THE USE OF INCOMPATIBILITY FOR THE PRODUCTION OF F_1 HYBRIDS IN FORAGE GRASSES

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SUMMARY

A possible method for the production of F_1 hybrid seed of forage grasses in commercial quantities is described. The method depends on the two-locus incompatibility system postulated by Lundqvist (1961). Inbred lines are made, each deriving from a single genotype, having a high degree of within-line incompatibility so that when two, or more, such lines are interplanted the seed produced will be largely of hybrid origin. The consequences of multi-locus incompatibility mechanisms are examined and a general method for calculating the expected composition, in terms of incompatibility groups, of an inbred line in any generation and for any number of incompatibility loci is given.

1. INTRODUCTION

FOSTER (1971*b*) has studied the theoretical expectation of F_1 hybridity resulting from bulk interpopulation hybridisation in herbage grasses and, in two other papers (Foster, 1971*a, c*), he has reported on the performance of such hybrids under spaced plant and sward conditions. He found that F_1 hybrids were not on average superior to the parental varieties but that certain F_1 's yielded up to 31 per cent more than their higher yielding parent under spaced plant conditions and up to 17 per cent more in swards. Wright (1972) has pointed out that with diploid inheritance, the mean performance of both semi-hybrid varieties of the type proposed by Foster and conventional synthetics is expected to be the same. Burton (1956) has discussed the value of F_1 hybrids in forage grasses and possible techniques for producing F_1 seed in commercial quantities; he concluded that the extra seed cost might well be justified by the increased yields of forage. McWilliam (1962) has shown the value of F_1 hybrids between species of *Phalaris*. It is tempting to consider the possibility of producing F_1 hybrids of forage grasses more nearly along the lines used in maize, sorghum and some other crops. Usually, the production of F_1 hybrids depends either on a rapid technique of mass emasculation or the use of male-sterility although sporophytic incompatibility has been used in brassicas (Thompson, 1957) and of course F_1 hybrids between pairs of self-incompatible genotypes are readily made and were used by both Burton (*loc. cit.*) and McWilliam (*loc. cit.*). The structure of the flowers of most forage grasses does not permit emasculation on the scale required and the effort involved in developing male-sterile lines, if such could be found, would be unlikely to be worth while. The two-locus self-incompatibility mechanism, proposed by Lundqvist (1961) for *Festuca pratensis* and stated by Weimark (1968) to operate in some other grasses including *Lolium perenne*, may provide a basis for the development of varieties consisting largely of F_1 hybrid genotypes and may also permit conventional combining ability tests to be made on inbred lines.

Hayward and Wright (1971) have produced some evidence that the incompatibility mechanism in *Lolium perenne* is rather less precise in its control than it was believed to be by Weimark and that it may depend on a multi- rather than a two-locus system.

In this paper schemes are developed for the production of F_1 hybrids by means of inbred lines having a high degree of within-line incompatibility. Two such lines when interplanted and flowering simultaneously will produce seed very largely of hybrid origin. The idea will first be explained in terms of Lundqvist's two-locus system and then extended to three- and multi-locus systems.

2. THE TWO-LOCUS SYSTEM

The system proposed by Lundqvist postulates two independently segregating loci, S and Z, with gametophytic control such that a compatible pollination is one in which, for at least one locus, the allele in the pollen is not matched by either allele in the stigma.

Consider a single genotype $S_1 S_2 Z_1 Z_2$ or for brevity 12.12. Assuming that it is possible to produce a progeny by selfing a plant with this genotype, then the expected composition of the first inbred generation, I1, will be as shown in the first two columns of table 1. Such a population will itself produce a pollen cloud consisting of equal proportions of the four possible pollen genotypes and will result in an A1 (first advance) population having the expected frequency composition shown in table 1.

There is for our purposes no need to consider each of the possible genotypes separately; instead they can be thought of as falling into three categories according to their degree of homozygosity. The genotypes in the I1 can be grouped into those which have no homozygous loci and those which have one or two loci which are homozygous; for brevity these are called hom^0 , hom^1 and hom^2 respectively. Each of these "genotype groups" is

TABLE 1
Frequency of genotypes in the I1 generation and in its A1 progeny

I1 ♀ genotype	I1 frequency	♂ Gamete compatibility				Frequency of progeny genotypes in the A1 within each ♀ genotype in the I1				
		1.1	1.2	2.1	2.2	11.12	12.11	12.12	12.22	22.12
11.11	$\frac{1}{16}$	—	+	+	+	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	—	—
11.12	$\frac{1}{8}$	—	—	+	+	—	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{4}$	—
11.22	$\frac{1}{16}$	+	—	+	+	$\frac{1}{3}$	—	$\frac{1}{3}$	$\frac{1}{3}$	—
12.11	$\frac{1}{8}$	—	+	—	+	$\frac{1}{4}$	—	$\frac{1}{2}$	—	$\frac{1}{4}$
12.12	$\frac{1}{4}$	—	—	—	—	—	—	—	—	—
12.22	$\frac{1}{8}$	+	—	+	—	$\frac{1}{4}$	—	$\frac{1}{2}$	—	$\frac{1}{4}$
22.11	$\frac{1}{16}$	+	+	—	+	—	$\frac{1}{3}$	$\frac{1}{3}$	—	$\frac{1}{3}$
22.12	$\frac{1}{8}$	+	+	—	—	—	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{4}$	—
22.22	$\frac{1}{16}$	+	+	+	—	—	—	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$
Overall frequency of progeny genotypes in A1 weighted for parental frequencies in I1						$\frac{5}{36}$	$\frac{5}{36}$	$\frac{16}{36}$	$\frac{5}{36}$	$\frac{5}{36}$

Note that the completely homozygous offspring do not occur in the A1 generation and that the completely heterozygous genotypes do not produce offspring.

expected to produce a pollen cloud in which the four possible types of male gamete are uniformly distributed and the male gametes produced by the population as a whole will therefore have a uniform distribution irrespective of the frequencies of hom^0 , hom^1 and hom^2 genotypes (this will only be true if the hom^1 and hom^2 groups themselves comprise equal proportions of their constituent genotypes, *e.g.* if the hom^1 group contains equal proportions of 11.12, 22.12, 12.11 and 12.22 genotypes). Assuming a uniform distribution of male gamete frequencies it is possible to construct from table 1 a 3×3 matrix **A** in which each column contains the expected frequencies of the three genotype groups in one generation which are produced by one of the groups in the preceding generation. This matrix occupies columns 2, 3 and 4 in table 2. The hom^1 used as female parents is expected to have no

TABLE 2

The matrix A for two-loci and the frequencies of the different genotype groups in successive generations

		f_n genotypes			Frequency of genotypes in I1 and subsequent generations		
		hom^2	hom^1	hom^0	I1	A1	A2
f_{n+1} genotypes	hom^2	0	0	0	$\frac{1}{4}$	0	0
	hom^1	$\frac{2}{3}$	$\frac{1}{2}$	0	$\frac{1}{2}$	$\frac{5}{12}$	$\frac{5}{24}$
	hom^0	$\frac{1}{3}$	$\frac{1}{2}$	0	$\frac{1}{4}$	$\frac{4}{12}$	$\frac{5}{24}$

Note 1—The I1 generation frequencies are assumed to be in binomial proportions.

Note 2—The frequencies in the A1 and A2 generations have not been normalised, the ratio between hom^1 and hom^0 frequencies in the total A1 population is of course $\frac{5}{6}$: $\frac{4}{6}$ and similarly in the A2 $\frac{1}{2}$: $\frac{1}{2}$.

offspring of type hom^2 and to have equal proportions of types hom^1 and hom^0 . If $\mathbf{f}_n$ is a column vector of the frequencies of the genotype groups in generation n , then $\mathbf{A}\mathbf{f}_n$ gives the expected frequencies in generation $n+1$. That is:

$$\mathbf{f}_{n+1} = \mathbf{A}\mathbf{f}_n$$

and starting with the expected frequencies in I1 as the elements of $\mathbf{f}_n$, it is possible to calculate the expected frequencies in any subsequent generation (table 2). The A2 and subsequent generations are expected to contain equal proportions of hom^1 and hom^0 types and the hom^2 type disappears from the A1 generation. The process is essentially that of a Markoff chain but the presence of a row and a column of zeros in the matrix means that, to obtain the actual frequencies for any generation, the column vector $\mathbf{f}$ has to be normalised so that the frequencies sum to unity; in a conventional Markoff chain they would do so anyway.

3. THREE OR MORE LOCI

As mentioned earlier, there is some evidence to suggest that the incompatibility system in ryegrass involves more than two loci. The process for finding the frequencies in any generation under multi-locus control is a simple extension of that used in the two-locus case and requires only the calculation of the expected frequencies in the I1 and the construction of the matrix **A**.

Consider the case of k independently segregating loci. The I1 generation will then consist of $k+1$ genotype groups of types hom^k , hom^{k-1} , ..., hom^0 with expected frequencies which can be calculated from the binomial $(a+b)^k$, where a represents homozygous loci and b heterozygous loci. Thus with three loci the possible classes are hom^3 , hom^2 , hom^1 and hom^0 with expected frequencies $\frac{1}{8}$, $\frac{3}{8}$, $\frac{3}{8}$, $\frac{1}{8}$ respectively.

The matrix $\mathbf{A}$ for any number of loci can be constructed as follows. Write down a $(k+1) \times (k+1)$ matrix $\mathbf{X}$ such that each column contains the binomial expansion $(a+b)^k$. Construct another $(k+1) \times (k+1)$ matrix $\mathbf{Y}$ such that each column contains the coefficients of the binomial $(a+b)^{j-1}$ (where j is the column) in its first j rows and zeros elsewhere. Let $\mathbf{Z} = \mathbf{X} - \mathbf{Y}$; then normalising the columns of $\mathbf{Z}$ gives $\mathbf{A}$, i.e. $a_{ij} = z_{ij}/z_{.j}$. The construction of $\mathbf{Z}$ for three loci is shown below.

$$\begin{array}{ccc} \mathbf{X} = \begin{array}{cccc} 1 & 1 & 1 & 1 \\ 3 & 3 & 3 & 3 \\ 3 & 3 & 3 & 3 \\ 1 & 1 & 1 & 1 \end{array} & \mathbf{Y} = \begin{array}{cccc} 1 & 1 & 1 & 1 \\ 0 & 1 & 2 & 3 \\ 0 & 0 & 1 & 3 \\ 0 & 0 & 0 & 1 \end{array} & \mathbf{Z} = \begin{array}{cccc} 0 & 0 & 0 & 0 \\ 3 & 2 & 1 & 0 \\ 3 & 3 & 2 & 0 \\ 1 & 1 & 1 & 0 \end{array} \end{array}$$

Note $\mathbf{Z} = \mathbf{X} - \mathbf{Y}$ and a_{ij} (i.e. each element of $\mathbf{A}$) = $z_{ij}/z_{.j}$.

The expected frequency compositions of successive generations for three and four loci are summarised in table 3. The progress towards constant frequency in successive generations is effectively complete by the A2 generation.

TABLE 3
Expected frequencies of genotype classes in successive generations for three- and four-locus systems

	I1	A1	A2	A3	A4
Three loci					
hom^3	0.1250	0.0000	0.0000	0.0000	0.0000
hom^2	0.4750	0.3112	0.2824	0.2801	0.2799
hom^1	0.4750	0.4898	0.5000	0.5000	0.5000
hom^0	0.1250	0.1990	0.2176	0.2199	0.2201
Four loci					
hom^4	0.0625	0.0000	0.0000	0.0000	0.0000
hom^3	0.2500	0.1749	0.1608	0.1595	0.1594
hom^2	0.3750	0.4076	0.4040	0.4032	0.4031
hom^1	0.2500	0.3273	0.3392	0.3405	0.3406
hom^0	0.0625	0.0902	0.0960	0.0969	0.0969

It is useful at this point to introduce the idea of the "within population compatibility" (or WIC) of the various female genotypes, that is the proportion of the gametes in the pollen cloud with which they are compatible. This can be calculated from the expression $\text{WIC} = 1 - (\frac{1}{2})^p$, where p is an integer representing the degree of homozygosity, e.g. the hom^2 genotypes will have a WIC of $\frac{3}{4}$ and the hom^0 a WIC of 0. WIC of less than one but more than zero does not imply a reduction in seed produced so long as the supply of pollen is not limiting; it merely imposes restrictions on the genotypes of the offspring from any female parent and is of importance in estimating the likely frequency of outcrossing in the presence of alien pollen.

4. MULTIPLICATION AND USE OF THE PARTIALLY INBRED LINES

The coefficient of inbreeding of the lines will be 0.5, assuming that the base populations from which the parents were drawn were themselves not inbred. Multiplication of course will not be expected to increase this. The rate of multiplication will, however, be affected to some degree by the effect of inbreeding depression on seed yields and by the fact that hom^0 individuals are effectively female sterile if exposed only to pollen from within their own population. At equilibrium, therefore, half the population in the two-locus case produce no seed and for three and four loci the proportions are about $\frac{1}{2}$ and $\frac{1}{10}$ respectively. Note that the more loci there are involved, the easier it is to multiply the inbred population.

A mixed planting of two inbred lines will produce a mixture of F_1 hybrids and inbred progeny. Assuming a pollen cloud consisting of equal amounts from the two sources the proportion of outbred seed produced by any genotype class can be found from the expression

$$\frac{1}{1 + \text{WIC}}$$

and the proportion of outbred seed produced by the population as a whole by summing over all genotypes after weighting for their frequencies. In two loci, for example, this will be

$$\left(\frac{1.0}{1.5} \times 0.5\right) + \left(\frac{1.0}{1.0} \times 0.5\right) = 0.83$$

and the values for three and four loci are 0.71 and 0.64. When there are eight loci the figure is 0.53, *i.e.* effectively no greater than would be the case with no incompatibility at all.

5. DISCUSSION

The method proposed for producing the parental lines depends to some degree on a contradiction. The assumption is made that a plant of constitution hom^0 can be selfed, *i.e.* that the incompatibility mechanism is less than completely effective, while the construction of the matrix A and hence the calculation of the expectations of frequencies in the A_1 and later generations depends on the assumption that it is completely so. It is well known that most grass plants will produce a little viable seed by selfing, *i.e.* when no truly compatible pollen is available. During the multiplication phases, although some nominally incompatible fertilisations will undoubtedly occur, especially with hom^0 female genotypes, the incompatible pollen will usually be in competition with compatible pollen with respect to any female genotype and "illegitimate" fertilisation will be rare.

The parental lines, being derived from single plants, will have an expected inbreeding coefficient of 0.5 and will not therefore provide a particularly good base for selection for combining ability between inbreds from the same base population, since the best cross cannot be better than the best full-sib family derived from the base population. If the inbreds come, however, from different base populations, the variance between crosses may be greater than the genotypic variance in either base population and it would be reasonable to select at least for general combining ability. The method

might be especially useful in producing hybrids between closely related species such as *Lolium perenne* and *Lolium multiflorum* with the aim of producing a uniform F_1 hybrid cultivar, the commercial seed itself being of hybrid origin. Maintenance of the inbred lines at an inbreeding coefficient of 0.5 should not be particularly difficult but multiplication of them to the stage at which they were available in sufficient quantity to be the immediate parents of seed in commercial quantities would present problems in providing isolation plots since quite low initial levels of contamination by alien pollen would rapidly increase, particularly because the homo⁰ genotypes are effectively female sterile towards pollen from their own population but fully fertile with foreign pollen. A certain amount of the multiplication process could be carried out by vegetative subdivision but this would obviously become uneconomic as the inbred populations increased in size, moreover it would not be worth while unless it enabled at least one generation of seed multiplication to be avoided. Multiplication of inbred lines would, of course, proceed in step with their evaluation as parents and many lines could be discarded before multiplication had been carried very far. The inevitable presence of inbred seed in the commercial stock would be a disadvantage; but this seed would certainly produce less vigorous seedlings which would be eliminated or suppressed in the sward during establishment.

6. REFERENCES

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