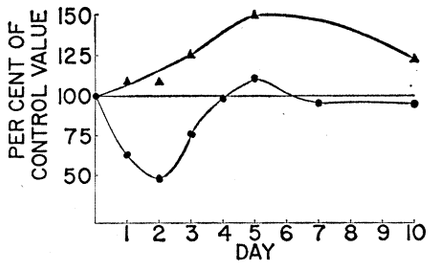

Fig. 1. Effect of DMBA on the incorporation of sodium C^{14}-formate into RNA in the rat mammary gland. DMBA was fed to rats on day 0. Incorporation is expressed as percentage of control values. Control values for female rats (●——●) were 7.9 count/min per microgram of RNA. Control values for male rats (▲——▲) were 6.3 count/min per microgram of RNA.

adenine and guanine accounted for 100.9 percent of the radioactivity. In a second experiment, male rats were killed 3 days after they were fed DMBA. The specific activity of RNA in the experimental animals increased 23 percent over that of control values. Adenine and guanine accounted for 98 percent of the radioactivity in the control animals and for 94 percent in the experimental animals. The data suggest that the specific activity is due to incorporation of C^{14}-formate into the RNA purines.

The effect of DMBA on the incorporation of formate into the RNA of the uterus in the castrated rats was also determined. Whereas C^{14}-formate incorporation into the uterine RNA of the castrated female rat was 13.67 ± 9.1 count/min per microgram of RNA, it was 15.02 ± 2.21 in rats treated with DMBA. DMBA appears to have little or no effect on uterine formate incorporation. When a single injection of estradiol-17β was given to other castrated rats, a two-fold increase in the formate incorporated into uterine RNA was observed (25.90 ± 9.89 count/min per microgram of RNA). The data suggest that a carcinogenic hydrocarbon like DMBA does not possess estrogenic activity.

The inhibitory effect of DMBA on the incorporation of formate is evidently a specific effect on the mammary gland. When incorporation of C^{14}-formate into mammary gland, liver, and kidney RNA from female rats treated with DMBA is measured, incorporation of formate into RNA is inhibited in the mammary gland; it is stimulated in liver and kidney (Table 2). These results are comparable to those that show increased incorporation of $C^{14}O_2$ into RNA uridine from the livers of rats fed 3-MC (*7*).

Under certain conditions one effect of chemical carcinogens on the target tissue is a decrease in macromolecular synthesis. There is a decrease in the amount of messenger RNA synthesized in the liver in animals 1 month after their treatment with 4′fluoro-4-dimethylaminoazobenzene (*8*). In addition to the decrease in total synthesis, there is a change in the pattern of countercurrent distribution. Hulten and Arrhenius (*9*) have observed a decrease in the ability of microsomes from the livers of rats treated with 2-aminofluorene to incorporate leucine into protein. This effect is apparent 4 hours after feeding, but only in rats that have been subjected to an experimental vitamin E deficiency. Although the adrenal cortex of the rat does not develop cancer after DMBA treatment, it is profoundly affected by this carcinogen. Thymidine incorporation is inhibited in the adrenal glands of rats treated with DMBA (*10*).

Similarly, DMBA affects the incorporation of C^{14}-formate into mammary gland RNA. In the female Sprague-Dawley rat, this incorporation is severely decreased within one day of the feeding to the animal of 20 mg of DMBA. When similar experiments are done with male control rats and male rats fed DMBA, incorporation of C^{14}-formate into mammary gland RNA of the experimental animals is stimulated. These effects are directly due to the sex hormones present in the animals (Table 1). Incorporation of C^{14}-formate is unchanged in castrated animals, whether or not the rats have been fed DMBA. In the presence of estradiol, DMBA feeding causes a marked decrease in incorporation; in the presence of testosterone, DMBA causes a slight increase in incorporation. It is unlikely that these effects are secondary to that of DMBA on the adrenal, since the effect on the adrenal is constant with animals of either sex (*11*).

Whether the differences between male and female rats in the incorporation of formate may be related to another physiological difference in the mammary gland, breast cancer in females and none in males after DMBA feeding, is not known. Likewise, it is not known whether a decrease in RNA synthesis may be related to tumorigenesis. It is of interest that there is a slight decrease in tumorigenesis in rats fed DMBA if the rats are concomitantly treated with either uracil or thymidine (*12*).

PAUL R. LIBBY
THOMAS L. DAO

Department of Breast Surgery, Roswell Park Memorial Institute, Buffalo, New York

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13 April 1966

Evolutionary Significance of Polyploidy in the Pteridophyta

Abstract. *Polyploidy occurs in the heterosporous and homosporous Pteridophyta, but with a much higher frequency in the latter. Ninety-six percent of the homosporous Pteridophyta show a gametic chromosome number greater than 27, whereas 90 percent of the heterosporous ones possess a gametic chromosome number less than 28. Ultrafrequent establishment of polyploidy in the homosporous Pteridophyta appears to be necessary to create and maintain genetic variation in the face of the homozygotizing effects of habitual self-fertilization in the monoecious gametophytes of these plants.*

The frequency with which interspecific hybrids are found and the allopolyploid origins which have been demonstrated for many fern species (*1*) has led to the assumption that ferns are typically outbreeding organisms and, consequently, have a tendency to-

ward heterozygosity at any gene locus. Investigations of the reproductive biology of several ferns, however, indicate that fern populations are characterized by greater homozygosity than that previously suspected.

A majority of the homosporous ferns are characterized by free-living, monoecious gametophytes. Since these gametophytes produce gametes mitotically, any sexual sporophyte resulting from the self-fertilization of an isolated gametophyte will be homozygous for every gene in the genome. Studies by Wilkie on the bracken fern, *Pteridium aquilinum* (L.) Kuhn, indicated that only 8 to 17 percent of the gametophytes of this fern could form complete homozygotes (*2*), the remainder requiring cross-fertilization for successful sporophyte production. Our experiments have confirmed the foregoing results in *Pteridium*, but investigation of other fern taxa has revealed that these low frequencies of selfing are not found in all homosporous ferns (*3*).

Techniques have been devised to compare the rates of inbreeding and outbreeding in populations of gametophytes under laboratory conditions. Spores are sown on a defined agar medium (*4*) and cultured under continuous illumination. After germination, the population of gametophytes is separated into three subpopulations: one of isolated gametophytes, each in a petri plate (60 by 15 mm), and two composite subpopulations in large petri plates (150 by 25 mm), each containing a known number of gametophytes. When gametangia begin to form, one of the composite subpopulations and that of isolated gametophytes are watered periodically to initiate sexual reproduction. The composite subpopulation which is not watered serves as a control, and any sporophytes produced by it are assumed to result from apogamy. If sporophytes do make their appearance in the control subpopulation, the species is eliminated from the experiment.

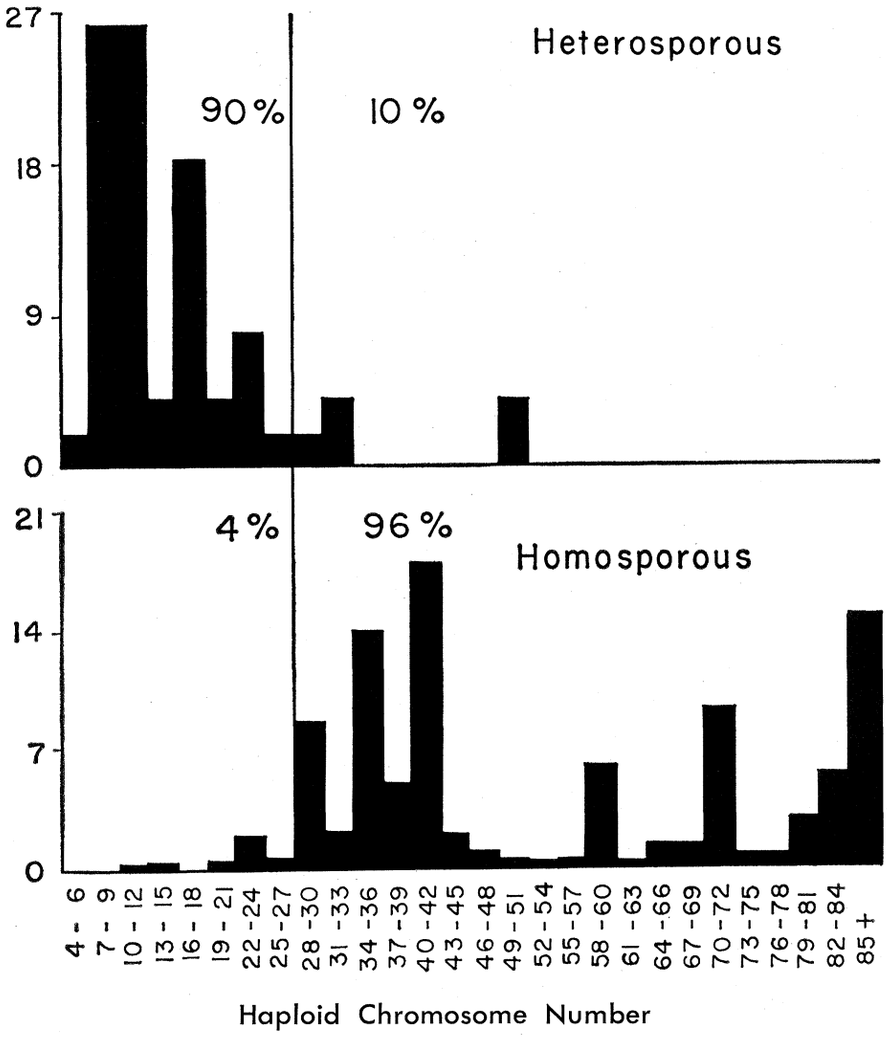

Fig. 1. Distribution of the haploid chromosome numbers of the Pteridophyta. The ordinate of each histogram represents the frequency expressed on a percentage basis. Heterosporous Pteridophyta (50 species) include the Selaginellaceae, Isoetaceae, Marsileaceae, and Salviniaceae. Homosporous Pteridophyta (1166 species) include the Psilotaceae, Lycopodiaceae, Equisetaceae, Ophioglossaceae, Marattiaceae, and all homosporous families of the Filicales. Data were compiled from Chiarugi (*8*) and Fabbri (*9*).

Six species have been experimentally studied with the above technique: *Thelypteris normalis* Moxley, *Thelypteris dentata* E. P. St. John, *Osmunda regalis* L., *Woodwardia fimbriata* Sm., *Microlepia speluncae* (L.) Moore, and *Pteridium aquilinum* (L.) Kuhn. Isolated gametophytes of the first five species formed sexual sporophytes (which are necessarily completely homozygous) as readily as gametophytes in the composite culture. No recessive sporophytic lethal genes were indicated in these ferns since all the isolated gametophytes yielded vigorous, morphologically normal sporophytes. By contrast, gametophytes of *Pteridium aquilinum* yielded a very low percentage of homozygous sporophytes, some of which exhibited various deficiencies that led to the death of the sporophyte. Wilkie reported a similar result (*2*).

Thus, in the almost obligatorily cross-fertilizing *Pteridium aquilinum*, deleterious recessive genes are present in the gene pool, whereas in the species that readily form complete homozygotes they do not seem to be present. In addition, studies have shown that vigorous homozygous sporophytes are freely produced by the following taxa: *Asplenium adiantum-nigrum* L., *Adiantum capillus-veneris* L., *Polystichum adiantiforme* J. Sm., and other species of *Thelypteris*. It may be a valid generalization that many, if not most, homosporous fern taxa have the capacity to produce complete homozygotes by self-fertilization and frequently do so in nature.

Morphological evidence may also support this generalization. In most leptosporangiate homosporous ferns the prothallia are monoecious or at least potentially so (*5*); this is also true for the Lycopodiaceae and Equisetaceae. In homosporous ferns, antheridia appear before archegonia and continue to develop while the archegonia are

mature (*6*); in an outbreeding system, archegonia might be expected to develop first. The necks of the archegonia of most of the Polypodiaceae (s.l.) are curved inward, away from the apical notch of the gametophyte (*6*), thereby pointing their mouths toward the antheridia and rhizoids. This appears to be an adaptation to self-fertilization. If this were a cross-fertilizing system, selection should favor archegonial necks curved in the opposite direction.

Thus, homosporous vascular plants are apparently characterized by degrees of homozygosity that would be unattainable in a heterosporous plant in many generations of selfing. The maintenance of heterozygosity at individual gene loci is unlikely in these homosporous plants because of continuing exposure of sporophytes to selection in the homozygous condition. The obvious evolutionary diversity and successful adaptation to diverse environments of these plants, however, raise the question of the means by which genetic variability (evolutionary potential) is maintained and realized.

On the average, the Pteridophyta have much higher chromosome numbers than the seed plants. The mean gametic chromosome number of those angiosperms whose numbers had been counted before 1963 is 15.99 (*7*). The mean gametic chromosome number of the Pteridophyta as a whole is calculated to be 55.27, based upon lists compiled by Chiarugi (*8*) and Fabbri (*9*). The mean gametic chromosome numbers of the heterosporous and homosporous Pteridophyta are 13.62 and 57.05, respectively. The magnitude of the chromosome number seems to be correlated with the occurrence of homospory or heterospory. In this connection, the close agreement of the mean gametic chromosome number for the angiosperms (which are heterosporous) and the heterosporous Pteridophyta (n = 15.99 and n = 13.62, respectively) in contrast to the homosporous plants (n = 57.05) is notable.

Figure 1 depicts the distribution of the known gametic chromosome numbers of 50 heterosporous and 1166 homosporous species of Pteridophyta. For comparative purposes the percentage of the total sample falling in each class of gametic chromosome numbers is plotted. Of the homosporous plants, 96 percent have a gametic chromosome number greater than 27, whereas 90 percent of the heterosporous ones have a gametic chromosome number less than 28. Unlike the homosporous species, complete homozygote formation in the heterosporous plants is highly unlikely, and the lower incidence of polyploidy in the latter may be related to this.

Polyploidy increases gene redundancy and, if dominant alleles are present, any recessive alleles of the same genes elsewhere in the genotype are kept from immediate exposure to selection even when the plants are homozygous at each locus. Because polyploidy increases the dosage of genes beyond the disomic condition, genes can mutate and take on new functions without depriving the organism of essential processes which are maintained by their former homologs.

This potential for genetic variation could gradually become expressed in the phenotype of the polyploid or could be released for recombination by occasional hybridization of gametophytes which possess duplicated genes that have mutated in different ways. The resulting sporophytes would be heterozygous and give rise to recombinant progeny, some of which might be adaptive. Intergenomic crossing-over is another possible mechanism for release of this kind of genetic variation.

Polyploidization may provide further selective advantages in the homosporous Pteridophyta by the production of immediate genotypic changes (especially where allopolyploids are produced). These new genotypes may be advantageous by reason of heterotic effects produced by intergenomic heterozygosity or the genetic change may directly result in a specific adaptation.

Thus polyploidization is a means by which homosporous Pteridophyta may maintain, create, and release genetic variability in spite of the production of homozygous sporophytes.

EDWARD J. KLEKOWSKI, JR.
HERBERT G. BAKER
Botany Department,
University of California, Berkeley

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16 March 1966

Fungicide Selective for Basidiomycetes

Abstract. *Concentrations of 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin lower than 8 parts per million prevented mycelial growth of a number of Basidiomycetes. By contrast, mycelial growth of various other fungi—Phycomycetes, Ascomycetes, and Deuteromycetes—was 50 percent inhibited only by concentrations of 32 ppm or higher. Two exceptions to this pattern of selective fungitoxicity were found: an isolate of* Rhizoctonia solani *was not as sensitive as other Basidiomycetes, and the deuteromycete* Verticillium albo-atrum *was inhibited by lower concentrations than affected other fungi in this group. Spore germination of two Basidiomycetes,* Uromyces phaseoli *and* Ustilago nuda, *was inhibited 95 percent or more at 10 ppm.*

The compound 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin (oxathiin) has recently proved effective in chemotherapy of bean rust and of loose smut of barley (*1*). Tests in this laboratory, however, indicated that the compound is relatively nontoxic to three ascomycetous fungi. This difference suggested selective toxicity to Basidiomycetes. To explore this apparent

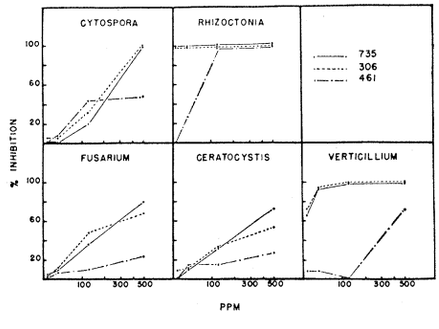

Fig. 1. Inhibition of growth of five fungi by 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin (*735*) and two of its isomers: 2,3-dihydro-5-(*N-m*-tolyl) carboxanilido-6-methyl-1,4-oxathiin (*306*) and 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin-4,4-dioxide (*461*).