

# SEED SIZE IN CROSSES BETWEEN DIPLOID AND AUTO-TETRAPLOID *NASTURTIUM OFFICINALE* AND ALLOTETRAPLOID *N. UNISERIATUM*

BY H. W. HOWARD, *Plant Breeding Institute, School of Agriculture, Cambridge*

(With Four Text-figures)

## 1. INTRODUCTION

In two previous papers (Howard, 1939, 1942) it was found in the genus *Brassica*, first, that in certain cases large numbers of good seeds could be obtained from crosses of the type tetraploid female  $\times$  diploid but not from those of the type diploid female  $\times$  tetraploid, and secondly, that triploid seeds from such crosses were only about 20 % of the weight of normal tetraploid seeds. These two results were explained by reference to the embryo : endosperm genom ratios (cf. Watkins, 1932). In the present paper a somewhat similar case to those previously studied in the genus *Brassica* is described for *Nasturtium* (watercress), another genus of the family Cruciferae.

## 2. MATERIAL

There are two distinct species of watercress: *Nasturtium officinale* R.Br., which has a somatic chromosome number of  $2n=32$ , and *N. uniseriatum* Howard & Manton, which has a somatic chromosome number of  $2n=64$ . This latter species is an allotetraploid, one of whose parent species is *N. officinale* (Howard & Manton, 1946). The two species can be easily distinguished by their fruits (see Fig. 1) and by their seeds (see Fig. 2). *N. officinale*

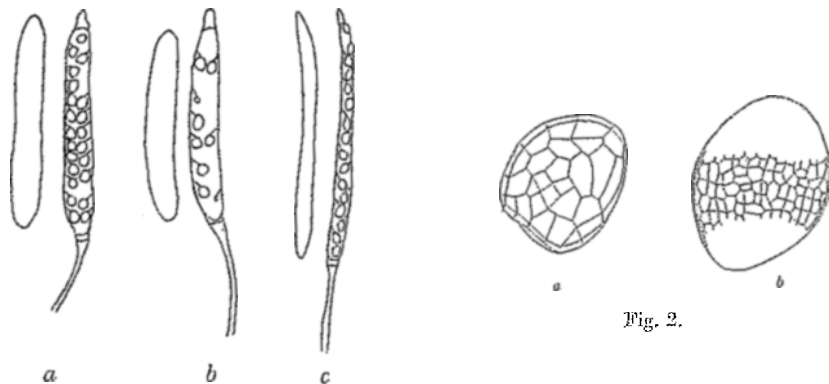


Fig. 1.

Fig. 1. Fruits of (a) diploid *N. officinale*, (b) autotetraploid *N. officinale* and (c) *N. uniseriatum*. (Drawn from photographs taken by Dr I. Manton.)

Fig. 2. Seeds of (a) *N. officinale* and (b) *N. uniseriatum*. (The autotetraploid *N. officinale* is similar to its diploid.)

has relatively broad fruits with biseriate arrangement of seeds, while *N. uniseriatum* has narrow fruits with more or less uniseriate arrangement of seeds. The seeds differ in the number and size of the alveoli on their testas.

## 112 Crosses between diploid, autotetraploid and allotetraploid *Nasturtium*

In addition to the two species of watercress there was also used in the crossing experiments an autotetraploid which had been produced by colchicine treatment from normal diploid *N. officinale*. A description of this autotetraploid has been given by Howard & Manton (1946).

### 3. COMPATIBILITY AND FERTILITY OF THE CROSSES

Both *N. officinale* and *N. wisneriatum* are self-compatible and seeds are easily obtained by selfing. The autotetraploid *N. officinale* is also self-compatible, but like all autotetraploids it has a reduced seed fertility which in this case is about 44 % of the diploid. This is a higher fertility than that found in autotetraploids of *Brassica oleracea*, *Datura Stramonium*, *Gossypium arboreum* and *Nasturtium wisneriatum*, but lower than that found in autotetraploids of *Hordeum sativum* and *Zea Mays* (see Table 1).

Table 1. *The seed fertilities of some representative autotetraploids*

Species	Reference	Relative seed fertility*		% seed fertility $4x/2x$
		$2x$	$4x$	
<i>Datura Stramonium</i>	Blakeslee, Belling & Farnham (1923)	352.6	70.2	20
<i>Lycopersicum esculentum</i>	Sansome (1933)	—	—	20
<i>Nasturtium wisneriatum</i>	Howard (unpub.)	39.0	6.2	21
<i>Gossypium arboreum</i>	Stephens (1942)	18	4	22
<i>Brassica oleracea</i>	Howard (1939)	43	15	36
<i>Nasturtium officinale</i>	Howard & Manton (1946)	25	11	44
<i>Hordeum sativum</i>	Karpechenko (1938)	19.6	11.3	58
<i>Zea Mays</i>	Randolph (1935)	—	—	80 to 95

\* Seeds per fruit (or per spike in *Hordeum*), except for *Brassica oleracea*, in which it is number of good seeds per 100 ovules.

The results of all the nine possible selfs and crosses between diploid *Nasturtium officinale*, autotetraploid *N. officinale* and allotetraploid *N. wisneriatum* are given in Table 2 and illustrated in Figs. 3 and 4. In all cases good development of the fruit and of a high

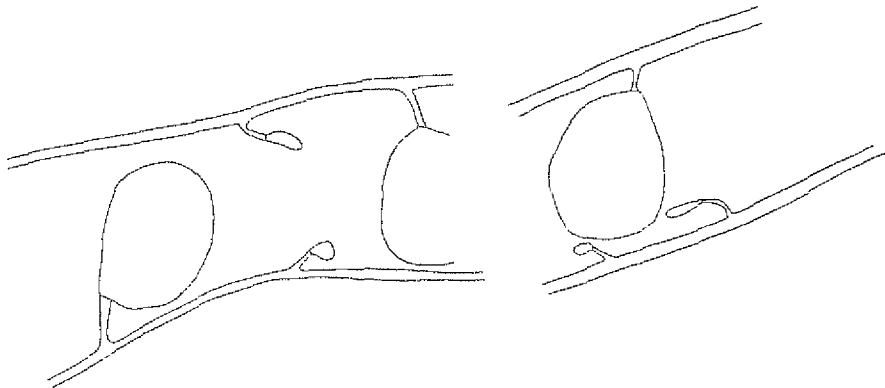


Fig. 3. Two portions of a fruit from the cross diploid *N. officinale* female  $\times$  *N. wisneriatum*. At the same magnification as Fig. 4. Note the very small size of undeveloped ovules.

percentage of the ovules takes place. This must mean that pollen-tube growth and fertilization plus some development of the embryo and endosperm occurs in all types of pollination. Good seed, however, is only produced by the three selfings and by the two crosses, autotetraploid *N. officinale* female  $\times$  *N. wisneriatum* and *N. wisneriatum* female  $\times$  diploid

*N. officinale*. The other four crosses produce empty 'seeds' which are large in the two crosses, diploid *N. officinale* female  $\times$  *N. uniseriatum* and *N. uniseriatum* female  $\times$  autotetraploid *N. officinale*, and small in the two crosses diploid *N. officinale  $\times$  autotetraploid and its reciprocal. As can be seen from a comparison of Figs. 3 and 4, even the small*

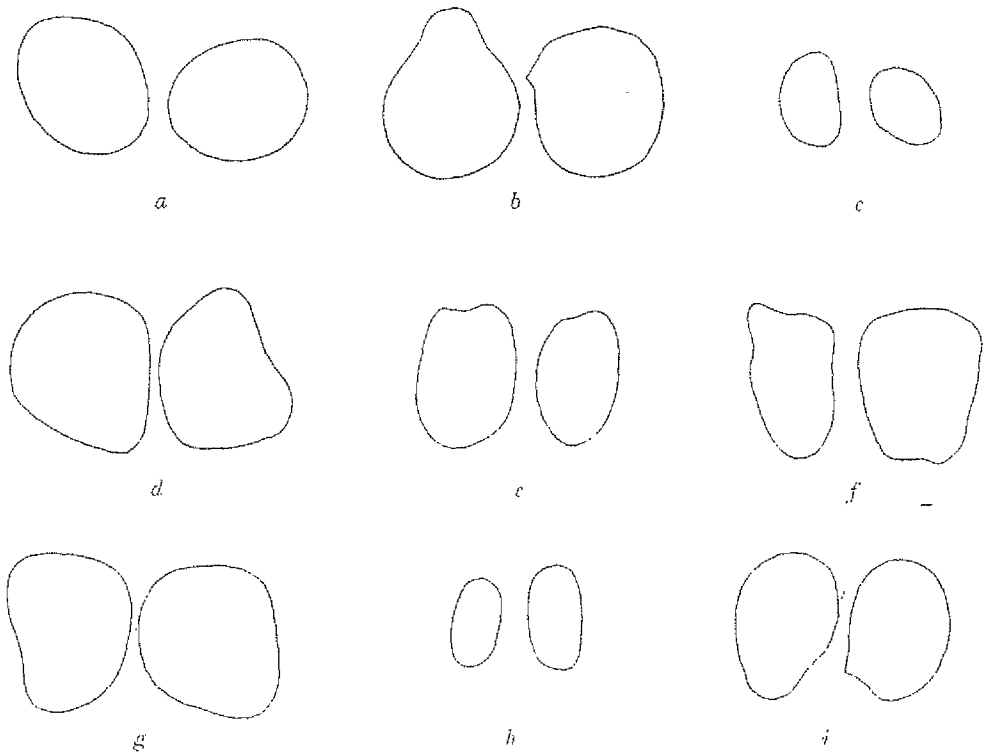


Fig. 4. Seed sizes. (a) Diploid *N. officinale* self. (b) and (c) Diploid *N. officinale* female  $\times$  *N. uniseriatum* and autotetraploid *N. officinale* respectively. (d) *N. uniseriatum* self. (e) and (f) *N. uniseriatum* female  $\times$  diploid and autotetraploid *N. officinale* respectively. (g) Autotetraploid *N. officinale* self. (h) and (i) Autotetraploid *N. officinale* female  $\times$  diploid *N. officinale* and *N. uniseriatum* respectively.

empty seeds represent considerable growth of ovules, and the large empty seeds are as big as normal seeds obtained by selfing. An explanation of these crossing results is given in the last part of this paper.

Table 2. Results of the crossing experiments with the various types of watercress

Female parent	Male parent	Result per fruit	Average seed weight mg.
Diploid <i>N. officinale</i>	Diploid <i>N. officinale</i>	26 large good seeds	0.235
Diploid <i>N. officinale</i>	<i>N. uniseriatum</i>	14 large empty seeds	—
Diploid <i>N. officinale</i>	Autotetraploid <i>N. officinale</i>	9 small empty seeds	—
<i>N. uniseriatum</i>	<i>N. uniseriatum</i>	29 large good seeds	0.208
<i>N. uniseriatum</i>	Diploid <i>N. officinale</i>	9 small good seeds	0.075
<i>N. uniseriatum</i>	Autotetraploid <i>N. officinale</i>	12 large empty seeds	—
Autotetraploid <i>N. officinale</i>	Autotetraploid <i>N. officinale</i>	11 large good seeds	0.324
Autotetraploid <i>N. officinale</i>	Diploid <i>N. officinale</i>	22 small empty seeds	—
Autotetraploid <i>N. officinale</i>	<i>N. uniseriatum</i>	15 small good seeds	0.124

## 4. SEED WEIGHTS

The seed weights for watercress were determined by Dr L. C. Luckwill of Aberdeen University using a Fabergé type of torsion balance. The seeds were weighed singly (see Table 3), and the average weights given in Table 2 are from samples of twenty seeds each, chosen at random (only sixteen seeds of *N. uniseriatum* × *N. officinale* were available for Dr Luckwill).

Table 3. *Weights in mg. of single seeds chosen at random*

Diploid <i>N. officinale</i> selfed	Autotetraploid <i>N. officinale</i> selfed	Autotetraploid <i>N. officinale</i> × <i>N. uniseriatum</i>	<i>N. uniseriatum</i> selfed	<i>N. uniseriatum</i> × diploid <i>N. officinale</i>
332	320	155	228	50
208	370	112	213	11
230	330	76	235	105
337	332	100	200	109
211	378	129	190	100
178	220	174	205	89
250	359	101	203	65
251	311	106	183	91
229	204	121	230	82
262	325	96	231	59
249	335	123	191	60
221	352	80	206	85
223	327	121	214	54
272	338	93	230	100
231	286	166	193	94
233	336	192	206	51
279	327	90	212	—
225	280	181	203	—
259	305	111	214	—
230	— 350	145	180	—
Mean	324.5	123.6	208.3	75.3
S.D.	22.8	34.4	16.3	26.8
S.D./Mean	9.3 %	27.8 %	7.8 %	35.3 %

(a) *Watercress*. It will be noticed first from Table 3 that the autotetraploids are considerably larger than the diploids and that only two of the autotetraploid seeds fall in the range of the diploids (i.e. the seeds weighing 220 and 280 mg. respectively). It is also possible that the small autotetraploid seed weighing 220 mg. was not that of a true autotetraploid but of an embryo with 63 or 65 chromosomes.

Secondly, it can be seen that the two hybrid types of seed are small and that even the largest of the hybrid seeds are not as large as the smallest of the seeds from the respective female parents selfed.

Thirdly, the standard deviations are of some interest. It can be seen from Table 3 that the seeds from all three self-pollinations give similar values for the percentage standard deviation of the mean seed weight, but that the two types of hybrid seeds give considerable higher values for this ratio. This may be due to an effect similar to that suggested by Fabergé (1936, p. 395) to account for the lower variability of autotetraploids as compared with diploids. During the action of genes determining size, circumstances may arise under which one gene fails to have full effect and under which the normal two would have a more complete effect.

(b) *Autotetraploids*. The seeds of autotetraploid *N. officinale* are considerably larger than the diploid and weigh about 138 % of the diploid. This increase in weight is similar to that found in other autotetraploids as is shown in Table 4. This increase in weight of autotetraploid seeds over diploids has sometimes been explained as being due to the

presence of fewer seeds per fruit in the autotetraploid. A comparison of Tables 1 and 4, however, suggests that this is not so, since an autotetraploid of high fertility such as maize shows as big an increase in size as one of low fertility such as *Lycopersicum*. Also direct experiments with *Brassica oleracea* diploids in which small numbers of seed per fruit were obtained by pollination with few pollen grains showed no increase in seed size for seeds from such fruits (Howard, unpublished).

Table 4. *The seed weights of some representative autotetraploids*

Species	Reference	Seed weights in mg.		
		2x	4x	4x/2x %
<i>Nasturtium officinale</i>	—	0.235	0.324	138
<i>Brassica oleracea</i>	Howard (1939)	4.08	5.34	130
<i>Hordeum sativum</i> var. Kolkhoza	Karpechenko (1938)	57.0	71.5	125
<i>H. sativum</i> var. Colechicum	Karpechenko (1938)	47.0	49.25	105
<i>Linum usitatissimum</i>	Kuhk (1943)	—	—	125-150
<i>Lycopersicum esculentum</i> , line 1, Seeds	Fabergé (1936)	2.44	3.49	143
<i>L. esculentum</i> , line 2, Seeds	Fabergé (1936)	2.67	3.18	119
<i>L. esculentum</i> , line 1, Embryos	Fabergé (1936)	0.97	1.38	142
<i>L. esculentum</i> , line 2, Embryos	Fabergé (1936)	1.00	1.25	125
<i>Petunia nyctaginiflora</i>	Hesse (1938)	0.079	0.124	158
<i>Zea Mays</i>	Randolph (1935)	222	330	149

(c) *Hybrid tetraploids*. In Table 5 the weights of seeds from the cross autotetraploid *N. officinale* female  $\times$  *N. uniseriatum* are compared with those from the two crosses amphidiploid *Brassica chinensis-carinata* female  $\times$  autotetraploid *B. chinensis* and its reciprocal. It will be seen that in the *Nasturtium* cross the seeds are very much more reduced in size than in the two *Brassica* crosses. This can be explained if we assume that allotetraploid *N. uniseriatum* is intermediate between diploid and autotetraploid *N. officinale* in its 'physiology of seed production'. This suggestion will be considered further in the last part of this paper.

Table 5. *Weights of hybrid tetraploid seeds*

Female parent	Male parent	Seed weights in mg.		
		Female	Hybrid	Hybrid/ female %
Autotetraploid <i>Nasturtium officinale</i>	<i>Nasturtium uniseriatum</i>	0.324	0.124	38
Amphidiploid <i>Brassica chinensis-carinata</i>	Autotetraploid <i>Brassica chinensis</i>	5.50	3.45	63
Autotetraploid <i>B. chinensis</i>	Amphidiploid <i>B. chinensis-carinata</i>	4.24	2.75	65

The *Brassica chinensis-carinata* results are from Howard (1942).

(d) *Triploid seeds*. The weights of triploid seeds from crosses of the type tetraploid female  $\times$  diploid are compared in Table 6. It will be seen that the seeds from the cross *N. uniseriatum* female  $\times$  diploid *N. officinale* are not so much reduced in size as those from the *Brassica* crosses. This can again be explained if we assume that allotetraploid *Nasturtium uniseriatum* is intermediate between diploid and autotetraploid *N. officinale* (see the last part of this paper and Table 7) in its 'seed physiology'.

## 5. EXPLANATION OF THE *NASTURTIIUM* RESULTS

As has already been suggested the *Nasturtium* crossing results can be explained if we suggest that *N. uniseriatum* has a 'physiology of seed production' intermediate between those of diploid *N. officinale* and autotetraploid *N. officinale*. This explanation is expanded

116 *Crosses between diploid, autotetraploid and allotetraploid Nasturtium*

in Table 7. In this table it is suggested that a single genom of *N. officinale* has a strength of 1.0 and a single genom of *N. uniseriatum* a strength of 1.41 (cf. Stephens (1942) for *Gossypium* crosses). This strength of 1.41 for a single genom of *N. uniseriatum* is less than a strength of 2.0 which might be expected since *N. uniseriatum* is an allotetraploid, one

Table 6. *Seed weights of triploid seeds*

Female parent	Male parent	Seed weights in mg.		
		Female	Hybrid	Hybrid/ female %
<i>Nasturtium uniseriatum</i>	<i>Nasturtium officinale</i>	0.208	0.075	36
Autotetraploid <i>Brassica oleracea</i>	<i>Brassica oleracea</i>	5.34	1.20	22
Amphidiploid <i>B. chinensis-carinata</i>	<i>B. chinensis</i>	5.50	0.775	14
Amphidiploid <i>B. chinensis-carinata</i>	<i>B. carinata</i>	5.50	1.40	25

The *Brassica oleracea* result is from Howard (1939) and the *B. chinensis-carinata* results from Howard (1942).

of whose parent species is *N. officinale* (Howard & Manton, 1946). This lower strength may be due to *N. uniseriatum* having evolved since its production part way to the diploid condition (cf. Darlington, 1937, p. 225 and also Howard, 1942, p. 110 and discussion). It might, however, be due to the strength of the other parent species of *N. uniseriatum* (this other parent is not known) having a low strength of genom for seed formation. The first alternative seems more reasonable.

Table 7. *Endosperm: embryo ratios for watercress selfs and crosses*

Female parent	Male parent	Embryo	Endosperm	Endosperm/ embryo ratio	Seed result
2x <i>N. officinale</i>	2x <i>N. officinale</i>	2x = 2.0	3x = 3.0	1.50	Large, full
2x <i>N. officinale</i>	<i>N. uniseriatum</i>	$x + y = 2.41$	$2x + y = 3.41$	1.41	Large, empty
2x <i>N. officinale</i>	4x <i>N. officinale</i>	3x = 3.0	4x = 4.0	1.33	Small, empty
<i>N. uniseriatum</i>	<i>N. uniseriatum</i>	2y = 2.83	3y = 4.24	1.50	Large, full
<i>N. uniseriatum</i>	2x <i>N. officinale</i>	$y + x = 2.41$	$2y + x = 3.83$	1.59	Small, full
<i>N. uniseriatum</i>	4x <i>N. officinale</i>	$y + 2x = 3.41$	$2y + 2x = 4.83$	1.42	Large, empty
4x <i>N. officinale</i>	4x <i>N. officinale</i>	4x = 4.0	6x = 6.0	1.50	Large, full
4x <i>N. officinale</i>	2x <i>N. officinale</i>	3x = 3.0	5x = 5.0	1.67	Small, empty
4x <i>N. officinale</i>	<i>N. uniseriatum</i>	$2x + y = 3.41$	$4x + y = 5.41$	1.58	Small, full

The value of 1.414 for the strength of a genom of *N. uniseriatum* was obtained from a consideration of the small full seeds from the two crosses *N. uniseriatum* female  $\times$  diploid *N. officinale* and autotetraploid *N. officinale* female  $\times$  *N. uniseriatum*. As is shown in Tables 5 and 6 these seeds weigh 36 and 38 % respectively of the selfed seeds from the female parents. Considering seed development to be determined only by embryo : endosperm relations (reasons for this being considered the most important relation in crosses where pollen tube growth is normal have been given by Watkins (1932) and Howard (1939)), the endosperm : embryo ratios for the two crosses were equated and the value of  $y$  in terms of  $x$  found. The calculation is from the equation  $2y + x : y + x = 4x + y : 2x + y$ . This gives  $y^2 = 2x^2$ .

An examination of Table 7 shows that large full seeds are obtained when the endosperm : embryo ratio equals 1.50, small full seeds when the ratio equals 1.58 or 1.59, large empty seeds when the ratio equals 1.41 or 1.42, and small empty seeds when the ratio departs most from the normal one of 1.50 to 1.33 and 1.67 respectively. Thus the suggestion that *N. uniseriatum* has a seed physiology intermediate between diploid *N. officinale*

( $x=1$ ) and autotetraploid *N. officinale* ( $n=2x=2$ ) does give a comprehensive explanation of all the *Nasturtium* results.

Finally, one may make a further comparison of the *Nasturtium* and *Brassica* results. In *Brassica* small, full seeds are obtained from crosses of the type tetraploid female  $\times$  diploid (i.e. with endosperm : embryo ratios of 1.67 : 1), and empty seeds from crosses of the type diploid female  $\times$  tetraploid (i.e. with ratios of 1.33 : 1). The *Brassica* results thus agree with the *Nasturtium* results as far as the crosses of the type diploid female  $\times$  tetraploid are concerned but only partly so when the reciprocal crosses are considered. It is, however, important to note first that the small good seeds in *Nasturtium* have a ratio of 1.58 : 1 which is on the same side of 1.50 : 1 as the *Brassica* ratio of 1.67 : 1, and secondly, that the small full seeds in *Nasturtium* (both triploid and hybrid tetraploid) are relatively heavier than the corresponding triploid *Brassica* seeds (see Tables 5 and 6).

#### 6. SUMMARY

1. Crosses between diploid *Nasturtium officinale*, allotetraploid *N. uniseriatum*, and autotetraploid *N. officinale* were studied for seed development and seed size.

2. Autotetraploid *N. officinale* has a seed fertility of 44 % of the diploid, and its seed weight is 138 % of the diploid.

3. The three self-pollinations produce large full seeds; the two crosses *N. uniseriatum* female  $\times$  diploid *N. officinale* and autotetraploid *N. officinale* female  $\times$  *N. uniseriatum* small full seeds; the two crosses diploid *N. officinale* female  $\times$  *N. uniseriatum* and *N. uniseriatum* female  $\times$  autotetraploid *N. officinale* large empty seeds; and the two crosses diploid *N. officinale* female  $\times$  autotetraploid *N. officinale* and its reciprocal small empty seeds.

4. The seed results in the crossing experiments are explained by reference to the endosperm : embryo relations.

5. It is suggested that the genom of *N. uniseriatum* (32 chromosomes) has a strength of 1.41 as compared with 1.00 for *N. officinale* (16 chromosomes) and not of 2.00 as might be expected in a tetraploid.

#### REFERENCES

- BLAKESLEE, A. F., BELLING, J. & FARNHAM, A. (1923). Inheritance in tetraploid *Daturas*. *Bot. Gaz.* **76**, 329-73.
- DARLINGTON, C. D. (1937). *Recent Advances in Cytology*, 2nd. ed. London.
- FABERGÉ, A. C. (1936). The physiological consequences of polyploidy. I. Growth and size in the tomato. II. The effect of polyploidy on variability in the tomato. *J. Genet.* **33**, 365-97.
- HESSE, R. (1938). Vergleichende Untersuchungen an diploiden und tetraploiden *Petunien*. *Z. indukt. Abstamm.- u. Vererb. Lehre*, **75**, 1-23.
- HOWARD, H. W. (1939). The size of seeds in diploid and autotetraploid *Brassica oleracea*. *J. Genet.* **38**, 325-40.
- HOWARD, H. W. (1942). The effect of polyploidy and hybridity on seed size in crosses between *Brassica chinensis*, *B. carinata*, amphidiploid *B. chinensis-carinata* and autotetraploid *B. chinensis*. *J. Genet.* **43**, 105-19.
- HOWARD, H. W. & MANTON, I. (1946). Autopolyploid and allopolyploid watercress with the description of a new species. *Ann. Bot., Lond., N.S.*, **10**, 1-13.
- KARPECHENKO, G. D. (1938). New tetraploid barleys—the hulled and the naked. *C.R. Acad. Sci. U.R.S.S.* **21**, 59-62.

- KUITE, R. (1943). Vergleichende Untersuchungen an di- und tetraploiden Lein (*Linum usitatissimum*).  
*Z. Pflanzenz.* 25, 92-111.
- RANDOLPH, L. F. (1935). Cytogenetics of tetraploid maize. *J. Agric. Res.* 50, 591-605.
- SANSONE, F. W. (1933). Chromatid segregation in *Solanum Lycopersicum*. *J. Genet.* 27, 105-32.
- STEPHENS, S. G. (1942). Colchicine-produced polyploids in *Gossypium*. I. An autotetraploid Asiatic  
cotton and certain of its hybrids with wild diploid species. *J. Genet.* 44, 272-95.
- WATKINS, A. E. (1932). Hybrid sterility and incompatibility. *J. Genet.* 25, 125-62.