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Screening Aquatic Ecosystems for Mutagens with Fern Bioassays

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Recent researches on the royal fern, *Osmunda regalis*, have documented a high incidence of post-zygotic mutational damage in a population growing in a river heavily polluted with paper processing wastes, whereas genetic studies of nearby populations in nonpolluted environments failed to detect mutational damage. Intensive genetic and cytogenetic studies of mutation in *O. regalis* indicates that natural populations of homosporous ferns may be useful *in situ* bioassay systems for monitoring the presence of mutagens in aquatic ecosystems. Since these organisms are long-lived perennials with an ontogenetic system which stores mutational damage, they can be manipulated to give an integrated estimate of mutational damage for specified blocks of time (in units of years). Thus, the fern bioassay may be an inexpensive means of detecting both chronic low dose and episodic high dose inputs of mutagenic pollutants into aquatic ecosystems.

The fern mutagen bioassay is based upon the detection of numerous categories of post-zygotic mutation load in natural fern populations. The frequency of sporophytic and embryonic lethals, leaf or root mutations, auxotrophic gametophytic mutations as well as numerous phenotypic alterations of gametophyte morphology can be routinely detected and quantified. In addition, various two-break chromosome aberrations (paracentric inversions, reciprocal translocations and ring chromosomes) can be readily screened for in the spore mother cells of many homosporous ferns.

Introduction to the Problem

The detection of dispersed mutagens in ecosystems involves overcoming a number of obstacles: generally the chemical nature of the mutagen or mutagens is unknown; these unknowns are quite often in low concentration and may be episodic with reference to their presence in the environment; and finally, the mutagenic material may be restricted to, or more concentrated in, a specific portion of the environment (for example, in an aquatic environment the mutagens may be in the water or bound to organic material or clay micelles). Because of these complexities the employment of a single assay protocol to screen ecosystems for dispersed mutagens runs a high risk of generating "false negatives," i.e., not detecting mutagenic activity when mutagens are present. Thus satisfactory screening of an ecosystem must be based upon diversity of assay protocols.

Four general approaches appear feasible for mutagens. These four assays either concentrate mutagens from an ecosystem by physical or biological means or detect mutational damage in test organisms by the dispersed mutagens. The following list will illustrate these assays: (1) sampling and concentrating a fraction of the environment (atmosphere, water, and soil) and assaying for mutagenicity with a laboratory based assay (*1*), (b) introducing the mutagen assay organism into the environment for a period of time and monitoring changes in mutation rate (*2*); (c) using indigenous bioconcentrators to accumulate material from the ecosystem and then testing extracts of these organisms with laboratory based mutagen assays (*3*, *4*); and (d) measuring genetic damage in selected populations of organisms naturally occurring in the ecosystem, an *in situ* bioassay (*5*). This latter approach will be the subject of this communication.

Requirements of an *In Situ* Bioassay

An *in situ* bioassay is based upon the detection of genetic damage in selected components of the indigenous biota of the ecosystem being studied. The organisms selected to function as bioassays should be capable of being manipulated to fulfill a number

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bioassays as they unambiguously detect recent mutational damage. Various categories of gametophytic mutations are extremely rare in natural populations of ferns. Since the gametophyte generation is independent of the sporophyte generation in these plants, mutations which either reduce the viability of this generation or prevent the formation of gametes will not establish polymorphisms in fern populations. A number of categories of gametophytic mutations have been documented in mutational studies (*5*, *7*).

Spore formation in ferns is preceded by meiosis; thus the immediate progenitor of the haploid spore is the diploid spore mother cell. Genetic studies have indicated that many of the gene products present in the spore mother cell are present also in the spore; thus the gene products present in the spore reflect the diploid genotype of the spore mother cell rather than the haploid spore nucleus. If a spore mother cell is heterozygous for various recessive mutations which control specific aspects of metabolism functional in both gametophyte and sporophyte, the meiotically derived spores that have these recessive mutations will germinate and mitotically divide a few times prior to cessation of growth. This is the result of gene products present in the cytoplasm of the spore which are the products of the wild type alleles in the spore mother cell being used up after a period of growth. Thus when the gametophyte starts to depend upon gene products coded by its own nuclear genotype, any mutational defects result in the termination of growth. The resulting phenotype is a very small gametophyte (3–10 cells). Such mutants have been called "presumptive auxotrophs" (*5*) and must represent post-zygotic mutations in the sporophyte. In addition to this mutant, gametophytes occur which lack the capacity for regular and organized growth. Rather than the normal cordate shape such gametophytes form an irregular calluslike mass and are considered post-zygotic mutations also.

Both of the above gametophyte mutations result in highly abnormal gametophyte phenotypes. Another kind of gametophytic mutation has been documented in mutational studies. Klekowski and Davis (*9*) reported a class of mutations which results in a smaller gametophyte with a normal morphology which exhibits a strong maternal effect in that sporophytes which form on this gametophyte lack leaves regardless of the genotype of the zygote. Since these mutations only occurred in a population of *Osmunda regalis* known to carry a high frequency of recently induced chromosomal mutations, it was concluded that this mutant phenotype was caused by deletions of chromosome material.

In addition to the various kinds of sporophytic and gametophytic mutations, many ferns can be screened readily for two-break chromosome mutations. Heterozygosity for chromosomal rearrangements such as paracentric inversions, reciprocal translocations, and ring chromosomes can be determined readily in many species of ferns (*5*). Since such chromosome rearrangements are rare in normal populations of ferns (there are no documented cases of such polymorphisms in spite of numerous cytological studies), their presence generally indicates a recent mutational origin.

A consideration of the morphology and anatomy of homosporous ferns reveals an organism almost ideal for the storage and perpetuation of various post-zygotic mutations. The apical meristem of this plant is based upon an apical cell with three cutting faces (*10*); this cell divides to give rise to daughter cells which continue to undergo mitosis. Thus all of the cells in the body of the rhizome are derived ultimately from the apical cell. A mutation in this cell eventually will be passed to all of the subsequent cells of the rhizome. The leaf primordia also are organized on an apical cell pattern; again, a mutation occurring in a leaf apical cell will be passed on to all of the subsequent cells of that organ. Thus the meristems of many homosporous ferns will perpetuate recessive sporophytic and dominant and recessive gametophytic mutations if they occur in the apical cells of the various meristems of the sporophyte.

The growth and development of the sporophyte of many homosporous ferns is extremely regular and patterned. Leaves originate in fairly predictable patterns and chronologies and the rhizomes dichotomize to form networks of interconnected apices. This regularity can be used to date when various mutations have occurred by analyzing the chimeric distribution of a given mutation in a sporophyte. This has been achieved for a population of the royal fern, *Osmunda regalis*, growing in a river in western Massachusetts in which mutagens are suspected (*8*). Sporophytes were shown to be chimeric for two-break chromosome mutations, recessive sporophytic lethals, and gametophytic mutations. These mutations were shown to be post-zygotic in origin and, at least for the chromosome mutations, it was found that three dating periods could be devised; prior to 6 months, 6 months to 5 years, and more than 5 years prior to meiosis. This chronology is based upon major ontogenetic events and how they relate to the distribution of chimeras. In this case they coincide with the differentiation of pinnae on the frond and the division of the apical cell leading to leaf primordia initials (*11*).

of biological criteria. A discussion of these criteria is warranted, as this will acknowledge the problems inherent in the development of such assays as well as give criteria to judge the merits of the fern assay.

Obviously the most important feature of the species selected as an assay is the ability to detect genetic damage in natural populations of this organism. The genetic damage should encompass a number of different genetic endpoints (both various chromosomal and genic mutational changes) and should be distinguishable from the components of genetic load normally present in populations of sexually reproducing organisms. Ideally, the genetic damage attributable to recent post-zygotic mutations should be detectable and distinguished from background mutational load. Other features of an organism used in this kind of bioassay include: a sessile life form to help document the origin and nature of the mutagens, a perennial habit with an ontogenetic system which allows the storage and accumulation of mutations in the somatic cells of the organism (this will result in a system sensitive to episodic inputs of mutagens), and a sufficiently well understood and regular ontogeny to help date the occurrence of mutations in terms of the chronology of the organism. Finally, if the organism is a bioconcentrator of promutagens and mutagens, possesses the necessary metabolism to activate promutagens to mutagens, and is ubiquitous ecologically, this organism will be an ideal candidate for an *in situ* bioassay system (unless the organism is highly insensitive to the effects of mutagens, i.e., can exclude mutagens from the cellular environment, can inactivate mutagens, or possesses highly efficient repair systems).

The Fern Assay

The use of fern species as *in situ* bioassays for mutagens dispersed in ecosystems is based primarily upon the numerous genetic endpoints that can be assessed for genetic damage. Any discussion of the relevance of this plant in such research therefore must begin with some understanding of the life cycle of this organism and how it can be manipulated. Since a detailed description of the methodologies involved in using this bioassay has been presented recently (*5*), the following discussion will be primarily an overview.

The life cycle of homosporous ferns is characterized by an alternation of independent autotrophic haploid (the gametophyte) and diploid (the sporophyte) generations. The dominant generation is the sporophyte generation which is complex morphologically and distinct from the gametophyte generation. Studies on the population biology of these plants have developed methods to screen for genetic load in these organisms (*6*). A sporophyte genotype can be screened readily for the presence of sporophytic and gametophytic mutations (lethals and various other detrimental phenotypes) because of the ease of culturing the fern gametophytes and their hermaphroditic nature. Fern gametophytes can be cultured on agar solidified inorganic nutrient and grown under continuous fluorescent illumination. They generally reach sexual maturity in two weeks to three months, depending upon the species. At sexual maturity the gametophyte bears male and female gametangia, and these gametangia form gametes through mitotic divisions; thus all of the gametes formed by a single gametophyte have identical genotypes. The self-fertilization of a gametophyte results in a completely homozygous zygote genotype being formed. If the gametophyte genotype contains a recessive mutation affecting genes expressed only in the sporophyte generation, that mutation will be homozygous and expressed. Where this mutation is a recessive sporophytic lethal, the zygote will abort. By the same reasoning, other kinds of mutations can result in sporophytes being formed with aberrant phenotypes. By capitalizing upon these genetic aspects of the life cycle, a fern sporophyte genotype can be screened readily for heterozygosity for recessive sporophytic lethals. [Experimental details of this analysis have been given elsewhere (*7*)]. Such recessive lethals may represent either the normal genetic load found in all species of sexually reproducing organisms or an additional component due to mutational damage from an offending mutagen. Since genetic load varies quantitatively both inter- and intraspecifically, this parameter is a meaningful estimate of mutational damage only when the normal genetic load background is low or nearly absent.

Recessive sporophytic lethals can be used in a more laborious test for mutational damage. This involves obtaining samples of meiotic products from different parts of the same sporophyte and comparing the genotypes present in these two spore samples. When recessive sporophytic lethals are present in one spore sample and absent in the other, these lethals represent post-zygotic mutations, and their frequency is a useful index of the mutagenic propensity of the environment. Details on the genetic procedures involved in this kind of analysis have been given by Klekowski (*8*).

Genetic endpoints which are attributable primarily to recent mutations are available and represent categories of mutational damage which generate aberrant phenotypes not encountered in routine genetic load studies in ferns. These phenotypes are important, especially in the use of ferns as *in situ*

Attributes and Limitations of the Fern Assay

Reconsidering the fern assay in light of the previously discussed requirements of *in situ* assays will illustrate the areas which need further research. *Osmunda regalis* is the most well studied in terms of the assessment of mutational damage and the understanding of ontogeny and dating of mutations. Unfortunately, this fern is restricted ecologically, whereas other fern taxa which are much more widespread ecologically and therefore potentially more useful are practically unknown genetically. With reference to screening aquatic habitats, species of *Acrostichum* and *Ceratopteris* should be useful in tropical and subtropical environments, whereas in the eastern United States *Onoclea sensibilis*, *Woodwardia areolata*, *W. virginica*, *Matteuccia struthiopteris*, and some *Thelypteris* species all have suitable ecologies. At the present time the majority of these species are practically unknown genetically (with the exception of *Ceratopteris*) but since the problems involved in developing *in situ* bioassays already have been outlined for *O. regalis*, the application of these ideas to these other taxa should not be difficult. If the results of these researches result in systems as tractable as *O. regalis*, ferns will have a very useful role in future *in situ* bioassays.

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REFERENCES

1. Pelon, W., Whitman, B. F., and Beasley, T. W. Reversion of histidine-dependent mutant strains of *Salmonella typhimurium* by Mississippi River water samples. Environ. Science Technol. 11: 619 (1977).
2. Schairer, L. A., et al. Exploratory monitoring of air pollutants for mutagenicity with the *Tradescantia* stamen hair system. Environ. Health Perspect. 27: 51 (1978).
3. Parry, J. M., Tweats, D. J., and Al-Mossawi, M. A. J. Monitoring the marine environment for mutagens. Nature 264: 538 (1976).
4. Barnes, W., and Klekowski, E. J., Jr. Testing the environment for dispersed mutagens: Use of plant bioconcentrators coupled with microbial mutagen assays. Environ. Health Perspect. 27: 61 (1978).
5. Klekowski, E. J., Jr. Detection of mutational damage in fern populations: An *in situ* bioassay for mutagens in aquatic ecosystems. In: Chemical Mutagens: Principles and Methods for Their Detection, Vol. 5, A. Hollaender and F. de Serres, Eds., Plenum Press, New York–London, 1978.
6. Klekowski, E. J., Jr. Genetic lead in *Osmunda regalis* populations. Am. J. Bot. 60: 146 (1973).
7. Klekowski, E. J., Jr. The genetics and reproductive biology of ferns. In: The Experimental Biology of Ferns, A. F. Dyer, Ed., Academic Press, New York, in press.
8. Klekowski, E. J., Jr. Mutational load in a fern population growing in a polluted environment. Am. J. Bot. 63: 1024 (1976).
9. Klekowski, E. J., Jr., and Davis, E. L. Genetic damage to a fern population growing in a polluted environment. Segregation and description of gametophyte mutants. Can. J. Bot. 55: 542 (1977).
10. Bierhorst, D. W. On the stem apex, leaf initiation and early leaf ontogeny in Filicalean ferns. Am. J. Bot. 64: 125 (1977).
11. Klekowski, E. J., Jr., and Berger, B. B. Chromosome mutations in a fern population growing in a polluted environment: a bioassay for mutagens in aquatic environments. Am. J. Bot. 63: 239 (1976).