

THE EFFECT OF POLYPLOIDY AND HYBRIDITY  
ON SEED SIZE IN CROSSES BETWEEN *BRASSICA*  
*CHINENSIS*, *B. CARINATA*, AMPHIDIPOID  
*B. CHINENSIS-CARINATA* AND AUTO-  
TETRAPLOID *B. CHINENSIS*

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(With Six Text-figures)

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I. INTRODUCTION

WATKINS (1932) suggested that it was a general rule in crosses of the type high-chromosome number  $\times$  low-chromosome number that better seed production was obtained with the high-chromosome number plant as female parent than with the low-chromosome number one as female. In some crosses, e.g. *Primula sinensis*, this is due to bad pollen tube growth in the cross diploid female  $\times$  autotetraploid male. In most cases, however, it appears to be due to the embryo : endosperm relations being different in the reciprocal crosses.

Crosses between diploid and autotetraploid *Brassica oleracea* were found to fit the Watkins rule (Howard, 1939). Thus in this species the cross diploid female  $\times$  autotetraploid produces only 'developed ovules', while the reciprocal cross with the autotetraploid as female parent produces a high set of good triploid seeds. It was also found that the triploid seeds from the cross autotetraploid female  $\times$  diploid were only about one-third the normal weight of *Brassica* seeds.

In this paper it is shown that crosses between an experimentally produced amphidiploid and its parent species behave in a similar way to the crosses between diploid and autotetraploid *B. oleracea*. Thus only

developed ovules were obtained from the crosses parent diploid female  $\times$  amphidiploid, and the triploid seeds from the crosses amphidiploid female  $\times$  parent diploid species were only about one-third the size of normal *Brassica* seeds.

## 2. THE PRODUCTION AND BEHAVIOUR OF AMPHIDIPOID

### *B. CHINENSIS-CARINATA*

(1) *Introduction.* According to Morinaga and other Japanese cytologists (summaries in Morinaga (1934) and U (1935)), if we denote the genomes of *B. campestris* ( $n=10$ ), *B. nigra* ( $n=8$ ) and *B. oleracea* ( $n=9$ ) as a, b and c respectively, then the genomes of the following species, *B. juncea* ( $n=18$ ), *B. napus* ( $n=19$ ) and *B. carinata* ( $n=17$ ), are ab, ac and bc respectively. The  $F_1$  hybrid between *B. chinensis* ( $n=10$ , i.e. belonging to the *campestris* group) and *B. carinata* has therefore the constitution abc on the above scheme.

Also, according to U (1935), the genomes a and c have some parts in common, but the genome b forms no bivalents with either a or c. Also no quadrivalents are normally found in *B. napus* (constitution aacc) or in the synthetic *napus* obtained by U from a cross between *campestris* and *oleracea*. Amphidiploids<sup>1</sup> from the cross *chinensis*  $\times$  *carinata* (constitution aabbcc) might therefore be expected to form 27 bivalents regularly, to breed true and to be fully fertile.

(2) *The production of the amphidiploids.*  $F_1$  seeds were obtained by crossing *chinensis* female with *carinata*. Somatic doubling of the  $F_1$  was easily obtained by colchicine treatment. One method used (this method has also been very successful with many other *Brassica* species) was to place drops of a 0.5% colchicine solution between the cotyledons of young seedlings. One drop per day and treatments of from one to five days were used. This treatment is successful in doubling the whole or parts of the growing point of the stem but avoids damaging the radicle such as happens when seeds are soaked in colchicine solutions.

In doubling the  $F_1$  hybrids in 1938 several diploid-tetraploid sectorial chimaeras were obtained. These were very easily recognized since the  $F_1$  hybrid (diploid) is sterile and only sets small fruits while the amphidiploid (doubled  $F_1$ ) is fertile and sets large fruits. A few octaploids were also obtained. These were almost completely sterile.

<sup>1</sup> It is suggested that amphidiploid is a useful term for describing such experimentally produced polyploids but that it should not be applied to allopolyploid species such as *B. napus*. These are better described as allotetraploids. The distinction is somewhat artificial but it can be useful.

The amphidiploids were selfed in 1938, and a second generation of amphidiploids was raised from this seed in 1939.

(3) *Cytology of the parents and  $F_1$  hybrid.* The chromosome number of *B. chinensis* is  $n=10$  (see Fig. 1) and of *B. carinata* ( $n=17$ ) (see Fig. 2).

Morinaga (1931) found that the  $F_1$  hybrid between *chinensis* and *carinata* formed 1-9 bivalents plus 25-9 univalents. According to U (1935) these bivalents would be due to pairing between the a and c genomes. A short examination of slides of  $F_1$  hybrids grown in Cambridge showed that, in addition to bivalents and univalents, trivalents and quadrivalents were also formed (see Fig. 3). It is interesting in this connexion to compare the results of Catcheside (1934) with those of Morinaga (1929) and U (1935). In the  $F_1$  hybrid *B. napus*  $\times$  a  $n=10$  chromosome species, Catcheside found up to 12 bivalents per nucleus and also trivalents, while Morinaga and U regularly observed 10 bivalents and never more per nucleus.

(4) *Cytology of the amphidiploid.* Only one plant of the second generation of amphidiploids grown in 1939 (*ch-ca* am 2) has been examined cytologically. This plant was found to have a chromosome number of  $2n=55$  instead of the true amphidiploid number of 54 (see Fig. 5 and Table 1). Only a few cells were observed at first metaphase and these were seen to contain about 27 bivalents. In one cell, however, a single ring quadrivalent was observed (see Fig. 4). The univalent also observed is to be expected if the chromosome number is 55. A few second metaphase plates have been counted (see Table 1). These show irregularities of numerical disjunction other than those caused by the somatic number of 55, i.e. there are plates with 26 and 29 chromosomes in addition to those with 27 and 28. The fact that *ch-ca* am 2 has an irregular chromosome number of 55 also shows that numerically irregular disjunctions occurred in the first generation of amphidiploids. Bridges were observed at first anaphase (they were also seen in the  $F_1$  hybrid), and a few of them were found to persist to second metaphase (see Fig. 6). Such persistent bridges have not been observed in other *Brassica* hybrids, and their persistence may be connected with the crowded plates in the amphidiploid.

(5) *Fertility of the amphidiploids.* The fertility of the first amphidiploids ( $A_1$  generation, i.e. those obtained from  $F_1$  seedlings by colchicine treatment) was not investigated. In 1939 four plants of an  $A_2$  generation were selfed and crossed among themselves to determine their seed fertility. Two *chinensis* and two *carinata* plants were also selfed for comparison with the amphidiploids. The results are given in Table 2. The

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cross *ch-ca* am 1  $\times$  *ch-ca* am 4, which produced 11.8 and 10.0 good seeds per fruit, suggests that some amphidiploids may be nearly fully fertile. Other amphidiploids, however, appear to have a seed fertility of only

Table 1. *Chromosome numbers of second metaphase plates of ch-ca am 2*

Chromosome no.	25	26	27	28	29
No. of plates	1	1	6	5	2
	2		11		2

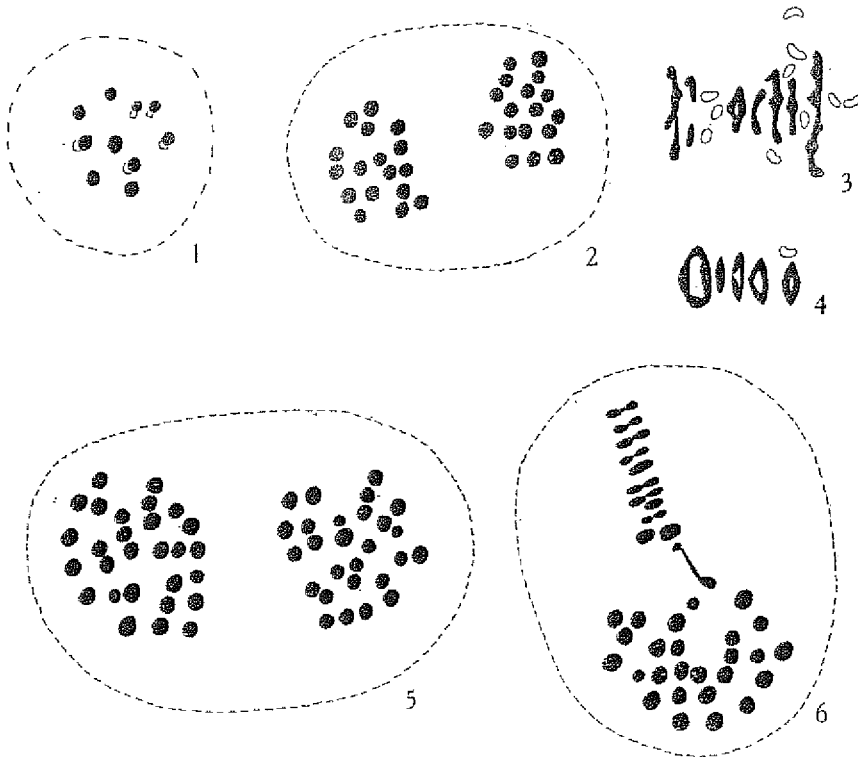


Fig. 1. *B. chinensis*—first metaphase,  $n=10$ .

Fig. 2. *B. carinata*—second metaphase,  $n=17$ .

Fig. 3. *B. chinensis*  $\times$  *carinata*  $F_1$ . Side view of first metaphase—1 quadrivalent, 1 trivalent, 5 bivalents, 10 univalents.

Figs. 4-6. *B. chinensis-carinata* amphidiploid plant 2. Fig. 4. First metaphase, part of a plate only—1 quadrivalent, 4 bivalents and 1 univalent. Fig. 5. Second metaphase, -28:27 segregation. Fig. 6. Second metaphase, persistent bridge from first anaphase.

about 50%. Such a low fertility might be expected for *ch-ca* am 2 which was found to have a chromosome number of 55 instead of the true amphidiploid one of 54.

The low fertility of the amphidiploid plants when selfed is probably partly due to self incompatibility which is found in the *chinensis* parent. The cut stigma treatment, see Table 2, makes self incompatible *Brassica*

Table 2. *The fertility of chinensis, carinata and amphidiploid chinensis-carinata plants*

Plant	Pollination	Good seeds per fruit
<i>chinensis</i> 1	Self	15/9 = 1.7
<i>chinensis</i> 1	Self, cut stigma	175/14 = 12.5
<i>chinensis</i> 2	Self	61/18 = 3.4; 49/10 = 4.9
<i>chinensis</i> 2	Self, cut stigma	75/9 = 8.3
<i>carinata</i> 1	Self	113/9 = 12.6; 123/10 = 12.3
<i>carinata</i> 1	× <i>carinata</i> 2	84/6 = 14.0
<i>carinata</i> 2	Self	122/10 = 12.2; 123/8 = 15.4
<i>ch-ca</i> am 1	Self	20/8 = 2.5; 29/7 = 4.1; 18/7 = 2.6
<i>ch-ca</i> am 1	× <i>ch-ca</i> am 2	47/10 = 4.7; 29/6 = 4.8
<i>ch-ca</i> am 1	× <i>ch-ca</i> am 4	118/10 = 11.8; 159/16 = 10.0
<i>ch-ca</i> am 2	Self	32/12 = 2.7; 31/6 = 5.2; 40/8 = 5.0
<i>ch-ca</i> am 2	× <i>ch-ca</i> am 1	43/8 = 5.4
<i>ch-ca</i> am 2	× <i>ch-ca</i> am 4	80/16 = 5.0
<i>ch-ca</i> am 3	Self	26/8 = 3.3; 41/14 = 2.9; 67/14 = 4.8
<i>ch-ca</i> am 3	× <i>ch-ca</i> am 4	34/8 = 4.3
<i>ch-ca</i> am 4	Self	52/17 = 3.1; 50/14 = 3.6; 47/9 = 5.2
<i>ch-ca</i> am 4	× <i>ch-ca</i> am 2	6/10 = 0.6

*ch-ca* am 1, etc. = *chinensis-carinata* amphidiploid plant 1, etc.

plants self fertile as was first shown by Sears (1937). In addition to good seeds, however, the amphidiploids also produced shrivelled seeds. Shrivelled seeds are not found in selfed fruits of *chinensis* and are due to embryo abortion. Also in many cases the good seeds from the amphidiploids had split testas. The seeds from the cross *ch-ca* am 1 × *ch-ca* am 4, however, were perfectly good ones with intact testas.

### 3. CROSSING RESULTS

(1) *General considerations.* In making the pollinations about ten flowers per inflorescence were emasculated and crossed. Most of the results are given in Table 3. It appeared from the size of fruit produced and from the presence of good seeds or 'developed ovules' that pollen-tube growth and fertilization took place in every cross. A possible exception is the cross *carinata* female × tetraploid *chinensis* which only set very small fruits. Developed ovules were always very much larger than obviously unfertilized ones, and in many cases could have been described as 'shrivelled seeds'. It is reasonable to assume that developed ovules have been fertilized, that an embryo and endosperm have started to develop but that the embryo has later aborted. This type of development has in fact been found to be common in species crosses in the genus *Brassica*. Pollen-tube growth and fertilization were also found to take

place in both crosses between diploid and autotetraploid *B. oleracea* (Howard, 1939).

(2) *Crosses between chinensis and carinata.* The cross *chinensis* female  $\times$  *carinata* produces a very high set of good seeds. As this cross is of the type diploid female  $\times$  allotetraploid, it might have been expected to produce only developed ovules (see Watkins (1932) and the introduction to the present paper). The result obtained is most easily explained by assuming that *carinata*, although still an allotetraploid in its chromosome number, has evolved since its formation to behave like a diploid in its physiology of seed production. This assumption will be made in the whole of the present paper.

It must, however, be emphasized that it is an assumption to suggest that *carinata* has a diploid seed-producing physiology and that the only evidence for this assumption is that explanations containing it seem to work quite well in explaining the crossing results and seed size results given in this paper. Also it must be pointed out that the *chinensis* genome (a) is not one of the constituents of the *carinata* genome (bc), and we might therefore have made the assumption that *chinensis* had a tetraploid seed-producing physiology (this would be a reasonable assumption since *chinensis* is probably a secondarily balanced form as is shown by secondary associations of chromosomes (Richharia, 1937)).

The suggestion that allotetraploids may evolve so as to resemble diploids in their physiology of seed production is, however, a reasonable one. Thus Wettstein (1938) has observed the gradual loss of gigas characters and a diminution in cell volume accompanied by an increase in fertility in an experimentally produced bivalent form of the moss, *Bryum caespiticium*. Such an evolution in physiology of seed production would also seem to have taken place in the genus *Paeonia*. Thus Saunders & Stebbins (1938) found that the cross *P. albiflora* (diploid)  $\times$  *P. tomentosa* (tetraploid) takes place much more easily with the former as ovulate parent, and the cross *P. albiflora* (diploid)  $\times$  *P. officinalis* (tetraploid) shows no reciprocal differences in compatibility, seeds being obtained from both crosses.

The cross *carinata* female  $\times$  *chinensis* has only produced a few good seeds. The significance of this is not known, but similar examples of incompatibility of species crosses in one direction only are common, e.g. the cross *Raphanus sativus*  $\times$  *Brassica oleracea* produces a good set of seeds only with *Raphanus sativus* as ovulate parent. Negative results in crosses would also appear to be of doubtful significance in many cases. Thus in the present investigation a high set of good seeds from the cross

amphidiploid female  $\times$  either parent species was obtained when *ch-ca* am 3 was used as female parent and not when *ch-ca* am 1, 2 and 4 were used. Similarly, the cross *Brassica pekinensis* female  $\times$  *carinata* has only produced developed ovules although *chinensis* and *pekinensis* are closely related species (Richharia, 1937).

(3) *Crosses involving the amphidiploid.* Since it has been assumed that *carinata* as well as *chinensis* has a diploid seed-producing physiology,

Table 3. *Crossing results (the results given are a typical selection)*

Female parent	Male parent	Result of pollination: Good seeds per fruit, etc.
<i>chinensis</i> 1	<i>carinata</i>	56/11 = 5.1; 136/12 = 11.3
<i>chinensis</i> 1	<i>ch-ca</i> am 1	Developed ovules only
<i>chinensis</i> 1	4x <i>chinensis</i>	Mostly developed ovules—3G, selfs?
<i>chinensis</i> 2	<i>carinata</i>	99/15 = 6.6; 124/18 = 6.9
<i>chinensis</i> 2	<i>ch-ca</i> am 4	Developed ovules only
<i>chinensis</i> 2	4x <i>chinensis</i>	Developed ovules only (1G)
<i>carinata</i> 1	<i>chinensis</i> 1.	Developed ovules only
<i>carinata</i> 1	<i>ch-ca</i> am 4	Developed ovules only
<i>carinata</i> 1	4x <i>chinensis</i>	Small fruits, only a few developed ovules
<i>carinata</i> 2	<i>chinensis</i> 1 and 2	Mostly developed ovules, 7G
<i>carinata</i> 2	<i>ch-ca</i> am 2	Developed ovules only
<i>carinata</i> 2	<i>ch-ca</i> am 4	Developed ovules only
<i>carinata</i> 2	4x <i>chinensis</i>	Only small fruits set
<i>ch-ca</i> am 1	4x <i>chinensis</i>	39/13 = 3.0
<i>ch-ca</i> am 1	<i>chinensis</i>	Developed ovules only
<i>ch-ca</i> am 1	<i>carinata</i>	Developed ovules only
<i>ch-ca</i> am 2	4x <i>chinensis</i>	54/15 = 3.5
<i>ch-ca</i> am 2	<i>chinensis</i>	Developed ovules only
<i>ch-ca</i> am 2	<i>carinata</i>	Developed ovules only
<i>ch-ca</i> am 3	4x <i>chinensis</i>	34/9 = 3.8; 17/14 = 1.2
<i>ch-ca</i> am 3	<i>chinensis</i>	39/14 = 2.8
<i>ch-ca</i> am 3	<i>carinata</i>	33/12 = 2.8
<i>ch-ca</i> am 4	<i>chinensis</i>	Developed ovules only
<i>ch-ca</i> am 4	<i>carinata</i>	Developed ovules only
4x <i>chinensis</i>	<i>chinensis</i>	Developed ovules only
4x <i>chinensis</i>	<i>carinata</i>	67/8 = 8.4
4x <i>chinensis</i>	<i>ch-ca</i> am 4	25/5 = 5.0; 6/6 = 1.0

it follows that the amphidiploids would be expected to behave as tetraploids in their physiology of seed production. This is in fact found to be so. The crosses *chinensis* or *carinata* female  $\times$  the amphidiploid produce only developed ovules while, when *ch-ca* am 3 is used as female parent, the crosses amphidiploid female  $\times$  either parent species produce a high set of good seeds per fruit. Also all three amphidiploids tested (*ch-ca* am 1, 2 and 3) produce a high set of good seeds when used as female parents in the cross with autotetraploid *chinensis*. In the reciprocal cross with tetraploid *chinensis* as female parent only one amphidiploid (*ch-ca* am 4) was tested as male parent. This cross also produced a high set of good

seeds. If *carinata* had a tetraploid seed-producing physiology, the amphidiploid would be expected to behave as an hexaploid and the cross tetraploid *chinensis* female  $\times$  the amphidiploid (i.e. tetraploid female  $\times$  supposed hexaploid) would only be expected to produce developed ovules.

(4) *Crosses involving autotetraploid chinensis*. As was to be expected the cross diploid *chinensis* female  $\times$  autotetraploid only produced developed ovules—an occasional good seed was obtained but these may be selfs or tetraploids (cf. Howard, 1939, p. 332). The reciprocal cross tetraploid female  $\times$  diploid also only produced developed ovules in contrast to the *B. oleracea* results of Howard (1939), where the cross tetraploid female  $\times$  diploid produced a high set of good seeds. Similar results to those obtained with *chinensis* have also been found in crosses between autotetraploids and diploids in *B. rapa*, *nigra* and *napus* and also in *Raphanus sativus* and *Nasturtium officinale*. The *Brassica oleracea* result thus appears to be the exceptional one.

The cross *carinata* female  $\times$  tetraploid *chinensis*, as would be expected both from the result of the cross *carinata* female  $\times$  diploid *chinensis* and from the fact that it is a cross of the type diploid female  $\times$  tetraploid, only produces developed ovules, and in this cross the development of the hybrid embryos appears to stop very early so that only small fruits are set. The cross tetraploid *chinensis* female  $\times$  *carinata* produces a high set of good seeds, and, if the suggestion that *carinata* has a diploid seed-producing physiology is correct, is another example of the cross tetraploid female  $\times$  diploid producing a high set of good seeds.

The crosses between autotetraploid *chinensis* and the amphidiploid *chinensis-carinata* plants have already been discussed.

#### 4. SEED SIZE RESULTS

(1) *Introduction*. In *Brassica oleracea* it was found that the triploid seeds from the cross tetraploid female  $\times$  diploid were quite full but small, having an average weight of only about one-third that of seeds from selfing the diploid (see Howard (1939), and also Table 4 of this paper). Such seeds are not in any way shrivelled and fully fill the testa. The small size of these triploid *B. oleracea* seeds was explained as being due to an abnormal embryo : endosperm relation, a triploid embryo developing in a pentaploid instead of in a 4.5-ploid endosperm (cf. the normal-sized seeds from both diploid selfed (diploid embryo in triploid endosperm) and tetraploid selfed (tetraploid embryo in hexaploid endosperm) which have normal embryo : endosperm ratios of 1 : 1.5). The small size of these triploid seeds can be described more shortly as due to the ' polyploidy



effect'. In several of the crosses described in this paper small seeds have also been obtained. Like the *B. oleracea* triploid seeds these small seeds are not in any way shrivelled.

The seeds were weighed in successive samples of ten seeds each. The weights are given in Table 4. Four sizes of seed can be recognized. The first size of seed is the normal size and has an average weight of about 4.5 mg. Seeds of this type were obtained from selfing diploid and tetraploid *chinensis*, from selfing *carinata*, and from crossing the amphidiploid *chinensis-carinata* plants among themselves. The second size of seed is somewhat smaller in size than normal, having an average weight of about 3.1 mg. Such seeds were obtained from crosses between the two

Table 4. *Weights of samples of ten seeds each*

Female parent	Male parent	Weights of samples of ten seeds in mg.
<i>chinensis</i> 1	Self	40, 41
<i>chinensis</i> 1	<i>carinata</i> 1	12, 14, 11, 13, 13
<i>chinensis</i> 2	Self, cut stigma	44, 45, 43
<i>chinensis</i> 2	Self	44, 47, 41, 43, 46
<i>chinensis</i> 2	<i>carinata</i> 2	17.5, 14, 15, 19.5, 18
<i>carinata</i> 1	Self	58, 56, 55
<i>carinata</i> 1	<i>carinata</i> 2	40, 47, 46, 39, 46
<i>carinata</i> 2	<i>chinensis</i> 1	wt. of 7=3; hence wt. of 10=4.3
<i>ch-ca</i> am 1	<i>ch-ca</i> am 4	39, 45, 37, 43, 47
<i>ch-ca</i> am 1	<i>ch-ca</i> am 4	40, 46, 45.5, 44, 46
<i>ch-ca</i> am 1	4x <i>chinensis</i>	37, 32, 36, 37
<i>ch-ca</i> am 3	<i>ch-ca</i> am 4	54, 53, 58
<i>ch-ca</i> am 3	<i>chinensis</i>	10, 12, 6, 7
<i>ch-ca</i> am 3	<i>carinata</i>	15, 13, 14
<i>ch-ca</i> am 3	4x <i>chinensis</i>	36, 36, 34, 32
4x <i>chinensis</i>	Self	41, 42, 44, 44, 41
4x <i>chinensis</i>	<i>carinata</i>	13, 13, 12, 12, 10
4x <i>chinensis</i>	<i>ch-ca</i> am 4	26, 29
2x <i>oleracea</i>	Self	41
4x <i>oleracea</i>	Self	53
4x <i>oleracea</i>	2x <i>oleracea</i>	12

tetraploids, tetraploid *chinensis* and amphidiploid *chinensis-carinata*. The third size of seed are the small ones of about 1.3 mg. weight. These correspond in size to the small triploid seeds obtained from the cross tetraploid *B. oleracea* × diploid. They were obtained from the following crosses: diploid *chinensis* female × *carinata*, tetraploid *chinensis* female × *carinata*, amphidiploid *chinensis-carinata* female × *chinensis* and amphidiploid *chinensis-carinata* female × *carinata*. Class four seeds are extremely small, weighing only 0.4 mg. and were obtained from the cross *carinata* female × *chinensis*. A summary of the seed sizes will also be found in the last column of Table 5.

(2) *Crosses between chinensis and carinata.* The good seeds from the cross *chinensis* female × *carinata* are small and only about one-third the

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weight of selfed seeds of either parent. This reduction in seed size can be explained to a certain extent by extending the idea of embryo : endosperm ratio from total genomes present to numbers of each type of genome present (see Table 5). Thus in the cross *chinensis* female  $\times$  *carinata*, 1 *chinensis* genome in the embryo is balanced against 2 instead of  $1\frac{1}{2}$  *chinensis* genomes in the endosperm, and 1 *carinata* genome in the embryo against 1 instead of  $1\frac{1}{2}$  *carinata* genomes in the endosperm. Such a disturbance of normal embryo : endosperm ratios must take place in every interspecific cross. It is convenient to describe the small size of seeds

Table 5. *Embryo : endosperm ratios*

Ch = *chinensis* genome, and Ca = *carinata* genome.

Cross, female parent first	Constitution of		Embryo : endosperm ratios for			Average weight of seeds mg.
	Embryo	Endosperm	Ch + Ca	Ch	Ca	
ChCh $\times$ ChCh	ChCh	ChChCh	2 : 3	2 : 3	—	4.2
CaCa $\times$ CaCa	CaCa	CaCaCa	2 : 3	—	2 : 3	4.8
ChChChCh $\times$ ChChChCh	ChChChCh	ChChChChChCh	4 : 6	4 : 6	—	4.2
ChChCaCa $\times$ ChChCaCa	ChChCaCa	ChChChCaCaCa	4 : 6	2 : 3	2 : 3	4.5
ChCh $\times$ CaCa	ChCa	ChChCa	3 : 3	1 : 2	1 : 1	1.5
ChChCaCa $\times$ ChCh	ChChCa	ChChChCaCa	3 : 5	2 : 3	1 : 2	1.0
ChChCaCa $\times$ CaCa	ChCaCa	ChChCaCaCa	3 : 5	1 : 2	2 : 3	1.4
ChChCaCa $\times$ ChChChCh	ChChChCa	ChChChChCaCa	4 : 6	3 : 4	1 : 2	3.4
ChChChCh $\times$ ChChCaCa	ChChChCa	ChChChChChCa	4 : 6	3 : 5	1 : 1	2.7
ChChChCh $\times$ CaCa	ChChCa	ChChChChCa	3 : 5	3 : 4	1 : 1	1.2

obtained from such crosses as due to a 'hybridity effect', just as it is convenient to describe the small size of triploid seeds as being due to a 'polyploidy effect'.

It would also be possible to explain the action of the hybridity and polyploidy effects in producing small seeds as due to disturbed mother plant : embryo or mother plant : endosperm relations. Watkins (1932) and Howard (1939) have, however, given reasons for suggesting that the most important relation is that between embryo and endosperm, and that relations involving the mother plant are of secondary importance.

The few good seeds obtained from the reciprocal cross with *carinata* as female parent were extremely small. No attempt has been made to germinate these seeds, and it is possible that they are not viable. At least 150 plants have been grown from the cross *chinensis* female  $\times$

*carinata*, and every plant was found to be hybrid. In this cross and all the others the sizes of seed produced rule out the possibility that selfs had been obtained because of bad technique.

Examples of the hybridity effect producing small seeds have been obtained in other *Brassica* crosses. Seeds from the cross autotetraploid *chinensis* female  $\times$  autotetraploid *oleracea* had an average weight of 0.95 mg., and seeds from the cross autotetraploid *chinensis* female  $\times$  autotetraploid *nigra* an average weight of 1.3 mg. The average weights of selfed seeds of all three parents in these two crosses are about 4.5 mg. It is also interesting to note that Beasley (1940*a*) found that the hybrid seeds from crosses between American 26-chromosome and Asiatic 13-chromosome species of cotton were very small. The small size of the hybrid cotton seeds may have been due to either the polyploidy or the hybridity effects or to a combination of the two effects.

(3) *Crosses between the amphidiploids and chinensis or carinata.* The seeds from the cross amphidiploid female  $\times$  a parent species (*chinensis* or *carinata*) would be expected to be small because of the polyploidy effect (cf. triploid *B. oleracea* seeds), and they were found to be small (see Table 4 or 5). These two crosses thus afford more evidence for the suggestion that in the genus *Brassica* triploid seeds from the cross tetraploid female  $\times$  diploid are always about one-third the weight of normal diploid seeds.

However, the cross amphidiploid  $\times$  a parent species is not so simple as the cross autotetraploid  $\times$  diploid. As can be seen from Table 5, in addition to the polyploidy effect there has also to be considered the hybridity effect in crosses between the amphidiploid and one of the parent species. The small sizes of the seeds from the two crosses amphidiploid  $\times$  either *chinensis* or *carinata* may, therefore, be due to either the polyploidy or hybridity effects or more likely to a combination of the two effects. A little evidence to suggest that the polyploidy effect is the more important one in the cross amphidiploid female  $\times$  *chinensis* can be obtained by comparing this cross with the cross amphidiploid female  $\times$  tetraploid *chinensis*. As is shown in Table 5, both these crosses have a 1 : 2 embryo : endosperm ratio for *carinata* genomes, and the cross amphidiploid  $\times$  diploid *chinensis* has a normal 2 : 3 embryo : endosperm ratio for *chinensis* genomes as compared with the abnormal 3 : 4 embryo : endosperm ratio for *chinensis* genomes in the cross amphidiploid  $\times$  tetraploid *chinensis*. But seeds from the cross amphidiploid  $\times$  tetraploid *chinensis* have an average weight of 3.4 mg. as compared with 1.0 mg. for seeds from the cross amphidiploid female  $\times$  diploid *chinensis*. The

small size of seeds from this latter cross, therefore, appears to be largely due to the polyploidy effect.

The fact that seeds from both backcrosses are of similar sizes is also of some interest. If *carinata* had a tetraploid seed-producing physiology in relation to the diploid one of *chinensis*, then the amphidiploid would behave as a hexaploid and we might expect the cross amphidiploid  $\times$  *carinata* (supposed hexaploid  $\times$  tetraploid) to produce seeds of a different size than the cross amphidiploid  $\times$  *chinensis* (supposed hexaploid  $\times$  diploid).

(4) *Crosses involving autotetraploid chinensis.* The cross *chinensis-carinata* amphidiploid female  $\times$  tetraploid *chinensis* and its reciprocal are both examples of the type tetraploid  $\times$  tetraploid, if we assume *carinata* to have a diploid seed-producing physiology. Seeds from these two crosses might, therefore, be expected to be of normal size. The results (see Tables 4 and 5) show that the seeds from these two crosses are certainly very much larger than triploid seeds but not quite as heavy as normal seeds. This reduction in size can be accounted for on the hybridity effect (see Table 5). The cross tetraploid *chinensis* female  $\times$  amphidiploid has an especially unbalanced embryo : endosperm ratio for *carinata* genomes, and it may be significant that seeds from this cross are smaller than those from the reciprocal.

It was rather surprising to obtain such a high set of good seeds from the cross tetraploid *chinensis* female  $\times$  *carinata*. The seeds from this cross would be expected to be small in size because of the polyploidy effect (again assuming *carinata* to have a diploid seed-producing physiology) and might also be expected to be further reduced in size by the hybridity effect (cf. diploid *chinensis* female  $\times$  *carinata*). Actually, the results (see Table 4 or 5) show that there is no double reduction in size, the seeds being about the same size as triploids and as hybrids from the cross diploid *chinensis*  $\times$  *carinata*.

##### 5. DISCUSSION: THE PHYSIOLOGY OF ALLOTETRAPLOID SPECIES

The results given in this paper suggest the following generalizations for crosses in the genus *Brassica*. First from crosses of the type diploid female  $\times$  tetraploid there is never obtained a high set of good seeds. Secondly, a high set of triploid seeds may be obtained from the cross tetraploid female  $\times$  diploid. Thirdly, triploid seeds from the cross tetraploid female  $\times$  diploid are always small in size, being about one-third the weight of normal seeds. Fourthly, seeds from crosses of the types diploid

$\times$  diploid and tetraploid  $\times$  tetraploid are of normal size. If these four generalizations are correct, then the results of crosses, including the size of seeds obtained, give a method, although it must be admitted that it is a rather indirect one, of investigating the comparative physiology of experimentally produced autotetraploids and amphidiploids and of allotetraploid species.

Darlington (1932, p. 207) suggests that pollen-grain size measurements show that in some genera tetraploids have evolved so as to resemble diploids again while in others no such evolution has occurred. Measurements of pollen-grain sizes of the allotetraploid species *B. juncea*, *B. carinata*, and *B. napus* seemed to suggest that the first species had not evolved so as to resemble a diploid but that the other two might now resemble diploids again in certain aspects of their physiology. A certain amount of indirect evidence has been given in this paper to support the view that *carinata* has a diploid seed-producing physiology.

It is possible that more direct evidence about the seed-producing physiologies of *B. juncea* and *napus* will soon be obtained. By crossing autotetraploid *chinensis* with autotetraploid *nigra*, an experimental amphidiploid, which should on the Japanese scheme be equivalent to artificial *juncea*, has been obtained. Artificial *napus* has also been obtained by crossing tetraploid *chinensis* with tetraploid *napus*. The artificial *napus* has not yet flowered.

The artificial *juncea* (amphidiploid *chinensis-nigra*), when used as female parent in the cross with *juncea*, produced a high set of good seeds which had an average weight of about three-quarters that of normal *juncea* seeds. Both *juncea* and *chinensis-nigra* amphidiploid therefore appear to have similar seed-producing physiologies. In other words, the species *B. juncea* still appears to have a tetraploid seed-producing physiology. Unfortunately, for some unknown reason, the amphidiploid produces only developed ovules when selfed or when used as male parent in the cross with *juncea*. This latter behaviour of the artificial *juncea* is very similar to that found for amphidiploids from the cross Asiatic 13-chromosome cotton  $\times$  American 13-chromosome cotton by both Beasley (1940*b*) and Harland (1940). The cotton amphidiploid is apparently male-sterile but produces a good set of seeds when used as female parent in the cross with American 26-chromosome species (these latter species are supposed to be allotetraploids from hybrids between Asiatic 13-chromosome and American 13-chromosome cottons). It is hoped that there will be no such complication with artificial *B. napus*. The *B. napus* case may also be more satisfactory in that good seeds are obtained from both

crosses between *B. napus* and a 10-chromosome species; this suggests that *napus* has a diploid seed-producing physiology.

I have also previously suggested that the results of crosses between diploid watercress, the artificial tetraploid and the naturally occurring tetraploid can be explained if we assume that the naturally occurring tetraploid has a seed-producing physiology intermediate between that of the diploid and that of the artificial autotetraploid (Howard, demonstration at the Seventh International Genetical Congress, August 1939). Since the watercress results were first discussed, however, the wild tetraploid has been shown to be an allotetraploid and not an autotetraploid (Howard & Manton, 1940). The explanation of the crossing and seed-size results is thus now more complicated.

#### 6. SUMMARY

1. Amphidiploid *Brassica chinensis-carinata* and autotetraploid *B. chinensis* plants were obtained by colchicine treatment.

2. The amphidiploid ( $2n=54$ ) has a fairly regular meiosis and is quite fertile.

3. A study was made of crosses between *B. chinensis*, *B. carinata*, the amphidiploid *B. chinensis-carinata* and autotetraploid *B. chinensis*. Pollen-tube growth appeared to be good in all crosses.

4. A high set of good seeds per fruit is produced in crosses of the types, diploid  $\times$  diploid, tetraploid  $\times$  tetraploid and tetraploid female  $\times$  diploid. Crosses of the type diploid female  $\times$  tetraploid never produced a high set of good seeds, developed ovules only being obtained from such crosses.

5. *B. carinata* ( $n=17$ ), although an allotetraploid species, appeared to behave as a diploid in its physiology of seed production.

6. Good seeds from crosses of the type tetraploid female  $\times$  diploid are only about one-third the size of normal seeds from selfing diploids. 'Hybridity' may also produce a reduction in seed size.

7. It is suggested that some allotetraploid species may have evolved so as to have a 'diploid physiology'.

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