

# THE SIZE OF SEEDS IN DIPLOID AND AUTO-TETRAPLOID *BRASSICA OLERACEA* L.

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(With Five Text-figures)

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## 1. INTRODUCTION

WATKINS (1932) gave a very useful summary and exposition of the factors influencing the successful production of seeds when a diploid and its autotetraploid are crossed. This often depends upon which plant is used as female parent. In some cases, e.g. *Primula sinensis*, diploid pollen will not grow down diploid styles and the cross therefore fails when the autotetraploid is used as male parent. In other cases, e.g. *Campanula persicifolia*, pollen-tube growth takes place in both crosses and it appears to be the development of the embryo and endosperm which is different. This is not surprising, since, as Watkins points out (and see also Diagram 1), the nuclear constitution of the endosperm is  $4n$  in the cross diploid female and  $5n$  in the cross tetraploid female. Watkins also gives evidence for showing that it is the relation endosperm: embryo which is important and not any relation involving the mother plant.

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*Brassica oleracea* and its autotetraploid are an example of the second class. Diploid pollen appears to be capable of growth down diploid styles, but the cross only succeeds when the tetraploid is used as female parent.

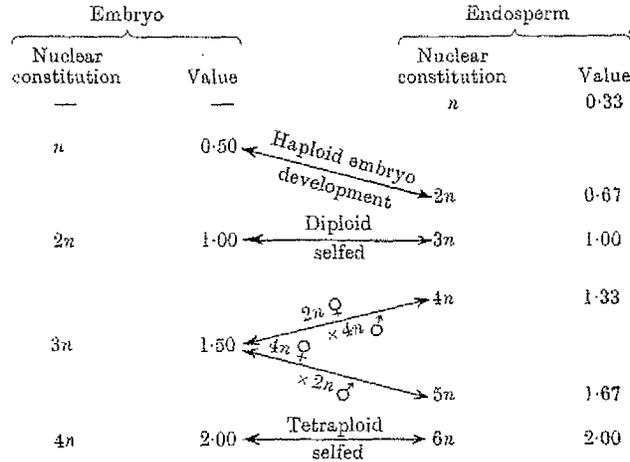


Diagram 1. Embryo: endosperm relations.

The data given in this paper support all the conclusions of Watkins. They are, however, more complete in one way than data for any other plants since it was possible to weigh *Brassica* seeds. Also, unlike *Campanula persicifolia* (Gairdner & Darlington, 1931) for which there is also considerable data, the seeds are non-endospermic.

2. SIZE OF SEEDS IN CONTROLLED EXPERIMENTS

The material used for these experiments consisted of callus cuttings, and cuttings from these callus cuttings, from one callus produced by decapitating a seedling of thousand-head kale. Among these cuttings there were some autotetraploids. The diploid and autotetraploid stocks thus differed only in chromosome number and not in geetical constitution. Fortunately the plants were self-compatible to a certain extent—though the seed set appears to be lower than the 80% found when other kale plants were selfed.

(a) *Seeds from selfing diploid kale*

When the diploid kale was selfed, a very uniform collection of seed was obtained in nearly every case. The weight of ten seeds is given in Table I a. In some cases, however, a few seeds very much smaller than the normal were obtained. Intermediate types of seed between the normal size and these very much smaller seeds did not occur (see § 3(a)).

(b) *Seeds from selfing autotetraploid kale*

When autotetraploid kale is selfed, the number of seeds obtained per fruit is very much less than that obtained from selfing the diploid. This is to be expected since autotetraploid kale shows meiotic irregularities. There are also produced in addition to the good seeds a fairly large number of various types of shrivelled seeds. These latter are presumably embryos of the type  $4n + 1$  and  $4n - 1$ , etc., but the chromosome counts made were only accurate to  $\pm 1$  chromosome.

TABLE I  
*Weight of seeds from diploid and autotetraploid  
thousand-head kale*

Seed	Wt. of single seeds in grams.			
	(a) Diploid selfed	(b) Tetraploid selfed	(c) Tetraploid ♀ × diploid ♂	(d) Diploid ♀ × tetraploid ♂
1	0.0046	0.0058	0.0014	0.0006
2	38	50	10	04
3	44	52	12	04
4	42	50	14	04
5	38	56	10	06
6	40	54	12	04
7	44	52	16	04
8	42	54	10	04
9	36	52	10	04
10	38	48	12	06
Mean	0.00408	0.00534	0.00120	0.00046

In all four cases, shrivelled seeds have been rejected and only well-developed ones weighed.

(c) Tetraploid ♀ × diploid ♂. Small but full seeds, 100% germination.

(d) Diploid ♀ × tetraploid ♂. Seeds same size as (c), but contain no embryos.

Two well-developed seeds were obtained from this cross which did contain embryos (see § 3(b)).

Autotetraploid kale forms an average of 4.0 quadrivalents per nucleus. Counts of second metaphase plates showed 57% with 18, 14% with 19, 20% with 17, and others with 20 and 16 chromosomes. It is not, therefore, surprising to find that the seed fertility of autotetraploid kale is only about 35% of diploid (see Table II; percentage good pollen is 98 (cf. autotetraploid *Datura*)).

Root tips were fixed from a few of the shrivelled types of seed found when autotetraploid kale is selfed. It was only possible to see that they had about 36 chromosomes (see Table III).

The "normal" seeds from selfing autotetraploid kale are considerably heavier, about 30%, than diploid seeds (see Table I). They are also recognizably bigger (see Fig. 1).

(c) *Seeds from crossing diploid ♀ × tetraploid ♂*

Nearly all the "seeds" (two exceptions) from this cross are very small (see Fig. 1). They are also very light (see Table I*d*), and contain no embryo. They therefore give 0% germination. The two exceptional seeds are discussed in § 3(*b*) of this paper.

It is not known whether these empty seeds are produced from fertilized ovules or just from unfertilized ovules. There are reasons for

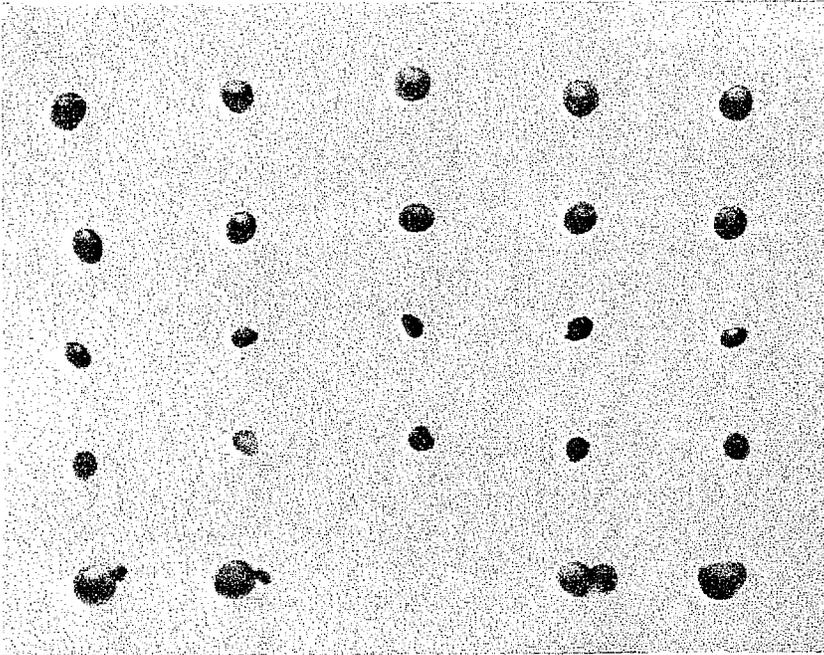


Fig. 1. Seeds of *Brassica oleracea* (on moist filter paper for one day). Row 1: tetraploid selfed. Row 2: diploid selfed. Row 3: tetraploid female × diploid male. Row 4: diploid female × tetraploid male. Row 5: four double seeds; in the two seeds on the left only one ovule has developed a large embryo—the other parts of the double seeds contained no recognizable embryos.

suggesting that they develop from fertilized ovules, or at least from ovules which have been penetrated by pollen tubes. The first reason for suggesting this is that there also occur ovules in the fruits from this cross of a much smaller size, which are the same size as all ovules in fruits obtained from flowers which have been emasculated and left unpollinated. The second reason is that the two exceptional seeds (see § 3(*b*)) do show that diploid pollen tubes can function in diploid styles. The development of the fruit also suggested that fertilization may have occurred.

It is not, however, possible to state definitely that fertilization occurs and that the abnormal development of ovules without an embryo of recognizable size is due to a disturbance in the embryo: endosperm relationship. It is intended to investigate this point further in the spring of 1939.

TABLE II

*Fertility of diploid and autotetraploid kale*

*A* = no development of ovules; *S + s* = shrivelled seeds; *G* = good seeds.

(a)  $2n$  selfs.

<i>A</i>	<i>S + s</i>	<i>G</i>	Total ovules	% <i>G</i>
281	7	212	500	42.2
255	4	222	481	46.3
171	7	137	305	40.1

This fertility is considerably less than the 77.2% found in another diploid kale plant in 1937.

Also XVII 24 F  $\times$  XVII 21 G ( $2n \times 2n$ ) gave this year—361 total ovules, 278 G; % *G* = 77%.

(b)  $4n$  selfs.

<i>A</i>	<i>S + s</i>	<i>G</i>	Total ovules	% <i>G</i>
203	39	55	297	18.5
306	15	72	393	18.2
455	36	72	563	12.8

Fertility of  $4n$  kale is, therefore, about  $\frac{1}{5}\%$  = 36% approx.

(c)  $4n$  female  $\times$   $2n$  male.

<i>A</i>	<i>S + s</i>	<i>G</i>	Total ovules	% <i>G</i>
71	27	39	137	28.5

(d)  $2n$  female  $\times$   $4n$  male.

<i>A</i>	<i>S + s</i>	<i>G</i>	Total ovules
200	71	0	271
131	64	0	195

The small seeds without any ovules, whose weights are given in Table Id, are classified among the "s" group.

TABLE III

*Small and shrivelled seeds from tetraploid selfed*

Parent	Description of seed	Weight of seed g.	Chromo- some no. $\pm 1$
XVII 24 B selfed	Medium full	0.0030	36
"	Medium shrivelled	18	36
"	Medium shrivelled	16	36
"	Medium shrivelled	12	36
"	Small full	14	36

Chromosome number of *B. oleracea* is  $n=9$ ,  $2n=18$ .

(d) *Seeds from crossing tetraploid* ♀  $\times$  *diploid* ♂

When the cross is made this way round, the number of seeds per fruit is about equal to that obtained by selfing the autotetraploid (see Table II). The seeds are very small (see Table I and Fig. 1). They are thus very

distinct from the seeds obtained by selfing either the diploid or autotetraploid. Their peculiar development is probably to be explained by the peculiar endosperm: embryo relation (see Diagram 1). In § 3(a) there is evidence for showing that the relations of neither embryo nor endosperm to the parent plant is of much importance.

About 150 of these small seeds were obtained by crossing autotetraploid ♀ × diploid ♂. Root tips have been fixed from seven only. All these seven were found to have  $27 \pm 1$  chromosomes, i.e. they are triploids as expected. Forty seeds have been placed on moist filter paper in Petri dishes and all forty germinated. The germination does, however, appear to be somewhat slower than that of diploids and tetraploids.

No large seeds in addition to these small triploids (about 150) were obtained from the crosses. It is reasonable to suggest that they probably will occur in future experiments. Diploid pollen grains are often found in small numbers in the normally haploid pollen from diploid plants. If these fertilize the diploid ovules of the tetraploid, then a tetraploid embryo and hexaploid endosperm will be produced. Such an ovule should grow into a normal-sized seed. It should also be possible to test this by pollinating the tetraploid with a mixture of haploid and diploid pollen. If, as it seems very reasonable to suppose, all triploid embryos grow into the very small type of seed and tetraploid into the large type, such experiments would afford a relatively easy method of comparing the growth of haploid and diploid pollen tubes in tetraploid styles.

It also seems possible that these small triploid seeds would be useful material for growth rate experiments. Fabergé (1936) found that tetraploid seedlings of *Lycopersicum esculentum* did not maintain their initial larger embryo size over the diploid. It would be interesting to see how far this initial very low embryo weight of the triploids showed itself in the size of the seedlings.

### 3. ABNORMAL SEEDS

#### (a) *Small seeds from diploid selfed*

When examining fruits from diploid selfed pollinations a few very small seeds were found. These were nearly the same size as those obtained from the cross tetraploid female × diploid male. They were not shrivelled seeds but were quite full and round. There were no intermediates (one exception, see Table IV) between these very small seeds and the normal size seed of the diploid. It was suspected that these small seeds were triploids.

The seeds were weighed (see Table IV) and germinated on moist filter paper in Petri dishes. Root tips were taken and fixed. The chromosome numbers given in the table are only accurate to  $\pm 1$ , i.e. whether the plants were about diploid or about triploid.

It will be seen from Table IV that if we exclude the one medium-sized seed there are eleven small seeds, and of these six are diploid and five triploid. It seems most probable that these triploid seeds are produced by diploid embryo-sacs being fertilized by haploid pollen. If this is so, it is then very interesting to note that these triploid seeds are of the same size as those produced from the cross tetraploid ♀  $\times$  diploid ♂, i.e. the mother plant is of little importance and the important relationship is that of embryo: endosperm as suggested by Watkins (1932). The occa-

TABLE IV

*Small seeds from diploid selfed, or diploid  $\times$  diploid*

Parents	Description of seed	Wt. of seed g.	Chromo- some no. $\pm 1$
XVII 24 F $\times$ XVII 21 G	Small, full	0.0022	18
"	"	18	18
"	"	16	27
"	"	12	27
"	"	08	27
XVII 24 F Self	"	0.0018	18
"	"	08	18
"	"	08	27
XVII 24 B (2n parts) Self	Medium, full	0.0030	18
	Small, full	14	18
	"	14	18
	"	12	27
Average weight of small seeds, embryos $2n = 18$ ,		0.00157 g.	
" " $3n = 27$ ,		0.00112 g.	

sional production of diploid embryo-sacs is to be expected since diploid pollen grains are found in *Brassica oleracea* and in most plants. The frequency of occurrence of diploid pollen grains appears to vary considerably, e.g. see Levan (1933) on *Allium oleraceum*.

The occurrence of the seven diploid small seeds is peculiar and their significance unknown. There appears to be no good reason for their occurrence. They are not just extremes in the normal variation of size of diploid *Brassica oleracea* seeds. One possible suggestion is that they are produced by the parthenogenetic development of ovules. This is perhaps unlikely as Noguchi (1928) found that in the cross *B. campestris* female  $\times$  *B. oleracea* male, parthenogenesis did not occur and that matroclinous embryos were produced only by pseudogamy. The size of such matroclinous seeds would be very interesting. The other point

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raised by these small diploid seeds is whether all the small seeds (seven only have been examined) from the cross tetraploid ♀ × diploid ♂ are triploids. Some might be diploids by parthenogenesis—Gairdner & Darlington found one such seed in the cross tetraploid *Campanula persicifolia* × diploid—and others might be tetraploids.

(b) *Two exceptional seeds from diploid ♀ × tetraploid ♂*

Besides the small seeds with no embryos obtained from this cross (see § 2(c)), two large seeds with embryos were obtained. Their weights were 0.0032 and 0.0026 g. They are thus more than twice as heavy as the seeds from the cross  $4n$  ♀ ×  $2n$  ♂. The chromosome numbers of these two plants were found to be  $36 \pm 1$ , i.e. the two seeds are tetraploids.

The obvious explanation of their occurrence is that they were formed by the fertilization of diploid embryo sacs. The occurrence of such embryo-sacs was also seen to occur in § 3(a). These two seeds would have a normal embryo: endosperm relationship and it can be seen from their weights that they have developed to near the normal diploid size. The occurrence of these two seeds also shows that diploid pollen tubes can function in diploid styles.

The two seeds do not weigh as much as normal diploid seeds. This is possibly significant. It may be due to the relations diploid mother plant: hexaploid endosperm: tetraploid embryo. It might also be due to the small numbers of seeds developing in each fruit. It would be interesting to know what size of seeds develop in fruits after pollinations with few pollen grains to produce only one or two seeds per fruit.

(c) *Double seeds*

(i) *Materials and methods.* A few seeds with two embryos were found by searching samples of the seed of different *Brassica* species, but as many as fifty-four were obtained from one special sample of marrow-stem kale (*B. oleracea*) seeds. This special sample was large screenings of seeds obtained from Gartons (of Warrington).

The samples of seeds were searched while still in the dry condition and also without the help of any magnifier. The seeds with two embryos are fairly obvious (see Fig. 2).

The seeds were germinated in Petri dishes on moist filter paper and then planted in 4 in. pots. After germination it was noted whether the two seedlings were of equal size and vigour or whether one was larger and stronger than the other (in one case it was noted that both seedlings were peculiar).

No root-tip counts were made. The occurrence of plants other than diploids was searched for when the plants flowered by examining their pollen. Tetraploids should have larger pollen than diploids; triploids and haploids should have considerably less than 98% good pollen. The

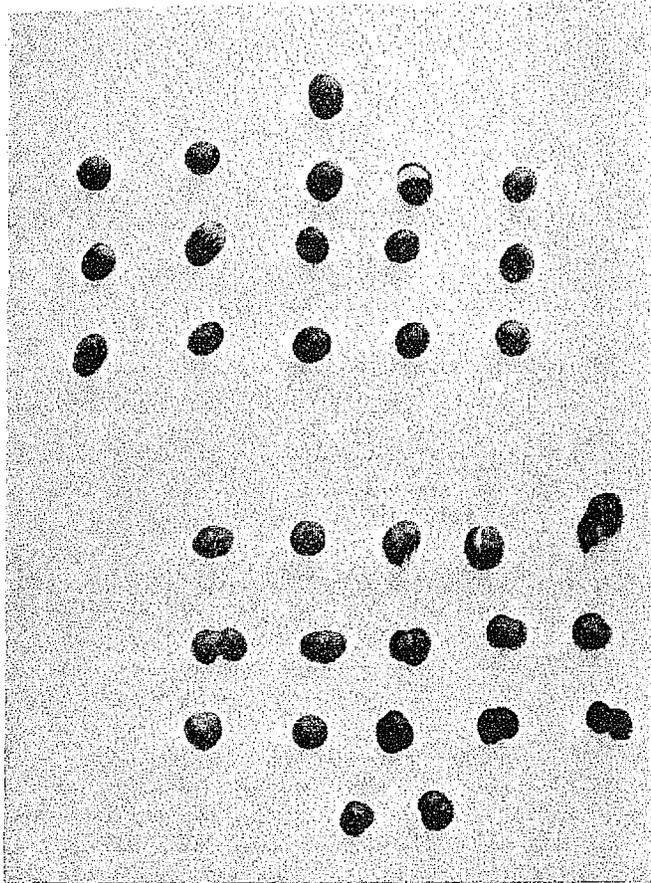


Fig. 2. Marrow-stem kale seeds (on moist filter paper for 1 day). (a) Top collection, single seeds. (b) Bottom collection, double seeds.

chromosome numbers of plants with more than 2% bad pollen were determined from temporary aceto-carminic smears of pollen mother cells.

It was possible to recognize two types of double seeds. One of these types, type A, appears to be the result of the fusion of two ovules during development (alternatively from a fission of one ovule into two). Good examples of type A ovules are to be found in Fig. 2:—e.g. second row of

double seeds, first seed on the left; third row of double seeds, last seed on the right. Other examples are the three double seeds on the left-hand side in Fig. 1. The other type of double seed, type B, is not so obviously double and looks as if it might originate from two embryo-sacs developing in the same ovule. Most of the double seeds in Fig. 2 are of this type. Also the double seed in the extreme right in Fig. 1 is of type B.

Type A double seeds are obviously formed from the fusion of two ovules (alternatively from a fission of one ovule into two) as is shown in Diagram 2, type 1. It was thought that type B double seed might result

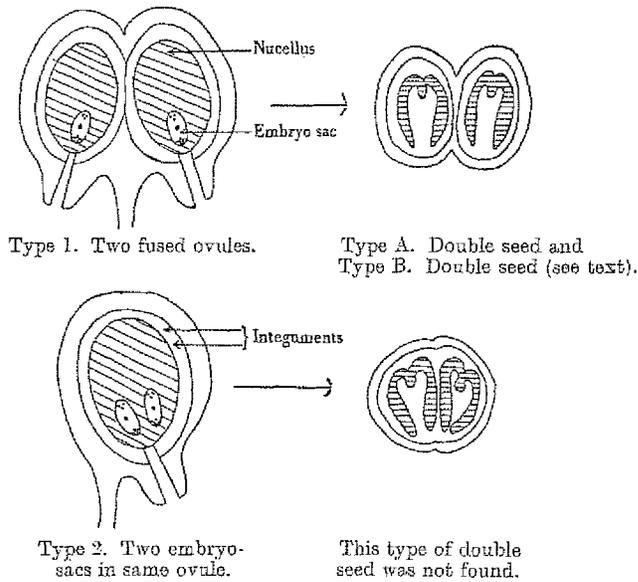


Diagram 2. Suggested origin for two different types of double seeds.

from two embryo-sacs developing in one ovule as shown in type 2 of Diagram 2. However, I found two double seeds of type B when examining the green fruits of *Raphano-Brassica* amphidiploids. These two seeds were embedded and sectioned. Fig. 3 is a drawing of a section through one of these seeds. It can be seen from the structure of the ovule walls that this seed results from the fusion of two ovules (or alternatively from the fission of one). There is a separate "pigmented" layer around each ovule. (A good description of the structure of the integuments of *Brassica oleracea* ovules is to be found in Thompson (1933).) It thus seems that both type A and type B double seeds originate from fused ovules, and that neither type is formed from two embryo-sacs in the same ovule.

Three double seeds were also obtained from the thousand-head kale plants of the stock used for the diploid-tetraploid investigations. One of these came from a tetraploid. It consisted of one large tetraploid embryo and a shrivelled seed. The double seed weighed 0.0074 g., i.e. it was much heavier than a normal tetraploid seed, although it contained only one embryo. The weights of two double seeds from the diploids, both of which contained two diploid embryos each, were 0.0060 and 0.0056 g. They are thus intermediate between the weight of a single seed and the combined weight of two seeds.

(ii) *Occurrence of one diploid-tetraploid seed.* A sample of the large screenings from marrow-stem kale seeds was obtained from Gartons (of

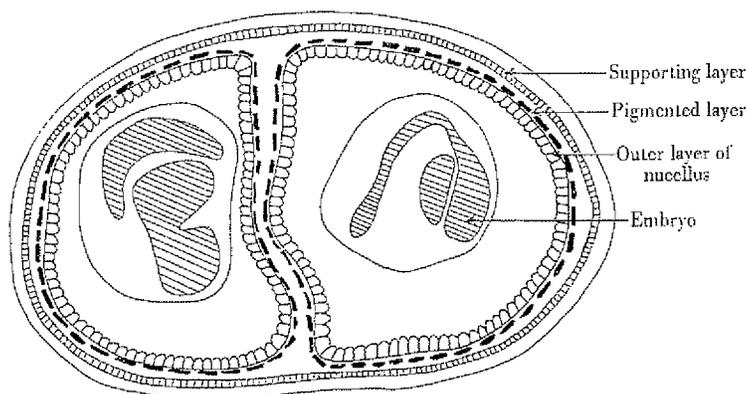


Fig. 3. Section through a double seed from a *Raphano-Brassica* amphidiploid. Both the supporting layer and the pigmented layer are parts of the integuments of the ovules (see Thompson, 1933).

Warrington). This sample was found by weighing the whole sample and three small samples from it of 500 seeds each to contain about 39,800 seeds. From the sample fifty-four double seeds were obtained, i.e. an average of 1.35 per 1000 seeds.

Fifty-four seeds were germinated and of these fourteen had one embryo either bigger or more vigorous than its twin. Of these 108 embryos, two died as seedlings and one was a very weak plant which was kept in a pot inside the greenhouse. The remaining 105 seedlings were planted outside in the autumn of 1937. Three of these were found to be missing in the spring of 1938. None of these three missing plants had been classified at time of germination as smaller or weaker than their twins. The remaining 102 plants all flowered in the spring of 1938.

Of these 102 plants all were diploids except one plant. This plant, 27 a, was a tetraploid. Its pollen was measured as  $11 \times 9$  in arbitrary units

as compared with the diploid size of  $9 \times 8$  in the same units. The tetraploid thousand-head kales had pollen of the  $11 \times 9$  size. The chromosome numbers of the two plants, 27*a* and 27*b*, were determined from smears of pollen mother cells. They were found to be 36 and 18 respectively. At the time of germination no difference in size or of vigour was noted between the two embryos. In other cases the more vigorous embryo was always labelled *a* and the smaller *b*. The one plant kept in the greenhouse did not flower.<sup>1</sup> All root tips taken from it were found to be diploid.

The one tetraploid embryo may have originated in two ways (*a*) fertilization of diploid embryo-sac by diploid pollen, (*b*) doubling of zygote nucleus or a nucleus of young embryo. The two seeds described in § 3 (*b*) suggest that hypothesis (*a*) is a reasonable one.

(iii) *Occurrence in Brassica species other than oleracea.* Five double seeds have been examined from other varieties of *B. oleracea* than those described above. All ten embryos were diploid. Two double seeds were found in *B. chinensis*; all four embryos were diploids.

One double seed was found in a very large sample of swede, *B. Napus*, seed. The larger embryo was diploid. The weaker one died before its chromosome number could be determined. It is interesting to note that Morinaga and Fukushima (1932) found haploid *B. napella* (variety of *B. Napus*) plants growing in crops. Perhaps these came from double seeds, as Harland (1936) found in sixteen seeds of Sea Island Cotton (an allopolyploid like *B. Napus*), two pairs of embryos which were diploid: diploid and fourteen pairs which were diploid: haploid. Harland also stated that haploids are very rare in seeds with one embryo only.

Four double seeds were also obtained among the seeds from a cross of *B. chinensis* ( $n = 10$ )  $\times$  *B. carinata* ( $n = 17$ ). Two were of type A and two of type B, see Diagram 2. All eight embryos were  $F_1$  hybrids with 27 chromosomes. This would be expected, if type B double seeds are fused ovules and capable of fertilization by two pollen tubes. If they arose from one ovule, we should have to conclude that one embryo had split into two very early in development as in identical twins in animals.

The differences in the occurrence of embryos other than diploids between the double seeds of *Brassica* and those of *Gossypium* (Harland, 1936) and of various members of the Gramineae (Müntzing, 1938) where triploids are very common, is very easily explained. The double seeds in *Brassica* examined in this investigation come from fused ovules. The seeds with two embryos in the other investigations are formed from the development of two embryo-sacs in the same ovule, Müntzing (1938).

<sup>1</sup> This plant flowered in April 1939 and was found to be a diploid.

Perhaps there do also occur in the genus *Brassica* seeds with two embryos formed from one ovule. These may be less obvious than the two-ovule type and perhaps only found by germinating large numbers of seeds.

#### 4. DISCUSSION

##### (a) *Size of embryos and seeds in other genera*

Müntzing (1938) states "... the twins with the deviating chromosome numbers are almost exclusively found among the so-called *b*-plants, representing those members of the twin pairs which are weak at the seedling stage". The plants with the deviating chromosome numbers which he found were seventy-seven triploids, eleven haploids, two tetraploids (and five other deviations, these five all being in *Poa pratensis*). This fits in with the *Brassica* data given here as far as triploids and haploids are found. These are expected to be smaller than diploids. But it may be that Müntzing, in only examining in his later work twin plants which are weaker than normal, is missing many tetraploids. This would certainly have been the case with the one tetraploid found in the *Brassica* double seeds.

Harland (1936) also found that one of the twins in the Sea Island Cotton seeds was very small. This is presumably the haploid. The haploid is expected to be smaller than the diploid embryo because it has a lower chromosomes number, i.e. in a similar manner to the expected "gigas" characters in autotetraploids. But the very small triploid seeds found in *Brassica oleracea* suggest that the small size of a haploid embryo will be accentuated by the abnormal embryo: endosperm relationship. As is shown in Diagram 1, the relation haploid embryo : diploid endosperm and triploid embryo : pentaploid endosperm are very similar. This also suggests that it might be possible to obtain haploids by searching the fruits of diploids for rare very small but quite full (and not shrivelled) seeds.

##### (b) *Size of seeds in species crosses*

Watkins (1932) was led to make his summary of the importance of embryo-endosperm relations in the development of seeds by his investigations in crossing tetraploid and hexaploid wheats. While he did find in his summary of the problem that his conclusions could often be extended to species crosses, it seems to the present author a rather dangerous procedure. The first reason is that when we are dealing with species crosses there are differences in kinds of genes not as with diploids and their autopolyploids where it is numbers of genes of the same kind only. Secondly, many allopolyploids have probably evolved to be similar to

the diploids from which they originated, e.g. in cell size (see Darlington, 1937, p. 224).

As an example of the above difficulties there is a good case in the genus *Brassica*. *B. chinensis* ( $n=10$ ) and *B. carinata* ( $n=17$ ) produce large numbers of quite good seeds whether the cross is made *B. chinensis* as female parent or with *B. carinata* as female parent. The seeds from the cross are very much the same size (very much smaller than both parents) whichever way the cross is made. A difference in chromosome number thus appears to be of no importance. It is also interesting to note that *B. carinata* has a pollen grain size only just larger than that of *B. chinensis*. Also the seeds from the cross *B. pekinensis* ( $n=10$ —a species very similar to *B. chinensis* (see Richharia, 1937)) female and *B. carinata* are very shrivelled and contain no embryos or very small ones. A genetic difference is here therefore of more importance than one of chromosome number.

(c) *Explanation of differences in seed size*

It is not possible to suggest why the abnormal embryo: endosperm relationship should produce the very small triploid seeds when tetraploid female is crossed by diploid male. Preliminary observations do not suggest that in *Brassica oleracea* it is due to a slower rate of cell division in the earlier stages. Embryos 13 days after pollination appear to be of the same size as judged by number of cells whether they are formed in fruits obtained by selfing diploids, by selfing tetraploids or by crossing tetraploid female by diploid male. It is also not known whether any triploid embryos at all are formed in the cross diploid ♀ × tetraploid ♂.

It is not, however, surprising to find that a change in the ratio of genes present in the endosperm to genes present in the embryo should have such a large effect on the development of the seed. Lawrence & Scott-Moncrieff (1935), for instance, showed very clearly that there was marked interaction and competition of the genes for flavone and anthocyanin production in *Dahlia* varying with the numbers of genes present.

## 5. SUMMARY

1. The mean weights of single *Brassica oleracea* seeds were:
  - (a) Diploids, 0.00408 g.
  - (b) Tetraploids, 0.00534 g.
  - (c) Triploids from cross tetraploid ♀ × diploid ♂, 0.00120 g.
  - (d) "Seeds" with no embryos from diploid ♀ × tetraploid ♂, 0.00046 g.

2. The very small size of the triploid seeds from the cross tetraploid female  $\times$  diploid male is caused by the abnormal endosperm: embryo relationship.

3. 11 very small seeds (weights from 0.0022 to 0.0008 g.) were obtained among the normal seeds from diploid selfs and diploid  $\times$  diploid pollinations. Six of these seeds were diploids and five triploids. It is suggested that the triploids were produced from diploid embryo-sacs fertilized by haploid pollen. Their size (average weight 0.0011 g.) is very similar to the size of triploids obtained in the cross tetraploid  $\text{♀} \times$  diploid  $\text{♂}$ . This suggests that the important relationship is embryo: endosperm and not a relationship involving the mother plant.

4. Besides the very small "seeds" from the cross diploid  $\text{♀} \times$  tetraploid  $\text{♂}$  which contained no embryos, there were two larger seeds (weights 0.0032 and 0.0026 g.). Both these seeds contained tetraploid embryos. They were presumably produced from diploid embryo-sacs fertilized by diploid pollen. The large size of these seeds again suggests that the important relationship is embryo: endosperm and that relationships involving the mother plant are of minor importance.

5. Fifty-four seeds were obtained in a sample of marrow-stem kale seeds. They are probably all formed from fused ovules. One embryo was found to be tetraploid. This embryo was the same size as its sister diploid embryo. All the other embryos which grew into large plants (104 out of 107) were diploids.

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