EVOLUTIONARY PATHWAYS OF CYTOPLASMIC MALE STERILITY IN SOLANUM¹

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CYTOPLASMIC factors interact with specific genes of *Solanum* species to lead to cytoplasmic male sterility. It is by now clear that such plasmon-gene interactions constitute a major force in the evolution of these potato species (KOOPMANS 1951, 1952, 1954, 1955, 1959; BUCK 1960; DIONNE 1961; GRUN, AUBERTIN, and RADLOW 1962). An earlier search for plasmon differences in a limited number of species brought to light four separate plasmon factors and a start was made toward determining the geographical distribution of each and the genes to which they are sensitive (GRUN, AUBERTIN, and RADLOW 1962).

The purpose of the study reported here was to analyze the number, types, and distribution of genes and plasmons that interact to lead to a specific sterility condition (indefecescence) as background for interpreting the steps involved in the establishment of the changed plasmon in natural populations. The specific questions asked here are: (1) What is the number of genes that can interact with the sensitive cytoplasm in leading to indefecescence? (2) What is the geographical distribution of individual genes to which the plasmon is sensitive? (3) Are there differences between sensitive strains of cytoplasm? (4) Can the apparent plasmon effects be interpreted as resulting from differential gamete selection? (5) Are restorer genes in operation in this material?

MATERIALS AND METHODS

The identities and places of origin of the plants used are listed in Table 1 in geographic order from north to south. Some background information on these species was described earlier (GRUN 1961). The classification system used follows the treatment of CORRELL (1962) which includes the classification of cultivated forms by DODDS. This involves a grouping of clones previously listed as *S. phureja* and *S. stenotomum* as *S. tuberosum* Groups Phureja and Stenotomum respectively.

The method of study consisted of production and comparison of F_1 and backcross progenies. Bud emasculation preceded all crossings and plants were handled in a standard fashion as described earlier (GRUN and RADLOW 1961). Crossings were made in the greenhouse and plants classified for dehiscence or indefecescence in the field. Nonrandomized plantings were used for this purpose since it has been found that the character is not influenced by ordinary field variations. A plant was classified as indefecescence when the anthers of a minimum of ten of its open flowers (five tested on each of two different days) failed to shed pollen when they were rapped against a clean slide. Plants having indefecescence and dehiscence anthers will be referred to as indefecescence and dehiscence plants respectively.

The fit of observed to calculated backcross ratios was determined using the chi-square test in those cases in which n for the smaller class was 14 or more. When n for the smaller class was

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less than 14, ratios which fit within the 95% confidence interval were determined from the binomial distribution (STEEL and TORRIE 1960).

The method of notation described earlier (GRUN, AUBERTIN, and RADLOW 1962) is used here with the change that the plasmon factor is bracketed to aid in distinguishing it from nuclear genes. Thus, respective forms of the cytoplasm are labelled with a superscript *s* for sensitive or superscript *r* for resistant. Cytoplasm sensitive to recessive genes is designated with the lower case letter. Thus, [*df^s*] is plasmon sensitive to recessive *df* genes, while [*Df^r*] is not sensitive. Cytoplasm sensitive to dominant genes is correspondingly designated with the upper case letter. [*In^s*] plasmon is sensitive to dominant *In* genes, while the [*in^r*] plasmon is resistant.

RESULTS

Genotypic bases for indehiscence: The sensitive cytoplasm, [*In^s*], has been localized only in the strain of *S. chacoense* f. *gibberulosum* (clones AA, AB, AC, and AD-1) which originated in the area near Manfredi, province of Cordoba, Argentina (GRUN, AUBERTIN, and RADLOW 1962). This plasmon is sensitive to dominant indehiscence-conditioning genes (*In*) which have previously been identified at three loci. (The resistant form of the plasmon, [*in^r*], produces normal plants having dehiscent anthers in the presence of either the *In* or *in* alleles.) To determine the number and geographic distribution of *In* alleles, pollen from parents carrying *In* was applied to stigmas of plants of known [*In^s*] plasmon strains that were homozygous recessive for indehiscence genes.

TABLE 1

Identification and places of origin of clones used

Species	PI Numbers*	Area of origin	Approximate latitude
<i>S. tuberosum</i>			
Group Phureja	195199 (BS)	Bogota, Colombia	4-5°N
	225706 (BZ)	Narino, Colombia	2°N
	225672 (BV)	Narino, Colombia	2°N
	225682 (BW)	Narino, Colombia	1°N
	195191 (AN)	Pichincha, Ecuador	0-1°S
Group Stenotomum	230512 (CX)	Chachapoyas, Peru	7°S
	230513 (CY)	Chachapoyas, Peru	7°S
	195214 (DK)	Huanuco, Peru	10°S
	195213 (DL)	Huancavelica, Peru	13°S
	205526 (CV)	Larecaja, Bolivia × Paucartambo, Peru	17°S 13°S
<i>S. chacoense</i>			
f. <i>gibberulosum</i>	133073 (AA)	Cordoba, Argentina	32°S
	133619 (AB)	Cordoba, Argentina	32°S
	133664 (AC)	Cordoba, Argentina	32°S
	195184 (AD)	Cordoba, Argentina	32°S
<i>S. kurtzianum</i>	133695 (AV)	NW Argentina	
	175435 (EU)	Mendoza, Argentina	33°S
	133687 (AU)	NW Argentina	
	175434 (AW)	Mendoza, Argentina	33°S

* Letter designations are those used for convenience in this report. When a collection contained more than one clone, each individual within the collection was designated by a number.

TABLE 2

Segregation ratios from crosses of pollen parents heterozygous for In genes with pistillate parents having [In⁸] plasmon

Cross number	<i>chacoense</i> pistillate parent	Pollen parent	Dehiscence		Ratios fit with P 0.05
			+	-	
1	AD-1	Stenotomum (CX-2) × <i>chacoense</i> (AC)	44	44	1:1
2	AC	Stenotomum (CY-1) × <i>kurtzianum</i> (AV)	57	45	1:1
3	AC	Sib	47	45	1:1
4	AC	Sib	49	53	1:1
5	AC	Sib	23	68	1:3
6	AC	Sib	21	81	1:3
7	AC	Sib	38	65	*
8	AB	Sib	13	58	1:3
9	AC	Phureja (BS) × <i>kurtzianum</i> (EU-4)	17	58	1:3
10	AC	Sib	9	57	1:7
11	AA	Sib	7	52	1:7, 1:15
12	AC	Sib	7	66	1:7, 1:15
13	AC	Sib	8	50	1:3, 1:7, 1:15
14	AC	Phureja (BZ-3) × Phureja (AN)	3	17	1:3, 1:7, 1:15
15	AC	Sib	5	59	1:7, 1:15, 1:31
16	AC	Stenotomum (CY-1) × Phureja (AN)	1	56	1:15, 1:31, 1:63
17	AC	Phureja (AN) × Stenotomum (CV-1)	2	63	1:15, 1:31, 1:63
18	AC	Sib	2	64	1:15, 1:31, 1:63
19	AC	Stenotomum (CY-1) × <i>kurtzianum</i> (AV)	4	98	1:15, 1:31, 1:63
20	AC	Phureja (BW-1) × Phureja (AN)	3	133	1:15, 1:31, 1:63
21	AC	Phureja (BW-1) × Stenotomum (CX-2)	3	207	1:31, 1:63

* This exceptional progeny did not fit any of the expected backcross ratios.

The ratios dehiscence:indehiscence are a measure of the number of *In* genes for which the pollen parents were heterozygous. A 1:1 ratio, such as that in crosses 1 to 4 of Table 2, indicated that the pollen parent was heterozygous for one *In* gene; a 1:3, that it was heterozygous for two; a 1:7 heterozygosity for three; 1:15 heterozygosity for four; 1:31 heterozygosity for five, and 1:63, heterozygosity for six. The larger the number of genes for indehiscence, the less sure was the determination of the correct ratio. This resulted from the fact that as *n* for the smaller class (dehiscence) decreased, the differences in P for fits to 1:15, 1:31, and 1:63 became less evident. In the preparation of Tables 2 and 4, fit of the ratios observed to alternative diploid ratios was tested, and only those ratios which the data would fit were listed. It is of equal significance to note the ratios that the data of certain ratios did not fit. For example, the ratio of Cross 10 in Table 2 did not fit a 1:3 or 1:15, but did fit a 1:7. Many of the ratios listed in Table 2 are for four to six *In* genes, a substantial increase over the one to three *In* loci found in the earlier report. This difference resulted from the fact that ratios of the earlier report were based on analysis of dominant parent × recessive parent backcrossed to recessive parent, while the present analysis was more often of the form dominant parent × dominant parent crossed onto the recessive parent. The former analysis would tend to minimize the number of dominant genes revealed par-

ticularly when the dominant parent was heterozygous. Dominant genes of the two parental populations of *S. tuberosum* and of *S. kurtzianum* were combined in the F_1 's tested in the present analysis, a procedure which would increase the number of *In* genes detected. The *In*-bearing pollen parents of a number of these progenies are sibs. In some cases the sib ratios are different (Crosses 2, 3, and 4 as compared with 5, 6, and 8; Cross 9 as compared with 10, 11, and 12), a result which suggests that the original parents were, in fact, heterozygous for at least one *In* gene.

Geographic and species distribution of individual In genes: *In* genes exist in plants of diploid populations of *S. tuberosum* Group Phureja native to Colombia and Ecuador, Group Stenotomum native to Peru, and in *S. kurtzianum* from Argentina, a total linear distance of at least 2700 miles. An attempt was made to learn whether the same *In* genes are widely distributed in plants in this area or whether the area plants contain different *In* genes each restricted in distribution. The following method was used: When the parents of the plants used as pollinators in Tables 2 to 4 contained the same *In* allele there was a likelihood that

TABLE 3

Testcrosses which did not segregate any dehiscence plants when tested on In^s plasmon

Cross Number	<i>chacoense</i> pistillate parent	Pollen parent	Number indehiscent
1	AC	Phureja (BW-1) × Phureja (AN)	105
2	AC	Sib	57
3	AC	Sib	77
4	AC	Sib	7
5	AC	Sib	72
6	AA	Same sib as Cross 5	79
7	AC	Sib	93
8	AC	Sib	94
		Total BW-1 × AN	584
9	AC	Phureja (BZ-3) × Phureja (AN)	106
10	AA	Sib	70
11	AC	Same sib as Cross 10	81
		Total BZ-3 × AN	257
12	AC	Phureja (AN) × Stenotomum (CY-1)	53
13	AC	Phureja (BW-1) × Stenotomum (CX-2)	54
14	AC	Sib	101
		Total BW-1 × CX-2	155
15	AC	Phureja (BZ-3) × Stenotomum (CX-2)	102
16	AC	Stenotomum (CV-5) × Stenotomum (CY-1)	49
17	AB	Same clone as Cross 16	30
18	AC	Sib	129
		Total CV-5 × CY-1	208
19	AC	Stenotomum (CY-1) × <i>kurtzianum</i> (AV)	97

TABLE 4

Comparison of dehiscence segregation ratios from the same pollen parent on different chacoense [In^s] pistillate parents

Cross number	<i>chacoense</i> pistillate parent	Pollen parents	Dehiscence		Ratios fit with P 0.05
			+	-	
1	AB	Stenotomum (CY-1) × Phureja (AN)	13	31	1:3
2	AA	Same clone	4	39	1:7, 1:15, 1:31
3	AC	Same clone	1	72	1:15, 1:31, 1:63
4	AB	Phureja (AN) × <i>chacoense</i> (AC)	72	81	1:1
5	AA	Same clone	65	202	1:3
6	AC	Same clone	10	83	1:7, 1:15
7	AA	<i>kurtzianum</i> (AU) × Stenotomum (DK)	4	55	1:7, 1:15, 1:31
8	AC	Same clone	10	60	1:7
9	AA	<i>kurtzianum</i> (AN) × Stenotomum (DK)	21	60	1:3
10	AC	Same clone	18	65	1:3
11	AA	Stenotomum (CY-1) × <i>kurtzianum</i> (AV)	39	49	1:1
12	AC	Same clone	54	44	1:1
13	AA	Stenotomum (DL-1) × <i>kurtzianum</i> (AW)	20	45	1:3
14	AC	Same clone	11	86	1:7, 1:15
15	AD-1	Stenotomum (CX-2) × <i>chacoense</i> (AC)	44	44	1:1
16	AC	Same clone	23	49	1:3

the pollen parent would be homozygous *In In*. This homozygous pollen parent would, when crossed onto the test plant having [*In^s*] plasmon, produce a non-segregating indehiscent progeny. The data in Table 3 summarize examples of such nonsegregating progeny. The proportions of various species combinations in Tables 2 to 4 that did and did not segregate are listed in Table 5. 53% of the crosses between *S. tuberosum* parents produced nonsegregating progeny, thereby suggesting that *In* genes are shared by biotypes of this species. By contrast, the progeny of only one cross out of 18 (6%) of *tuberosum* × *kurtzianum* failed to segregate, and even that one progeny is in doubt since it consisted of only 97 plants. This evidence could be interpreted as suggesting that individual *In* genes

TABLE 5

Proportion of various species combinations that did or did not segregate dehiscent plants when tested on In^s plasmon

Combination	Segregating	Nonsegregating
Group Phureja × Group Phureja	3	9
Group Stenotomum × Group Stenotomum	0	2
Group Phureja × Group Stenotomum	5	4
Total <i>S. tuberosum</i> × <i>S. tuberosum</i>	8	15
<i>S. tuberosum</i> (Both Groups) × <i>kurtzianum</i>	17	1

of *S. tuberosum* are relatively widely distributed within the range of the species through Colombia and Peru. They are not, by and large, allelic with *In* genes of *S. kurtzianum*. The sharing of alleles among plants of the closely related groups of *S. tuberosum* agrees with the earlier findings of DODDS and PAXMAN (1962) who studied alleles of a number of other genes. It is admittedly possible, however, that failure of segregation in progenies in Table 3 resulted from the presence in the pollen parent of five or more *In* genes in the heterozygous condition rather than from *In* homozygosity. Infrequent dehiscent segregants could, under these conditions, not have been picked up. Populations of the size that would have had to be raised to detect with certainty the one plant in 128 to be expected from a segregation of, for example, seven *In* genes in the heterozygous condition would have outstripped the field facilities available.

Differences among [In^s] plasmon clones: The data in Table 4 list comparisons of cases in which a given pollen-parent hybrid which had *In* gene(s) was crossed onto more than one *S. chacoense* f. *gibberulosum* test plant having [*In^s*] plasmon. Some of the segregation ratios (Crosses 7–8, 9–10, and 11–12) were the same when different [*In^s*] pistillate parents were used. In a number of the examples, however, ratios produced in different [*In^s*] plasmons were different even though all progenies segregated dehiscent and indehiscent offspring. There was a consistency, moreover, in that the proportion of indehiscent segregants was highest among progenies tested on *S. chacoense* f. *gibberulosum*, clone AC, intermediate on those tested on clone AA, and lowest on clone AB.

Three alternative interpretations of the differences in backcross ratios are: (1) The *chacoense* [*In^s*] tester clones may differ in content of restorer genes which can counteract the indehiscence effect of one or more of the *In* genes; (2) the *chacoense* testers may differ in stylar make-up so that certain pollen tubes having *In* genes do not grow normally or are unsuccessful in competition with *in* tubes; (3) the [*In^s*] plasmon of the *chacoense* pistillate parents may not be constant, but may exist in various forms which differ in sensitivity. In order to determine which of the hypotheses is correct, an analysis of Crosses 4 to 6 and 15 to 16 of Table 4 was undertaken. This was done by taking a sample of normal dehiscent plants from among the progeny of Crosses 4, 5, and 15 and crossing them reciprocally with the sensitive clone AC. The following predictions were made:

(1) The restorer gene interpretation would require, in the case of crosses 4 to 6, that at least three *In* genes be present in the pollen parent (Phureja (AN) × *chacoense* (AC) in the heterozygous condition. In the backcross of this pollen parent onto AC (Cross 6) all three *In* genes would segregate and be expressed as the 1:7 ratio recorded. (For the purposes of this interpretation it is assumed that the 10:83 ratio recorded in Cross 6 is a 1:7 and not a 1:15. The addition of a fourth *In* gene, which would be required for a 1:15 ratio, would not change the logic of the test used.) In the cross of the same pollen parent onto AA (Cross 5) only two of the *In* genes would be expressed, the function of the third having been restored by a dominant restorer gene (*R*) from clone AA. In the cross onto AB (Cross 4) only one *In* gene would be expressed since two were restored by epistatic interaction with restorer gene(s) present in clone AB. Some of the normal

dehiscent plants of Crosses 4 and 5 of Table 4 would, therefore, be heterozygous for *In* and the restorer. Since AC is homozygous recessive for both genes, according to this interpretation, such double heterozygotes would produce indehiscent segregants in progeny of both reciprocal crosses with AC.

(2) The stylar inhibition interpretation would again require that there be three *In* genes in the heterozygous condition in the pollen parent. AC would have no pollen tube inhibitors while AA had an inhibitor for any pollen tubes bearing one of the *In* genes, and AB had inhibitors for tubes bearing either of two of the *In* genes. None of the normal dehiscent plants of Crosses 4 and 5 of Table 4 would, according to this interpretation, contain any *In* genes, all *In* genes having been expressed. Progeny following reciprocal crossing of such dehiscent plants with AC would therefore not include indehiscent.

(3) According to the differential plasmon sensitivity interpretation, the three *In* genes would segregate, the [*In*^s] plasmon of plant AC being sensitive to all three, that of AA sensitive to two of them, and that of AB sensitive only to one. This would mean that some normal dehiscent progeny of Crosses 4 and 5 contained unexpressed *In* genes. When such progeny were crossed with AC using AC as the pistillate parent, indehiscent offspring should appear, although the reciprocals, using AC as pollen parent, should be entirely dehiscent.

The data in Table 6 list progeny of crosses between dehiscent plants from Crosses 4, 5, and 15 of Table 4 and sensitive clone AC. Data in the left half of the table list progeny of such crosses when AC was the pistillate parent and those on the right side are their reciprocals. Some progenies (Crosses 3, 11, and 12)

TABLE 6

Results of reciprocal crossings of dehiscent plants from Crosses 4, 5, and 15 of Table 4 with S. chacoense f. gibberulosum, clone AC. The two progenies listed for each cross number are results of reciprocal crossings of the same plants

Cross Number	Plasmon parent	Dehiscence		Ratios fit with P 0.05	Plasmon parent	Dehiscence		Ratio fit with P 0.05
		+	-			+	-	
Progeny of Cross 4								
1	AC	84	15	7:1	AB	84	11	7:1
2	AC	78	23	3:1	AB	57	38	1:1
Progeny of Cross 5								
3	AC	98	2*	1:0	AA	103	0	1:0
4	AC	56	22	3:1	AA	68	19	3:1
5	AC	76	23	3:1	AA	84	18	3:1, 7:1
6	AC	13	3	..	AA	32	49	1:1
7	AC	8	3	..	AA	82	19	3:1, 7:1
Progeny of Cross 15								
8	AC	90	2*	1:0	AD-1	70	26	3:1
9	AC	76	1*	1:0	AD-1	80	20	3:1, 7:1
10	AC	87	0	1:0	AD-1	63	27	3:1
11	AC	82	0	1:0	AD-1	93	1*	1:0
12	AC	88	0	1:0	AD-1	91	0	1:0

* These few indehiscent plants could have resulted from misclassification or from genetic-environmental causes other than those considered here.

essentially lack indehiscent plants, a result to be expected since some of the dehiscent segregants in Table 4 may lack *In* genes by any of the three interpretations. Indehiscent plants appear in varying ratios, however, among most of the progenies listed in Table 6. The stylar inhibition interpretation, therefore, seems unlikely. The evidence of Table 6 supports, unexpectedly, both of the other two interpretations.

According to the restorer gene interpretation, indehiscent and dehiscent segregants should appear in both the reciprocal progenies. Such occurred in Crosses 1, 2, 4, 5, 6, and 7 of Table 6. The ratios recorded are those to be expected based upon the operation of varying numbers of dominant restorer genes in conjunction with *In* genes. Thus, the double heterozygote, *In in R r* backcrossed with *in in r r* would segregate three dehiscent to one indehiscent, the indehiscent being *In in r r*. A 7:1 ratio, such as appears in both reciprocals of Cross 1 would result from a backcross of a plant heterozygous for two restorer genes either of which could restore the function of the given *In*, i.e. *In in R r R₁ r₁* $\times$ *in in r r r₁ r₁*. The indehiscent plant would be *In in r r r₁ r₁*. The ratios of reciprocals of the same crosses should, according to the restorer-gene interpretation, be the same. This occurred in Crosses 1, 4 and 5. The population sizes when clone AC was plasmon parent of Crosses 6 and 7 were too small to be interpretable for this purpose. The ratios may be different in reciprocals of Cross 2.

The data for progeny of Cross 15 of Table 4 indicate that there are differences in sensitivity of different [*In^s*] plasmon strains, since Crosses 8, 9, and 10 of Table 6 demonstrate consistent differences between reciprocals. The ratios of progenies having clone AD-1 as plasmon parent are those to be expected from an interaction involving one *In* gene and one dominant restorer gene. The AC [*In^s*] sensitive plasmon is not sensitive to this particular *In* gene, while the AD-1 [*In^s*] plasmon is sensitive to it.

While the progeny of Cross 15 are unambiguous in showing difference in sensitivity of AC and AD-1 plasmon, they raise other questions that are yet to be answered. The crosses 8 to 12 of Table 6 were originally made to determine, as outlined above, the reason that more indehiscent plants appeared in AC than AD-1 plasmon. The Table 6 results show, contrary to expectation, indehiscent plants in AD-1, but not in AC plasmon. Further progeny must be raised before this reversal can be understood.

DISCUSSION

The data in this report bear on three interrelated questions: (1) What were the steps in the establishment in native populations of the genes with which the plasmon interacts? (2) What were the steps leading to the establishment of a new plasmon form? (3) Could a change in plasmon act as an isolation barrier?

Evidence has been cited (GRUN, AUBERTIN, and RADLOW 1962) to show that the ancestral plasmon forms are the resistant ones. Which, then, was the ancestral allele of the gene? The data in Tables 2 to 4 taken with those of the earlier paper, show the existence of a minimum of five independent *In* genes (to which [*In^s*] plasmon is sensitive) over a range of 2700 miles. Some *In* genes occur in popu-

lations native through Colombia and Peru far from the [*In*^s] populations of *S. chacoense* in Argentina. In a similar way [*Lb*^s], a plasmon which conditions a sterility expressed as lobed microspores, occurs in *S. verrucosum* near Mexico City, while the *Lb* gene (to which the plasmon is sensitive) is the established allele in other *Solanum* species native throughout South America (Table 7, GRUN, AUBERTIN, and RADLOW 1962). The fact that sensitive genes occur far from the area of the plasmon factors with which they can interact has also been established for *Epilobium* (MICHAELIS and BAKKER 1948) and *Oenothera* (STINSON 1960). This evidence makes unlikely the hypothesis that the sensitive allele increased in native populations as a result of reinforcement, i.e., selection for its activities in producing a male sterility isolating barrier.

The following interpretation could explain the evolutionary changes in *In* and *Lb* genes in native populations: As in any differentiation process, a specialized series of reaction steps lead under normal conditions to the differentiation of anthers and pollen. These steps are conditioned by enzymes initially coded by genes such as *In* and *Lb*. These specific genes are so-called "plasmon-sensitive genes" (CASPARI 1948) so changes in plasmon factors could lead to blockage of the synthetic steps controlled by them. Thus, the four plasmon factors uncovered to date in *Solanum*, [*In*^s], [*Lb*^s], [*df*^s], and [*pl*^s], could be considered capable of blocking four independent steps in the pathway normally conditioned by the genes *In*, *Lb*, *df*, and *pl*. The five different *In* genes share sensitivity to the same plasmon change and interact with this plasmon change to produce a similar phenotypic expression of sterility. It therefore seems likely that their products are involved in the same biosynthetic pathway. The five genes are not functionally identical, however, for they include differences in interactions with restorer genes and in interactions with the two forms of the [*In*^s] plasmon. Mutation of the respective genes to *in*, *lb*, *Df*, and *Pl* can serve to bypass the plasmon-induced blockage, restoring normal fertility.

What, then, could have been the steps leading to the establishment of an altered plasmon? At the start, according to present evidence, the ancestral plasmon was [*in*^r] and genotype *In*-. In the population of *S. chacoense* f. *gibberulosum*, at Manfredi, Argentina, a cytoplasmic change introduced [*In*^s], thereby producing [*In*^s]-*In*-indehiscent plants. Such plants, while having indehiscent anthers, were entirely normal in other respects. The establishment of the new plasmon not only could have been sympatric with the old, but it would have required the old as a source of pollen. Since indehiscent plants are female fertile, offspring would have been produced in free competition with [*in*^r] dehiscent plants. As pointed out by LEWIS (1941) such indehiscent (female) plants would increase in the population if they had even a very slight selective advantage until the supply of pollen in the population became limiting. Without a selective advantage, the new plasmon would maintain its initial proportion in the population (WATSON and CASPARI 1960). Accordingly, establishment of the new [*In*^s] plasmon would be dependent on the vigor, disease resistance, etc. of the harboring plant and would be independent of its effects on male fertility.

At some time during the establishment of such indehiscent plants, a mutation

of gene *In* to *in* or of *r* to *R* occurred producing a return to male fertility. The mutant form of either gene would have a strong selective advantage (WATSON and CASPARI 1960) such that it should become fixed producing a normal male fertile population of the type to be found at Manfredi.

Would such a population be isolated from its parental stock? WATSON and CASPARI (1960) came to the conclusion, based upon a mathematical model, that the new and old populations would, upon being brought together, produce a "unified Mendelian population." This conclusion would certainly apply to the new plasmon type during early establishment stages when it depended upon pollen from the ancestral population for seed set. After the new plasmon became established as a pollen fertile population, it seems possible, however, that the plasmon differentiation could operate as an isolation mechanism in the following way: If a [*In^s*]-*in in* population should come in contact with a [*in^r*]-*In-* population, the hybrids would be a mixture of male sterile and male fertile plants depending on the direction of the cross. The male sterile hybrids would produce a certain proportion of male sterile backcross progeny. Thus the population at the meeting place of the parental forms would have a reduced male reproductive potential. This male sterility at the meeting zone would prove a quantitative barrier to migration of genes between populations. The barrier would break down if the [*in^r*] population genotype changed from *In* to *in*. WATSON and CASPARI (1960) have shown that if random breeding occurs, *In* will, in fact, be displaced by *in*. In the case postulated here, however, random breeding is ruled out by the spatial separation of the two parental populations. There would be no direct selection against *In* genes except at the meeting zone itself. Whether the two populations ever became one would depend upon the relationship between the rate of selection against *In* genes at the meeting zone and the rate of migration of new *In* as well as *in* alleles into the meeting zone.

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SUMMARY

A minimum of five alternative independent *In* genes can lead to indehiscence when they are combined with [*In^s*] plasmon strains. The *In* genes occur in populations of *S. tuberosum*, Groups Phureja and Stenotomum, and in *S. kurtzianum*. Plants bearing *In* genes occur from central Argentina, where the [*In^s*] plasmon population exists, to Colombia, a linear distance of at least 2700 miles. At least two dominant restorer genes exist in the *S. chacoense* [*In^s*] plasmon strains. These are epistatic to *In* and produce normal fertility in plants that would otherwise be indehiscent. Restorer genes are specific rather than general in their effects, a given restorer counteracting only specific *In* gene(s). It is therefore concluded that the *In* genes are not identical in function. The [*In^s*] plasmon exists in at

least two forms that differ in their *In* gene sensitivity. These observations are used as a basis for an interpretation of steps in establishment of a changed plasmon in a natural population.

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