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Review Article

Structural and Functional stability and asymmetry among organelles and Organs Appear Regulated by The Specific Genes in Stem cells. I. Emanation of Chromatin From Chromosomes is Also an Inherent Genomic Mechanism Among Eukaryotes

Hit. Kishore Goswami[1]*

[1]Department of Botany & Genetics, Retired Professor, Bhopal, Madhya Pradesh, India

***Corresponding Author:** Hit. Kishore Goswami, Department of Botany & Genetics, Retired Professor, Bhopal, Madhya Pradesh, India

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Abstract

The release of chromatin dots from various chromosomes from within the cells has been observed several times over five decades; sometimes even the detaching fibril has been clearly observed. Mitotic and meiotic cells observed from plants, animals and human lymphocyte cultured cells have confirmed our consistent observations. Earlier we had named these chromatin emanations as marker dots. Plants of a few species of Ophioglossum L, Isoetes L and some ferns in natural populations have been found to consistently possess “marker dots” (? Microchromosomes; 2 to 3 micron in size) in many cells of some species. A few chromatin dots of variable sizes were also reported in cells of brain tumour (medulloblastoma) tissues studied during the 1970s. These chromatin dots remain within the new cells even after several cell divisions, obviously due to passing through meiotic barriers and moving with anaphasic separations in mitotic and meiotic cell divisions.Furthermore, release of chromatin dots is often related to phenotypic as well as genotypic expression. Not only plants which show chromatin dots and exhibit some rare and abnormal features, but also human cultured lymphocytes revealed positive correlation with some or the other pathological conditions. Many new traits suddenly appearing in some plants resemble exactly those features which were once present in the phylogenetic history dating several million years.. Certainly, chromatin emanation from some or the other chromosome (s) appears a natural, evolutionarily conserved epigenetic molecular activity of specific genes housed within stem cells.. These observations suggest that there must have been some very specific stem cells inherently present since the origin of “Basic Eukaryotic genome” whose functions and specific controlled regulation has remained unaltered. Obviously, structural and functional organization of organelles and organs are fundamentally controlled by the polygenic system localised within specific stem cells. Earlier hypothesis of the author that the DNA molecule ever since evolved, is ceaselessly replicating and is being randomly distributed, becomes justified because chromatin emanation, fission and fusion are the backbone events of all kinds of chromosomal evolution among genomes

Keywords: Chromatin emanation from chromosomes, Expelled chromatin dots and abnormal features; Role of stem cells; chromatin emanation in Eukaryotes; Pathological conditions in man; Evolutionary epigenetics, Concept of the basic eukaryotic genome

Introduction

The release of chromatin dots from various chromosomes from within the cells has been observed several times over five decades; sometimes even the detaching fibril has been clearly observed. Mitotic and meiotic cells observed from plants and animals and human lymphocyte cultured cells have confirmed our consistent observations [1-2-3-4]. This communication lays special emphasis on microchromosomes in plants and more so among those species which

are exposed to variable adaptive strategies. Furthermore, it is also hypothesized that these chromatin dots are precursors of B chromosomes [5-6] certain chromatin dots being emanated from chromosomes are larger in size more than 3 or even 4 micron; [7]. Such dots are retained within cells las has been proved by [8] among vertebrates. According to them, microchromosomes are building blocks of bird, reptile and mammal chromosomes.

Such chromatin dots are often seen in variable numbers but are not present in all cells of the plant. I have critically observed the presence of these chromatin dots (? microchromosomes) regularly in mitotic as well as meiotic cells of a few species of Ophioglossum and some other plants [4-9]. Expelled chromatin dots of variable sizes have often been observed to be present in mitotic as well as meiotic cell divisions in a few more recently discovered plant species hence it is becoming worthwhile to consider them as microchromosomes.Initially, chromatin dots range in size but congregation of some microchromosomes can not be ruled out as we have even observed presence of new chromosomes without microchromosomes or larger chromatin dots or fragments. Sometimes chromatin structures emanated from chromosomes in human cells are large in size (3 to 4 micron) and these are being designated as small supernumerary chromosomes [10-11-12] Many workers by modern molecular cytogenetic studies [13-14-15] have identified the original abode of these structures in respective chromosomes. Certain chromatin dots being emanated from chromosomes are larger in size more than 3 or even 4 micron; [7].

Such dots are retained within cells has been proved by [8] among vertebrates. According to them, microchromosomes are building blocks of bird, reptile and mammal chromosomes.

There is every possibility that chromatin structures of variable sizes are being sporadically expelled from some or the other chromosomes at any developing stages of cell divisions by selective attenuation. Obviously this intra-chromosomal molecular phenomenon may be an inherent epigenetic molecular mechanism 5; [16] within genomes.

This communication lays special emphasis on frequent presence of chromatin dots of variable sizes in mitotically (root tip cells) and meiotically dividing cells (spore mother cells in the heterosporangia of some plants) along with the origin of new chromosomes [3-5-10-17-18] By the unique mechanisms of “fission and fusion” the genome appears to have been enriched by evolving a new path of chromosome evolution. For example, Isoetes pantii is the only species in the world flora of the genus to possess 2n=48 chromosomes (n=12) while all species so far known have 2n=22 and multiples of n=11 (except Isoetes hystrix: n=10; [19-20-21-22-23].

It appears that the chromatin emanations and also chromosome breakage--fragments may sometimes fuse to evolve new chromosomes [24] within a genome. Some observations are discussed hereunder.

Material and Methods

Plant Materials (Figures: 1-5)

While reporting more than 1400 chromosomes in Ophioglossum. reticulatum [21] had also mentioned about ten fragments. No specific attention thereafter, was paid to these small chromatin dots by most authors [22-25-26].

Observations on morphological variations in the developmental stages of tropophyll, corm and spike as well as presence of types of spores among different species of the genus Ophioglossum L have been searched since 1970 during plant collections from a large number of localities in Indian subcontinent. Diagnosis of different species has been based on poly-diagnostic approaches involving morphological (always by SEM of spores, comparing exine ornamentations) anatomical, chromosomal as well as rbcL based phylogenetic assessments as per standard procedures which have been presented earlier in detail by various authors [9-23-27-28-29-30-31]

Chromosome Study:

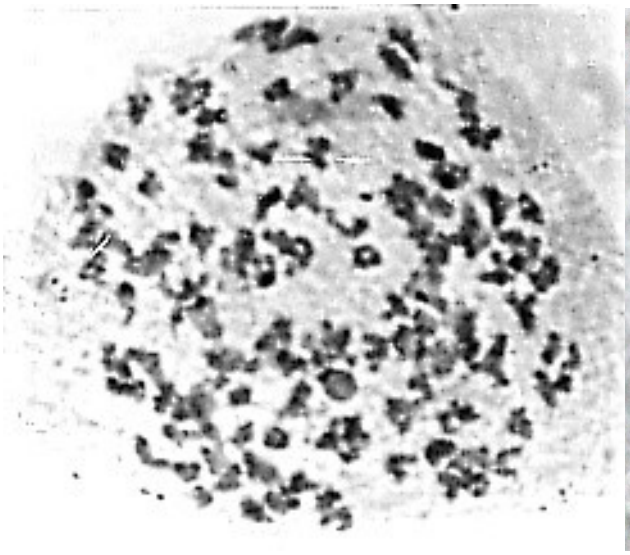
Chromosome studies have been a routine during 1970-2004 by simple squash preparations staining with 1.5 to 2 % acetic orcein or sometimes by Feulgen’s approach (Figures. 1,3, 4) despite the fact that chromosomal count may not be of great relevance in diagnosing a species. For Ophioglossum plants both young roots and spikes were properly processed for chromosome studies.

Mitotic and meiotic studies [4-9-28] have confirmed interspecific and intraspecific variations in chromosome numbers indicating thereby that chromosome count does not help in taxonomic identification among these species.

However, species show variations in the presence or absence of microchromosomes; many of them possess microchromosomes and express rare morphological teratologies (Figures. 1,2).

All plants belonging to Isoetes pantii collected from the original locality in Narsinghgarh (MP, India) and lately, also collected from a locality in Gujarat possess heterosporangia (microspores and megaspores develop within one and the same sporangium [32].

Such sporangia are now termed as “heterosporangia”;Goswami, 2014). Micro-sporemother cells and megaspore mother cells were squashed from heterosporangia to yield meiotic divisions. Chromosome preparations were also obtained several times from root tip mitosis, Squashing was also attempted on sporangia of a few ferns (Figure.5). Relevant studies have been reviewed from time to time [17- 33-34-35].



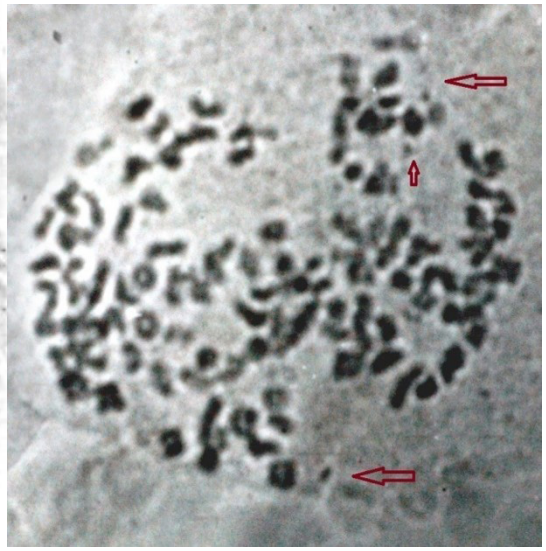
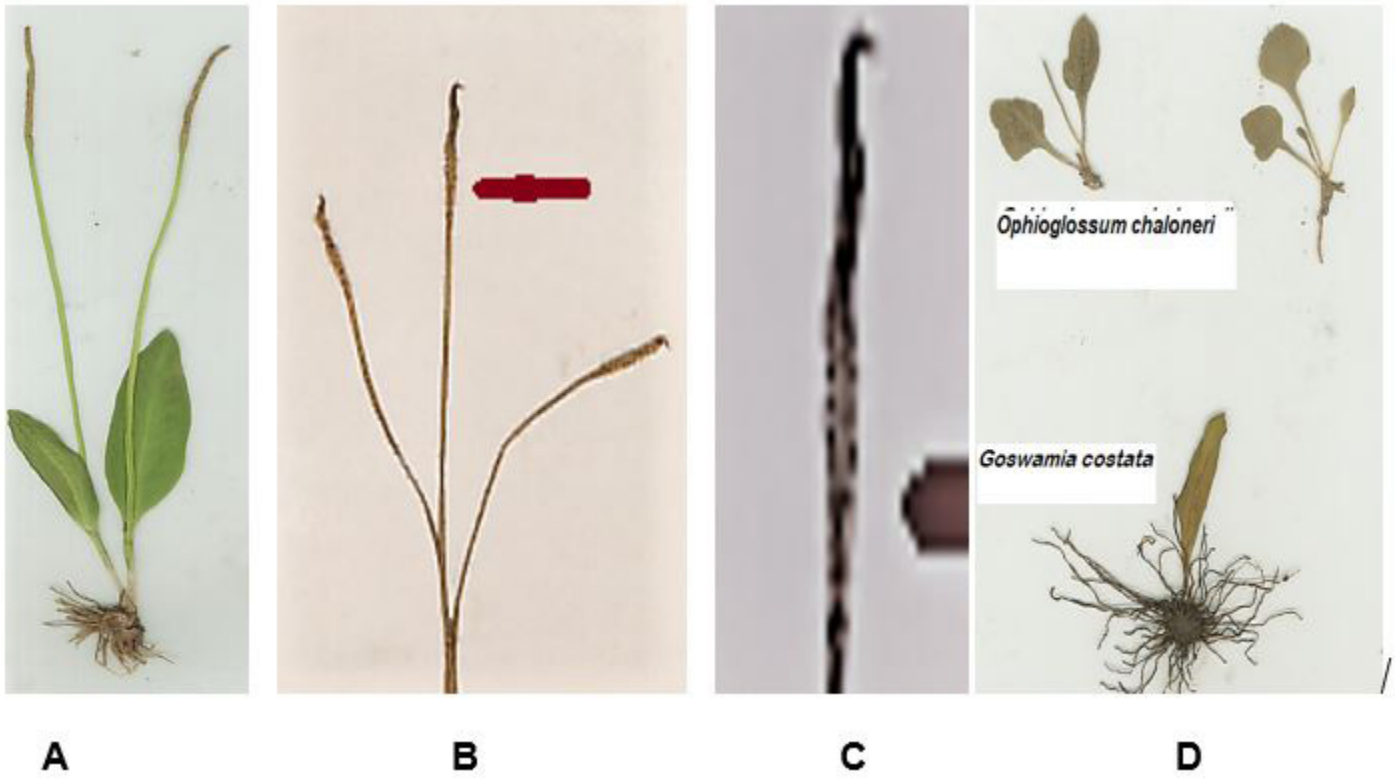


Figure.1:A ***Figure.1:B***

Figure:A Diakinesis in O. chaloneri showing 105-1110 bivalents with more than six chromatin dots (arrowed); this slide also exhibited more than 130 bivalents with many overlapping stages;

Figure: B. Diakinesis in O.eliminatum (now Goswamia eliminata) showing about 90 bivalents with 03 chromain dots (marker dots) (x1500)



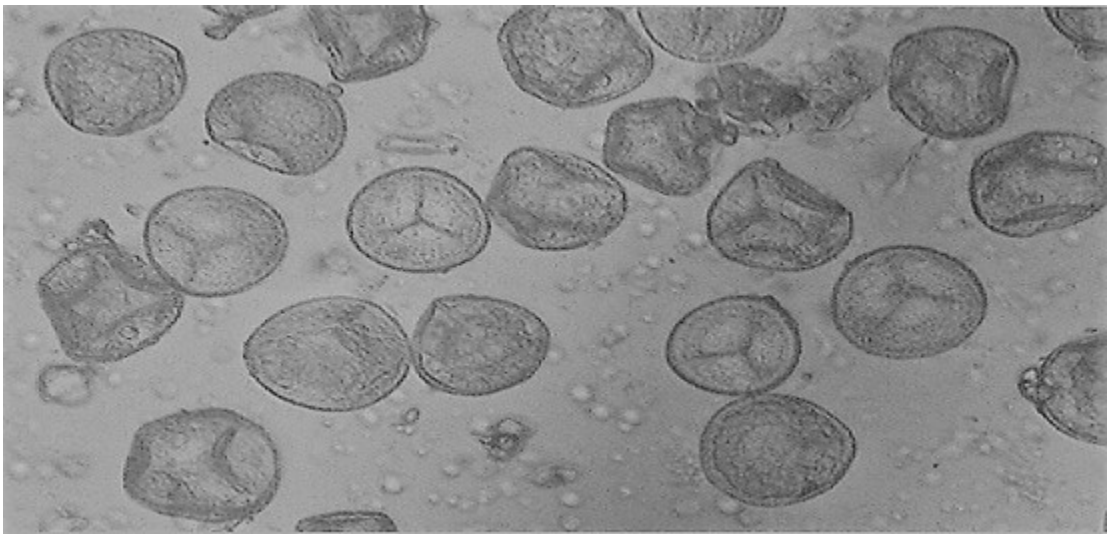


Figure: 2. A. Normal plant (Goswamia costata= Ophioglossum costatum)

Figure: B-E. O. Chaloneri An extremely rare variant showing leafless trifurcated petiole or tropophore bearing fertile spikes (sporophores)

Figure: C ; A sporophore enlarged to show normal fertile bearing sporangia;

Figure: D; Small plants for comparison of O. chaloneri and Goswamia costata;

Figure: E. spores released were normal mixed with deshaped spores

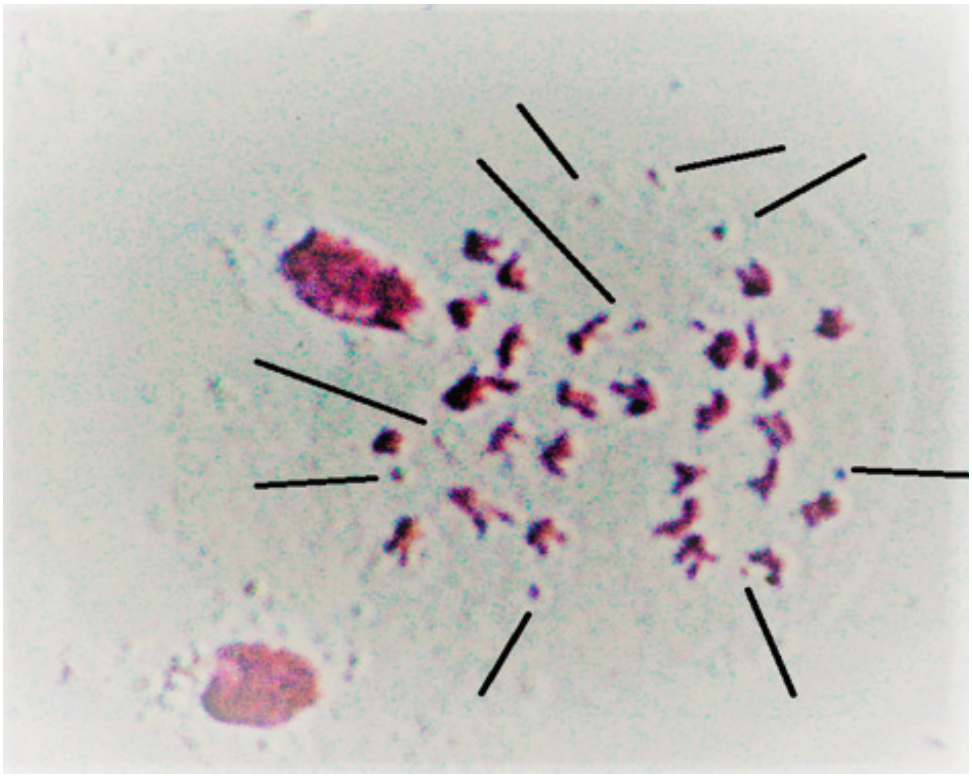


Figure: 3 A.

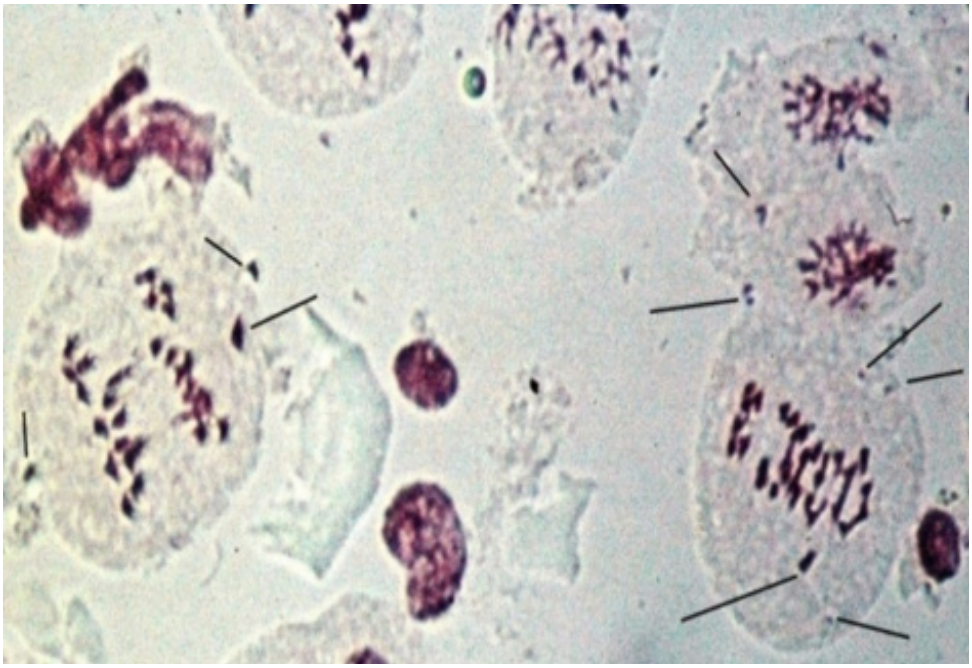


Figure: 3 Meiosis in Isoetes pantii A. A megaspore mother cell at an early anaphase I in a heterosporangium revealed many chromatin dots which appear to have arisen due to fragmentation of chromosomes and also chromatin attenuation of a few chromosomes. The anaphasic separation shows reduced chromosome number , 14 at one and 13 chromosomes moving towards the respective poles (Photographed at 100 x 10) ;

Figure: B . Microsporogenesis within the same heterosporangium shows microsporemother cells exhibiting anaphase I (left: lagging B or an X chromosome (15 X 40, enlarged)

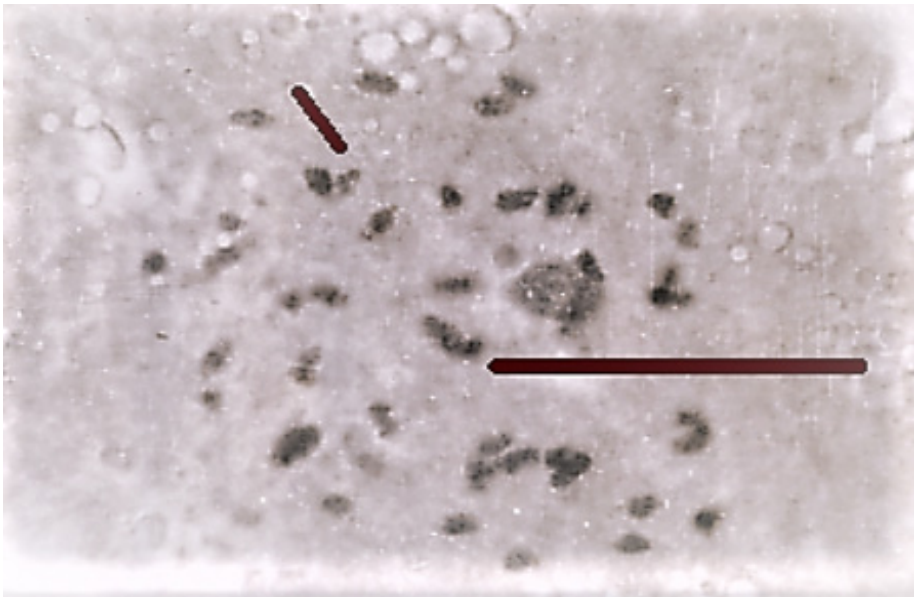


Figure: C

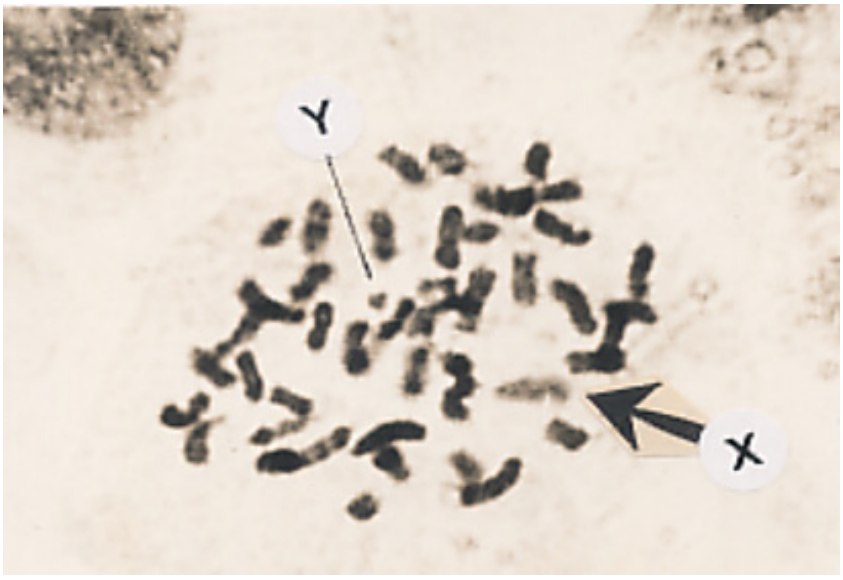


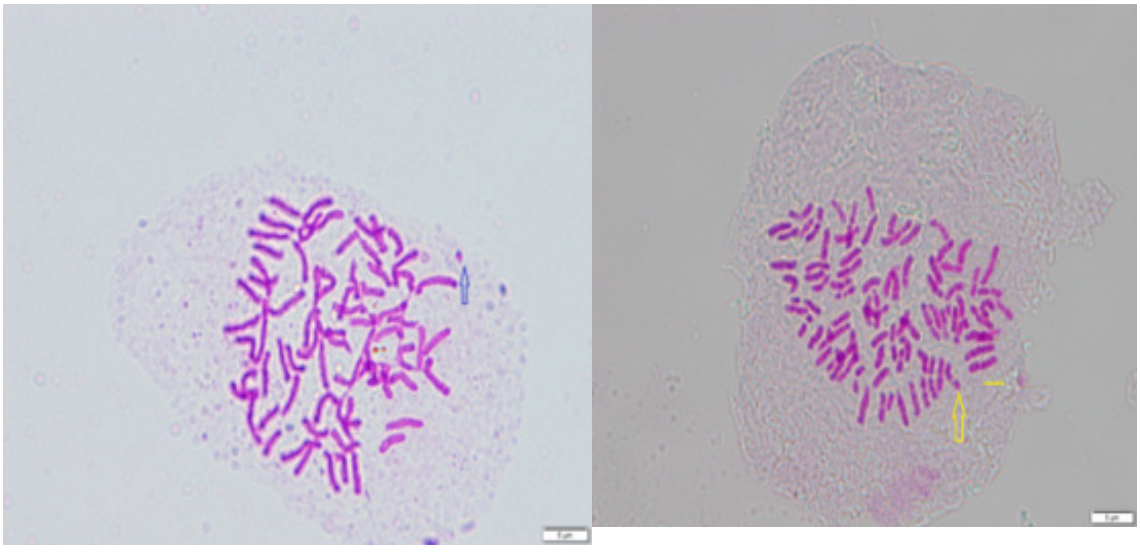
Figure D

Figure: 4 A & B Fragmentation and release of chromatin dots (as many as 08 Marker Dots; [33] In megaspore mother cells of Isoetes pantii. Variable counts of chromosomes and frequent presence of chromatin dots or Marker dots (arroed in A & B) have been observed in plant populations of Isoetes pantii. Such plants have always been loaded with abnormal megaspores resembling fossil lycopods along with normal megaspores in the heterosporangia (NOT in megasporangia; [17-33-35-81].

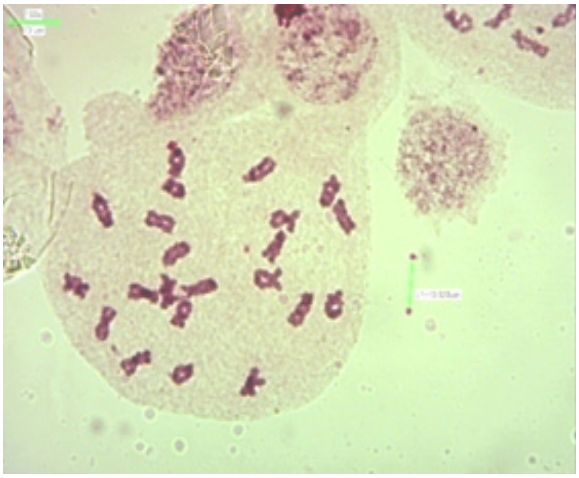
Figure: C & D. In early 1970s most mitotic and meiotic preparations also revealed 2n=48 chromosomes which included a large submetacentric, heterochromatic chromosome; C. Is the picture, published in in 1975 of a spore mother cell with such an X chromosome and D. Is a root tip mitosis showing clear X and Y chromosome (exactly same chromosome as known and published many times; see [42]. One or two B chromosomes were also identified as laggards in anaphasic segregations (B &C)

Population cytogenetic data have emphasized that plants with clear count of 2n=48 chromosomes include 1 0r 2 B chromosomes and X and Y chromosome never possess any release of chromatin material thereby suggesting a role of congregational mechanisms for the evolution of New Chromosomes.

Figure : Microchromosomes (arrowed) in some Ferns



A. Lepisorus bicolour ***B. Lepisorus angustus***



C. Polystichum ichangensis

Figure: C, Polystichum Ichangensis

Figures A-C, Courstsey of Dr. Zhenlong

Figure D, courtesy of Dr Iruduraj

Figure: 5 Meiosis I. A- B Ferns ; C Early Diakinesis in a fern Osmunda hugelina: A bivalent showing attachment of a chromatid at the terminal end of a chromosome (chromatid end) another round small dot is indicated as attached to a chromosome. (Courstey: Dr Iruduraj)

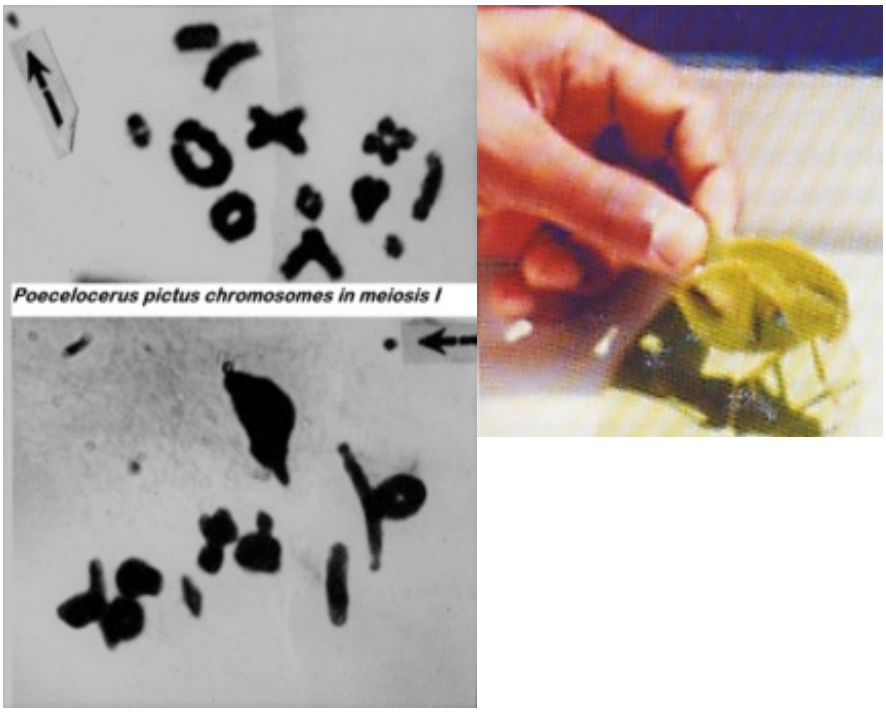


Figure 6 B Silkworm testis reveals 8 (n=8) bivalents along with small microchromosomes

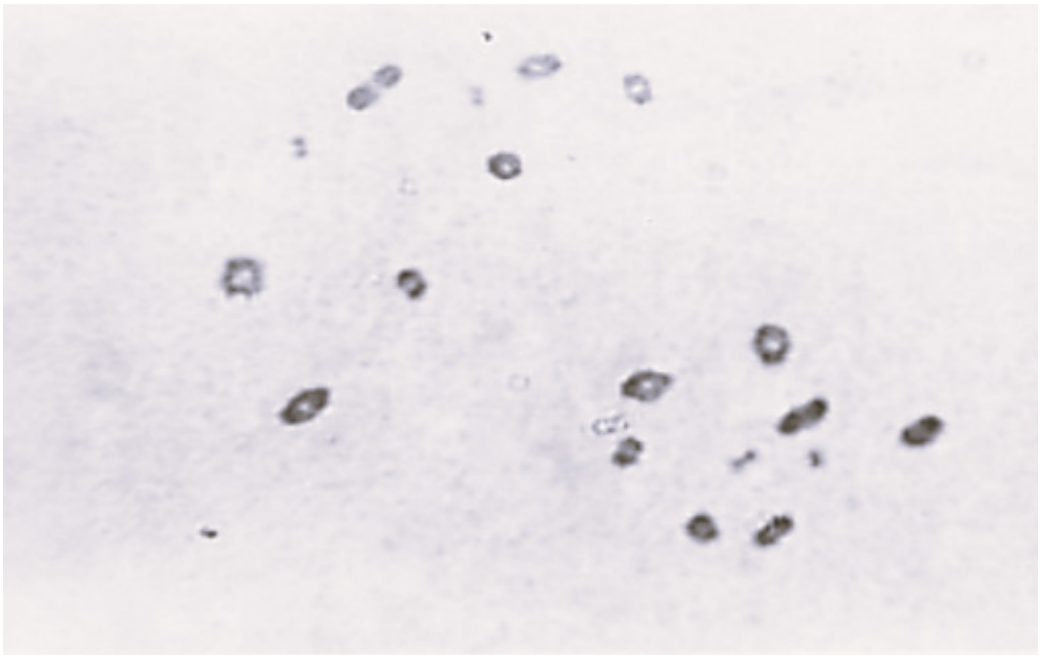
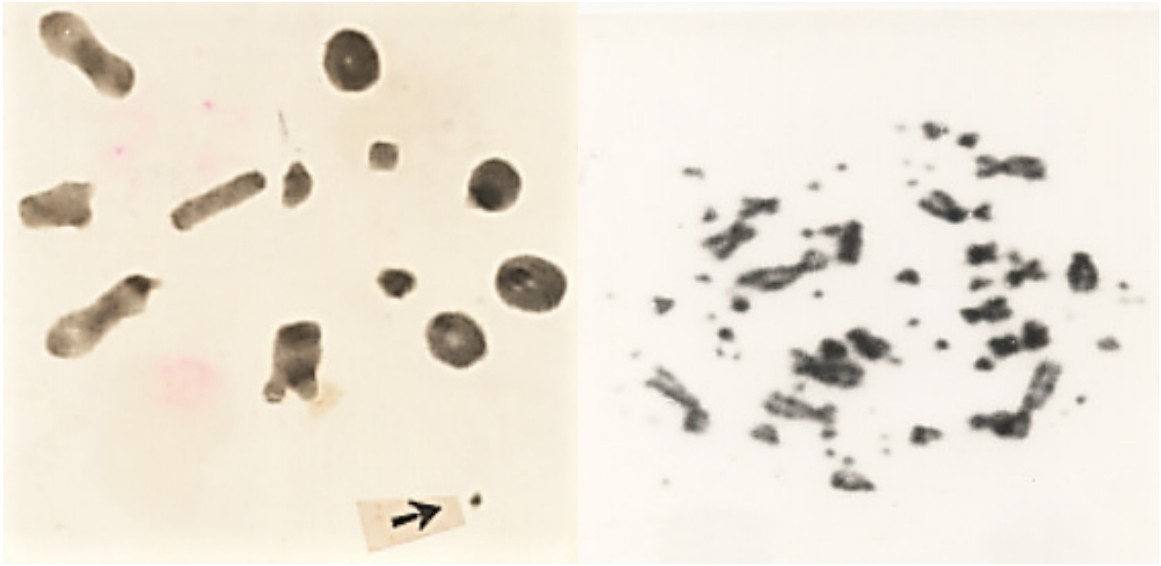
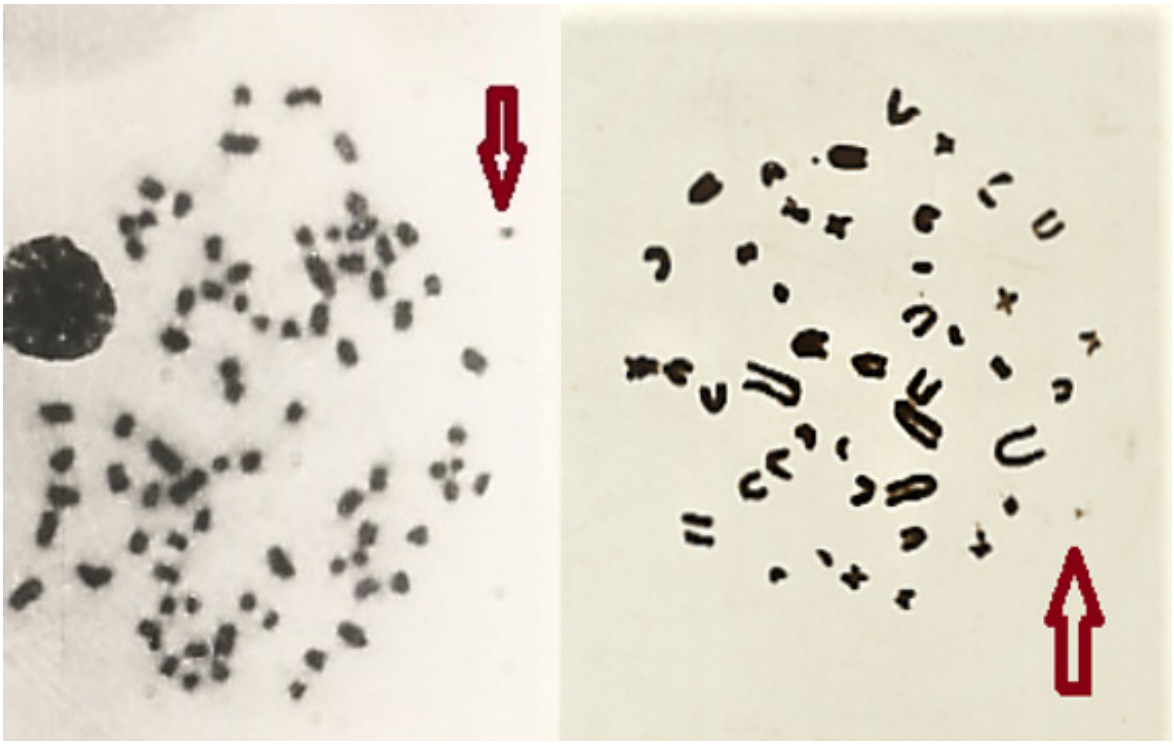


Figure 6 B

Figure: B Silkworm testis reveals 8 (n=8) bivalents along with small microchromosomes



(A)Tylotropidus voricomis ***(B) Passer domesticus (House sparrow)***



(C)Cannis (Dog) ***(D) Vandicoot bengalensis***

Figure 7: Marker dots are seen in A, C & D; (B) Birds and reptiles always possess many microchromosomes

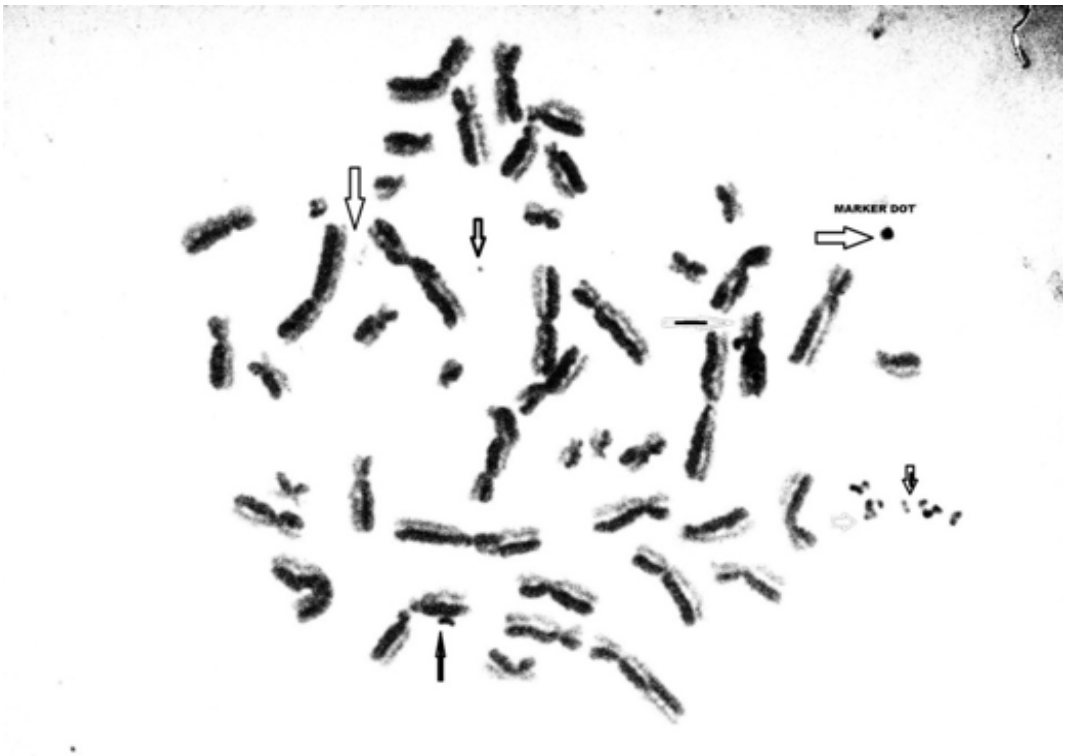


Figure: 8A

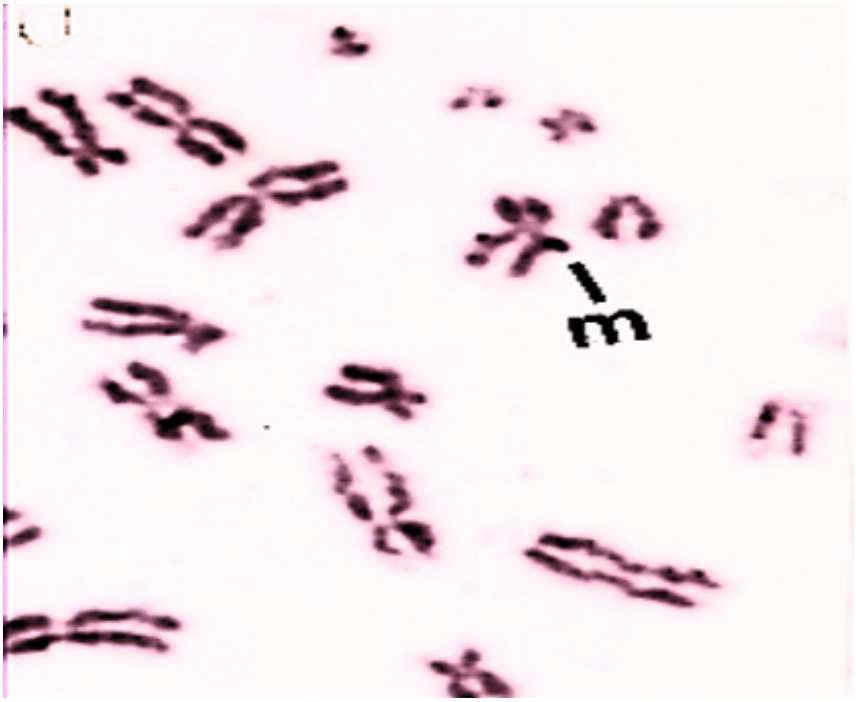


Figure: 8 B

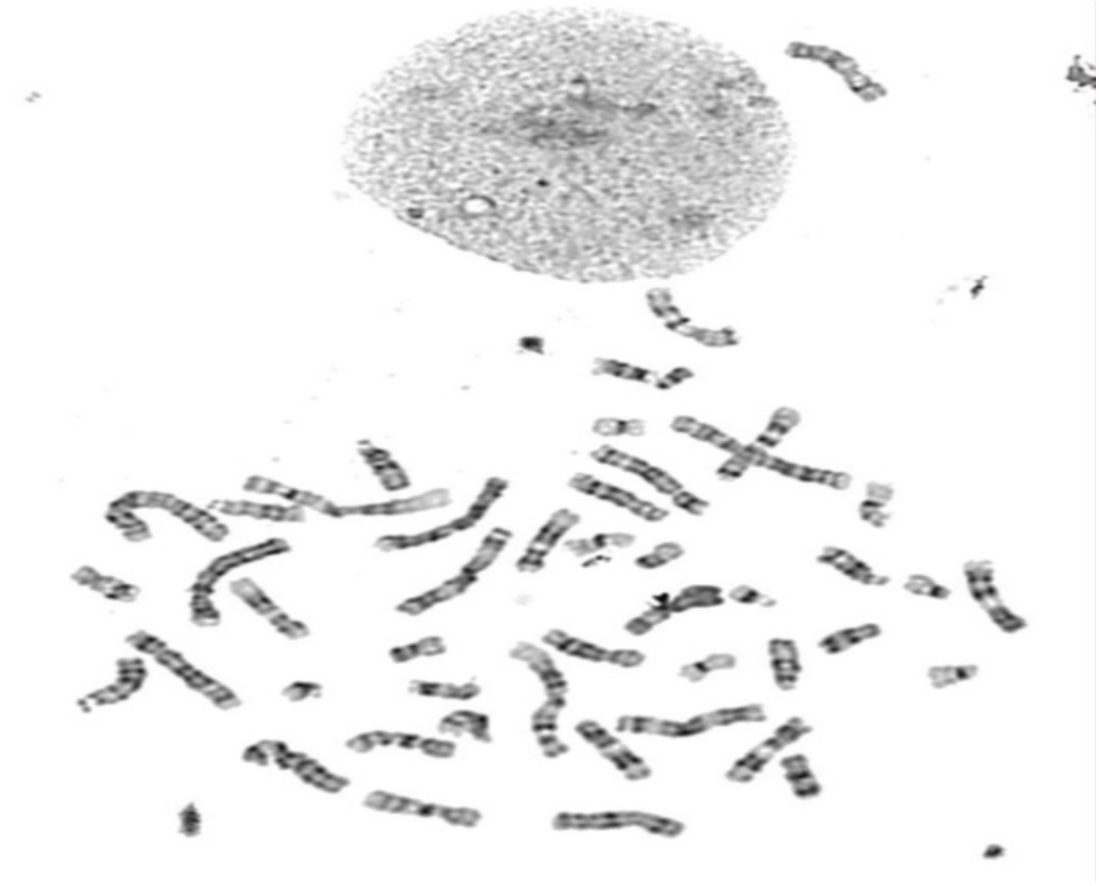
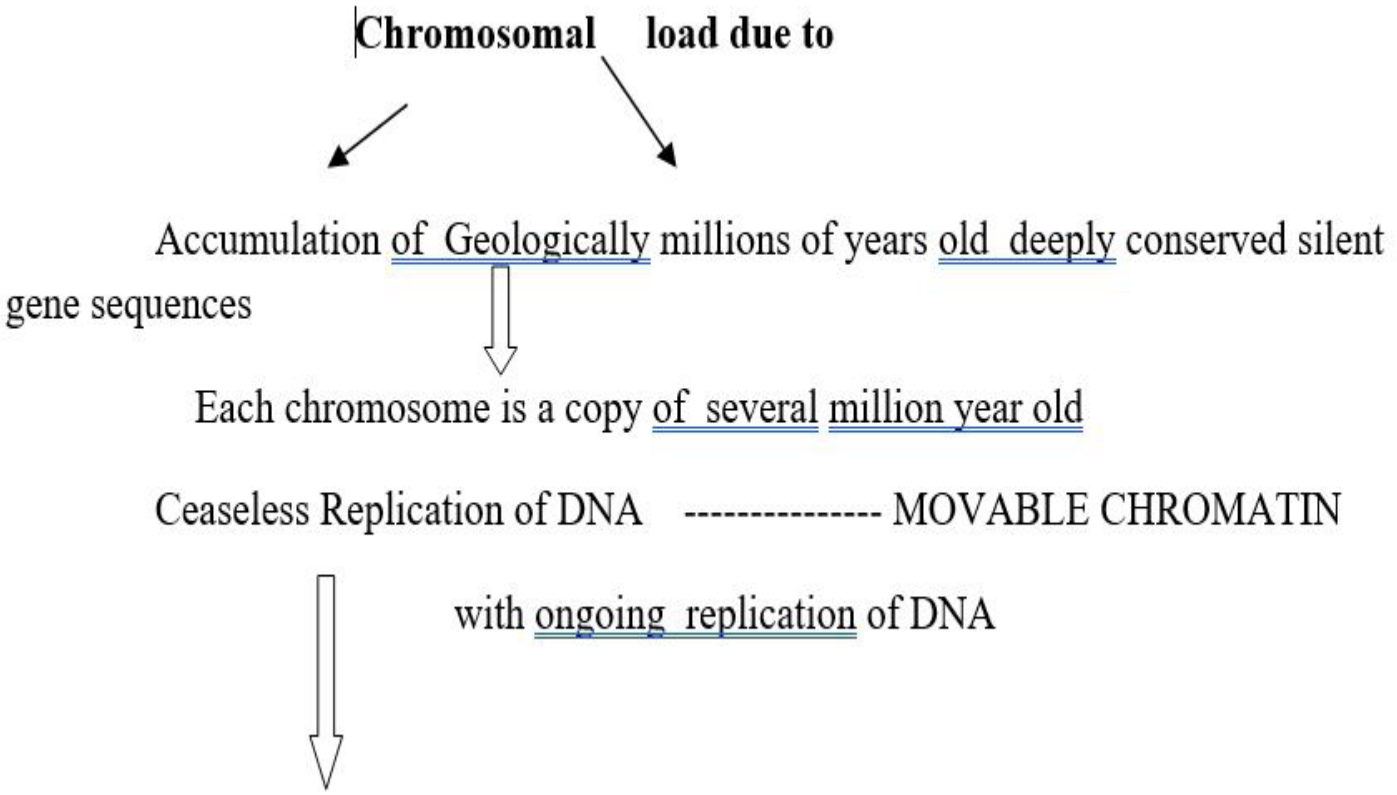


Figure: 9. Giemsa banded metaphase chromosomes indicating origin of a small chromatin structure (mini chromosome) as if transcribed

near the centromere of chromosome (Arrowed) 20; Linear chromatin from chromosomes 16, 19, 20 Small fragments are also seen The woman aged 50 had exhibited two abortions and one stillbirth.



A large number of chromatin dots have been observed in cells of many plants, animal and human cell divisions which may be first detected in after the S phase of mitosis. BUT only few are retained in mitotic metaphases on maturity.

Expulsion of chromatin might be based on genetic load on each chromosome (intrachromosomal load) which may be different for each chromosome (See Text)

Figuure 10. Hypothetical explanation of chromatin dots (marker dots) from a eukaryotic chromosome during S phase of mitotic divisionit

Name of species	Mi ch	Rare Traits	Remarks/ References
1.Goswamia costata Zhang & Zhang	1-2 per cell	I.Petiole may be absent; tropophyll (leaf) arising directly from rhizome; II.Leaf shape and size variable in each plant	Chromatin dots sometimes observed emanating from chromosomes in mitotically dividing cells in root tips
(Ophioglossum costatum)	1-3/ cell	III. Some plants possess canals in the mesophyll IV. Parallel veins 3 or 4 on both sides of thickened mid vein in some plants (Resemble fossil leaf of glossopterid leaf) V.Sometimes swollen structures are seen on the mid vein of variant leaves on the same plant	
2.Goswamia eliminata Zhang & Zhang (2020) . (Ophioglossum eliminatum; Khandewal & Goswami (1984)	1-3 /cell	I.Hyadothode like strctures in the mesophyll and natural tearing of leaves Leading to canal formation II. Many plants have exhibited development of spore-mass on margins of leaf (without a sporangium) III. A few plants were collected to have flat thin leaf from the base of tropophyll replacing development of a sporophore.	Goswami et al 1989, Goswami, 2007 Goswami & Khandelwal 1980
		III.Microchromosome always present in meiotic cells in such aberrant plants	Figure 3 C

Table:1 Extremely rare features among plants possessing microchromosomes /Attenuated Expelled Chromatin dots (based on minimum of 40 observations during 1975- 2020)

Persons with number of	**Number of times persons investigated**			
Metaphases	**Once**	**Twice**		**Thrice**
Apparently Normal (12500 metaphases)	170	80	56	Total 306
Recurrent Abortions (4544 metaphases)	67	140	18	225
Syndromes (4287 metaphase)	--	09	12	21
Malignancies (1130) metaphses	14	05	02	21
Methyl isocyanate gas exposed (11,886 metaphases)	14	12	56	82
Suspected pathology/control of cancer patients (1253 metaphases)	08	11	04	23
Totals	**273**	**257**	**148**	**678**

Table: 2. Human Chromosomes :Persons investigated, their category with Approximate number of Metaphases scored

MARKER DOTS

Chromosome No. of screened			Marker dots emanating from		
Number	Metaphases	Near Centromere/	Telomeric end/	Arm p/	Arm q
1	117	05	51	04	--
2	126	--	117	--	--
3	125	--	43	--	--
4	850	03	502	--	18
5	850	03	502	--	18
8	180	--	45	49	--
9	120	--	73	--	17
11	165	--	70	12	--
12	47	--	29	--	--
13	132	--	41	18	73
16	87	10	45	--	01
17	140	--	37	--	58
Y	17	--	12	--	27

Table: 3. Human Chromosomes II. SEARCH FOR INVOLVEMENT OF CHROMOSOME-LOCUS IN RELEASING

Expulsion of chromatin might be based on genetic load on each chromosome (intrachromosomal load) which may be different for each chromosome (See Text)

Animal materials (Figures: 6 &7)

Cytogenetic studies on animal materials [38] were conducted as per standard techniques and staining schedules had included haematoxylin, acetic orcein and Feulgen techniques. Chromosomes were prepared from testicular (Figs.6, 7) and bone marrow processing in dogs, (Cannis domesticus) Vandicoot bengalensis; and house sparrow (Passer domesticus). Additionally a few insects also were dissected and meiotic preparations yielded excellent chromosome plates. While occurrence of microchromosomes is a regular phenomenon in birds, reptiles and other lower vertebrates, one or two dot chromosomes were sometimes observed in the dog and Vandicoot chromosome preparations.

Human Chromosomes (Figures:8-10)

A few chromatin dots of variable sizes were reported in squashed cells of many brain tumour tissues [1-16-36]. Later lymphocyte cultures studies by standard lymphocyte cultures and staining with Giemsa, G and C banding and Feulgen's approaches on more than 600 persons revealed definite origin of larger chromatin dots of variable sizes which appeared as an outcome of some triggered molecular mechanism operative on chromosomes . We had published clear pictures of chromosomes, observed in many cases revealing direct detachment of these dots, termed as "Marker Dots (MDs). These emanating marker dots [33-35-37-39-40-41-42] varied in origin from different chromosomes (as identified by G banding; see Tables 1, 2).

OBSERVATIONS

Morphological Comments in Plants

Many of the morphological traits described in Ophioglossum eliminatum now Goswamia eliminata, [81] and O. chaloneri are quite new in plant morphology [4-16-28-35-43-44]. Exactly similar and detailed observations on species of Isoetes have been followed since 1966 and papers have been published describing hitherto unknown features among many species [32-45-16-17-42-34-35]. This has been a consistent observation that the presence of microchromosomes, (persistent chromatin dots) has been found to be positively correlated with highly unusual morphological and anatomical features, some of them have been described earlier; [9-27-28]. Additional extreme and specific variations need a brief mention.

The sporophore (fertile organ/ spike) arises conventionally from the basal groove or just at the base line of the tropophyll (leaf) in almost all species of Ophioglossum and the stalk of

(Fig. 2 A) spike is often long but we have been collecting such variants in O.vulgatum where too short stunted spike develops as an abnormal feature. Spores from such sporophores are normal as well as highly deshaped or malformed. In Ophioglossum chaloneri more than sixty percent plants possess highly displaced origin of fertile sporophores (spikes). It is still strange that the same rhizome may produce two or three tropohphylls (leaves) one exhibiting displaced origin of the sporophore . Even a sporophore may originate directly from the rhizome. We have already suspected elsewhere that O.chaloneri [35] may be a hybrid between O. vulgatum and Goswamia costata Zhang & Zhang (=O. costatum).

Recurrent teratological observations which may be even too rare, should not be ignored because such developmental errors often have involvement of genetic factors, quite likely, these may be hidden traits within the genome of the genus [46-47]. For example, Goswamia eliminata Zhang & Zhang and Ophioglossum chaloneri Goswami et al exhibit classical presence of many microchromosomes and also demonstrate expressions of extremely rare morphological aberrations (eg. origin of spikes from the rhizome; presence of clusters of spores on the margins of the leaf etc). Above all, the most unique is the occurrence of leafless (no trophophore / trophophyll) two plants among the O.chaloneri collections over a decade.

These plants (Figure. 2 B-D) possess a long, erect thin structure rising from the rhizome, designated as stalk which trifurcates into three peduncles, which in turn, each one bears a fertile spike (sporophore).. Spores released from the sporangia of sporophores from this plant have presented mixed occurrences of normal and deshaped spores (Figure. 2 E) as also observed in normal plants of O. chaloneri. A few simple leafless plants have been known for quite some time,[26-52] but we have recorded one specimen each in Ophioglossum eliminatum (now, Goswamia eliminata Zhang & Zhang) and Ophioglossum costatum (now, Goswamia costata). However such trifurcated leafless specimens have never been reported in any species of ophioglossaceae.

Emanation or expulsion of chromatin dots has been frequently observed in chromosome preparations of many ferns by many other workers (see Figure. 4) including some species of moss and angiosperms.

Chromosomal features in Isoetes pantii Goswami & Arya

Chromosome studies on Isoetes pantii during the 1970s [3-17] had indicated the origin of chromosome variations and the presence of an X chromosome along with two B chromosomes. These observations had suggested that chromosome evolution in this species is more likely to be associated with the new evolutionary path and also for possession of heterosporous sporangia [18-32] as the fixed normal feature of the species. This is worth mentioning that mitotic and meiotic studies on plants of Isoetes pantii , Isoetes coromandeliana, I. sampathkumaranii, I. fuchsii, I. bhimii and Isoetes narsinghgarensis have been carried out for several years but repeated different counts of chromosomes among plant populations of Isoetes pantii have been unique and exclusive. But growing in the same pond the plants of of Isoetes coromandelina and I. sampathkumaranii did reveal as expected (known for quite some time) exact count of 2n= 22+1 and 2n=66+1 chromosomes respectively [34] but never with any variation.

Chromosome attenuation, fragmentation and origin of new chromosomes

It becomes logical to opine that chromosome breaks and translocations observed in earlier populations of Isoetes pantii from the same locality-pond have been replaced by the “appearance” of new chromosomes within the genome; [10-17-18-48] finally attempting to fix up X-Y (Figure.3) chromosome mechanism. C banding of chromosomes has perfectly confirmed large submetacentric chromosome to be heterochromatic which is being observed for several years (see Figures 3 C, D) Anaphasic movements have often confirmed lagging chromosomes to correspond with B , X and Y chromosome behaviour during both mitotic and meiotic cell divisions. Consistent observations of 2n=48 chromosomes in some plants in the same population confirm that many breaks and fragments (Figure. 3 A) in some plants might have been responsible for gradually restoring chromosome stability and the origin of sex chromosomes within the genome. So, it can be hypothesized that appearance of plants with variable chromosome numbers in the same population of plants is directly correlated with the appearance of some relic [18] features as frequent breaks and many chromatin dots might have been the consequences of chromosomal reshuffle. This intrachromosomal triggering within the genome has to be responsible for the origin of sex chromosomes and a new path of speciation. The population data on yearvise chromosome studies had indicated the decline of plants with variable numbers [16] and also, the surviving majority of plants with 2n=48 chromosomes does not show any abnormal spores except that the heterosporangium does possess microspores and megaspores within the heterosporangium.

Above reasoning is further supported by the find of new species exhibiting chromosome number based on n=12 (2n=48, 60, & 72 chromosomes).. These new species have been grouped within the “pantii complex” (viz. I. fuchsii, I. narsinghgarensis, I. bhimii possess 2n= 60 chromosomes [34-48]. Whatever be the cause of origin, change in chromosome numbers of species in natural populations influences gene recombinations and fertility. Changes due to hybridizations or any other chromosomal mechanisms (viz. polyploidy, gross chromosomal aberrations etc) result in low or reduced fertility and many of the genotypes are exposed to rigorous tests of environmental factors. We already know that the

new chromosomes arise within a genome by the chromosomes "within the genome" and this intragenomic inherent epigenetic mechanism is assisted by natural hybridization (introducing foreign DNA) and chromosomal aberrations (deletions, translocations etc) be it a plant [18-50-42-51-53-54] or animal species including human chromosomes [13-11-14-15-43-49-55-56-57-58-59-60]

Microchromosomes in small Insects, Grasshoppers

Several small insects, grasshoppers and small dying animals were dissected during our routine classroom execrcises and small chromatin dots were reported [6-38]; Figs 6 & 7). This is important to visualize that the chromatin emanation must be inherent in many natural populations of animals; hence additional search is required.

DISCUSSION

Ejection of chromatin dots from chromosomes may be associated with DNA hypomethylation or heterochromatinization but also often observed associated with chromosome translocations as ascertained by repeated observations on plant, animal and human cells. Since these dots persist in cell populations of the same individual plant or animal the author has had termed these as marker dots [33-37] These are G-C rich chromatin bodies and do have genes [8-58-39-61]. But the size of emanated chromatin material becomes of importance. Some such expelled chromatin measuring 03 micron or more is often being referred as small supernumerary marker chromosomes. Such expelled chromatin dots are also being observed by most modern molecular approaches including FISH and DNA hybridisation techniques by various workers. Nevertheless, Marker Dots or supernumerary marker chromosomes are those chromatin -structures which are expelled from a chromatid of a specific chromosome (s) as the consequence of "molecular triggering of the specific loci within a particular chromosome and any chromosome can be molecularly affected and involved in the process of chromatin attenuation [5-33-35-37-47-61-62]. There are also evidences published by other workers [13-14-15-42-43-57-63-65] that appearance of supernumerary marker chromosome is a definite denominator of chromosomal involvement representing onset of some or the other pathological condition.

Obviously, the nature of a pathological condition would depend mainly on the "genic" content of the chromatin dot / microchromosome /marker dot / small supernumerary marker chromosome and the site of chromatin attenuation at the chromosome from where the chromatin-log has been de-saddled (dislodged from or near centromere, telomere or any other site). There is every possibility that marker dots and small marker chromosomes may be the same structures "produced" by the same mechanism of expulsion of chromatin, the main difference being the size of the emanated chromatin mass. Since marker dots have been observed and described as being detached from specific locus [5-33-37-39-62] of a chromosome, the "marker-dot emanation " should be listed as a definite kind of chromosomal aberration to be tagged with other standard aberrations (deletion, duplication, inversion and translocation). Formation or appearance of SMCs also is due to chromatin expelled from some or the other chromosome [10-11-14-42-57] see [35-61]. It is quite reasonable to opine that the chromosomal DNA goes on passively expelling chromatin and also receiving exchanges of DNA sequences (intrusion). For example, as a rare instance [64] have reported insertion of mtDNA at the breakpoint junction of a reciprocal constitutional translocation.

Rare teratologies associated with chromatin emanation in plants

The presence of 6 to 7 microchromosomes in O. chaloneri Goswami et al. appears to be a chromosomal anomaly. Although, this is difficult to prove that microchromosomes are responsible for these or other phenotypic aberrations in natural populations, however this is also imperative to emphasize that such a possibility cannot be ruled out. We have shown that release of chromatin dots from the chromosomes is a natural inherent part of the genome of every plant and animal species including human chromosomes [39-35-58-65].

Extensive and repeated expeditions from the same and different localities for observing variations in natural populations reveal the evolutionary processes going on in order to shape the evolving genomes. Leafless (without trophophylls) plants, showing rhizome, giving rise to erect stalk and the peduncle bearing a fertile organ (sporophore) have been collected three times during past eight years, once in O.gramineum and two times in Goswamia costata (=O. costatum) thereby suggesting that the set of genes responsible for leafless plants and spikes being produced directly from rhizome are inherent within the genome of Ophioglossum. According to earlier studies [52] peculiarly enough O. Kawamurae, O. simplex, O. lineare and O. ramosi do not have trophophyll. However, a specimen as presented here (Fig. 2; trifurcated stalk bearing three independent peduncles and sporophores)..is a unique find giving a glimpse of a "Rhynioid" specimen .

Be it any chromosome (plant or animal), mainly due to any of evolutionary designed molecular mechanisms [65]. like DNA hypomethylation or heterochromatinization or may be even by any chromosomal aberration (eg translocation; [39-61], a small part of chromatin is exudated from any locus of a chromosome.. Certainly, ejection of chromatin dots from chromosomes is an inherent epigenetic phenomenon [58]. Theoretically, the function of these chromatin dots should be like a "macro--TEs" and the genetic impact or gene actions of these dots would depend [35-58] on the parent chromosome from where the dots have been expelled. Since these

dots persist in the cell populations of the individuals of the species, these can be considered to be microchromosomes. These microchromosomes appear to be associated with adaptive responses in Goswamia costata and O. chaloneri because these plants can inhabit and survive in any locality within even in semiarid conditions. O. chaloneri survives almost throughout the year (July to April) except hardy summer.

New Chromosomes: Marker Dots / Supernumerary Marker chromosomes in humans

Though ignored by workers on modern molecular chromosomal techniques (in situ hybridization etc) we had already reported in 1973 and in 1986 that small chromatin dots, small or larger ones do emanate from certain chromosomes (Fig 8; Tables 1 & 2) which, sometimes were clearly seen attached with a fine fibril and identity of each chromosome which was involved in this special epigenetic molecular mechanism, was verified by G banding and Feulgen's staining procedures. So, identification of chromosomes was not ambiguous at all. Recent workers have also reported association of these extra chromosome structures (marker dots / supernumerary chromosomes) with infertility, recurrent abortions and various pathological anomalies including some syndromes and mental retardation which indirectly confirm our earlier observations. Nevertheless, this is almost certain that release of chromatin from one or more chromosomes appearing as supernumerary marker chromosome (Fig. 8) is positively correlated with many pathological conditions including spontaneous abortions or infertility.

Evolutionary importance of Chromatin emanation: A reference to Chromosomal Load

The evolution of chromosome size, structure and shape, number, and the change in DNA composition suggest the high plasticity of nuclear genomes at the chromosomal level. The structure and shape of chromosomes can be altered by chromosome rearrangement, including insertion, duplication, deletion, centric split and fusion, inversion, and translocation. Comparative cytogenetic studies have revealed extensive chromosome rearrangements in many plant [50] and animal species [49-66] including man [55]. The differences in the structure, shape, and numbers of chromosomes in related species, both in animals and plants, are due to the syntenic blocks being assembled in different combinations. Blocks that are fused together in one species can be separated on different chromosomes in another. Segments within blocks can be duplicated, lost, or inverted. Repetitive DNA sequences, which represent a conspicuous fraction of every eukaryotic genome, particularly in plants, are found to be tightly linked with plant chromosome evolution. A large number of studies [49-59-60-65) the possible connections between evolution and epigenetic alterations in chromosome structure and influencing heterochromatin formation, centromere function, and epigenetic-associated transposable element inactivation. Different classes of repetitive sequences have distinct distribution patterns on the chromosomes and the entire assemblage supports epigenetic control on various molecular mechanisms. [67-68] have concluded on the basis of extensive studies on human lymphocyte cultures that the rate of chromosome breakage is age dependent in lymphocytes of adult controls. They also suggested that chromatid exchanges between non-homologous segments also indicate chromosomal instability. The current karyotype of a given species is formed by complex chromosomal rearrangement usually combining two or more rearranging events, and the process is still going on. By and large, most species have a fixed basic and haploid number and each chromosome within the sub-genome of a species is loaded with as many genes as could have been housed in the abode/locus. Each chromosome also has many genes which are not active [68] many genes produce deleterious effects and also there are fixed cycles of generations for mutability of each gene. In other words, each gene has a different span of mutation period [39-50-61-69-70-71-72].

From evolutionary point of view, many chromosomes within the specific genome must also be loaded with geologically too old (for millions of years) DNA sequences and fundamentally, making most chromosomes as copies of earlier chromosomes. The assemblage and load of such relic genes as well as mutation and recombinational loads cumulatively constitute chromosomal load (see Figure.10). This cumulative impact must be the inherent evolutionary compulsion to remove certain sequences at some time form some specific chromosome; not all chromosomes at one time as revealed by a follow-up study on human chromosomes [5]. Role of specific stem cells and some exclusive genes cannot be rulled out. Expulsion of chromatin material as a l dot or a short filament r, can be from any domain of a chromosome (see, Table 2 &3) thereby offering a "seat" for another future incumbent.

Chromosomal DNA goes on replicating as long as the cell lives and this ceaseless replication tendency has been going on since the earliest phase of evolution [61]. Many moving DNA small fragments are known to be housed at some locus on a specific chromosome [47-58- 73] even DNA sequence from mitochondrial genome in man is known to be translocated to chromosomal sequences. Such findings also provide evidence for a previously unrecognized insertional mechanism in humans, by which non-mobile extra-chromosomal fragments can be inserted into the genome at DSB repair junctions. A team of investigators see, [64] have reported breakpoints occurring within an L1-type repeat element at 9p24 and at the 3'-end of an Alu sequence at 11q23. At the breakpoint junction of derivative chromosome 9, they discovered an unusually large 41-bp insertion, which showed 100% identity to 12S mitochondrial DNA (mtDNA) between

nucleotides 896 and 936 of the mtDNA sequence. Analysis of the human genome failed to show the pre-existence of the inserted sequence at normal chromosomes 9 and 11 breakpoint junctions or elsewhere in the genome. Willett et al. strongly opined that the insertion was derived from human mtDNA and captured into the junction during the DSB repair process. These observations are believed to be the first report of spontaneous germ line insertion of modern human mtDNA sequences and suggest that DSB repair may play a role in inter-organellar gene transfer in vivo.

Frequent Breaks and Transfer of Genes/ Chromatin

The molecular phenomenon of expulsion of chromatin from a eukaryote chromosome appears the same but the size of emanated chromatin makes a difference. Chromatin dot about less than 02 micron is a microchromosome; less than 3 micron can be a marker dot (MD) and a little larger with a definite chromosome shape should be referred to as a supernumerary marker chromosome (SMC). Naturally, chromatin dots emanating from chromosomes within the genome must be packed with a few genes and the phenotypic expression of the traits will obviously depend on their specific gene contents and expressions. Certainly, chromatin emanation from some or the other chromosome (s) appears a natural, evolutionarily conserved intracellular epigenetic molecular activity randomly operative within (Fig 3) chromosomes in eukaryotes. Occasional translocations also appear as essential events within a genome. Earlier hypothesis that the DNA molecule ever since evolved, is ceaselessly replicating and is being randomly distributed, becomes justified because expulsion, fission and fusion are the backbone events of chromosomal evolution among genomes [49-55-74-75]. One of the best examples is the human Y chromosome evolution which depicts an evolutionary demonstration of fissions, fusions and heterochromatinization of chromosome segments and subsequent translocations from a few autosomes of early hominids [55-74-76-77-78]. It has been possible to assume that DNA sequences can be continued to ceaselessly multiply, be copied, be fused and also spliced so as to generate new gene-chromosome pathways and amplify within the genome of evolving organisms [42-46-59-64-79- 80].

This is worth mentioning that in all cells whenever many “marker dots” have been found there always have been some or the other kind of chromosomal reshuffle (Fig.3 & 4). This cannot be ascertained whether this is the cause or a consequence but repeated observations in several populations justify the association between frequent emanation and chromosome changes.

Conclusion

Our publications during past five decades have offered clear pictures of chromosomes observed in many plant animal and human materials revealing direct detachment of chromatin dots, termed as “Marker Dots”.. These expelled chromatin may be either one or two or many in number but in many cases some of these are retained within cells and have been repeatedly observed in many plants including human cultured lymphocytes. The emanating marker dots varied in origin from different loci of chromosomes (terminal centromeric or interstitial) Quite often these are associated with the appearance of very unusual traits in some individual organisms. Present and also other authors have also described chromatin structures being emanated from specific chromosomes to be associated with some pathological features in certain human patients more commonly with malignancies and recurrent abortions. Such inferences have been based on modern molecular approaches including FISH and DNA hybridisation techniques.

Precisely, these marker dots or small supernumerary marker chromosomes are those chromatin -structures which are expelled from a specific chromosome (s) as the consequence of “molecular triggering” at the specific loci within a particular chromosome. This epigenetic mechanism appears to be related with “chromosomal load” evolutionarily inherent within all eukaryotic chromosomes and also this selective molecular mechanism should be a pathway for the chromosomal evolution within the genome. Theoretically, entire such mechanisms which are deeply conserved within the basic eukaryotic genome appear to be regulated by the very special stem cells conserved and transmitted from generations for millions of years.

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