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Electrophoretic Evidence for Genetic Diploidy in the Bracken Fern (*Pteridium aquilinum*)

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Analysis of isozyme variability demonstrates that bracken fern (*Pteridium aquilinum*) has a diploid genetic system and expresses solely disomic inheritance patterns. Electrophoretic data indicate that genetically variable progeny are produced in natural populations after intergametophytic mating rather than by a process involving recombination between duplicated unlinked loci. Although some enzymes are encoded by more than one locus, this has resulted from subcellular compartmentalization of isozymes, and there is no evidence of extensive gene duplication resulting from polyploidy. The conclusions reached in this report differ from those which propose polyploidy as an adaptive mechanism for maintaining genetic variability in *Pteridium* and other homosporous pteridophytes.

THERE ARE TWO OPPOSING THEORIES to explain the production of genetically variable offspring in homosporous pteridophytes. The more conventional theory proposes that outcrossing generates heterozygosity and subsequent meiotic segregation leads to variable progeny (*1–4*). However, the unique life-cycle characteristics of homosporous pteridophytes inspired Klekowski and Baker (*5*) to formulate an alternative theory concerning the genetic behavior of these plants. The homosporous pteridophytes differ from other vascular land plants in producing wholly independent, potentially bisexual gametophytes. If these gametophytes self-fertilize, they give rise to sporophytes that are homozygous at all genetic loci. The potential for this reproductive process led to the proposal that homosporous pteridophytes are primarily inbreeding (*5*). A second distinctive feature of homosporous pteridophytes is that they typically have high chromosome numbers, suggesting that they are highly polyploid. According to Klekowski and Baker (*5*), such polyploidy could be a genetic adaptation required to overcome the extreme homozygosity imposed by recurrent inbreeding. To release variability from these homologously homozygous plants, Klekowski proposed that homoeologous chromosomes (those from different genomes within a polyploid) pair during meiosis (*6*). Such pairing would result in recombination between different genomes and the subsequent release of genetic variability among progeny.

Several approaches, including cytological studies (*7*), segregation of morphological markers (*8*), and segregation of electrophoretically detectable genetic markers (*9*), have been used to detect the results of homoeologous pairing. However, more recently, several studies have questioned the universality of Klekowski's hypotheses by demonstrating that some homosporous pteridophytes are outcrossing (*2–4*) and genetically diploid (*10, 11*). These studies, however, did not include the species that had been used to demonstrate homoeologous pairing. By means of electrophoretic analysis of enzyme variability, Chapman *et al.* (*9*) found that in bracken fern (*Pteridium aquilinum*) several enzymes were expressed as multiple bands. Chapman *et al.* (*9*) interpreted these results as evidence that there were duplications in the coding genes resulting from polyploidy. Furthermore, the variability expressed among siblings was attributed to homoeologous recombination in the parental generations.

Although these results seemed conclusive at the time, it has been shown that multiple-banded isozyme patterns do not always indicate polyploidy. Use of an inappropriate grinding buffer during preparation can lead to enzyme breakdown and subsequent ghost banding or poor band resolution (*12*). Multiple bands can also result from assaying enzymes composed of more than one subunit. For example, a dimeric enzyme appears as one band in homozygotes but as three bands in heterozygotes (*13*). Also, many diploid plants have been shown to express several isozymes for certain enzymes, each isozyme encoded by a separate genetic locus within a single genome (*14*). These isozymes are active in different compartments (for example, chloroplasts or cytosol) within the cell (*15*). Multimeric isozymes and enzyme compartmentalization were not discussed by Chapman *et al.*, and the grinding buffer they used was a simple one (*9*), which may have resulted in enzyme breakdown.

The present study reassesses the inheritance patterns of polymorphic structural genes in *P. aquilinum* by means of updated protocols for horizontal starch gel electrophoresis (*16*). This study also differs from that of Chapman *et al.* (*9*) in that we directly analyzed gametophytic progeny (representing individual meiotic products), rather than examining sporophytes arising from self-fertilized gametophytes. Thirty-nine single-frond spore samples were collected from wild sporophytes across the United States, Mexico, and Europe (*17*). Each spore sample was sown separately onto nutrient agar medium (*18*) and cultured under standard conditions (*19*). When gametophytes were 3 weeks old, prior to maturation of gametangia, they were harvested for electrophoretic analysis. At least ten gametophytes from each spore sample (giving a total of

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Table 1. Buffer systems used and number of isozymes detected for each enzyme surveyed, with comparisons to expectations for diploid seed plants (*14*); ND, data not available. Dashes indicate enzymes not discussed in (*14*).

Enzyme	Acronym	Buffer system (*16*)	No. of isozymes detected	Expected diploid number (*14*)	Isozyme detected in chloroplasts*
Aspartate aminotransferase	AAT	8	1	3, 4	AAT
Fluorescent esterase	FE	8	2	—	FE-1
Glutamate dehydrogenase	GDH	11	1	1	ND
Isocitrate dehydrogenase	IDH	11	1	—	None
Malate dehydrogenase	MDH	11	4	3, 4	MDH-2
Malic enzyme	ME	8, 7	1	—	None
Phosphoglucoisomerase	PGI	8, 6	2	2	PGI-2
Phosphoglucomutase	PGM	11, 6	2 (3)†	2	PGM-1
6-Phosphogluconate dehydrogenase	6-PGD	11	2	2	6-PGD-2
Shikimate dehydrogenase	SKDH	11	1	—	SKDH
Triosephosphate isomerase	TPI	8, 6	3	2, 3	TPI-2

*Isozymes are numbered sequentially from the fastest migrating (most anodal) bands. †A possible third isozyme was detected only in some plants (see text).

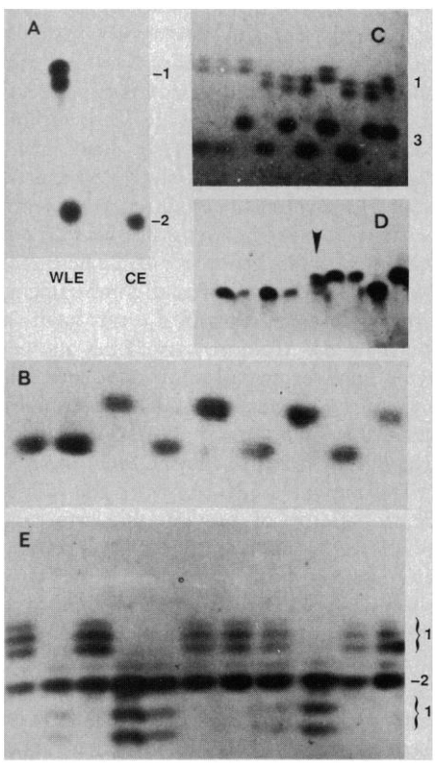

Fig. 1. Photographs of representative starch gels showing enzyme banding patterns of *Pteridium aquilinum*. Numbers refer to isozymes sequenced from the fastest migrating (anodal) band. See Table 1 for enzyme abbreviations. (**A**) Comparison of whole-leaf extract (WLE) and chloroplast extract (CE) of MDH from a sporophyte. The slower migrating MDH-3 and MDH-4 are not shown. (**B**) Segregation of SKDH allozymes among sibling gametophytes from a heterozygous parent. (**C**) Independent segregation of two PGM isozymes among gametophytic progeny. Splitting of the PGM-1 allozymes is an artifact resulting from the buffer system used. (**D**) Progeny array of gametophytes from a plant (AZtr8) heterozygous for *Idh*. The gametophyte in the central lane (indicated by arrowhead) expresses the parental three-banded phenotype. (**E**) Segregation of allozymes among sibling gametophytes for TPI.

800 progeny) were tested. Eleven enzymes were assayed (Table 1) and segregation patterns among sibling gametophytes enabled interpretation of the number of coding genes, and therefore isozymes, of each enzyme (Table 1). To test for enzyme compartmentalization, intact chloroplasts were isolated and subjected to electrophoretic analysis alongside whole-leaf extracts (*20*).

Our results differed markedly from those reported by Chapman *et al.* (*9*). Five of the 11 enzymes were encoded by a single locus and 6 were encoded by several loci and were thus expressed as more than one isozyme. Electrophoretic analysis of chloroplast extracts demonstrated that for multilocus enzymes, some of the isozymes were active only in the chloroplasts (Table 1 and Fig. 1A). When a parent sporophyte was heterozygous at a locus coding a particular isozyme, the two constituent allozymes were segregated among the gametophytic progeny (Fig. 1B) in approximately 1:1 ratios. If a sporophyte was heterozygous at two loci coding for the same enzyme, then segregation occurred independently for each isozyme (Fig. 1C). All 39 parent sporophytes were heterozygous for at least one locus and yielded a total of 62 segregation patterns of which 56 (90%) were not significantly different from the expected 1:1 ratio (G test, goodness of fit; $P < 0.05$) (*21*). One exceptional plant (AZtr8) yielded gametophytic progeny of which 9% (11 out of 120) expressed the parental heterozygous phenotype for isocitrate dehydrogenase (IDH) (Fig. 1D). This result could be considered as evidence for low levels of recombination between an *Idh* gene and a homoeologous gene at which the alleles are null (not expressed) (*9*). A second possibility is that the heterozygous gametophytes had arisen from a nonreductional meiotic division (*22*) and were therefore diploid. Further analysis involved screening for heterozygous gametophytes such that only a portion of the thallus was analyzed electrophoretically. Subsequent cytological examination (*23*) of the remaining portions revealed that the heterozygous gametophytes had 104 chromosomes, twice the number in the homozygous gametophytes ($n = 52$). Gametophytic heterozygosity is therefore most easily explained by production of unreduced spores, and there is no need to invoke homoeologous recombination as an explanation.

Gottlieb (*14*) proposed that the number of isozymes of each enzyme is highly conserved in diploid plants and that gene duplication and polyploidy can increase the number. In the present study, *P. aquilinum* fits closely the predictions for a diploid plant. Furthermore, subcellular compartmentalization of isozymes indicates that the genes coding multilocus enzymes are within the same genome and are not the result of genome duplication (polyploidy). The only increase in isozyme number over the expected diploid pattern was a faint extra band for phosphoglucomutase, which was expressed in 18 of the parent sporophytes, but only rarely and even more faintly in the gametophytes. Since it was not detected in all plants, it could represent a recent gene duplication (*24*) or an ancient duplication that has been partly silenced (*25*). In addition, the extra band was invariable in its relative mobility so that its genetic control is yet undetermined, and it could be an artifact or ghost band. A possible third isozyme of triosephosphate isomerase (TPI) was also evident but it was not under distinct genetic control; comigration of allozyme bands occurred always for two of the three TPI isozymes (Fig. 1E). This pattern could be caused by post-transcriptional modification of the enzyme (*26*) or a tandem duplication resulting in tight linkage (no recombinants were recovered) (*11*). Neither of these conditions involves a polyploid genetic system.

The data presented here show that *P. aquilinum* is genetically diploid. Furthermore, because all parent sporophytes yielded progeny that segregated for at least one enzyme locus, they must have been generated by mating between two genetically different gametophytes (intergametophytic mating) (*6*). Recombinant genotypes were therefore produced in the sample populations by outcrossing (*1, 3, 4, 7, 27*) and not via homoeologous pairing (*5, 9*). Thus, a single haploid spore cannot give rise to a genetically heterogeneous population of this colonizing species (*9*). In this study we find no evidence of polyploidy in *Pteridium* and so support the contention that homosporous

ferns with high chromosome numbers may be diploid (*11*). The results also call into question evidence supporting a selective value of polyploidy in maintaining genetic variability in *Pteridium* and other homosporous pteridophytes (*5, 9*).

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Effect of Lizards on Spider Populations: Manipulative Reconstruction of a Natural Experiment

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Which species affect one another, how intensely, and the mechanisms of those effects are crucial data for understanding how ecological communities work. Tropical islands without lizards, the major top predators, have about ten times as dense web spider populations as those with lizards; processes responsible for this effect were experimentally simulated by removing lizards from randomly selected mainland plots. Spider densities in removal plots averaged 2.5 times as high as controls. Spider survival, prey abundance, and prey consumption were all negatively affected by lizards. Contrary to most studies, predator removal caused an increase in the number of spider species.

INSECTIVOROUS VERTEBRATES ARE POtentially both predators upon and competitors of insectivorous arthropods. Persistence of such arthropods in places having vertebrates may therefore be quite tenuous. In many tropical areas, lizards are the dominant vertebrate insectivore; these feed upon spiders and eat many of the same prey types as spiders eat (*1, 2*). In 1983 we reported (*3*) that orb spider densities are about ten times high on islands without lizards than on those with lizards. Also, numbers of spider species are about twice as high on islands without lizards. These data, from about 100 islands, strongly suggest that lizards reduce spider abundance and species diversity; however, rather than directly demonstrating the causal factor, they are correlative. Furthermore, such data in themselves shed limited light on the mechanism of the effect. Is it predation, competition, or both? We report now a manipulation of lizard populations that was designed to test directly the causal agent and elucidate the mechanisms responsible for drastically smaller spider populations on lizard-inhabited islands. Our experiment is similar to a less extensive one performed by Pacala and Roughgarden (*4*) in a different tropical system; ours both confirms and extends their results.

Our study site was Staniel Cay, Bahamas, a very large island (hence a "mainland") in the midst of the smaller islands used in the 1983 study. Initially, we staked out nine 83.6-m^2 plots whose mean vegetation height ranged from 0.14 to 0.53 m and whose vegetation consisted primarily of sea grape (*Coccoloba*). Plots were divided into three groups, with high, medium and low vegetation, called blocks (*5*); each block had three plots. This experimental design corrects for the effect of plot heterogeneity, in this case in vegetation height, which may otherwise mask the effect of the manipulation. Enclosures were built on two plots in each block, leaving the third plot unenclosed. Then one enclosure in each block was randomly chosen for lizard removal. Thus, there were three replicates of three treatments: lizard-removal enclosures, control enclosures where lizards were not removed, and unenclosed plots where lizards were not removed. Comparisons between lizard and control enclosures were used to determine the effect of lizards. Comparisons between control enclosures and unenclosed plots were used to determine the effect of enclosures. Enclosures were constructed between 29 April and 5 May 1985 and generally followed the design of Pacala *et al.* (*6*); wood-framed fences with one-eighth inch hardware cloth were sunk 0.31 m into the ground and stood 0.93 m high, with 0.41-m sheets of plastic mounted horizontally on top to prevent passage by lizards. Most lizards on the study site were *Anolis sagrei*, with some *Anolis carolinensis* and *Ameiva festiva*. Because lizard and spider densities were reduced during enclosure construction, the control enclosures and open plots were restocked with lizards to about their natural densities (0.06 to 0.10/m^2) before the first census (11 May), and all plots were restocked immediately after the first census with three female and two male *Metepeira datona* (the commonest spider species). Plots were censused at about 4-week intervals for slightly more than a year; numbers and species identities of web spiders and lizards (*7*) were recorded, and four (22 cm by 14 cm tanglefoot) sticky traps were placed for 24 hours in each plot to estimate abundance of other arthropods, the potential prey of both lizards and spiders.

Lizard removal dramatically increased web spider density (Fig. 1). The first mar-

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