CHROMOSOME ABERRATIONS AND MUTATIONS IN NATURE

(Miss) SUSAN THOMAS

Botany Department, Kerala University, Trivandrum

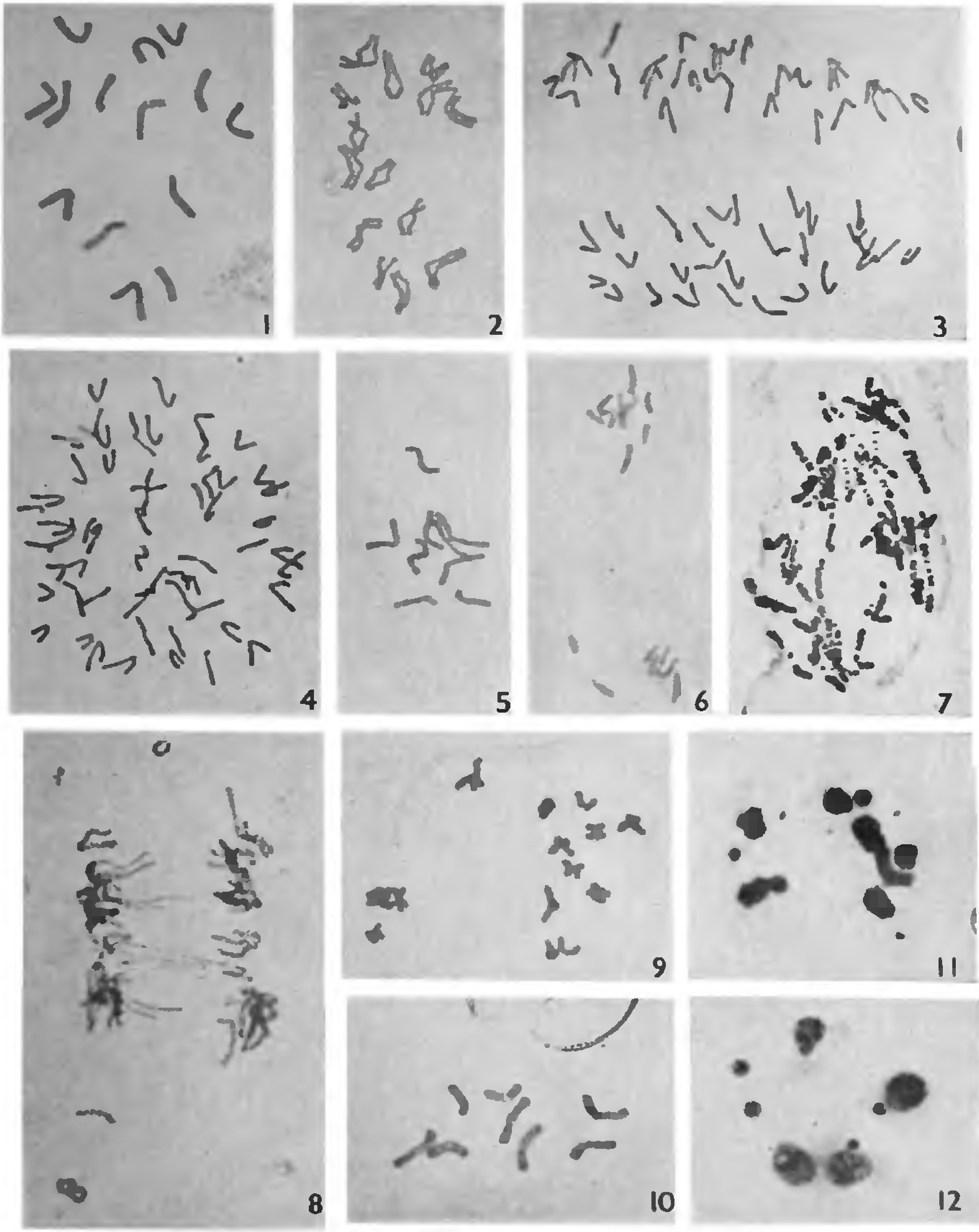
Spontaneous occurrence of gene mutations and structural changes as well as numerical variation of chromosomes have been observed in nature. But the exact factors responsible for these changes have not been fully understood. In certain investigations on artificial induction of mutations conducted during the last three years it has been found that decaying organic substances normally used in agricultural practice, like compost and oil-cakes, have a marked effect on the structure and behaviour of chromosomes when applied in certain high concentrations.

In the course of their studies on mutation, Swaminathan and Natarajan[4] found that immersion of seeds in vegetable oils can cause breakage in chromosomes. The cytological and genetical effects of certain vegetable oils have been more fully reported on recently by them. They have suggested that the unsaturated fatty acid components constitute the probable mutagenic fraction of the vegetable oil. Studies on the effect of oils carried out in this laboratory have not only confirmed their cytological observations, but have also shown that Bassia oil (from seeds of *Bassia latifolia* Roxb., family Sapotaceæ) is capable of inducing these changes very effectively in plants (unpublished). It has also been observed that oil-cakes are capable of inducing similar changes. Oil-cakes from which all traces of oil have been removed by Soxhelt method, and garden compost made from leaves and animal refuse have been found to produce the same effect. The fact that deoiled cakes and compost when mixed with sand in certain high concentrations produce these changes goes to show that there are other factors operative in bringing about these changes besides the constituents of the oil.

Experiments showed that very high concentrations of Bassia oil-cake (1 oil-cake : 5 sand) inhibited growth of the plant. Similar results were obtained with groundnut-cake, castor-cake and gingelly oil-cake, though the concentrations needed for effecting various types of chromosomal changes varied with the oil-cake used. Such inhibition in growth was not observed in the case of compost even when used without mixing with sand. The cytological effects induced by oil-cakes and compost have been studied in detail in *Chlorophytum heyneanum*, *Allium cepa*, *Trigonella fœnum-grœcum* and *Typhonium flagelliforme*. The plants were allowed to grow in pots containing varying concentrations of any one oil-cake or compost mixed with sand. An equal number of plants grown in pure sand or ordinary soil was kept as controls. The effect of vegetable cakes and compost on the process of cell division appears to be almost similar in all these cases.

Cell division was normal in the root-tips of control plants, and in the case of *Chlorophytum heyneanum* 14 chromosomes were observed (Fig. 1). In the treated plants the chromosomes in many cells do not form a metaphase plate, but are scattered throughout the cells. They split and often lie parallel to one another as in materials treated with C-mitotic agents (Fig. 2). But unlike in typical C-mitosis, where the centromere division is delayed,[2] in this case the centromeres divide at about the same time or slightly earlier as indicated by the diverging of the daughter chromosomes from each other at this region only. It may be pointed out that according to Schrader[3] a spindle consists of two elements, an external spindle which arises by the activity of the centrosome or other polarising factors and an internal one formed in close connection with the centromere. The action of colchicine and other C-mitotic agents has been regarded as inactivating both the external and internal spindles (Levan,[2] Barber and Callan[1]). It seems that an inactivated external spindle alone produces the type of chromosome divisions as observed in Fig. 2. The formation of a restitution nucleus after such doubling gives rise to tetraploid nuclei (Fig. 3). Frequently this process is repeated and giant polyploid cells are formed (Fig. 4). The occurrence of multipolar and partially active spindles and somatic reduction (Fig. 5) may be responsible for the production of numerous aneuploid cells in the treated plants (Fig. 6). Considerable erosion of chromatin and fragmentation of chromosomes have also been observed in many cells (Fig. 7). Breakage of chromosomes followed by fusion usually results in the formation of ring chromosomes, chromatid bridges and acentric fragments at anaphase (Fig. 8).

The frequency of aberrations induced by Bassia cake in root-tip cells of onion and their



FIGS. 1-12. (× 750). Fig. 1. Somatic metaphase in the control plant of *Chlorophytum heyneanum* ($2x = 14$). Figs. 2-5 and 9-12 from *C. heyneanum* grown in compost unmixed with sand. Fig. 2. Chromosome doubling in root-tip cell showing early splitting of the centromere region. Fig. 3. Anaphase in root-tip cell showing doubled number of chromosomes ($4x = 28$). Fig. 4. A polyploid cell ($8x = 56$). Fig 5. Metaphase in root tip cell showing 9 chromosomes instead of 14. Fig. 6. Somatic segregation in root-tip cell of *Typhonium flagelliforme* grown in compost unmixed with sand. Figs. 7 and 8 from *Allium cepa* grown in Bassia cake and sand (1 : 15). Fig 7. Root-tip cell showing fragmentation of chromosomes and erosion of chromatin. Fig. 8. Anaphase in root-tip cell showing chromatid bridges, ring chromosomes and acentric fragments. Fig. 9. Metaphase II in a PMC showing the unreduced number of chromosomes. Fig. 10. Pollen mitosis showing 8 chromosomes instead of the normal 7. Fig. 11. Diakinesis in a PMC showing fragments. Fig. 12. Tetrad stage in PMC showing four micronuclei.

relative frequency at various concentrations of the substance in the soil are recorded in Table I

TABLE I

Frequency of cells showing chromosome changes at metaphase in roots of onion treated with varying concentrations of Bassia cake in sand

Nature of treatment	Total cells analysed	Number of normal cells	Number showing fragments	Number showing 'C-metaphase'	Number showing polyploidy	Number showing somatic segregation	Number of aneuploid cells	Percentage of cells showing aberrations
Bassia cake and sand (1 : 10 by weight) ..	154	106	16	20	4	7	1	31·2
(1 : 15 ") ..	264	198	20	22	4	8	12	25·0
(1 : 20 ") ..	148	116	18	5	1	6	2	21·6
(1 : 25 ") ..	242	190	16	14	6	6	10	21·5
(1 : 30 ") ..	127	111	8	2	..	4	2	12·6
(1 : 35 ") ..	248	236	5	3	..	2	2	4·8

It may be noted that the frequency of aberrations tends to increase approximately in direct proportion to the increase in the concentration of oil-cake in soil. In concentrations above 1 : 10 (by weight) mitosis is totally inhibited and no roots are produced. The effect of duration of treatment on the frequency of aberrations induced in *Chlorophytum heyneanum* grown in compost unmixed with sand is shown in Table II.

TABLE II

Frequency of cells showing chromosome changes at metaphase in root-tip cells of Chlorophytum heyneanum *grown in compost unmixed with sand for different periods*

Duration of treatment	Total cells analysed	Number of normal cells	Number showing polyploidy	Number showing 'C-metaphase'	Number showing somatic segregation	Number showing fragments	Number of aneuploid cells	Percentage of cells showing aberrations
1 Month ..	104	55	32	8	1	3	5	47·1
2 Months ..	148	105	17	9	1	5	11	29·1
3 " ..	131	98	18	3	4	2	6	25·2
4 " ..	138	110	8	6	6	4	4	20·3
5 " ..	250	215	10	8	6	5	6	14·0
6 " ..	264	230	14	6	4	4	6	12·9
7 " ..	218	200	2	..	8	2	6	8·3

It can be seen that there is a gradual decrease in the percentage of aberrations observed as the period of treatment increases, this relation being found in the pollen mother cells also. This decrease can probably be attributed to the lowering of the nitrogen content of the compost, or the decrease in the concentration of any other substance causing these aberrations.

During meiosis, non-disjunction at anaphase I followed by the formation of a single interphase nucleus leads to the formation of cells with unreduced number of chromosomes at metaphase II (Fig. 9). Irregular distribution of chromosomes at anaphase I and II results in pollen with varying chromosome numbers (Fig. 10). Fragmentation of chromosomes (Fig. 11), inversion leading to the formation of chromatid bridges and fragments at anaphase, translocation, cytomixis, formation of dyads at the tetrad stage, clumping of chromosomes, etc., were observed in several cells. The presence of one or more micronuclei is a frequent feature in these plants (Fig. 12). All these abnormalities result in the production of an appreciable proportion of sterile pollen.

In another investigation using nitrogenous fertilizers like ammonium sulphate and urea, it has been found that solutions of these substances with nitrogen content varying from 0·15 to 0·02% produced almost identical results as regards chromosomal aberrations. It seems possible therefore that in oil-cakes and compost also the active agent in producing aberrations may be the nitrogen present in these substances. In the absence of adequate amounts of other essential elements like phosphorus and potassium, high levels of nitrogen exert a toxic effect on growth. This is borne out by the fact that plants grow well in compost even when it is not mixed with sand, while growth is inhibited when they are planted in a mixture of sand and oil-cakes or nitrogenous fertilizers containing the same level of nitrogen as in pure compost of the type used in the present study. The extent to which other nutrients interfere with nitrogen in affecting chromosomal aberrations are being investigated.

The present investigation has clearly shown that decaying organic matter when present in soil in high concentrations can induce chromosomal mutations of different magnitudes. While all chromosomal changes need not lead to viable mutations, the evidence from various studies and observations in this laboratory also show that some of them at least pass on to the next generation through vegetative as well as sexual reproduction. This indicates a partial answer to the wide occurrence of mutations in nature.

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1. Barber, H. N. and Callan, G. H., *Proc. Roy. Soc.*, London, 1942, **131 B**, 258.
2. Levan, A., *Hereditas*, 1938, **24**, 471.
3. Schrader, F., *Mitosis*, New York, 1944.
4. Swaminathan, M. S. and Natarajan, A. T., *Curr. Sci.*, 1956, **25**, 382.
5. — and —, *Jour. Hered.*, 1959, **50**, 177.

ECHO-I—THE US BALLOON SATELLITE

THE United States National Aeronautics and Space Administration (NASA), launched successfully on August 12, 1960, the largest balloon satellite *Echo-I*, in an experiment designed to test the feasibility of global communications by satellite reflections.

Echo is a 100-ft. sphere of aluminized plastic mylar, 0·0005" thick, and weighs about 125 lb. It was rocketed aloft deflated and packed in a 26½" canister in the nose cone of a Thor-Delta rocket. Upon ejection from the launcher, the two hemispheres of the canister separated and the plastic sphere was released free. Crystals of a subliming solid that had previously been injected into the sphere inflated the balloon; the sublimation process was to continue for a month or so to compensate for micrometeorite punctures.

The *Echo* was programmed to orbit 1000 miles up at 16000 miles per hour. Initially the orbit is inclined at 47° to the equator, and the period is 118·2 minutes. The orbit is nearly circular with a perigee distance of 1530 km. and an apogee distance of 1690 km.

Communications *via* the satellite were established during *Echo's* second revolution round the earth. The linking stations are Bell Telephone Laboratory's Station at Holmdel, N. J., and the Radio Astronomy Station at Goldstone, Calif. A special horn-reflector antenna recently completed at Holmdel is the eastern terminus of the satellite link. Goldstone is using its 85-ft. dish at the western terminus. Transmission frequency is 960 Mc westbound, about 2·39 Gc eastbound. F-m is used with a deviation of ± 150 Kc. at the highest modulation frequency of 3 Kc. *Echo* thus serves as a first step towards a worldwide communications system which would be independent of ionospheric disturbances. According to NASA report the communications satellite *Echo* is expected to remain in orbit for about a year.