

POLLEN GERMINATION IN *BRASSICA CHINENSIS* × *RAPHANUS SATIVUS* F<sub>1</sub> HYBRIDS

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IN 1934 plants of *Brassica chinensis* (Chinese cabbage) were pollinated by *Raphanus sativus* (radish). A few seeds were obtained and five hybrid seedlings were raised in 1935—Nos. 1, 2 and 3 from the family *Brassica chinensis* 45 × Radish 39 and Nos. 4 and 5 from *B. chinensis* 44 × Radish 39. It was obvious from vegetative characters, and later from flowers and fruit, that the plants were intermediate between the parents; and chromosome counts confirmed that they were diploid hybrids with 19 somatic chromosomes, that is 10 from *B. chinensis* plus 9 from radish.

At the end of the heterotype division, besides tetrads, a considerable number of dyads was formed. These indicated the occurrence of restitution nuclei and the formation of pollen grains with the unreduced number of chromosomes. This gave the opportunity of studying the behaviour of diploid pollen on diploid styles, and pollination was carried out between the hybrid plants.

Usually pairs of flowers were pollinated on the day of opening. About 24 hours later the pistils were removed and fixed in a mixture of 70 per cent alcohol (100 c.c.) with formalin (7 c.c. of 40 per cent). Longitudinal free-hand sections were cut, stained by warming in lacto-phenol cotton blue, and mounted in lacto-phenol. The pollen grains on the stigma were observed and the numbers of ungerminated and germinated grains counted. The rest of the section—style, and septum in the ovary—also was searched for pollen tubes, but if germination had proceeded so far that tubes were in the ovary their pollen grains were emptied, and thus unstained, and could be counted on the stigmatic papillae amongst the stained unemptied grains.

A few observations were made on fresh pollen. One anther was taken from each of three opening buds and the pollen mixed in a drop of acetocarmine. The counts of large full grains and empty smaller ones (of varied size) gave these results:

Date	Plant No.	Full	Empty	Full grains %
22. vi. 35	1	17	230	6.9
	2	236	598	39.4
25. vi. 35	3	87	606	14.4
	3	144	255	36.2
9. vii. 35	4	12	399	3.0
3. vii. 35	5	110	627	17.5

Thus there was considerable variation from day to day and probably from plant to plant. This also appears from the figures quoted below for actual observations on the stigmas.

Fixation No.	Pollination		Plant No.		Large full	Small and empty	Large full %	Large germinated %	Emptied %
	Date	Hours	♀	♂					
					$F_1 \times F_1$				
207	22. vi	24	1	1	9	62	13	? 11	0
					4	13	24	0	0
					6	32	19	? 50	0
208	22. vi	24	1	2	41	43	49	51	0
					35	37	49	40	0
					93	105	47	39	? 3 No tubes
259	6. viii	25	1	3	18	115	13.5	? 28	0
					5	20	20	0	0
206	22. vi	24	2	1	1	21	5	0	0
					7	118	6	0	0
					81	208	28	? 22	0
202	21. vi	24	2	2	41	62	40	63	0
217	28. vi	24½	2	2	20	16	55	50	0
					25	30	45	32	0
205	22. vi	23½	2	2	23	98	19	26	0
					15	35	30	13	0
					28	70	29	18	0
218	28. vi	24½	2	5	5	55	8	0	0
					12	110	10	17	0
264	7. viii	25	2	4	2	15	12	? 50	0
					0	3	0	0	0
214	27. vi	24	5	2	136	146	48	81	10
					36	42	46	80	3
					73	56	56	90	11
213	27. vi	24	5	5	0	34	0	0	0
					4	53	7	0	0
245	3. vii	26¾	5	5	20	90	18	15	0
					12	106	10	? 8	0
					$F_1 \times$ other plants				
221	28. vi	24½	5	Radish 59	109	—	100	88	11 tubes
					138	—	100	91	11 "
242 a	3. vii	26¾	5	Radish 59	264	—	100	76	26 "
					174	—	100	88	29 "
220	28. vi	24½	5	<i>chinensis</i>	216	—	100	93	? 17 "
					313	—	100	96	6 tubes
243	3. vii	26¾	5	<i>chinensis</i>	159	—	100	77	53 "
					72	—	100	78	25 "
244	3. vii	26¾	5	Swede 44	116	—	100	91	45 "
					214	—	100	80	48 "
219	28. vi	24½	5	Turnip 36	16	41	28	87	15 ? tubes
				× radish 73	2	32	6	100	0
					Other species and crosses × $F_1$				
228	2. vii	26½	Radish 59	5	16	44	27	88	0
					5	49	9	80	0
227	2. vii	26½	Radish 59	2	9	61	13	89	? 22
					4	52	7	75	? 25
225	2. vii	26½	<i>carinata</i> 56	2	4	26	13	50	0
					7	10	41	86	0
226	2. vii	26½	<i>carinata</i> 56	5	28	43	39	82	? 7
					18	174	9	33	0

Fixation No.	Pollination		Plant No.		Large full	Small and empty	Large full %	Large germinated %	Emptied %
	Date	Hours	♀	♂					
Other species and crosses $\times F_1$									
216	27. vi	24½	Swede 44	1	28	42	40	96	37 style
215	27. vi	24½	Swede 44	2	10	7	59	90	11
					132	171	43	77	44 tubes
223	1. vii	24	<i>carinata</i> $\times$ radish) $F_1$	2	74	73	50	91	48 "
					12	39	24	92	25 "
224	1. vii	24½	<i>carinata</i> $\times$ radish) $F_1$	5	8	11	42	88	? 12.5
					10	22	31	100	30
229	2. vii	26	<i>carinata</i> $\times$ cabbage) $F_1$	2	1	24	? 4	0	0
					46	45	50	89	50 tubes
230	2. vii	26	<i>carinata</i> $\times$ cabbage) $F_1$	5	36	51	41	94	64 "
					44	85	34	55	50 "
231	2. vii	26	<i>carinata</i> $\times$ cabbage) $F_1$	Self	45	95	32	62	43 "
					36	73	33	92	22 "
					15	55	21	87	40

At the beginning plant 2 appeared to have more abundant pollen in its anthers than the other plants, and from the counts it seems that this was also connected with a higher proportion of full pollen grains. This plant therefore was used more freely for pollination than the others.

When pollen was fairly abundant only one anther was used on each stigma, but if pollen was not set free easily two or more anthers were used.

*Percentages of full pollen on different dates*

(Extracted from table above)

Plant No.	June				July			August	
	21	22	27	28	1	2	3	6	7
1	—	13, 24, 19, 5, 6, 28	40, 59	—	—	—	—	—	—
2	40	49, 49, 47, 19, 30, 21	48, 46, 56, 43, 50	55, 45	24, 42	13, 7, 13, 41, 50, 41	—	—	—
3	—	—	—	—	—	—	—	13.5 20	—
4	—	—	—	—	—	—	—	—	12.0?
5	—	—	0, 7	8, 10	31, 4	27, 9, 39, 9, 32	18 10	—	—

After pollination within the  $F_1$  family a total of 28 stigmas from 9 combinations—3 selfs and 6 crosses—was examined. On 8 stigmas on which there were very few full grains no full grains germinated. On the others germination varied from 8 to 90 per cent (and the members of each set of two or three agreed reasonably well), but in all except one set the germ tubes were only short and had not entered the papillae, and the grains were still full of protoplasm. On the three stigmas of set 214, plants  $5 \times 2$ , however, 3, 10 and 11 per cent of the germinated

grains were completely emptied of their contents, and a few pollen tubes could be seen in the ovaries. Even the germ tubes of the partially emptied grains looked more normal than those on the other stigmas, on which they were only about as long as the pollen-grain diameter, and many were somewhat twisted.

Thus in general the pollen of these diploid hybrids is not successful on their diploid styles, but occasionally a few grains do germinate better and enter the stigma and style. It might be suggested that perhaps the successful tubes came from pollen grains with approximately the haploid number of chromosomes, but this does not seem to be the only reason, or occasional tubes would have entered other stigmas, as it is likely that the pollen in any one anther would possess a range of chromosome numbers.

In hybrids in which some good pollen is produced it would seem that the failure of these roughly diploid pollen grains to germinate on the diploid stigmas is itself a powerful factor in producing the characteristic sterility of the hybrids, for only occasional pistils would set a few seed even if all were fully pollinated.

Our plants were largely exposed to open pollination. They stood in a group, and bees were frequently observed visiting their flowers. Other species and hybrids of *Brassica* were growing near and may also have contributed pollen. Comparatively few pods were set and most of them did not contain seeds. Rough counts were made on branches picked at random of the number of pods and of the remains of flowers and flower stalks at the end of the season (on 28. viii. 35) when flowering was finished.

Plant	Pods	Flowers	Pods as % of flowers
1	42	310	13.5
2	24	329	7.3
3	24	304	7.9
4	31	320	9.7

If even imperfect pod development is the result of pollen tube growth and perhaps fertilization of at least one ovule, this count would mean that pollen tubes only entered about one stigma in ten as in the experiments described.

In order to obtain further evidence of the viability of their pollen grains and of the suitability of their stigmas for pollen germination, the *chinensis* × radish hybrids were also used as male parents in pollination on other species and hybrids with different chromosome numbers, and were themselves pollinated by their parent species, and also by swede and an  $F_1$  hybrid from turnip × radish.

On Radish 59 pollen from plants 2 and 5 gave a high percentage germination, although the actual numbers were small, but only a few grains in set 227 were doubtfully emptied and only one tube was found nearly as far as the base of a papilla in set 228.

A large proportion of pollen from the same two hybrids also germinated on the stigmas of *B. carinata* 56 ( $2n=34$ ), but only two grains were possibly emptied and no tubes were found in the pistil.

On Swede 44, however, pollen from hybrids 1 and 2 germinated well. About 40 per cent of the germinated grains were emptied of their contents and a number of tubes were found in the stigmas, styles, and ovaries. Later a few more flowers were pollinated and developed small pods in which several ovules had undergone some development, thus indicating again the entrance of the hybrid pollen tubes.

An  $F_1$  hybrid between *B. carinata*  $\times$  radish ( $2n=17+9=26$ ) was pollinated by plants 2 and 5. On three out of four stigmas germination was good, some grains were emptied and one tube was seen well down the ovary.

The same two *chinensis*  $\times$  radish plants were used also to pollinate an  $F_1$  hybrid between *B. carinata* and *B. oleracea* (cabbage). Many grains germinated and a number of tubes were found in stigma and style and ovary.

Thus from the results on swede and the *carinata*  $\times$  radish and *carinata*  $\times$  cabbage hybrids it is obvious that most of the pollen grains of the *B. chinensis*  $\times$  radish hybrids were capable of germination and the production of vigorous pollen tubes. The failure on styles of their own family will be discussed below.

Several crosses showed that other pollen was able to grow in the styles of the *chinensis*  $\times$  radish  $F_1$  hybrids. Plant 5 was pollinated by Radish 59 on two days. On all four stigmas germination was high and many pollen tubes were seen as far as the ovaries. The actual number of pollen grains was much higher than in the reciprocal cross, but even so the difference in pollen tube growth was striking.

Pollination by *B. chinensis* gave a similar result, and a number of tubes were found well down in the ovaries. Swede pollen also germinated freely and the germ tubes were found all the way down into the ovaries.

The failure of the diploid pollen on the stigmas of their own family must then be the result of incompatibility factors or the departure from the usual balance of  $1n$  pollen tubes in  $2n$  styles.

For several reasons it is not likely that incompatibility factors would be involved to such an extent. Our plants of *B. chinensis* and radish

generally have set some seed from artificial self pollination or from bagged inflorescences, and two different plants of *B. chinensis* were parents of these  $F_1$  hybrids. Pollen from the  $F_1$  turnip  $\times$  radish, with the same number of chromosomes but different parent plants, was equally unsuccessful on the  $F_1$  stigmas of *chinensis*  $\times$  radish, in spite of the possible identity of self sterility factors in *chinensis* and turnip (or other species of the  $n=10$  group). In addition it is now being found at Cambridge that tetraploid hybrids from *R. sativus*  $\times$  *B. oleracea* will self quite readily, in spite of the marked self incompatibility of *B. oleracea*.

Normally in radish, pollen grains with 9 chromosomes germinate on, and their germ tubes grow in, styles with 18 chromosomes. Similarly in *B. chinensis* 10-chromosome pollen tubes grow in 20-chromosome styles. In the  $F_1$  hybrids between these species the pollen grains may have from 9 to 19 chromosomes—probably more nearly 19—and they have to germinate on 19-chromosome styles. The balance is disturbed and only a few succeed in entering the papillae and growing. If these few succeed because of low chromosome numbers the theory of Watkins (1932) is fully supported. The progeny, now growing, should then have considerably less than 38 ( $=2 \times 19$ ) somatic chromosomes.

It seems more likely to be the result of ordinary fluctuations—genetic, physiological, or environmental—that produce exceptions to the rule, and some evidence in support of this is appearing from the behaviour of pure species pollen in some other *Brassica* crosses.

With radish ( $2n=18$ ) as female parent and *chinensis*  $\times$  radish hybrid pollen the balance also was unusual, and growth not successful, but with radish pollen ( $n=9$ ) and *chinensis* pollen ( $n=10$ ) on the hybrid stigmas ( $2n=19$ ) the normal relationship was more closely reached, and more pollen tubes entered the stigma, as would be expected on Watkins' view.

When other species are crossed presumably other factors are introduced, and with swede ( $2n=36$ ) pollination was partially successful in both directions. The hybrid pollen did not do well on *B. carinata* styles ( $2n=34$ ), but the pollen tubes were able to grow on  $F_1$  stigmas of *B. carinata*  $\times$  *B. oleracea* ( $2n=26$ ) and of *B. carinata*  $\times$  radish ( $2n=26$ ), so that when species other than the parents of the hybrid are involved the relation between the chromosome numbers of pollen tubes and styles may be upset by other factors.

The interest of these results is that this sterile  $F_1$  produces a large amount of diploid pollen, which is normally functional in several crosses, but is unable to grow except rarely on the stigmas of the diploid  $F_1$  itself. In any genus in which this effect occurs the artificial or natural produc-

tion of polyploids may clearly be restricted. The possibility is not unexpected since Watkins has already pointed out (1932) that it does occur in some genera that diploid pollen, as a result of the disturbance of the normal relation between pollen tube and style, may fail to function on diploids, although it behaves normally on tetraploids. The correctness of this explanation is probably confirmed by the results of Terasawa, who has obtained constant amphidiploid hybrids of this cross, *B. chinensis*  $\times$  *R. sativus* ( $2n=38$ ). His  $F_1$  plants (1932) gave only a few seeds, and thus resembled ours. The amphidiploid  $F_4$  plants were highly fruitful from selfing and gave many seeds: in one experiment (1933) 128 flowers gave 102 seeds. This certainly appears to show that the diploid hybrid pollen did grow more successfully on the pistils of the amphidiploids than on those of the diploids, in accordance with the expectations of Watkins.

#### SUMMARY

Diploid pollen, with 19 chromosomes, from the  $F_1$  hybrid plants ( $2n=19$ ) of *B. chinensis*  $\times$  *R. sativus* usually failed to grow on the  $F_1$  stigmas. It was, however, fully capable of germination on the stigmas of swede ( $2n=36$ ) and of some other crosses. Also pollen of radish, *B. chinensis*, and swede was able to grow on the  $F_1$  stigmas.

The sterility of the  $F_1$  plants is attributed to the alteration of the chromosome balance between pollen and style from the usual  $1n:2n$  to  $2n:2n$ .

The work described was carried out at the Plant Breeding Institute, School of Agriculture, Cambridge.

#### REFERENCES

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