CENTRIC FRAGMENTS AND POLLEN-PART MUTATION OF INCOMPATIBILITY ALLELES IN PETUNIA[1]

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Received November 24, 1959

THE incompatibility (*S*) alleles of flowering plants have two functions—one determining pollen phenotype, the other shaping the stylar phenotype. That these two functions can be separated as the result of mutation was observed by LEWIS (1949), who obtained self-fertile mutants in which an *S* allele's pollen activity alone had been altered. Similar pollen-part mutants have predominated among spontaneous and radiation-induced mutants obtained in subsequent investigations (LEWIS and CROWE 1954; PANDEY 1956; BREWBAKER and SHAPIRO 1959).

Self-fertility is known to appear in many self-incompatible species as the result of induced tetraploidy (LEWIS 1947; BREWBAKER 1954). This breakdown of incompatibility is based on a novel *S* gene interaction termed "competition" which follows when two different alleles occur in the same pollen grain. Such interaction characterizes all *S* allele heterozygotes tested in *Petunia inflata* Fries, the species employed in this study (BREWBAKER and SHAPIRO, unpublished data).

Centric chromosome fragments occur widely in plants, often as euchromatic and genetically inert "B" chromosomes. Other supernumeraries evidently are derived by more or less recent fragmentation from the standard "A" genome (GRUN 1959; MUNTZING 1958). Irradiation is a notoriously effective tool for the induction of such fragmentation.

Centric fragments, competition and pollen-part change are linked in the present study to afford a cytogenetic basis for pollen-part mutation and to suggest a possible reason for the establishment and distribution of fragments in some flowering plants.

MATERIALS AND METHODS

All mutants were obtained following irradiation of a highly self-incompatible seed stock of *Petunia inflata* Fries ($2n = 14$). Incompatible matings to screen for *S* mutants were made exclusively on unirradiated pistils. Testers included *S* allele homozygotes of garden hybrid origin to which the alleles had been transferred from *P. inflata*. Mutant seeds were obtained only rarely from homozygotes, while they were recovered frequently from the heterozygotes, particularly from buds irradiated shortly prior to meiosis (BREWBAKER and SHAPIRO 1959).

The 3100 pollinations from which data are summarized here were made in

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[1] Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

bee-proof greenhouses. Flowers were emasculated in late bud stage on all self-fertile and most self-sterile plants. Fertile crosses averaged over 150 seeds per pollination, while incompatible matings averaged 0.024 seed/pollination using homozygous male testers and 0.204 seed/pollination using heterozygotes. Cytological observations were made on root-tip smears stained with aceto-orcein and on PMC's and pollen tubes stained with propionocarmine.

RESULTS

General survey of S *mutants*

At the time of writing, 68 plants had been grown successfully from X_1 "mutant" seed of *P. inflata* and identified genotypically. Eighteen of these X_1 plants were derived from homozygous self-incompatible (SI) parents, and each of these found to be of the same genotype as its parent, i.e., nonmutant.

Similarly, 20 of the 50 X_1 plants derived from heterozygous parents were self-incompatible, reacting phenotypically just as the parents. Plants of this nonmutant genotype have been found in all studies of *S* gene mutation. Among nine additional X_1 mutants derived from heterozygotes. four were female sterile and five were both female and male sterile. This sterility was exceedingly variable and associated with *S* gene change in only two instances. The remaining 21 self-fertile X_1 mutants included 15 in which the pollen activity alone had been altered.

Pollen-part mutants

Each of the 15 self-fertile X_1 plants categorized as a pollen-part mutant was fully self-fertile (SF), universally cross-fertile as male (F as ♂) and identical to its S_1S_2 parent as female (S_1S_2 as ♀). None of the mutant plants reacted as if homozygous for *S* alleles, a significant discrepancy from the expected 50 percent. The plants chosen for cytogenetic studies to be reported were obtained by X-irradiation (500 or 1000r) of the clone S_1S_2 prior to premeiotic interphase, and each derived from a separate mating. All progenies were self-pollinated, crossed as females to S_1S_1, S_2S_2, S_5S_5, S_1S_2 and S_1S_5 testers and crossed as males to appropriate tester genotypes. Pollen viability *in vitro* of all plants exceeded 75 percent, comparing favorably with the controls. Cytogenetic studies are reported here for five of the pollen-part mutants and their progenies.

(1) X9-4: Sixteen plants were grown from the cross of pollen-part mutant X9-4 (SF, F as ♂, S_1S_2 as ♀) with the homozygote S_5S_5 (Table 1). Seven plants were SI, segregating six S_1S_5 and one S_2S_5, indicating that X9-4 carried nonmutant chromosomes bearing S_1 and S_2. A unique *S* genotype, $S_1S_2S_5$ as ♀, was obtained among the nine SF plants. Six of these plants were universally cross-fertile as males but cross-sterile as females to S_1S_1, S_2S_2, S_5S_5, S_1S_2, S_1S_5, and S_2S_5 testers, and could be considered only as of the triallelic $S_1S_2S_5$ genotype as female. The three remaining SF plants reacted as S_2S_5 females.

A study of the mitotic chromosomes of six of the self-fertile plants (four $S_1S_2S_5$, two S_2S_5) revealed the presence in each of a small centric fragment (Figures 1–4). No such fragments could be observed in the three self-sterile plants studied. The fragments showed no irregularities in behavior, and could be discerned readily during meiosis and pollen tube mitosis (Figures 1–4).

TABLE 1

Incompatibility reactions and cytology of progenies from pollen-part mutants

Family	Number of plants	Self-fertility	Incompatibility reaction as:		Cytology
			male	female	
X9-4 × S_5S_5	6	SI•	S_1S_5	S_1S_5	14 (3 plts.)
	1	SI*	S_2S_5	S_2S_5	......
	6	SF*	F*	$S_1S_2S_5$	14 + frag* (4 plts.)
	3	SF*	F*	S_2S_5	14 + frag (2 plts.)
X10-1 × S_5S_5	4	SI	S_1S_5	S_1S_5	14 (1 plt.)
	12	SF	F	$S_1S_2S_5$	14 + frag (4 plts.)
	1	SF	F	S_1S_5	......
	1	SF	F	S_2S_5	......
X18-1 × S_5S_5	4	SI	S_1S_5	S_1S_5	14 (1 plt.)
	3	SI	S_2S_5	S_2S_5	14 (1 plt.)
	5	SF	F	$S_1S_2S_5$	14 + frag (1 plt.)
	1	SF	F	S_2S_5	14 + frag
	1	SF	F	F	......
X19–1 selfed	12	SF	F	S_1S_2	14 + frag (2 plts.)
S_1S_2 × X19-1	13	SF	F	S_1S_2	......
X19-1 × S_5S_5	8	SI	S_2S_5	S_2S_5	14 (3 plts.)
	5	SF	F	$S_1S_2S_5$	14 + frag (3 plts.)
	1	SF	F	S_5S_5	14 + frag
	1	SF	F	F	14 + frag
X22-2 × S_5S_5	2	SI	S_1S_5	S_1S_5	......
	6	SI	S_2S_5	S_2S_5	......
	2	SF	F	S_1S_5	......
	1	SF	F	$S_1S_2S_5$	......

* SI = self-incompatible. SF = self-fertile. F = fertile. frag = centric fragment.

The results of this and succeeding families are explained satisfactorily by the hypothesis that a fragment carrying an *S* allele was induced by radiation. As a consequence of competition interaction, fragment-bearing heterogenic pollen would be uninhibited in pollen tube growth, accounting for the observed self-fertility. The cross X9-4 (S_1S_2 + fragment S_2) × S_5S_5 would be expected on this hypothesis to produce ¼ S_1S_5: ¼ S_2S_5: ¼ S_1S_5 + frag S_2: ¼ S_2S_5 + frag S_2, an expectation with which the observed data were in accord.

(2) X10-1: Twenty plants were grown from the cross X10-1 × S_5S_5, of which 18 were classified genotypically (Table 1). Rather too much variability was observed among the 780 selfs and crosses made in this family to permit unequivocal classification of all plants, since many plants were poor seed setters. Four of those classified were SI, S_1S_5, while the remaining 14 were SF. The SF, F as ♂ and $S_1S_2S_5$ as ♀ class appeared again in this family and comprised 12 plants. Two other SF plants were classified as S_1S_5 as ♀ and S_2S_5 as ♀, respectively.

Cytological examination again revealed a small centric fragment in four of the SF plants and none in the SI plant tested. In this case, it was possible also to test the SF parent, X10-1, and rewarding to find the fragment present as expected. It is suggested that X10-1 was S_1S_1 + fragment S_2. The two unexpected SF geno-

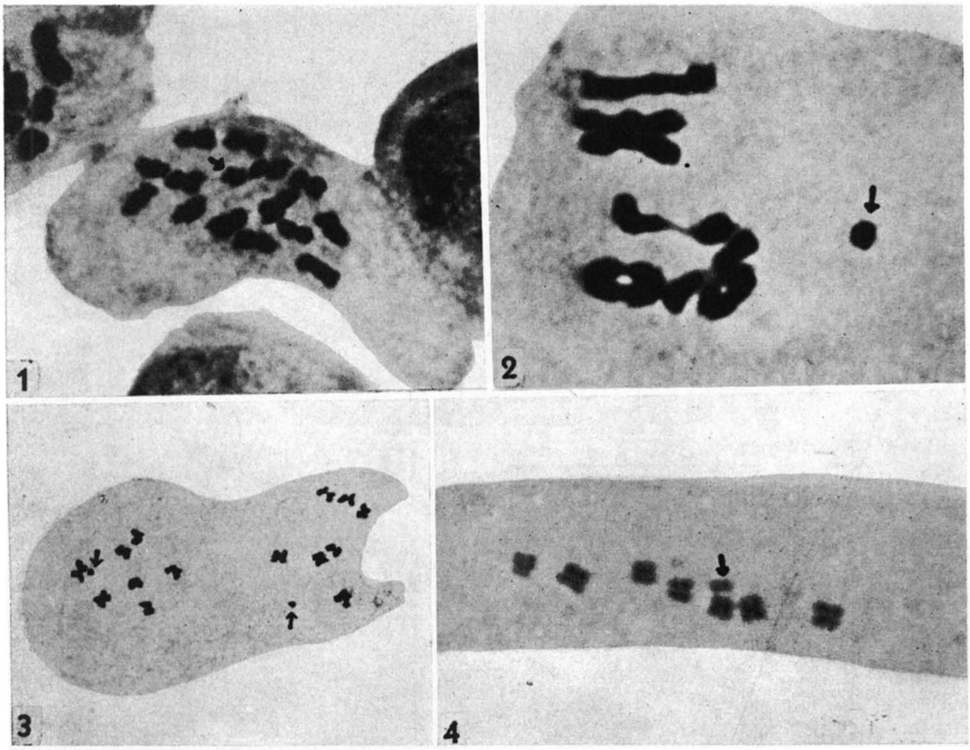

FIGURE 1–4.—Cytological behavior of the centric fragment. FIGURE 1.—Somatic chromosomes of a mutant showing 14 chromosomes and the centric fragment in a root-tip squash. FIGURE 2.—First metaphase in a PMC, showing seven bivalents and the fragment. FIGURE 3.—First anaphase groupings, showing seven chromosomes plus the fragment on each pole. FIGURE 4.—Pollen tube mitosis showing seven chromosomes and the fragment.

types deserve further test although the S_1S_5 as ♀ could be expected as a result of crossing over.

(3) X18-1: Fourteen plants were studied from the cross X18-1 × S_5S_5, of which seven were SI, including four S_1S_5 and three S_2S_5 (Table 1). The seven SF plants comprised five $S_1S_2S_5$ as ♀ and one S_2S_5 as ♀. An additional SF plant set seed reciprocally with every tester genotype (including homozygotes) and was classified as SF, F as ♀. Similar plants have been obtained as direct X-mutants or segregants on six separate occasions and are the subject of continued study.

Cytological studies revealed single fragments in each of two SF plants tested as well as in the parent clone, while two tested SI plants presented the normal 2n = 14 complement. The data are satisfactorily explained by assuming that X18-1 was S_1S_2 + fragment S_2.

(4) X19-1: Three families were grown from X19-1 (Table 1). The selfed family comprised plants of only one *S* phenotype—that being the same as the parent X19-1 (SF, F as ♂, S_1S_2 as ♀). Similarly the backcross of X19-1 as male to its parent produced plants of only one *S* phenotype, again that of X19-1. Seventeen plants were grown from the cross X19-1 × S_5S_5, and these segregated into

four groups. Ten of these were SI, of which the eight tested plants were S_2S_5. $S_1S_2S_5$ plants again prevailed among the SF group, while two additional SF plants—the subject of some 140 crosses—were of the unexpected phenotypes (1) SF, F as ♂, F as ♀ and (2) SF, F as ♂ and S_5S_5 as ♀.

Fragments were observed in each of the seven tested SF plants of these three families, as well as in the parent X19-1. None of the three SI plants tested carried fragments.

(5) X22-2: Plant X22-2 was a self-fertile pollen-part mutant differing from those considered previously in the unreliability of its behavior as a female. In a first set of pollinations X22-2 was SF, F as ♂ and S_1S_2 as ♀; in a second season, it was SF, F as ♂ and F as ♀ (perhaps affording a key to the origin of this latter phenotype). X22-2 represented an odd family; its sibs from the same mutant capsule included a prostrate sterile dwarf and three pollen-part mutant plants, of which one was SF, F as ♂ and S_1S_2 as ♀, one SF, F as ♂ and F as ♀ and one female sterile, F as ♂.

The 11 plants grown from X22-2 × S_5S_5 were rather more conventional in behavior, and similar to families reported previously (Table 1). The results are explained satisfactorily by assuming that X22-2, which carried a fragment, was genotypically S_1S_2 + fragment S_1.

DISCUSSION

The five pollen-part mutants studied have been shown to be self-fertile by reason of their inclusion of *S* gene bearing fragment chromosomes and production of the uninhibited heterogenic competition pollen type, S_1S_2 (a genotype which has also been studied in 3n and 4n lines). The karyotypes proposed for the five mutants were as follows:

X10-1	S_1S_1 + fragment S_2
X19-1	S_2S_2 + fragment S_1
X22-2	S_1S_2 + fragment S_1
X9-4 and X18-1	S_1S_2 + fragment S_2

All four combinations are thus represented, and the expected 1:1 ratio of homozygous to heterozygous progeny satisfied. The fragments were of relatively constant size (about 1/5 length of chromosome) and with median centromeres. They clearly did not affect pollen viability or activity, e.g., the expected 1:1 segregation of seven and seven + fragment pollen types was realized satisfactorily by the test-cross segregation of 36 SI:40 SF plants.

No cytological studies have been reported previously for pollen-part mutants, nor have the extremely elucidative homozygotes been employed as testers for X_1 plants. LEWIS (1949) proposed that independent loss of pollen activity proved that the *S* gene was bipartite, having independently mutable pollen and stylar components. Subsequent studies have concurred without lending particular support to this hypothesis (LEWIS and CROWE 1954; PANDEY 1956). Some if not all of the pollen-part mutants of these other plants (Oenothera, Prunus and Trifolium spp.) can be satisfactorily explained on a fragment or *S* gene translocation basis.

An exception is the class of nine X_1 plants concluded by PANDEY (1956) to be pollen-part mutant homozygotes. These nine plants could also be interpreted as *S* gene deletions which are lethal when homozygous, placing them more realistically with their sibs, in which *S* deletion prevailed (28 of 29 plants classified).

The origin of self-fertility in a self-incompatible species as the result of the addition of a chromosome fragment arouses speculation concerning the origin of centric fragments in general. *S* gene bearing fragments have the immediate advantage, when competition occurs, of indifference to an incompatibility screen. They could be expected to spread widely in a self-sterile population, since competition grains carrying fragments would be universally uninhibited. Self-fertility *per se* would confer a considerable survival advantage to the fragment and assist in its establishment in the species. Subsequent *S* gene loss (heterochromatization) by the fragment would not be entirely unexpected if inbreeding depression later endowed it with selective disadvantage.

SUMMARY

Genetic analyses of five pollen-part incompatibility allele mutants in Petunia indicated that all resulted from the addition of an *S* gene bearing centric fragment to the normal genome. Fragment-carrying heterogenic pollen grains were uninhibited in all matings as a result of competition interaction (observed similarly for heterogenic diploid grains of the corresponding tetraploids). It is suggested that the induction of self-fertility may provide a selective mechanism for the establishment and distribution of supernumerary centric fragments in a species.

ACKNOWLEDGMENTS

The authors wish to acknowledge particularly the assistance of MR. NATHAN SHAPIRO. The junior author is grateful to the Rockefeller Foundation for an award of a postdoctoral fellowship during the course of this study.

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