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Ceratopteris: A Model Plant for the 90s

Ferns don't garner much respect within the plant science community. Of no importance to agriculture, and valued mainly for their ornamental varieties, ferns are often viewed as primitive plants that have little to add to our understanding of higher plant developmental processes. But it may be time for that view to be revised: in the last few years, it has become evident that the fern *Ceratopteris richardii* provides remarkable opportunities for developmental, physiological, genetic, and, with any luck, molecular studies of plant processes as diverse as sex determination, pattern formation, photomorphogenesis, and mechanisms for coping with environmental stresses.

Ceratopteris grows terrestrially throughout the tropics, but it is well known to tropical fish fanciers as a common aquarium plant because it is able to grow submerged in water. Until 15 years ago, when Leslie Hickok, now a professor of botany at the University of Tennessee, noticed a big, ugly fern in a greenhouse at Smith College, Ceratopteris was not especially well studied. Hickok was investigating the then-current idea that ferns—most of which have many chromosomes—are polyploid, and he began to examine whether Ceratopteris had duplicated loci. Hickok's work, and that of other investigators, showed that Ceratopteris, and, for that matter, many other ferns, are not as genetically complex as their chromosome content might imply: Ceratopteris is diploid at many of its loci. The proof of that—the fact that it is possible to isolate Ceratopteris mutants in which a wide variety of processes are altered—has become the basis of Hickok's subsequent work on this fern.

A number of features of the Ceratopteris life cycle make genetic analysis of this fern feasible. Like all ferns, and, indeed, all plants, Ceratopteris alternates between sporophyte and gametophyte generations. The diploid sporophyte, which bears the familiar green fronds, releases haploid spores that germinate to give rise to tiny, free-living gametophytes (see photograph). Ceratopteris gametophytes can be hermaphroditic or male. The hermaphrodite gametophyte is a two-dimensional, heart-shaped sheet of cells, with a meristematic region in the crook of the heart. It differentiates archegonia (which produce the eggs), antheridia (sperm-producing structures), and rhizoids (anchoring and absorbing organs). The smaller male gametophyte lacks a meristem and differentiates antheridia but not archegonia.

If a hermaphrodite fertilizes itself, a homozygous sporophyte will result. Sperm from a male gametophyte can also cross-fertilize the egg of a hermaphrodite, producing a heterozygous sporophyte. The sporophyte can also reproduce vegetatively by means of leaf buds. If these buds are removed and replanted, they will develop into new sporophyte individuals. Ceratopteris has further reproductive tricks that can be turned to the geneticist's advantage: sporophytes can produce diploid gametophytes directly, in a process called apospory, and gametophytes can be induced to develop directly into haploid sporophytes in a process called apogamy.

The existence of a free-living, haploid gametophyte is a boon to fern geneticists because mutations can be assessed directly in the haploid gametophyte, and both recessive and dominant mutations can be identified. Genetic analysis is difficult to do in organisms with long life cycles, however, and many ferns have exceedingly long generation times, in some cases over 2 years. The generation time of Ceratopteris, by contrast, is very short, making genetic analysis simple and rapid. A germinated spore takes only 10 to

Twelve-day-old Ceratopteris gametophytes. The larger ones are hermaphrodites, and the smaller ones are males. Males are approximately 0.5 mm in length.

12 days to develop into a sexually mature gametophyte, and in just 3 months the fertilized egg develops into a sporophyte that is competent to produce spores. Less than 2 weeks after spores are mutagenized (both x-rays and ethylmethanesulfonate have been used as mutagens), M_1 gametophytes can be screened. Because the spore contains just one cell that will give rise to all the cells of the gametophyte, few if any gametophytes will be mosaics. Gametophytes carrying potentially interesting mutations can be selfed and the effect of the mutation in homozygous M_1 sporophytes assessed. Once the sporophytes produce spores, M_2 mutant gametophytes can then be retested.

In addition to reproducing quickly, Ceratopteris—at least the gametophyte—is very small, which makes large-scale

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mutant hunts relatively simple. Ten thousand spores can germinate on a single Petri plate, and it is easy to screen a million gametophytes in a selection screen. Large numbers of sporophytes can be grown as well; although the sporophytes can grow to be quite large, they reach reproductive maturity when they are still small. The ease with which Ceratopteris can be screened places it in an enviable position that few plants can match.

The opportunity to examine the effects of mutations on the free-living Ceratopteris gametophyte is invaluable for studying processes such as gametophyte development, gametogenesis, and fertilization, which are harder to study in the less accessible gametophytes of seed plants. For example, Hickok and his collaborators have isolated a number of mutants in which the pattern of gametophyte growth is altered. Some mutants grow in an indeterminate filamentous form, and another has multiple meristematic regions rather than just one. The meristem of yet another mutant is located centrally, rather than at the margin, and the resulting gametophyte is tube shaped rather than two-dimensional. A different class of mutations affects sperm development: mutant gametophytes produce slow or nonmotile sperm.

A particularly interesting aspect of gametophyte development is sex determination. The sex of gametophytes is determined by pheromones known as antheridiogens, which promote the development of male gametophytes. In the absence of antheridiogens, hermaphrodite development ensues. Antheridiogens of some fern species are known to be similar to gibberellins; the structure of the Ceratopteris antheridiogen had appeared gibberellin-like as well because when Hickok and his colleague Thomas Warne treated gametophytes with agents that block gibberellin biosynthesis, the male phenotype was eliminated. More recently, however, Hisakazu Yamane and his collaborators at the University of Tokyo have obtained preliminary structural evidence suggesting that the Ceratopteris

antheridiogen may be a sesquiterpene, a compound that shares chemical intermediates with gibberellin.

The antheridiogen signal is apparently secreted by the more rapidly growing gametophytes in a population, which develop as hermaphrodites. Once sufficient antheridiogen has accumulated in the culture medium, some of the younger (i.e., more slowly developing) gametophytes develop as males. Jo Ann Banks of Purdue University has found that the window of antheridiogen sensitivity occurs approximately 4 days after the spore germinates and lasts for only a day or so. If a gametophyte is exposed to antheridiogen during this sensitive phase, it may become a male; otherwise, it will become a hermaphrodite.

Although antheridiogen appears to be necessary and sufficient for sex determination, the potential involvement of abscisic acid (ABA) complicates the process. The effect of adding ABA to a population of gametophytes is opposite to that of adding antheridiogen: ABA inhibits male development. Spores contain high concentrations of ABA—on the order of 40 μM, according to work of Warne and Hickok—which drop precipitately in the days following germination. The decline in ABA concentration, therefore, may determine the onset of the antheridiogen-sensitive period. Whether ABA is also involved in the reestablishment of antheridiogen insensitivity is not yet known.

Sensitivity to both antheridiogen and ABA is genetically determined and varies among Ceratopteris strains. As a consequence, the ratio of male to hermaphrodite gametophytes may differ from strain to strain. Genetic screens have yielded additional mutants in which the process of sex determination is altered. For instance, Banks has identified several her (hermaphroditic) mutations; gametophytes hemizygous for one of these mutations always develop as hermaphrodites, even in the presence of antheridiogen. By screening for second-site suppressors of the her phenotype, Banks has obtained putative tra (transformer) mutations that cause all

gametophytes to develop as males. In addition, gametophytes carrying a feminizing (fem) mutation lack antheridia and develop exclusively as females. Gametophytes carrying yet another mutation develop initially as males in the presence of antheridiogen, but within a week they all transform into hermaphrodites. With these sorts of mutations, a pathway of gene activities in sex determination should eventually be constructed, and cloning of the genes may reveal how the pheromone signal is transduced into the gametophyte's response.

Photomorphogenesis, another process central to the development of the Ceratopteris gametophyte, can also be altered by mutation, as Hickok, Todd Cooke of the University of Maryland, and Michizo Sugai of Toyama University are finding. Spores normally require exposure to red light to germinate, but one fascinating mutation causes spores to germinate in the dark. Not only that, but light actually suppresses germination of spores carrying this mutation. A selection for gametophytes that grow as if they are exposed to light even when kept in the dark has turned up mutants that resemble the de-etiolated (det) mutants of Arabidopsis and other flowering plants: wild-type gametophytes grown in the dark are elongated, whereas dark-grown mutants have a normal morphology.

Another set of Ceratopteris mutants includes those with altered sensitivity to environmental stresses. Ferns in general are remarkable in their tolerance to adversity—they are so armed with defensive compounds that they are rarely subject to attack by certain types of pathogens, for example—but they are, nonetheless, sensitive to herbicides and to extreme environmental conditions. In a series of selection screens, Hickok and his collaborators have isolated mutants that are tolerant to normally toxic levels of the herbicides paraquat, glyphosate, and acifluorfen. In the case of the paraquat-tolerant mutant, some evidence suggests that the herbicide fails to gain access to the chloroplast, perhaps

because the mutation causes it to be sequestered in another cellular compartment.

Tolerance to environmental stresses of many sorts, such as high soil salt, is often caused by mutations at multiple loci. Nevertheless, Hickok and his colleagues germinated spores on medium containing high NaCl concentrations (200 mM) and were able to identify single-gene mutations that confer partial NaCl tolerance on gametophytes. By subsequently mutagenizing a strain carrying the salt tolerance mutation *stl1* and reselecting for growth on NaCl, they identified an additional salt tolerance mutation, *stl2*. Double mutant strains have an even more pronounced resistance to salt—that is, they grow better than either single mutant on 100 or 200 mM NaCl (wild-type gametophytes, by contrast, begin to die when subject to only 75 mM NaCl).

Although sporophytes homozygous for both the *stl1* and *stl2* mutations are tolerant to increased levels of NaCl, they are not nearly as salt resistant as gametophytes, perhaps because the vascular system of the sporophyte concentrates the salt. Recently, Hickok has identified a mutation that enhances the ability of *stl2* mutant sporophytes to tolerate even more salt. He selected this mutation by allowing gametophytes derived from mutagenized *stl2* spores to cross-fertilize and then screening for sporophytes that could grow on high concentrations of NaCl. Such selection screens, especially multiple or serial selections, should do an admirable job of identifying genes that contribute to salt tolerance in Ceratopteris—genes that may be useful for the eventual introduction of salt tolerance into crop plants.

With the identification of genes that are involved in processes such as sex determination, photomorphogenesis, paraquat resistance, and salt tolerance, an important next step is to try to understand at a biochemical level how the products—both wild type and mutant—of these genes function. The molecular biology of Ceratopteris is largely unexplored, but the haploid genome is large—on the order of 5 × 10⁹ bp are divided among 39 chromosomes—and gene isolation will be more difficult than it is for plants with small genomes, such as Arabidopsis. Differential cDNA screening might be a way to isolate Ceratopteris genes whose expression is disrupted by mutation. Banks is hopeful that maize transposable elements will turn out to be active in Ceratopteris; if so, genes disrupted by transposons will be easy to clone. There is, as yet, no established method for Ceratopteris transformation. Banks has been able to detect the expression of a GUS gene that she introduced into gametophytes by the biolistic method, however, and she believes it is only a matter of time before transformation becomes routine.

Once interesting Ceratopteris genes have been isolated, it will be important to ask whether seed plants contain similar genes and whether the functions of these genes are conserved. The converse approach—asking whether Ceratopteris has genes that are homologous in sequence and function to other plant genes—is possible as well and may help plant biologists understand how plant developmental systems have evolved. For example, a family of Arabidopsis genes shares sequences with the floral patterning gene *agamous*. Using a DNA probe from the *ag* locus, Banks has found that Ceratopteris contains just one *ag*-related sequence. By studying this gene, she may be able to glean the original function of the ancestral *ag* locus—information that may help explain the roles of the *ag* family members of higher plants.

With its straightforward genetics and the ease of genetic screens and selections (particularly of the haploid gametophyte, but also of the diploid sporophyte), Ceratopteris is poised to become an important model plant system—especially once transformation and gene tagging systems are developed. Not only Ceratopteris but also other seedless plants, such as the moss *Physcomitrella patens*, which is also being used for genetic studies of plant development, will provide information about the evolution of developmental systems and allow plant biologists to study processes that cannot be studied easily in seed plants. If the kinds of experiments that are now being done with Ceratopteris are any guide, these model systems may one day make great contributions to our understanding of a wide range of developmental processes in plants.

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