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TRANSPOSABLE ELEMENTS AND GENETIC INSTABILITIES
IN CROP PLANTS

(controlling elements, recurrent mutations, hybrid dysgenesis, somatic mutations, tissue culture)

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SUMMARY

Transposable elements have long been associated with certain unstable loci in maize and have been intensively studied by McClintock and others. It is known that a transposable element can control the expression of the structural genes at the locus where it resides. These controlling elements in maize are now beginning to be studied at the molecular level. Using recombinant molecular probes we have been able to describe the changes induced by the controlling element *Ds* at the shrunken locus. *Ds* elements appear to be large and dissimilar insertions into the wild-type locus -- two elements actually map within the transcribed region of the gene.

Genetic instabilities have been described in other economically important plants but the bases for these phenomena have not been understood. We believe that it is likely that some of these instabilities are the result of transposable element activity much as in the case of maize.

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In this paper we would like to briefly describe our work with the maize controlling element *Ds* (Dissociation) and then turn to a more general discussion of genetic instabilities that have been observed in other crop plants. We have particularly selected instances which suggest strong analogies to transposable element phenomena in eukaryotes. There are three types of genetic instabilities that are especially intriguing: (1) The first concerns recurrent variants that continue to appear in established inbred lines, often of long standing. Such behavior could be expected, for example, if the excision of a transposable element from a selected mutant locus occurred leading to a restoration of "wild-type" activity. (2) Crosses between teosinte and maize and crosses between *Nicotiana* species frequently result in a high rate of spontaneous and often unstable mutations. These effects show distinct parallels to

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what has been termed "hybrid dysgenesis" in *Drosophila* which is now known to be associated with transposable elements. (3) The process of plant tissue culture has been used to produce a wide gamut of variants. It may be that plants regenerated from culture have been subjected to a "shock" to the genome sufficient to cause controlling elements to spontaneously transpose and might account for some of these variants.

THE MAIZE CONTROLLING ELEMENTS

Emerson's account of variegated pericarp that appeared in 1914 comprises the first published account of an unstable locus in maize (EMERSON 1914). It was, however, McCLINTOCK (1950) who showed (for the first time in any organism) that the instabilities were caused by transposable units that were associated with the loci in question. A lucid and well-illustrated summary of the two best-studied maize transposable element systems, *Ac-Ds* (*Activator-Dissociation*) and *Spm* (*Suppressor-mutator*), appears in McCLINTOCK's 1965 paper. Transposable elements have subsequently been discovered and described in bacteria, yeast, and *Drosophila*; DNA sequences with similar properties have also been reported in a number of animals. At the present time a great deal more is known about the molecular structure of the transposable elements in organisms other than maize. Nevertheless, the maize elements retain their attractiveness and importance because of the intensive genetic studies that have finely dissected their behavior and their ability to control the activities of structural genes.

1. THE MOLECULAR DESCRIPTION OF *Ds*

We elected to begin our studies of the maize controlling elements by examining *Ds*-induced mutations at the *Shrunken* (*Sh*) locus. This locus encodes the enzyme sucrose synthetase (CHOUREY and NELSON 1976) and conditions a collapsed crown of the kernel in the recessive condition. Several *Ds*-induced mutations were selected at this locus by McCLINTOCK (1952, 1953). In the presence of a *trans*-acting regulatory component, *Ac*, *Ds* elements break chromosomes at the site of their insertion, cause adjacent deletions, and give rise to infrequent germinal *Sh* revertants. If *Ac* is removed from the genome, the *Ds* mutations behave as stable alleles.

We initially constructed and identified a cDNA clone from a fraction enriched for sucrose synthetase mRNA. The probe was used to show that the gene was unique and that structural changes in the locus were associated with *Ds* intervention (BURR and BURR 1981). In unpublished work we have since cloned the genomic sequence for sucrose synthetase as a 19 kilobase *Bam*HI restriction fragment from the McClintock standard wild-type DNA. The transcribed region and the direction of transcribed region and the direction of transcription in the cloned insert were determined. Examination of heteroduplexes of the genomic clone with sucrose synthetase mRNA reveal that there are no large intervening sequences although several small ones may exist. Subclones of the genomic clone were used to analyze

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four *Ds* -induced mutations. Two *Ds*'s are insertions of 20 kilobases that map within the transcribed region of the gene; two others map at the 5' end of the gene (Fig. 1). There appear to be no other structural rearrangements of the locus associated with these mutations other than the very large insertions. All of the *Ds*'s examined show a *Bam*HI site close to each end which may be indicative of short terminal repeats. Aside from this, however, the four *Ds*'s analyzed show profound

FIGURE 1. Structure of a wild-type *Shrunken* locus of maize. The heavy bar delimits the transcribed region; transcription is from right to left. The arrows indicate the sites of insertion of *Ds* elements.

differences in their restriction maps. This was unexpected as these *Ds*'s share a common genetic origin and have been separated from one another by only a few generations. Comparison of one pair of closely related *Ds*'s suggests that the differences might be due to extensive internal rearrangements. Non-reciprocal exchanges with other silent copies residing in the genome, however, cannot be ruled out. We believe that the rearrangements occur during transposition or "change-of-state" and are mediated by *Ac*.

2. GENETIC PROPERTIES OF THE MAIZE CONTROLLING ELEMENTS

The maize genetic elements exhibit two major functions: transposition and control of gene activity. We have been able to show that *Ds* elements, like other transposable elements, are discrete sequences that insert into a locus. In so doing they apparently inhibit or modulate gene activity. The maize elements clearly transpose by a different mechanism than that which has been envisaged thus far for prokaryotic transposons. *Mu* and *Tn3* are probably the best characterized prokaryotic elements. Although the actual mechanism of their transposition is still under debate (SHAPIRO 1979; HERSHEY and BUKHARI 1981),

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both apparently transpose by replication. When transposition occurs between two replicons they fuse to form an intermediate cointegrated structure. After "resolution" is completed, copies of the element are found at both the new and the old positions.

McCLINTOCK (1951) followed *Ds* genetically and cytologically and showed that when it moved from one location to another no genetically active material could be detected at the original site. In maize, therefore, unlike the prokaryotic examples just considered, transposition appears to be accompanied by excision of the element. McCLINTOCK (1951) also described two abnormal transposition events that resulted in a duplication of the chromosomal sequences between the original *Ds* site and the new site of insertion. From this she concluded that transposition normally occurs during chromosomal replication. Furthermore, the structures described by McCLINTOCK (1951) also require breakage of both chromosomal strands on either side of *Ds*. Studies on variegated pericarp involving *Ac* at the *P* locus have supported the idea that excision accompanies transposition and that transposition takes place during replication of the chromosomes (GREENBLATT and BRINK 1962).

McCLINTOCK (1956) coined the term "controlling elements" for the maize transposable entities because of their ability to both positively and negatively modulate gene activity. Perhaps the most remarkable illustration of this feature is provided by an allele of *Bronze* (*Bz*) under *Ds* control (DOONER 1981). In *bz-m4* mutant plants (grown in the absence of *Ac*) the kernels exhibit much higher than normal levels of the *Bz* enzyme UFGT (uridine flavonoid glucosyl transferase) early in development but lower than normal levels later in development. The enzyme is involved with anthocyanin biosynthesis which in the kernel is located in the outermost layer of the endosperm, the aleurone, and pigment production ensues appearance of the enzyme. In the case of *bz-m4*, however, coloration of the kernels does not accompany the enzyme synthesis even though enzyme activity was greater in *bz-m4* at a time when normal kernels begin to show color. Dooner found that in the mutant the enzyme was being expressed not in the aleurone, as it normally is, but in the internal cells of the endosperm. Association of *Ds* at the *Bz* locus had thus changed both the time and the tissue in which the enzyme was being made. Furthermore *Bz* is normally not expressed when the plant is homozygous for mutant alleles at either the *C* or *R* loci. In the case of *bz-m4* this epistatic control no longer exists. McCLINTOCK (1961) has pointed out how the regulation of structural gene activity by controlling elements can lead to new developmental patterns of gene expression. The maize controlling elements that have been so intensively studied as genetic entities currently provide the best examples of gene control by transposable elements. This aspect is only just beginning to receive attention in other organisms (ROEDER et al. 1980; BINGHAM 1981, and unpublished data).

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RECURRENT OFF-TYPES IN INBRED STRAINS

JENSEN (1965) and PEARSON (1969) have reviewed a problem that has long perplexed plant breeders -- namely the repeated appearance in long established lines of particular off-types or "rogues". These have sometimes been ascribed to seed contamination even though the accompanying unaffected characters resemble those of the parental lines. Moreover as the biological basis for this phenomenon has not been understood, it is suspected that many instances have gone unreported. In asexually reproducing plants, or in plants with several inflorescences, the "mutational" character is often detected as a change in morphology during development. These events occur at high frequency, usually at the rate of 1×10^{-3} and cannot be eliminated from a population even by assiduous roguing. Strain variation is often a factor in the frequency with which such rogues appear.

Some of these cases clearly have a chromosomal rather than a mutational basis. RILEY and KIMBER (1961), for example, showed that nondisjunction leading to aneuploidy was commonly found in wheat lines and resulting off-types were often monosomics. Other rogues were noted to appear following environmental stress. An example of this is the loss of apical dominance which shows up in certain European varieties of tomato in response to high temperatures during germination (LEWIS 1953). Although the trait is not heritable, the predisposition to apical dominance loss may be a recessive character.

In commercial sorghum lines in the United States, short stature has been selected to facilitate mechanical harvesting. The dwarf nature of the plant is conditioned by recessive alleles at four major loci. In plantings of some varieties of sorghum, tall rogue plants are observed repeatedly (QUINBY and SCHERTZ 1970). These rogues have been found to be caused by the mutation of the unstable recessive *dw3* allele to a dominant form. Another dwarf gene, either *dw2* or *dw3*, is unstable in certain sorghum lines (SCHERTZ, personal communication). The very high rate at which spontaneous mutants for resistance to the toxin of the root rot organism *Periconia circinata* appear in many varieties of sorghum (SCHERTZ and TAI 1969) may indicate another unstable locus in the species.

Snap bean varieties exhibit several common types of deleterious off-types that affect quality. The "flat pod" rogue occurs at a frequency of 0.1 to 0.2% in some varieties and constitutes a serious problem for seed producers. "Flat pod" is a simple dominant allele with F_1 plants showing an intermediate phenotype (ATKIN and ROBINSON 1972). Because snap beans are naturally self-pollinated, the rogue plants are typically homozygous but the factors behave as simple mendelian traits and segregate 1:2:1 in an F_2 generation. KERR (1971) observed mutations of the rogue-type back to round-type but at a much lower frequency than mutations to rogue. The "stringy pod" rogue (ATKIN 1972) and another rogue with mosaic-like crinkled leaf variegation in the Stringless Refugee variety (COYNE 1969) are less commonly encountered off-types. Both behave as simple

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recessive alleles. The frequency of the mosaic rogues is suppressed by temperatures above 80° F or when reciprocal crosses of the rogue in the Stringless Refugee background are made with the Bush Blue Lake variety.

One of the best known recurrent off-types is the "rabbit ears" rogue of garden peas that produces plants with pointed leaflets, upward-curving pods with fewer seeds, and narrow stipules (BATESON and PELLEW 1920). Self-fertilized rogue plants produce only rogues and all progeny of reciprocal crosses of rogue to "crop-type" are rogues. Selves of these progeny continue to maintain the rogue phenotype. Progeny of backcrosses, however, begin development with an intermediate phenotype but progressively change to "rabbit ears" in successively higher nodes. In most cases the change to rogue character is completed before formation of the floral nodes but in occasional plants flowers from the basipetal inflorescences transmit both the rogue and crop-type traits. In the same flower transmission of the crop-type phenotype is more frequently passed through the female gametes than by the pollen.

In 1923 BROTHERTON crossed "rabbit ears" rogues in the variety "Gradus" by the "English Mummy" variety, a line which never produces rogues. From these crosses and subsequent F₂ generations it became evident that the rogue plants were distinguished from crop-type plants by a single dominant mendelian factor. Even in heterozygotes with "English Mummy" there was some indication of a conversion of the recessive allele to the rogue character but at a much lower rate than observed in "Gradus". The tendency of the second allele to be converted following mutation of the initial allele is a common feature of some recurrent off-types in other species.

MANGELSDORF (1974) listed a number of reports of recurrent mutations that had been noted in established inbred strains of maize. As Mangelsdorf had studied mutations that arose as a result of corn-teosinte hybrids, he ascribed these mutations to introgressed teosinte germplasm residues remaining in the maize lines. SINGLETON (1943) studied a reduced stature variant identified in P39, a well-established sweet corn inbred. Although he did not stipulate that it was a recurrent mutation in P39, our own observations suggest that this is likely to be the case. The variant appeared to behave as a simple recessive trait but it showed an unexpected heterotic effect compared with the parental line when outcrossed to two other lines. SINGLETON (1914) also reported that when new inbred lines were "extracted" from crosses of reduced plants back to P39, a number of novel mutations were found -- all showed mendelian inheritance. Similar variants with simple inheritance were also examined by JONES (1945) in other inbred lines. It is unclear whether any of these were recurrent mutations, but at least one inbred, CI Kr 187-2, had a high propensity for producing variants. Jones found that all six variants he studied exhibited a heterotic effect when backcrossed to the parental line. Later SCHULER (1954) re-examined the question of heterosis resulting from single gene differences and concluded that in all cases the variants were likely to have also differed

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from their parents at other loci.

In the majority of cases we have considered, the recurrent mutation affects a character selected as an agronomically desirable trait and the mutation often restores the wild-type phenotype. The rogues are therefore behaving as genetic revertants of selected mutants. The spontaneous reversion of mutant alleles is not unexpected if transposable elements had inserted at these loci and were the original mutagenic agent. As an example of this the standard colorless allele at the A locus of maize was used as a stable recessive for years before RHOADES (1938) discovered that it reverted to full color in the presence of the *Dt* controlling element. In an analogous situation BINGHAM and JUDD (1981) have found that the *white-apricot* eye color mutant of *Drosophila*, discovered by Heustis in 1923, is the result of the insertion of a *copia* transposable element into the *White* locus. Insertional mutations may revert to wild-type if the element transposes away and leaves the locus intact. At least in maize, transpositions may be under the control of other *trans*-acting elements in the genome. Their presence in some stocks and not in others may explain why mutant alleles show marked instability in some strains while maintaining stability in others.

The recurrence of recessive mutations can also be explained by the action of transposable elements. An example is provided by the work of LIEBMAN et al. (1981) who investigated a mutator strain of yeast that frequently lost three linked loci. It was found that these loci were flanked by Tyl elements that repeatedly caused deletions of the intervening genes but not the flanking sequences. These deletions were associated with rearrangements of the transposable elements themselves. The genes that were deleted could be transposed to another chromosomal location (STYLES et al. 1981). It is now known that maize transposable elements can also cause deletions of adjacent loci (DOONER 1981; BURR and BURR 1981). Certain interconvertible states can be controlled by transposable element inversion as occurs in the alteration of expression of *Salmonella* flagellar antigens (ZIEG et al. 1977) or host range of bacteriophage Mu (KAMP et al. 1978).

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In an earlier paper in these Symposia series McCLINTOCK (1978) pointed out that wide species crosses were among the stresses that might trigger reorganization of parental genomes. Demonstration of this comes from extensive work on interspecific hybridization in the genus *Nicotiana*. SMITH (1968) has reviewed the literature on this subject and only a few aspects will be mentioned here. One of the most remarkable consequences of interspecific crosses between different sections of the genus *Nicotiana* is the formation of genetic tumors on aerial parts of the plant. Tissues excised from these tumors and grown in tissue culture are able to synthesize their own phytohormones in contrast to comparable cultures of normal tissues which require hormonal supplementation. (Unlike the

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crown gall tumors induced by *Agrobacterium tumefaciens*, these genetic tumors do not appear to have any bacterial, viral, or plasmid agent associated with the transformed tissues.) Tumors develop in all cases when reciprocal crosses are made. AHUJA (1968) discovered that by repeatedly backcrossing a hybrid of *N. longiflora* x *N. debneyi-tabacum* to the *N. debneyi-tabacum* parent tumor formation could be shown to be correlated with a specific fragment of a single *longiflora* chromosome. In backcrosses to the other parent he found that tumor formation was lost when only a few *debneyi-tabacum* chromosomes were left in a *longiflorum* background. Ahuja suggested that *N. longiflorum* and other members of the *Alatae* section of the genus possessed a factor, I, that caused tumor formation when put into a favorable genetic background. Such backgrounds that would allow expression, he suggested, exist in species in other sections of the genus. SMITH (personal communication), however, has found that a single *N. glauca* (Section *Paniculate*) chromosome in an otherwise *N. langsdorffii* (Section *Alatae*) background causes tumor formation. This finding is contrary to what had been predicted by Ahuja. The basic idea is nonetheless interesting because it anticipates the mechanism of hybrid dysgenesis occurring in *Drosophila* (KIDWELL et al. 1977; PICARD 1979).

SMITH (1968) has cited many other manifestations of instabilities arising in interspecific hybrids in the genus *Nicotiana*. These include variability in the pigmentation and shape of flowers and the lack of uniformity in the growth and habit of F₁ plants. Mutable alleles at two loci controlling flower color have been described (SMITH and SAND 1957; SAND 1976). Three alleles at one locus appeared to reversibly change state but did not mutate to either a stable recessive to a stable full color condition. In the presence of a regulatory element at the second locus, however, the timing of somatic sector formation is modified and germinal mutations to full color can be found. For unknown reasons the unlinked regulatory element could not be recovered from the revertants.

In hybrids of *N. tabacum* x *N. otophora* and subsequent backcrosses to *N. tabacum*, the dominant allele from *N. otophora* specifying carmine flower color becomes unstable and can mutate to coral (BURNS and GERSTEL 1967). Somatic sectoring and loss of carmine color was associated with the partial or complete loss of a heterochromatic block by chromosome breakage. In addition to chromosome breakage large megachromosomes are occasionally observed in these hybrids (GERSTEL and BURNS 1976).

Teosinte (*Zea mexicana*) is the closest relative of maize. In studying possible evolutionary schemes for maize MANGELSDORF (1958) and his colleagues found that maize-teosinte hybrids and their backcrosses to maize were mutagenic with over 2% of the F₁ progeny and later generations exhibiting mutations. Even in plants containing a single teosinte chromosome, mutations were induced on both the maize and teosinte homologues as well as on other maize chromosomes. Both stable and unstable mutations were found that affected the endosperm as well as other plant characters and all were recessive, simply

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inherited traits. BIANCHI (1958) had found that a number of mutations occurred repeatedly from different teosinte crosses prompting Mangelsdorf to express the opinion that a limited number of loci might be targets.

The induction of unstable mutations in interspecific crosses of *Nicotiana* and maize-teosinte hybrids resembles certain aspects of the hybrid dysgenesis syndrome in *Drosophila* (KIDWELL et al. 1977). This phenomenon has recently received considerable attention. The principal consequence of the syndrome is that when crosses of laboratory strains of *Drosophila* are crossed to flies captured in the wild, female infertility frequently results in the F₁ generation. Other aberrations include male recombination, chromosomal abnormalities, and a high rate of mutagenesis. The mutations generated are often unstable. Such phenomena were observed even if only a portion of a wild strain chromosome in an otherwise laboratory strain genome was used in a cross (ENGELS 1981). The direction in which the cross is performed determines whether or not the dysgenic symptoms are expressed -- they are only observed when wild flies are used as the male parents and laboratory strains as the female parents. Genetic studies have shown that the chromosomes of the wild strains carry two independent families of factors called I and P. When I and P males are crossed to laboratory females lacking these elements, and designated R or M, hybrid dysgenesis results. BINGHAM (personal communication) and his colleagues have recently found that P factors are discrete insertions of 300 to 3000 nucleotides present as repeated copies in P, but not M, strains. These P elements are found at unstable loci induced by hybrid dysgenesis and are at the break points of inversions and translocations in dysgenic hybrids. The effect of maternal cytoplasm in this phenomenon has been likened to zygotic induction of temperate phages in prokaryotes (ENGELS 1979).

Another way of looking at hybrid instability in general comes from the study of nomadic genes in *Drosophila* (YOUNG and SCHWARTZ 1981). These transposable elements constitute many families. Although they are found at different chromosomal locations in various strains, they manage to preserve a constant number of copies (except in tissue cultures) that is characteristic of each family. The implication of this finding is that if one or a few members of a transposable element family is introduced into a new genome, it will continuously replicate and transpose until it reaches a maximal number. Depending on the mechanism by which these elements limit their number, the cytoplasm can have a greater or lesser effect on transposable element activity.

SOMATICALLY INDUCED VARIANTS FROM TISSUE CULTURE

Variants and mutant lines are often found in plant tissue culture and in plants regenerated from these cultures (SKIRVIN 1978). Occasionally agronomically or horticulturally interesting cultivars have been identified among these regenerates. NICKELL and HEINZ (1973) reported that cultivars of sugar cane

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derived from plants regenerated from single cells differed morphologically and developmentally and some exhibited resistance to mosaic virus, a trait not found in parental lines. SHEPARD et al. (1980) established clones from potato plants regenerated from leaf cell protoplasts that displayed a more compact growth habit, earlier tuber set, different tuber morphology, shorter daylength requirements for flowering, and novel resistance to blights caused by *Alternaria solani* and *Phytophthora infestans*. These variants were stable for at least three somatic generations in different environments. A more detailed analysis (SECOR and SHEPARD 1981) indicates that field grown clones differed from the progenitor Russet Burbank line in 22 characters. The expression of some of these characters was influenced by environmental conditions. All clones were distinguished from the parental line in at least one character and some showed as many as seventeen. The basis for this variation is not known although aneuploidy does not appear to be the cause (SHEPARD et al. 1980). The Burbank potato is a tetraploid and has been maintained by asexual propagation for more than a century. The meristems of higher plants give rise to two ontogenetically distinct layers. The *tunica* is represented by one or more superficial layers that ultimately forms the epidermis and a portion of the cortical tissues. The *corpus* differentiates into the fascicular and ground tissue systems. Since both are present in apical meristems, asexually propagated cuttings or buds can maintain tissue chimeras indefinitely. Therefore many possibilities can exist for the segregation of inherent heterozygosity or clonal differences.

We do not intend to minimize the effect of aneuploidy in causing variability. Chromosome imbalance is a frequent trait of regenerated plants (ORTON 1980). Even in diploids such as *Datura* (BLAKESLEE 1934) the primary trisomics of each of the chromosomes lends a unique phenotype to the seed capsule. Nevertheless, there are cases where gene mutation may be the basis for somatically induced variability. Plants regenerated from maize callus are typically diploid (GREEN and PHILLIPS 1975). Among progeny of regenerated maize plants numerous simply inherited mutations have been observed for plant and endosperm characters (EDALLO et al. 1981). These variants resemble those previously observed in spontaneous mutations of maize. It is interesting to note that plants derived from the same subculture did not bear the same mutations indicating that once released, mutational activity continued. Despite the fact that only 2 out of 110 regenerates were aneuploid, most showed some degree of pollen sterility possibly as a manifestation of chromosomal rearrangements. TING et al. (1979) also reported some variation in maize plants derived from anther culture. Haploid plantlets are obtained by this process but after chromosome doubling fertile plants can be regenerated. Ting and his associates also noted a high degree of pollen sterility, fragmentation of the nucleolus in the microsporocytes and sporocyte fusions. Bridges and fragments at the first, but not the second, anaphase division of meiosis were also observed suggestive of possible chromosomal inversions.

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(1973) have shown that the maize controlling elements exist as stable components of the genome until a shock is applied that allows them to transpose and be expressed. In the cases studied this shock has always been associated with chromosome breakage, but other agents are not excluded. It seems quite possible that the processes of tissue culture and regeneration that we have considered here may constitute similar shocks to the genome and liberate previously silent copies of transposable elements. As a consequence they may leave loci or reinsert themselves into functional genes and thereby give rise to new mutations. The activated transposable elements could also effect changes by bringing about deletions, inversions, translocations and duplications of chromosomal segments.

LITERATURE CITED

- AHUJA, J. D. 1968 An hypothesis and evidence concerning the genetic components controlling tumor formation in *Nicotiana*. *Molec Gen. Genetics* 103: 176-184.
- ATKIN, J. D. 1972 Nature of the stringy pod rogue of snap beans, *Phaseolus vulgaris*. *Search, Agriculture* 2(9): 1-3. N.Y. State Agri. Exp. Sta., Geneva.
- ATKIN, J. D. and W. B. ROBINSON 1972 Nature of the flat pod rogue of snap beans, *Phaseolus vulgaris*. *Search, Agriculture* 2(9): 4-9. N.Y. State Agri. Exp. Sta., Geneva.
- BATĒSON, W. and C. PELLEW 1920 The genetics of rogues among culinary peas (*Pisum sativum*). *Proc. Roy. Soc., Ser. B* 91: 186-195.
- BIANCHI, A. 1958 Defective endosperm factors from maize-teosinte derivatives. *Maize Genetics Coop. Newsletter* 32: 11.
- BIANCHI, A., F. SALAMINI, and R. PANLAVECCHIO 1969 On the origin of controlling elements in maize. *Genetica Agrarica* 22: 335-344.
- BINGHAM, P. M. 1981 A novel dominant mutant allele at the *white* locus of *Drosophila melanogaster* is mutable. *Cold Spring Symp. Quant. Biol.* 45: 519-525.
- BINGHAM, P. M. and B. H. JUDD 1981 A copy of the *copia* transposable element is very tightly linked to the *w^a* allele at the *white* locus of *D. melanogaster*. *Cell* 25: 705-711.
- BLAKESLEE, A. F. 1934 New Jimson weeds from old chromosomes. *J. Hered.* 25: 80-108.
- BROTHERTON, W. 1923 Further studies of the inheritance of "rogue" type in garden peas (*Pisum sativum* L.). *J. Agri. Res.* 24: 815-852.
- BURNS, J. A. and D. U. GERSTEL 1967 Flower color variegation and instability of a block of heterochromatin in *Nicotiana*. *Genetics* 57: 155-167.
- BURR, B. and F. A. BURR 1981 Controlling-element events at the shrunken locus in maize. *Genetics* 98: 143-156.
- CHOUREY, P. S. and O. E. NELSON 1976 The enzymatic deficiency conditioned by the *shrunken-1* mutations in maize. *Biochem. Genet.* 14: 1041-1055.
- COYNE, D. P. 1969 Breeding behavior and effect of temperature on expression of a variegated rogue in green beans. *J. Am. Soc. Hort. Sci.* 94: 488-491.

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- DOERSCHUG, E. B. 1973 Studies of Dotted, a regulatory element in maize. *Theor. Appl. Genet.* 43: 182-189.
- DOONER, H. K. 1981 Regulation of the enzyme UFGT by the controlling element *Ds* in *bz-m4*, an unstable mutant in maize. Cold Spring Harbor Symp. Quant. Biol. 45: 457-462.
- EDALO, S., C. ZUCCHINALI, M. PERENZIN, and F. SALAMINI 1981 Chromosomal variation and frequency of spontaneous mutation associated with *in vitro* culture and plant regeneration in maize. *Maydica* 26: 39-56.
- EMERSON, R. A. 1914 The inheritance of a recurring somatic variation of variegated ears of maize. *Am. Nat.* 48: 87-115.
- ENGELS, W. R. 1979 Extrachromosomal control of mutability in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. USA* 76: 4011-4015.
- ENGELS, W. R. 1981 Germline mutability in *Drosophila* and its relation to hybrid dysgenesis and cytotype. *Genetics* 98: 565-587.
- GERSTEL, D. U. AND J. A. BURNS 1976 Enlarged euchromatic chromosomes ("megachromosomes") in hybrids between *Nicotiana tabacum* and *N. plumbaginifolia*. *Genetica* 46: 139-153.
- GREEN, C. E. and R. L. PHILLIPS 1975 Plant regeneration from tissue cultures of maize (*Zea mays* L.). *Crop Sci.* 15: 417-421.
- GREENBLATT, I. M. and R. A. BRINK 1962 Twin mutations in medium variegated pericarp maize. *Genetics* 47: 489-501.
- HARSHEY, R. M. and A. I. BUKHARI 1981 A model of DNA transposition. *Proc. Nat. Acad. Sci.* 78: 1090-1094.
- JENSEN, N. F. 1965 Population variability in small grains. *Agronomy J.* 57: 153-162.
- JONES, D. F. 1945 Heterosis resulting from degenerative changes. *Genetics* 30: 527-542.
- KAMP, D., R. KAHMANN, D. ZIPSER, T. R. BROKER, and L. T. CHOW 1978 Inversion of the G DNA segment of phage Mu controls phage infectivity. *Nature* 271: 577-580.
- KERR, L. B. 1971 The flat pod rogue in snap beans (*Phaseolus vulgaris* L.). Ph.D. Thesis. Utah State University.
- KIDWELL, M. G., J. F. KIDWELL, and J. A. SVED 1977 Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility, and male recombination. *Genetics* 86: 813-833.
- LEWIS, D. 1953 The rogue tomato: a problem in nuclear, cytoplasmic and environmental control. *Heredity* 7: 337-359.
- LIEBMAN, S., P. SHALIT, and S. PICOLOGLOU 1981 Ty elements are involved in the formation of deletions in *DEL 1* strains of *Saccharomyces cerevisiae*. *Cell* 26: 401-409.
- MANGELSDORF, P. C. 1958 The mutagenic effect of hybridizing maize and teosinte. Cold Spring Harbor Symp. Quant. Biol. 23: 409-421.
- MANGELSDORF, P. C. 1974 Corn, Its Origin Evolution and Improvement. Harvard. pp. 139-140.
- McCLINTOCK, B. 1950 The origin and behavior of mutable loci in maize. *Proc. Nat. Acad. Sci.* 36: 344-355.
- McCLINTOCK, B. 1951 Chromosome organization and genetic expression. Cold Spring Harbor Symp. Quant. Biol. 19: 13-47.
- McCLINTOCK, B. 1952 Mutable loci in maize. Carnegie Inst. Wash. Year Book 51: 212-219.
- McCLINTOCK, B. 1953 Mutation in maize. Carnegie Inst. Wash.

GENETIC INSTABILITIES

- Year Book 52: 227-237.
- McCLINTOCK, B. 1956 Controlling elements and the gene. Cold Spring Harbor Symp. Quant. Biol. 21: 197-216.
- McCLINTOCK, B. 1961 Some parallels between gene control systems in maize and in bacteria. Am. Nat. 95: 265-277.
- McCLINTOCK, B. 1962 Topographical relations between elements of control systems in maize. Carnegie Inst. Wash. Year Book 61: 448-461.
- McCLINTOCK, B. 1965 The control of gene action in maize. Brookhaven Symp. Biol. 18: 162-182.
- McCLINTOCK, B. 1978 Mechanisms that rapidly reorganize the genome. Stadler Symp. 10: 25-47.
- NICKEL, L. G. and D. J. HEINZ 1973 Potential of cell and tissue culture techniques as aids in economic plant improvement. In: A. SRB (Ed.) Genes, Enzymes, and Populations. Plenum, New York. pp. 109-128.
- ORTON, T. J. 1980 Chromosomal variability in tissue cultures and regenerated plants of *Hordeum*. Theor. Appl. Genet. 56: 101-112.
- PEARSON, O. H. 1968 Unstable gene systems in vegetable crops and implications for selection. Hort. Science 3: 271-274.
- PICARD, G. 1979 Non-mendelian female sterility in *Drosophila melanogaster*: principal characteristics of chromosomes from inducer and reactive origin after chromosomal contamination. Genetics 91: 455-471.
- QUIMBY, J. R. and K. F. SCHERTZ 1970 Sorghum genetics, breeding, and hybrid seed production. In: J. S. WALL and W. M. ROSS Sorghum Production and Utilization. Avi Publishing Co., pp. 73-117.
- ROEDER, G. S., P. J. FARABAUGH, D. T. CHALEFF, and G. R. FINK 1980 The origins of gene instability in yeast. Science 209: 1375-1380.
- RHOADES, M. M. 1938 Effect of the *Dt* gene on the mutability of the *a1* allele in maize. Genetics 23: 377-397.
- RILEY, R. and G. KIMBER 1961 Aleuploids and the cytogenetic structure of wheat varietal populations. Heredity 16: 275-290.
- SAND, S. A. 1976 Genetic control of gene expression: Independent location of *Flt* (3) and its interactions with the mutable *V* locus in *Nicotiana*. Genetics 83: 719-736.
- SCHERTZ, K. F. and Y. P. TAI 1969 Inheritance of reaction of *Sorghum bicolor* (L.) Moench to toxin produced by *Periconia circinata* (Mang.) Sacc. Crop Sci. 9: 621-624.
- SCHULER, J. F. 1954 Natural mutations in inbred lines of maize and their heterotic effect. I. Comparison of parent, mutant and their F₁ hybrid in a highly inbred background. Genetics 39: 908-922.
- SECOR, G. A. and J. F. SHEPARD 1981 Variability of protoplast-derived potato clones. Crop Sci. 21: 102-105.
- SHAPIRO, J. A. 1979 Molecular model for the transposition and replication of bacteriophage Mu and other transposable elements. Proc. Nat. Acad. Sci. USA 76: 1933-1937.
- SHEPARD, J. F., D. BIDNEY, and E. SHUHIN 1980 Potato protoplasts in crop improvement. Science 208: 17-24.
- SINGLETON, W. R. 1943 Breeding behavior of C30 a diminutive P39 mutant whose hybrids show increased vigor. Genetics 28: 89.

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- SINGLETON, W. R. 1947 Mutations in maize inbreds. *Genetics* 32: 104.
- SKIRVIN, R. M. 1978 Natural and induced variation in tissue culture. *Euphytica* 27: 241-266.
- SMITH, H. H. 1968 Recent cytogenetic studies in the genus *Nicotiana*. *Adv. Genet.* 14: 1-54.
- SMITH, H. H. 1972 Plant genetic tumors. *Prog. Exp. Tumor Res.* 15: 138-164.
- SMITH, H. H. and S. A. SAND 1957 Genetic studies on somatic instability in cultures derived from hybrids between *Nicotiana langsdorffii* and *N. sanderae*. *Genetics* 42: 560-582.
- STILES, J. I., L. R. FRIEDMAN, C. HELMS, S. CONSAUL, and F. SHERMAN 1981 Transposition of the gene cluster *CYC1 - OSM1 - RAD7* in yeast. *J. Mol. Biol.* 148: 331-346.
- TING, Y. P., G. MINGGUANG, G. LIGUAN, Y. BENLIANG, and H. JIAOXIANG 1979 Meiotic chromosome behavior of the H₂ progeny of maize pollen-plants. *Acta Genet. Sinica* 6: 159-164.
- YOUNG, M. W. and H. E. SCHWARTZ 1981 Nomadic gene families in *Drosophila*. *Cold Spring Harbor Symp. Quant. Biol.* 45: 629-640.
- ZIEG, J. M., M. SILVERMAN, M. HILMEN, and M. SIMON 1977 Recombinational switch for gene expression. *Science* 196: 170-172.

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