

Radiation Effects on the One Locus-Gametophytic System of Self-Incompatibility in Higher Plants*

(A Review)

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Summary. A brief review is presented of the temporary and permanent effects which are usually observed after irradiation treatment of self-incompatible plants with a one locus-system of gametophytic incompatibility and attempts are made to demonstrate that the study and exploitation of such effects can lead to important advances in the fields of mutation breeding, radiobiology *per se* and biochemistry.

The fact that irradiation treatment has only negative effects on the self-incompatibility system (inactivation of the incompatibility reaction and/or genetic losses at the *S* locus) is discussed in relation to the positive changes (formation of new specificities) which are observed during inbreeding. In this connection, the suggestion is made that the self-incompatible plant may perhaps be equipped with a switch system or a mutagenic mechanism which enables it to display a new specificity when this new specificity is needed for increasing the level of genetic polymorphism in the population to which the plant belongs.

1. Introduction

The importance of the incompatibility locus of higher plants in mutation research has been clearly underlined by LEWIS (1949b) who pointed out its unique advantages for refined studies in genetics, radiobiology and evolution. Several authors (LEWIS and CROWE, 1953, 1954; BREWBAKER and NATARAJAN, 1960; BREWBAKER and SHAPIRO, 1959; PANDEY, 1956; SHARMA and BOYES, 1961) have also demonstrated the efficiency of radiation techniques for inducing temporary or permanent changes which allow self-fertilization to take place and which, in turn, render possible the study and the exploitation of inbreeding effects in otherwise self-incompatible species.

Irradiation studies on self-incompatible organisms are therefore contributing to many different fields of applied and fundamental research among which the most important can be briefly defined as follows:

Applied mutagenesis: According to the model of LEWIS and CROWE (1958), induction of self-compatibility in cross-pollinated species may be the first prerequisite to the removal of pre-zygotic barriers between different species. Hence, mutations towards self-compatibility do not only modify the breeding system within the species but may also affect the interspecific relationship within the whole genus. Independently of these effects on species barriers, the induction of self-compatibility in self-incompatible cultivars also leads, as was stated above, to the interesting possibility of inbreeding and selecting for valuable agronomic traits which may never ex-

press themselves otherwise in the highly heterozygous background of cross-pollinated individuals. The reverse effect, that is to say the induction of self-incompatibility in a self-compatible species, would also be highly desirable because it would automatically lead to obligate cross-fertilization and, eventually, to the spontaneous formation of heterotic hybrids between different inbred lines grown side by side in the same field. The technical difficulties involved in the detection of a self-incompatible mutation are such, however, that this attractive project appears almost impossible to realize.

Radiobiology per se: As was stressed by LEWIS (1949a) the induced mutation towards self-compatibility constitutes a very interesting criterium of sensitivity for the radiobiologist who can rely on the style barrier for automatic selection of all self-compatible mutations. The system is particularly advantageous because, like in microbial genetics, extremely large numbers of individuals (microspores) can be screened for a specific and well defined irradiation effect. The objection that radiobiologists often orientate their investigations towards the field of Health Physics and are, consequently, not interested in plant material, does not withstand an objective examination. The radiation work of EHRENBURG and ERIKSSON with the *waxy* locus in maize (EHRENBURG and ERIKSSON, 1966) and the excellent review in READ and GRAY (1959), not to say anything about the very numerous theories and biological laws which have been developed from plant material and extended to the animal kingdom afterwards, demonstrate beyond doubt that the higher plant in general and the gametophyte in particular can be considered as reliable material for fundamental research in radio-

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biology. This is especially true of the S system of gametophytic incompatibility which appears to have much in common with the immunogenetic mechanisms studied in man (LINSKENS, 1961; HAVEZ, 1967).

Biochemical genetics: Biochemical studies on the self-incompatibility system are essentially based, at the moment, on the determination of quantitative and qualitative differences between the self-compatible and the self-incompatible reactions. It would therefore be highly desirable to provide the biochemist with different mutants which display a specific behaviour at different steps of the reaction and which could eventually allow a classification of the various events taking place in the pollen grain, in the pollen tube and in the style. The analysis of the direct effect of irradiation upon the incompatibility reaction itself (LINSKENS *et al.*, 1960; HOPPER and PELOQUIN, 1968) also constitutes an interesting technique for an appraisal of time-sensitivity relationships and of the critical doses at which the incompatibility reaction can be inhibited. The specific radiosensitivity of a chemical substance being a function of its structural composition and of the type of radiation used, the biochemist should be able to use such sensitivity data for testing his hypotheses on the identity and the time of action of the different chemical entities which participate in the incompatibility reaction.

Since the great majority of radiobiological studies have been restricted to the one locus-gametophytic system of self-incompatibility the present survey shall only consider the radiation responses of this system. For a review of radiation effects on other systems the reader is referred to ERNST (1957) and to SHARMA and BOYES (1961) (sporophytic self-incompatibility) and to the articles of BREWBAKER and EMERY (1962), and DAVIES and WALL (1961) (interspecific incompatibility). For a clear survey on the general aspects of self-incompatibility in angiosperms, consultation should be made of the reviews by BATEMAN (1952), LUNDQUIST (1965), LINDER (1967) and ARASU (1968).

2. Test-Plants most commonly used for Radiation Research on the One-Locus Gametophytic System of Self-Incompatibility

The ideal test-plant for research on self-incompatibility has been partly described by LEWIS (1948) and should, no doubt, fulfill all of the following requirements:

- Agricultural value.
- Diploid status (in most cases).
- Absence of environment-induced pseudo-compatibility.
- Style sufficiently large for biochemical analyses.
- Stigma sufficiently large for application of high numbers of pollen grains.

Incompatibility reaction easily detectable by means of examination of pollen tube growth in the style.

High numbers of ovules in the ovary and a natural tendency towards parthenocarpy.

Large chromosomes which can be easily observed in PMC's, pollen tubes and root tips.

A well established linkage map with several seedling markers which could eventually be used for mapping the S locus.

A tendency towards parthenogenesis or androgenesis so that homozygosity at the S locus in a completely isogenic background could eventually be obtained by doubling the chromosome number in spontaneous haploids.

A capacity for vegetative multiplication which permits clonal propagation of rare genotypes and tester stocks.

As such an ideal species does not exist, most research workers have selected as test material the species which fulfilled some of the most vital requirements in their research programme. Among the plant genera most commonly used in radiation research one must list *Lilium* (large style), *Trifolium* (almost complete absence of pseudo-compatibility), *Nicotiana* and *Petunia* (high number of seeds per pod), *Oenothera* (large chromosomes) and *Lycopersicum* (agricultural value and extremely good capacity for vegetative propagation).

3. Description of Irradiation Effects

An attempt has been made, in Figure 1, to classify irradiation effects in terms of the duration and the nature of the induced responses. One can see that these effects are described as physiological (action on the metabolic activity of the whole plant or of a whole organ) or genetical (inhibition or modification of the S locus itself). Whereas the physiological effects are always temporary and cannot be trans-

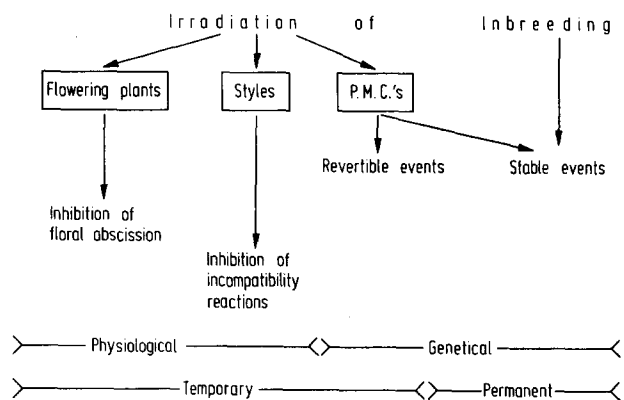


Fig. 1. Classification of radiobiological and inbreeding effects on the gametophytic system of self-incompatibility

mitted from the treated generation to the next, genetical effects are either temporary (reversible events) or permanent (stable mutations). Mention is also made, on Figure 1, of the inbreeding effects which have been observed by a number of researchers (DENWARD, 1963; HOGENBOOM, 1968; MARTIN, 1968; DE NETTANCOURT and ECOCHARD, 1969) and which greatly increase the complexity of the problem. Such inbreeding effects are not necessarily connected to irradiation treatment and may perhaps appear irrelevant to the subject treated in this review. Yet, the feeling prevailed that inbreeding, which is a direct consequence of radio-induced self-compatibility, was too intimately related to the modification and breakdown of self-incompatibility for being discarded from the present analysis.

I. Physiological Effects of Irradiation

A. *Chronic Irradiation of Flowering Plants.* A very indirect effect of irradiation upon the physiology of self-incompatibility has recently been described in *Lycopersicon peruvianum* by DE NETTANCOURT and ECOCHARD (1968). In this case, the irradiation treatment applied did not appear to mutate the S locus or to inhibit the incompatibility reaction but simply reduced the frequency of floral abscission and thereby increased the probability that a fruit would be formed. The probability that such a fruit contained seed (pseudo-compatibility) being usually unaffected by the treatment, the overall seed-set per plant was greatly increased by irradiation. The nature of this irradiation effect is not known but it is probable that it inhibited the formation of the abscission layer which controls flower-dropping in self-incompatible species of tomatoes. A number of slow growing pseudo-compatible pollen tubes (escapes) were therefore able to assume their fertilizing functions in the ovary of a flower which would normally have fallen down several days earlier.

B. *Acute Irradiation of Styles.* That irradiation treatment could act directly upon the self-incompatibility reaction in the style was most beautifully demonstrated in *Petunia* by LINSKENS and his coworkers (1960). These authors were able to show that a dose of 2000 rad of X-rays applied to the style immediately before selfing could break down the incompatibility reaction and induced approximately 50% of the treated flowers to yield seeds. Only a weak effect (pollen-tube growth) was observed when pollination was done 24 hours after style irradiation whereas no effect could be recorded when pollination was carried out 20 hours before irradiation.

The finding that a relatively low dose was required to produce the desired effect together with the fact that maximum sensitivity occurred when pollination immediately followed irradiation treatment is highly suggestive of a gene inactivation process which was

temporarily induced in the style. Direct destruction of incompatibility substances in the style would not be expected to take place at such low doses (VASIL'EV, 1962) and at a stage where the signal contact between mature pollen grains and the incompatible style has not yet been established. Results very similar to those of LINSKENS *et al.* have recently been reported by HOPPER and PELOQUIN (1968) who irradiated styles of *Lilium longiflorum* with different dosages. The data of HOPPER and PELOQUIN indicate, however, that the dose required to inhibit the incompatibility reaction in *Lilium* styles ranges from 6000 to 24000 rad and is therefore much higher than the optimum dose of 2000 rad estimated by LINSKENS *et al.* Among other explanations, HOPPER and PELOQUIN suggest that the observed difference in sensitivity between *Lilium* and *Petunia* may be due to variations in stylar thickness, *Petunia* having a thin solid style and *Lilium* a thick hollow style. Such a hypothesis does not appear founded because the penetrating power of 250 kV of X-rays, which were used in both the experiments of LINSKENS *et al.* and of HOPPER and PELOQUIN, greatly exceeds the few millimeters in thickness of a *Lilium* style. The alternative explanations of HOPPER and PELOQUIN (fundamental difference in target or target sensitivity between the two species) appears much more appropriate and may be related to large variations in cell numbers between *Petunia* and *Lilium* styles or to a size difference between the S loci of the two species. HOPPER and PELOQUIN underline the fact that the results obtained after style irradiation are very similar to those which follow heat treatment and suggest enzyme inactivation as a possible cause of the radiation effect.

II. Genetical Effects of Irradiation

A. *Determination of the sensitive stage.* LEWIS (1949a) was the first to find that mutations induced at the S locus could not express themselves in the pollen if irradiation treatment was applied after meiosis. This author concluded that the incompatibility reaction is laid down in the cytoplasm at the time of tetrad formation. Whereas this conclusion is very probably valid, one must not forget that LINSKENS *et al.* (1960) observed irradiation effects on self-incompatibility immediately after irradiation of pollen grains and one must also remember that another gametophytic gene, the *waxy* locus, has been observed to express mutations in the treated generation after irradiation of microspores which had not yet undergone the last DNA synthesis before pollen maturation (DE NETTANCOURT and ERIKSSON, 1968). Yet, it is possible that the effect described by LINSKENS *et al.* (1960) was physiological and not mutational and it is even more likely that the *waxy* gene and the S locus do not carry on their coding functions at the same stage of microsporogenesis.

As it is obvious, furthermore, that irradiation of megasporogenetic stages is very impractical and leads to eventual mutations in self-compatibility which are shielded by the style barrier and which cannot be selected for in the treated generation, the great majority of experimentators have therefore restricted their studies to irradiation of Pollen Mother Cells and earlier stages during anther formation. A summary of the results obtained under such conditions is given in the two following sections.

B. Reversible events. After irradiation of PMC's or of still earlier stages many workers (LEWIS, 1951; LEWIS and CROWE, 1953; PANDEY, 1956; DAVIES and WALL, 1961) have found that some of the pollen which is subsequently formed appears to have lost its specificity and is able to grow down the incompatible style and to carry on its fertilizing function. At the following generation, however, self-compatibility is no longer observable and the irradiation effect is demonstrated to have been only temporary.

LEWIS (1951) and PANDEY (1956, 1959) who have reviewed the considerable literature dealing with the subject of pseudo-compatibility have both concluded that these revertible events which occur spontaneously or after irradiation treatment must not be ascribed, in the majority of cases, to environmental or artificial pseudo-compatibility but rather to specific genetic phenomena which lead to a temporary loss of activity at the S locus. The arguments which LEWIS (1951) used to demonstrate the genetic nature of revertible events as well as the four hypotheses which this author considered as possible explanations to such

a genetic phenomenon are listed in Figure 2. Because all recorded data fitted best the AUERBACH Theory (1950) of Reversible Mutations, LEWIS concluded that the revertible events which he observed in his material (irradiated and non-irradiated plants of *Oenothera organensis*) were probably due to a labile premutation of the S locus which subsequently revert, after a certain number of cell divisions, to the original allelomorph. The evidence of LEWIS is essentially based on the fact that revertibility was found to occur, before pollination, at a frequency which is closely related to the number of cell divisions taking place after irradiation treatment. LEWIS (1951), however, did not entirely dismiss the hypothesis of a cytoplasmic modification which would lead to the loss of large numbers of cytoplasmic components. At the time he reported these conclusions, LEWIS was considering the S locus to be composed of both a pollen and a style part. It is obvious that revertibility can be readily explained if one assumes, as LEWIS (1960) did later on, that the S locus comprises an activity cistron controlling antigen activity in the pollen (P) and in the style (S) and a specificity cistron common to both the pollen and the style reaction. Any stable and constructive change in the specificity cistron will result into the formation of a new specificity which shall enable the mutated pollen grain to grow after selfing. At the next generation the new specificity will be active in both the pollen and the style and self-incompatibility will reappear. In this case, however, the new specificity should be detectable by means of back-cross tests to the original stock and this has never been observed by LEWIS (1951).

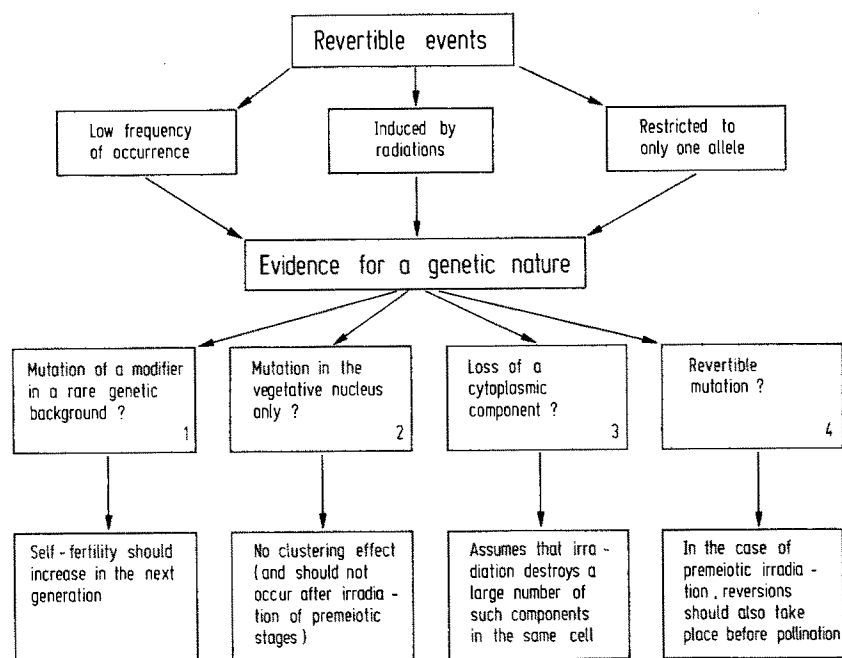


Fig. 2. Incomplete summary of LEWIS' argumentation for supporting the hypothesis that revertible events correspond to revertible mutations (see LEWIS, 1951)

C. Stable mutations. Whereas revertible events have only been searched for and found in the pollen, three types of stable mutations are described in the literature: 1. pollen reaction mutations, 2. style reaction mutations and, 3. both pollen and style reaction mutations. All these mutations are referred to as losses or permanent inactivation and no changes in specificity have ever been reported to occur after radiation treatment. Such a finding is rather unexpected if one considers, as LEWIS did in 1951, that the S gene, 'with its many alleles each having a specific and different effect appears to be pregnant with possibilities for constructive mutations'. Whereas the most obvious conclusion to LEWIS' statement is that irradiation, being

essentially a deleting agent, cannot be expected to induce constructive mutations, the possibility also exists, as was hinted by LEWIS, that the *S* gene is a very special gene which cannot be studied by means of conventional techniques. Discussion of inbreeding effects in the last section of this paper may help to throw some light on the matter.

An attempt has been made, in Figure 3, to classify the various types of stable mutations reported so far and to establish, for each type, the various radio-induced events which may have given rise to the observed modification in self-incompatibility. In each case, the results are considered on the basis of the two hypotheses of LEWIS (1960) (1: pollen part — style part; 2: activity part — specificity part) on the structure of the *S* genes. For sake of clarity more complex models of the *S* locus (LINSKENS, 1965; ASCHER, 1966) have not been incorporated in Figure 3 and shall be discussed in a next section. One can see from Figure 3 that pollen reaction mutations (LEWIS, 1947a, 1947b, 1949a, 1949b, 1961; BREWBAKER, 1954; BREWBAKER and SHAPIRO, 1959; BREWBAKER and NATARAJAN, 1960; PANDEY, 1956, 1959) and pollen + style mutations (PANDEY, 1956) can readily be explained in terms of cytological events which all lead to a duplication of the *S* locus (competition effect in the pollen, as demonstrated by BREWBAKER and NATARAJAN in 1960) or to a deletion or a crossing-over event at the incompatibility locus (genetic losses suggested by LEWIS, 1951, and PANDEY, 1956). These two interpretations are not necessarily conflicting but one must remember that PANDEY (1965) has clearly shown that the usual presence of a centric fragment in pollen part mutants of *Petunia* and *Nicotiana* needed not to be attributed to a competition effect between two *S* alleles and could equally well be explained by the assumption that the centric fragment restitutes to the pollen a vital gene which is linked to the incompatibility locus and which was deleted or inactivated at the time the *S* mutation took place. PANDEY's arguments, and the fact that centric fragments in pollen part mutants have never been observed outside the *Solanaceae* family, strongly indicate that most pollen part mutations must be ascribed to genetic losses rather than to competition effects in the pollen grain. Depending upon the size of the induced change and regardless of which of the two *S* models of LEWIS (1960) is considered, deletion or unequal crossing-over can lead to a permanent loss of the pollen reaction alone, the style reaction alone, or of both (Fig. 3). In those cases, however, where as reported for *Prunus* (LEWIS and CROWE, 1954) and for *Trifolium* by PANDEY (1956) only the style reaction is inhibited, it is difficult to understand how the mutation can be selected for after bud irradiation, both the unmutated pollen reaction in the pollen grain and the unmutated style acting as a screen against fertilization. In order to account for this objection, the assumption is made

in Figure 3 that a mutation affecting the style reaction alone must have been coupled, in the same pollen grain, to a revertible mutation affecting the pollen reaction. This revertible mutation is supposed to have opened the way to the germinating pollen grain and to have reverted once the style barrier had been passed (LEWIS and CROWE, 1953).

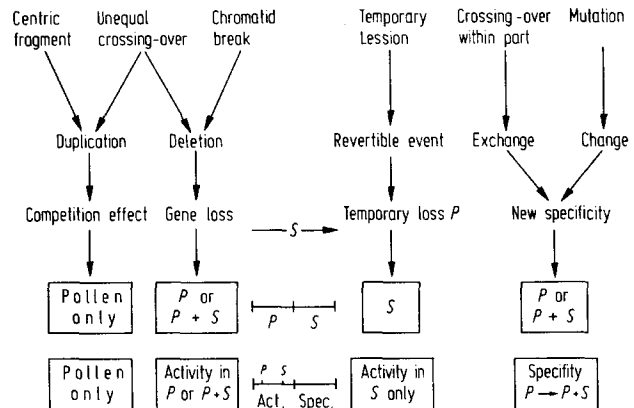


Fig. 3. Radio-induced events which may lead to stable changes at the *S* locus. Except for within-part crossing-over and true point-mutations, all events result into a loss of the pollen or style specificity reactions (1st gene model of LEWIS) or into a loss of the pollen or style activity reactions (2nd model of LEWIS)

Another puzzling element is that the frequency and the distribution of the three types of stable genetic losses described above appears to be highly dependent upon the test species involved, *Trifolium repens* ($2n = 32$) being apparently the only species in which all three categories of mutations can be induced by irradiation (PANDEY, 1956). Both stylar and pollen mutations were occasionally recorded by LEWIS and CROWE (1954) in *Prunus avium* whereas all radio-induced changes were restricted to the pollen reaction in *Petunia* and *Oenothera*. The situation is even more disappointing in *Lycopersicum* where neither pollen part nor style part mutations have been reported by the various workers (DAVIES and WALL, 1961; HOGENBOOM, 1968; DE NETTANCOURT and ECOCHARD, 1968) who have attempted to use irradiation for inducing self-compatibility. Independently of the fact that the structure and the mutability of the *S* locus may not necessarily be the same in all species with a gametophytic type of self-incompatibility, these great variations in effects can also be explained by the simple assumption that close linkage may exist, in some plant species, between certain parts of the *S* locus and a vital gene regulating pollen tube growth or embryonic survival. Any mutation affecting both this vital gene and the *S* locus would not be viable in a true diploid species. The situation is probably not that simple, however, since LEWIS found that sensitivity not only varied from species to species (1949a) but also from allele to allele, only

two out of four alleles tested being either revertibly or permanently mutated by X-ray treatment in *Oenothera organensis* (1951). LEWIS observed, in this case, that the allele which accompanied the mutating allele appeared to influence the direction of mutation. Such a finding definitely opens the way to interesting speculations on the high specificity of the genetic mechanisms which govern radio-sensitivity and repair processes.

Another discovery of LEWIS (1949a) is that none of the pollen reaction losses which were induced by X-rays at the *S* locus had any deleterious effect, even when homozygous, on the viability and fertility of the diploid plant or on the male and female gametophytes. This observation, explained by LEWIS on the basis of a very efficient process of haplontic selection, demonstrates that mutations can be induced by radiations which are extremely discrete and restricted to only part of the *S* locus. Hence, the *S* system constitutes for the radiogeneticist a material which not only leads to automatic transmission of the mutation to the next generation but also selects exclusively, when irradiation is applied to PMC's, for intragenic mutations or very minute deficiencies. The elaborate experiments of LEWIS clearly show, furthermore, that the self-compatible mutation is a reliable criterium for refined studies in radiobiology. Because he was handling millions of individuals (pollen grains) rather than a few thousands mice or hamsters, LEWIS (1949a) was able to demonstrate, some 20 years ago, in the course of a single experiment with *Prunus avium* and *Oenothera organensis*:

1. The bipartite structure of a genetic locus.
2. The influence of temperature on the radiosensitivity of certain meiotic stages.
3. The stage-sensitivity relationship.
4. The linearity of dose-responses.
5. Variations in the radiation responses of different alleles.
6. An interallelic dependance on mutability.

With the possible exception of the *waxy* locus in cereals, no other system than the *S* gene in higher plants appears to provide more attractive possibilities for mutation research on multicellular organisms.

Figure 3 also makes allowance for the two mechanisms (true point mutations or crossing-over within the gene) which could eventually lead to the formation of new specificities (PANDEY, 1956; LUNDQUIST, 1965). The occurrence of such events remains of course highly speculative since no report has ever been made of the radio-induction of a new specificity at the *S* locus.

III. Consistency between Radiobiological Data and the Biochemical Models of Self Incompatibility

As was stated in the beginning of this review, radiobiology can in many ways contribute to the biochemistry of self-incompatibility. One aspect of this contribution lies, at the moment, in the fact that the

elaboration of a biochemical model of self-incompatibility must be consistent with the data obtained by the experimentators who have irradiated specific stages of the incompatibility reaction and have observed precise patterns of physiological and genetical reactions. In other words, the biochemical model of self-incompatibility must account for the fact that:

1. Irradiation treatment, when applied on the style immediately before pollination, can inhibit the incompatibility reaction (LINSKENS *et al.*, 1960; HOPPER and PELOQUIN, 1968).
2. The optimum radiation doses which induce this effect are relatively low and, at any rate, well below the dosages required for breaking down a molecular structure.
3. Duplication of genetic material in the pollen leads to a competition effect which, in turns, inhibits the incompatibility reaction (STOUT and CHANDLER, 1944; LEWIS, 1947a, 1960; ATWOOD and BREWBAKER, 1953; BREWBAKER, 1954; BREWBAKER and SHAPIRO, 1959; BREWBAKER and NATARAJAN, 1960).
4. Duplication of genetic material in the style does not appear to impair the incompatibility reaction.
5. Mutations can be induced which may involve only the pollen reaction or the style reaction and which therefore suggest that different coding units in the pollen and in the style are participating to the incompatibility reaction.

Whereas it is up to the biochemist to estimate the usefulness of such information and to request, eventually, additional radiation work in the field, one may perhaps, before concluding this chapter, briefly describe some of the biochemical models which have been proposed in the past and attempt to establish a relationship between these models and the radiobiological information available to date. The establishment of such relationships is of course still highly speculative but constitutes, nevertheless, an obvious approach towards a better understanding of both the radiation effect and the biochemistry of self-incompatibility.

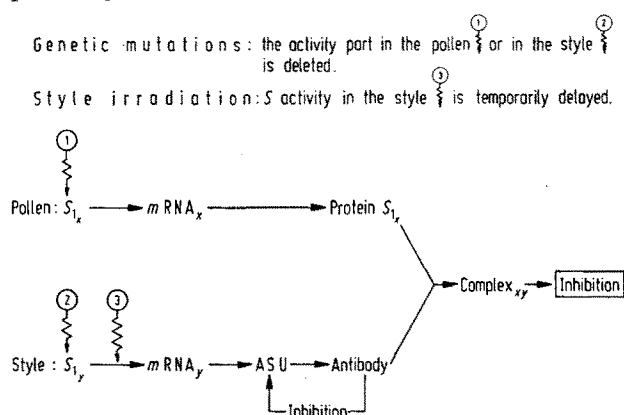


Fig. 4. Consistency between radiobiological data and LINSKENS' model (1965) of the self-incompatibility reaction

Many biochemical models of the self-incompatibility reaction have been discussed and proposed in the past (for a review, see LINSKENS, 1961; PANDEY, 1962; LEWIS, 1965; HAVEZ, 1967; LINSKENS, 1967; PANDEY, 1967) but one of the best known is probably that of LINSKENS (1965) who adapted, on the basis of the genetical evidence obtained by LEWIS (1960), TALMAGE's hypothesis (1959) on the existence of an 'antibody synthesizing unit' (ASU). LINSKENS suggests (Fig. 4) that the *S* allele in the style codes, via this antibody-synthesizing unit, an Y antibody which can, when in excess, inhibit its own production (cross-pollination) or combine with an X protein in the pollen tube to form an inhibiting X-Y complex (self-pollination). It is simple, when LINSKENS' model is considered, to explain irradiation effects in the following manner:

1. *Style inhibition*: irradiation shortly prior self-pollination inactivates the *S* alleles in the style so that antibody production can no longer take place at the time the pollen germinates (Fig. 4). The X-Y complex is not formed and pollen tube growth proceeds unimpaired. If, however, the pollen is applied on the flower several hours before irradiation, the X-Y complex is already produced when irradiation treatment inactivates the *S* locus in the style and pollen tube growth remains inhibited. On the other hand, when pollination is made 20 hours after irradiation LINSKENS only observes a weak irradiation effect because, gene inactivation being only temporary, the *S* locus has resumed its function after the first few hours which followed treatment.

2. *Genetic mutations*: LINSKENS' model is compatible with the finding that a mutation can be revertible (inactivation of the *S* locus in pollen) or permanent (gene loss). Pollen mutations would derive from the loss of the pollen part on the activity cistron; as a result of this loss no X proteins would be formed which could combine with the stylar antibody. Similarly, a style mutation would derive from a loss of the style part on the activity cistron which would enable it to control the synthesis of the antibody in the style.

Another biochemical model of the incompatibility reaction has been published by ASCHER (1966) which also appears to be consistent with the radiobiological data available. ASCHER's model is based on the operator-regulator system of JACOB and MONOD (1961) and makes provision for three different sites of genetic activity in the pollen (Fig. 5):

— A low velocity operon which controls germination and early pollen growth and which may eventually be switched off when the pollen reserves are exhausted.

— A high velocity operon, responsible for pollen growth through the stylar tissue and which is switched on by the stylar metabolites.

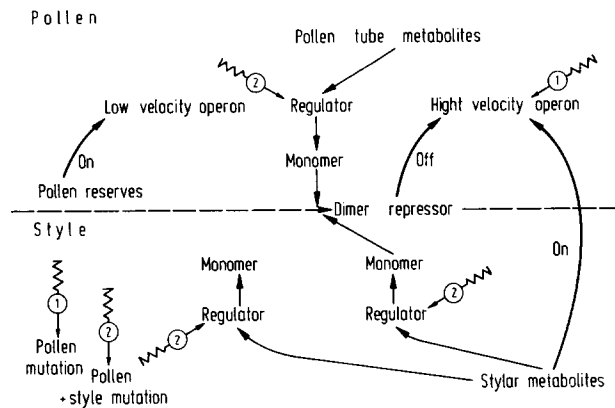


Fig. 5. Consistency between radiobiological data and ASCHER's model (1966) of the self-incompatibility reaction. Any mistake or inaccuracy in the readaptation of ASCHER's model is the sole responsibility of the present author

— A regulator which contains the necessary information for coding a specific monomer in the pollen tube.

In the style (Fig. 5), ASCHER considers only two sites of genetic activity, namely the two regulators which each code a specific monomer. When the monomer in the pollen tube is identical to one of the monomers in the style a dimer repressor is formed which switches off the high velocity operon in the pollen and, consequently, prevents pollen tube growth to proceed. The incompatibility reaction has taken place. As pointed out by ASCHER himself this highly speculative but attractive model is consistent with most radiobiological facts. Irradiation effects on styles can be explained, like in the case of LINSKENS' model, by a temporary inactivation of the regulator whereas pollen part mutations can readily be accounted for by a transformation of the high velocity operon which would become tolerant to the dimer repressor. Style part mutations are, however, more difficult to explain on the basis of ASCHER's model unless one assumes that a bipartite locus (with a style part and a pollen part in the activity cistron) composes the regulator allele. In this connection, and if a parenthesis may be allowed at this stage of the discussion, it might perhaps be desirable to indicate that the terminology of LEWIS (1960) in reference to an activity cistron composed of a pollen part and a style part appears to be misleading. As defined by the CIS-TRANS test, a cistron is a functional unit (BENZER, 1955) which cannot be subdivided into further units of function. It should therefore be more appropriate to refer to the activity cistron of LEWIS in terms of a coding complex or of a bipartite segment of activity regulation.

4. Inbreeding Effects

As stated earlier, a discussion of inbreeding effects in a review paper devoted to the radiogenetics and

the radiobiology of self-incompatibility may perhaps appear to be out of place. Yet, one must not forget that inbreeding is the normal consequence to a mutation breeding programme for self-compatibility and one must also take into account the fact that inbreeding alone appears to be a powerful tool for inducing drastic changes in the behaviour of the *S* gene (DENWARD, 1963; DE NETTANCOURT and ECOCHARD, 1969).

Inbreeding effects, as described in the literature, can be classified in two categories: 1) progressive loss of self-incompatibility, 2) formation of new specificities.

It is relatively simple to explain the emergence of self-compatibility during inbreeding on the basis of recombinations of modifier genes which inhibit activity at the *S* locus. ATWOOD (1942), PANDEY (1960), LUNDQUIST (1961), RICK (1963), MARTIN (1963) and HOGENBOOM (1968) have clearly demonstrated, among others, the influence of the polygenic background upon the strength of the incompatibility reaction (for a discussion, see LUNDQUIST, 1965). A confirmation of this finding was recently provided by MARTIN (1968) who reported that the specificity of the incompatibility reaction in a wild tomato species is progressively lost when the *S* alleles are transferred into a self-fertile species. MARTIN observed that this loss appeared to be controlled by many genes and suggested that, in the tomato, self-incompatibility may have evolved towards self-compatibility by means of mutagenic changes in the genetic background.

What is, however, more difficult to understand is the formation of new specificities during inbreeding. The occurrences of such new specificities have occasionally been reported in the segregating progenies of self-incompatible individuals and usually attributed to a possible contamination by stray pollen (ATWOOD, 1942; LEWIS, 1951; WILLIAMS, 1951; PANDEY, 1956; MARTIN, 1961). DENWARD (1963) was really the first to report that the phenomenon was not necessarily due to accidental crossing and that new specificities occur regularly during inbreeding which become stabilized after a few numbers of generations. DENWARD's explanation to his observations is that the action of the *S* gene is strongly dependent upon the general genetic background of the cell and that the specificity of the incompatibility reaction is determined by the entire genotype of the plant. Inbreeding modifies the overall genetic background and therefore affects the behaviour of the *S* locus. LUNDQUIST (1965) compared this hypothesis to those of BATEMAN (1952) and PANDEY (1959), who attributed the formation of new specificities to recombination between very closely linked genes with cumulative effects, and indicated that FISHER (1961) gives the theory for such generation of *S* alleles upon

recombination. Whereas LUNDQUIST (1965) did not exactly dismiss the possibility that the *S* segment could be looked upon as a group of linked (BATEMAN, 1952) or unlinked (DENWARD, 1963) modifiers, he carefully underlined the fact that the specifying segment of the *S* locus forms a functional unit which as such should not be composed of different genetic entities. There is, indeed, a considerable bulk of genetic and biochemical evidence which indicates that the incompatibility substance is probably a specific protein coded by only one or few genetic units (LEWIS, 1960, 1965; LINSKENS *et al.*, 1960; LINSKENS, 1961, 1965; PANDEY, 1959, 1962; ASCHER, 1966). Modifier genes may repress the activity of the *S* locus but can hardly be expected to induce a qualitative change in the coding specificity of the locus itself. If recombination is responsible for the formation of new specificities one must assume that it derives only from intracistron crossing-over and not from intergenic exchanges.

The hypothesis of intra-cistronic or intra-genic recombination as an origin to the formation of new specificities implies, however, that the phenomenon should take place at random and only sporadically. This is not at all what has been observed by DE NETTANCOURT and ECOCHARD (1969, and unpublished) who found that new specificities in inbred individuals of *Lycopersicon peruvianum* occur in sudden waves on the plant and at very high frequencies.

In spite of the fact that many more investigations with highly inbred material or isogenic lines are still needed for elucidating the mechanisms involved, it therefore appears that the occurrence of new *S* specificities cannot be entirely explained on the sole basis of recombination or variation in the genetic background. In the search for additional explanations, it may be interesting, if not premature, to establish a connection between the inbreeding effects described above and a recent theory (EDSTRÖM, 1968) which supposes that chromosomes have the capacity to store information on each different allele present in the population. This exchange of information is assumed to take place between homologous chromomeres at meiosis and to result, under good conditions of outbreeding, into a situation where every member of the population carries on its chromosomes a duplicate copy of the different alleles segregating in the community. This very ingenious hypothesis, which is supported by only meagre evidence, could perhaps provide a clue to the occurrence of new specificities in an inbred population. Inbreeding, by means of an unknown mechanism, which may be controlled by both the external environment and the genetic background, would inactivate the *S* gene in charge and would switch-on one of the allelic copies previously stored during outbreeding. The hypothesis is essentially speculative and somewhat fantastic but may perhaps deserve further testing.

5. Conclusion

This brief review of irradiation and inbreeding effects on the gametophytic system of self-incompatibility in higher plants underlines the interest of the system for both fundamental and applied research in radiobiology.

In addition to the fact that irradiation treatment greatly contributes to plant-breeding sciences by providing the geneticist with self-compatible lines derived from a material which is naturally self-incompatible and unfitted for intensive selection, irradiation experiments also lead to a better understanding of the basic mechanisms involved in self-incompatibility. Much light has been shed on the time of action and on the bipartite structure of the S locus and a clearer knowledge has been gained of both the revertible and permanent mutations which modify the breeding systems of allogamous species. Undoubtedly, the time is now ripe for a detailed biochemical analysis of the radio-induced mutants which have been detected and isolated by the radiobiologists. The range of different mutations which could be placed at the disposal of the biochemist is still narrow but would nevertheless include all possible types of reaction losses in the pollen, the style and in both the pollen and the style.

It now remains for the radiobiologist to find an answer, among many others, to the puzzling fact that irradiation does not seem to have the property of inducing any true point-mutations which could result into a functional change of the specificity. Whereas a logical explanation appears to be that irradiation is essentially a deleting agent which cannot reconstruct within the gene (see discussion in SOBELS, 1968), one must also take into account LEWIS' suggestion (1951) that the S gene may be so structured that a constructive mutation cannot be induced or detected by means of conventional methods. The results obtained in inbreeding experiments suggest, at any rate, that the self-incompatible plant can handle a technique of its own for elaborating a new specificity when an increase in genetic polymorphism is required. Whether or not the process involved is controlled by modifier genes, recombination events at the S locus or activation of another allele on the chromosome remains, however, an open question for future research.

Zusammenfassung

Es wird ein Überblick gegeben über die temporären und permanenten Nachwirkungen einer Strahlenbehandlung von selbstinkompatiblen Pflanzen mit gametophytischer Determination. Die Untersuchung und Auswertung solcher Effekte kann zu Fortschritten auf dem Gebiete der Mutationszüchtung, der allgemeinen Strahlenbiologie und der Biochemie beitragen.

Die Tatsache, daß die Strahlenbehandlung lediglich negative Wirkungen hat (Inaktivierung der Inkompatibilitätsreaktion und/oder genetische Störungen am S-Locus), wird vergleichend diskutiert mit den positiven Änderungen (Auftreten neuer Spezifitäten) bei anschließender Inzucht.

Es wird in diesem Zusammenhang vorgeschlagen anzunehmen, daß die selbstinkompatible Pflanze möglicherweise mit einem Schalt-System oder einem mutagenen Mechanismus ausgerüstet ist, die sie in die Lage versetzen, eine neue Spezifität zu entwickeln, wenn diese zur Erhöhung des Niveaus des genetischen Polymorphismus in der Population notwendig ist.

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