out *S*-adenosyl-L-methionine demonstrated saturable binding of [^{3}H]dihydroalprenolol. Scatchard analysis of saturation curves on ghosts previously incubated with *S*-adenosyl-L-methionine revealed an increase in the number of binding sites, with no change in their affinity for [^{3}H]dihydroalprenolol (Fig. 1). *S*-Adenosyl-L-homocysteine inhibits the methyltransferases (*1*). Introduction of 200 μM *S*-adenosyl-L-homocysteine and 200 μM *S*-adenosyl-L-methionine into reticulocyte ghosts inhibited both the formation of phosphatidylcholine and the appearance of new β-adrenergic receptor sites approximately 55 percent (Fig. 2).

To determine whether one or both methyltransferases were responsible for the increase in the number of β-adrenergic receptors, we incubated reticulocytes with varying concentrations of *S*-adenosyl-L-methionine. After 60 minutes of incubation, we measured phosphatidyl-*N*-monomethylethanolamine, phosphatidylcholine, and the number of β-adrenergic receptor binding sites. The number of [^{3}H]dihydroalprenolol binding sites increased with the synthesis of phosphatidylcholine, but not with the synthesis of phosphatidyl-*N*-monomethylethanolamine (Fig. 3). These observations indicated that the increase in the number of β-adrenergic receptors that were made available for binding was related to the increased synthesis of phosphatidylcholine.

After incubation of reticulocytes for 60 minutes at 38°C, adenosine 3′,5′-monophosphate (cyclic AMP) accumulation with isoproterenol or sodium fluoride was reduced tenfold compared to unincubated controls. Thus the incubation conditions needed to increase the number of β-adrenergic receptors made the measurement of coupling between the unmasked receptors and adenylate cyclase technically infeasible.

The mechanism by which phospholipid methylation uncovers hidden receptors is unknown. Since de novo synthesis of new receptors is not possible in a purified membrane preparation, the appearance of new receptor sites may involve changes in charge or microenvironment of the membrane surrounding the receptor binding site. Depending on the physiological conditions, it has been shown that the number of β-adrenergic receptor sites can be rapidly increased or decreased in the rat pineal gland (*6*) and frog erythrocyte (*7*) even after inhibition of protein synthesis. Previous studies have shown that hydrolysis of membrane phospholipids reduces the number of available β-adrenergic receptors (*8*). The activities of certain membrane-bound enzymes, such as Ca^{2+}- and Mg^{2+}-dependent adenosinetriphosphatase and Na^+- and K^+-dependent adenosinetriphosphatase have been found to depend on the presence of phospholipids (*9*) and may also be influenced by phospholipid methylation.

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Homoeologous Heterozygosity and Recombination in the Fern *Pteridium aquilinum*

Abstract. *The bracken fern,* Pteridium aquilinum, *which can form completely homozygous zygotes in a single generation of self-fertilization, has a genetic system that allows the storage and release of genetic variability in spite of this homozygosity. Analysis of the distribution of electrophoretically demonstrable genetic markers demonstrates that this system is based on recombination between duplicated, unlinked loci.*

Among vascular plants, homosporous pteridophytes appear to be the only ones whose spores may give rise to gametophytes, each of which may form both male and female gametes. Because the gametes arise through mitotic cell division, all those produced by an individual gametophyte have identical genotypes. Consequently, self-fertilization of a gametophyte (intragametophytic selfing) produces zygotes with completely homozygous genotypes (*1*). It has been suggested (*2*) that, in adaptive relationship to this aspect of their life cycle, homosporous ferns have evolved a genetic system which permits the expression of genetic variability in the meiotic products (spores) formed by sporophytes that are homozygous in the conventional sense. In these ferns, which are polyploid (*1*), genetic variation is believed to be stored in the form of duplicated, unlinked loci (homoeologous heterozygosity), and released through the process of homoeologous pairing of chromosomes at meiosis. Thus, sporophytes produced through intragametophytic selfing are homozygous at the homologous-gene level but can carry homoeologous heterozygosity (*3*).

Evidence supporting this hypothesis has been of three kinds: the expression of chromosomal aberrations in homozygous spore-mother cells formed by sexual and apogamous species (*4*), the correspondence of segregation ratios of morphological marker genes to those predicted by models of tetrasomic inheritance (*5*), and the segregation of gametophytic mutants (*6*). But none of these lines of evidence unequivocally demonstrates homoeologous heterozygosity or recombination. We now present evidence derived from the demonstration of heterozygosity and recombination at polymorphic structural genes in homozygous sporophytes, and the inheritance of these genetic markers in families of the bracken fern, *Pteridium aquilinum*.

Pteridium aquilinum is a diverse species found throughout the world in temperate and tropical areas. Taxonomically it is polytypic, with two subspecies further subdivided into 12 varieties (*7*). Although much subspecific taxonomic variability is present, most taxa have the same chromosome number, $n = 52$, $2n = 104$ (*8*). Homozygous sporophytes representing both subspecies of *P. aquilinum* were grown from collections of spores made in Florida, Mexico, Colombia, and England (*9*). To ensure a

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common parentage, we used a single frond sampled from a natural population to establish each family of sib sporophytes. Sporophytes having homozygous genotypes were generated from these spores by standard laboratory culture procedures (*10*).

Enzymes used as markers were examined by horizontal starch-gel electrophoresis (*11*). Clear resolution was obtained for six polymorphic enzymes: glutamic oxaloacetic transaminase (GOT), phosphoglucose isomerase (PGI), esterase (EST), peroxidase (PER), catalase (CAT), and malate dehydrogenase (MDH). Different banding patterns were interpreted as representing different genotypes.

Table 1 shows the bands on gels in each group of homozygous sib sporophytes; these are provisionally equated with alleles (*12*). The occurrence of two different alleles in the genotype of a homozygous sporophyte indicates the presence of duplicated genes, a condition established for each of the six polymorphic enzyme systems surveyed. Some 71 (36 percent) of 198 enzyme genotypes examined were heterozygotes (*13*).

Since each family of homozygous sporophytes was generated from the spores of a single parental frond, analysis of the genotypes represented in a family allows inferences to be drawn regarding the number of loci and alleles present in the parental genotype and the extent to which homoeologous pairing of chromosomes was responsible for the generation of the parental genotype. The data in Table 1 can be generalized into four patterns (*14*). In the simplest case, three alleles, A^1, A^2, and A^3, are present, and only two genotypes are represented in the sporophytes: $A^1A^1A^2A^2$ and $A^1A^1A^3A^3$. This is the case for GOT in family MF3; and we conclude that the gene encoding GOT was present in four doses in the parental genotype, which was a homoeologous heterozygote for the locus. In a more complicated situation, two alleles, A^1 and A^2, are present, and the genotypes $A^1A^1A^1A^1$ and $A^1A^1A^2A^2$ occur. Family MG1 exhibits this condition for enzymes GOT, PGI, and PER; family MF1 for GOT, PGI, EST, and MDH; family MF2 for EST and MDH; family MF3 for PGI; family SA for PGI and MDH; and family ENG2 for PGI and CAT. The parental genotype for these families presumably was A^1A^1/A^2A^2. Since the same allele is present on both pairs of homologs, the parental genotype was generated through homoeologous pairing in the grandparent sporophyte, if a single mutational origin for the A^1 allele is assumed (*15*). Conversely, the parental genotype could have been A^1A^2/A^1A^2, with the genotypes in the family of homozygotes being formed directly through homoeologous pairing.

In a related situation, three alleles, A^1, A^2, and A^3, and the four homozygous genotypes occur in a family: $A^1A^1A^1A^1$, $A^2A^2A^2A^2$, $A^1A^1A^3A^3$, and $A^2A^2A^3A^3$. The criterion for defining this condition is the occurrence of one homozygote and various heterozygotes involving two of the three alleles. The inferred parental genotype is either A^1A^2/A^1A^3 (in which case homoeologous pairing generated the meiotic products that formed the homozygous genotypes) or A^1A^1/A^2A^3. On the basis of the assumptions discussed in the previous example, we conclude that the latter genotype was formed by homoeologous pairing in the grandparental sporophyte generation. The families illustrating this situation are MF2 for the enzyme PG1, and MF3 for enzyme PER.

Cases in which three detectable alleles are represented in a family and the array of phenotypes suggests the segregation of null alleles occurs in family MG1 for CAT. Three phenotypes occurred in the homozygotes: F, S, and MS. Thus the original parent had the alleles F, S, and M. In order to form the array of meiotic products in the family, we postulate that the parental genotype had four alleles (F, S, M, and Null) which could thus form the spore genotypes FM, FS, MS, F Null, M Null, and S Null. Selfing would then produce the following sporophyte phenotypes: FM, FS, MS, F, M, and S. Similar patterns are found in families MF1 for CAT, in MF2 for CAT, and in SA for GOT. Family MF4 for GOT can be similarly interpreted, but in this case four different phenotypes occurred in the offspring: S, M, MS, and FS. We assume that the original parental sporophyte had the alleles S, M, F, and Null and that they experienced homoeologous recombination to form the meiotic genotypes represented in the family. Family SA for CAT is a slightly more complicated case since four detectable alleles are documented in the offspring: XS, F, M, and S. Thus, the parent carried the alleles XS, F, M, and S, together with two or four null alleles, and was therefore, either 6X or 8X for this gene locus. Homoeologous chromosome pairing must have

Table 1. Electrophoretic phenotypes for six enzymes in homozygous families of *Pteridium aquilinum* (*12*).

Family	Genotype	Enzyme					
		GOT	PGI	EST	PER	CAT	MDH
MG1	1	S	F, M	F	S	F	S
	2	S	F, M	F	F, S	S	S
	3	F, S	F, M	F	F, S	M, S	S
	4	S	M	F	S	S	S
MG2	1	M	F	F	F, S	S	S
MF1	1	M	F, M	F	S	M, S	F, S
	2	M, S	F, M	F	S	M	F, S
	3	M	F	F	S	XS	S
	4	M	F, M	F, S	S	XS	S
MF2	1	M, S	M, S	F	S	S	F
	2	F, S	F	F, S	F	M, S	F
	3	M, S	F	F	S	S	F, S
	4	M, S	F, S	F, S	S	F	F, S
MF3	1	M, S	F, M	F	F, S	XS	S
	2	F, S	F	F	S	XS	S
	3	F, S	F, M	F	F, M	XS	S
MF4	1	S	F	F	S	F	F
	2	M, S	F, M, S	F	S	S	F
	3	M	M, S	F	S	S	F
	4	F, S	F, M	F	S	S	F
MB3	1	S	M	F	F, S	M, S	F, S
MD1	1	F, S	F	F	S	M, S	S
SA	1	F, M	F	F	F, M	XS	F, S
	2	S	F	F	S	XS	S
	3	M	F, M	F	S	XS	S
	4	F, M	F	F	F, S	F, M	F, S
	5	M	F	F	M	S	S
	6	S	F	F	S	S	F, S
ENG2	1	F, S	F	S	F	F, M	F
	2	F, S	F, M	S	F	F	F
MC	1	F, S	F	F	S	S	S
GW1-11	1	F, S	F	F	F	F, M	S
TUR2-11	1	F, S	F, M	F	F, S	F, M	S

occurred in this parental genotype (regardless of whether it was 6X or 8X) to obtain the array of meiotic genotypes documented. Some parental alleles may not have been present in the homozygous sporophytes examined for each family. Consequently, the genic diversity shown in Table 1 may be an underestimate.

The foregoing data and interpretations suggest that *P. aquilinum* is an ancient tetraploid ($n = 52, x = 26$), and that duplicated loci, homoeologous heterozygosity, and homoeologous pairing of chromosomes occur (*16*). Perhaps the most surprising finding is that the basic chromosome complement of $x = 26$ may also be of polyploid origin. Note that the enzyme PGI in family MF4 shows three alleles, and that one individual has all three present. Since the sporophyte genotypes are homozygous, each allele must be present an even number of times; therefore, the conclusion follows that the parental sporophyte genotype was at least hexaploid for this gene.

Homoeologous recombination has several interesting consequences in relation to the population biology of *P. aquilinum*, which is an aggressive weed throughout the temperate and tropical regions of the world. When a single *Pteridium* spore, reaching a suitable site, develops into a gametophyte, intragametophytic selfing is likely to occur, producing a sporophyte with complete homologous homozygosity. With strictly homologous pairing of chromosomes, offspring of the colonizing sporophyte would be genetically uniform and homozygous; but with homoeologous pairing and crossing-over, an individual sporophyte can produce a genetically variable population. The more successful genotypes can then reproduce asexually by means of rhizomes, thereby perpetuating adaptive combinations of alleles. Hence, *Pteridium* has both the self-compatibility adaptive for a colonizer and the capacity for high levels of recombination usually associated with outcrossing.

Pteridium retains the self-compatibility of a colonizer (*17*), but, because of its capacity for homoeologous recombination, the loss of heterozygosity normally associated with inbreeding is greatly slowed. The reproductive strategy of this fern may combine the advantages of recombination usually associated with sexual reproduction and the advantages of the perpetuation of a successful genotype associated with asexual reproduction (*18*). The question of whether it is even adaptive cannot be answered with certainty at the present time.

Because duplicated loci are recombining, the normal practice of determining the amount of heterozygosity per locus is meaningless. The effective heterozygosity includes all alleles at all duplicate loci encoding a particular gene product. Depending on the degree of homoeologous pairing and the amount of homoeologous heterozygosity, the effective heterozygosity of natural populations would be higher than that expected from the binomial distribution, even if all other conditions for this expectation were met. Because there is evidence that homoeologous recombination occurs in other homosporous ferns (*3, 4*), caution should be exercised in using genotypic frequencies in homosporous pteridophytes to estimate dimensions of population structure. For example, the "conspicuous excess of heterozygotes" reported by Levin and Crepet (*19*) for populations of *Lycopodium lucidulum* could be at least partially accounted for by homoeologous recombination.

Although the actual frequency of selfing in natural populations of *Pteridium* is unknown, the high levels of heterozygosity reflected in Table 1 suggest that it is low, the species being normally outcrossed (*18*). However, the special characteristics of the genetic system of *P. aquilinum*, including the ability to produce a genetically heterogeneous population from a single spore, obviously might be advantageous to a colonizing species like the bracken fern. How widespread homoeologous recombination is among the homosporous ferns and polyploid plants in general remains to be determined.

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2. E. J. Klekowski, Jr., *Ann. Mo. Bot. Gard.* **59**, 138 (1972); *Am. J. Bot.* **60**, 535 (1973).
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9. Spore collections: MG1, MG2, MF1, MF2, MF3, and MF4 from a series of discontinuous populations in pastures along Highway 180 between La Venta and Coatzacoalcos, Mexico; MB3 from KM35 on Highway 261, Xacab, Mexico; MD1 and MC1 from Palenque, Mexico; GW1-11 from the vicinity of Miami, Florida; TUR2-11 from San Andreas Tuxtla, Mexico; SA from Cauna, Colombia (collected by Dr. G. Wilder); and ENG2 from an isolated plant in a door yard across from the Jodrell Gate, Royal Botanic Gardens, Kew, England.
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11. R. K. Selander, M. H. Smith, S. Y. Yang, W. E. Johnson, J. B. Gentry "Studies in Genetics VI," *University of Texas Publ. 7103* (1971), p. 49. Extracts for electrophoresis were prepared by grinding sporophyte tissue in a 1:1 (by volume) mixture of acetate buffer (*p*H 7.2) and a 2*M* polyvinylpyrrolidone solution. Extracts were centrifuged at 17,000 rev/min for 30 minutes, and the supernatants were stored at −70°C.
12. The bands on gels (termed electromorphs) are designated in relation to the relative distance of their migration from the origin, as fast (F), medium (M), slow (S), and extra slow (XS). Heterodimeric bands were not scored.
13. Thirty-three sporophytes were screened for the six enzyme systems.
14. Instead of using various combinations of fast (F), medium (M), slow (S), and extra slow (XS) for the electromorphs of a given enzyme locus, we have designated them A^1, A^2, A^3, A^4. For example, the presence of three bands for a given enzyme system in a family of homozygotes is shown by A^1, A^2, and A^3, regardless of what combination of bands is present.
15. Where two alleles, A^1 and A^2, occur and the genotypes $A^1A^1A^1A^1$ and $A^1A^1A^2A^2$ are represented in the homozygotes, it is possible that a mutation to allele A^2 occurred in each pair of homologous chromosomes. Therefore the segregation of homozygotes and homoeologous heterozygotes is possible without recourse to homoeologous pairing. However, because of the high incidence of these genotypes (14 out of 48 enzyme systems) we consider this hypothesis insufficient to account for observation.
16. Since meiosis in this plant usually involves the formation of bivalents, the high frequency of homoeologous recombination detected in these families clearly suggest that both homologous and homoeologous bivalents occur. Whether pairing occurs randomly within a set of *Pteridium* chromosomes is unknown. The use of the term "homoeologous recombination" in this context needs clarification. *Pteridium aquilinum* is the only extant species of the genus *Pteridium*. The diploid chromosome number of 104 suggests an evolutionary history involving polyploidy. Originally these were true homoeologs in the genome, but as the data in this paper show, homoeologous recombination occurs. This process will result in a loss of homoeology and the generation of four new homologs. The term homoeologous recombination in the present context refers to both the original recombination of the homoeologs and the present recombination of the new homologs.
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18. Characteristics of its mating system indicates that *P. aquilinum* is normally outbreeding. Ontogenetic studies of gametophytes in culture indicates that initially male and female gametophytes are present. The presence of males is related to antheridogen, a diffusible substance produced by the female gametophytes [W. Döpp, *Ber. Dtsch. Bot. Ges.* **63**, 193 (1950); U. Näf, *Growth* **20**, 91 (1956); B. R. Voeller, *Colloq. Int. C.N.R.S.* **123**, 665 (1964)].
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