiou and Farrar 1997, Hooper and Haufler 1997, Chiou et al. 1998) demonstrate that (a) tropical systems are complex and pteridophytes in those regions feature a broad range of breeding systems, from inbreeding and mixed mating (Ranker 1992a) to strongly outcrossed ones (Hooper and Haufler 1997); (b) populations of tropical pteridophytes harbor highly diverse individuals and may be capable of rapid recombinational radiation; and (c) future studies should endeavor to consider more species from tropical regions. Having high levels of genetic variation may provide tropical species with more opportunities for recombination, which can influence genetic structure of individuals and populations and thereby promote diversification.

Genetics of populations: Summary and implications

The study of the population biology of homosporous plants is still in its infancy. As discussed here, however, available databases and ongoing analyses have already provided us with some fascinating insights into the mechanisms that control homosporous plant evolution. First, by considering the partitioning of protein variants within and between populations, we may conclude that most genetically diploid homosporous plant species breed randomly in nature. Just as in angiosperms, inbreeding may be a specialized trait confined to colonizing species.

Spores can bring about remarkable gene movement in homosporous plants. Not only is this important in thinking about the population biology of pteridophytes, but it also relates to the maintenance of barriers between species. We

know that in most cases homosporous plants lack prefertilization mechanisms to prevent the fusion of gametes from any two individuals (Schneller 1981), that is, homosporous species do not have the specialized pollination mechanisms so well developed in many angiosperms. Judging from the available results, we may also conclude that minute homosporous plant spores do travel long distances and therefore generate high rates of gene flow. Cousens and colleagues (1985) suggested that habitats suitable for the growth of gametophytes (safe sites) are rare and may lead to the clustering of many different species in a single small space. If spores from separate species are regularly dispersed to small safe sites, then it is likely that their sperms and eggs will frequently come into contact. This situation may lead to the development of mechanisms to prevent the zygotes formed by interspecific hybridization from developing into fertile plants. Populational studies indicate that these mechanisms, called "postzygotic genetic incompatibility," should be the primary force that isolates one species from another. Thus, if homosporous plant species are regularly developing genetic differences that will prevent the formation of fertile interspecific hybrids, it is not surprising to find that the average genetic identity between congeneric homosporous plant species (0.38) is much lower than that between angiosperm congeners (0.67) (Soltis and Soltis 1989).

On the other hand, the vagility of spores may be overemphasized. More studies of gametophyte biology can provide a better-balanced profile of homosporous species' capacities for colonization and interbreeding (Chiou et al. 1998, Dassler and Farrar 2001). Also, the inclusion of more tropical species may change the statistics, because some of them show little genetic interspecific differentiation (average interspecific genetic identity among tropical *Pleopeltis* congeners = 0.849 [Haufler et al. 2000]). In such cases, it is likely that mechanisms other than postzygotic incompatibility are operating to maintain species distinctness. It is clear that improving our understanding of the evolutionary and ecological mechanisms operating within and between homosporous plant populations can be of great importance in clarifying the patterns and processes of evolution in these unique organisms.

Current data concerning the genetics of individuals and populations indicate that continued coordination of studies on natural sporophyte populations with laboratory analyses of gametophytes will enable us to develop a more complete understanding of homosporous plant breeding systems. Whereas studies of cultured gametophytes can show us what a given species might be doing in nature, analyses of sporophytic populations can demonstrate whether this potential is being realized. One set of studies without the other only generates a partial picture of evolutionary modes and mechanisms. Although we have already come a long way, additional studies of individuals and populations, especially in tropical habitats, will complement and extend the available data, thereby improving our appreciation of the variety of ways in which homosporous plants interact and generate novelty.

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