

The incompatibility loci as indicators of conserved linkage groups in the Poaceae

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Linkage between an incompatibility locus and the locus of the gene encoding the enzyme glucose phosphoisomerase has been demonstrated for the grasses *Alopecurus myosuroides*, *Phalaris coerulescens*, *Festuca pratensis*, *Holcus lanatus* and *Secale cereale* suggesting the conservation of a common chromosomal element in the evolution of the Poaceae.

INTRODUCTION

Gametophytic self-incompatibility controlled by genes at two loci, first described by Lundqvist (1954, 1956) in *Secale cereale* and Hayman (1956) in *Phalaris coerulescens* has subsequently been found in all genetically analysed self-incompatible species belonging to the Poaceae (see Leach 1983 for references). This system is unique to the grasses and is presumed to have arisen only once. The two loci (*S* and *Z*) assort independently and each have a large number of different alleles, e.g., *Festuca pratensis* *S* = 14 and *Z* = 13 (Lundqvist, 1969). Identity between pollen and pistil for the alleles at each locus leads to incompatibility. Thus an $S_{1.2}Z_{1.2}$ style rejects all pollen of S_1Z_1 , S_1Z_2 , S_2Z_1 , S_2Z_2 genotypes; specifically that produced by anthers of a plant of that genotype but will accept pollen differing by at least one *S* or *Z* allele.

$$\begin{array}{c} \text{e.g. } S_{1.2}Z_{1.2} \times S_{1.3}Z_{1.2} \\ \downarrow \\ S_1Z_1 \quad S_1Z_2 \quad S_3Z_1 \quad S_3Z_2 \end{array}$$

i.e. 50 per cent of pollen in such crosses would be compatible. Genotype combinations in which 75 per cent and 100 per cent of pollen is compatible are possible. A completely self-incompatible plant sets no seed with its own pollen and so crosses between plants of different incompatibility genotypes may be made by simply bagging heads together. The compatibility relationship between plants can be established from pollination tests.

As a result of the potential for differential transmission of different types of pollen, it follows that

genes which are linked to the self-incompatibility genes may have a disturbed rate of transmission. Consider a pair of plants which are reciprocally 50 per cent incompatible and each of GPI-2 (glucose phosphoisomerase) type 1-3 with *Gpi-2* linked to the *S* locus. These could have genotypes

$$(\text{♀}) \frac{S_2P_1}{S_3P_3} Z_{1.3} \times (\text{♂}) \frac{S_2P_1}{S_1P_3} Z_{1.3}$$

(where P_1 is used to represent *Gpi-2-1* etc.) and when crossed they would give the following gametes:

	non-recombinant	recombinant
	$S_2P_1Z_1$	$S_2P_3Z_1$
	$S_3P_3Z_1$	$S_3P_1Z_1$
	$S_2P_1Z_3$	$S_2P_3Z_3$
	$S_3P_3Z_3$	$S_3P_1Z_3$
freq. of each	$\frac{1-r}{4}$	$\frac{r}{4}$

	non-recombinant	recombinant
	$(S_2P_1Z_1)$	$(S_2P_3Z_1)$
	$S_1P_3Z_1$	$S_1P_1Z_1^*$
	$(S_2P_1Z_3)$	$(S_2P_3Z_3)$
	$S_1P_3Z_3$	$S_1P_1Z_3^*$
freq. of each	$\frac{1-r}{4}$	$\frac{r}{4}$

where r = recombination frequency between the *S* locus and *Gpi-2* (*P*) locus.

All pollen types S_2Z_1 and S_2Z_3 (circled) are incompatible and so *Gpi-2-1* is transmitted only in recombinant gametes $S_1P_1Z_1$ and $S_1P_1Z_3$ (*). The female gametes carry *Gpi-2-1* and *Gpi-2-3* (indicated as P_1 and P_3) equally frequently leading to an expected proportion of $r/2$ GPI type 1-1 in the progeny of this cross. GPI types 1-3 and 3-3 have expectations of $\frac{1}{2}$ and $(1-r)/2$ respectively. Other sorts of crosses may also lead to disturbed segregations but this cross is one from which the recombination fraction may be calculated using maximum likelihood estimation. Loci not linked to the incompatibility loci will not give disturbed segregation ratios.

ANALYSIS

The grasses are classified into 23 tribes, have a basic chromosome number of $n=5$ or $n=7$ and fossil records of grass pollen are found in the Paleocene (65 million years) (Muller, 1981). The chromosomes of species with a basic number of 7 are generally metacentric and form on average one chiasma per arm at metaphase. Very few plants are well mapped genetically; and gene mapping techniques are restricted since it is not possible to G band plant chromosomes in the same way as animal chromosomes or to set up somatic cell lines. However, the behaviour of the incompatibility system means that the linkage groups in which the incompatibility genes are located may be readily identified.

Linkage of *PGI-2* (*Gpi-2*) (E.5.1.3.9) to one of the loci determining gametophytic self-incompatibility has been reported in *Lolium perenne* (Cornish *et al.*, 1980) and in the closely related *Lolium multiflorum* (Fearon *et al.*, 1983). These observations suggest the question "Is there evidence for the conservation of this linkage in other grasses with the same incompatibility system?"

Five species of grasses were chosen for study (table 1). The two locus incompatibility system has been demonstrated in three of these, *Secale cereale* (*loc. cit.*), *Phalaris coerulea* (*loc. cit.*) and *Festuca pratensis* (Lundqvist, 1955; 1961). *Holcus lanatus* is highly self-incompatible, shows gametophytic determination of incompatibility and differences in the compatibility status between reciprocal crosses (Weimark, 1968). *Alopecurus myosuroides* is highly self-incompatible and shows gametophytic determination of incompatibility (Leach, unpublished). Both of these species are likely to have the same incompatibility system as is found in all other grasses. All species chosen

were diploid with $n=7$ (Darlington *et al.*, 1955) and each comes from taxonomically different tribes well separated on an evolutionary time scale (Stebbins, 1971).

With the exception of *S. cereale* all the species are perennials and so the procedure generally adopted to acquire plants of suitable incompatibility relationships has been to backcross progeny plants to parents. Where the original acquisitions were known to be sibs or half sibs pollination tests were carried out to establish pairs of plants suitable for crossing. *S. cereale* is an annual and so only sib crosses are possible. Many of the introductions of this species tested exhibited a degree of self-compatibility and so an independent marker had to be used as an indicator of crossing. Table 1 shows the results of the linkage analyses carried out.

Table 1 Estimates of the recombination frequency between the *S* locus and *Gpi-2* locus

Species	Recombination frequency	Standard error	Sample size
<i>L. perenne</i> (Cornish <i>et al.</i> , 1980)	0.1538	0.0252	
<i>L. multiflorum</i> (Fearon <i>et al.</i> , 1983)	0.2414	0.0795	
<i>A. myosuroides</i>	0.1852	0.0449	150
<i>P. coerulea</i>	0.1134	0.0312	207
<i>F. pratensis</i>	0.2381	0.0933	42
<i>H. lanatus</i>	0.2987	0.0563	132
<i>S. cereale</i> *	1) 0.0714 2) 0.1902	0.0668	28 30

* Estimate 1) is from a single head from a full sib cross and 2) is an upper limit based on observing no recombinant in a collection of related full sib progenies where these plants were from *bona fide* crosses.

The estimate of the recombination frequency from *H. lanatus* is significantly greater than that for *L. perenne*, *P. coerulea* and *S. cereale*. No other pair of these values is significantly different. These estimates are based upon the disturbance in the segregation ratio. Viability effects for GPI segregation were shown to be negligible since in all species undisturbed segregation ratios have been found in crosses of similar compatibility status. Thus the disturbed segregations observed are the result of linkage to the incompatibility factors and not due to viability effects. Whilst it is not possible to prove that it is the same self-incompatibility locus linked to *Gpi*, the close linkage values suggest that a unit has been conserved and that the same loci are involved in each species.

Both *Alopecurus myosuroides* and *Holcus lanatus* show the same pattern of disturbed segregation and a similar linkage value. The observations strongly suggest that they also possess the two locus system of incompatibility.

CONCLUSION

These data should be of interest not only to workers on evolution, but also to plant breeders as it might be possible to extrapolate from the results of linkage studies in one grass species to possible linkage relationships in another. Such limited linkage data as are available certainly support this view (Miller, 1984; Brown, 1983).

A theoretical analysis of the effect, in terms of linkage disequilibrium (Leach *et al.*, 1986), that a single locus gametophytic self-incompatibility system has on genes linked to it reveals that the enforced heterozygosity of the system does not shelter lethal mutations. Thus it is reasonable to assume that genes of economic importance closely linked to the incompatibility loci are neither protected nor less readily available for selection. However it was found that where an incompatibility system is operating selection intensities (for removing deleterious genes) of less than unity are more effective at lowering gene frequency than is complete selection.

Acknowledgements We acknowledge financial support from the Australian Research Grants Scheme. Mr Clive Chesson gave valuable technical advice.

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