From these observations, it is evident that the oocytes have the ability to differentiate and are more prone to oncogenesis, whereas the spermatogonia tend to show rapid proliferation to give rise to nullipotent cells or organoid formations mimicking the early blastoderm, suggesting a directional differentiation. As tumor cells mimic and retain the properties of the cells of origin, it can be concluded from this study, that the ovum contributes the differentiating functions while the spermatozoon initiates organisation and proliferation in the zygote. The differing properties of the germ cells are related to their location within the genital ridge and to the local interactions and feedbacks.

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Orange II induced cytogenetical changes in albino mice

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Summary. Daily feeding of the common food color Orange II to mice in doses of up to 3.0 g/kg b.wt for 180 days had deleterious effects on somatic and spermatogonial chromosomes. The chromosomal abnormalities induced were breaks, gaps, constrictions, centric fusion, fragments of unknown origin, translocation, deletion, stickiness, ring chromosomes, pyknosis and other bizarre configurations.

Food colors are used extensively in a variety of ways to make food more appealing and acceptable to the consumer. Orange II [Monoazo: C.I. Acid orange 7 (15510)] is the sodium salt of p-[(2-hydroxy-1-naphthyl) azo] benzene sulphonic acid and has been toxicologically classified under the category C II by the Joint FAO/WHO Expert Committee on Food Additives[2], indicating that virtually no information on long term toxicity of this color is available. It is most commonly used in bakery products, beverages, ice cream, soft drinks, confectionery, milk products, sausages, snack foods, pet foods, cereals and multicolored medicinal tablets.

40 male ICR/Swiss mice (average weight, 20–25 g; age, 6–8 weeks old) were used for the study. They were divided into 4 groups and each group was force-fed orally with 0.0 (control), 0.1, 0.5 and 3.0 g/kg b.wt Orange II (superior

Table 1. Effect of Orange II feeding on the bone marrow cells of male mice as shown by chromosomal abnormalities

Sample No.	Treatments	Normal cells	Abnormal cells (=%)	Type of abnormality						
				Breaks	Fragmentation	Centric-fusion	Translocation	Deletion	Ring-formation	Short, thick, stumpy
1	Control mice fed with ordinary lab chow	98	2	1	–	1	–	–	–	–
2	Mice fed with 0.1 g/kg b.wt Orange II	99	1	–	–	1	–	–	–	–
3	Mice fed with 0.5 g/kg b.wt Orange II	98	2	1	–	–	–	–	–	1
4	Mice fed with 3.0 g/kg b.wt Orange II	81	19	1	2	10	1	1	2	2

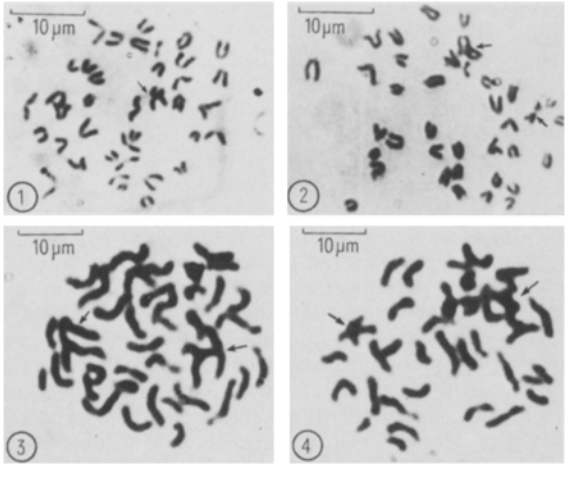
Table 2. Effect of Orange II feeding on the meiotic cells of male mouse as shown by chromosomal abnormalities

Sample No.	Treatments	Normal cells	Abnormal cells (=%)	Type of abnormality Breaks	Fragmentation	Fusion	Pyknosis	Stickiness	Pulverization	Bizarre configuration
1	Control mice fed with ordinary lab chow	98	2	1	1	–	–	–	–	–
2	Mice fed with 0.1 g/kg b.wt Orange II	98	2	–	1	–	–	–	1	–
3	Mice fed with 0.5 g/kg b.wt Orange II	99	1	–	1	–	–	–	–	–
4	Mice fed with 3.0 g/kg b.wt Orange II	58	42	7	8	7	10	3	5	2

quality, procured from M/s. Dadajee Dhakjee & Co., Vugadi, Bombay, India). Each dose was dissolved in 1 ml water and the solution was administered forcibly by opening the mouth with the help of blunt end droppers, or the color was mixed into ground feed and small pellets of the feed were force-fed to the mice. They consumed 10–15 g rat feed (procured from M/s. Hind Lever Ltd, Bombay, India) daily, and tolerated the force-fed doses of the food dye. Water was given ad libitum. Temperatures were 24 ± 1 °C, and the mice were kept under normal conditions.

After 180 days of feeding, no change was observed in the body weights. Those mice which were fed with 3.0 g/kg b.wt Orange II were lethargic compared to controls. Mice were killed by cervical dislocation. Upon biopsy, liver and spleen were enlarged. All other organs appeared normal.

Colchicine, an inhibitor of mitotic spindle formation was injected into the mice at 0.5 mg/kg b.wt in order to arrest and accumulate the chromosomes of somatic and meiotic cells, 6 h before killing through the i.p. route. Bone marrow cells were collected from the femur and meiotic cells from the seminiferous tubules. Both were put into a prewarmed (37 °C) hypotonic solution of potassium chloride for 10 min. Both types of cells were fixed in methanol and acetic acid in the ratio of 3:1. Dispersion and spreading of chromosomes was accomplished by the air-drying flame method. Staining was done using the May-Grunwald Giemsa stain[3]. Photomicrographs were taken with the help of a Leitz Panphot camera.



Somatic and spermatogonial metaphase chromosomes. Figure 1. 2 chromosomes showing centric fusion (arrows), probably a dicentric chromosome. Figure 2. Somatic chromosomes showing fusion in various ways. Figure 3. Bizarre configuration of the chromosomes at metaphase. Figure 4. Spermatogonial chromosomes showing centric fusion, fusion at various places, translocation and deletion.

Study of the bone marrow cells revealed 40 chromosomes in controls, all acrocentric. Mice fed with 0.0, 0.1 and 0.5 g/kg b.wt Orange II showed 1–2% chromosomal abnormalities but mice fed with 3.0 g/kg b.wt Orange II showed 19% chromosomal abnormalities with breaks, gaps, fragmentation, centric fusion, translocation, deletion, ring formation and sometimes short, thick and stumpy chromosomes (figs 1 and 2).

Chromosomes at the spermatogonial metaphase in controls appeared as highly condensed and discrete units. (fig. 1). The diploid number of 40 chromosomes was easily detectable. Mice fed with higher doses of Orange II displayed 42% chromosomal abnormalities in the form of breaks, gaps, fragmentation, pulverization, joining in various ways, pyknosis, stickiness and bizarre configurations (figs 3 and 4). A detailed analysis of the various types of chromosomal abnormalities induced by Orange II is summarized in tables 1 and 2.

Various researchers have investigated chromosomal abnormalities induced by insecticides[4,5], drugs[6,7], phenolic compounds[8], metals[9], preservatives[10], antioxidants[11], artificial sweeteners[12] and chemosterillants[13] but such a study of food colors has not yet been done. It was on these grounds that the present study was undertaken.

The kinetic response of the testis to Orange II in rats has been studied by Singh and Khanna[14], indicating testicular degeneration. Hematological studies by Prasad and Rastogi[15] revealed occurrence of acute lymphocytic leukaemia after feeding this colour for 90 days. A histopathological study showed testicular degeneration also. No cytogenetic reports were, however, available on this color. It was thus thought proper to study the toxicity effects of this common food color on somatic and spermatogonial chromosomes.

It is well known that centric fusions and dissociations lead to chromosomal breakages and joinings while cellular degeneration leads to stickiness and fragmentation of chromosomes. Normal disjunction of the chromosomes during

cell division is disturbed by breakage, fusion and pyknosis. It is clear that these changes in the chromosomes result from alterations in the synthesis of DNA and RNA. Inhibition of DNA synthesis leads to pyknosis while chromosomal breaks and acentric fragments are caused by RNA metabolism due to Orange II feeding. Actually, protein synthesis is first affected by this chemical, in turn altering the DNA synthesis of the chromosomes.

Although at low concentrations this food color does not produce any significant chromosomal abnormalities, at the high concentration of 3.0 g/kg b.wt, many chromosomal abnormalities were induced. Other pathological studies including histopathology and hematology and further tests to evaluate the mutagenicity of this chemical are in progress.

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Reticulate evolution and cladism: Tests for the direction of evolution

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Summary. Reticulate evolution between ancestral-descendant lineages can be critically tested for by investigating the relationship between out-group and mid-point roots on numerically derived cladograms. The western grass-snake provides a worked example for the theoretical test.

Numerical cladistic techniques are used to study supraspecific affinities but it could be argued that reticulate evolution renders a cladistic approach to intraspecific affinities inappropriate. Wagner cladograms[1] are a widely used numerical cladistic method. Initially a Wagner network is obtained by computing the most parsimonious connection between taxa. Conversion to a tree or cladogram is then achieved by mid-point or outgroup rooting[1].

This paper suggests tests for detecting reticulate evolution based on statistical investigations of the patristic distance between the mid-point and out-group roots of Wagner trees to decide the correct direction of evolution. Analysis of a test case indicates that reticulate evolution can be detected by these tests and does not perturb Wagner networks. It does, however, perturb the root required for conversion to a tree. Consequently, prior to this conversion, one should test for reticulate evolution and if necessary find the correct roots.

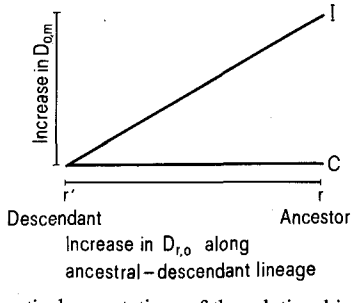

Figure 1. Theoretical expectations of the relationship between $D_{o,m}$ and $D_{r,o}$ at any given cladistic level in an ancestral-descendant lineage if time of divergence is well correlated to extent of divergence. C is the expection if the putative root (r) is correct whilst I is the expectation if evolution is in the opposite direction and r' is the correct ancestral root. See text for explanation.

When these tests on the direction of evolution are clearly decisive one can confirm that the cause of the pattern of population affinities is phylogenesis rather than adaption to current environmental gradients. How then can one test for reticulate evolution?

If the extent of patristic divergence is well correlated to the time of divergence the mid-point root (m) and the out-group root (o) on any one cladistic level of a Wagner tree will tend to coincide. Consequently, in an ancestral to descendant lineage the patristic distance (D) between the outroot and the midroot ($D_{o,m}$) will tend to zero at any cladistic level and will tend not to be highly correlated with the patristic distance ($D_{r,o}$) between the putative root (r) of the total lineage and the outroot at the various levels. This is illustrated as line C in figure 1 where the hypothesized direction of evolution ($r \rightarrow r'$) is correct.

If however the putative root r is erroneous (and if in fact r' is the correct ancestral root) then $D_{o,m}$ should be highly inversely correlated with $D_{r,o}$ and $D_{o,m}$ should only tend to zero at r'. In other words when the direction of evolution is incorrect the further one is from the real root of the lineage (in toto) then the greater the distance between the outroot and midroot at a given cladistic level. The outroot and midroot should only coincide at r' where $D_{o,m}$ should obviously tend to zero irrespective of whether the direction of evolution is correct. This is line I in figure 1 where the hypothesized direction of evolution ($r \rightarrow r'$) is incorrect.

If the extent of divergence is not well correlated to time of divergence $D_{o,m}$ may not tend to zero and there may be a significant regression of $D_{o,m}$ against $D_{r,o}$. Nevertheless the