Theor Appl Genet (1988) 75:882–888

Detection and estimation of linkage for a co-dominant structural gene locus linked to a gametophytic self-incompatibility locus

C. R. Leach
Department of Genetics, University of Adelaide, South Australia 5000, Australia

Received September 10, 1987; Accepted November 11, 1987
Communicated by P. L. Pfahler

Summary. The operation of gametophytic self-incompatibility systems may lead to disturbed segregation ratios for genes at loci linked to the self-incompatibility loci. An exhaustive consideration of the types of crosses, methods of linkage estimation, progeny sizes and controls needed for accurate analysis of disturbed segregation ratios is presented. Examples of the application of these methods are included.

Key words: Gametophytic self-incompatibility – Linkage – Disturbed segregations

Introduction

Incompatibility systems in angiosperms have been attracting attention in recent years, both because of their importance in practical plant breeding and as a result of their role as examples of cellular interaction and recognition (Heslop-Harrison 1983). Self-incompatibility may be defined as the failure of self- or cross-pollination by reason of genetic similarity within an otherwise freely interbreeding group (Darlington and Mather 1949).

Incompatibility systems are classified as heteromorphic or homomorphic according to whether or not they are associated with differences in floral structure. Homomorphic systems may be further classified according to the genetic control of pollen phenotype. Systems are described as being sporophytic if pollen behaviour is determined by the genotype of the plant producing it and gametophytic if pollen behaviour is determined by the gene(s) carried by the pollen. The genetic control of gametophytic self-incompatibility is by genes at one, two, or rarely, three or more multi-allelic loci. Matings are incompatible if the allele(s) in the pollen are matched by the alleles expressed in the diploid tissue of the pistil (see Frankel and Galun 1977).

The operation of a gametophytic self-incompatibility system in appropriate crosses leads to differential transmission of pollen grains of different incompatibility genotypes (Breiger and Mangelsdorf 1926). Consequently, genes which are linked to self-incompatibility genes may show disturbed segregation ratios.

Simmonds (1966) derived theoretical expectations and variances of linkage estimates for a locus linked to the self-incompatibility locus from both backcross and F_2 segregations. His analysis was restricted to phenotypes determined with complete dominance.

Segregation ratios for isozyme loci differing between reciprocal crosses are increasingly being used to indicate linkage to the gene(s) controlling gametophytic self-incompatibility (Labroche et al. 1983; Wendel and Parks 1984; Tanksley and Loaiza-Figuera 1985; Van Dijk 1985; Wricke and Wehling 1985; Leach and Hayman 1987). In addition, Polans and Allard (1985) and Figueiras et al. (1985) reported disturbed segregation ratios which are, most likely, also examples of linkage to the incompatibility genes.

The increasing amount of work being carried out in the area of self-incompatibility suggests that it is an appropriate time for an exhaustive consideration of the types of crosses, methods of linkage estimation, progeny sizes and controls needed for accurate analysis of disturbed segregation ratios.

The analysis is restricted to gametophytically determined self-incompatibility systems since, as pointed out by Thompson and Taylor (1965), linkage to a sporophytically determined self-incompatibility system does not lead to disturbed segregation ratios.

Materials and methods

Theoretical methods

The method applied involves a consideration of the array of crosses possible for plants segregating for the isozyme structural gene that have been shown by pollination tests (Watkins 1931) to have an appropriate percentage of compatible pollen. Alleles at the isozyme structural locus are denoted A_1, A_2, etc. The recombination frequency is represented by the symbol r. The female parent is written first in all crosses. Maximum likelihood methods are used for all derivations of the recombination frequency estimate and its variance.

A number of cases are considered involving linkage of an isozyme-determining structural gene to gametophytic self-incompatibility controlled by genes at one or more loci.

Gametophytic self-incompatibility controlled by a single multi-allelic S-locus

Consider a cross in which the male parent is 50% compatible with the female and heterozygous for an isozyme determining gene for which the female is homozygous, e.g. $\frac{S_1A_1}{S_2A_1} \times \frac{S_1A_1}{S_3A_2}$. All pollen carrying the S_1 allele will be ineffective in fertilization (East and Mangelsdorf 1925) and so the A_1 allele will be transmitted only as a result of a recombination event between the S and A loci. Thus, pollen grains carrying S_3A_1 and S_3A_2 will fertilize S_1A_1 and S_2A_1 bearing ovules in the ratio of $r:1-r$ leading to isozyme genotypes A_1A_1 and A_1A_2 in the progeny in a ratio of $r:1-r$.

The reciprocal cross yields A_1A_1 and A_1A_2 in a ratio of 1 : 1. If the observed numbers of A_1A_1 and A_1A_2, say y_1 and y_2, ($y_1 + y_2 = n$), depart significantly from the expected $\frac{n}{2}$ in the original cross but not in the reciprocal, it may be concluded that the isozyme locus is linked to the incompatibility locus.

The observed proportion $\frac{y_1}{n}$ of A_1A_1 in the progeny of the first cross gives a direct estimate $\hat{r}$ of r. The variance of this estimate $V(\hat{r})$ is $\frac{\hat{r}(1-\hat{r})}{n}$.

The sample size necessary to have a 95% probability that the observed proportion of one of the genotypes, e.g. $\frac{y_1}{n} = r$, differs significantly from an expectation of 0.5 may be calculated from the solution of the equation

$$n = \frac{Z^2 V(r)}{(r - 0.5)^2}$$

where Z is the value from tables of the normal distribution for a probability of 5%, i.e. 1.96 (Fisher and Yates 1963). For small values of r ($r < 0.2$) a value from Students' t distribution (Fisher and Yates 1963) should be used instead of Z. In these cases the t_1 value of 12.7 gives an upper limit for the estimate of n.

The method of sample size estimation considered here is not that generally used to distinguish two segregation ratios (Mather 1957) but is the standard approach (Snedocor and Cochran 1980) based on the assumption that there is interest in detecting a departure from a parameter, i.e. that this value does not lie in the 95% confidence limits of the recombination frequency estimated from the data.

The appropriate controls required to confirm linkage are: (1) demonstrating that the cross is 50% compatible using a pollination test which rules out the possibility of differential transmission due to certation (Heribert Nilsson 1916); and (2) observing undisturbed segregation ratios in crosses with the same percentage compatible pollen indicating that disturbances are not likely to be due to viability differences associated in some way with isozyme genotypes.

Gamethophytic self-incompatibility controlled by two multi-allelic loci S and Z

Where gametophytic self-incompatibility is controlled by two or more loci there may be differences in compatibility of reciprocal crosses. Further, for two loci there are three classes of compatible pollinations (Hayman 1956). Identity between pollen and pistil for the alleles at each locus leads to incompatibility (Lundqvist 1954). Thus, an $S_{1.2}Z_{1.2}$ style rejects all pollen of types S_1Z_1, S_1Z_2, S_2Z_1 and S_2Z_2, specifically the pollen produced by anthers of a plant of that genotype, but will accept pollen differing by at least one S or Z allele, e.g. pollen from a plant of genotype $S_{3.4}Z_{1.2}$.

An array of crosses where the parents have differing percentages of compatible pollen, isozyme loci linked to either or both of the incompatibility loci and the incompatibility loci themselves linked is elaborated.

Sample size estimation follows the same principle as above and the appropriate controls are indicated.

Results

Gametophytic self-incompatibility controlled by a single multi-allelic S-locus

Three types of pollination can be recognised. These are incompatible ($S_1S_2 \times S_1S_2$), 50% compatible ($S_1S_2 \times S_1S_3$) and fully compatible ($S_1S_2 \times S_3S_4$). There are no differences between reciprocal crosses in incompatibility relationships. Only crosses which are 50% compatible may lead to disturbed segregation ratios.

The estimates of the recombination frequency and its variance between an isozyme-determining gene and an incompatibility gene are shown in Table 1.

The sample sizes required to have a 95% probability of detecting a significant departure from an expectation of $r = 0.5$ for a given recombination frequency are listed in Table 2.

Gametophytic self-incompatibility controlled by two multi-allelic loci S and Z

When two loci determine the gametophytic self-incompatibility a number of different features may occur.

1 Four classes of crosses may be detected by scoring pollination tests (Hayman 1956): (1) fully compatible e.g. $S_{1.2}Z_{1.2} \times S_{3.4}Z_{3.4}$, (2) 75% compatible e.g. $S_{1.2}Z_{1.2} \times S_{2.3}Z_{2.3}$, (3) 50% compatible e.g. $S_{1.2}Z_{1.2} \times S_{1.3}Z_{1.2}$, and (4) fully incompatible e.g. $S_{1.2}Z_{1.2} \times S_{1.2}Z_{1.2}$.

2 There may be reciprocal differences in compatibility between the parents:

$S_{1.1}Z_{1.2} \times S_{1.2}Z_{1.2}$ 50% compatible
$S_{1.2}Z_{1.2} \times S_{1.1}Z_{1.2}$ incompatible.

Table 1. Estimates of the recombination frequency and its variance between an isozyme-determining gene and an incompatibility gene

Cross ♀	Cross ♂	Pollen compatibility		Progeny A_1A_1	Progeny A_1A_2	Progeny A_2A_2	Total	Estimate of recombination frequency $\hat{r}$	Variance ($\hat{r}$)
1) $\frac{S_1A_1}{S_2A_1}$	$\times\frac{S_1A_1}{S_3A_2}$	50%	Exp.	r	1 − r		1	$\frac{y_1}{y_1+y_2}$	$\frac{y_1y_2}{y_1+y_2}=\frac{\hat{r}(1-\hat{r})}{n}$
			Obs.	y_1	y_2		n		
2) $\frac{S_1A_1}{S_3A_2}$	$\times\frac{S_1A_1}{S_2A_1}$	50%	Exp.	$\frac{1}{2}$	$\frac{1}{2}$		1	–	–
			Obs.	y_1	y_2		n		
3) $\frac{S_1A_1}{S_2A_2}$	$\times\frac{S_1A_1}{S_3A_2}$	50%	Exp.	$\frac{r}{2}$	$\frac{1}{2}$	$\frac{1-r}{2}$	1	$\frac{y_1}{y_1+y_3}$	$\frac{2y_1(y_2+y_3)}{y_1+y_2+y_3}=\frac{2\hat{r}(1-\hat{r})}{n}$
			Obs.	y_1	y_2	y_3	n		

Table 2. Sample sizes necessary to have 95% probability of detecting a significant departure from expectation of r = 0.5 for a given recombination frequency (r) for a locus linked to the incompatibility locus

Recombination frequency (r)	Sample size n Cross type (see Table 1)	
	2	3
0.1	7	14
0.2	23	46
0.3	21	42
0.4	93	186

Table 3. Outcome of crosses in which the incompatibility loci are linked

Genotypes of parents ♀	Genotypes of parents ♂		Frequency of pollen genotypes in terms of recombination frequency r S_1Z_1	S_2Z_2	S_1Z_2	S_2Z_1
1)[a] $\frac{S_1Z_1}{S_2Z_3}$	$\times\frac{S_1Z_1}{S_2Z_2}$		$\frac{1-r}{2}$	$\frac{1-r}{2}$	$\frac{r}{2}$	$\frac{r}{2}$
2) $\frac{S_1Z_1}{S_2Z_3}$	$\times\frac{S_1Z_2}{S_2Z_1}$		$\frac{r}{2}$	$\frac{r}{2}$	$\frac{1-r}{2}$	$\frac{1-r}{2}$
		Compatibility of pollen	–	+	+	–
3)[a] $\frac{S_1Z_1}{S_3Z_4}$	$\times\frac{S_1Z_1}{S_2Z_2}$		$\frac{1-r}{2}$	$\frac{1-r}{2}$	$\frac{r}{2}$	$\frac{r}{2}$
4) $\frac{S_1Z_1}{S_3Z_4}$	$\times\frac{S_1Z_2}{S_2Z_1}$		$\frac{r}{2}$	$\frac{r}{2}$	$\frac{1-r}{2}$	$\frac{1-r}{2}$
		Compatibility of pollen	–	+	+	+

[a] The linkage phase of the female parent does not influence the argument

Table 4. Percentage of compatible pollen for a range of recombination frequencies from a cross of the form $\frac{S_1Z_1}{S_3Z_4}$ × 3) $\frac{S_1Z_1}{S_2Z_2}$ or 4) $\frac{S_1Z_2}{S_2Z_1}$

Recombination frequency	% compatible pollen	
	Cross 3	Cross 4
0.05	52.5	97.5
0.1	55	95
0.2	60	90
0.3	65	85
0.4	70	80
0.45	72.5	77.5
0.5	75	75

3 The isozyme locus may be linked to either incompatibility locus:

$$\frac{S_1A_1}{S_1A_1}Z_{1.2}\times\frac{S_1A_1}{S_2A_2}Z_{1.2}$$

or

$$S_{1.1}\frac{Z_1A_1}{Z_2A_1}\times S_{1.2}\frac{Z_1A_1}{Z_2A_2}.$$

4 Different isozyme loci may be linked to each incompatibility locus:

$$\frac{S_1A_1}{S_1A_1}\frac{Z_1B_1}{Z_2B_1}\times\frac{S_1A_1}{S_2A_2}\frac{Z_1B_1}{Z_2B_2}.$$

5 The incompatibility loci may themselves be linked. If this is the case the question arises as to whether it is possible to classify pollinations as 50% and 75% compatible. As illustrated in Table 3, the outcome for cases 3 and 4 depends upon the value of the recombination frequency between the loci. The expected percentage of compatible pollen for a range of recombination frequencies is shown in Table 4.

Table 5. Estimates of recombination frequency and variance for crosses in which the male parent is 50% compatible with the female

Cross ♀	Cross ♂		Progeny				Estimate of recombination frequency $\hat{r}$	Variance ($\hat{r}$)
1)[a] $\frac{S_1A_1}{S_1A_1}Z_{1.2}$	$\times \frac{S_1A_1}{S_2A_2}Z_{1.2}$	Exp.	A_1A_1 r	A_1A_2 $1-r$			$\frac{y_1}{y_1+y_2}$	$\frac{y_1y_2}{y_1+y_2}$
		Obs.	y_1	y_2				
2)[a] $\frac{S_1A_1}{S_1A_2}Z_{1.2}$	$\times \frac{S_1A_1}{S_2A_2}Z_{1.2}$	Exp.	A_1A_1 $\frac{r}{2}$	A_1A_2 $\frac{1}{2}$	A_2A_2 $\frac{1-r}{2}$		$\frac{y_1}{y_1+y_3}$	$\frac{2y_1(y_2+y_3)}{y_1+y_2+y_3}$
		Obs.	y_1	y_2	y_3			
3)[a] $\frac{S_1A_1}{S_1A_2}Z_{1.2}$	$\times \frac{S_1A_3}{S_2A_4}Z_{1.2}$	Exp.	A_1A_3 $\frac{r}{2}$	A_1A_4 $\frac{1-r}{2}$	A_2A_3 $\frac{r}{2}$	A_2A_4 $\frac{1-r}{2}$	$\frac{y_1+y_3}{y_1+y_2+y_3+y_4}$	$\frac{(y_1+y_3)(y_2+y_4)}{y_1+y_2+y_3+y_4}$
		Obs.	y_1	y_2	y_3	y_4		
4)[b] $\frac{S_1A_1}{S_1A_2}\frac{Z_1B_1}{Z_2B_2}$	$\times \frac{S_1A_1}{S_2A_2}\frac{Z_1B_1}{Z_2B_2}$	Exp.	A_1A_1 $\frac{r}{2}$	A_1A_2 $\frac{1}{2}$	A_2A_2 $\frac{1-r}{2}$		as for 2) above	
		Exp.	B_1B_1 $\frac{1}{4}$	B_1B_2 $\frac{1}{2}$	B_2B_2 $\frac{1}{4}$			

[a] The situation of linkage to the S locus is illustrated. It is exactly the same for the Z locus
[b] It is only possible to detect and estimate linkage for an isozyme gene locus linked to either the S or Z locus, not both

Table 6. Estimates of recombination frequency and variance for crosses in which the male parent is 75% compatible with the female

Cross ♀	Cross ♂		Progeny				Total	Estimate of recombination frequency $\hat{r}$	Variance ($\hat{r}$)
1) $\frac{S_1A_1}{S_2A_1}Z_{1.2}$	$\times \frac{S_2A_1}{S_3A_2}Z_{2.3}$	Exp.	A_1A_1 $\frac{1+r}{3}$	A_1A_2 $\frac{2-r}{3}$			1	$\frac{2y_1-y_2}{y_1+y_2}$	$\frac{(1+\hat{r})(2-\hat{r})}{n}$
		Obs.	y_1	y_2			n		
2) $\frac{S_1A_1}{S_1A_2}Z_{1.2}$	$\times \frac{S_2A_1}{S_3A_2}Z_{2.3}$	Exp.	A_1A_1 $\frac{1+r}{6}$	A_1A_2 $\frac{1}{2}$	A_2A_2 $\frac{2-r}{6}$		1	$\frac{2y_1-y_3}{y_1+y_3}$	$\frac{2(1+\hat{r})(2-\hat{r})}{n}$
		Obs.	y_1	y_2	y_3		n		
3)[a] $\frac{S_1A_1}{S_2A_1}\frac{Z_1B_1}{Z_2B_1}$	$\times \frac{S_2A_1}{S_3A_2}\frac{Z_2B_1}{Z_2B_2}$	Exp.	A_1A_1 $\frac{1+r_1}{3}$	A_1A_2 $\frac{2-r_1}{3}$			1	$\hat{r}_1=\frac{2y_1-y_2}{y_1+y_2}$	$\frac{2(1-\hat{r}_1)(2-\hat{r}_1)}{n}$
		Obs.	y_1	y_2			n		
		Exp.	B_1B_1 $\frac{1+r_2}{3}$	B_1B_2 $\frac{2-r_2}{3}$			1	$\hat{r}_2=\frac{2z_1-z_2}{z_1+z_2}$	$\frac{2(1-\hat{r}_2)(2-\hat{r}_2)}{n}$
		Obs.	z_1	z_2			n		
4)[a] $\frac{S_1A_1}{S_2A_2}\frac{Z_1B_1}{Z_2B_2}$	$\times \frac{S_2A_1}{S_3A_3}\frac{Z_2B_1}{Z_2B_2}$		as above 2) for A locus similarly in r_2 and z for B locus						

[a] It is possible to detect and estimate linkage for two isozyme loci, one linked to S and the other to Z

Thus, if the incompatibility loci are tightly linked ($r < 0.1$) it would be possible to distinguish only three types of pollination. This situation would be indistinguishable from the case where self-incompatibility was under the control of a single locus. For recombination frequencies in the range $0.2 - 0.3$, a continuity of the percentage of compatible pollen would be expected. However, as workers have classified pollinations as 75% compatible (e.g. Hayman 1956; Weimark 1968), it is reasonable to deduce that, in the case of the Poaceae, if the loci are linked this linkage must be loose. The results which follow for 75% compatible crosses involving linked loci are only true if this is the case. In considering a single linked isozyme locus the order of the loci must be considered, i.e. $S - A - Z$, $A - S - Z$, $A - Z - S$.

6 *Combinations of situations 2) – 4) may occur.*

Independent assortment of the incompatibility loci

The estimates of the recombination frequency and its variance for crosses in which the male parent is 50% compatible with the female are given in Table 5. The sample sizes required to have 95% probability of detecting significant departures from expectation for a given recombination frequency are given in Table 2.

Appropriate controls involve finding an undisturbed segregation ratio for the same isozyme in a cross with 50% compatible pollen. This rules out both certation and diploid viability effects.

The estimates of the recombination frequency and its variance for crosses in which the male parent is 75% compatible with the female are given in Table 6.

The sample sizes required to be 95% sure of detecting significant departures from expectation for a given recombination frequency are listed in Table 7.

It is not possible to have an undisturbed segregation for a gene which is linked to one of the incompatibility genes if the male parent is 75% compatible with the female and so an appropriate control must be found in a cross with 50% compatible pollen.

Table 7. Sample sizes necessary to have 95% probability of detecting departure due to various recombination frequencies (r) when the male parent is 75% compatible with the female

Recombination frequency (r)	Sample size (n)
0.1	51
0.2	93
0.3	213
0.4	861

Linkage of the incompatibility loci

The results illustrated assume no interference. The recombination frequency between the first pair of loci is denoted by r_1 and between the second pair by r_2. The contribution to the isozyme genotype of the progeny by the female parent will be identical from a homozygote or $\frac{1}{2}A_1$ and $\frac{1}{2}A_2$ from a heterozygote. A summary of the contribution by the male parent for all possible genotype arrangements is shown in Table 8.

Table 8. Frequencies of male gametes effecting fertilization for all possible arrangements of linked loci

	♀	×	50% compatible male			
1)	$\frac{S_1 A_1 Z_1}{S_1 A_1 Z_2}$	×	$\frac{S_1 A_1 Z_1}{S_2 A_2 Z_2}$	$\frac{S_1 A_2 Z_1}{S_2 A_1 Z_2}$	$\frac{S_1 A_2 Z_2}{S_2 A_1 Z_1}$	$\frac{S_1 A_1 Z_2}{S_2 A_2 Z_1}$
		A_1	r_1	$1 - r_1$	$1 - r_1$	r_1
		A_2	$1 - r_1$	r_1	r_1	$1 - r_1$
	♀	×	75% compatible male			
2)	$\frac{S_1 A_1 Z_1}{S_2 A_1 Z_2}$	×	$\frac{S_2 A_1 Z_2}{S_3 A_2 Z_3}$	$\frac{S_2 A_2 Z_2}{S_3 A_1 Z_3}$	$\frac{S_2 A_2 Z_3}{S_3 A_1 Z_2}$	$\frac{S_2 A_1 Z_3}{S_3 A_2 Z_3}$
		A_1	$\frac{r_1 + r_2}{2}$	$1 - \frac{r_1 + r_2}{2}$	$\frac{1 - r_1 + r_2}{2}$	$\frac{1 + r_1 - r_2}{2}$
		A_2	$1 - \frac{r_1 + r_2}{2}$	$\frac{r_1 + r_2}{2}$	$\frac{1 + r_1 - r_2}{2}$	$\frac{1 - r_1 + r_2}{2}$
3)	$\frac{A_1 S_1 Z_1}{A_1 S_2 Z_2}$	×	$\frac{A_1 S_2 Z_2}{A_2 S_3 Z_3}$	$\frac{A_2 S_2 Z_2}{A_1 S_3 Z_3}$	$\frac{A_2 S_3 Z_3}{A_1 S_3 Z_2}$	$\frac{A_1 S_2 Z_3}{A_2 S_3 Z_2}$
		A_1	$\frac{2r_1 + r_2 - 2r_1 r_2}{2}$	$1 - \frac{2r_1 + r_2 - 2r_1 r_2}{2}$	$\frac{1 + r_2 - 2r_1 r_2}{2}$	$\frac{1 - r_2 + 2r_1 r_2}{2}$
		A_2	$1 - \frac{2r_1 + r_2 - 2r_1 r_2}{2}$	$\frac{2r_1 + r_2 - 2r_1 r_2}{2}$	$\frac{1 - r_2 + 2r_1 r_2}{2}$	$\frac{1 + r_2 - 2r_1 r_2}{2}$

In the case where the male is 50% compatible the outcome is the same for all orders of the loci and for a cross of the form $S_{1.2}Z_{1.1} \times S_{1.2}Z_{1.2}$ the proportions of A_1 and A_2 would be in terms of r_2. The progeny sizes may be determined from Table 2.

In the case where the male is 75% compatible, linkage detection and estimation is not possible. The order $A - Z - S$ is the same as case 3 in Table 8.

Application to experimental results

Disturbed segregation ratios have been used to estimate the recombination frequency between genes at an incompatibility locus and the gene encoding the enzyme glucose phospoisomerase for five different grass species (Leach and Hayman 1987).

Further examples of the use of disturbed segregation ratios as an indicator of linkage to the genes controlling gametophytic self-incompatibility are found in Labroche et al. (1983), Wendel and Parks (1984), Tanksley and Loaiza-Figuera (1985), Van Dijk (1985) and Wricke and Wehling (1985). In addition, Polans and Allard (1985) reported a segregation ratio for the isozyme peroxidase in *Lolium multiflorum* of 52:62:7 when the expected ratio was 1:2:1. These data lead to an estimate of the recombination fraction between the peroxidase and incompatibility loci of $r_{S\text{-}PER} = 0.12 \pm 0.04$. Figueiras et al. (1985) also report a disturbed segregation ratio for phosphoglucose isomerase in *Secale cereale*. The observations of 54:47:22 cf. 1:2:1 lead to an estimate of the recombination fraction between the phosphoglucose isomerase and incompatibility loci of $r_{S\text{-}PGI} = 0.29 \pm 0.06$.

Discussion

A completely self-incompatible plant sets no seed with its own pollen and so crosses between plants of different incompatibility genotypes may be made easily. The operation of this type of incompatibility system means that it is technically possible to perform genetic analyses on a diverse range of species without the tedium and technical problems of hand emasculation of plants. Thus, using these features of the incompatibility system considerably extends the species that may be investigated beyond the relatively restricted group of plants of agricultural importance.

A greater awareness of the types of disturbance that may occur as a result of linkage associations to self-incompatibility loci as documented here should prove useful in the interpretation of data involving isozyme segregations. Indeed, with the appropriate application of such methods it might prove possible to establish the number of loci involved in the control of self-incompatibility in species such as *Beta vulgaris* (Larsen 1978), *Ranunculus acris* (Lundqvist et al. 1973; Osterbye 1977) and *Borago officinalis* (Crowe 1971), all of which are reported to have multiple loci involved in the determination of self-incompatibility, but for which the exact number of these loci is unknown.

Acknowledgements. Much of this work was included in a Ph.D. thesis submitted to the University of Adelaide. Dr. D. L. Hayman was a supervisor and his help and guidance is acknowledged. I would also like to express my thanks to Dr. O. Mayo for his critical appraisal of the work.

References

Breiger FG, Mangelsdorf AJ (1926) Linkage between a flower color factor and self-sterility factors. Proc Natl Acad Sci USA 12:248–255

Crowe LK (1971) The polygenic control of outbreeding in *Borago officinalis*. Heredity 64:111–118

Darlington CD, Mather K (1949) The elements of genetics. Allen and Unwin, London

East EM, Mangelsdorf AJ (1925) A new interpretation of the hereditary behaviour of self-sterile plants. Proc Natl Acad Sci USA 11:166–171

Figueiras AM, Gonzalez-Jaen MT, Salinas J, Benito C (1985) Association of isozymes with a reciprocal translocation in cultivated rye (*Secale cereale* L.). Genetics 109:177–193

Fisher RA, Yates F (1963) Statistical tables for biological, agricultural and medical research, 6th edn. Oliver and Boyd, London

Frankel R, Galun E (1977) Pollination mechanisms and plant breeding. Springer, Berlin Heidelberg New York

Hayman DL (1956) The genetical control of incompatibility in *Phalaris coerulescens* Desf. Aust J Biol Sci 9:321–331

Heribert Nilsson N (1916) Populationsanalysen und Erblichkeitsversuche über die Selbstfertilität und Sterilität bei dem Roggen. Z Pflanzenzücht 4:1–44

Heslop-Harrison J (1983) Self-incompatibility: phenomenology and physiology. Proc R Soc London, Ser B 218:371–395

Labroche Ph, Poirier-Hamon S, Pernes J (1983) Inheritance of leaf peroxidase isoenzymes in *Nicotiana alata* and linkage with the S-incompatibility locus. Theor Appl Genet 65: 163–170

Larsen K (1978) Self-incompatibility in *Beta vulgaris* L. I. Four gametophytic, complementary S-loci in sugar beet. Hereditas 85:227–248

Leach CR, Hayman DL (1987) The incompatibility loci as indicators of conserved linkage groups in the Poaceae. Heredity 58:303–305

Lundqvist A (1954) Studies of self-sterility in rye *Secale cereale* L. Hereditas 40:278–294

Lundquist A, Østerbye U, Larsen K, Linde-Laursen IB (1973) Complex self-incompatibility systems in *Ranunculus acris* L. and *Beta vulgaris* L. Hereditas 74:161–168

Mather K (1957) The measurement of linkage in heredity, 2nd ed. Methuen, London

Østerbye U (1977) Self-incompatibility in *Ranunculus acris* L. II. Four S-loci in a German population. Hereditas 87:173–178

Polans NO, Allard RW (1985) Inheritance of electrophoretically detectable variation in rye grass. J Hered 76:61–62

Simmonds NW (1966) Linkage to the S-locus in diploid potatoes. Heredity 21:473–479

Snedocor GW, Cochran WG (1980) Statistical methods, 7th ed. Iowa State University Press, pp 53–58

Tanksley SD, Loaiza-Figuera F (1985) Gametophytic self-incompatibility is controlled by a single major locus on chromosome 1 in *Lycopersicon peruvianum*. Proc Natl Acad Sci USA 82:5093–5096

Thompson KF, Taylor JP (1965) Linkage between S alleles and pigmentation in marrow-stem kale, *Brassica oleracea*. Euphytica 14:285–292

Van Dijk H (1985) Allozyme genetics, self-incompatibility and male sterility in *Plantago lanceolata*. Heredity 54:53–63

Watkins AE (1931) Genetical and cytological studies in wheat. J Genet 15:323–366

Weimark A (1968) Self-incompatibility in the Gramineae. Hereditas 60:157–166

Wendel JF, Parks CR (1984) Distorted segregation and linkage of alcohol dehydrogenase genes in *Camellia japonica* L. (Theaceae). Biochem Genet 22:739–748

Wricke G, Wehling P (1985) Linkage between an incompatibility locus and peroxidase locus (*Prx* 7) in rye. Theor Appl Genet 71:289–291