## DREUX DE NETTANCOURT Incompatibility and Incongruity in Wild and Cultivated Plants

2nd Edition

Springer-Verlag Berlin Heidelberg GmbH

# Dreux de Nettancourt

# Incompatibility and Incongruity in Wild and Cultivated Plants

Second, totally revised and enlarged edition

With 41 Figures in 67 separate Illustrations and 19 Tables



Professor DREUX DE NETTANCOURT Université Catholique de Louvain Faculté des sciences agronomiques Unité de biochimie physiologique Croix du Sud 2/20 B-1348 Louvain-la-Neuve, Belgium

*Cover illustration:* Cross section of an incompatible pollen tube in the intercellular space of the stylar conducting tissue of *Lycopersicum peruvianum* (courtesy University of Siena). The endoplasmic reticulum shows a concentric parallel configuration (de Nettancourt et al. 1973 a)

The first edition was published under the title "Incompatibility in Angiosperms" as Vol. 3 of the Series "Monographs on Theoretical and Applied Genetics", Springer 1977.

#### ISBN 978-3-642-08457-7

Library of Congress Cataloging-in-Publication Data De Nettancourt, D., 1933 – Incompatibility and incongruity in wild and cultivated plants/Dreux de Nettancourt. – 2nd, totally rev. and enlarged ed. p. cm. Rev. ed. of: Incompatibility in angiosperms. 1977. Includes bibliographical references (p.). ISBN 978-3-642-08457-7 ISBN 978-3-662-04502-2 (eBook) DOI 10.1007/978-3-662-04502-2 1. Angiosperms. 2. Plant genetics. 3. Pollination. 4. Plant breeding. I. De Nettancourt, D., 1933 – Incompatibility in angiosperms. QK495.A1 D46 2001 581.3'5 – dc21

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilm or in any other way, and storage in data banks. Duplication of this publication or parts thereof is permitted only under the provisions of the German Copyright Law of September 9, 1965, in its current version, and permission for use must always be obtained from Springer-Verlag. Violations are liable for prosecution under the German Copyright Law.

© Springer-Verlag Berlin Heidelberg 2001 Originally published by Springer-Verlag Berlin Heidelberg New York in 2001 Softcover reprint of the hardcover 2nd edition 2001

The use of general descriptive names, registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

Production: Klemens Schwind Cover design: design & production GmbH, D-69121 Heidelberg Typesetting: K+V Fotosatz GmbH, Beerfelden

SPIN 10695077 31/3136 5 4 3 2 1 0 – Printed on acid-free paper

To Gabrielle, my wife, for her encouragement, patience and everlasting guidance regarding the word processor

## Preface

The aim of this book is to provide a picture, as complete as possible, of the current state of knowledge of pollen-pistil barriers in flowering plants and of the ability of man to silence, mutate or transfer the genes that control them. The work was conducted in two phases. The first one essentially consisted of a review of early work, such as it had been summarized in a monograph I wrote in 1977 regarding the main features, origin and classification of self-incompatibility systems, their distribution among the angiosperms, the modalities of their inheritance at the scale of individuals and populations, the mutability of SI genes, and their possible involvement in the control of barriers between species. The major part of this information was derived from the 1977 monograph and systematically enriched (when material was available) by an account of more recent or contemporary research on the distribution, genetics and population genetics of SI. The second phase, by far the most challenging, consisted of the description and review of the very impressive and abundant research results that have accumulated, since the early 1980s, on the cellular and molecular biology of pollen-pistil interactions. The nature of this research, although the ultimate objectives (understanding and control of pollen-pistil barriers) remained unchanged, was radically modified by the new ability of man to perform analyses of life at the molecular level and to exploit all the techniques of modern biology to study the structure, action, function, mutation, reconstruction and evolution of the genes and mechanisms that participate in the recognition and rejection of incompatible pollen grains and pollen tubes.

A major difficulty in the preparation of a book of this type, which covers the interactions of many different disciplines, deals with the classification and distribution of information. It is necessary to compromise between repetition and overlap, essential if each chapter is to be considered separately, and crossreferencing, required for the unity of the work. Furthermore, I have not been able to avoid the creation of an opening chapter devoted to a general presentation of pollen-pistil barriers; it is not intended to summarize the book but to provide the perspective necessary for direct access to the matter covered by the other chapters. Again, I had to choose between cross-referencing and the duplication of information.

### The Role of Professor Linskens

The person to blame is Professor H.F. Linskens. Not only did he suggest the first edition of this book approximately 25 years ago, he proposed this "bis repetita placent" episode of pollenpistil tribulations. However, he meant well. I must say that Professor Linskens tried to make up for his impulses through his constant support and the regular transmission of a very impressive amount of information. I not only owe him the text of articles by Correns (1913), Sutton (1918), Brieger (1930) and Sears (1937) but also articles discussing the latest state of the art (to the end 1999) of pollen-part mutations, the dimer hypothesis, pollen-tube-directed differential ovule development and the biochemistry of haplotype dominance. Through him, from several of his six working addresses in Europe and America, I received extensive collections of abstracts from recent papers that dealt with all possible aspects of pollen-pistil interactions. None of my mistakes, misinterpretations or omissions can be attributed to him, but he must be credited for his support and the suggestions that enabled me to improve my work.

## Help from the Scientific Community at Large and from Colleagues at Louvain-la-Neuve

Other people, in addition to Professor Linskens, gave advice and information. Dr. V.E. Franklin-Tong kindly provided explanations of the functional significance of the interaction between stigmatic S-proteins and SPB, a pollen protein, in the field poppy (Jordan et al. 1999, but unpublished at the time). She informed me, on this occasion, of the discovery, by a Birmingham group (N.N. Jordan and co-workers), of the first evidence that programmed cell death was involved in the rejection phase of SI. Professor B.A. McClure kindly commented on the research results at his laboratory and provided unpublished information and an illustration (Fig. 5.3) regarding work dealing with unilateral inter-species incompatibility. Professor Wehling described the results of his attempts to test the Bm2 probe of *Phalaris coerulescens*, supplied by Professor Langridge, as a candidate for the S-gene in rye. Professor M. Cresti contributed the unpublished micrographs of incompatible pollen tubes, which are now Fig. 3.1 and Fig. 5.1.

I thank all of them wholeheartedly, and I also express my deep gratitude to the scientists and the publishers who so kindly contributed photographs, drawings or tables from early or recent work and authorized me to use them in my book. Their very kind cooperation is acknowledged in the captions of figures and tables.

Finally, I want to thank the University of Louvain and the Unit for Physiological Biochemistry (FYSA) for the hospitality and the help that was generously and kindly given to me. My gratitude goes, in particular, to Professor A. Goffeau, former director of FYSA, and to Professor M. Boutry, who replaced him after his retirement. I appreciate the instructive discussions I had with them on the expression, cloning, sequencing and reconstruction of genes from yeast and higher plants.

Mr. Philip Menier competently advised and participated in the treatment and harmonization of 1200 references from several bibliographies, which Mrs. C. Durand reconstituted in part and which, at a later stage, Mrs. M. Rochat processed, checked and finalized with admirable patience. They cannot be blamed, of course, for flaws in the raw material provided to them. Mrs. A.M. Faber skillfully prepared the assembly of microphotographs and the computer drawing of diagrams.

D. DE NETTANCOURT

Emeritus Professor, Physiological Biochemistry Unit, Catholic University of Louvain

## Contents

1	The Basic Features of Self-Incompatibility	1
1.1	A Definition	1
1.2	Nature of the SI Reaction	2
1.3	Classification of SI Systems	3
1.3.1	The Time of Gene Action in the Pistil	3
1.3.2	The Time of Gene Action in the Stamen	4
1.3.2.1	Determination of the Pollen Phenotype	
	in Gametophytic Systems	4
1.3.2.2	Determination of the Pollen Phenotype	
	in Sporophytic Systems	6
1.3.3	The Association with Floral Polymorphism	7
1.3.3.1	The Distylic Condition	8
1.3.3.2	Tristyly	9
1.3.4	The Site of Gene Expression	10
1.3.4.1	Stigmatic Inhibition	10
1.3.4.2	Stylar Inhibition	11
1.3.4.3	Ovarian Inhibition	12
1.3.5	The Number of Genetic Loci	
	and the Involvement of Polyallelic Series	13
1.3.5.1	The Genetic Basis of Recognition	13
1.3.5.1.1	Control by a Single but Complex Locus	13
1.3.5.1.2	Recognition by Two Unlinked Loci	
	in the Grasses	14
1.3.5.1.3	Recognition by Two or More Loci	
	in Several Other Families	14
1.3.5.2	Polyallelism at the Incompatibility Loci	14
1.3.5.3	How Many Genes are Involved	
	in the Rejection Process?	15
1.3.5.3.1	Stigmatic SSI	15
1.3.5.3.2	Stigmatic GSI	15
1.3.5.3.3	Stylar GSI	15

1.4	Recapitulation on the Classification of SI Systems	16
1.5	The Distribution of SI Systems	17
1.5.1	Incidence of SI in the Families	17
1.5.2	Distribution of SI among Species Important for Agriculture	21
1.6	Chronology of Early Researches on SI	23
2	The Genetics of Self-Incompatibility	25
2.1	Sporophytic Heteromorphic Systems	25
2.1.1	Distyly	25
2.1.1.1	A Supergene	26
2.1.1.2	Within Which Recombination Occurs	27
2.1.1.3	The Supergene is Controlled	
	by Modifier Genes	28
2.1.2	Tristyly	28
2.1.2.1	Homomorphic Variants and Supergenes	
	in Tristyly	30
2.1.2.2	One Genotype, Two Phenotypes	30
2.1.2.3	Dominance Change in O. articula	30
2.1.2.4	Breeding Behavior Can be Independent	
	of Floral Heteromorphism	31
2.1.3	Multi-Allelic Series in Species	
	with Incomplete Heterostyly?	31
<b>ว</b> ว	Charaphytic Homomorphic Stigmatic Control	21
2.2	Two Di Allelie Leei	21
2.2.1	A Single Leave with Delvellelie Series	51
2.2.2	A Single Locus with Polyanence Series,	
	the Practice Type	37
2221	The Brassica Haplotunos	34
2.2.2.1	Class I Haplotypes	34
2.2.2.1.1	Class II Haplotypes	35
2.2.2.1.2	Extension of the Hanlotype Concent to Other	55
4.4.4.4	Canas Other Families and Other Systems	36
2222	Schools-Related (SLR) Canas in <i>Brassica</i>	30
2.2.2.3	Many Genes in the S-Linbage Group	37
2.2.2.4 ))	A Single Sporophytic Stigmatic Locus	57
4.4.J	with Multiple Alleles but without Dominance	
	and Competitive Interaction	38
224	Three or Four Polyallelic Loci in Fruca sativa	38
2.2.T	Thee of four foryunche Loci in Lincu sullin.	50

and the second se

2.3	Gametophytic Homomorphic S Systems with Polvallelic Series	39
2.3.1	One-Locus Stigmatic Control:	
	the Case of the Style-Less Field Poppy	39
2.3.1.1	Genetics of SI Polymorphism in P. rhoeas	
	and Other Species with Polyallelic,	
	Monofactorial SI	40
2.3.1.2	The Number of S Alleles in <i>P. rhoeas</i>	42
2.3.2	Two Loci-Stigmatic Control in the Grasses	42
2.3.2.1	Breeding Efficiency of the S–Z System	43
2.3.2.2	The Size of Polyallelic Series	43
2.3.3	Four-Loci Stigmatic Gametophytic Control	
	in the Ranunculaceae, the Chenopodiaceae	
	and the Liliaceae	45
2.3.3.1	Few Alleles per Locus in Tetra-Factorial	
	Stigmatic GSI	46
2.3.3.2	Linkage Between the Four SI Genes	47
2.3.3.3	SI is Maintained in Tetraploid R. repens	47
2.3.4	Monofactorial Stylar GSI	
	with Polyallelic Series	47
2.3.4.1	The Size of Polyallelic Series	
	in Stylar Monofactorial GSI	48
2.3.4.2	The Structure of the S Locus	
	in Stylar Monofactorial GSI	49
2.3.4.3	Identification of S-Bearing Chromosomes	50
2.3.5	Bifactorial Stylar GSI	
	with Epistatic Relationships	50
2.3.6	Three or Four S Loci in a Complementary	
	System of Lotus tenuis	52
2.3.7	Ovarian Gametophytic SI	52
2.3.7.1	Post-Zygotic OSI?	53
2.3.7.2	Cyclic, Post-Zygotic, Polygenic SI	54
2.3.7.3	Incompatible Pollen Tubes That Prevent	
	Ovule Development	54
2.4	Sporophytic-Gametophytic Systems	55
2.4.1	Three Genes Participate in the Ovarian	
	Gametophytic-Sporophytic System	
	of Theobroma cacao	55
2.4.2	Sporophytic Stigmatic SI Revisited	
	in the Cruciferae and the Compositae	56
2.4.3	A One-Locus Sporophytic System	
	with Traces of Gametophytic Pollen Control	
	in the Caryophyllaceae	58

2.5	Genes Involved in the Rejection Phase of SI	59
2.5.1	In Stigmatic SI Systems	59
2.5.2	The Rejection Phase in Species	
	with Stylar GSI	61
2.5.3	SI in the Ovary	61
2.6	SI in Polyploids	62
2.7	Fauilibrium Frequencies of SL Alleles	62
2.7.1	Two Alleles at One Locus	
	in a Sporophytic System	62
2.7.2	Trimorphism	63
2.7.3	One Polyallelic Locus	
	in a Sporophytic System	63
2.7.4	Polyallelic Series in a Monofactorial	
	Gametophytic System	64
2.7.5	Two Polyallelic Gametophytic Loci	64
2.7.6	The Number of Possible Allelic Combinations	
	in Theobroma	65
2.8	The Maintenance and Efficiency	
	of Incompatibility Systems	65
2.8.1	Population Sizes and Numbers	
	of Incompatibility Alleles	66
2.8.1.1	Smallest Numbers of Alleles Required	66
2.8.1.2	"Molecular Restraints to the Coding Capacity	
	of the S Gene in <i>Papaver</i> "?	66
2.8.1.3	Consequences of High Numbers of Alleles	
	at the SI Loci	67
2.8.1.4	Linkage Effect as a Main Cause to Unequal	
	S-Allele Frequencies in British Populations	
	of P. rhoeas	67
2.8.2	The Selection of Rare Alleles	
	and Replacement Processes	67
2.8.3	Explanations to the Large Numbers of Alleles	
	Found in Oenothera, Trifolium, Carthamus	
	and <i>Lolium</i>	68
2.8.3.1	High Mutation Rates	68
2.8.3.2	Subdivisions of Populations	69
2.8.3.3	Migration and Hard Seed Carryover	69
2.8.4	The Efficiency of SI Mechanisms for Preventing	
	Unions Between Near Relatives	70
2.8.4.1	A Comparison of Parent-Offspring	
	Relationships in Heterostylic	
	and Gametophytic Systems	70

the second second second second

2.8.5	Effects of Pollen and Seed Dispersal, Overlapping Generations and Plant-Size Variations in Populations at Equilibrium	71
2.8.6	A New Mathematical Approach to SI Polymorphism in a One-Locus Gametophytic	
2.8.7	System The Concept of a Frequency-Equivalent	71
	Population	72
3	Cellular and Molecular Biology	
	of Self-Incompatibility	73
3.1	Heteromorphic Incompatibility	74
3.1.1	A System of Its Own	74
3.1.1.1	The Research Approaches are Different	74
3.1.1.2	Several Rejection Sites	74
3.1.1.3	Rejection Cascades	75
3.1.1.4	Stigmatic Rejection May Occur on Wet Stigmas	76
3.1.1.5	The Rejection Sites Are Not Always Typical	
	of a Sporophytic System	76
3.1.1.6	The Incompatibility Loci Are Usually Di-Allelic	76
3.1.1.7	Role of the Internal Environment	
	in the Specificity of Incompatibility Products .	77
3.1.2	Occurrence and Function of Stigma	
	and Pollen Polymorphism	78
3.1.2.1	Analyses of Pollen Walls	79
3.1.2.2	The Role of Pollen and stigma Dimorphism	
	on Pollen Affixation and Pollen Metabolism	81
31221	Effects of Differences in the Morphology	01
5.1.2.2.1	of the Stigmatic Cuticle and in the Sculpturing	
	of Pollen Exine	81
31222	Function of the Pollen Exine in <i>Jensonia</i>	83
31222	Availability of Evudate on the Stigma Surface	83
3.1.2.2.3 3.1.2.2.4	Variations in Osmotic Pressures	83
313	The Molecular Biology of Heteromorphic SI	84
2121	Identification of S Decognition Eactors	Q1
2122	In There a Fundamental Difference Between SI	04
5.1.5.2	in Heteromorphic Species and SI	
	in Species and St	05
	in sporophytic-Homomorphic Systems:	85
3.2	Homomorphic Sporophytic Stigmatic SI:	
	the Brassica Type	87
3.2.1	Morphology and Structure of Stigma	
	and Pollen Surfaces	87
3.2.1.1	The Stigma Surface	87
3.2.1.2	The Pollen Exine and the Pollen Coating	88

3.2.1.2.1	Differences in the Pollen Exine Sculpturing	
	Between SSI and GSI	89
3.2.2	The Route of the Compatible Pollen Tube	
	Through the Stigma	89
3.2.2.1	Self-Incompatible Brassica oleracea	89
3.2.2.2	Self-Compatible Arabidopsis thaliana	90
3.2.3	Stigmatic Proteins Involved in the Recognition	
	of Incompatible Pollen	91
3.2.3.1	Immunological Detection and Purification	
	of SLG	91
3.2.3.1.1	Purification of SLG	92
3.2.3.2	Essential Features of SLG	92
3.2.3.2.1	Cloning of the Gene Encoding SLG	92
3.2.3.2.2	The SLG Sequence	93
3.2.3.2.3	Structure of SLG and Homologies	
	Between Alleles	94
3.2.3.2.4	Nature, Origin and Frequency of Sequence	
	Variations Between Different Alleles	94
3.2.3.2.5	Co-Evolution of SLG and SRK	94
3.2.3.2.6	Structural and Functional Distinctness of SLG	
	in Class-II Haplotypes	95
3.2.3.3	The S-Receptor Kinase Gene, SRK	95
3.2.3.3.1	Essential Features of SRK	96
3.2.3.4	A Direct Method for the Cloning	
	of S Haplotypes	98
3.2.3.5	SLG and SRK Are Present, Often as Traces,	
	in Other Parts of the Brassica and Transgenic	
	Nicotiana Flowers	99
3.2.3.5.1	In the Pollen	99
3.2.3.5.2	In Anther Walls	99
3.2.3.5.3	In the Transmitting Tissue of the Stigma,	
	Style and Ovary	99
3.2.3.5.4	In Transgenic Tobacco	99
3.2.3.6	A Putative Receptor Kinase Gene	
	in Ipomoea trifida	99
3.2.3.7	SLG and SRK Have Many Relatives	100
3.2.3.7.1	Members of the S Multi-Gene Family	
	That Are Linked to the S Locus	101
3.2.3.7.2	S-Locus-Related Sequences in Arabidopsis	102
3.2.3.7.3	Relationship of SRK/SLG to the Putative Kinase	
	Receptor (ZmPK1) from Maize	102
3.2.3.7.4	ARC1, a Putative Downstream Effector for SRK	102
3.2.4	The S-Specific Pollen Determinant	102
3.2.4.1	Expected Features of the S Determinants	102
3.2.4.1.1	Allelism to the SRK or SLG Genes?	102

And the second second second

3.2.4.1.2	Likelihood of a Dimer Mechanism in SI Systems	
	of the Brassica Type	103
3.2.4.1.3	Linkage of Pollen and Stigma Determinants	
	to the S Haplotype	103
3.2.4.1.4	Sporophytic Expression	104
3.2.4.2	Contribution of the Tapetum to the Pollen Coat	
	and to SI	104
3.2.4.2.1	Contribution to Pollen Coating	104
3.2.4.2.2	Evidence That the Pollen Coating Carries	
	the Pollen S Determinant	104
3.2.4.2.3	Tapetal Origin of Pollen S Determinants?	105
3.2.4.3	The Search for the Pollen S Determinant:	
	Recent History	105
3.2.4.3.1	The S-Glycoprotein-Like Anther Protein	106
3.2.4.3.2	The S-Locus Anther	106
3.2.4.3.3	Pollen-Coat Protein Class A	106
3.2.4.3.4	Pollen-Coat Protein A2	107
3.2.4.3.5	SLL2-S9 and S-Locus Anther-Expressed	
	S9 Gene	107
3.2.4.3.6	The Systematic Analysis of S	
	and S-Related Regions	107
3.2.4.4	Finding the Pollen Determinant	108
3.2.4.4.1	The Gene Fulfils the Requirements	
	for the Hypothesized Pollen Determinant	108
3.2.4.4.2	SCR Is a Relative of PCPs	108
3.2.4.4.3	Origin (Sporophytic and Tapetal) of SCR	108
3.2.5	What Happens After an Incompatible	
	Pollination?	109
3.2.5.1	Pollen Capture by the Stigma	109
3.2.5.2	Relationships Between Pollen Hydration	
	and SI	110
3.2.5.3	Stigmatic S Glycoproteins Are Glycosylated	110
3.2.5.4	The Recognition of Incompatible Pollen	110
3.2.5.5	Rejection of Incompatible Pollen	112
3.2.5.6	The Role of Callose	112
3.3	Stigmatic Monofactorial Multiallelic GSI	
	in Papaver rhoeas	113
3.3.1	Compatible and Incompatible Pollinations	113
3.3.1.1	Morphology and Growth of Compatible Pollen	
		113
5.5.1.2	Incompatible Pollen Grains and Pollen lubes .	113
5.5.2	An in vitro Bioassay for the Study of Stigmatic	
	S Proteins – Pollen Metabolism and Pollen-	
	Stigma Interactions after Self-Pollination	114

3.3.3	Characterization of Stigmatic S Proteins	
	and Cloning of the Stigmatic S Gene	114
3.3.3.1	Isolation and Characterization	
	of the Stigmatic S Proteins	114
3.3.3.1.1	Isolation and Testing of Function	114
3.3.3.1.2	Co-Segregation with S Alleles	114
3.3.3.1.3	Characteristics of the Protein	115
3.3.3.1.4	S Activity, S Specificity and the Role	
	of Glycosylation	115
3.3.3.1.5	Polymorphism of S Sequences	115
3.3.3.1.6	The S Proteins Are Not Major Proteins	
	of the Stigma	115
3.3.3.1.7	The S Protein Is Not a Ribonuclease	116
3.3.3.2	Cloning and Nucleotide Sequencing	
	of the Stigmatic S Gene	116
3.3.3.3	Biological Activity of Mutant Derivatives	
	of the S Protein	116
3.3.3.4	Large Numbers of ORFs with Homology	
	to the Stigmatic S Gene of Papaver Are Present	
	in the Arabidopsis Genome	117
3.3.4	Pollen Genes That Participate in the SI	
	Response	117
3.3.4.1	Inhibition of Incompatible Pollen Tubes	
	Depends on Pollen-Gene Expression	117
3.3.4.2	Involvement of a Signal-Transduction	
	Mechanism in the SI Response	118
3.3.4.3	A Membrane Glycoprotein That Binds Stigmatic	
	S Proteins in Pollen	119
3.3.4.4	Programmed Cell Death Is the End Point	
	of the SI Response in <i>Papaver rhoeas</i>	121
2.4		
3.4	Stigmatic Bi-Factorial GSI in the Grasses	121
3.4.1	Flowers and Pollination	122
3.4.1.1	Stigma and Pollen	122
3.4.1.2		122
3.4.1.3	Self-Pollination	122
3.4.2	SI in Phalaris coerulescens	123
3.4.2.1	Identification of Restriction Fragments	100
	Linked to the Pollen S Gene	123
3.4.2.2	Bm2 is not the S Gene	123
3.4.2.3	Involvement of Inforedoxins in the SI	124
2 4 2		124
5.4.5 2 4 2 1	SI III Kye	125
5.4.5.1	Describer that the SI Mechanism Involves	125
2 1 2 1 1	ritosphorylation and is Ca Dependent	125
J.4.J.1.1		123

A REAL PROPERTY AND A REAL PROPERTY.

3.4.3.1.2	Gel Electrophoresis of Pollen Phosphoproteins	125
3.4.3.1.3	Effects of Inhibitors	126
3.4.3.2	A Model for the SI Mechanism in Rye	126
3.4.4	Applicability of the Model to All SI Species	
	of Grasses	127
3.4.4.1	The S and Z Loci Are Not Interchangeable	127
3.4.4.2	Conserved S Sequences of Brassica Amplify	
	S-Linked Fragments in Rye	127
3.5	Monofactorial Stylar GSI with Multiple Alleles:	
	the <i>Nicotiana</i> Type	128
3.5.1	Pollen-tube Morphology and Growth	
	in Compatible Styles	128
3.5.1.1	Observation under the Light Microscope	128
3.5.1.1.1	Role and Specificity of the Stigmatic Exudate .	129
3.5.1.1.2	Mitosis in the Generative Nucleus	
	of Petunia hybrida	129
3.5.1.2	Electron Microscopy	129
3.5.2	Morphology and Growth	
	of Incompatible Tubes	130
3.5.2.1	Incompatible Tubes of <i>N. alata</i>	
	under Epifluorescence Illumination	130
3.5.2.2	Electron Microscopy	131
3.5.2.3	The Role of Callose	132
3.5.2.4	Mitosis in the Generative Nucleus	
	of P. hybrida	132
3.5.3	Early Research on the Nature	
	of the SI Reaction	133
3.5.3.1	SI as a Process of Growth Inhibition	133
3.5.3.2	Is the S Phenotype of Mature Styles Determined	
	before Pollination?	133
3.5.3.2.1	Evidence from In Vitro Tests	133
3.5.3.2.2	Diverging Results	133
3.5.3.3	First Models of the Gametophytic Stylar	
	SI Mechanism	134
3.5.3.4	Towards the Detection of Stylar S Proteins	135
3.5.4	Isolation, Cloning and Sequencing of a Stylar	
	Protein Segregating with the S2 Allele	
	of <i>N. alata</i>	136
3.5.5	The S-Associated Glycoproteins Are Ribo-	
	nucleases, and SI Involves the Degradation	
	of Pollen RNA	137
3.5.6	Evidence that the S Proteins of Petunia and	
	Nicotiana Are Responsible for the S-Allele-	
	Specific Recognition and Rejection of Self	
	Pollen	138

3.5.6.1	Induction of Loss and Gain of Functions	
	at the S Locus of <i>P. inflata</i>	138
3.5.6.2	S-Allele-Specific Pollen-Tube Rejection	
	in Transgenic Nicotiana	139
3.5.6.3	Proof that Ribonuclease Is Involved	
	in the Rejection of Self Pollen	139
3.5.7	Main Features of the S-Ribonuclease Gene	
	and of Ribonucleases	139
3.5.7.1	Distribution and Structural Features	
	of the Gene	139
3.5.7.1.1	Solanaceae	140
3.5.7.1.2	Rosaceae	141
3.5.7.1.3	Scrophulariaceae	141
3.5.7.2	What Are the Effects of S Ribonucleases	
	on rRNA and mRNA?	141
3.5.7.3	Are the Effects of Ribonucleases Irreversible? .	142
3.5.7.4	Why Pollen Tubes Are Not Inhibited	
	in the Stigma	143
3.5.7.5	What Determines the S Specificity	
	of Stylar Ribonucleases?	143
3.5.7.5.1	The Role of the Carbohydrate Moiety	143
3.5.7.5.2	The Role of HV Regions	144
3.5.8	S-Gene Products in Pollen Grains	
	and Pollen-Pistil Recognition	145
3.5.8.1	The S-Ribonuclease Gene Is Expressed	
	in Developing Pollen Grains	145
3.5.8.1.1	N. alata	145
3.5.8.1.2	P. hybrida	145
3.5.8.1.3	<i>L. peruvianum</i>	145
3.5.8.2	but Pollen S Ribonucleases Do Not Determine	
	the Pollen S Phenotype	145
3.5.8.3	Involvement of Protein Kinase?	146
3.5.8.3.1	A Pollen Receptor-Like Kinase 1 in P. inflata	146
3.5.8.3.2	In Vitro Phosphorylation of the S Ribonucleases	
	from <i>N. alata</i>	147
3.5.8.4	The Role of Pollen Determinants	147
3.5.8.5	Current Research Regarding the Identification	
	of Pollen S Determinants	149
3.5.8.5.1	A Functional Genome Approach to Search	
	for the Pollen S Gene of <i>P. inflata</i>	149
3.5.8.5.2	Towards the Fine-Scale Mapping of the S Locus	
	in Petunia hybrida	149
3.5.8.5.3	Use of a Two-Hybrid System to Identify	
	the Pollen S Component in S. chacoense	149

to any contract of the last state

4	Breakdown of the Self-Incompatibility Character, S Mutations and the Evolution of Self-Incompatible Systems	151
4.1	The Physiological Breakdown of SI	152
4.1.1	Age Factors	152
4.1.1.1	Bud Pollination	152
4.1.1.2	Delayed Pollination, Use of Stored Pollen	
	and End-of-Season Effects	153
4.1.2	Irradiation	153
4.1.2.1	Chronic Exposure to Low Dose Rates	
	of Radiation	154
4.1.2.2	Acute Irradiation of Styles	154
4.1.2.3	High Temperatures	155
4.1.3	Application of $CO_2$	156
4.1.4	Hormones and Inhibitors	156
4.1.4.1	$\alpha$ -Naphthalene Acetic Acid	
	and Indole Acetic Acid	156
4.1.4.2	Effects of Transcription	
	and Translation Inhibitors	157
4.1.4.3	Effects of Proteinase and Tunicamycin	158
4.1.4.4	Effects of Inhibitors of Protein Phosphatase	158
4.1.5	Pistil Grafting	159
4.1.6	Mutilations, Injections and the Effects	
	of Castration on Pollen-Tube Growth	159
4.1.7	Mentor Effects	160
4.1.7.1	Mentor Pollen Is More Efficient	
	When Inactivated or Killed	160
4.1.7.2	Nature of the Mentor Effects	161
4.2	Genetic Breakdown of SI and S-Gene Mutations	161
4.2.1	Loss of S Function in Pollen Grains of Species	
	with SSI	161
4.2.2	Loss of S Function in the Pollen of Species	
	with GSI	163
4.2.2.1	Function Loss of the Pollen Determinant	
	Associated with the Presence of a Free Centric	
	Fragment	163
4.2.2.1.1	Competitive Interaction	163
4.2.2.1.2	Complementation	165
4.2.2.1.3	Restitution	165
4.2.2.1.4	Likelihood of the Three Hypotheses	166
4.2.2.1.5	Origin of the Centric Fragment in "Pollen-Part"	
	SC Mutants of N. alata	166

4.2.2.1.6	Current Approaches to the Biomolecular Study of PPMs Associated with Additional	
	Chromosomal Material	166
1 2 2 2	Function Loss of the Pollon Determinant	100
4.2.2.2	Not Accordiated with the Dressnes	
	of a Contria Engement	160
4222	The Energy of C Materians Leading	100
4.2.2.3	the Frequency of S Mutations Leading	1.0
	to the Loss of SI Function in Pollen Grains	169
4.2.2.4	Production of Cultivars with Modified	
	Breeding Regimes: Examples of Iraditional	0
	and Molecular Approaches	170
4.2.2.4.1	Cherry Stella	170
4.2.2.4.2	Elstar	170
4.2.2.5	The Use of "Pollen-Part" Mutations	
	for the Production of F1 Hybrid Seed	171
4.2.3	Loss of S Function in the Stigmas of Species	
	with SSI	172
4.2.3.1	Utility of SC Stylar Mutations and of Silencing	
	Studies for the Understanding and Exploitation	
	of SI	172
4.2.3.1.1	Breakdown of SI Through Silencing Effects	172
4.2.3.1.2	Why Gene Silencing Occurs	173
4.2.3.1.3	Scientific Interest of S-Gene Silencing	173
4.2.3.1.4	The Importance of Specific S-Function Losses	
	for Basic and Applied Research	174
4.2.4	Loss and Gain of S-Function Approaches	
	in the Stigma or Style of Species with GSI	174
4.2.4.1	SC Mutants Arising Spontaneously	
	or from Conventional Mutagenic Treatment	174
4.2.4.2	Genetic Constructs and Ablations of S-Gene	
	Products Leading to SC and their Importance	
	for SL Research	175
42421	How to Induce Function Loss	170
1.2. 1.2.1	Through the Use of Anti-Sense DNA	175
42422	Loss of Function and Gain of Function	175
1.2.1.2.2	Approaches Are Complementary	176
12123	Competition Effects Occurring in the Styles	170
7.2.7.2.3	of <i>Detunia</i> Plants with a Tri-Allelic S2-S3-S3×	
	Cenotype	177
1 2 1 2	Dresonce and Expression of S Dibonucleases	1//
4.2.4.3	in Self Compatible Lines	177
1211	In Self-Compatible Lines	1//
4.2.4.4	of Dianta with Mono Eastorial Styler CSI	170
1 2 5	or Franks with Mono-Factorial Stylar GS1	1/0
4.2.3	SU Infough Generic Unanges Occurring	170
4 2 5 1	Uniside the 5 Locus	1/ð
4.2.3.1	In sporophytic systems	1/9

and other states and the states

\_

4.2.5.2	In Gametophytic Systems	180
4.2.5.2.1	Mutations of Major Genes	180
4.2.5.2.2	Action of Polygenes	180
4.2.5.2.3	S Alleles Trapped in Translocation Rings	181
4.2.6	SC in Polyploids	182
4.2.6.1	Tetraploid Forms and Tetraploid Species	
	Are Often Self-Compatible	182
4262	Competitive Interaction in Diploid	
1.2.0.2	Hetero-Allelic Pollen	182
4263	Effects of Polyploidy on SL in Monocots	102
1.2.0.3	and Certain Primitive Dicots	183
127	The Generation of New SL Alleles	18/
4.2.7	Conflicting Evidence Degarding the Dole	104
4.2.7.1	of HV Degions in the Solona cone?	101
4 2 7 1 1	Only Equip A As Are Deepensible for the Differ	104
4.2.7.1.1	Only Four AAS Are Responsible for the Differ-	
	Alleles of Colouring discourses	104
4 2 7 1 2	Alleles of Solanum chacoense	184
4.2.7.1.2	In Petunia and Nicotiana, the Ribonuclease	
	Sequences Responsible for Pollen Recognition	
	Appear to be Scattered Inroughout the	105
		185
4.2.7.2	New S Alleles Appear in Inbred Populations	187
4.2.7.3	Origin of New Specifities	188
4.2.7.4	The Dual Specificity of New S Alleles May Play	
	a Key Role in the Generation of New S Alleles	188
4.2.7.5	The Role of the Genetic Background	189
4.2.7.6	Methods for a Rapid and Reliable	
	Identification of S Alleles in Plant Breeding	189
43	Evolution of SI	190
431	Allelic Diversity	190
4311	Origin Distribution and Extent of Divergences	170
1.3.1.1	among Functional S Alleles in the GSI System	
	of the Solanaceae	101
13111	Intragenic Crossing Over or Accumulation	171
4.3.1.1.1	of Single BD Changes?	101
12112	Distribution and Variability of S Alleles	102
4.3.1.1.2	Inter Species Variation in S Allele Age	192
4.5.1.1.5	and Number	102
4212	C Alleles in Other Femilies with a Diheruslasse	192
4.3.1.2	S Alleles in Other Families with a Ribonuclease	102
4 2 1 2 1		192
4.3.1.2.1	Differences Between the Scrophulariaceae	102
4 2 1 2 2	and the Solanaceae	192
4.3.1.2.2	Differences Between the Rosaceae	100
	and the Solanaceae	193
4.3.1.2.3	S-RNase Polymorphism in the Rosaceae	193

4.3.1.3	Homology or Convergence Among S	
	Ribonucleases?	193
4.3.1.3.1	What Happens in Legumes?	194
4.3.1.4	SLG and SRK Allelic Divergences	
	in the Brassicaceae	194
4.3.1.4.1	Hyper-Mutability of the S Locus	194
4.3.1.4.2	The S Locus is not a Hot Spot	
	of Recombination	195
4.3.1.4.3	Distribution and Extent of Variations Between	
	S Alleles	196
4.3.1.4.4	Extent of the Divergence Between SLG and SRK	
	of a Same S Haplotype	197
4.3.1.4.5	How is Sequence Similarity Usually Maintained	
	Between SRK and SLG in Brassica Haplotypes?	197
4.3.1.5	S Alleles Are Very Old	197
4.3.1.5.1	S-Ribonuclease Polymorphism in the Solanaceae	
	Arose Before the Emergence of Nicotiana,	
	Petunia and Solanum	198
4.3.1.5.2	The Origin of SLG and SRK	199
4.3.1.6	PCR Methods for Assessing Divergence	
	Among S Alleles	201
4.3.1.6.1	In the Crucifers	201
4.3.1.6.2	In Solanaceous Species	201
4.3.2	The Multiple Origins of SI Systems	202
4.3.2.1	Early Views	202
4.3.2.1.1	SI is a Primitive Outbreeding Mechanism	
	That Promoted the Expansion of Angiosperms	202
4.3.2.1.2	Gametophytic Poly-Allelic Incompatibility Is the	
	Ancestral System and Occurred Only Once	203
4.3.2.2	Current Thoughts	203
4.3.2.2.1	Towards a General Agreement Regarding	
	the Multiple Origins of SI	203
4.3.2.2.2	Multiple Gene Systems as an Origin	
	of Mono-Factorial GSI?	204
4.3.2.2.3	S Ribonucleases Could Be Operating	
	in a Very Vast Majority of Species	
	from the Dicot Families	205
4.3.3	Origin of the Different Homomorphic SI	
	Systems and Their Relationships	206
4.3.3.1	Origin of Stylar Mono-Factorial GSI	
	and Properties of Allelic Genealogies	
	at the GSI Locus	206
4.3.3.2	The Origin of SSI	207
4.3.3.3	Evolution of Inbreeding Depression	
	and Its Importance for S-Allele Invasion	207
4.3.3.4	Transitions Between GSI and SSI	207

and the second second

4.3.3.5	Co-Existence of SI and SC Alleles	
	or Breakdown of the System?	208
4.3.4	The Origin of Heteromorphic Incompatibility .	208
4.3.4.1	Arguments against Evolutionary Relationships	
	with Homomorphic SI	209
4.3.4.1.1	Homomorphic SI Systems Probably Have	
	Multiple Origins	209
4.3.4.1.2	Heteromorphic Incompatibility	
	is Scattered Among the Angiosperms	
	and Has Poly-Phyletic Origins	209
4.3.4.1.3	There Are Basic Differences Between	
	Heteromorphic and Homomorphic SI	209
4.3.4.2	Which Came First, Heteromorphy	
	or Incompatibility?	210
4.3.4.3	Evolution of Tristyly	211
4.3.4.4	The Evolutionary Breakdown or Transformation	
	of Heteromorphy	211
4.3.5	SC as the "Paradox of Evolution"	212
4.3.5.1	The Derived Condition of SC	212
4.3.5.1.1	More Recent Arguments	213
4.3.5.2	Reasons for the Expansion of Self-Fertilizers	213
4.3.5.2.1	Outbreeding is not Always Essential	
10101211	Once the Environment has been Captured	214
4.3.5.2.2	Inbreeding-Outbreeding Alternations Provide	~
1.5.5.2.2.2	Fertility Insurance	214
4.3.5.2.3	SC Facilitates the Establishment	
10101210	of Colonies after the Long-Distance Dispersal	
	of Single Seeds	214
4.3.5.2.4	Self-Fertilizers Are Qualified Colonizers	214
4.3.5.2.5	Inbred Populations Display High Levels	~
110101210	of Genetic Diversity	215
4.3.6	The Origin of Inter-Species Incompatibility	215
1.010		210
5	Incompatibility and incongruity Barriers	015
		217
5.1	Inter-Species Incompatibility Under the Control	
	of the S Locus	217
5.1.1	The SI×SC Rule	217
5.1.1.1	Distribution of the Barrier	218
5.1.1.1.1	In the Solanaceae	218
5.1.1.1.2	In Sporophytic Systems	218
5.1.1.1.3	The SI×SC Rule in the Grasses	219
5.1.2	Many Exceptions to the SI×SC Rule	219
5.1.2.1	They Occur Essentially in the Case of SI×SI	
	Pollination	219

5.1.2.2	SIדSc" Crosses	220
5.1.3	Differences Between the SI Reaction	
	and Inter-Species Rejection Processes	221
5.1.3.1	Situation in the Solanaceae	221
5.1.3.1.1	Observations with the Light Microscope	221
5.1.3.1.2	Electron Microscopy	222
5.1.3.2	In the Brassicaceae	222
5.1.4	The Involvement of the S Locus	224
5.1.4.1	Unilateral Pre-Zygotic Isolation Is an Active	
	Process in <i>Brassica</i>	224
5.1.4.2	SE a Class of S Alleles That Clearly Display	
	a Dual Function	224
5.1.4.3	Unilateral Pre-Zygotic Isolation Requires	
011110	the Action of S-Ribonucleases in <i>Nicotiana</i>	225
515	S-Recognition Structures Participating	
5.1.5	in Inter-Species III	227
5151	The Antigen-Antibody Model	228
51511	SIX SI Crosses	220
51512	SIXSI Crosses and ScXSC Crosses	220
51513	Actuality of the Model	220
5152	The "Area Hypothesis"	22)
5153	The S Locus as a Cluster of Primary	22)
5.1.5.5	and Secondary Specificities	230
5151	Why SLVSI Crosses Often Fail to Follow	230
5.1.5.4	the SLXSC Dule	221
516	Other Constic Loci Also Participate	231
5.1.0	in Inter Species Incompatibility	222
5161	The D Legue of Solanum	222
5.1.0.1	A Switch Cono in Lucopersister	232
5.1.0.2	The Two Dower Competition Hypothesis	232
5.1.0.5	Non Eurotional and "Conditionally" Eurotional	234
5.1.0.4	S Alleles in a SC Cultiver of Detuning	224
E 1 6 E	An Internation Detwoon the S. Leave	234
5.1.0.5	An Interaction between the 5 Locus	225
	and Major Genes in Lycopersicum penneulit	233
5.2	Incongruity Between the Pollen and Pistil	236
5.2.1	What is Incongruity?	236
5 2 1 1	When Does it Start?	236
5212	When Does it End?	230
5.2.1.2	Constic Basis of Incongruity	237
5.2.2	Origin of Tissues and Conomos Involved	230
5.2.5	in Incongruity	220
524	In incongruity	237
J.2.4	Arguments in Support of the Usersthesis	240
5.2.4.1	Dellan Distil Derriero Detween SC Species	240
5.2.4.1.1	Polien-Pistil Barriers Between SC Species	241

And the second se

5.2.4.1.2	The Conditions that Overcome SI Often	
	Have No Effect on Incongruity	241
5.2.4.1.3	The Genetics of Acceptance	
	and Non-Acceptance	241
5.2.4.2	Questions Remain	242
5.3	The Removal of Pollen–Pistil Barriers	
	Between Species	243
5.3.1	Intra-Species Inbreeding	244
5.3.2	Induced Mutations	245
5.3.3	Effects of Mentor Pollen	246
5.3.3.1	Can Mentor Effects on Self Pollen Consolidate	
	Reproductive Barriers Between Species?	246
5.3.3.2	Nature of the Mentor Effects	246
5.3.4	Bud Pollination and the Action	
	of Protein Inhibitors	247
5.4	Transfer of the S Gene to Autogamous	
	Species	247
5.4.1	Introduction of the Brassica SLG and SRK	
	Genes in SC Species	248
5.4.1.1	Transfer of SLG to B. napus	248
5.4.1.2	Transfer of SLG and SRK to A. thaliana	
	and <i>N. tabacum</i>	248
5.4.2	Transfer and Expression of the S-Ribonuclease Gene in SC Species and SC Inter-Species	
	Hybrids of <i>Nicotiana</i>	249
5.4.3	Other Genes Should Be Transferred	
	with the S Gene	250
5 5	Reconstruction of Multigenic SI	
515	in SC Species	250
		200
5.6	Crop Improvement Through the Transfer	
	of Individual Genes	251
6	Conclusions	253
61	High-Quality Research and Abundance	
0.1	of Achievements	252
611	High_Auglity Research	255
612	An Abundance of Achievements:	255
0.1.2	Classification Distribution and Inheritance	
	of Solf Incompatible Systems	252
	or sen-incompatible systems	233

6.1.3	Fine-Structure Studies of Pollen and Pollen	
	Tubes in Compatible and Incompatible	
	Surroundings	254
6.1.4	Identification of S Genes Active in the Pistil	254
6.1.5	Discovery of a Putative Pollen Determinant	
	in the Cabbage Family	254
6.1.6	Progress Towards the Understanding	
	of S Specificity	254
6.1.7	Advances in Cellular and Molecular Surgery	255
6.1.8	New Information Regarding the Evolution	
	of SI Systems	255
619	Bypassing Pre-Zygotic Inter-Species Barriers	256
0.1.9	Bypussing the Bygotte inter species buttlets	250
6.2	There Are Still Numerous Gaps	
	in Our Knowledge and Skill	256
6.2.1	Unraveling the S-Gene Family	256
6211	Genetic Control is More Complex	200
0.2.1.1	Than Expected	256
6212	The S-Gene Family of Brassica	250
0.2.1.2	is Surprisingly Large	257
6213	S Locus Complexity Has Also Been Found	257
0.2.1.5	in the Solonocono	257
6214	The S Alleles of Deterry May Ales Polong	231
0.2.1.4	The S Alleles of Pupaver May Also belong	257
(22	to a Large Family	257
0.2.2	Analysis of Recognition and Rejection	257
6.2.2.1	Identification and Function of S and S-Related	255
(	Proteins in the Pistil	257
6.2.2.2	The Search for Pollen Determinants	258
6.2.2.3	Identification of the Genes and Processes	
	Affected by the Rejection Phase of SI	258
6.2.3	The Molecular Biology of SI	
	in Heteromorphic Species	259
6.2.4	Barriers to the Expression of Transferred Genes	259
6.2.5	Evolution of SI	260
6.2.5.1	The Multiple Origin of Homomorphic SI	260
6.2.5.2	A Relationship Between GSI	
	and Sporophytic SI?	260
6.2.5.3	A Multiple Gene System as the Starting Point	
	for GSI	260
6.2.5.4	Is the Emergence of New S Alleles a Gradual	
	Process?	261
6.2.5.5	Nature of the Relationship (Homology or	
	Convergence) Between the S Proteins of the	
	Different Families of Plants That Share	
	a Common SI System	261
6.2.5.6	Evolution of Heteromorphic SI	261
	-	

6.2.6 6.2.6.1	Inter-Species Incompatibility and Incongruity . The Complexity of Pollen–Pistil Barriers	262
6.2.6.2	Between Species The Need for Further Research on the Melocular Biology of Bro Zurotic Barriors	262
6.2.6.3	Between Species	262 263
References		265
Subject Index		309