CHROMOSOME AND CELL GENETICS

Edited by

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GORDON AND BREACH SCIENCE PUBLISHERS
New York London Paris Montreux Tokyo

Published in India by Oxford & IBH Publishing Co.

Published outside of India by Gordon and Breach Science Publishers S.A.
P.O. Box 161, 1820 Montreux 2, Switzerland

Gordon and Breach Science Publishers

P.O. Box 786
Cooper Station
New York, NY 10276
United States of America

P.O. Box 197
London WC 2E 9PX
England

58, rue Lhomond
75005 Paris
France

14-9 Okubo 3-chome
Shinjuku-ku
Tokyo 160
Japan

Library of Congress Cataloging in Publication Data
Main entry under title:

Chromosome and cell genetics.

Includes index.
1. Chromosomes. 2. Cytogenetics. I. Sharma, Arun Kumar, 1923—
II. Sharma, Archana Mookerjea.
QH600.C49 1985 574.87'322 85-5496
ISBN: 2-88124-038-0 (Primlani)

ISBN: 2-88124-038-0

Printed by Printsman Press, Faridabad, India

Preface

The last decade has witnessed significant developments in the study of heredity, mainly due to the remarkable inputs from cell, molecular and organismic genetics. The techniques of cell fusion, molecular hybridization and cell and tissue culture, coupled with mutagenesis, have emerged as powerful tools in the understanding of the events at the subcellular and molecular levels. Marked refinements of the separation techniques, involving both column and density gradient separation, as well as the use of restriction enzymes, have led to the identification of individual segments and sequence complexity in the chromosome architecture. The manipulation of genes, their cloning and utilization through appropriate vectors, are just the byproducts of this explosion of genetic methods.

The developments have covered a wide spectrum, ranging from the simplest microbe to the most complex eukaryote, *Homo sapiens*. The progress has been too phenomenal and too diverse for complete comprehension by any researcher working in any particular discipline of genetics. There has undoubtedly been a continuous assessment of these advances, both in the eukaryotic and prokaryotic systems in different forms, especially during the last International Congress of Genetics and the subsequent symposia. The remarkable findings based on the new methodologies mentioned earlier call for their dissemination amongst all interested in gene and genetics in general, and research workers in particular. Hardly any textbook, written at one particular time, can do full justice to this science when the explosion often leads to a complete reorientation of the basic concepts. The best example has been the split gene concept so elegantly demonstrated in recent years. Reviews on advances in genetics, published in peer-reviewed journals, undoubtedly meet the requirements of a specialist but not necessarily of a student or a researcher interested in learning about overall advances in genetics. The presentation of the latest developments in a comprehensible form by eminent experts in the field, both for the student and the advanced scholar, is the principal objective of this volume.

The canvas is too wide to be covered in a single volume and it is intended to have the other facets of genetics discussed in subsequent publications of this series. The editors sincerely hope that this series will be of benefit to those interested in having an overall comprehension of the continuing progress

of this unifying discipline. The authors are grateful to the experts who have contributed articles for this volume.

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1. Polyploidy and Its Role in the Evolution of Higher Plants

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1. Introduction

Polyploidy was and still is an important factor in the evolution of higher plants. About 70 per cent of all higher plants are estimated to be polyploids. Most of them belong to the very heterogeneous group of allopolyploids whereas the autopolyploid status has been evidenced so far only for a small number of species. The segment-allopolyploids occupy an intermediate position between auto- and allopolyploids. The proportion of polyploids is especially high in the *Gramineae* whereas it is very low in the gymnosperms. The importance of polyploidy research becomes evident from the fact that about 10,000 papers may have been published in this field during the past decades. Reviews with many references have been given by Stebbins [178–180], Schwanitz [164], Grant [65], Gottschalk [57] and Lewis [111] among others.

The analysis of the origin of polyploid species from their progenitors with lower genome levels has succeeded so far in a relatively small number of cases only. These investigations are not only carried out by means of the classical methods of this discipline such as morphology, taxonomy, plant geography, genetics, cytology, cytogenetics and plant breeding, but to an increasing extent also by means of biochemical methods. Very young branches of this field are tissue culture and somatic hybridization. The near future will show whether relevant problems of polyploidy research, which could not be studied by means of the classical methods, can be solved using these modern procedures.

Under consideration of the present status, even the optimal utilization of the methods available, in most cases, only facilitates to draw hypothetical conclusions without being able to confirm the concepts through the resynthesis of the respective species. The difficulties, which have to be overcome, are mainly related to the methodological possibilities of producing interspecific or intergeneric hybrids. The fusion of protoplasts of different origin and the development of somatic hybrids from the fusion products may be a successful way in some cases in which this aim cannot be reached via fusion of gametes. New impulses in experimental evolution research can also be expected from sterile cultures of hybrid embryos. But as long as these methods cannot be used on a broad scale investigations have to be continued using the classical cytogenetic methods, mainly Kihara's genome analysis. This is the main reason why many excellent results of polyploidy research have already been obtained some decades ago. Therefore, they have to be considered in a review on the present status of this field.

2. Methods for Increasing the Chromosome Number

The application of colchicine, introduced into mutation research in the 30's by Nebel [138] in *Tradescantia* and by Dermen [26] in *Rhoeo*, is still the most frequently used method nowadays for increasing the chromosome number. This holds true for both the production of auto- and of allopolyploid plants.

Apart from a few exceptional cases, no principally new results were obtained in this field during the past decades. It is therefore not necessary to review these well-known methods in detail. The advantage of the treatment of shoot tips consists in the fact that the very susceptible root system remains intact, but very often, chimerical plants arise having branches of different genome levels. This disadvantage can be avoided by treating seeds. Following this method, however, many of the young plantlets are unable to form a functioning root system and they die in very early stages of ontogenetic development. By germinating diploid seeds on colchicine-agar, not only tetraploid plants but plants with higher ploidy levels were obtained [50, 184]. A very effective method in cereals consists in floating the seedlings on the surface of the colchicine solution, the coleoptiles being immersed. This procedure has low lethality and leads reliably to polyploidization [104]. A modified method can be used in grasses for producing polyploid sectors by treating vegetative tillers [121]. This method can successfully be utilized in sterile hybrids and in plants with low seed production in which not enough material for seed treatment is available.

Since a few years, the methods of callus culture in combination with colchicine application are being used for producing polyploids. In *Hemerocallis flava*, for instance, tetra- and octaploid cells were obtained in this way from which tetraploid plants regenerated in high frequency [19]. This holds also true for producing allopolyploid plants. Colchicine treated callus tissue of the intergeneric hybrid ***Triticum crassum* × *Hordeum vulgare*** gave rise to chimeras having parts with doubled chromosome number [136]. It is still too early to judge the prospects of this method for polyploidy research. It should, however, be emphasized that the genotypic constitution of the initial material, used for tissue culturing, could play an important role with regard to the intensity of regeneration. Findings in this field are not yet available in polyploids but they have been obtained in different mutants of *Pisum sativum* which differ strikingly from each other in regeneration capacity (Jacobsen, unpub).

The action of some colchicine derivatives such as iso-colchicine, colchamine and trimethyl colchicinic acid is essentially lower than that of colchicine. Acenaphthene, introduced into polyploidy research by Shmuck [167] and Kostoff [102], in its action similar to colchicine, is only little used. Its application is difficult because it is not soluble in water.

Some natural compounds, partly used in daily life, were not only found to induce gene or chromosome mutations but also anomalies at the genome level comparable to the action of colchicine. This holds true for certain herbicides and insecticides, distinct alkaloids and ingredients of medicinal plants. Polyploidizing processes are also induced in low frequencies by X- and gamma rays, also by some mutagenic chemicals which are widely used for inducing gene and chromosome mutations.

In some families of the plant kingdom, the induction of polyploidy by means of colchicine makes considerable difficulties. Many genera of the *Leguminosae* are an instructive example for such an unusual behaviour. It is therefore of interest that nitrous oxide was found to be much more effective than colchicine in different *Trifolium* species [189].

A fascinating method for producing allopolyploid plants is the somatic hybridization. If the protoplasts of somatic cells of two different diploid species are fused, allotetraploid plants can arise from the fusion products. In some genera, for instance in *Nicotiana*, *Solanum* and in different *Cruciferae*, this method is used with great success as far as the number of regenerated shoots is concerned.

The limits of all the methods available are mostly reached with the simple doubling of the chromosome number, i.e. with the level of $4n$ in diploid species. The optimum of the physiological efficiency of most plant species is generally correlated with the "normal" genome level of the respective taxa. The experimental increase of the number of genomes leads mostly to a reduction of the selection value of the plants. This holds already true for the transition from $2n$ to $4n$. Higher levels are rarely realized. The respective plants show either manifold monstrosities and are sterile if they are at all able to produce flowers; very often, they are semilethal. These negative effects appear in phylogenetically polyploid species more drastically than in diploid ones. So far, only few exceptions from this widely valid rule are known.

The highest autopolyploid level, which has been reached experimentally, is $16n$ in a lateral branch of an octaploid tomato [53]. In allopolyploids, the levels of $8n$ to $12n$ are realized a little more easily than in autopolyploids, especially in *Gramineae*. The highest levels known in this group are $14n$-plants from the cross *Bromus carinatus* × *trinii* and $16n$-plants from different species of the *Bromus carinatus* complex [176, 182]. These results are in strong contradiction to the high levels known for many polyploid species of the plant kingdom.

3. Autopolyploid Plants

A tremendous amount of work has been done during the past decades characterizing the morphology, physiology, fertility, vitality and the meiotic behaviour of experimentally produced autopolyploid plants in comparison to their diploid initial lines. Knowledge on the evolutionary importance of autopolyploidy, on the other hand, is very restricted as the autopolyploid status could be evidenced so far only for a small number of species in higher plants.

3.1. The Fertility of Experimentally Produced Autopolyploids

The transition from $2n$ to $4n$ is in general combined with a reduction of the fertility. This is the main reason for the reduced selection value of the auto-

tetraploids in all those cases in which the species studied propagate sexually. This holds also true for the autotetraploid strains of those diploid or amphiploid crops which are grown because of their seed production such as cereals, oil crops and most of the legumes.

The degree of the reduction in fertility varies widely in different species. One should therefore avoid to generalize findings obtained only in a small number of species. On the contrary, only a very broad spectrum of data, collected from a great number of different species, gives that objective survey which is necessary for judging the influence of the increased number of genomes on the fertility of the plants. This becomes clear from the two graphs of Fig. 1, in which the pollen fertility of autotetraploid plants of 135 species and the seed fertility of 154 species are presented. In the majority, seed and pollen fertility were studied in the same species. The values were collected from a great number of publications; they are related to the control values of the diploid initial material.

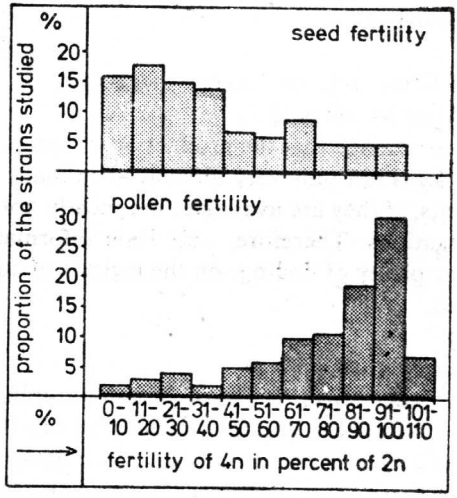

Fig. 1. The seed and pollen fertility of autotetraploid strains as related to the control values of the diploid initial lines.
Seed fertility: 154 species.
Pollen fertility: 135 species.

With regard to the seed fertility, the reaction of the autotetraploids is very negative. Only 5 per cent of the 154 strains tested were found to be approximately competitive to their diploid parental strains. In about 60 per cent of the material studied, the seed production ranged from 0 to 40 per cent of the diploid control values. The values for the pollen fertility are not so negative. About 30 per cent of the strains were similar to their diploid mother lines; 7 per cent were even better from reasons not known.

It may be surprising that the two graphs of Fig. 1 do not show a higher degree of conformity. This can, however, not be expected if the background for these two kinds of fertility is considered. The pollen fertility is mainly governed by the course of microsporogenesis. The seed fertility, however, is a very complex phenomenon depending not only on the degree of anomalies during macrosporogenesis. The low seed production is often the result of a summarizing effect of several negative factors. A diploid tomato plant, for instance, produces about 40 fruits with 40–100 seeds per fruit. An autotetraploid plant has only four to five fruits with about 10 seeds each.

Even the findings obtained from such a broad basis as illustrated in Fig. 1 cannot be generalized. Values of autotetraploid strains of "diploid" species have been used for constructing these two graphs irrespective of the fact whether the initial material is really diploid or phylogenetically polyploid. The reduction of the fertility of plants with doubled chromosome number is much higher in polyploid species than in diploid ones. This has clearly been demonstrated by Westergaard [202] studying autotetraploids of 21 diploid, tetra- and hexaploid *Solanum* species.

3.2. The Meiotic Behaviour of Experimentally Produced Autopolyploids

It was already mentioned that the production of autopolyploid plants with levels higher than $4n$ is not only very difficult or in many species impossible, but that these plants, if they are available, are mostly not suited for detailed cytological investigations. Therefore, only little information is available in this group, whereas plenty of findings on the meiosis of autotetraploid plants have been obtained.

3.2.1. The meiosis of autotetraploids

In the pollen mother cells (PMCs) of an autotetraploid plant, each chromosome of the complement is present in the form of four homologues. They show a characteristic pairing behaviour: either one quadrivalent or two bivalents are formed during zygotene and pachytene. It was already demonstrated by Darlington [22] that the close pairing of homologous chromosomes, as it is observed in the PMCs of diploid plants, occurs principally only between two chromosomal units irrespective of the fact how many homologues are present in the nucleus. This phenomenon is called "primary pairing". In an autotetraploid nucleus, the primary pairing can happen in two different modifications (Fig. 2).

—Two normal bivalents arise each of them showing close chromosome pairing at pachytene.

—The four homologous chromosomes are united to a cross-shaped configuration, a quadrivalent, which shows primary pairing in its four arms.

As the four chromosomes of each group are fully homologous and struc-

turally identical, they can replace each other during the course of pairing. The two arms of such a chromosome do not necessarily pair with corresponding arms of a single second chromosome but they can pair with the arms of two different chromosomes of the group. In this way, a partner exchange with regard to the course of primary pairing occurs giving rise to the cross-shaped quadrivalent just mentioned (Fig. 2).

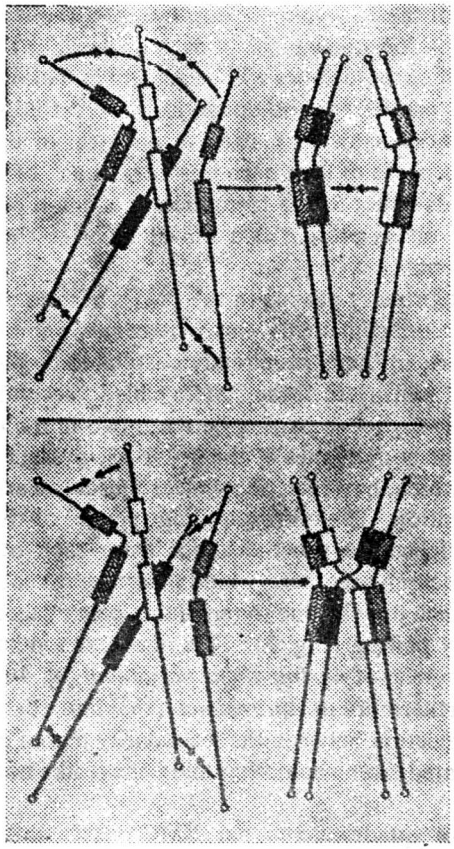

Fig. 2. Above: The occurrence of two bivalents showing secondary association from four homologous chromosomes in the zygotene and pachytene of an autotetraploid plant.
Below: The occurrence of a cross-shaped partner exchange figure giving rise to a quadrivalent.

Normally, only the results of these two possibilities can be observed by evaluating the frequency of bivalents and quadrivalents in metaphase I. In some cytologically favourable species, however, the course of pairing can be

directly observed. The spatial location of the four homologous chromosomes within the nucleus seems to be responsible for which of the configurations will arise. If they are distant from each other, there is only little probability that they are united in single pairing configuration; two bivalents arise. Lie they, however, closer together, it is obviously a matter of chance whether two bivalents or one quadrivalent are formed depending on the details of the course of pairing as illustrated by the arrows in Fig. 2. These procedures are clearly analysable in the pachytene of autotetraploid tomatoes [48].

If chiasma formation takes place in each of the four arms of the quadrivalent a ring of four chromosomes will be present in diakinesis and metaphase I which is easily detectable microscopically also in cytologically less favourable species. Lack of chiasma formation in one arm of the pachytene configuration results in a chain of four chromosomes. Lack of chiasma formation in several arms leads either to the formation of a chain of three homologues whereas the fourth one is present as a univalent or to the formation of two rodlike open bivalents.

So far, only the primary pairing in the PMCs of autotetraploid plants was discussed which occurs principally only between two homologous chromosomes or between parts of them. As already postulated by Darlington [22] there is another mode of pairing in these PMCs called "secondary pairing". This is not a close but a very loose pairing between two homologous bivalents. In these cases, the four homologues of the group are present in the form of two normal bivalents which, however, show clear mutual relations with regard to their spatial orientation. The corresponding regions of the two bivalents lie more or less parallelic in a short distance from each other demonstrating thereby that they belong together. A kind of a "distant conjugation" between them is realised which may be due to remaining pairing forces which were not needed for primary pairing (Fig. 2). This phenomenon has been clearly observed in the pachytene of 4*n* *Impatiens balsamina* [9], 4*n* *Lycopersicon esculentum* [48, 66], furthermore in *Antirrhinum* [183] and *Digitalis* [112]. There is no substantial connection between the secondarily paired bivalents; therefore, they remain separated from each other and behave like normal bivalents until metaphase I.

The number of quadrivalents per PMC varies not only considerably between different species but also between different varieties of the same species. Examples are autotetraploids of *Lycopersicon esculentum*, *Asparagus officinalis*, *Oryza sativa*, *Secale cereale* and many others. Very high values were found in 4*n* *Allyssum maritinum* and *Glycine max*. Unusually low quadrivalent frequencies are known in autotetraploids of *Cuminum cyminum*, *Raphanus sativus*, *Lotus corniculatus*, *Vaccinium corymbosum* among others. In some species, there is no quadrivalent formation at all in autotetraploid plants. This holds for instance true for *Solanum rybinii* [161], *Agathaea coelestis* [157], *Tagetes erecta* [10] and for distinct strains or species which show

otherwise normal quadrivalent frequencies such as *Lycopersicon esculentum* mentioned above.

The causes of the differences just given are not yet fully known. In some cases, they are certainly genetically conditioned. In others, however, they could be due to diverging environmental conditions as the chiasma frequency depends highly on temperature, the degree of water supply of the plants and on other external factors. These findings show that it is not justified to associate a distinct quadrivalent frequency with the autotetraploids of each species. According to Morrison and Rajhathy [123], about two-thirds of all the chromosomes present in autotetraploids of 12 different species were found to unite to quadrivalents. This statement is only valid under consideration of the restrictions just given.

In the PMCs of most autotetraploid plants there is a normal distribution of the chromosomes from the rings in anaphase I followed by an undisturbed continuation of the course of meiosis. Genomatically balanced diploid microspores arise giving rise to fully functional diploid gametes. Misdistributions from the rings or chains, but especially the presence of tri- and univalents, however, lead to the formation of unbalanced gametes. They cause the occurrence of aneuploid plants in the progenies of the autotetraploids which reduce the seed production of the tetraploid strain as a whole. Their frequency can be very high, almost 50 per cent in 4*n* *Sesamum annuum* and *Lolium perenne* [91, 168].

In a small number of tetraploid PMCs, the course of meiosis is altered in such a way that triploid and haploid microspores are formed instead of the diploid ones. In self-propagating species, diploid plants can arise in the offspring of tetraploid ones due to the union of double reduced male and female germ cells. Investigations in autopolyploid tomatoes have shown that these reversions are combined with the separation of whole genomes. The distribution of $36+12$ chromosomes ($=3n+n$) in anaphase I of a tetraploid PMC is not a random event but a distinct regularity, the mechanism of which has not yet been clarified. In other words, the 12 chromosomes of one of the two interphase nuclei of the tetraploid PMCs represent a complete genome. This becomes also clear from triploid and hypertriploid PMCs which are found in small frequencies in tetraploid anthers. Three diploid microspores instead of four aneuploid are formed in triploid PMCs as a consequence of a modified course of meiosis resulting in genomatically balanced nuclei deriving from unbalanced mother cells. In hypertriploid PMCs, three diploid microspores are formed whereas the remaining chromosomes are united into a micronucleus which degenerates [49–58, 64].

3.2.2. The meiosis of autopolyploids with higher levels

Only a few autopolyploid plants with higher genome levels were found to produce flowers with more or less normal sex organs which can be used to

investigate the meiotic behaviour. The small number of findings available in this field can therefore not be generalized. Hexaploid plants of *Tabernaemontana divaricata*, an ornamental apocynacea, had a markedly regular meiosis with low multivalent frequency resulting in a pollen fertility of 79 per cent [18]. Octaploid petunias and millets have a strongly reduced number of PMCs in their pollen sacs; their meiosis is very difficult to study. The frequency of hexa- and octavalents in metaphase I is low.

The meiosis of octaploid tomatoes could be completely analysed. The details of pachytene pairing are similar to those in autotetraploid plants. Also here, primary pairing occurs only between two chromosomal units. The eight homologous chromosomes of each group are mostly present in the form of four bivalents which often show secondary pairing. As a consequence of partner exchange during primary pairing, very complicated pachytene configurations can arise in which six, rarely even all the eight homologues are united to a common figure. Only about 20 per cent of all the chromosomes present were found to be involved in configurations of more than four homologues. Manifold irregularities were observed in the later stages due to misdistributions from the various metaphase configurations and to the great number of univalents present [53]. As a consequence of additional anomalies, preferably of irregularities in spindle formation, the production of numerically balanced tetraploid gametes cannot be expected; the octaploid tomatoes are sterile.

If the meiotic behaviour of $4n$, $6n$, $8n$ plants is compared, it becomes obvious that the degree of meiotic anomalies increases with increasing ploidy level. Moreover, the cytological stability decreases. In a certain proportion of PMCs there is a tendency to reduce the chromosome number down to lower, but more stable levels. These processes occur not only during meiosis but also in the archespore cells of the octaploid anthers giving rise to tetraploid or diploid PMCs [52, 55, 64].

3.2.3. The relationship between meiosis and fertility

It is evident that the meiotic anomalies of autopolyploid plants of higher levels are responsible for the reduction of the proportion of functionable germ cells. The reduced fertility, however, appears also in autotetraploids which show only little meiotic disturbances. It is therefore the opinion of many cytologists, that the reduced fertility is not a cytological but a genetic problem. The genetic constitution of many polyploid strains tested seems to be responsible for a relatively normal or a reduced fertility rather than the degree of meiotic irregularities.

The seed production of autopolyploids can be improved by hybridizing different strains followed by sharp selection. In some species, this process was found to be associated with a reduction of the degree of meiotic anomalies, mainly with the reduction of multivalent frequency indicating a meiotic cause of the reduced fertility. This has been demonstrated for distinct strains of $4n$