widespread in the Hawaiian flora, but *Myrica faya* appears unique in its ability to establish populations in young volcanic sites (*9*). Similar effects of biological invasions on ecosystem dynamics are likely to prove most common on oceanic islands; their native biota is relatively depauperate, and successful invasions are frequent (*1, 2*). Biological invasions of continents are less common, but they can be equally disruptive (*6, 22*).

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17. The major sources are the lichen *Stereocaulon volcani*, blue-green algae associated with mats of liverworts, decomposing *Metrosideros* leaves, and decaying wood. Fixation of N by these sources was estimated as for *Myrica*.
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Discovery of Transposable Element Activity Among Progeny of Tissue Culture–Derived Maize Plants

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Tissue culture–derived plants of many species have often been observed to possess both genetic and cytogenetic abnormalities. A high frequency of structurally altered chromosomes in maize (*Zea mays* L.) plants regenerated from tissue culture led to the prediction that newly activated transposable elements could be detected in regenerated plants. Testcrosses of 1200 progeny from 301 regenerated maize plants confirmed that ten regenerated plants from two independent embryo cell lines contained an active *Ac* transposable element. No active *Ac* elements were present in the explant sources. Recovery of transposable element activity in regenerated plants indicates that some tissue culture–derived genetic variability may be the result of insertion or excision of transposable elements, or both.

ORIGINALLY, THE TISSUE CULTURE of plants was assumed to be a means of asexually propagating many genetically identical individuals. However, heritable changes are frequently recovered in regenerated plants and their progeny. In maize, more than 45 tissue culture–induced mutant phenotypes have been observed, including defective kernel mutants, dwarfs, albinos, many kinds of leaf striping and variegation, and changes from Texas cytoplasmic male sterility to fertility (*1–5*). Cytological aberrations are commonly observed in regenerated maize plants. Interchanges and deletions, which require chromosome breakage, are the most frequent types of rearrangements (*2–4, 6*). In some cases, phenotypic variation is coincident with cytological aberrations (*7*), but usually the phenotypic variation cannot be explained solely on the basis of detectable changes in chromosome structure or number (*2, 3*). The observation that visible changes in chromosome number or structure can account for only a portion of the variation observed in regenerated maize lines suggests that other mechanisms must be involved. Larkin and Scowcroft (*8*) have proposed a number of alternatives, including the release or activation of transposable genetic elements.

Transposable elements in maize cause a variety of changes in gene expression and chromosome structure. McClintock (*9*) discovered transposable elements in the progeny of self-pollinated plants that had undergone a cycle of chromosome breakage, joining of broken ends, and rebreakage (*10*). Chromosome breakage initiated by special chromosomal stocks, ultraviolet light, and x-rays can cause the release or activation of transposable elements (*11, 12*). Such studies indicated that transposable elements exist in an inactive form within the "normal" maize genome and can be released under unusual circumstances. This prediction was confirmed by DNA analysis that revealed four to eight copies of an *Activator*-like sequence in several maize inbreds lacking the transposable element activity characteristic of the *Activator-Dissociation* (*Ac-Ds*) system (*13*).

McClintock (*14, 15*) ascribed the release or activation of transposable elements by chromosome breakage to the "genomic stress" that a broken chromosome causes within a cell. She speculated that such stresses may lead to a variety of genetic alterations on which natural or artificial selection might

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then be imposed. Burr and Burr (*16*) and McClintock (*15*) have suggested that the tissue culture environment may also be a "genomic stress," thereby causing transposable element activation. The resulting changes may account for at least a portion of the somaclonal variation so frequently observed (*8, 16*). The primary objective of the present study was to determine whether the transposable element *Ac* could be released or activated by the process of tissue culture and plant regeneration, as a first step in determining whether transposable elements are a source of somaclonal variation in maize.

Regenerated plants (R_0 generation) and their progeny (R_1 and R_2 generations) were obtained from cultures initiated from immature embryos of three inbred backgrounds (A188, B73, and Oh43) (*17*). The parental genotypes and regenerated lines had already been genetically and cytologically characterized. Plants were regenerated 4 to 22 months after culture initiation, and some plants were tested directly for *Ac* element activity by crossing them as males to the *Ds* tester (Fig. 1). Most plants were self-pollinated or crossed with the inbred A188, and, subsequently, a sample of about five R_1 progeny was tested for *Ac* activity. In a few cases R_2 plants were tested, but in all cases the plants tested could be traced back to a specific regenerated plant and source embryo.

One half of each source ear was used for embryo isolation and culture initiation; the other half was left on the plant to mature. These ears provided kernels that could later be used as noncultured controls to determine whether transposable elements had been present in the donor plant before culturing. If a regenerated plant gave evidence of transposable element activity, the corresponding control kernels were planted and tested the following year. The inbred lines B73, A188, and Oh43 were also included in the tests for transposable element activity.

The presence of an active *Ac* element was tested directly in crosses to two tester stocks containing the corresponding nonautonomous element *Ds*, which transposes only in the presence of *Ac*. These stocks also contained genes required for the pigmentation of the aleurone, which is the outer layer of endosperm. This triploid tissue receives two doses of each chromosome from the female parent and one from the male, and is ideal for observing transposable element effects because color changes within it do not markedly affect kernel viability. Each testcross produces up to several hundred kernels that can be readily scored. The first series of tests used a stock containing *C*, a dominant gene necessary for aleurone color, and a *Ds* element about 33 map units proximal to *C* (Fig. 1). *C* and *Ds* are located on chromosome 9 in the tester stock, originally provided by McClintock. The *Ds* in this material is the "double-*Ds*" type, which can cause chromosome breakage in the presence of *Ac* and, therefore, loss of the genes distal to *Ds* (*18*). All other genes necessary for aleurone color (*C2, A, A2*, and *R*) were present in the tester in a homozygous dominant condition.

Regenerated plants (301 total) or their R_1 and R_2 progeny lines were tested for *Ac* activity by crossing them as males to the tester stock. Among approximately 1200 testcross ears, 56 ears segregated for variegated kernels (Fig. 1). The 56 ears could be traced back to 11 R_0 plants from three embryo cell lines designated (*3*) 4-41, 1-42, and 8-27.

We planted some variegated kernels from these testcrosses for confirmation of *Ac* activity, using a second tester stock homozy-

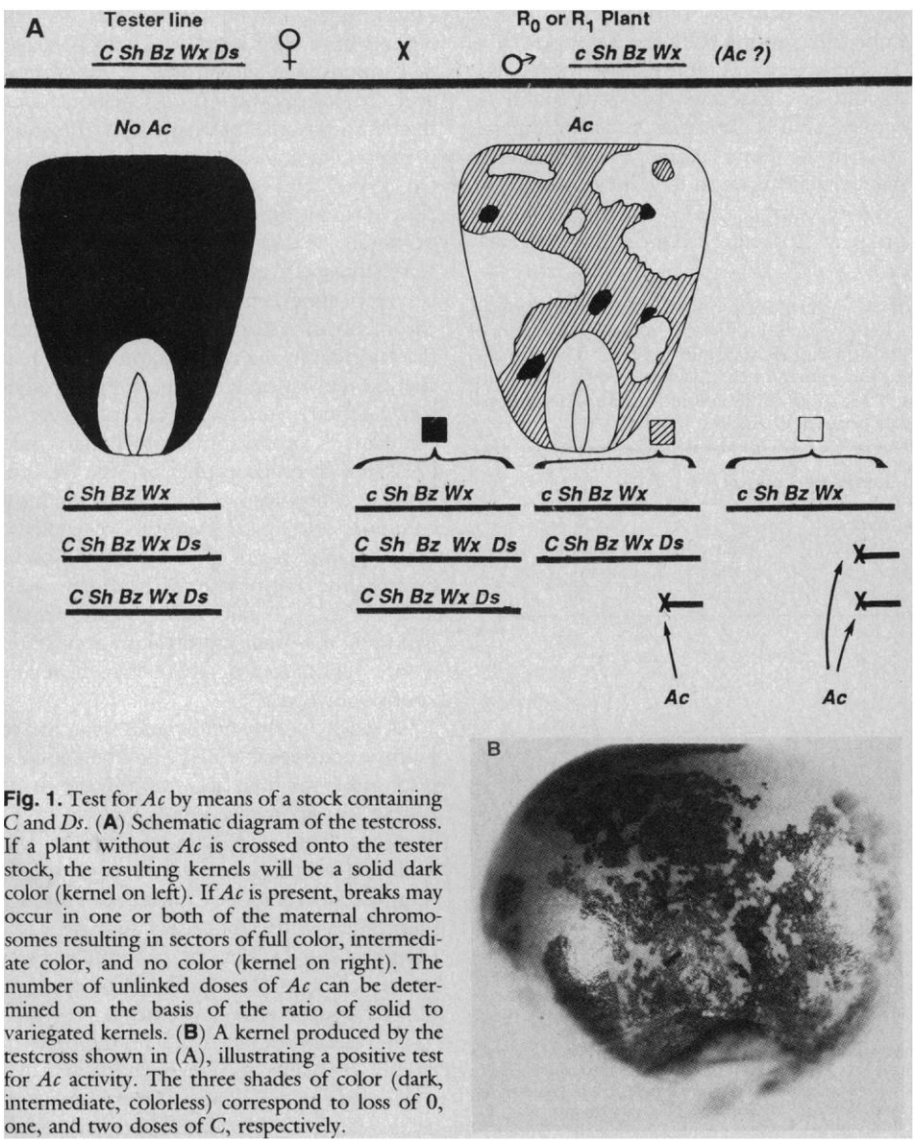

Fig. 1. Test for *Ac* by means of a stock containing *C* and *Ds*. (**A**) Schematic diagram of the testcross. If a plant without *Ac* is crossed onto the tester stock, the resulting kernels will be a solid dark color (kernel on left). If *Ac* is present, breaks may occur in one or both of the maternal chromosomes resulting in sectors of full color, intermediate color, and no color (kernel on right). The number of unlinked doses of *Ac* can be determined on the basis of the ratio of solid to variegated kernels. (**B**) A kernel produced by the testcross shown in (A), illustrating a positive test for *Ac* activity. The three shades of color (dark, intermediate, colorless) correspond to loss of 0, one, and two doses of *C*, respectively.

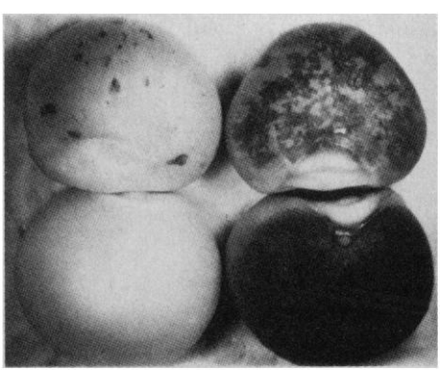

Fig. 2. Four kernels from an ear produced by a testcross of a plant grown from a variegated kernel such as the one in Fig. 1B (presumed to be a result of one dose of *Ac*) to the *c-m2* tester. These kernels illustrate the four phenotypes expected from such a cross if an active *Ac* element is present. In the absence of *Ac* activity, only fully colorless and fully colored kernels should be produced. The ratio of the four kernel types on this particular ear indicated that one dose of *Ac* was present (Table 1).

gous for the mutable allele *c-m2*. This stock has a different *Ds* element inserted into the *C* locus. In this case, *Ac* activity causes transposition of *Ds* from the locus (without chromosome breakage) and is recognized by small colored sectors of aleurone (Fig. 2). Eight of the nine *c-m2* tests of cell line 4-41 confirmed that the variegated kernels did contain *Ac* activity (Table 1). In six of these tests, *Ac* segregated as expected if it was unlinked to the *C* locus. In the other three tests, the frequency of variegated kernels was lower than expected, and in the testcross with the fewest progeny, no variegated kernels were observed. These data may indicate a low frequency of transmission of *Ac* through the pollen parent, possibly as a result of linkage of the *Ac* to a chromosomal abnormality, which is not unexpected in tissue culture–derived materials. Two out of three tests of cell line 1-42 indicated *Ac* activity closely linked to the *C* locus. The third testcross of this set was deficient for variegated kernels, which indicates that linkage was not involved but *Ac* transmission was reduced. Testcrosses of plants from line 8-27 have also consistently produced only 5 to 10% variegated kernels, which may indicate that an *Ac* element is present but does not transmit well through pollen.

Ac activity has not been found in noncultured control kernels for any of the three cell lines now under investigation (30 testcrosses), remnant seed from the parental rows (22 testcrosses), or any of the inbred lines involved (49 testcrosses). Additional evidence for the absence of *Ac* before culture initiation is that each of the cell lines demonstrating *Ac* activity was heterogeneous; an average of 30% of the regenerated plants from the positive cell lines had *Ac* activity while the remaining 70% did not (Table 2). If an active *Ac* had been present in the embryos before the tissue cultures were initiated (for example, from stray pollen) most, if not all, of the plants from those cell lines would have expressed *Ac*.

Any given regenerated plant was typically represented by four to eight progeny testcrosses. For 9 out of the 11 regenerated plants initially scored as containing *Ac* activity, two or more positive testcrosses were produced, making pollen contamination during the testcrossing an unlikely explanation for the results obtained.

Nine regenerated plants from a single cell line (4-41) had *Ac* activity, indicating that activation occurred during tissue culture rather than during plant regeneration (Table 2). The one *Ac*-containing plant from line 1-42 did not appear to be sectored for *Ac* activity, so it is likely that *Ac* activation took place in the tissue culture some time before plant regeneration in this line as well.

Close linkage of *Ac* to the *c* allele in some progeny of plant 2 from cell line 1-42 may indicate that the *Ac* had its origin in chromosome 9; McClintock (*19*) and Bianchi *et al.* (*12*) were able to activate *Ac* by inducing breakage of this chromosome. *Ac* might have originated elsewhere in the genome and transposed to this position. However, studies of the elements *Ac* and *En* show that they usually transpose relatively short distances (*20*).

Transposable element activation in regenerated maize plants has been observed by Evola *et al.* (*21*), who reported activation of both *Ac* and *Spm*. Groose and Bingham (*22*) have observed an unstable flower color allele in tissue culture–derived alfalfa (*Medicago sativa* L.), which behaves as though controlled by a transposable element.

One mechanism that may be responsible for chromosome breaks in tissue culture and thus possibly leads to transposable element activation involves the late-replicating nature of heterochromatin. Late-replicating heterochromatin has been observed in maize (*23*), oats (*24*), and many other species (*25*). Furthermore, late-replicating chromosome regions have been associated with breakage of chromosomes in cultures of *Crepis capillaris* (*26*). McCoy *et al.* (*27*) demonstrated that chromosome breakage was the most common cytological change in regenerated oat plants; these breakage events occurred close to the centromere, a region known to be highly heterochromatic (*24*). These researchers speculated that there might be late-replicating pericentromeric heterochromatin in oats that replicates even later than normal in the tissue culture cells. This delayed replication would hold the chromosomes together at anaphase, centromere to centromere, producing a stress that could break the chromosomes at or near the centromere. Chromosome breakage is the most common cytological event in regenerated maize plants; many of these breaks occur between the centromere and heterochromatic knobs (*2, 3*). This chromosome region would be the predicted breakpoint position if late replication of the heterochromatic knobs is involved.

Changes in the methylation pattern of inactive transposable elements may also be responsible for their activation in the tissue culture environment. DNA analysis of *Ac* elements that cycle between activity and inactivity indicates that, when active, the *Ac* is unmethylated at one or more sites that are methylated when the *Ac* is inactive (*28*). Chandler and Walbot (*29*) reported a correlation between DNA modification and loss of activity of maize *Mu* elements.

The variability produced by the in vitro culture of plants has been of interest and concern to plant scientists for a number of years. Studies of maize and many other

Table 1. Results of crosses of selected progeny plants from cell lines 4-41, 1-42, and 8-27 to *c-m2* tester. Each line represents one testcross for the plant listed. The probabilities shown are based on χ^2 tests of the goodness-of-fit of these data to a model in which one dose of *Ac* was unlinked to *C*. The expected frequencies from cross *c-m2/c-m2* × *CDs/c Ac/+* were, for colored with colorless sectors, 0.165; for colorless with colored sectors, 0.25; for fully colorless, 0.25; and for fully colored, 0.335. The expected ratio is different from 1:1:1:1 because of crossing over between *C* and *Ds*.

Cell line and R_0 plant	Progeny plant tested	Kernel phenotypes				*P**
		Colored with colorless sectors	Colorless with colored sectors	Fully colorless	Fully colored	
4-41 plant (11)	A	0	0	6	6	*
(18)	A	2	7	5	10	N.S.
(27)	A	5	6	32	21	**
	B	7	8	7	14	N.S.
	C	3	3	5	3	N.S.
	D	21	42	40	49	N.S.
(33)	A	1	1	22	35	**
(41)	A	22	26	39	33	N.S.
	B	7	9	10	14	N.S.
1-42 plant (2)	37-9†	3	170	6	182	**
	37-9†	2	53	11	51	**
	37-4‡	0	5	81	67	**
8-27 plant (2)	69-2‡	1	1	22	30	**
	69-1‡	4	2	142	140	**

*,**Significant at $P < 0.05$ and $P < 0.01$, respectively, N.S. indicates that the χ^2 value was not significant at $P = 0.05$. †Ratio of kernel types from these plants indicates linkage to *C*. The majority of kernels carrying *Ac* have a colorless background indicating that they are recessive for *C*. Likewise, most colored kernels (dominant for *C*) do not carry *Ac*. ‡The three plants represented in these crosses all came from ears on which only 5 to 10% of the kernels were variegated. However, additional tests of progeny of plant 37-4 showed the presence of normally segregating *Ac* activity (*30*). This indicates that the *Ac* may have been linked to a chromosomal aberration causing the pollen carrying it to be less competitive than normal, and that this linkage was eventually broken. A similar event may explain the results observed for plants 69-1 and 69-2.

Table 2. Frequency of *Ac*-containing regenerated (R_0) plants among total R_0 plants from *Ac*-containing embryo cell lines 4-41 and 1-42.

Embryo cell line	Number with *Ac* activity/total R_0 plants tested	Percent
4-41	9/26	34.6
1-42	1/7	14.3

species have demonstrated the occurrence of both chromosomal abnormalities and heritable phenotypic changes. This study has revealed a third type of change, the activation of transposable elements. Researchers who have observed both chromosomal alterations and phenotypic mutants in their materials have generally been unable to directly correlate these occurrences. Activation of transposable elements by chromosome breakage may provide a link between the chromosome aberrations and mutations. A transposable element, once released, could insert into a gene locus rendering it inactive or otherwise altered. Elucidation of the mechanisms governing somaclonal variation may enable researchers to better control the amount, and perhaps the type, of variability produced by the in vitro culture of plants.

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(continued from page 730)

bundles similar to those that occur naturally. A new computational approach to protein folding was described by F. Richards (Yale), who determined all possible amino acid sequences compatible with a given protein backbone fold and found relatively few sequences that can fit a given backbone conformation.

Another difficult question is how amino acid replacements in a given protein change the structure over evolutionary time. This question was addressed by C. Chothia and A. Lesk (Cambridge, United Kingdom) by comparing structures of families of proteins such as the globins and the immunoglobulins. They find that α helices change their directions by a few degrees and that β sheets experience local changes in conformation, with atoms moving up to a few angstroms. However, the structural changes in different regions of each protein tend to be coupled to maintain the geometry at the binding sites. These examples of how protein structures can adapt to amino acid replacements give some insight into the capacity of proteins for evolutionary change.

A striking example of convergent evolution of lysozymes was presented by C.-B. Stewart (University of California, Berkeley). Sequence data were used to suggest that the bacteriolytic lysozyme c was recruited twice during evolution as a digestive enzyme in the stomachs of foregut-fermenting mammals, occurring once in the cow and once in the tree-dwelling, leaf-eating langur monkey. Analysis of evolutionary trees suggests that after foregut fermentation arose in monkeys, the langur stomach lysozyme gained sequence similarity to the cow stomach lysozyme, permitting it to adapt to the acidic environment of the stomach fluid.

Another session dealt with the construction of evolutionary trees based on sequence information. G. Olsen (Indiana) groups thermophilic, methanogenic, and halophilic archaebacteria together—to the exclusion of eukaryotes and eubacteria. On the basis of unequal rates of sequence change, J. Lake (UCLA) suggests that the eukaryotic RNA genes evolved from those of sulfur-metabolizing prokaryotes.

The correlation between protein domains and exons in genes of contemporary organisms was emphasized by W. Gilbert (Harvard) in hypothesizing that genes evolved in an RNA world as RNA's containing single exons that encoded short functional polypeptide domains. Genes then evolved by recombination into DNA. This scenario implies that introns are ancestral remnants of primordial genes and that the general absence of introns in the genes of prokaryotes reflects their loss. This is contested by others (T. Cavalier-Smith, Kings College, London), who propose that introns were inserted into prokaryotic-type genes. At the heart of this problem is the question of the evolutionary lineage relations between eukaryotes and prokaryotes (F. W. Doolittle, Dalhousie).

The many deep mysteries concerning evolution of catalytic function include how enzymes arose, how amino acid replacements affect structure and function, and how present-day catalysts are descended from more primitive ones. However, the recent discovery of RNA catalysis has provided at least one of the missing links and it gives hope that we are on the path to solving some of the questions obscured by the 3.5 billion years of birth, change, and death since the emergence of cells.