Genet. Res., Camb. (1973), **21**, *pp.* 221–228
Printed in Great Britain

Interspecific aneuploidy in *Cucurbita**

By W. P. BEMIS

Committee on Genetics, University of Arizona, Tucson, Arizona, U.S.A. 85721

(*Received* 23 *October* 1972)

SUMMARY

Interspecific aneuploids were made in the genus *Cucurbita* by pollinating the autoallotroploid (diploid *C. moschata* – haploid *C. palmata*) with pollen from diploid *C. moschata*. Seventy-eight, or 0·36 %, of the potential ovules showed some degree of continued development after pollination. The high frequency of continued ovule and embryo development indicated a preferential distribution or loss of *C. palmata* univalents during meiosis in the autoallotriploid and that megagametes aneuploid for more than one *C. palmata* chromosome were capable of initiating continued ovule development after fertilization. Lethal effects of interspecific aneuploidy were expressed by complete embryo abortion to sterility of mature plants. Nine plants developed to maturity and seven were cytologically identified. Four were trisomic and two were monosomic for single *C. palmata* chromosomes, i.e. they were $2n+1$ and $2n+19$ ($n = 20$) respectively. One plant was $2n$ and phenotypically identical to the *C. moschata* parent. One $2n+1$ plant was fertile and the single *C. palmata* chromosome was transferred in the succeeding generation.

1. INTRODUCTION

A method of transferring a homologous pair of chromosomes of one species into the genome of another species was described by O'Mara (1940). O'Mara transferred homologous pairs of rye chromosomes into the wheat genome by creating the amphiploid of the rye-wheat hybrid and back-crossing to the wheat parent. This produced a plant that was diploid wheat–haploid rye, i.e. its metaphase I configuration was 21 II and 7 I. This plant was self-fertile, and plants disomic for rye having 22 II were isolated from its progeny. Gerstel (1945) used essentially the same method in adding chromosomes of *Nicotiana glutinosa* into the genome of *N. tabacum*. In both instances the amphiploids and their derivatives were reasonably fertile.

This method of transferring chromosomes from one species to another was attempted using *Cucurbita* species which are diploid, $2n = 40$, and produce relatively large numbers of ovules in each fruit. The interspecific hybrid between *C. moschata* cv. 'Butternut', a domesticated species, and *C. palmata*, a wild xerophytic species, was described by Bemis (1963) as being completely sterile. There was very little homology between the *C. moschata* and *C. palmata* genomes,

* Journal Paper No. 2001 of the Arizona Agricultural Experiment Station.

as indicated by bivalent formation at metaphase I, which ranged from 6 to 9 instead of the normal 20. The degree of homology between the *C. moschata* and *C. palmata* genomes was comparable to that reported for *C. moschata* and *C. foetidissima* (Bemis, 1970).

2. MATERIALS AND METHODS

Autotetraploids of the parent species *C. moschata* and *C. palmata* were created and the interspecific hybrid was made at the tetraploid level. The resulting amphiploid was female-fertile but male-sterile, even though 40 bivalents were observed at metaphase I. The male sterility of the amphiploid was genic, not chromosomal. The amphiploid was pollinated with pollen from its diploid *C. moschata* parent, resulting in an autoallotriploid containing two *C. moschata* genomes and a single *C. palmata* genome. Metaphase I configurations of this autoallotriploid usually consisted of 20 bivalents (*C. moschata*) and 20 univalents (*C. palmata*), although occasionally one or two trivalents would be observed. These autoallotriploids were the pistillate parent in the formation of interspecific aneuploids. The autoallotriploid was also genic male sterile as indicated by the failure of the androecium to develop as in the amphiploid. It would also be expected to have a very high degree of chromosomal sterility.

The following schematic diagram shows the steps in the formation of interspecific aneuploids:

M = *C. moschata* genome P = *C. palmata* genome

MM double → MMMM × PPPP → double PP

↓

MMPP × MM

↓

MMP × MM

↓

Interspecific aneuploids MM ± P

The autoallotriploids (MMP) produced vigorous vines under greenhouse conditions, each vine being capable of supporting the development of about 20 fruit. Four vines were grown and 95 hand-pollinations using pollen from diploid *C. moschata* were made, resulting in the formation of 65 fruit. A random sample of seven fruit was taken and the mean number of ovules per fruit was 330 ± 24. Consequently the 65 fruit probably represented $21\,450 \pm 194$ ovules or megagametes.

The 65 hand-pollinated fruit were allowed to develop for 30 days on the vine. They were then harvested and stored at room temperature for an additional 60 days to permit full maturation of any seed they might contain. They were then cut open and any well-developed seed-coats were removed and room-dried. The dried seed coats were examined for embryo development and all detectable embryos were excised. The excised embryos were then placed in a germinator (30 °C) and scored for viability. Those embryos capable of continued development in the germinator were transferred to vials. As development continued the seedlings

were transferred to a soil medium and eventually to the ground bed of the greenhouse. Only nine developed into mature plants.

3. RESULTS AND DISCUSSION

The frequency of interspecific aneuploids depends on the frequency of aneuploid megagametes, since the microgamete is haploid *C. moschata*. If the 20 *C. palmata* chromosomes are univalents and distribute randomly to the poles at anaphase I, then the frequency of various megagametes can be calculated by the expansion of a binomial (Table 1).

Table 1. *Calculated frequencies of various megagametes of an autoallotriploid having $n = 20$ and having 20 univalents distributed randomly*

Megagametes	Expected frequency
n or $2n$	$0{\cdot}095 \times 10^{-5}$
$n+1$ or $2n-1$	$1{\cdot}9 \times 10^{-5}$
$n+2$ or $2n-2$	$18{\cdot}1 \times 10^{-5}$
$n+3$ or $2n-3$	$108{\cdot}7 \times 10^{-5}$

Table 2. *The distribution of ovules in individual fruit and their final developmental stages*

((A) Well-developed empty seed coats, (B) non-viable embryos, (C) viable, non-developing embryos, (D) developing embryos failing to produce seedlings, (E) seedlings failing to produce mature plants, (F) mature plants.)

Fruit no.	Total developing ovules	Final developmental stage A	B	C	D	E	F
1	10	3	2	2	1	—	2
2	10	3	4	1	—	1	1
3	10	1	6	3	—	—	—
4	9	1	1	5	1	—	1
5	7	4	2	—	—	—	1
6	7	2	2	1	1	1	—
7	5	3	—	—	1	1	—
8	3	1	1	—	—	—	1
9	3	1	1	1	—	—	—
10	3	1	2	—	—	—	—
11	2	1	—	—	—	—	1
12	2	1	—	—	—	—	1
13	2	—	—	1	—	—	1
14	1	—	—	—	1	—	—
15	1	1	—	—	—	—	—
16	1	1	—	—	—	—	—
17	1	1	—	—	—	—	—
18	1	1	—	—	—	—	—
19–65	0	—	—	—	—	—	—
Total	78	26	21	14	5	3	9
Observed frequency ($\times 10^4$)	36·4	12·1	9·8	6·5	2·3	1·4	4·2

https://doi.org/10.1017/S0016672300013410 Published online by Cambridge University Press

Seventy-eight developing ovules were removed from the 65 fruit (Table 2) and nine plants developed to maturity, although most were abnormal as will be discussed later. Seven of the nine plants were cytologically identified. Four were $2n+1$, 2 were $2n+19$ and 1 was $2n$ which developed from $n+1$, $2n-1$ and n megagametes respectively. The expected frequency of $n+1$ or $2n-1$ gametes is $1{\cdot}9 \times 10^{-5}$ or approximately 1 out of 50000. The observed frequency of the six plants identified as being $2n+1$ or $2n+19$ was $2{\cdot}8 \times 10^{-4}$ or approximately 1 out of 3500, which is slightly over 14 times the expected frequency. If we consider the two unidentified plants as being aneuploid for only one *C. palmata* chromosome and combine them with the six known plants, the observed frequency is $3{\cdot}8 \times 10^{-4}$ or about 20 times the expected frequency. The probability of recovering the $2n$ plant from a population of 21000 potential gametes is 0·02, suggesting that the frequency of recovery is higher than expected.

Table 3. *Goodness of fit test to a Poisson distribution for total developing ovules per fruit and embryo development per fruit*

	Total developing ovules		Embryo development	
Class	Observed	Calculated	Observed	Calculated
0	47	19·6	51	29·2
1	5	23·5	3	23·4
2	3	14·1	5	9·3
3	3	5·6	1	2·5
4	0	1·7	0	
5	1		1	
6	0		0	
7	2	0·5	2	0·6
8	0		1	
9	1		1	
10	3		0	
	$\chi^2 = 149$, $P = < 0{\cdot}001$		$\chi^2 = 69{\cdot}3$, $P = < 0{\cdot}001$	

These data indicate that (1) there is preferential distribution or loss of some of the 20 *C. palmata* univalents, and (2) megagametes that are aneuploid for more than one *C. palmata* chromosome are capable of initiating ovule development after fertilization.

Data on the number and degree of ovule development in the 65 fruit from the autoallotriploid plants are given in Table 2. Forty-seven of the fruit contained no evidence of ovule development, i.e. the integuments were still rudimentary. The numbers of ovules developing in the remaining 18 fruit were highly variable. The distribution of ovule development in the population of 65 fruit was tested for goodness of fit to a calculated Poisson distribution (Table 3). The results show that the observed distributions for total developing ovules and for embryo development do not fit the calculated Poisson distributions, indicating that ovule development is a non-random process. Six of the 65 fruit contained 68 % of the total developing ovules and these same six fruit contained 75 % of ovules having some degree of

embryo development. These data suggest that a fertilized and developing ovule has a stimulating effect on ovules in the same fruit and results in preferential ovule development. If this supposition is true it would also tend to explain the relatively high observed frequency of ovule development in the entire population of fruit.

(i) *Lethal effects of interspecific aneuploidy*

The data in Table 2 show that aneuploidy results in a wide range of embryo and seedling development before death of the organism. The most frequent form of lethality is complete ovule abortion, in which the integuments remain in a rudimentary stage of development. These accounted for 99·64 % of the total ovules. The remaining 0·36 %, which showed a marked degree of development beyond the rudimentary integument stage, were grouped into six classes (Table 2):

Table 4. *Fifty-two excised embryos subjectively rated as percentage of normal development of* C. moschata *embryo and numbers of those that were non-viable, viable but non-developing, failed to produce seedlings, lethal in seedling stage and those producing mature plants*

		Numbers classified as				
Excised embryos (% of normal *C. moschata* embryo)	Total no.	Non-viable	Non-developing	Failed to produce seedlings	Seedling lethal	Mature plants
95	2	—	—	—	—	2
90	1	—	—	—	1	—
75	3	—	—	—	2	1
50	5	3	1	1	—	—
45	4	—	—	2	—	2
40	4	1	—	1	—	2
35	3	1	2	—	—	—
30	4	1	1	1	—	1
25	7	3	4	—	—	—
20	14	10	3	—	—	1
15	5	2	3	—	—	—
Total	52	21	14	5	3	9
Mean	35·6	26·7 (*a*)	25·4 (*a*)	42·0 (*b*)	80·0 (*c*)	53·9 (*bc*)

Means followed by identical letters are not significantly different at the 0·05 probability level.

(A) well-developed empty seed coats; (B) non-viable embryos; (C) viable, non-developing embryos; (D) developing embryos failing to produce seedlings; (E) seedlings failing to produce mature plants; and (F) mature plants. The well-developed empty seed coats were characteristic of complete integument development but with complete embryo abortion soon after fertilization, resulting in no visual evidence of embryo development at maturity. These accounted for one-third of the developed ovules, the rest showing some measurable degree of embryo development.

The embryos were excised from the dried seed and subjectively rated as percentage of development (size) of a typical *C. moschata* embryo. They were placed in the five classes previously listed and are shown as they relate to percentage development in Table 4. Non-viable embryos were those that failed to show evidence of chlorophyll formation when placed in the germinator. The viable, non-developing embryos showed cotyledon enlargement and chlorophyll development but the radicle and plumule failed to develop. Those failing to produce seedlings had very weak radicle and plumule growth which was soon arrested and growth completely stopped. Three embryos developed into seedlings but died after 3–4 leaves had developed. Nine embryos continued to develop into mature plants.

The subjective ratings of embryo development in the seed ranged from 15 to 95 % and had a mean value of 35·6 %. The non-viable and non-developing embryos had mean percentage development values of 26·7 and 25·4 % respectively, while the later three classes had mean percentage development values of 42·0, 80·0 and 53·9 % respectively.

The Kolmogorov–Smirnov two-sample statistic test was applied comparing the populations of the five classes of embryos as each related to percentage development of the excised embryos. The non-viable and non-developing embryos were not statistically different ($P = 0{\cdot}05$) from each other but were statistically different from the other three classes. If embryo development is arrested at an early stage resulting in a small underdeveloped embryo it is less likely to continue development during the germination process.

The range in degree of development of embryos that ultimately produced mature plants was considerably greater than expected. The nine mature plants were produced from embryos that ranged in development from 20 to 95 % of a normal *C. moschata* embryo. The lethal effects of the aneuploidy were manifested in the mature plants as complete sterility in all but one of the aneuploids. The fertile aneuploid was a $2n+1$ plant that developed from a near normal developed embryo (95 %).

(ii) *Characteristics of the nine mature plants*

Some characteristics of the nine plants produced in this study are shown in Table 5. With the exception of plants nos. 8 and 1, all were sterile. The staminate flowers aborted before blooming or were devoid of any androecium at blooming. The pistillate flowers, when pollinated with *C. moschata* pollen, would occasionally produce a fruit, but all fruit produced were completely seedless. In some cases rudimentary seed coats would develop, but there was no evidence of continued ovule development after pollination. Plant no. 8 was phenotypically identical to the *C. moschata* parent and was cytologically identified as a diploid. The occurrence of this plant emphasizes the high degree of non-homology between the *C. moschata* genome and the *C. palmata* genome as there was no indication of any *C. palmata* germ plasm being associated with the *C. moschata* genome. The *C. moschata* parent line used in this study was highly homozygous and therefore any deviation from the *C. moschata* phenotype would be attributed to that portion of the *C. palmata* genome any individual plant contained.

Plants nos. 15 and 19 which were cytologically identified as $2n+19$, i.e. diploid *C. moschata*, but were monosomic for one of the *C. palmata* chromosomes. Even though these plants differed from the autoallotriploid parent line (MMP) by the loss of a single *C. palmata* chromosome, they were phenotypically quite distinct from the autoallotriploid.

Table 5. *Characteristics of the nine mature plants*

Plant no.	Excised embryo (% development)	Chromosome no.	Fertility status	Days to first pistillate flower
8	95	$2n$	Fertile	76
1	95	$2n+1$	Fertile	88
15	75	$2n+19$	Sterile	105
19	45	$2n+19$	Sterile	103
21	45	?	Sterile	116
9	40	$2n+1$	Sterile	150
23	40	$2n+1$	Sterile	95
12	30	?	Sterile	106
5	20	$2n+1$	Sterile	137

The aneuploid plants were retarded in their development as indicated by the number of days to the first pistillate flower (Table 5). Plant no. 8, the diploid, had the first pistillate flower reach anthesis in 76 days. The eight other plants, including the two non-confirmed aneuploids, ranged from 88 to 150 days before anthesis of the pistillate flowers. The four aneuploid plants having one *C. palmata* chromosome in a homozygous diploid *C. moschata* background (interspecific trisomics) show an extreme effect on leaf shape. This effect on leaf shape caused by a single *C. palmata* chromosome is probably the result of a 'trisomic effect' as well as the specific loci for leaf shape on the extra chromosome. It appears that the extra *C. palmata* chromosome in each of the four plants represents a different chromosome from the *C. palmata* genome.

A limited population of 12 plants were grown from selfed seed of plant no. 1 (MM + 1P). Based on their phenotype, seven were normal and presumably diploid and five were aneuploid.

Cucurbita, in which no natural polyploidy is known to occur, can be induced to various ploidy levels without disrupting normal growth. The polyploids MMMM, PPPP, MMPP and MMP used in this study were as vigorous as the diploids MM and PP. There was a reduction in fertility in the autotetraploids and the amphiploid and the autoallotriploid is highly sterile as has been discussed. However, the aneuploids, either $2n+1$P or $2n+19$P, all exhibited abnormal growth patterns and with the exception of one $2n+1$P plant were completely sterile. This disruption in normal growth is attributed to imbalance of the *C. palmata* genome.

REFERENCES

O'MARA, J. G. (1940). Cytogenetic studies on Triticale. I. A method for determining the effects of individual *Secale* chromosomes on *Triticum*. *Genetics* **25**, 401–408.

GERSTEL, D. U. (1945). Inheritance in *Nicotiana tabacum*. XX. The addition of *Nicotinana glutinosa* chromosomes to tobacco. *Journal of Heredity* **36**, 197–206.

BEMIS, W. P. (1963). Interspecific hybridization in *Cucurbita*. II. *C. moschata* Poir x xerophytic species of *Cucurbita*. *Journal of Heredity* **54**, 285–289.

BEMIS, W. P. (1970). Polyploid hybrids from the cross *Cucurbita moschata* Poir x *C. foetidissima* HBK. *Journal of the American Society for Horticultural Science* **95**, 529–531.