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Colchicine Induced Quadrivalent Formation in *Helianthus*: Evidence of Ancient Polyploidy

R. C. Jackson
Department of Biological Sciences, Texas Tech University, Lubbock, Texas (USA)

B. G. Murray
Department of Botany and Biochemistry, Westfield College, University of London, London (England)

Summary. The premeiotic treatment of microsporocytes of *Helianthus annuus* and *H. laciniatus* ($x = 17$) results in the formation of multivalent chromosome configurations at meiosis in these 'diploid' species. This is interpreted to mean that colchicine has disrupted the normal attachment of genomes to the nuclear membrane and allowed synapsis of the ancestral genomes, indicating the polyploid origin of these 'diploid' species. This technique should be of importance in generating intergenomal recombination in classical allopolyploid agronomic species because it could produce new genotypic combinations normally impossible to obtain.

Key words: *Helianthus* – Colchicine – Induced quadrivalents – Cryptic polyploidy

Introduction

The effects of the alkaloid colchicine on many aspects of chromosome behaviour are well known. Colchicine is commonly used in the induction of polyploidy, but it can be used to modify meiotic chromosome pairing (Driscoll et al. 1967; Dover and Riley 1973). Using very low concentrations of colchicine (0.01%) Dover and Riley (1973) were able to increase the frequency of bivalent and trivalent formation in hybrids between *Triticum aestivum* and *Secale cereale,* but untreated material had a very high frequency of univalents. Thus, treatment of meiocytes, prior to the onset of meiosis, with low concentrations of colchicine resulted in extensive intergenomal pairing among the three *Triticum* genomes and also among them and the *Secale* genome. This technique, therefore, allows the expression of cryptic genome homology and could provide a mechanism for the exchange of genetic material between previously isolated genomes. Driscoll and Darvey (1970) were able to show that colchicine only affects the spatial relationships of homologues and not the process of chiasma formation. Colchicine reduced chiasma formation in normal bivalents but had no effect on the interarm chiasma frequency of an isochromosome.

It is common practice to describe as diploid the bivalent forming species of a genus that have the lowest chromosome number of which other taxa may have multiples. Although this is a logical procedure, it does result in situations where the diploid species of a genus may have very high chromosome numbers compared to related genera. Without genetic data it is usually not possible to confirm or deny this diploid status, but by utilizing dilute colchicine on premeiotic cells it may be possible to show that these diploids contain more than two homologous genomes. To test this idea, we have chosen to study the effects of premeiotic colchicine treatment on two 'diploid' species, *Helianthus annuus* L. and *H. laciniatus* A. Gray (Compositae) both of which have $2n = 34$.

Materials and Methods

Young inflorescences were removed from plants by cutting the stems under water and were placed in a 0.5×10^{-4} M solution of colchicine for 68 h prior to fixation. Material treated for periods of more than 68 h showed a high frequency of tetraploid pollen mother cells. Treatment for periods of less than 68 h only resulted in post anaphase I irregularities. Control material was fixed without any pretreatment. Meiotic preparations were made using FLP orcein (Jackson 1973) and meiotic configurations and chiasma frequency were scored in 50 pollen mother cells per plant.

Results

In both *H. annuus* and *H. laciniatus* the control material showed only bivalent formation; univalents and multivalents were absent (Table 1). However, the

Table 1. Total and mean meiotic configurations in plants of *Helianthus laciniatus* and *H. annuus* treated with 0.5×10^{-4} M colchicine solution and their untreated controls. Values are for 50 cells per plant unless stated otherwise. c = chain, o = ring

Species	Configurations								Chiasmata
	I	cII	oII	III	cIV	oIV	cVI	oVI	
H. laciniatus									
1 treated	5	670	156	5	5	2	0	0	1015
	0.10	13.40	3.12	0.10	0.10	0.04	0	0	20.30
control	0	612	238	0	0	0	0	0	1088
	0	12.24	4.76	0	0	0	0	0	21.76
2 treated	7	569	237	1	10	8	1	0	1083
	0.14	11.38	4.74	0.02	0.02	0.16	0.02	0	22.20
control	0	671	179	0	0	0	0	0	1110
	0	13.42	3.58	0	0	0	0	0	20.58
H. annuus									
1 treated	4	396	427	0	6	2	3	0	1289
	0.08	7.92	8.54	0	0.12	0.04	0.06	0	25.78
control (34 cells)	0	308	270	0	0	0	0	0	848
	0	9.06	7.94	0	0	0	0	0	24.94
2 treated	2	462	352	2	10	3	2	0	1222
	0.04	9.24	7.04	0.04	0.20	0.06	0.04	0	24.44
control	0	425	425	0	0	0	0	0	1275
	0	8.50	8.50	0	0	0	0	0	25.50
3 treated	1	455	366	1	9	3	1	0	1233
	0.02	9.10	7.32	0.02	0.18	0.06	0.02	0	24.66
control	0	464	386	0	0	0	0	0	1236
	0	9.28	7.72	0	0	0	0	0	24.72
4[a] treated	2	447	397	0	7	3	1	0	1243
	0.04	8.94	7.94	0	0.14	0.06	0.02	0	24.86
5[a] treated	0	507	310	0	10	2	1	2	1182
	0	10.14	6.20	0	0.20	0.04	0.02	0.04	23.64

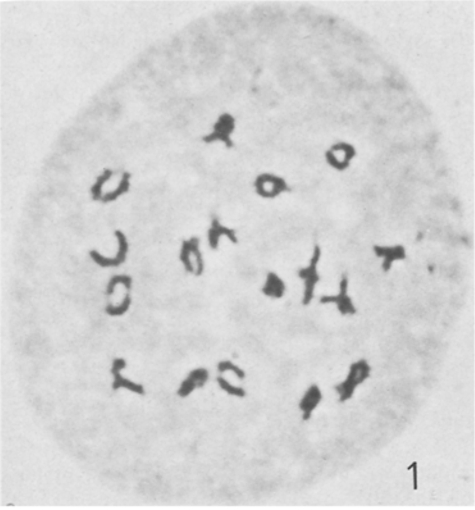
[a] Control material was not obtained for 4 and 5, but these were the same strains as the other three plants

colchicine treated material of both species showed low frequencies of chain trivalents, chain and ring quadrivalents and chain and ring hexavalents (Figs. 1, 2, 3, 4). In all cases univalent frequency was low and the dose of colchicine appeared to have little effect on chiasma frequency. The variation in chiasma frequency can be accounted for by the variation in greenhouse conditions. One plant treated with 0.5×10^{-4} M Velban (vinblastine sulphate; Lilly) formed eight chain quadrivalents and one ring quadrivalent in a sample of 34 cells. The amount of intergenomal pairing is greater than the observed multivalent frequency because it has been shown that the number of pachytene quadrivalents is equal to the number of pachytene bivalents in a two chiasma per bivalent system (Jackson and Casey 1982; Jackson and Hauber 1982).

Discussion

The results of this investigation show that the pattern of meiotic chromosome pairing in these two *Helianthus* species is modified by the application of a dilute solution of colchicine to the meiocytes after the last premeiotic mitosis. The appearence of quadrivalents in both species indicates that some, if not all, of the chromosomes are quadruplicated in the 'diploid' *Helianthus* species. Thus, *Helianthus* is probably of polyploid origin as has been suggested by Heiser and Smith (1955); they proposed *Viguiera* species with $n = 8$ and $n = 9$ as probable progenitors of this allopolyploid genus. The formation of hexavalents in the colchicine treated material indicates either that there were translocation differences between the two ancestral genomes or that they occured after the origin of the genus.

At present we can only speculate about how colchicine treatment can alter the pattern of meiotic pairing. It is becoming clear that chromosomes are not distributed at random in nuclei but are present in an orderly arrangement throughout the cell cycle (Ashley and Pocock 1981; Avivi and Feldman 1980; Bennett 1982). Bennett et al. (1974) have shown that chromatin is attached to the nuclear membrane in pollen mother cells of wheat, and in *Lilium* Hotta and Shepard (1973)



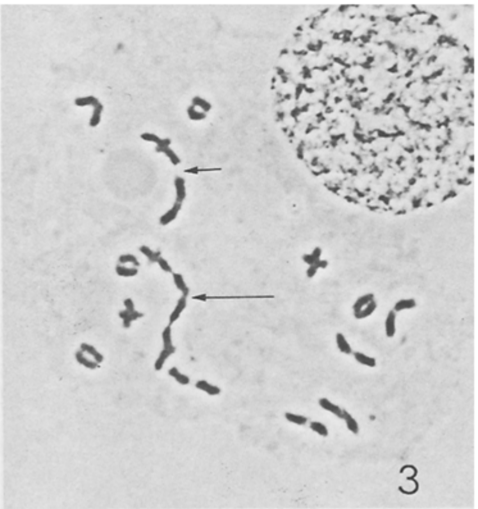


Fig. 1. Diakinesis in a control plant of *H. annuus* with 17 bivalents

Fig. 2. Diakinesis in colchicine treated *H. annuus*. Note ring hexavalent (arrow) and 14 bivalents

Fig. 3. Diakinesis in colchicine treated *H. annuus*. Note chain hexavalent (long arrow), chain quadrivalent (short arrow) and 12 bivalents

Fig. 4. Diakinesis in colchicine treated *H. laciniatus* with a ring quadrivalent (arrow) and 15 bivalents

found that colchicine caused a reduction of a membrane associated DNA binding protein which normally appears during meiotic prophase. Feldman and Avivi (1973) suggest that in wheat somatic association of strict homologues may be responsible for the regular formation of bivalents and that somatic association is brought about and maintained by the attachment of chromosomes to the nuclear membrane by microtubules or other proteins susceptible to the action of colchicine or similar alkaloids (Feldman et al. 1966; Avivi et al. 1969). We suggest that chromosome positioning may be of vital importance in the regulation of homologous/homoeologous chromosome pairing at meiosis and that colchicine disrupts the orderly arrangement of chromosomes on the nuclear membrane permitting the expression of cryptic genome homologies. We would suggest that *Triticum* and *Helianthus* have a comparable mechanism to promote bivalent formation since both groups yield multivalents when treated with colchicine.

We therefore believe that there is good evidence to support Heiser and Smith's (1955) hypothesis that the genus *Helianthus* is of polyploid origin, and we suggest that Ph-like or somatic association genes are responsible for the observed bivalent formation in untreated plants.

In addition to the theoretical significance of our work, these findings may have practical value. In crop plants new genetic combinations provide the raw material for selective breeding programmes. Colchicine induction of intergenomal recombination in classical allopolyploids may give rise to a large number of new recombinant genotypes which could have considerable agronomic potential.

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Dr. Brian Murray
Department of Botany and Biochemistry
Westfield College
University of London
Kidderpore Avenue
London, NW3 7ST (England)