FREQUENCY AND DISTRIBUTION OF SELF-INCOMPATIBILITY ALLELES IN *RAPHANUS RAPHANISTRUM*[1]

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ESTIMATES of the number of S alleles in species of flowering plants with gametophytic self-incompatibility run to several hundreds. PAXMAN (1963), for example, estimated 192 different S alleles in one population of *Trifolium pratense* and 215 in a second population, with lower 5% fiducial limits of 149 and 110 respectively.

In contrast, a study to determine the number of loci controlling self-incompatibility in *Raphanus raphanistrum* (SAMPSON 1964) disclosed a frequent recurrence of certain S alleles in the initial research material. *R. raphanistrum*, a wild radish, has a sporophytic type of incompatibility determined by S alleles at one locus. The allele distribution found in the initial material from two isolated populations in Nova Scotia, Canada and from a third population in Poland are given here, together with the results from sampling a New Brunswick population and a second Polish population for nine S alleles.

Having determined the frequency of nine alleles in the wild, the question arose as to what theoretical frequency to expect. The answer would be simple for species with the gametophytic type of self-incompatibility, where, if N is the number of S alleles, the equilibrium frequency for all alleles (deterministic model) is 1/N. However, in species with the sporophytic type of incompatibility three types of S allele interaction occur (recessive, dominant, independent) and the pollen phenotype appears to be independent of the stigma phenotype, giving nine possible types of alleles each with a different frequency at equilibrium. Four of these types were present among the sampled S alleles of *R. raphanistrum:* pollen recessive–stigma dominant (S_1, S_6, S_{13}); pollen dominant–stigma recessive (S_9, S_{14}); pollen dominant–stigma dominant (S_{11}, S_{12}); pollen dominant–stigma independent (S_2, S_4) (SAMPSON 1964).

The task of writing the comprehensive mathematical formula to determine equilibrium frequencies for the nine possible types of S alleles was not attempted. Rather, the specific type of recessiveness found in the stigma of *R. raphanistrum* was analysed in one computer program, using a deterministic model. The more general type of recessiveness, found in the pollen, was analysed with separate programs.

R. raphanistrum is an annual and thrives on disturbed soil that is open to the sun. It is probably native to the Mediterranean area, but it has been a weed of

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cultivated land throughout Europe for centuries and has been introduced to all other continents. It is established on both sides of North America but not in the interior. Whereas the large populations of Nova Scotia, New Brunswick and New England are pure *R. raphanistrum*, the western populations, extending from California to the Frazer Valley of British Columbia, have been introgressed by genes from the cultivated *R. sativus*.

For an aggressive weed, *R. raphanistrum* has the uncommon combination of annual habit with self-incompatibility and insect pollination. Possibly its seed dormancy, associated with the bony husk that encloses each seed, insures survival during the years when pollination is poor.

MATERIALS AND METHODS

The study began with 19 plants belonging to four families. Each family was grown from the seed of a single silique in the spring of 1961. These siliques had been collected in the wild (field weeds) at Nappan, N.S. (families 1 and 3) and at Sheffield Mills, N.S. (families 4 and 5) by Mr. G. A. MULLIGAN of the Plant Research Institute, Ottawa. Nappan and Sheffield Mills are about 45 aerial miles apart. Population 62 contained ten plants which I transplanted from an area about 30 feet long and 10 feet wide on a roadside near Fredericton, N.B., in July 1962. Fredericton lies about 120 aerial miles west of both Nappan and Sheffield Mills.

Although it may be too idealistic to look for equilibrium frequencies in populations that depend for their sites upon the plow and bulldozer, these three populations may have had 200 to 300 years during which to approach equilibrium. However, botanical records were not kept during the first centuries of settlement. The earliest reference to *R. raphanistrum* in Nova Scotia (locality not given) is in COCHRAN's list (in Haliburton, T.C., 1829, *An Historical and Statistical Account of Nova Scotia*). DR. COCHRAN taught at King's College, Windsor, N.S., about 20 miles from the Sheffield Mills population which today forms part of the largest concentration of the species in Nova Scotia. The National Museum of Canada has a herbarium specimen from Amherst, N.S. (5 miles from Nappan) collected by BALL in 1876. *R. raphanistrum* is listed as a troublesome weed at Fredericton in *Fowler's List of New Brunswick Plants*, 1878.

Of the two wild Polish populations, No. 14 contained six plants grown in the spring of 1962 from seed collected in the Danzig (Gdansk) district, and No. 15 contained ten plants from Warsaw district seed, grown in the fall of 1962. The Polish seed was obtained through the Botanic Garden of the University of Warsaw and the kindness of DR. A. PUTRAMENT. As the siliques were fractured, it was impossible to know whether any two Polish seeds came from the same plant.

The plants were grown in pots in a screened greenhouse. Pollen-stigma compatibilities were observed directly by microscopic examination of stigma squashes (see SAMPSON, 1964, for details). Most of the initial Nova Scotian plants had died by 1962, but the identities of nine alleles were maintained through progeny plants (SAMPSON 1964). Danzig plants were tested for eight of these alleles in both pollen and stigma. The stigmas of plants from Warsaw and Fredericton were tested for eight alleles from Nova Scotia and for S_{14} from Danzig. Their pollen was tested for S_9 and S_{14}, the only two alleles known to be stigma recessives.

S allele frequencies were calculated on the assumption that the 90 chromosomes bearing the *S* locus in the 45 tested plants represented a random sample of the species. However, the 19 Nova Scotian plants came from four seed parents, each of which could contribute the same alleles more than once to its progeny, resulting in a nonrandom sample. Therefore, all but one of the chromosomes with such an allele were sacrificed when calculating the allele frequency.

Some plants could not be tested for all test alleles. When this happened the number of untested chromosomes carrying known alleles other than the test allele was added to the sample size of the test allele. For example, only one Nova Scotian plant was tested directly for S_{14} from Poland, but progeny tests showed that S_{14} could not be identical with any of the 13 Nova Scotian alleles

except S_3, S_7, S_8, or S_{10}. Consequently, at least 11 Nappan chromosomes and at least eight Sheffield Mills chromosomes did not have S_{14}. These adjustments give conservative estimates of allele frequencies, but result in different sample sizes for different alleles. Five percent (.025 and .975 probability) fiducial limits for the allele frequencies were calculated by the method of STEVENS (1942).

Computation of theoretical frequencies of S *Alleles:* An IBM 1620 computer was used to obtain "expected" equilibrium frequencies for 27 theoretical populations to compare with the frequencies observed in the wild populations. Three computer programs were written to describe the mean behavior of stigma recessive alleles and, separately, of pollen recessive alleles together with that of the corresponding dominant alleles. The essentials of the three programs are:

(1) Two alleles, both recessive in the stigma to two other alleles (in *R. raphanistrum* S_9 and S_{14} are both recessive to S_{11} and S_{12} in the stigma) but acting independently of all other alleles; a variable number of other alleles, all independent; no pollen recessives. Equilibrium frequencies were computed for three populations with this program.

(2) No stigma recessive alleles; three pollen recessive alleles, each at a different level of dominance ($S_1 > S_6 > S_{13}$ in *R. raphanistrum*) and these three are recessive to all other alleles; the other alleles act independently of each other and are variable in number. Equilibrium frequencies were computed for nine populations with this program.

(3) Like (2) except that the pollen recessives (4, 6, or 8 in number) belong to one dominance level, that is, to simplify the calculations the recessive alleles are allowed to act independently of each other. Equilibrium frequencies were computed for six populations with four recessive alleles, for four populations with six recessive alleles and for five populations with eight recessive alleles.

The ideal program would treat stigma recessives and pollen recessives simultaneously. However, there are too many unknown variables in *R. raphanistrum* (the numbers of stigma recessive and stigma dominant alleles, for example) to warrant other than the limiting cases that were analysed.

The computations were based on deterministic models that assumed populations of infinite size and nonoverlapping generations. The latter assumption ignores the effects of some delayed germination in *R. raphanistrum*. Computation time was reduced by using the mean frequency of all the alleles at a given dominance level rather than by treating each allele separately. Thus, beginning with arbitrary initial values, the mean frequency of the dominant alleles, the mean frequency of the recessive alleles, and for program (1), the mean frequency of independent alleles were computed from generation to generation, until stable values—the mean equilibrium frequencies—were reached. No selection (in the usual sense) was introduced, the differences in mean frequency between the types of *S* alleles were caused entirely by the mechanism of the mating system.

RESULTS AND DISCUSSION

Nova Scotian populations: The nine Nappan and ten Sheffield Mills plants were selfed and crossed in all but 47 of the 361 possible combinations. Progenies from plants 1-2, 1-3, 3-5, 4-4, 5-2, 5-4 and 5-5 were analyzed also (SAMPSON 1964). Thirteen different *S* alleles were identified in this material and they occupied 31 of the 38 chromosomes. In detail, the genotypes found in family 1 were S_1S_3, S_4S_{13} and, in two plants, S_1S_2. The genotypes of family 3 were S_1S_{13}, S_5S_{13}, S_6S_{13}, S_{6-} and S_{7-}. Family 4 contained one plant that was S_1S_6 and four that were S_{8-}. The genotypes of family 5 were S_9S_{10}, S_9S_{11}, S_9S_{12}, S_9S_{13} and S_{10-}. The 19 plants were tested for activity of 12 alleles in both pollen and stigma. However, S_{13}, which was recessive in the pollen to the other 12 alleles from Nova Scotia, was not discovered until progenies from plants 5-2 (S_9S_{13}) and 3-5 (S_6S_{13}) were studied in

late 1961. Most of the 19 initial plants were dead by then so that S_{13} could have been present on any of the seven unknown chromosomes.

Danzig population: The six plants of family 14 were selfed and crossed in diallel in the spring of 1962. All were self-incompatible and plants 14-3, 14-4 and 14-5 were mutally cross-incompatible (because of S_9). The pollen and stigmas of each were tested for S_1, S_2, S_5, S_6, S_9, S_{11}, S_{12} and S_{13} (Figure 1A, B). Crosses involving S_2, S_6 and S_9 were incompatible. In addition, plants 14-3 and 14-4 gave intermediate reactions with S_6 pollen but this is not accepted as evidence that the two have S_6. These reactions and the unexpected incompatibility of pollen from plants 14-4 and 14-5 on tester plant 7-11 (Figure 1B) were discussed previously (SAMPSON 1964).

Analysis of four progenies from plant 14-1 showed that the S_6 of this plant had

♀ \ ♂		9·11	2·9	2·12	6·6	5(13)	1(13)	11·12	13·13
		7-11	10-12	10-7	11-6	3-1	41-2	7-1	9-12
6·14	14-1	+	+	+	−	+	+	+	+
2−	14-2	+	−	−	+	+	+	+	+
9−	14-3	−	−	+	±	+	+	+	+
9−	14-4	−	−	+	±	+		+	+
9−	14-5	−	−	+	+	+	+	+	+
−−	14-6	+	+	+	+	+		+	+

Ⓐ

♀ \ ♂		1(6)	4·9	9·11	6(13)	2·12	13·13	(13)14
		53-7	16-2	7-11	56-3	10-20	16-4	56-2
1−	15-1	±	+	+	+	+	+	+
9−	15-3	+	−	−	+	+	+	+
6−	15-4	+	+	+	−	+	+	+
6−	15-5	+	+	+	−	+	+	+
6−	15-9	+	+	+	−	+	+	+

Ⓒ

♀ \ ♂		(6)14	2−	9−	9−	9−	−−
		14-1	14-2	14-3	14-4	14-5	14-6
9·13	9-4	+	+	−	−	−	+
9·6	12-1	+	+	−	−	−	+
(9)11	7-11	+	+	+	−	−	+
2·9	10-12	+	−	−	−	−	+
2·11	18-11	+	−	+	+	+	+
6·6	11-6	+	+	+	+	+	+
5·13	3-1	+	+	+	+	+	+
1·12	10-5	+	+	+	+	+	+

Ⓑ

♀ \ ♂		2.12	13.13	6(13)	1(6)	4.9	9.11	(13)14
		10-20	16-4	56-3	53-7	16-2	7-11	56-2
12(14)	62-3	−	+	+	+	+	+	+
12−	62-4	−	+	+	+	+	+	+
13−	62-5	+	−	+	+	+	+	+
6−	62-6	+	+	−	+	+	+	+
1−	62-9	+	+	+	−	+	+	+
13−	62-10	+	−	+	+	+	+	+

Ⓓ

FIGURE 1.—Pollen-stigma compatibility (+ = compatible, i.e., test allele absent; − = incompatible, i.e., test allele present; ± = intermediate) of *Raphanus raphanistrum* plants when crossed with known genotypes. Numbers in the first column and row designate *S* genotypes with the number for each recessive allele in parentheses. Plant code numbers in the second column and row are hyphenated and consist of the family number followed by the individual's number. A.—stigma reactions of Danzig plants; B.—pollen reactions of Danzig plants; C.—stigma reactions of five Warsaw plants; five others (not shown) gave only compatible reactions; D.—stigma reactions of six Fredericton plants; four others (not shown) gave only compatible reactions.

the same dominance relations as S_6 from the two Nova Scotian populations. The other allele of plant 14-1 was designated S_{14} but it could be one of S_3, S_7, S_8 or S_{10} because no testers for these were alive when plant 14-1 flowered.

Fredericton population: The stigmas of ten plants were tested for S_1, S_2, S_4, S_6, S_9, S_{11}, S_{12}, S_{13} and S_{14} (Figure 1D) and the pollen of each was tested for S_9 and S_{14} (plant 53-2). The ten plants proved to be self-incompatible and six plants had five different test alleles on seven chromosomes (Figure 1D).

Each incompatible pollination was repeated and also confirmed by using other test plants when available. Reciprocal crosses showed that S_{12} was active in the pollen and stigma, S_{14} in the pollen only and S_1, S_6 and S_{13} in the stigma only of the Fredericton plants.

Warsaw population: Ten plants were tested for the same nine alleles and with the same test plants as were used for the Fredericton population. Three of the alleles were located in five plants (Figure 1C). Reciprocal tests showed that S_9 was active in both stigmas and pollen, S_1 was active in stigmas only whereas S_6 was active in the stigmas only of two plants but in both pollen and stigma of a third plant. Plant 15-1 gave an inconclusive reaction for S_1 with pollen of plant 53-7 (Figure 1C) but was quite incompatible with the pollen of plant 59-6 (S_1S_6). All ten plants were self-incompatible.

Pollen mixing: Data from Nova Scotian plants, grown from the seed of individual siliques, demonstrated the effectiveness of insects in mixing pollen from several plants. It is assumed that the most frequent allele in a family came from the seed parent, and that the pollen parent contributed any other allele that is present in the same heterozygote with the most frequent allele.

Accordingly, the seed parent of family 1 contributed S_1 together with S_4 or S_{13} and the pollen contributed three alleles. The seed parent of family 3 contributed S_{13} so that S_1, S_5 and S_6 came from pollen parents whereas S_7 could have come from either parent. The seed parent of family 5 contributed S_9 together with the unknown allele of plant 5-3, and the pollen contributed four alleles. Thus in three of the four cases the pollen that fertilized a single flower came from two plants at least. EMERSON (1939) made similar observations on self-incompatible *Oenothera organensis*.

These observations were used to correct for the over-representation of certain chromosomes from the four seed parents. Two chromosomes with S_1 in family 1, two chromosomes with S_3 in family 3, three chromosomes with S_3 in family 4 and three chromosomes with S_9 in family 5 were omitted before calculating allele frequencies.

Observed allele frequencies: Seven of the nine *S* alleles that were tested for most widely occurred in two or more populations. Five were found in both Poland and Canada (Table 1). Because small samples were adequate to show the widespread distribution of these *S* alleles, the total number of *S* alleles in *R. raphanistrum* appears to be relatively small. However, each local population probably has most of the alleles of the species. For example, 13 different alleles, plus the unknown alleles on seven chromosomes, were present in 19 Nova Scotian plants (SAMPSON 1964).

TABLE 1

Occurrence and frequency of nine self-incompatibility alleles in five populations

Allele	Phenotypes		Nappan, N.S.	Sheffield Mills, N.S.	Fredericton, N.B.	Danzig, Poland	Warsaw Poland	Sample size (No. of tests)	Frequency	5% Fiducial limits	
	Stigma	Pollen								Lower	Upper
2	Ind.	Dom.	2	0	0	1	0	80	.038	.008	.110
4	Ind.	Dom.	1	0	0	0	0	73	.014	.0003	.076
11	Dom.	Dom.	0	1	0	0	0	79	.013	.0003	.071
12	Dom.	Dom.	0	1	2	0	0	80	.038	.008	.110
Combined				(8 chromosomes)				312	.026	.011	.050
9	Rec.	Dom.	0	1	0	3	1	79	.063	.021	.138
14	Rec.	Dom.	0	0	1	1	0	71	.028	.003	.095
Combined				(7 chromosomes)				150	.047	.019	.094
2, 4, 11, 12		Dom.									
9 and 14		Dom.		(15 chromosomes)				462	.032	.018	.053
1	Ind.	Rec.	2	1	1	0	1	77	.065	.022	.142
6	Ind.	Rec.	2	1	1	1	3	80	.100	.044	.188
13	Ind.	Rec.	2	1	2	0	0	72	.069	.023	.152
Combined				(18 chromosomes)				229	.079	.047	.121

Ind.: independent. Dom: dominant. Rec.: recessive.

An examination of the frequency of the different classes of alleles shows that the stigma independents S_2 and S_4 had almost the same mean frequency as that of the stigma dominant alleles S_{11} and S_{12}, namely .026. The two stigma recessives had a mean of .047 and the three pollen recessives had a mean of .079 (Table 1). The three-fold difference between the four dominants or independents and the three pollen recessives was highly significant ($t = 3.0$) whereas the mean for the stigma recessives did not differ significantly from the other two means ($t = 1.3$ for both tests). Combining the stigma recessives with the four dominants or independents gave a mean of .032 for these six "pollen dominants", which differed significantly from the mean of the three pollen recessives ($t = 2.8$).

A precise estimate of the total number of S alleles in the species can not be made from these mean frequencies without knowing the proportions of the several classes of alleles in the species. Allele frequencies were computed for theoretical populations in order to learn how change in the proportions of the different types of S alleles altered the expected mean frequencies.

Theoretical allele frequencies: Stigma recessives will be considered first, then pollen recessives. The lower observed mean frequency of the stigma recessives (.047) than that of the pollen recessives (.079) is what one intuitively would expect because the stigma recessives are recessive to only a few other alleles whereas the pollen recessives in *R. raphanistrum* are recessive to most other alleles. Thus the theoretical population structure for stigma recessives is the more complicated, in that it must include variables for independent alleles.

Equilibrium frequencies of stigma recessive alleles were obtained for three

theoretical populations each with two recessive and two dominant alleles and respectively 6, 16 or 36 independent alleles (computer program 1). The mean frequencies for the stigma recessive, stigma dominant and independent alleles, respectively, were .1203, .0970 and .0942 for the population with ten alleles; .0552, .0497 and .0494 for the population with 20 alleles and .0263, .0250 and .0249 for the population with 40 alleles. The slight difference between the stigma dominant and independent alleles justifies combining the observed frequencies of these two types, that is, combining S_2 and S_4 with S_{11} and S_{12} (Table 1).

The observed difference in mean frequency between the two stigma recessives on one hand and the four dominants and independents on the other was .021 ± .016 (Table 1) whereas, in the theoretical populations, this difference decreased from .0254 to .0058 to .00135 as the total number of alleles increased from 10 to 20 to 40.

Probably *R. raphanistrum* has between 20 and 40 different *S* alleles (discussed later) so that the stigma recessives may be expected to have nearly the same frequency as that of the dominants and independents. However, there are two unknowns that affect this conclusion—the numbers of stigma recessives and of stigma dominants in *R. raphanistrum*. It is expected by analogy with pollen recessives, but not proven by computation, that the difference between the stigma recessives and dominants will decrease if *R. raphanistrum* has more than two stigma recessives, but increase with more than two stigma dominant alleles.

The effect on *S* allele equilibrium frequency of the general recessiveness characteristic of the pollen of *R. raphanistrum* was determined in 24 theoretical populations, using computer programs (2) and (3). The results with both programs followed regular patterns so that the frequencies in intermediate populations can be predicted from Figure 2.

Within each of the nine theoretical populations with three pollen recessive alleles and from seven to 82 pollen dominant alleles, the three recessives had nearly the same frequency so that only the means are plotted (Figure 2). For example, $S_1 = .1575$, $S_2 = .1643$ and $S_3 = .1678$ for the population with ten alleles and $S_1 = .0777$, $S_2 = .0767$ and $S_3 = .0742$ for the population with 50 alleles. Although it is not pertinent to the discussion, it is interesting to note that previous results (BATEMAN 1952; COPE 1962) showed an inverse relation between the frequency of an allele and its position on the dominance sequence, whereas the opposite was obtained here for the three recessives in populations with 25 or more alleles.

The nine populations with three pollen recessive alleles were alike in that each showed a large difference in mean frequency between the recessive and dominant alleles (Figure 2). Means for populations with 67 and 82 dominant alleles are not plotted in Figure 2. They are, for the recessives and dominants, .0648 with .0120 and .0592 with .0100, respectively. The recessives were 2.24 times more frequent in the population with 10 alleles, 3.08 times with 20 alleles, and 4.65 times with 50 alleles, compared with 2.42 times in the actual sample of *R. raphanistrum*.

The results with four and six pollen recessive alleles showed a reduction in the

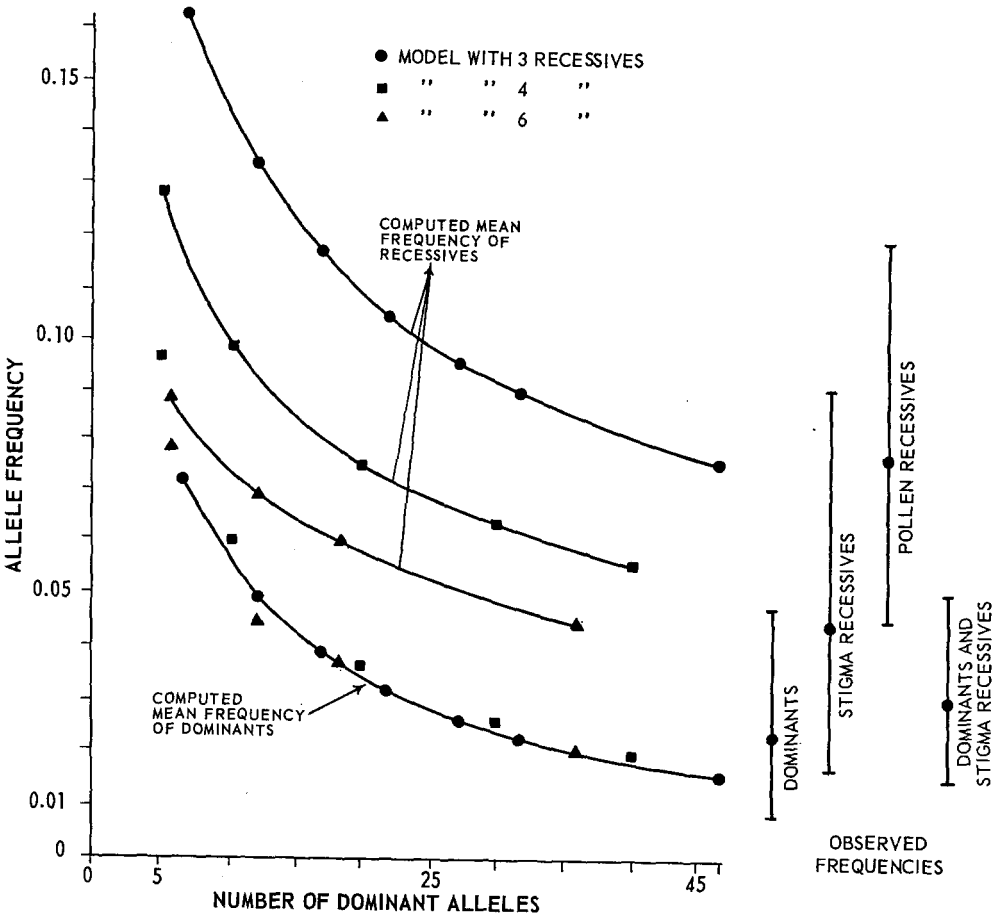

FIGURE 2.—Mean equilibrium frequency of pollen recessive *S* alleles and of dominant *S* alleles in 16 theoretical populations (with 3, 4 or 6 recessive and 5 to 47 dominant alleles) compared with the means and 5% fiducial limits (P = .025 to .975) observed for three types of *S* alleles in *R. raphanistrum*. For clarity, the curves for the dominant alleles in models with four and six recessive alleles are not shown.

mean frequency of recessives as the number of recessive alleles increased, but compared with the results with three recessives, the frequency of the dominant alleles remained about the same (Figure 2). That is, the total frequency of recessive alleles is nearly constant whether it be divided among three, four or six different alleles, provided that the number of dominant alleles remains the same. (Means for the population with four recessive and 60 dominant alleles are not plotted. They are .0460 for the recessives and .0136 for the dominants).

Results for the five populations with eight pollen recessive alleles (not plotted in Figure 2) show a continued reduction of the difference in frequency between the recessive and dominant alleles as the number of recessive alleles increases. Indeed the recesssives in the population with eight recessive and five dominant

alleles had a lower mean frequency than that of the dominants (recessive = .0755, dominant = .0792). This is because the usual higher frequency of recessive alleles results from their small numbers as well as from their recessiveness (SAMPSON, unpublished). The mean equilibrium frequencies for recessive and dominant alleles respectively for other populations with eight recessive alleles are .0608 and .0514 for 10 dominants, .0526 and .0386 for 15 dominants, .0472 and .0311 for 20 dominants and .0432 and .0262 for 25 dominants.

Estimate of the number of alleles: The observed mean frequencies may be compared singly with the theoretical frequencies by inspection (Figure 2). Thus the observed mean frequency of pollen recessives best fits the four populations with 3 recessive + 43 dominant alleles, or 4 + 17, or 6 + 8, or 8 + 5 alleles. The observed mean frequency of pollen dominants (Figure 2, "dominants and stigma recessives") best fits the curve for about 20 dominant alleles and 3, 4, 6 or 8 pollen recessives.

Simultaneous fitting of the two observed values to the two theoretical values was achieved by using the original raw data and chi-square analysis. The observed mean frequency for pollen recessives represents 18 tests that showed the presence of and 211 tests that showed the absence of the three test alleles. For the six pollen dominant alleles that were sampled, 15 tests showed presence and 447 absence (Table 1). These observed ratios of 18:211 and 15:447 compare with the expected numbers 17.2:211.8 and 16.2:445.8 ($\chi^2 = 0.13$ with 2 degrees of freedom) for the theoretical population with four pollen recessive and 20 dominant alleles, for example. This chi-square value and those for the other theoretical populations were then plotted and the best fitting number of dominant alleles together with the range of values within which the observations for 95% of subsequent samples should lie (Table 2) were determined by inspection (± 1 allele).

This estimate of the number of alleles is biased by errors both in the expected and the observed frequencies. Both kinds of errors relate to stigma recessives. First, the theoretical populations, because they ignore the stigma recessive—pollen dominant class, contain slightly more pollen dominant alleles than they should. Second, combining the data from the stigma recessives, S_9 and S_{14}, with

TABLE 2

Changes in the estimated number of pollen dominant S alleles as the number of pollen recessive alleles is increased

Number of pollen recessive alleles	Number of pollen dominant alleles		
	Most probable	Lower limit (P = .025)	Upper limit (P = .025)
3	25* (31)†	13 (14)	47 (79)
4	21 (20)	10 (10)	42 (57)
6	18 (17)	7 (8)	33 (35)
8	15 (15)	7 (7)	24 (27)

* Underestimates obtained by including the observed data on stigma recessives in the pollen dominant class.
† Overestimates obtained by omitting the data on stigma recessives.

those of S_2, S_4, S_{11} and S_{12} probably results in an overestimate of mean frequency and thus an underestimate of the number of pollen dominant alleles. The latter error has been over corrected in a second estimate of the number of alleles by omitting the observations on S_9 and S_{14} (Table 2, estimates in parenthesis).

The observed data are too few to decide conclusively on the most probable combination of pollen recessive and pollen dominant in *R. raphanistrum* but the combinations of three with 31 and four with 21 alleles gave the best fits. The most likely numbers of alleles in the species is thus between 25 and 34 with lower and upper limits to this estimate of 15 and 82 alleles (Table 2).

Estimates of the same order of magnitude are suggested for two other species of the Cruciferae, both with sporophytic self-incompatibility. BATEMAN (1954) using a different sampling procedure, arrived at 22 as an underestimate of the number of *S* alleles in a natural population of *Iberis amara*. THOMPSON (1961), isolating *S* homozygotes to be used for hybrid seed production in marrow-stem kale (*Brassica oleracea*), concluded that the total number of alleles was limited. Newly isolated homozygotes usually had one of the 29 identified alleles rather than a new one (THOMPSON, personal communication, February 2, 1965).

The findings of THOMPSON and TAYLOR (1965) suggest even fewer *S* alleles in some horticultural varieties of *Brassica oleracea*. Five recessives occupied at least 35 of 96 chromosomes sampled, a mean frequency of .0729. At equilibrium, five recessive alleles at this frequency would be accompanied by about 15 dominant alleles (Figure 2) if the effects of some self-compatibility in *B. oleracea* are ignored. Thus the moderate numbers of alleles found in Iberis, Brassica and Raphanus contrast with the hundreds of alleles that have been estimated for some species with gametophytic self-incompatibility.

SUMMARY

Plants from three populations in Eastern Canada and from two in Poland were tested for the presence of nine different *S* alleles. Each of seven alleles was found in two or more populations; five alleles were found in both Canada and Poland. When the five populations were treated as samples from a panmictic species, the four dominant or independent test alleles had an observed mean frequency of .026; the two stigma recessives of .047 and the three pollen recessives had a mean frequency of .079.—Numerical analysis of theoretical populations (deterministic models) showed that at equilibrium the general-recessive type of *S* alleles have higher frequencies than the specific recessive *S* alleles, and the fewer of each type the higher their mean frequency. The number of recessive *S* alleles in the population had little influence on the mean equilibrium frequency of dominant *S* alleles. Comparisons of theoretical results with observed frequencies suggest that *R. raphanistrum* has between 25 and 34 different *S* alleles (depending on the number that are recessive). The upper and lower limits of this estimate, between which 95% of subsequent samples should lie, are 15 and 82 alleles.

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