northern part of the "super-giant" (>100 trillion cubic feet) Urengoy gas field (e in Fig. 2) (27, 28). In the case of the Tyumen hole the purpose is to evaluate the Mesozoic and Paleozoic sections for oil and gas, determine seismic velocities, study lithologic properties, and determine zones of overpressure and underpressure.

Six deep holes, as opposed to ultra-deep holes, are planned in oil-bearing regions (Dnieper-Donets, Astrakhan arch, and Timan-Pechora) and mining regions (Muruntau gold area in Uzbek S.S.R., Noril'sk nickel province, and Krivoi Rog iron region).

The depths to be reached in the deepest holes planned are on the order of 13 to 15 km and hence cannot provide any corroboration of interpretations made of DSS data that probe to between 80 and 100 km. The ultra-deep holes nonetheless do enhance interpretation of shallow data, whether garnered from conventional high explosive or other seismic sources or from nuclear explosions. Some of the proposed ultra-deep holes are in fact close to nuclear DSS lines (Fig. 2).

Conclusions

The Soviet program of deep seismic sounding is designed to explore the vast expanses of that country with underground nuclear explosions augmented by ultra-deep drill holes. The fact that the program has been operating since 1971 and shows every promise of continuing is testimony to its successes and usefulness to Soviet objectives. Such a notable program has been possible despite an apparent dearth of digital recording equipment and advanced analytical tools. The use of large seismic sources has allowed elucidation of crustal detail and mantle structure down to a depth of 200 km. Analysis of data has revealed gross inhomogeneities in the mantle underlying the Soviet Union. Comparable structures have not been described on other continents.

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The Significance of Responses of the Genome to Challenge

Barbara McClintock

There are "shocks" that a genome must face repeatedly, and for which it is prepared to respond in a programmed manner. Examples are the "heat shock" responses in eukaryotic organisms and the "SOS" responses in bacteria. Each of these initiates a highly programmed sequence of events within the cell that serves to cushion the effects of the shock. Some sensing mechanism must be present in these instances to alert the cell to imminent danger, and to set in motion the orderly sequence of events that will mitigate this danger. But there are also responses of genomes to unanticipated challenges that are not so precisely programmed. The genome is unprepared for these shocks. Nevertheless, they are sensed, and the genome responds in a discernible but initially unforseen manner.

An experiment conducted in the mid-1940's prepared me to expect unusual responses of a genome to challenges that the genome is unprepared to meet in an orderly, programmed manner. In most known instances of such challenges, the types of response are not predictable in advance of initial observations of them. Moreover, it is necessary to subject the genome repeatedly to the same challenge in order to observe and appreciate the nature of the changes it induces. Familiar examples are the production of mutation by x-rays and by some mutagenic agents.

It is the purpose of this discussion to consider some observations from my early studies that revealed programmed responses to threats that are initiated within the genome itself, as well as oth-

ers similarly initiated, that lead to new and irreversible genomic modifications. These latter responses, now known to occur in many organisms, are significant for appreciating how a genome may reorganize itself when faced with a difficulty for which it is unprepared. Conditions known to provoke such responses are many. A few of these will be considered, along with several examples from nature implying that rapid reorganizations of genomes may underlie some species formations. Our present knowledge would suggest that these reorganizations originated from some "shock" that forced the genome to restructure itself in order to overcome a threat to its survival.

Because I became actively involved in the subject of genetics only 21 years after the rediscovery, in 1900, of Mendel's principles of heredity, and at a stage when acceptance of these principles was not general among biologists, I have had the pleasure of witnessing and experiencing the excitement created by revolutionary changes in genetic concepts that have occurred over the past sixty-odd years. I believe we are again experiencing such a revolution. It is altering our concepts of the genome: its component parts, their organizations, mobilities, and their modes of operation. Also, we are now better able to integrate activities of nuclear genomes with those of other components of a cell. Unquestionably, we will emerge from this revolutionary period with modified views of components of cells and how they operate, but only however, to await the emergence of the next revolutionary phase that again will bring startling changes in concepts.

Experiment with *Zea mays* in the Summer of 1944 and Its Consequences

The experiment that alerted me to the mobility of specific components of genomes involved the entrance of a newly ruptured end of a chromosome into a telophase nucleus. This experiment commenced with the growing of approximately 450 plants in the summer of 1944, each of which had started its develop-

ment with a zygote that had received from each parent a chromosome with a newly ruptured end of one of its arms. The design of the experiment required that each plant be self-pollinated. This was in order to isolate from the self-pollinated progeny new mutants that were expected to appear, and to be confined to locations within the arm of a chromosome whose end had been ruptured. Each mutant was expected to reveal the phenotype produced by a minute homozygous deficiency and to segregate in a manner resembling that of a recessive in an F_2 progeny. Their modes of origin could be projected from the known behavior of broken ends of chromosomes in successive mitoses. In order to observe those mutants that might express an altered seedling character, 40 kernels from each self-pollinated ear were sown in a seedling bench in the greenhouse during the winter of 1944–1945.

Some seedling mutants of the type expected did segregate, but they were overshadowed by totally unexpected segregants exhibiting bizarre phenotypes. These segregants were variegated for type and degree of expression of a gene. Those variegated expressions given by genes associated with chlorophyll development were startlingly conspicuous. Within any one progeny chlorophyll intensities, and their pattern of distribution in the seedling leaves, were alike. Between progenies, however, both the type and the pattern differed widely. Variegated seedlings from the different progenies were transferred to pots in order to observe the variegated phenomenon in the later developing, larger leaves.

It soon became apparent that modified patterns of gene expression were being produced, and that these were confined to sharply defined sectors in a leaf. Thus, the modified expression appeared to relate to an event that had occurred in the ancestor cell that gave rise to the sector. It was this event that was responsible for altering the pattern or type (or both) of gene expression in descendant cells, often many cell generations removed from the event. It was soon evident that the event was related to some cell component that had been unequally segregated at a mitosis. Twin sectors appeared in which the patterns of gene expression in the two side-by-side sectors were reciprocals of each other. For example, one sector might have a reduced number of uniformly distributed fine green streaks in a white background in comparison to the number and distribution of such streaks initially appearing in the seedling

and showing elsewhere on the same leaf. The twin, on the other hand, had a much increased number of such streaks. Because these twin sectors were side-by-side they were assumed to have arisen from daughter cells following a mitosis in which each daughter had been modified in a manner that would differentially regulate the pattern of gene expression in their progeny cells.

After observing many such twin sectors, I concluded that regulation of pattern of gene expression in these instances was associated with an event occurring at a mitosis in which one daughter cell had gained something that the other daughter cell had lost. Believing that I was viewing a basic genetic phenomenon, all attention was given, thereafter, to determining just what it was that one cell had gained that the other cell had lost. These proved to be transposable elements that could regulate gene expressions in precise ways. Because of this I called them "controlling elements." Their origins and their actions were a focus of my research for many years thereafter. It is their origin that is important for this discussion, and it is extraordinary. I doubt if this could have been anticipated before the 1944 experiment. It had to be discovered accidentally.

Early Observations of the Effect of X-rays on Chromosomes

The 1944 experiment took place 13 years after I had begun to examine the behavior of broken ends of chromosomes. It was knowledge gained in these years that led me to conceive this experiment. Initial studies of broken ends of chromosomes began in the summer of 1931. At that time our knowledge of chromosomes and genes was limited. In retrospect we might call it primitive. Genes were "beads" arranged in linear order on the chromosome "string." By 1931, however, means of studying the "string" in some detail was provided by newly developed methods of examining the ten chromosomes of the maize complement in microsporocytes at the pachytene stage of meiosis. At this stage the ten bivalent chromosomes are much elongated in comparison to their metaphase lengths. Each chromosome is identifiable by its relative length, by the location of its centromere, which is readily observed at the pachytene stage, and by the individuality of the chromomeres strung along the length of each chromosome. At that time maize provided the best material for locating known

genes along a chromosome arm, and also for precisely determining the break points in chromosomes that had undergone various types of rearrangement, such as translocations and inversions. The usefulness of the salivary gland chromosomes of *Drosophila* for such purposes had not yet been recognized. This came several years later. In the interim, maize chromosomes were revealing, for the first time, some distinctive aspects of chromosome organization and behavior. One of these was the extraordinary effect of x-rays on chromosomes.

The reports of Muller in 1927 and 1928 (*1*, *2*) and of Hanson in 1928 (*3*) on the use of x-rays for obtaining mutations in *Drosophila*, and similarly that of Stadler in 1928 (*4*) with the barley plant, had a profound effect on geneticists. Here was a way of obtaining mutations at will. One did not need to await their spontaneous appearances. Many persons over many years continued to use x-rays for such purposes. But x-rays did not fulfill initial expectations of their usefulness. For other purposes, however, they have been most valuable, particularly for obtaining various types of structural reorganizations of the genome, from minute deficiencies to multiple rearrangements of chromosomes. It was to observe the effects of x-rays on chromosomes of maize that brought me to the University of Missouri at Columbia in the summer of 1931.

Prior to 1931 Stadler had been using x-rays to obtain mutations in maize. He had developed techniques for isolating those mutations that occur at selected gene loci. One method was to irradiate pollen grains. Pollen grains carry the haploid male gametes. The irradiated male gametes in Stadler's experiments carried wild-type alleles of known recessive mutants. Irradiated pollen was placed on the silks of ears of plants that were homozygous for one or more recessive alleles located in known linkage groups. An x-ray–induced mutation altering the expression of the wild-type allele of one of these recessives should be identifiable in an individual plant derived from such a cross. By the summer of 1931, Stadler had many plants in his field at Columbia, Missouri, from which one could choose those that exhibited one or another of these recessive phenotypes. Stadler had asked me if I would be willing to examine such plants at the meiotic stages to determine what types of events might be responsible for these recessive expressions. I was delighted to do so as this would be a very new experience. After my arrival at Columbia

in June 1931 plants were selected whose chromosomes were to be examined. The knowledge gained from these observations was new and impressive. Descriptions and photographs summarizing these observations appeared in a bulletin published by the University of Missouri Agricultural College and Experiment Station (*5*).

None of the recessive phenotypes in the examined plants arose from "gene mutation." Each reflected loss of a segment of a chromosome that carried the wild-type allele, and x-rays were responsible for inducing these deficiencies. They also were responsible for producing other types of chromosome rearrangements, some of them unexpectedly complex. A conclusion of basic significance could be drawn from these observations: broken ends of chromosomes will fuse, two-by-two, and any broken end with any other broken end. This principle has been amply proved in a series of experiments conducted over the years. In all such instances the break must sever both strands of the DNA double helix. This is a "double-strand break" in modern terminology. That two such broken ends entering a telophase nucleus will find each other and fuse, regardless of the initial distance that separates them, soon became apparent.

After returning to Cornell University in the fall of 1931, I received a reprint from geneticists located at the University of California, Berkeley. The authors described a pattern of variegation in *Nicotiana* plants that was produced by loss of a fragment chromosome during plant development. The fragment carried the dominant allele of a known recessive present in the normal homologues. Loss of the dominant allele allowed the recessive phenotype to be expressed in the descendants of those cells that had lost this fragment.

It occurred to me that the fragment could be a ring chromosome, and that losses of the fragment were caused by an exchange between sister chromatids after replication of the ring. This would produce a double-sized ring with two centromeres. In the following anaphase, passage of the centromeres to opposite poles would produce two chromatid bridges. This, I thought, could prevent the chromosome from being included in either telophase nucleus. I sent my suggestion to the geneticists at Berkeley who then sent me an amused reply. My suggestion, however, was not without logical support.

During the summer of 1931 I had seen plants in the maize field that showed variegation patterns resembling the one described for *Nicotiana*. The chromo-

somes in these plants had not been examined. I then wrote to Stadler asking if he would be willing to grow more of the same material in the summer of 1932 that had been grown in the summer of 1931. If so, I would like to select the variegated plants to determine the presence of a ring chromosome in each. Thus, in the summer of 1932 with Stadler's generous cooperation, I had the opportunity to examine such plants. Each plant did have a ring chromosome. It was the behavior of this ring that proved to be significant. It revealed several basic phenomena.

1) In most mitoses, replication of the ring chromosome produced two chromatids that were completely free from each other, and thus could separate without difficulty in the following anaphase.

2) Sister strand exchanges do occur between replicated or replicating chromatids, and the frequency of such events increases with increase in the size of the ring. These exchanges produce a double-size ring with two centromeres.

3) Mechanical rupture occurs in each of the two chromatid bridges formed at anaphase by passage of the two centromeres on the double-size ring to opposite poles of the mitotic spindle.

4) The location of a break could be at any one position along any one bridge.

5) The two broken ends entering a telophase nucleus then fuse.

6) The size and content of each newly constructed ring depend on the position of the rupture that had occurred in each bridge (*6–8*).

The conclusion seems inescapable that cells are able to sense the presence in their nuclei of ruptured ends of chromosomes and then to activate a mechanism that will bring together and then unite these ends, one with another. And this will occur, regardless of the initial distance in a telophase nucleus that separated the ruptured ends. The ability of a cell to sense these broken ends, to direct them toward each other, and then to unite them so that the union of the two DNA strands is correctly oriented, is a particularly revealing example of the sensitivity of cells to all that is going on within them.

Evidence from x-rays, ring chromosomes, and that obtained in later experiments (*9–12*), gives unequivocal support for the conclusion that broken ends will find each other and fuse. The challenge is met by a programmed response. This may be necessary, as both accidental breaks and programmed breaks may be frequent. If not repaired, such breaks could lead to genomic deficiencies having serious consequences.

Entrance into a Telophase Nucleus of a Broken End of a Chromosome

In the mid-1930's another event inducing chromosome rupture was discovered. It revealed why crossing over should be suppressed between the centromere and the nucleolus organizer in organisms in which chiasmata terminalize (that is, move from the initial location of a crossover to the end of the arm of the chromosome). In maize, terminalization occurs at the diplotene stage of meiosis. This is before the nucleolus breaks up, which it does at a later stage in the first meiotic prophase. It is known that the force responsible for terminalization is strong enough to induce chromosome breakage should the terminalization process be blocked before the terminalizing chiasma reaches the end of the arm of a chromosome. In maize the centromere and the nucleolus organizer on the nucleolus chromosome are relatively close together. No crossovers have been noted to occur between them. However, if a plant is homozygous for a translocation that places the centromere on the nucleolus chromosome some distance from its nucleolus organizer, crossing over does occur in the interval between them (10). A chiasma so located starts its terminalization process to reach the end of the arm. It is stopped, however, at the nucleolus border. The terminalizing chromatid strands cannot pass through the nucleolus. Instead, the two chromatids are ruptured at this border. Fusions then occur between the ruptured ends, establishing, thereby, a dicentric chromosome deficient for all of the chromatin that runs through the nucleolus and continues beyond to the end of the arm. At the meiotic anaphase, passage of the two centromeres of the dicentric chromosome to opposite poles of the spindle produces a bridge. This bridge is ruptured and again the rupture can occur at any one location along the bridge. Now a *single* ruptured end of a chromosome enters the telophase nucleus. How, then, does the cell deal with this novel situation?

In order to determine how a cell responds to the presence of a single ruptured end of a chromosome in its nucleus, tests were conducted with plants that were heterozygous for a relatively long inversion in the long arm of chromosome 4 of maize. It had been known for some time that a crossover within the inverted segment in plants that are heterozygous for an inversion in one arm of a chromosome would result in a dicentric chromosome, and also an acentric fragment composed of all the chromatin from the distal breakpoint of the inversion to the end of the arm. A chromatin bridge would form at the meiotic anaphase by passage of the two centromeres on the dicentric chromosome to opposite poles of the spindle. Mechanical rupture of this bridge as the spindle elongated would introduce a single broken end into the telophase nucleus, as illustrated in Fig. 1, a to d.

The intent of this experiment was to observe this chromosome in the following mitotic division in order to determine the fate of its ruptured end. This could be accomplished readily by observing the first mitotic division in the microspore. Meiosis on the male side gives rise to four haploid spores, termed microspores. Each spore enlarges. Its nucleus and nucleolus also enlarge. Approximately 7 days after completion of meiosis this very enlarged cell prepares for the first postmeiotic mitosis. This mitosis produces two cells, a very large cell with a large, active nucleus and nucleolus, and a small cell with compact chromatin in a small nucleus, surrounded by

Fig. 1. Photographs illustrating the behavior of a newly ruptured end of a chromosome at the meiotic mitoses in microsporocytes and in the postmeiotic mitosis in the microspore. (a) Chromatin bridge at the first meiotic anaphase, with accompanying acentric fragment. Note the thin region in the bridge where rupture probably would have occurred at a slightly later stage. (b) Two sister cells at a very late prophase of the second meiotic mitosis. The rupture of the chromatid bridge that occurred at the previous anaphase severed the bridge at a nonmedian position. The larger segment so produced appears in the upper cell and opposite to the shorter segment in the lower cell. Their locations away from the other divalent chromatids relate to late entrances into the previous telophase nuclei caused by tension on the bridge before its rupture. Their placements show the positions they occupied in these telophase nuclei. The acentric fragment is in the lower cell, close to the cell membrane that was formed at the end of the first meiotic mitosis. (c) Anaphase of the second meiotic mitosis. The chromatid with a ruptured end is located closer to the wall in each cell that was formed after the first meiotic mitoses than are the other chromatids, for reasons given in (b). Note dissimilar lengths of the arms with ruptured ends. The acentric fragment is near the middle of the spindle in the upper cell. (d) Telophase of the second mitotic mitosis with extensions in two of the four nuclei pointing toward each other, one in the upper left nucleus and one in the lower right nucleus. The shapes of these nuclei reflect the off-positioning of chromatids having a newly ruptured end. Such off-positioning starts with the first meiotic telophase, continues throughout interphase and into the prophase, metaphase, and anaphase of the second meiotic mitosis and then into telophase, as shown here. The acentric fragment is adjacent to but not within the nucleus to the upper right. Note formation of the cell plate in each cell that anticipates the four spores that are products of meiosis. (e) Mitotic anaphase in the microspore showing a chromatid bridge produced by "fusion" of the replicated broken end. (f) Same as (e) but a slightly later stage showing rupture of the bridge. [Photographs adapted from *Missouri Agricultural Experiment Station Research Bulletin 290* (1938)]
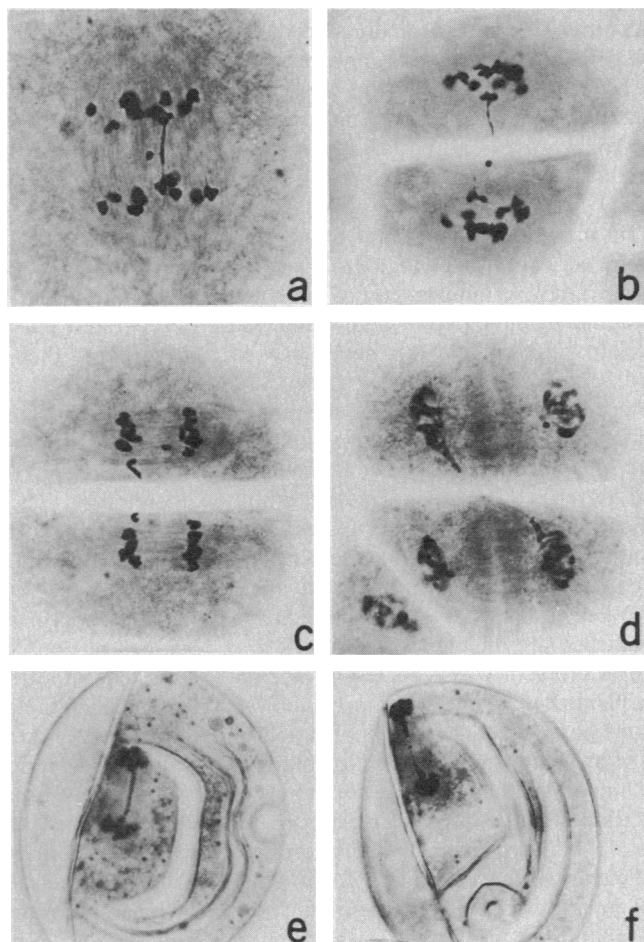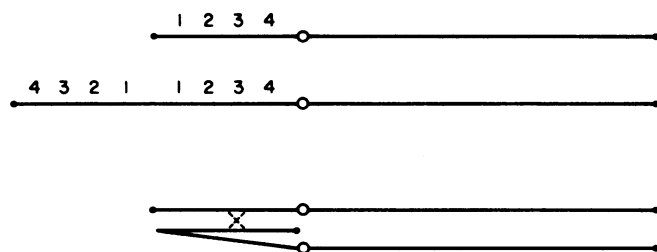
Fig. 2. Stylized representation of a crossover between a chromosome 9 with a normal short arm, upper line, and one with a duplication of this arm in reverse orientation, line below. In the lower two lines an exchange between two homologously associated arms is indicated by the cross. Such an exchange would give rise to a dicentric chromosome that simulates two normal chromosomes 9 attached together at the ends of their short arms, plus a small acentric fragment composed of the short arm of chromosome 9. The open circles represent centromeres. Telomeres are depicted as small knobs at the ends of chromosomes.

a thin layer of cytoplasm. This is the generative cell. Sometime later it undergoes a mitosis that will produce two condensed sperm cells. With completion of this division the pollen grain is nearly ready to function. The first division in the microspore may be observed readily merely by using a squash technique. The division of the generative cell, on the other hand, is obscured by the densely packed starch grains that accumulate during the interval between the two mitoses.

Examination of the first mitotic division in the microspore revealed a strange behavior of the single broken end that had entered a meiotic telophase nucleus. The replicated chromosome again was dicentric. The two chromatids produced by the replication process appeared to be fused at the location of the break that had occurred at the previous meiotic anaphase. In the spore, passage to opposite poles of the two centromeres of this newly created dicentric chromosome again produced a chromatid bridge that again was ruptured (Fig. 1, e and f). Thus, a newly ruptured end of the chromosome again entered each telophase nucleus. How would this newly broken end behave in subsequent mitoses? To determine this requires that the pollen grain with nuclei having such a ruptured end of a chromosome be functional. In the described instance, pollen grains whose nuclei had such a ruptured end would be deficient for a large terminal segment of the long arm of chromosome 4. Pollen grains whose nuclei have such a deficiency are unable to function.

The problem was resolved by obtaining plants having one chromosome of the maize complement with a duplication of all of its short arm in reverse orientation; its homologue had either a normal organization of its short arm, or better in the test to be performed, a short terminal deficiency of this arm that will not allow pollen grains receiving this chromosome to function. A crossover at the meiotic prophase, as shown in Fig. 2, produces a dicentric chromosome that simulates two normal chromosomes attached together at the ends of their short arms, and a fragment chromosome with telomeres at both ends. The dicentric chromosome produced by the crossover initiates the chromatid type of breakage-fusion-bridge cycle. This cycle, initially detected at the first mitosis in a microspore, could now be followed in subsequent mitoses. This is because the location of breaks in some of the anaphase bridges gave rise to chromosomes with at least a full complement of genes necessary for pollen functioning. Such functional pollen grains falling on the silks of ears will deliver their two sperm cells to the embryo sac inside a kernel-to-be. One sperm will contribute to the development of the embryo and the other will contribute to the development of the endosperm.

On the female side only a single cell in the kernel-to-be undergoes meiosis, and the embryo sac arises from only one of the four spores produced by the two meiotic mitoses. The other three spores degenerate. This one haploid cell, the megaspore, then undergoes three successive mitoses to form the embryo sac, or female gametophyte. Of the cells in the embryo sac only the egg cell and the much enlarged central cell need be considered here. The very large central cell has two haploid nuclei positioned close to each other and near the egg cell. After delivery of the two sperms to the embryo sac, one sperm nucleus fuses with the egg cell nucleus to form the diploid zygote. The other sperm nucleus and the two nuclei in the central cell fuse to form the primary endosperm nucleus, which is triploid. (The term "double fertilization" is commonly used in referring to these events.) Thus, the embryo and endosperm are formed separately, although both share the same genes, one set from each parent for the embryo, and two sets from the female parent and one set from the male parent for the endosperm. Although developing separately, the two structures are placed side-by-side in the mature kernel (Fig. 3).

It was soon learned that the chromatid type of breakage-fusion-bridge cycle, initiated at a meiotic anaphase, will continue during development of the pollen grain and the embryo sac. Whenever a sperm nucleus contributes a chromosome with a newly broken end to the primary endosperm nucleus, this cycle will continue throughout mitoses in the developing endosperm. Similarly, if the two nuclei in the central cell each have such a ruptured end of a chromosome, either the chromosome or chromatid type of breakage-fusion-bridge will occur throughout endosperm development. When, however, a single ruptured end of a chromosome is delivered to the zygote nucleus by either the egg or the sperm nucleus, the ruptured end will "heal" subsequently; the cycle ceases in the developing embryo. Although not yet proven at the molecular level, it is altogether likely that the healing process represents the formation of a new telomere at the ruptured end. This assures that the special requirement for DNA replication at free ends of chromosomes will be satisfied. This new telomere functions normally thereafter. It is as stable in this regard as any other telomere of the maize complement, and tests of this cover many cell and plant generations.

A cell capable of repairing a ruptured end of a chromosome must sense the presence of this end in its nucleus. This sensing activates a mechanism that is required for replacing the ruptured end with a functional telomere. That such a mechanism must exist was revealed by a mutant that arose in my stocks. When homozygous, this mutant would not allow the repair mechanism to operate in the cells of the plant. Entrance of a newly ruptured end of a chromosome into the zygote is followed by the chromatid type of breakage-fusion-bridge cycle throughout mitoses in the developing plant. This suggests that the repair mechanism in the maize strains I had been using is repressed in cells producing the male and female gametophytes and also in the endosperm, but is activated in the embryo. Although all of this was known before the 1944 experiment was conducted, the extent of trauma perceived by cells whose nuclei receive a *single* newly ruptured end of a chromosome that the cell cannot repair, and the speed with which this trauma is registered, was not appreciated until the winter of 1944–1945.

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Initiation of Activations of Previously Silent Genomic Elements

By 1947 it was learned that the bizarre variegated phenotypes that segregated in many of the self-pollinated progenies grown on the seedling bench in the fall and winter of 1944–1945 were due to the action of transposable elements. It seemed clear that these elements must have been present in the genome, and in a silent state previous to an event that activated one or another of them. To my knowledge, no progenies derived from self-pollination of plants of the same strain, or related strains, had ever been reported to have produced so many distinctly different variegated expressions of different genes as had appeared in the progenies of these closely related plants that were grown in the summer of 1944. It was concluded that some traumatic event was responsible for these activations. The unique event in the history of these plants relates to their origin. Both parents of the plants, grown in the summer of 1944, had contributed a chromosome with a newly ruptured end to the zygote that gave rise to each of these plants. The rupture occurred, in the first instance, at a meiotic anaphase in each parent, and the ruptured end then underwent the succession of mitotic anaphase breaks associated with the chromatid type of breakage-fusion-bridge cycle during development of the male and female gametophytes—the pollen grain and the embryo sac. I suspected that an activating event could occur at some time during this phase of the life history of the parent plants. I decided, then, to test if this might be so.

The newly activated elements, isolated from the initial experiment, were observed to regulate gene expression after insertion of an element, or one of its derivatives, at a gene locus (13–15). In some instances the general mode of regulation resembled that produced by the *Dotted* gene on the standard recessive allele, *a*, of the *A* gene. This *a* allele represents the first recognized gene among a number of others whose action is required for production of anthocyanin pigment, either red or purple, in plant tissues and in several tissues of the kernel (16). In the mid-1930's Rhoades discovered this *Dotted* (*Dt*) element in a strain of Black Mexican sweet corn (17, 18). It behaved as a dominant gene that caused the otherwise very stable but nonfunctional *a* allele to mutate to new alleles that allowed anthocyanin pigment to be formed in both plant and kernel. The name *Dotted* given to it refers to the

pattern of mutations that is expressed in plants and kernels homozygous for the *a* allele on chromosome 3 and having a *Dt* element located elsewhere in the chromosome complement. Small streaks of red or purple pigment appear in plants; the kernels have dots of this pigment distributed over the aleurone layer. (The aleurone layer is the outermost layer of the endosperm.)

In that this *Dt* may have originated from activation of a previously silent element in the maize genome, and in that such silent elements should be present in all maize genomes, it was decided to test whether the breakage-fusion-bridge cycle would activate one such silent *Dt*

Fig. 3. Longitudinal section through a mature maize kernel to show its parts. The cut surface was treated with an iodine-potassium iodide solution to stain amylose in the starch granules of individual cells, and to indicate by density of stain the relative amounts of amylose in these cells. The narrow outer layer of the kernel is the pericarp, a maternal tissue. The embryo and endosperm are side-by-side but clearly delimited from each other. The endosperm is above and to the right of the embryo. In this photograph, four parts of the embryo may be noted. To the left and adjacent to the endosperm is the scutellum with its canals. The shoot, to the upper left, and the primary root below it, are connected to each other by the scutellar node. The different staining intensities in the endosperm cells reflect different amounts of amylose in them. These differences relate to the presence and action of a transposable *Ac* element at the *Wx* locus (23). The *Wx* gene is responsible for conversion of amylopectin to amylose, but only in the endosperm, not in the embryo.

element. My stocks that were homozygous for the *a* allele had never given any indication of *Dt* action. Therefore, these stocks were used to test if a presumed silent *Dt* element could be activated by the chromatid type of breakage-fusion-bridge cycle. Plants homozygous for *a* and having a chromosome 9 constitution similar to that described for Fig. 2 were used as pollen parents in crosses to plants that also were homozygous for *a*. These pollen parents had the duplication of the short arm as shown in Fig. 2, but its homologue was deficient for a terminal segment of this arm that would not allow pollen grains having it to function. It was determined that 70 to 95 percent of the *functional* pollen grains produced by these plants carried sperms having a chromosome 9 with a newly ruptured end of its short arm, the initial rupture having occurred at the previous meiotic anaphase. Thus, most of the embryos and endosperms in the kernels on ears produced by the described cross started development with a newly ruptured end of the short arm of chromosome 9 in both embryo and endosperm. These kernels were searched for dots of pigment in their aleurone layer. A number of kernels had such dots. Many of these dots were confined to a restricted area of the aleurone layer, suggesting that this area represented a sector derived from a single cell in which a silent *Dt* element had been activated. One kernel had dots distributed over all of the aleurone layer, suggesting that the sperm nucleus contributing to the primary endosperm nucleus already had an activated *Dt* element in it. Tests of the plant arising from this kernel indicated that the sister sperm nucleus that had fused with the egg nucleus did not have such an activated element. Apparently, the activating event had occurred in the nucleus of only one of the two sperms. Significantly, this is only two mitoses removed from initiation of the chromatid type of breakage-fusion-bridge cycle. As was mentioned earlier, this cycle continues during successive mitoses in the development of the endosperm. This continuing cycle could explain the presence in some kernels of sectors with pigmented dots, and this, in turn, would imply that activations of silent elements could occur at any time that this cycle remained in operation (19–21).

A similar test was conducted some years later by Doerschug (22), using the same constitution of the pollen parent as that just described. He obtained similar results. In his tests, however, two kernels with spots of pigment distributed

over the entire aleurone layer proved to have an activated *Dt* element in the plant grown from each of these kernels. The behavior of these two newly activated *Dt* elements was extensively studied by Doerschug. The two elements differed from each other not only in their location in the chromosome complement, but also in their mode of control of the time and place of change in *a* gene action. We now know that such differences in performance of these elements are expected.

Doerschug's two *Dt* isolates are most significant for appreciating the speed of response of a genome to entrance of a newly ruptured end of a chromosome into a telophase nucleus. Each *Dt* element must have been activated in the microspore nucleus or not later than the generative nucleus produced by division of the microspore nucleus. The unexpected event probably is sensed and acted upon from the initial entrance of a single ruptured end of a chromosome into a telophase nucleus, and in each subsequent nucleus that receives such a newly ruptured end. It is recognized that *Dt* is only one among a number of silent but potentially transposable elements that are present in maize genomes. Most probably some of these other silent elements were activated during the described test, but they were not able to be recognized as were activations of *Dt* elements.

A similar approach could be used to detect such activations if a proper indicator stock were chosen for the test. Detection of silent elements is now made possible with the aid of DNA cloning techniques. Silent *Ac* (*Activator*) elements, as well as modified derivatives of it, have already been detected in several strains of maize (*23*). When other transposable elements are cloned it will be possible to compare their structural and numerical differences among various strains of maize. Present evidence suggests that wide differences may be found in this regard, as they have been found for middle and highly repetitious DNA sequences (*24*). In any one strain of maize the number of silent but potentially transposable elements, as well as other repetitious DNA's, may change, and most probably in response to challenges not yet recognized.

There are clear distinctions in the comportment of ends of chromosomes on entering telophase nuclei. These relate to (i) all chromosomes having normal ends, (ii) two chromosomes, each with a single ruptured end, or one chromosome with both ends ruptured, and

(iii) one chromosome with a single broken end. Both ends of normal, unbroken chromosomes have a normal telomere, and no difficulties are experienced. Two ruptured ends, neither with a telomere, will find each other and fuse. In these instances there is no immediate telomere problem. A single broken end has no telomere, and no other broken end with which to fuse. If the cell cannot make a new telomere, which is the case in the maize gametophytes and the endosperm, the evidence indicates that a special trauma is experienced. Telomeres are especially adapted to replicate the free ends of chromosomes. When no telomere is present, attempts to replicate this uncapped end may be responsible for the apparent "fusions" of the replicated chromatids at the position of the previous break, and be responsible for perpetuating the chromatid type of breakage-fusion-bridge cycle in successive mitoses. Activation of potentially transposable elements, as well as other structural modifications of the chromosomes not considered here, are recognizable consequences of the cell's response to the continuing trauma.

Further Examples of Response of Genomes to Stress

Cells must be prepared to respond to many sources of stress. Mishaps that affect the operation of a cell must be occurring continuously. Sensing these and instigating repair systems are essential. We are aware of some of the mishaps affecting DNA, and also of their repair mechanisms, but many others could be difficult to recognize. Homeostatic adjustments to various accidents would be required if these accidents occur frequently. Many such mishaps and their adjustments would not be detected unless some event or observation directed attention to them. Some, however, are so conspicuous that they cannot fail to be noted. For example, in *Drosophila*, some sensing device recognizes when the amount of ribosomal DNA is above or below the standard amount, and then sets in motion the system that will make the proper adjustment. Similarly, amitotic divisions of macronuclei in ciliates may result in unequal distributions of DNA to daughter nuclei. These deviations are sensed in each daughter cell. To make adjustments, one cell may respond by increasing its DNA content to reach the standard amount. The other cell may discard the excess DNA. There must be numerous homeostatic adjust-

ments required of cells. The sensing devices and the signals that initiate these adjustments are beyond our present ability to fathom. A goal for the future would be to determine the extent of knowledge the cell has of itself and how it utilizes this knowledge in a "thoughtful" manner when challenged.

One class of programmed responses to stress has received very little attention by biologists. Here a stress signal induces the cells of a plant to make a wholly new plant structure, and this to house and feed a developing insect, from egg to the emerging adult. A single *Vitis* plant, for example, may have on its leaves three or more distinctly different galls, each housing a different insect species. The stimulus associated with placement of the insect egg into the leaf will initiate reprogramming of the plant's genome, forcing it to make a unique structure adapted to the needs of the developing insect. The precise structural organization of a gall that gives it individuality must start with an initial stimulus, and each species provides its own specific stimulus. For each insect species the same distinctive reprogramming of the plant genome is seen to occur year-after-year. Some of the most interesting and elaborate plant galls house developing wasps. Each wasp species selects its own responding oak species, and the gall structure that is produced is special for each wasp to oak combination. All of these galls are precisely structured, externally and internally, as a rapid examination of them will show.

The galls on roots of legumes that are associated with nitrogen-fixing bacteria are readily available for examination. They illustrate in their own way an example of reprogramming of the plant genome by a stimulus received from a foreign organism. The induction of such reprogrammings by insects, bacteria, fungi, and other organisms that are not a required response of the plant genome at some stage in its life history is quite astounding. But this is no more astounding, it would seem, than the sharing of a single genome by two brilliantly designed organisms, the caterpillar and the moth. It is becoming increasingly apparent that we know little of the potentials of a genome. Nevertheless, much evidence tells us that they must be vast.

Many known and explored responses of genomes to stress are not so precisely programmed. Activation of potentially transposable elements in maize is one of these. We do not know when any particular element will be activated. Some responses to stress are especially signifi-

cant for illustrating how a genome may modify itself when confronted with unfamiliar conditions. Changes induced in genomes when cells are removed from their normal locations and placed in tissue culture surroundings are outstanding examples of this.

The establishment of a successful tissue culture from animal cells, such as those of rat or mouse, is accompanied by readily observed genomic restructuring. None of these animal tissue cultures has given rise to a new animal. Thus, the significance of these changes for the organism as a whole is not yet directly testable. The ability to determine this significance is a distinct advantage of plant tissue cultures. Many plant tissue cultures have developed new plants and, in some instances, many plants from a single initial cell or tissue isolate. A reason for this difference in behavior of animal and plant tissue cultures is not difficult to find. In many animals the germline is set aside early in cleavage, allowing the soma—a dead-end structure—to develop by any means, including genome restructuring and nonreversible programming. In higher plants, each fertile flower has the equivalent of a "germline" in it. The flower makes the gametes and initiates embryo formation. In this regard, consider the many flowers that may be produced by a bush or a tree. Some system must operate to reprogram the genome in those cells of the flower that will produce the gametes and establish the zygote. This implies that the specific programming sequences, earlier initiated and required for flower production, must be "erased" in order to return the genome to its very early state. If this occurs in so many places in a bush or a tree, then it is not surprising that it may occur in a plant cell or a cluster of cells not within a flower. Also, in many plants such resettings are a common means of initiating new individuals from somatic cells. In these instances, however, the process of resetting is regulated, and the genome is not permanently restructured. This is not true for plants arising from many tissue cultures. The treatment, from isolation of the cell or cells of a plant, to callus formation, and then to production of new plants from the cells of these calluses, must inflict on the cells a succession of traumatic experiences. Resetting of the genome, in these instances, may not follow the same orderly sequence that occurs under natural conditions. Instead, the genome is abnormally reprogrammed or decidedly restructured. These restructurings can give rise to a

wide range of altered phenotypic expressions. Some of the altered phenotypes are readily observed in the newly produced plants themselves. Others appear in their progeny. Some initially displayed altered phenotypes do not reappear in the progeny. Their association with genomic change remains problematic. Other altered phenotypes clearly reflect genomic restructuring, and various levels of this have been observed. It may be safe to state that no two of the callus derived plants are exactly alike, and none is just like the plant that donated the cell or cells for the tissue culture. The many levels of genomic modification that already are known and expressed as changed genotypes and phenotypes could be potent sources for selection by the plant breeder, and incidentally, for theoretical ponderings by the biologist.

Modifications in gene expression may be induced in plants when they are infected with an RNA virus. Instances of this may be detected merely by viewing infected plants in the field. For example, patterns of anthocyanin pigment distribution, normally highly regulated and prominently displayed in the flowers of a plant, may appear grossly distorted in those parts of a plant that clearly reveal the virus infection. Recently, it was learned that infection of maize plants with barley stripe mosaic virus, an RNA virus, may traumatize cells to respond by activating potentially transposable elements. These, in turn, may then enter a gene locus and modify its expression (25). Such changes in expression of known genes may be exhibited in the self-pollinated progeny of infected plants. More often they are detected in later generations. Yet, no virus genome has been detected in the immediate progeny of infected plants or in those plants shown to have a transposable element newly inserted at a known gene locus.

Species crosses are another potent source of genomic modification. Plants have provided many excellent examples of this. The advantage of plants is the ease of making crosses to obtain hybrids, the simplicity of growing them, the ready availability of their chromosomes, and the ability to obtain progeny in quantities, if necessary. The alterations produced when the genomes of two species are combined reflect their basic incompatibilities. Evidence of this is the appearance of the same types of genome change whenever the same two species are crossed. Expressions of incompatibilities do differ, but their nature is always in accordance with the particular

two species whose genomes are combined. The genus Nicotiana has a large number of species that differ from each other in chromosome number, chromosome organization, and phenotypic expressions. Genome incompatibilities have been observed in a large number of two-by-two combinations of species. An illustration is the behavior of chromosomes in the hybrid plant produced by the cross of N. tabacum by N. plumbaginifolia. The chromosomes of plumbaginifolia are lost during development of this hybrid plant. Although whole chromosome losses appear to be common, other irregularities in chromosome behavior also occur. These are chromosome fragments, chromosome bridges in somatic anaphases, and the appearance in an occasional metaphase plate of a single, very much elongated chromosome, termed a "megachromosome." The presence of one or two such hugely elongated chromosomes, in some somatic metaphase plates, characterizes the hybrid derived from the cross of N. tabacum × N. otophora. In this instance it is known that heterochromatic segments in chromosomes of the otophora set contribute to these linear amplifications (26, 27). Hybrids produced by crosses of distantly related Nicotiana species are known to give rise to tumors, some of which resemble teratomas. In one instance it was shown that tumor production relates to a single heritable modification which was initiated in the hybrid (28, 29).

Major restructuring of chromosome components may arise in a hybrid plant and continue to arise in its progeny, sometimes over successive plant generations. The restructuring may range from apparently simple to obviously complex. These are associated with translocations, inversions, deficiencies, duplications, and the like, which are simple in some instances or variously intercalated in others. New stable or relatively stable "species" or "genera" have been derived from such initial hybrids. The commercially useful plant, Triticale, is an example. Wheat (Triticum) and rye (Secale) were crossed and the combined set of chromosomes was doubled to provide reproductive stability. Nevertheless, this genome was not altogether stable. Selections continued in later generations for better performances with considerable success, even though instabilities were not eliminated altogether. Some species of Triticum undoubtedly arose by a comparable mechanism as that outlined for Triticale, and different related genera made their contribution to some of these

Triticum species. Evidence for this is exceptionally clear (*30*).

Undoubtedly, new species can arise quite suddenly as the aftermath of accidental hybridizations between two species belonging to different genera. All evidence suggests that genomic modifications of some type would accompany formation of such new species. Some modifications may be slight and involve little more than reassortments of repetitious DNA's, about which we know so little. (The adjective "slight" refers to the apparent simplicity of the restructuring mechanism rather than the significance of its consequences.) Major genome restructuring most certainly accompanied formation of some species. Studies of genomes of many different species and genera indicate this. Appreciation of the various degrees of reassortment of components of a genome, that appear during and after various types of genome shock, allows degrees of freedom in considering such origins. It is difficult to resist concluding that some specific "genome shock" was responsible for origins of new species in the two instances described below.

The organization of chromosomes in many closely related species may resemble one another when viewed by light microscopy. Only genetic and molecular analyses would detect those differences in their genomes that could distinguish them as species. In some instances of this type distinctions relate to the assortment of repetitious DNA's over the genome, as if a response to shock had initiated mobilities of these elements. In other instances, distinctions between related species are readily observed at the light microscope level, such as polyploidizations that are common in plants, or amplifications of DNA that do not alter chromosome number or basic metaphase morphologies. Others relate to chromosome fusions or fragmentations, or readily observed differences in the placement of specific DNA segments. There are many descriptions of differences in chromosome organization among the species of a genus. Two instances of these latter differences warrant special consideration here, because the observed differences in chromosome organization suggest origins from a response to a single event. One response gave rise to extensive fusions of chromosomes. The other placed heterochromatic segments at new locations within the chromosomes of the set.

That such multiple chromosome changes may relate to some initial event, occurring in a cell of the germline, is proposed and defended in a review by King (*31*). An example that would fit his proposal is the organization of chromosomes of the Indian muntjac deer (*Muntiacus muntjak*) (*32*) when compared with its closely related species, *M. reevesi*, the Chinese muntjac. The latter species has 46 chromosomes as the diploid number, whereas the Indian muntjac has 6 chromosomes in the female and 7 chromosomes in the male, and these chromosomes are huge in comparison with those of the Chinese muntjac. Observations of the chromosomes in the hybrid between these two species strongly supports chromosome fusion as the mechanism of origin of the reduced number and huge size of the Indian muntjac chromosomes (*33*). In general, evidence of fusion of chromosomes is plentiful. When two or three chromosomes of a set appear to have arisen by fusion, the question of simultaneous or sequential events responsible for these fusions cannot be determined with certainty. In the case of the Indian muntjac, it is difficult to avoid the conclusion that the fusions of so many chromosomes resulted from some initial shocking event that activated a fusion mechanism already known to apply to fusions of individual chromosomes in many other organisms (Robertsonian translocations). Whatever the cause, the changed chromosome organization is stunning.

Another stunning example of differences in chromosome organization between species is reported by Beermann in an extraordinarily thorough and fascinating account (*34*). This report describes the chromosome organization in three species of the copepod genus *Cyclops*. The main differences among them to be considered here relate to distributions of conspicuous heterochromatic blocks in the chromosomes of each species. In one species, these blocks are confined to the ends of chromosomes. In another species, blocks of heterochromatin are at the ends of chromosomes, but also positioned to each side of the centromere. In the third species, blocks of heterochromatin are distributed all along the chromosomes. An additional feature of this heterochromatin is its unchanged presence in cells of the germline in contrast to its elimination at cleavages specific for each species and in cells destined to produce the soma. The elimination process is associated with formation of rings of DNA cut from the heterochromatin (*35*). Again it is difficult to avoid concluding that these distinctive distributions of heterochromatin relate to unusual and disturbing events, and that these events have activated mechanisms that can redistribute heterochromatin to specific sites.

Concluding Statement

The purpose of this discussion has been to outline several simple experiments conducted in my laboratory that revealed how a genome may react to conditions for which it is unprepared, but to which it responds in a totally unexpected manner. Among these is the extraordinary response of the maize genome to entrance of a single ruptured end of a chromosome into a telophase nucleus. It was this event that, basically, was responsible for activations of potentially transposable elements that are carried in a silent state in the maize genome. The mobility of these activated elements allows them to enter different gene loci and to take over control of action of the gene wherever one may enter. Because the broken end of a chromosome entering a telophase nucleus can initiate activations of a number of different potentially transposable elements, the modifications these elements induce in the genome may be explored readily. In addition to modifying gene action, these elements can restructure the genome at various levels, from small changes involving a few nucleotides, to gross modifications involving large segments of chromosomes, such as duplications, deficiencies, inversions, and other more complex reorganizations.

It was these various effects of an initial traumatic event that alerted me to anticipate unusual responses of a genome to various shocks it might receive, either produced by accidents occurring within the cell itself, or imposed from without, such as virus infections, species crosses, poisons of various sorts, or even altered surroundings such as those imposed by tissue culture. Time does not allow even a modest listing of known responses of genomes to stress that could or should be included in a discussion aimed at the significance of responses of genomes to challenge. The examples chosen illustrate the importance of stress in instigating genome modifications by mobilizing available cell mechanisms that can restructure genomes, and do so in quite different ways. A few illustrations from nature are included because they support the conclusion that stress, and the genome's reactions to it, may underlie many formations of new species.

In the future, attention undoubtedly will be centered on the genome, with greater appreciation of its significance as a highly sensitive organ of the cell that monitors genomic activities and corrects common errors, senses unusual and unexpected events, and responds to them, often by restructuring the genome. We

know about the components of genomes that could be made available for such restructuring. We know nothing, however, about how the cell senses danger and instigates responses to it that often are truly remarkable.

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The Population Factor in Africa's Development Dilemma

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Africa, south of the Sahara, consists of 45 independent majority-ruled states. These range in size from small island states like Sao Tome and Principe and Seychelles, with populations of 86,000 and 66,000, respectively, to large countries with relatively sparse populations like Sudan, with a land area of 2.5 million square kilometers and a population of about 19 million, to densely populated countries like Nigeria, with a land area of 924,000 km² and a population of 85 million (Fig. 1). The region as a whole is the least developed of the world's inhabited geographical areas. Twenty-three of the 35 least developed countries are in Africa. Despite years of intensive efforts, many of these countries appear to be standing still or even going backward when several accepted indexes of development such as gross national product, education, infant mortality, general health status, and industrial output are considered.

Demographic Features

Africa, south of the Sahara, with an estimated population in 1983 of 400 million, has the fastest growing population in the world. The average growth rate is about 3 percent per year, with a range of 1.5 percent in Gabon to 4.1 in Kenya (1). The region is growing at a faster rate than the historical peaks achieved in Europe during its most rapid growth.

Summary. Despite several decades of international effort, the development problems of sub-Saharan Africa remain acute. By many of the quality of life indexes, the majority of African countries are standing still or moving backward. A rapidly growing population with an adverse dependency ratio places demands on services that present development strategies cannot satisfy. A reorientation of development is necessary to establish realistic population policies and to implement comprehensive family planning programs.

The crude birth rates are between 40 and 52 per 1000, and the crude death rates from 10 to 18 per 1000 (1). While mortality has been declining slowly, particularly during the 1960's and 1970's, the fertility rates are remaining fairly constant or even showing slight rises in some countries. In a few countries, with problems of sub- and infertility (Zaire, Gabon, and the Central African Republic, for example), the potential for increases in the fertility rates in the short term is high. The populations are rather young: 20 to 22 percent are under 5 years, and 45 to 50 percent are under the age of 15. The dependency ratio is there-fore very high and contrasts sharply with the ratios of the more developed countries (Table 1). The demographic profile suggests some immediate development needs, including education for the ever increasing numbers of children, more and improved health services, particularly those for mothers, infants, and young children.

Vital statistics in Africa are rather poor, and planners must depend on survey data, figures from a few registration centers in each country, or on pure guesses. However reliable they may be, the available data suggest a rather poor state of affairs. Infant mortality, with few exceptions (Kenya and some urban centers), averages more than 100 per 1000 live births. There are some countries or remote rural areas in which the figures are between 200 and 250 per 1000. Infant mortality rates have been found to be a rather sensitive index to the level of general development of a community and valuable as a measure of the quality of life. Infant mortality rates in Africa are about 5 to 20 times those for the more advanced countries. Young child (1 to 4 years) mortality in Africa ranges between 25 and 30 per 1000 (2); European and North American rates are usually

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