TWIN AND TRIPLET PEPPER SEEDLINGS

A Study of Polyembryony in *Capsicum frutescens*

D. T. Morgan, Jr. and R. D. Rappleye

NUMEROUS instances of plural embryo formation are recorded in the literature for many species of the angiosperms. In most plants where the sexual process is regular as reflected by the consistent production of sexual monoembryonic seeds, polyembryonic seedlings are of limited and sporadic occurrence. However, a relatively high percentage of the seedlings of a few species, primarily polyploid and apomictic, have been established as polyembryonic in origin. Since 1933, when haploid plants were found in twin seedlings of *Linum*[9] and *Oryza*[12], polyembryonic seedlings have been recognized as a source of variants in chromosome number. Subsequently, various combinations of haploid, diploid, polyploid and aneuploid plural seedlings have been reported in a number of species. Since multiple embryo formation is ordinarily an infrequent event, such reports are often based on a relatively small number of polyembryonic individuals. It is the purpose of this study to determine the different polyembryonic types and their frequencies in a relatively large population of a sexual diploid, *Capsicum frutescens*.

Materials and Methods

The seeds employed in this study were obtained from plants grown under relatively uniform environmental conditions in large commercial fields. Occasional seeds with fused or partially fused testas were observed in the large groups of seeds collected for analysis. Such seeds, obviously derived from separate ovules, were discarded. The remaining seeds were evenly spaced in groups of 100 on moist paper toweling by means of a vacuum operated seed counting plate. Seedling counts were made at intervals of seven and twelve days after placing the seeds in germinating chambers maintained at 30 degrees centigrade during the day and 20 degrees centigrade at night. Polyembryonic seedlings were selected, transplanted and grown to maturity. The chromosome number for each plant was determined from two separate root tips fixed in Navaschins', embedded, sectioned and subsequently stained by the crystal violet technique.

Results

The data relative to the frequency of polyembryony are summarized in Table I. A total of 294 polyembryonic seedlings was obtained from a population of 78,005 germinated seeds. Of the 294 seeds containing multiple embryos, 291 produced twin seedlings and 3 produced triplets. Statistically significant differences in the frequencies of polyembryony were observed for three varieties tested. The variety Pimento had the lowest frequency of polyembryony and Goliath the highest. An intermediate frequency was observed for the variety California Wonder. Varieties other than Goliath, California Wonder and Pimento are recorded as miscellaneous varieties in Table I due to the comparatively small samples tested for each individual variety.

The different polyembryonic types and their frequencies of occurrence are recorded in Table II. Chromosome counts were obtained for both members of 139 twin pairs and for all the seedlings resulting from the three triplets. Frequently one member of a twin was less vigorous than the other member and failed to survive. In such cases the chromosome number of the viable member is recorded in Table II under the heading "Survivor." Due to temporarily unfavorable growing conditions, many of the twin seedlings of the variety California Wonder did not survive. However, it was possible to grow to maturity all members of 96 of the 157 polyembryonic seedlings obtained from the variety Goliath. As shown in Table II, haploid-haploid, haploid-diploid, diploid-diploid and diploid-tetraploid twins were observed. The diploid-tetraploid and haploid-haploid twins occurred infrequently in comparison with the other types. Conjoined or partially fused diploid-diploid twins were observed but less frequently than diploid-diploid twins of the unattached type. No conjoined twins occurred in which the two plants had different chromosome numbers. All of the members of the triplets were diploid. Several of the polyembryonic types are illustrated in

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Figure 4

A-D show polyembryonic seedlings as they were removed from the germinating chamber. *E-H* show such seedlings at a later phase of growth. The nature of the twins and triplets shown is as follows: *A*—Haploid-diploid twin seedling. The smaller embryo was haploid. *B*—Unattached diploid-diploid twin. *C*—Conjoined diploid-diploid twin. *D*—Diploid-diploid-diploid triplet. *E*—Haploid-diploid twin: the smaller plant was haploid. *F*—Unattached diploid-diploid twin. *G*—Conjoined diploid-diploid twin. *H*—Diploid-diploid-diploid triplet with the two large members conjoined.

Figure 4. No aneuploids were observed and all of the diploid plants were highly fertile as shown by extensive seed formation. Of the 354 mature plants originating from polyembryonic seeds, 48 (13.6 percent) were haploids. The haploid plants were characterized phenotypically by their sterility, small size and elongate leaves. A haploid chromosome complement of 12 was observed confirming the reports of previous investigators.[3,8,16]

Discussion

The extensive literature in reference to polyembryony has been summarized previously by Webber.[17] Christensen and Bamford,[3] who found haploid plants in a population of twin seedlings of *C. frutescens,* have discussed most of the pertinent literature in regard to the present study. Consequently, a survey of the literature will not be attempted in this paper.

The data of Table I show varietal differences in the frequencies of polyembryony. Plural seedlings are approximately ten times as frequent in the variety Goliath as in the variety Pimento. Varying frequencies of polyembryony may be attributed to differences in environmental conditions and varietal genotypes. However, since the seeds employed in this study were collected from plants grown under relatively uniform environmental conditions, it seems likely that differences in polyembryonic frequency of the magnitude reported in Table I could not be due primarily to varying environmental factors. Moreover, since *C. frutescens* is characteristically a self-pollinating species with numerous clearly differentiated varieties, it would appear that the several frequencies of polyembryony reflect differences in varietal genotypes. Previous investigators have found intraspecific differences in frequencies of polyembryony for several species.[10,14,17] In *Linum,* Kappert[9] obtained lines with high frequencies of polyembryony by selection from the progeny of crosses between strains with low frequencies. Recently, varying frequencies of polyembrony have been reported for different genetic strains of *Nicotiana tabacum*[2] and *Asparagus officinalis*.[13] Perhaps the most convincing evidence for the effect of the genotype on polyembryonic frequency in *C. frutescens* will develop from the analysis of crosses between highly homozygous lines exhibiting extreme differences in frequencies of polyembrony. Homozygous diploids have been produced by colchicine treatment of the haploids obtained in this study for use in the further analysis of the effect of the genotype on polyembryony.

In the absence of specific morphological or genetic information any classification of the polyembryonic types in regard to origin must necessarily be speculative. Webber[17] states that polyembryony may often be a complex and variable process. However, it seems likely that the conjoined diploid-diploid twins arose from an embryonic split during ontogeny and would be classified under

TABLE I. Varietal frequencies of polyembryony

Variety	Dormant seeds	Germinated seeds.	Monoembryonic seeds	Polyembryonic seeds Twins	Triplets	% Polyembryony
California Wonder	45,574	39,562	39,450	112	0	0.28
Goliath	31,654	24,182	24,025	154	3	0.65
Pimento	7,800	6,608	6,604	4	0	0.06
Misc. varieties	14,583	7,653	7,632	21	0	0.27
Total, all varieties	99,611	78,005	77,711	291	3	0.38

TABLE II. Frequencies of polyembryonic types

Variety	Twins N-N	N-2N	2N-2N (1)	2N-2N (2)	2N-4N	Triplets 2N-2N-2N (3)	2N-2N-2N (1)	Survivors N	2N
California Wonder	1	7	24	3	0	0	0	1	28
Goliath	1	32	44	15	1	2	1	2	25
Pimento	0	2	2	0	0	0	0	0	0
Misc. Varieties	0	0	6	1	0	0	0	0	11
Total, all varieties	2	41	76	19	1	2	1	3	64

(1) Unattached.
(2) Conjoined
(3) Two members conjoined; one separate

Webber's category of cleavage polyembryony. Fusion or natural grafting between two separate diploid embryos offers an alternative explanation for the origin of the conjoined types. However, since both members of the conjoined pairs were invariably diploid, it would appear that embryonic fusion, if it occurred at all, was necessarily restricted to embryos of the same chromosomal constitution. The unattached triplet and diploid-diploid twins may represent examples of cleavage polyembryony at an earlier developmental stage than the conjoined twins or may conceivably be derived from different combinations of apomictic and sexual embryos originating from one or more embryo sacs. Preliminary results from further research on the origin of the polyembryonic types indicate that the majority of the diploid-diploid twins are sexual in origin. Cooper[5] in several species of *Lilium* observed the embryological development of haploid-diploid twins and concluded that the haploid embryo arose from a synergid stimulated to divide following fertilization. Androgenetic haploids, usually following interspecific and intergeneric pollinations, and haploids arising from somatic reduction in integumental cells of an apomictic species have also been reported.[4,6] However, since *C. frutescens* is ordinarily a self-pollinating sexual diploid, it seems likely that the haploid plants in the twin seedlings arose from reduced cells of the female gametophyte.

Various investigators have commented on the use of haploids in plant breeding studies.[1,3,7,11,13,14,15] Toole and Bamford[15] obtained homozygous diploids by colchicine treatment of *C. frutescens* haploids. Similarly colchicine treatment has doubled the chromosome complements of thirty of the haploids obtained during the present study. Since haploid plants may be readily obtained and their chromosome complement conveniently doubled, a technique for their utilization in the development of new varieties became apparent during the present study. Haploids originating from the gametophytes of the F_1 following intervarietal crosses could be obtained and subsequently treated with colchicine. The resultant doubled haploids would have different combinations of characters due to meiotic segregation in the F_1 hybrid. In a self-pollinated diploid species like *C. frutescens,* selection of desired types from the different doubled haploids would immediately result in the isolation of new homozygous varieties. If one parental variety possessed the majority of favorable characters, one or more generations of backcrossing to the superior variety prior to the isolation of the haploids would presumably greatly increase the chance of obtaining the desired type of double haploid.

Summary

1. Varieties of *C. frutescens* having high, low and intermediate frequencies of polyembryony were observed. The several frequencies are believed to reflect intrinsic differences in varietal genotypes.

2. Three triplet and 291 twin seedlings were obtained from 78,005 germinated seeds. Chromosome counts disclosed the following twin types which are listed in ascending order of their frequencies: diploid-tetraploid, haploid-haploid, conjoined diploid-diploid, haploid-diploid, and unattached diploid-diploid. All members of the triplet seedlings were diploid. Possible modes of origin of several of the polyembryonic types are discussed.

3. Thirty of the haploid plants from the polyembryonic seedlings have been treated with colchicine to produce homozygous diploids. The doubled haploids thus obtained are being employed in genetic and plant breeding studies.

4. A method is discussed for the utilization of haploids in developing new homozygous varieties.

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THE CHROMOSOMES OF VERTEBRATES

A REVIEW

THE author's purpose in writing this book* is clearly stated in the introduction. It is to assemble and critically examine the information which has accumulated on the cytology of vertebrates. This project was an ambitious one, but one long overdue. The result is a book which is all that can be desired in a work of this kind. Professor Matthey has not only brought his subject up to date, he has also been most successful in "sifting the grain from the chaff."

The book is free of gross errors and the omissions are deliberate. The Latin names of animals are quoted without authorities and plates lack dimensional scales. These have both been omitted to avoid possible error, since so many workers fail to be specific on these points. In any case, references are always given and workers may, if they wish, refer to the original works and form their own conclusions.

Four chapters, an appendix of thirty-five plates, a complete bibliography and a zoological index make up the book.

Chapter I is titled "Technique." The various methods of fixation are appraised and the value of the more important summarized in tabular form. Only two paragraphs are used to discuss stains; these however, constitute a good resume of the subject which is probably all it warrants in a book of this type. The remainder of the chapter is devoted to methods of examination, pointing out the difficulties and pitfalls to be avoided in the examination and interpretation of the preparations. The chapter, while brief (34 pages) should prove an invaluable guide to beginners as well as established workers.

The second chapter "Historical" is a critical review of the general contributions and conclusions of most of the vertebrate cytologists from the time of Schottländer (1888) to the present time. The available general information on the chromosome make-up of the five vertebrate classes is well covered. Sweeping generalizations are not only avoided but those made by some workers are pointed out and numerous errors and misinterpretations corrected.

Chapter III is devoted to "Sex Chromosomes." The subject is given the most careful and critical treatment. Whenever necessary, the author does not hesitate to interpret cytological data in the light of information supplied by genetics. However, the approach remains primarily a cytological one. The cytological basis of sexuality in mammals is covered by a summary of the contributions of Winiwater, Painter, Minouchi, Oguma, Makino, Koller and Matthey; then by reviewing the work of a number of authors on various mammals including man. The danger of all-inclusive generalizations is again brought out and illustrated by the work of Matthey and his pupil R. Bovery with *Sorex araneus*.

The final chapter "Chromosome Evolution, Phylogeny and Systematic Relationships" deals first with observed and hypothetical mechanisms of chromosome evolution, drawing freely from the more abundantly available information provided by insect and plant cytology. Finally, an extensive treatment is given to the problem of the evolution of chromosome systems in vertebrates, based on comparative studies made within and between the various classes.

The body of the text is illustrated with nicely drawn figures. The appendix consisting of thirty-five plates grouping 442 figures shows the same painstaking workmanship and careful detail.

In *Les Chromosomes Des Vertebres* Professor Matthey has succeeded in preparing not only an atlas of vertebrate chromosomes, he has also brought together most of the pertinent information on the subject as well as its many theoretical considerations. Besides being a valuable source of information for workers in vertebrate cytology, it should also be of more than passing interest to those interested in genetics, evolution and the other allied subjects.

L. A. DIONNE
University of Wisconsin

*MATTHEY, ROBERT, *Les Chromosomes Des Vertebres.* Librairie de l'universite, F. Rouge Lausanne. 1949.