

Cytogenetical Studies in Genus *Citrus* IV. Evolution in genus *Citrus*

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Introduction

Genus *Citrus* belong to subfamily Aurantoideae which constitutes one of the important subdivisions of family Rutaceae. Swingle (1948) recognises sixteen species under genus *Citrus*. While Tanaka (1937, 1954) has given much wider classification of genus *Citrus*. He has included one hundred and forty five species in this genus. Many of these species have been considered as bud sports, chance seedlings or hybrids by Swingle. As such he did not recognize them as valid species. *Citrus* species are known to hybridize among themselves without much difficulty and numerous instances of naturally occurring hybrids have been reported (Swingle 1948). Many of these hybrids produce apparently normal seeds that reproduce the hybrid plant true to the type. This is due to nucellar embryony.

Strasburger (1907) reported eight as the haploid number for genus *Citrus* but later findings of Longley (1925, 1926), Frost (1925a, 1925b, 1926, 1927, 1930, 1938) and Nakamura (1929, 1934a, 1934b) have established beyond doubt nine to be the haploid number. Krug (1943) published chromosome numbers in subfamily Aurantoideae with special reference to genus *Citrus*. His list of chromosome numbers in genus *Citrus* covers the findings of previous workers.

Considering the great diversity of characters in subfamily Aurantoideae considerable variation in chromosome number might also be anticipated. But the chromosome number is relatively constant for all the members of this genus and nearly all the cultivated forms are diploid ($2n=18$). The polyploidy in *Citrus* range from triploid to hexaploid. But reports of polyploidy beyond tetraploidy are rare. Furusato (1952) has reported in *Citrus Natsudaoidai* Hayata some polyploid forms.

The present studies were undertaken to determine the chromosomal basis of speciation in genus *Citrus*.

Materials and methods

The materials of the following members of genus *Citrus* were collected from the orchards of Naini Agricultural Institute, Allahabad.

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|---|--|
| 1. <i>Citrus penovisculata</i> | 13. <i>Citrus sinensis</i> var. Mosambi |
| 2. <i>Citrus aurantifolia</i> var. Kagzi lime | 14. <i>Citrus sinensis</i> var. Malta |
| 3. Rangpur lime | 15. <i>Citrus reticulata</i> var. Nagpur sangtra |
| 4. <i>Citrus medica</i> var. Citron bajoura | 16. <i>Citrus reticulata</i> var. Hill orange |
| 5. <i>Citrus limonia</i> var. Hill lemon | 17. <i>Citrus reticulata</i> var. Lyalpur orange |
| 6. <i>Citrus limonia</i> var. Kagzi Kalan | 18. <i>Citrus reticulata</i> var. Satsuma Mikan |
| 7. <i>Citrus limonia</i> var. Nepalese oblong | 19. <i>Citrus rugulosa</i> |
| 8. <i>Citrus limonia</i> var. Italian oblong | 20. <i>Citrus grandis</i> var. Red flesh |
| 9. <i>Citrus limonia</i> var. Italian round | 21. <i>Citrus paradisi</i> var. Ruby |
| 10. Mayer lemon | 22. <i>Citrus paradisi</i> var. Marsh |
| 11. <i>Citrus Karna</i> | 23. Poorman orange |
| 12. <i>Citrus limette</i> var. Metha | 24. <i>Citrus Assamensis</i> |
| | 25. <i>Citrus microcarpa</i> var. Hazara |

Besides these species of genus *Citrus* Rusk, an intergeneric hybrid between *Citrus* and *Poncirus trifoliata* was also studied. The details of technique followed for chromosomal studies have been given in an earlier publication (Naithani and Raghuvanshi 1962a).

Observations

The details of cyto-genetical studies of all these members have already been published (Naithani and Raghuvanshi 1958a, 1958b, 1961a, 1962b, 1962c, Raghuvanshi 1958, 1962a). The present paper deals with the conclusions drawn on the basis of these observations.

1. *Somatic chromosomes*

The normal chromosome number of all the species investigated has been found to be $2n=18$. The size of the somatic chromosomes is short and show gradual variation in size. Some of the longer chromosomes show secondary constrictions also. The karyotypic investigations on these various species of *Citrus* clearly reveal the part played by structural changes of chromosomes in the origin of new species.

2. *Bud mutations in Citrus*

The cells of somatic tissue of most of the species occasionally show tetraploid cells, chromatin bridges and lagging chromosomes etc. (Figs. 1, 2, 3). The significance of these anomalies in somatic tissue becomes clear when we take into consideration the fact that most of these members are being propagated vegetatively. The altered nuclei produced due to various irregularities in mitosis enter the growing region. These growing regions ultimately form new shoots, which may vary greatly from the parent plant. Thus a

new type may be produced in such a short duration.

Bud sports are very common in *Citrus*. The various varieties of grape fruits (*C. paradiso*) now under cultivation have originated as bud mutations (Webber 1948). The variety Marsh seedless probably originated as bud sports from the seedling having normal seedy fruits. It is a perfectly seedless variety. Marsh by bud mutation gave rise to Thompson which in turn gave through bud mutation the red fleshed variety Ruby, which has normal seedy fruits. About 52% PMCs in Marsh at metaphase show normal bivalent formation (Naithani and Raghuvanshi 1962c) while rest of the nuclei have two to four univalents (Fig. 4). But there is occasional failure of spindle mechanism at metaphase II (Fig. 5).

The variety Ruby originated as bud sport of Marsh. Ruby is a seedy variety and the pollen sterility is 4% and it shows normal meiosis (Fig. 6). Here we have an instance where a seedless variety Marsh, which originated from normal seedy type through bud variation, ultimately again produced a seedy variety, the Ruby. The occasional presence of two to four univalents in Marsh may be due to some imperceptible structural changes in the chromosomes. But the high sterility in Marsh appears to be genic.

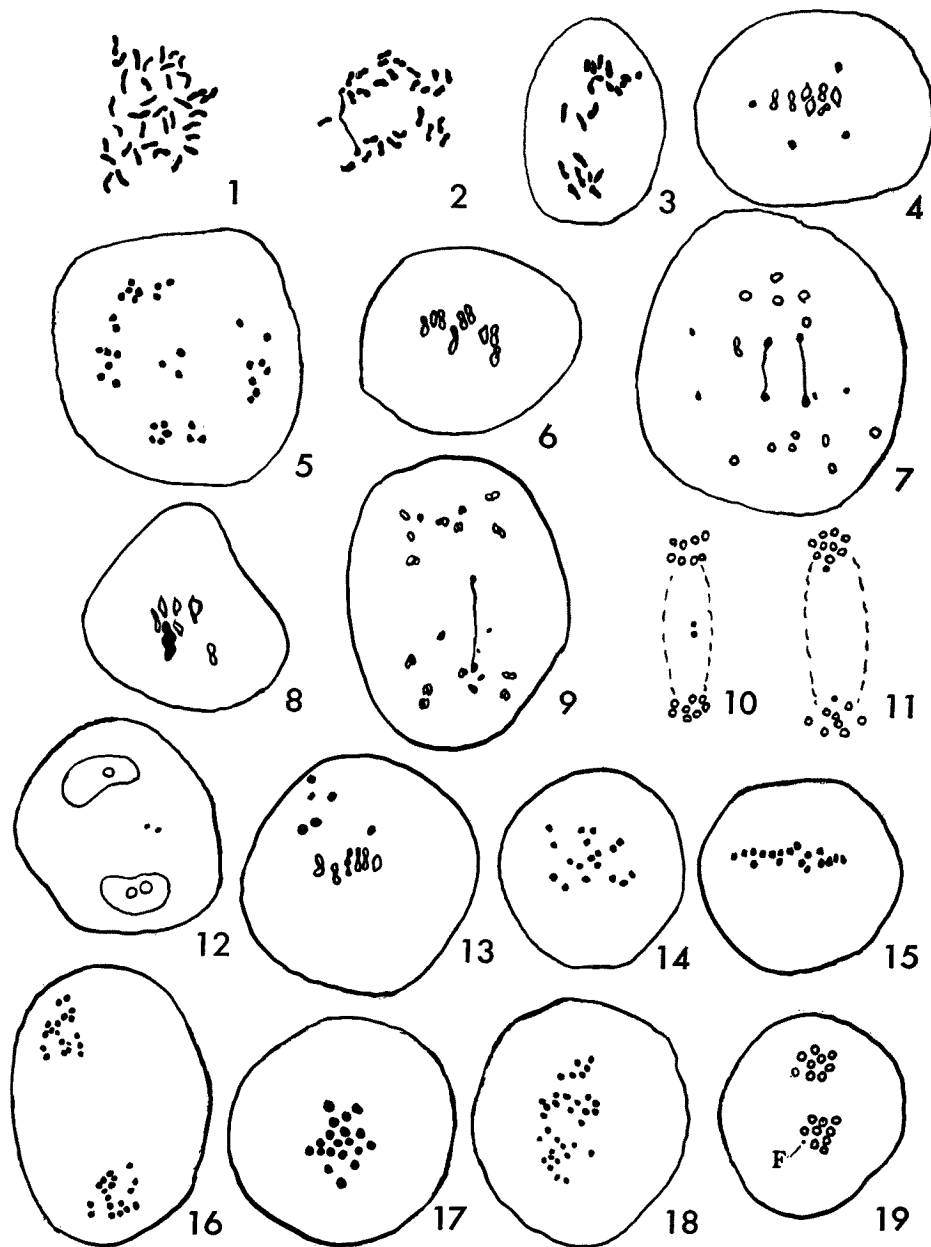
Thus the structural and genic alteration survive in *Citrus* species which are mainly propagated through vegetative means. This provides a wide scope for the origin of new forms because in the plants reproducing through the normal sexual means such chromosomal alterations cannot survive. Most of the bud variations in *Citrus* have diploid chromosome number ($2n=18$) though tetraploid cells have also been observed in somatic tissue. The chances of survival and entrance into daughter shoots of structural alterations are much more than the numerical ones because the tetraploid cells are comparatively slow in division and as such would not be able to compete with the cells having normal diploid number. This explains why there are comparatively few instances of polyploid bud mutations.

3. *Inversion heterozygosity*

The bridge and fragment at metaphase I and II are indications of inversion heterozygosity. Out of twenty four members studied eight show the bridge at anaphase in varying proportions.

The frequency of bridge formation in any species depends on three factors, chiasma frequency, number of inversions and size of inversions. Upcott (1937) has classified the inversion heterozygotes into three groups. The group one

Figs. 1-19. 1, tetraploid cell in somatic tissue (*C. aurantifolia* var. Kagzi lime). 2, chromatin bridge at anaphase of mitosis (*C. aurantifolia* var. Kagzi lime). 3, laggards at somatic anaphase (*C. limette* var. Metha). 4, metaphase I (Marsh grape fruit). 5, PMC showing failure of spindle mechanism (Marsh grape fruit). 6, metaphase I (Ruby grape fruit). 7, anaphase I showing two bridges, two fragments and two half univalents oriented opposite each others (Kagzi Kalan lemon). 8, metaphase I showing a ring of six chromosomes (*C. Assamensis*). 9, anaphase I showing dividing univalent (Malta). 10, anaphase I showing inclusion of half univalents in anaphasic group. (Malta). 11, two half univalents excluded



from the nuclei at interkinesis (Malta). 12, metaphase I showing five univalents and a trivalent (*C. Assamensis*). 13, restitution nucleus (Malta). 14, PMC showing eighteen univalents oriented on a single spindle (Malta). 15, PMC showing two spindles having eighteen elements each (Malta). 16, metaphase in binucleate PMC (*C. penvisculata*). 17, binucleate PMC showing failure of metaphase I spindle (*C. aurantifolia* var. Kagzi lime). 18, anaphase showing inclusion of a fragment in a telophasic plate (*C. penvisculata*) (Figs. 1-3 $\times 1416$; Figs. 4-19 $\times 810$).

shows more than ten percent bridges, the group two less than ten percent bridges and group three shows no bridge formation. The three members, *Citrus penvisculata*, var. Italian round lemon and Kagzi Kalan lemon come under first category. While *C. Assamensis*, Mayer lemon, Lyalpur orange, Citron bajoura and *C. Karna* come under second category showing less than ten percent bridge formation. The rest of the species (Table 1) come under the third category.

Table 1

Name	Average univalents per PMC at metaphase I	Percentage of bridges at anaphase	Percentage of pollen sterility
1. <i>C. penvisculata</i>	0.64	11	45
2. <i>C. aurantifolia</i> var. Kagzi lime	0.32	—	24
3. Rangpur lime	0.24	—	15
4. Citron bajoura	0.16	Rare	20
5. <i>C. limonia</i> var. Hill lemon	0.08	—	17
6. <i>C. limonia</i> var. Kagzi Kalan	1.2	11	48
7. <i>C. limonia</i> var. Nepalese oblong	0.4	—	12
8. <i>C. limonia</i> var. Italian oblong	0.32	—	18
9. <i>C. limonia</i> var. Italian round	0.16	11	22
10. Mayer lemon	0.28	3	15
11. <i>C. Karna</i>	1.52	Rare	30
12. <i>C. limatte</i> var. Metha (Sweet lime)	0.54	—	15
13. <i>C. sinensis</i> var. Mosambi	0.08	—	8
14. <i>C. sinensis</i> var. Malta	0.20	—	19
15. <i>C. reticulata</i> var. Nagpur Sangtra	—	—	8
16. <i>C. reticulata</i> var. Hill orange	—	—	4
17. <i>C. reticulata</i> var. Lyalpur orange	0.16	Rare	17
18. <i>C. reticulata</i> var. Satsuma Mikan	—	—	3
19. <i>C. rugulosa</i>	—	—	3
20. <i>C. grandis</i> var. Red flesh	—	—	5
21. <i>C. paradisi</i> var. Marsh	1.12	—	86
22. <i>C. paradisi</i> var. Ruby	—	—	4
23. Poorman orange	—	—	4
24. <i>C. Assamensis</i>	0.44	8	45
25. <i>C. microcarpa</i>	—	—	8
26. Rusk	1.08	—	52

In all the taxa showing inversion heterozygosity only one bivalent is involved in inversion. Two bivalents showing bridge formation have been observed in Kagzi Kalan lemon (Fig. 7). The percentage of bridge formation in this case is also fairly high. The Kagzi Kalan lemon shows the maximum amount of inversion heterozygosity among all the members studied.

The question arises whether the inversions are present only in the taxa showing bridge fragment configuration. The two genera *Paris* and *Paeonia* have among the largest chromosomes in the plant kingdom and they exceed

all the others in the presence of detectable inversions. It is because the inverted segments of the chromosomes in these cases which are relatively short in relation to the length of the chromosomes would nevertheless be long when compared to total length of chromosomes in *Citrus* species. It is possible, therefore, that the small inverted segments in the chromosomes of *Citrus* would never form chiasma when paired with a normal segment. So the bridge fragment configurations in *Citrus* may not reveal all the inversions for which *Citrus* species may be heterozygous.

The inversions are known to appear from time to time in most species. Various meiotic anomalies are quite common in species of *Citrus* so the production of inversions may also be quite frequent. If by chance these become associated with gene combination of selective value they may become established as permanent acquisitions of species populations, either in homozygous condition, in which they promote racial differentiation or in heterozygous condition. In the *Citrus* species having nucellar embryony there are every chances that such structural differences can survive.

4. *Translocation heterozygosity*

The presence of translocations in a diploid member is revealed by the association of more than two chromosomes resulting in formations of rings and chains. Such rings and chains of more than two chromosomes have been observed in *Citrus Assamensis* (Naithani and Raghuvanshi 1958b, Raghuvanshi 1962a). This presumably is the first record of multivalent formation in ordinary diploid species of *Citrus*. The highest association observed is a ring of six chromosomes (Fig. 8). This species shows bridge and fragment configuration also (Fig. 9). It is of great significance from evolutionary point of view because it clearly indicates the role played by both translocations and inversions in speciation. Here is an instance where both the structural changes, translocations and inversions, of major evolutionary importance in plant species, occur in the same individual.

In nature the translocation heterozygotes may arise due to crossing between two species or races or due to structural rearrangement of chromosomes in a homozygous free mating population. The translocation heterozygotes are subjected to negative selection pressure tending to decrease their frequency and eventually to eliminate them altogether. More than one embryo has been observed in *C. Assamensis*. Besides it always comes true to the type when propagated through seeds. This may be due to nucellar embryos. Under such circumstances translocations can multiply and become established somewhat more easily than in obligatory cross fertilizing forms.

5. *Univalents*

The presence of univalents appear to be fairly common among various species of *Citrus*. Out of 25 members studied as many as 17 show univalents in different proportions (Table 1). The average univalent per PMC at metaphase

is maximum in case of *C. Karna* (1.52%) followed by Kagzi Kalan lemon and Marsh grape fruit. The rest of 14 members have comparatively less percentage of univalents.

In *Citrus* species the univalent chromosomes arise in two ways. It is either a chromosome which has never undergone pairing at zygotene or it is one which paired to form a bivalent but whose two component chromosomes separated at diplotene owing to the fact that no chiasma was formed between them. The paired univalents, arising due to precocious separation at metaphase do not effect the normal segregation.

There are two different degrees of variation in univalent behaviour at meiosis in various members of genus *Citrus*.

1. Delayed equatorial orientation followed by division, as in *Triticum Aegilops* hybrids (Kihara 1931).

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|---------------------------------------|--|
| 1. <i>C. limonia</i> var. Kagzi Kalan | 5. <i>C. paradisi</i> var. Marsh grape fruit |
| 2. Meyer lemon | |
| 3. <i>C. Karna</i> | 6. <i>C. Assamensis</i> |
| 4. <i>C. limette</i> | |

2. Delayed self orientation not followed by division, as in trisomics *Mecostethus* (Callan 1941).

- | | |
|---|--|
| 1. <i>C. penviculata</i> | 7. <i>C. limonia</i> var. Italian oblong |
| 2. <i>C. aurantifolia</i> var. Kagzi lime | 8. <i>C. limonia</i> var. Italian round |
| 3. Rangpur lime | 9. <i>C. sinensis</i> var. Mosambi |
| 4. <i>C. medica</i> var. Citron bajoura | 10. <i>C. sinensis</i> var. Malta |
| 5. <i>C. limonia</i> var. Hill lemon | 11. <i>C. reticulata</i> var. Lyalpur orange |
| 6. <i>C. limonia</i> var. Nepalese oblong | |

Such behaviour of the univalents is due to their possessing only one undivided centromere. Where there is delayed equational orientation of univalents followed by the division (Fig. 10) the half univalents follow either of the two courses i.e. either they move to the two poles, though tardly yet reach the poles in time to be included in the telophasic plates (Fig. 11) or they remain lying in the cytoplasm (Fig. 12). As the half univalents do not divide at the second division they remain distributed at random to the two poles.

The factors that could be responsible for the presence of univalent chromosomes in various members of genus *Citrus* appear to be inversions, translocations and hybridization. The part played by segmental interchange in the presence of univalents is comparatively small. Out of all the members studied only *C. Assamensis* shows multivalent formation. The maximum number of univalents observed in this case is five (Fig. 13) though frequently only two univalents are present. *C. Assamensis* is also an inversion heterozygote (Fig. 9). So in this case the univalents may arise both as a result of inversions and translocations.

The inversion heterozygosity is quite prevalent in *C. penviculata*, Italian

round, Kagzi Kalan lemon, Mayer lemon, and to some extent in *C. Karna*, Lyalpur orange and Citron bajoura. The presence of inversions in these taxa obstruct the regular pairing and chiasma formation, thus giving rise to univalents.

Some members under investigation like *C. Karna*, Mayer lemon and Rangpur lime are known to be the result of natural hybridization between various members of genus *Citrus*. The two members *C. Karna* and Mayer lemon also show inversion heterozygosity which might be the consequence of hybridization. In these taxa the presence of univalents appears to be due to their hybrid origin. The variation in the percentage of univalents, in these various natural occurring hybrids, might be explained on the basis of comparative homology that existed between the chromosomes of the parent species and also probably on the time interval that might have elapsed since the hybridization took place. It is well known that the nature always tends towards stability. The hybrids may after passing through successive sexual generations come to have fairly regular meiosis. *C. Karna* shows the maximum univalency followed by Mayer lemon and Rangpur lime.

Besides lack of homology a whole series of environmental and genetical factors might effect the conjugation of chromosomes (Gowen 1928, Beadle 1930, Berger *et al* 1934, Katayama 1931). Though special care was taken to collect the material from healthy plants and under normal environmental condition yet these environmental factor might have also caused univalency to certain extent in various species of *Citrus*.

6. *Double division of the univalents*

During the course of meiotic studies in *C. sinensis* var. Malta it was observed that though there is generally regular bivalent formation yet the first metaphase spindle may break down resulting in a restitution nucleus (Fig. 14). These eighteen chromosomes again orient on a single spindle (Fig. 15) and later on the two group of separated half univalents again divide (Fig. 16). Thus the univalents undergo division twice. Such double division of univalents has been observed by Sikka (1940) in *Brassica* hybrids and Morinaga (1929a, 1929b) in hybrids of *Brassica napella*. Meurman (1928) found in *Ribes* that double division of chromosomes was characteristic of certain cells which showed no pairing of chromosomes while others with more or less bivalents did not show this phenomenon. Darlington (1930) points out that lack of pairing among the chromosomes is apparently a condition equal to the suppression of first division which ultimately leads to double division of chromosomes. Lack of pairing may be a necessary condition but that it is not a cause of double division of chromosomes is shown by the fact that many other members of genus *Citrus* like *C. penvisculata*, *C. Karna* etc. (Table 1) have much higher percentage of the univalents but they do not show double division of chromosomes. The cause of such double division might be genetical or physiological.

7. *Restitution nuclei*

The restitution nuclei have been observed in Kagzi lime, Marsh grape fruit, sweet lime, Italian round lemon and Malta (Fig. 14). The restitution nucleus was for the first time reported by Rosenberg (1927) in *Hieracium*. Since then it has been reported both in plants and animals but nothing definite is known as regards the cause of restitution nucleus formation. Darlington (1930) suggested that restitution nuclei in *Prunus* was formed by lagging of univalents at the second division. Sapehin (1933), however, suggested that it was due to 'operation' of Mendelian factors.

In *Citrus* species showing restitution nuclei the univalents do not appear to be the main cause because many plants having higher percentage of univalence do not show restitution nucleus. Most of the *Citrus* taxa showing restitution nuclei have fairly regular bivalent formation. The cause of formation of restitution nuclei seems to lie mainly in failure of spindle mechanism. Thus though the daughter bivalents separate yet they are unable to move to the poles and all of them are ultimately included in a single membrane.

The evolutionary significance of restitution nuclei lie in the production of diploid gametes which may give rise to polyploid individuals.

8. *Binucleate pollen mother cells*

The binucleate PMCs have been observed in *C. rugulosa*, *C. Assamensis*, Kagzi lime, Italian round lemon, sweet lime (Metha) and *C. penvisculata* (Fig. 17). Generally meiosis in binucleate PMCs is regular and a metaphase plate with eighteen bivalents at the equator is formed (Fig. 17). The failure of spindle mechanism at the first division of binucleate PMC is indicated in very rare cases (Fig. 18). In all the species showing binucleate PMCs giant-pollen grains have also been observed.

The binucleate PMCs produce diploid gametes, which ultimately produce polyploid forms. Thus if a diploid pollen fertilizes a haploid ovule a triploid will result. The triploids are of great value in horticulture because they are unbalanced types, as such due to irregularities in meiosis they are more or less sterile. This results in seedlessness, which is of great economic value in *Citrus*. Such triploids are generally propagated either through cuttings or budding. Some well known triploids of economic importance are Tahiti lime and Bearss lime (Webber 1948).

9. *Pollen sterility*

The percentage of pollen abortion appears to be fairly high among various taxa of *Citrus* (Table 1). The taxa showing normal meiosis have the pollen sterility ranging from 3 to 8% while the other taxa show much higher pollen sterility. Dobzhansky (1933, 1941, p, 292) has classified sterility as genic and chromosomal. In genic sterility are included all types which are produced by failure of sex organs to develop upto the point where meiosis can take place or certain genically controlled anomalies of spindle behaviour and genically controlled asynapsis or desynapsis (Clarke 1940, Li, Pao and Li 1945). The

chromosomal sterility on the other hand is marked by lack of homology between the chromosomes of the individual which results in higher percentage of univalent chromosomes.

The Marsh grape fruit shows the maximum sterility and is seedless. There is fairly regular bivalent formation but the main meiotic anomaly is the failure of second division spindle which hinders the formation of normal telophasic plates. Thus the sterility in Marsh grape fruit appears to be genic.

In most of the *Citrus* species univalents have been observed in various proportions (Table 1). Due to uncertain behaviour of univalents the telophasic plates come to possess unequal number of chromosomes. The univalents are also sometimes left in the cytoplasm which leads to deficiency of chromatin material in some nuclei. Also due to irregular separation some chromosomes may be duplicated in the same pole, while the other becomes deficient for one. These various anomalies lead to pollen sterility.

The inversion heterozygosity is quite common in various species of *Citrus* (Table 1). The segmental interchange has been observed in *C. Assamensis*. According to Upcott (1937) the structural hybridity and fertility of plant are in the reverse relation to each other in *Tulipa*. The frequency of aborted pollens reflects the amount and the kind of crossing over within the inversion loop. The maximum percentage of bridge formation has been observed in *C. limonia* var. Kagzi Kalan, *C. penvisculata* and *C. Assamensis* and consequently they also show high pollen sterility. Sometimes the fragment may be included in one of the telophasic plates (Fig. 19) and the chromatin of the acentric fragment may permit the normal development of the pollen grain.

Besides these factors the cryptic structural hybridity of Stebbins (1945) may also be present in *Citrus* chromosomes. But as the size of the chromosomes in *Citrus* is small it is not possible to demonstrate it.

Thus it is clear that the pollen sterility in genus *Citrus* is both genic and chromosomal.

Evolution in genus *Citrus*

The gene mutations, chromosome changes restriction of population size, natural selection and development of isolating mechanism have dominated the evolutionary history of most of the genera. In certain genera, polyploidy self-fertilization, apogamy and asexual reproduction result in many complications.

The diploidy, $2n=18$, appears to be physiologically optimum condition in genus *Citrus*. There are comparatively few instances of naturally occurring polyploids of some importance. No doubt, the polyploid forms are known to occur in most breeding cultures. As has already been mentioned the occurrence of polyploid gametes, is a common phenomenon in some species. The sudden appearance of polyploid bud mutants have also been noted, which could arise due to entry of polyploid somatic cell into the growing point of the shoot. Besides ordinary diploid forms are known to produce polyploid

nucellar embryos. Despite the fact that polyploid forms in *Citrus* could originate in a number of ways, as indicated, they are not able to compete with their diploid counterparts in struggle for existence. The leaves of polyploid forms in *Citrus* tend to be broader, thicker, and darker growth is slower, and tree size is consequently smaller and less erect. There is less tendency to bloom and set fruits. The fruits have thicker rind, larger and often more prominent oil glands stouter juice vesicles and more fibrous internal structure. Most of these characters place polyploid forms in *Citrus* at a disadvantage in comparison with their diploid counterparts and as such they are ultimately eliminated.

In absence of any important role of polyploidy, the question arises as to on what lines evolution has proceeded in this genus. The karyological and meiotic studies have clearly revealed that the structural changes have played a dominant role. It is interesting to speculate as to how this marked structural hybridity has arisen in various species of *Citrus*. The structural changes, when present in an organism, are known to occur quite independently in homologous chromosomes. They develop a condition of hybridity, within the organism, in no way different from that produced by crossing different individuals. Thus structural hybridity of an organism is dependent on the structural differentiation of the homologues, when they are derived from the same parental chromosomes, or from different chromosomes in the same parent, or again from widely separate crosses. Out of all the species showing marked structural hybridity, Meyer lemon is known to be a natural hybrid between *C. sinensis* and *C. limonia*. At least in this case the structural hybridity may have arisen due to crossing of two different species. While in *C. limonia* var. Italian round, it appears to be mainly due to structural differentiation of homologues, that are derived from the same parent because the only other variety of Italian lemon does not show inversion heterozygosity. Nothing definite is known regarding the inter-relationship of *C. penvisculata*, *C. limonia* var Kagzi Kalan and *C. Assamensis*, with other species of *Citrus*. Under such circumstances, the structural hybridity in these cases may be the consequence of hybridization or simply due to structural differentiation of homologous chromosomes, in a natural wild mating population.

The structural changes of chromosomes, as a mechanism of variation, in *Citrus* is of great significance. As opposed to gene mutations, their chief importance lies in effecting rapid evolutionary changes, provided the new type has the survival value. In a apogamic complex, like *Citrus*, all the structural changes in the chromosomes could survive to some extent, depending on the percentage of nucellar embryony in the particular case.

The effect of these structural changes, in *Citrus* species, is that they inhibit the pairing of relatively changed chromosomes, which ultimately leads to inter-sterility. Thus, sometimes, the rearrangement of the genes, in the chromosome set, may occur without effecting the phenotype but thereby makes

it more or less incompatible with the type. Gates (1938) is of the opinion that, as a result of this inter-sterility, the new chromosome type, as a fresh centre of breeding and mutation, is placed on the road to becoming a new species.

In genus *Citrus*, of all the structural changes to which chromosomes are liable, inversion and segmental interchange seem to have played the most important role in speciation. The universal importance of these structural changes could be realized from the fact that both ring forming plants and inversion heterozygotes occur in nature spontaneously. The various natural races in *Drosophila*, have been distinguished, on the basis of inversion heterozygosity (Sturtevant 1926, Koller 1935).

One of the factors concerned in effecting the variation, due to inversion in various species of *Citrus*, is the length of the reversed segment and the frequency of crossing over in it. The long inversions produce deleterious effects on the organism as they allow frequent crossing over in the inverted segment. This leads to bridge formation, which results in unequal distribution of the chromatin material to the two nuclei and finally to sterility. This gradual accumulation of sterility, in the long run, leads to complete suppression of sexual reproduction and the species has to resort to vegetative reproduction for its existence. Many *Citrus* forms are known to be exclusively cultivated through vegetative means. The detailed cytological studies, in such forms, have not been undertaken. So, may be, these are highly heterozygous forms, in which sexual reproduction has been completely suppressed. On the other hand short inversions, in the chromosomes of *Citrus* species, may pair straight with the corresponding normal segments by torsion pairing. Such suppression of crossing over in small inverted segments results in group of genes, located in the region, being inherited as a unit. Sturtevant (1926) has inferred the genetical suppression of crossing over in certain regions of X chromosomes, in *Drosophila*, to be due to such inverted segments. The short segments do not reduce the fertility appreciably, but produce the genetical isolation of the reversed segments, on account of torsion pairing and failure of crossing over. According to Darlington (1937) if there arise gene mutation in such segment, they become centre of discontinuity in the species. Richardson (1936) further suggests, that crossing over taking place in the nonhomologous segments of the chromosomes, structurally differentiated due to inversion, produce new chromosomes that produce duplications or deficiencies. In consequence the plant, homozygous for new chromosomes, is produced owing to the self-fertilization of the individuals, that are heterozygous for new chromosomes.

One of the most significant feature of evolution in genus *Citrus* is the weak development of isolation mechanism. Most of its members easily hybridize among themselves, as pointed out by Swingle (1948, p. 387).

Even the allied genera *Fortunella* and *Poncirus* are known to hybridize

with *Citrus* without much difficulty. The nucellar embryony in most forms reduces the chances of production of hybrids. Thus by reducing the opportunity for inter-breeding, through substitution of asexual reproduction, nucellar embryony acts as a agent of partial isolation (Dobzhansky 1937) and, therefore, it probably has favoured evolutionary differentiation of genetic types in *Citrus*.

Though the role of gene mutations in evolution of *Citrus* has not been studied by the author, yet, the findings of Frost (1948, p. 838) clearly reveal frequent occurrence of gene mutations in most of *Citrus* species.

According to Darlington (1937) in the structural and numerical hybrids, the mechanism of reproduction and laws of heredity partially break down. In such cases both meiosis and mitosis suffer abnormality.

The breakdown of mitosis, in some of the species of *Citrus*, is marked by the presence of lagging chromosomes and chromatin bridges at anaphase. Such anomalies seem to lead to the formation of polyploid nucleus. The possibility of the origin of tetraploid bud variants, due to entry of such tetraploid cells into growing shoots, has already been discussed. Also due to these various mitotic anomalies, nuclei with different genetical constitution are produced. If such nuclei get into the growing tissue, than a new type, quite different from the parent, is produced in no time.

The breakdown of meiosis is also evident in most of *Citrus* species. The univalent chromosomes, in various proportions, have been observed in seventeen out of twenty five taxa. These univalents arise both as a result of lack of synapsis as well as precocious separation. In some cases these univalents divide at the first division while at the second division in others. The univalents, that are not included in the telophasic plates, are ultimately lost in the cytoplasm. The micronuclei have been observed in *Citrus Assamensis* and Marsh grape fruit. At least in some cases the structural differences, in the pairing chromosomes, appear to be the main cause of failure of pairing. The inversion heterozygosity has been observed, in various degrees, in eight members. While segmental interchange has been observed only in *C. Assamensis*. On the other hand, some members like Rangpur lime and *C. Karna* etc. are known to be natural hybrids.

The breakdown of first or second division spindle has been observed in Kagzi lime, Marsh grape fruit, Sweet lime, Italian round lemon, Malta and *C. Assamensis*. This breakdown of spindle leads to the formation of restitution nucleus. Such restitution nuclei ultimately give rise to polyploid gametes. The breakdown of spindle appears to be genotypically controlled in Malta orange. In this case quite regular bivalent formation is observed and also all the bivalents orient themselves normally on the equator. The daughter bivalents separate out but they do not move to the poles and ultimately a restitution nucleus is formed. Malta orange also shows double division of chromosomes. All the eighteen chromosomes, after interkinesis

in the first division restitution nucleus, orient on a single spindle, divide, and separate out to the two poles. The eighteen chromosomes at the two poles again orient themselves on the spindle and undergo division. Thus, the chromosomes divide twice.

Another interesting feature of meiosis is the presence of binucleate PMCs in *C. penvisculata*, Kagzi lime, Italian round lemon, Mayer lemon, Sweet lime, *C. rugulosa* and *C. Assamensis*. The phenomenon of cytomixis, which has been observed in this genus for the first time (Naithani and Raghuvanshi 1958a) is quite significant because some workers have considered it to be a pathological condition.

The various meiotic anomalies clearly show the abundance of heterozygosis in various *Citrus* species.

It is known that failure of meiosis and special genetical properties, may in certain instances, independently determine the nucellar embryony. In such cases it is clear that special genetic properties are necessary for allowing the development of nucellar embryos in absence of sexual embryos. This property appears to be characteristic of genus *Citrus*. The breakdown of meiosis, as observed in most species, is of great significance when we study the evolution of nucellar embryony in *Citrus*.

The sexual embryos may develop in most of *Citrus* varieties, but then arises competition between the sexual and the apogamic embryos. The chances of elimination of one or the other type must depend largely on the number of nucellar embryos that start, and on the position and relative age and vigour of the two class of embryos. Thus sexual embryos, that are inferior in vigour to nucellar embryos are eliminated. With the abundant heterozygosis, as observed in most species, the gametic embryos of *Citrus* will have frequently unbalanced chromosome complements, which will certainly make them less viable. Thus these gametic embryos would be at a disadvantage in competition with the nucellar embryos, which possess the balanced chromosome complement of the parent plant.

Under whatever circumstances nucellar embryony might have originated, it has had significant effect on the evolutionary trends in *Citrus*. The reproduction through nucellar embryos like artificial vegetative reproduction, preserve any state of heterozygosis, that may have been produced either by hybridization or mutation.

In *Citrus* the evolutionary interaction of heterozygosis and nucellar embryony, probably has favoured not only gradual elimination of successful gametic reproduction, but also preservation and intensification of the tendency to produce nucellar embryos, from the time when nucellar embryony first appeared. According to Muller (1918) a considerable total rate of mutation with frequent cross pollination should tend to accumulation of unfavourable recessive genes, even in absence of asexual reproduction. If under such circumstances nucellar embryony originated in *Citrus*, forms with new charac-

ters would tend to be preserved by natural selection.

Thus gene mutations, hybridization, structural re-arrangement of the chromosomes and weak development of isolating mechanism are the main factors, which have dominated the evolutionary history in genus *Citrus*.

Summary

The detailed cytogenetical studies have been undertaken in 25 members of genus *Citrus*, for the first time to determine the chromosomal basis of speciation.

Inversion heterozygosity has been observed in *C. peninsculata*. This is the first record of inversion heterozygosity in genus *Citrus*.

Cytomixis is being reported for the first time in this genus.

Binucleate PMCs have been observed in some species, their significance in evolution of polyploid forms has been discussed.

C. limonia var. Italian round shows inversion bridges, while the other variety, I. oblong does not show any bridge and fragment configuration. This clearly shows the role of inversion in evolution of new forms in *Citrus*.

C. sinensis var. Malta shows occasional breakdown of first division spindle leading to formation of restitution nucleus. The double division of chromosomes have also been observed in Malta.

The high pollen sterility (86%) in Marsh grape fruit is genic because fairly regular bivalent formation has been observed.

C. Assamensis is a translocation heterozygote showing trivalents, quadrivalents and sexivalent at meiosis. Inversion bridges have also been observed in this case. This, presumably, is the first record of translocation heterozygosity in genus *Citrus*. A univalent bridge has been observed at anaphase I. The origin of this species has been discussed.

The somatic chromosomes in *Citrus* species vary between 3.5μ and 1μ . Karyotypes differ in different species.

The importance of mitotic anomalies, in the production of bud variants, has been emphasized.

The inversion heterozygosity has been observed in 8 out of 25 members of genus *Citrus*.

The univalent chromosomes occur in 17 out of 25 members.

The pollen sterility in *Citrus* species varies between 86% (Marsh grape fruit) and 3% (*C. rugulosa*). The structural hybridity and presence of univalent chromosomes appear to be the main cause of pollen sterility.

The evolution in genus *Citrus* has been discussed in detail. The polyploidy has not played any significant role in evolution. The structural changes, gene mutations and hybridization appear to have dominated the evolutionary history of genus *Citrus*. The breakdown of meiosis and its significance, with regard to the origin and evolution of nucellar embryony, has been emphasized.

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