

Instability in Plants and the Ghost of Lamarck

The repetitive DNA sequences in the plant genome make a major contribution to genetic instability and variability in plants

Early this month plant breeders and molecular biologists gathered at the 11th annual Minnesota Plant Biology Symposium* to consider some recent findings about the plant genome and their possible impact on plant breeding. If it is safe to say that the breeders are often skeptical that molecular biology will have any impact at all on their work, it is also safe to say that the research is producing new insights into the organization and function of the plant genome. At the very least, this should help to account for some previously unexplained variations that have been made by plant breeders. "The important thing," says Edwin Bingham of the University of Wisconsin (Madison), "is that molecular biology has the potential to explain a lot of genetic instabilities we couldn't explain in the past."

One message that came across very clearly at the symposium is that the plant genome is remarkably unstable, capable of undergoing change and generating variability. A large part of this instability may be caused by changes in the repeated DNA sequences that are even more common in plants than in animals. According to Richard Flavell of the Plant Breeding Institute (PBI) in Cambridge, England, more than 75 percent of all DNA sequences that are 50 base pairs or longer is repetitive DNA. "It seems particularly prone to change," he notes. "And there might lie the source of interesting variation."

Repeated sequences are especially prone to undergo loss or gain because they can promote the incorrect pairing of chromosomes during meiosis. Recombination may then result in the loss of repeats from one member of a chromosome pair and their gain by the other. In addition, if there are multiple copies of a gene, one copy may undergo mutations, possibly leading to a product with a new function, while the previous function is maintained by the remaining members of the family. Finally, many of the repeats that are dispersed throughout the genome have the characteristics of trans-

posable elements which, as Barbara McClintock of Cold Spring Harbor Laboratory has established, are important generators of mutations.

Changes in repeated sequences have been linked by Christopher Cullis of the John Innes Institute in Norwich, England, to one of those previously unexplained—not to say unbelievable—genetic instabilities that have been identified by plant breeders. Nearly 30 years ago, Alan Durrant of the University College of Wales in Aberystwyth grew seeds of an inbred flax strain in a number of different concentrations of the nutrients potassium, nitrogen, and phosphorus. Although individual plants of an inbred strain ought to be genetically homogeneous and very similar, if not identical, it was not surprising that the resulting plants looked abnormal. Their development had been influenced by the different nutrient concentrations, all of which were stressful to the plants.

What happened when seeds obtained from these plants were grown, this time under normal conditions, was unexpected, however. The progeny of plants from any one treatment, although similar to each other, differed both from the original parental stock and also from the progeny of plants exposed to the other treatments. One group was large and highly branched, for example, whereas another was small and spindly. Moreover, these characteristics were passed on to their progeny. "In one generation, there was a change that became heritable and did not reverse when conditions were reversed," Cullis says. Because the observation was reminiscent of the Lamarckian concept of evolution—a change acquired in response to an altered environment became hereditary—it did not meet with much acceptance.

More recently, however, Cullis has found that the different stressful conditions induce changes in the repetitive DNA of the plants. A few of the sequences increase in number but most decrease. Overall, there is a 15 percent difference in total DNA content between the two groups with the most different characteristics. The repeated DNA's that are affected include those coding for the RNA's of the ribosomes, the small

particles on which protein synthesis occurs, and other RNA's of unknown function.

The DNA alterations arise during mitosis when the original strains are growing in the stressful environments, Cullis says. This produces mosaic plants. The DNA in the bottom quarter or so of the stem has the original composition, with the altered patterns appearing in the upper regions and the seeds.

How gain or loss of repetitive DNA sequences influences the development of the flax plant is unclear, but there are precedents to suggest that the changes are not limited to flax and can have developmental and evolutionary consequences. For example, a few years ago, Michael Bennett of PBI and Perry Gustafson of the University of Missouri in Columbia found an effect of repeated sequences on the development of the seeds of triticale, a hybrid of wheat and rye. The ends of rye chromosomes usually consist of heterochromatin, dark-staining regions that are composed of repeated sequences. Wheat chromosomes do not have comparable heterochromatic regions on their ends.

In any event, triticale seeds are often abnormal and shriveled. Bennett and Gustafson identified some lines that showed less shriveling. The rye chromosomes of those lines had lost heterochromatin and the extent of the loss correlated with the diminution of shriveling. "You can conclude that blocks of repeated sequences can influence development, especially if you change the genetic background in which they occur," Flavell told the symposium participants.

Another surprising example of plant variability, this time in doubled haploids, may be related to heterochromatin alterations, according to Earl Wernsman of North Carolina State University. Doubled haploid plants are completely homozygous at all gene loci. They may be generated when germ cells, which have only a single (haploid) set of chromosomes, are used to develop plants in which the single set has been doubled. The final chromosome complement consists of identical pairs of chromosomes.

Wernsman finds that when doubled haploid lines of tobacco plants are de-

*The symposium "Implications of Molecular Genetics in Plant Breeding" was held at the St. Paul campus of the University of Minnesota on 4 to 6 June.

rived from anthers, which contain the male germ tissue, they differ markedly from the parental strain in color and structure and are much less vigorous and productive. In the second generation, the doubled haploid plants show further variability and even greater reductions in vigor and productivity. "We established that we are inducing variability somewhere that is not caused by parental heterozygosity," Wernsman asserts.

There are two possible reasons why the male-derived doubled haploids undergo these changes, Wernsman says. They develop not from the male gametes, the equivalents of sperm in animals, but from a haploid vegetative cell that forms the pollen tube and may not develop normally. Also the anther tissue must be grown in culture, which as a number of investigators have noted, can induce variability. For example, Cullis finds that culturing flax cells may induce changes in repetitive DNA similar to those generated by growing whole plants under stressful conditions. Wernsman reports that some of the anther-derived lines of doubled haploids have increased amounts of heterochromatin.

Although most of the symposium was devoted to plant systems, there were also presentations on the effects of repeated sequence changes on fruit fly development which may serve as models for the plant work. According to Kenneth Tartof of the Institute for Cancer Research in Philadelphia, the number of ribosomal genes in the fruit fly *Drosophila melanogaster* can be increased or decreased by genetic methods. A loss of the genes can produce the *bobbed* mutant, which displays a number of abnormalities, including short—*bobbed*—abdominal bristles. This mutant has about half of the approximately 250 ribosomal gene copies of normal flies.

Another fruit fly, *Drosophila mercatorum*, displays the "abnormal abdomen" syndrome, which may be equivalent to the *bobbed* mutation. Alan Templeton of Washington University has shown that the syndrome is caused by a lack of juvenile hormone esterase, the enzyme that normally destroys juvenile hormone at the appropriate time during development. As a result, the concentrations remain high in the flies and they retain a juvenile-type cuticle, which resembles that of *bobbed* mutants.

According to Robert DeSalle, who presented the Templeton group results at the symposium, the abnormal flies have an alteration in their ribosomal genes that may have the same net effect as the reduced gene number in the *bobbed* mutants. In fruit flies with the syndrome,

most of the gene copies of the larger of the two major ribosomal RNA's carry inserted DNA sequences that are not found in the normal flies. The genes with the inserts are preferentially transcribed in the fat body, the tissue that synthesizes juvenile hormone esterase. This apparently results in a lack of good copies of the RNA, a deficit in the ribosomes, and a failure to synthesize the esterase. Presumably, deficiencies in other proteins that are synthesized in the fat body may also contribute to the abnormal abdomen syndrome.

Changes in repetitive sequences have the potential of altering gene expression by less direct routes than by causing a ribosomal deficit. The repeated genes coding for ribosomal RNA's are arranged in long tandem arrays that are separated by spacer sequences that may



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Flax in bloom

themselves contain repeating DNA segments. Flavell and his colleagues have detected in wheat ribosomal spacers a repeated sequence that may help to regulate gene expression.

Other investigators had previously identified a similar structure in promoters or enhancers of gene transcription into RNA. An especially pertinent example has been provided by Ronald Reeder of the Fred Hutchinson Cancer Research Center in Seattle and his colleagues. They found the sequence as a repeated unit in the ribosomal spacer of the frog *Xenopus laevis* and showed that it acts as an enhancer, a regulatory sequence that increases the expression of genes under its influence. Reeder proposes that the enhancers work by binding factors needed for transcription.

The evidence suggests that ribosomal genes with many copies of the enhancer are transcribed in preference to genes with fewer copies, presumably because

the former can outcompete the latter for scarce transcription factors. This implies that a change in number of the repeated enhancer can affect transcription not just of the genes immediately under its control but also of other genes, if the change leads to an increase or decrease in the availability of transcription factors.

It may not be necessary to invoke mutations or changes in gene regulation to explain all examples of plant variability, according to John Dudley of the University of Illinois. Dudley reported on an experiment that was begun in 1896 by the late Cyril Hopkins in which corn has been selected over many generations for either high or low oil production or high or low protein production. The Illinois workers are finding, Dudley says, that "we are in the 85th generation and we have yet to exhaust the genetic variability for oil or protein." The concentrations of both are still increasing or decreasing, depending on which is being selected for, although the decrease in oil content has been leveling off as zero concentration is approached.

According to Dudley, analysis by standard genetic methods shows that both traits are controlled by many genes, 50 in the case of oil content and some 120 for that of protein. The genetic analysis suggests that the selection is simply increasing or decreasing the proportion of favorable genes and that new mutations need not be postulated. "I don't think it is unusual," Dudley concludes. "It is what to expect." In discussion of the corn results, Ronald Phillips of the University of Minnesota suggested that new variability could be released due to the behavior of repeated DNA sequences.

Plants clearly have a high degree of genetic instability and variability, which is grist for the mill of the plant breeders. This instability may reflect the greater need of plants to adapt. Higher animals have greater control over their environments, but plants are always at the mercy of climate and weather.

The surprise may be that plants are not even more unstable. "The combination of repeated sequences and all of the enzymes capable of responding to them are biological dynamite," as Flavell puts it. They may be mechanisms for maintaining the stability of the genome that can be disrupted by what McClintock has called genome shock. Sometimes the shock is provided by the environment. And sometimes it is provided by plant breeders who alter genetic backgrounds by producing hybrids, expose plant cells to culture, or submit plants to stress and other selection pressures.

—JEAN L. MARX