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Quantitatively determined self-incompatibility. 5. Detection of multi-locus systems

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Abstract. Multi-locus self-incompatibility systems may be distinguished from single-locus systems by reciprocal differences in backcrosses and between crossed progeny of individual clearly compatible crosses. Such crosses are extremely laborious, so other methods have been suggested. In this note, it is shown that the coefficient of crossability is not a useful discriminant of self-incompatibility, as indeed should be expected from the properties of multi-locus systems, and that linkage methods are also unlikely to be successful. Until more self-incompatibility genes have had their sequences characterised, there is no substitute for the traditional genetical methods.

Key words: Bioassay – Coefficient of crossability – Self incompatibility – Linkage estimation

Introduction

Lundqvist (e.g. 1990 a, b, c; 1991) and others have detected multi-locus gametophytically determined self-incompatibility across a wide range of taxa. If self-incompatibility can evolve in the manner proposed by Uyenoyama (1991), then there is no limit to the number of additional loci that can accumulate by duplication and subsequent increase in frequency through the advantage of additional cross-compatibility. Further, as is illustrated here and has been shown before, additional loci over about three impose little additional maintenance load on their possessor. Hence, while they may be expected to persist, they will provide little equilibrial advantage over systems possessing fewer loci. (See also Charlesworth 1979.)

The investigation of self-incompatibility is extremely laborious, so that a method has often been sought that allows its detection and evaluation through simple trials such as the initial diallels. In this paper, we examine one proposed method, that of Anderson et al. (1989). We also consider the possibility of using the linkage analysis method of Leach (1988) to discriminate between different systems of self-incompatibility.

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Properties of multi-locus gametophytic systems

Using the method of Mayo (1966), we have simulated gametophytically determined self-incompatibility systems having one, two, three, four or five loci. Results for populations of effective size $N_e = 100$ are shown in Tables 1 and 2. (Note that the standard errors have been calculated as if every potential cross were independent of every other, and similarly for every potential mutation event; neither assumption can be correct in successive generations in a small population. This means that the standard errors presented are too low, but it is not clear how to correct them for dependence.) These results are exemplary only; N_e is not known for any relevant species. The key points to note are that with more than about three loci the degree of increased cross-compatibility is trivial and that the cost of maintenance in terms of mutation similarly rises substantially only between one and three loci provided that the number of alleles per locus is not very small. Mutations to new specificities have never been unambiguously observed (e.g. Hayman and Richter 1992), so maintenance of an incompatibility system in this way is unlikely; in large populations, much lower mutation rates are in any case required to maintain a given level of variability.

One possible conclusion is that such systems should be quite widespread; but the labour of searching for them is very great. The other conclusion is that the level of

Table 1. Proportion of incompatible matings in a population of size 100. The estimate (followed by binomial standard error) derives from 2000 generations after the population is at equilibrium)

Number of loci	Number of alleles/locus	Proportion of incompatible pollinations	
1	3	0.6738	0.0006
	4	0.5098	0.0008
	5	0.4120	0.0008
	10	0.2413	0.0008
	20	0.1608	0.0008
	40	0.1106	0.0006
2	3	0.3833	0.0009
	4	0.2374	0.0008
	5	0.1790	0.0008
	10	0.1011	0.0006
	20	0.0543	0.0005
	40	0.0276	0.0004
3	3	0.2228	0.0008
	4	0.1343	0.0007
	5	0.1126	0.0007
	10	0.0580	0.0005
	20	0.0285	0.0004
	40	0.0145	0.0003
4	3	0.0763	0.0004
	4	0.0543	0.0003
	5	0.0408	0.0003
	10	0.0180	0.0002
	20	0.0071	0.0001
	40	0.0046	0.0001
5	3	0.0586	0.0004
	4	0.0403	0.0003
	5	0.0294	0.0003
	10	0.0131	0.0002
	20	0.0054	0.0001
	40	0.0049	0.0001

Table 2. Mutation rate ($\times 10^5$; followed by standard error) necessary to maintain the number of alleles shown in a population of size 100. Estimation over 2000 generations after equilibrium was reached

Number of loci	Number of alleles/locus	Mutation rate	
1	3	0	
	4	0	
	5	0	
	10	35.99	4.00
	20	536.36	11.52
	40	2,551.92	24.61
2	3	0	
	4	0	
	5	2.33	0.44
	10	204.90	5.06
	20	976.10	10.99
	40	3,147.41	19.52
3	3	0	
	4	6.08	0.71
	5	46.25	1.96
	10	338.08	5.30
	20	1,225.17	10.04
	40	3,558.92	16.91
4	3	0	
	4	32.25	1.42
	5	73.19	2.14
	10	393.25	4.95
	20	1,242.19	8.76
	40	3,380.50	14.29
5	3	5.25	0.51
	4	45.60	1.51
	5	90.15	2.12
	10	426.15	4.61
	20	1,277.22	7.94
	40	3,476.02	12.95

cross-incompatibility to be found in random samples from a neutral population will be no guide to the breeding system.

Coefficient of crossability

Anderson et al. (1989) presented a method designed to detect self-incompatibility by distinguishing the pattern of seed set in a diallel cross that is to be expected from self-incompatibility from that of other outbreeding mechanisms. (It is important to note that it is the pattern of seed set that Anderson et al. consider, as the overall level of seed set should be no lower in compatible crosses than in comparable species lacking a self-incompatibility system; see Cornish et al. 1980; Fearon et al. 1983.) They define the coefficient of crossability (CC) as actual seed set/potential seed set, where potential seed set is "the uppermost amount of seed set supported by a plant (e.g. highest seed set when outcrossed, open-pollinated, bulk outcrossed, or crossed to an unrelated S tester)". For data from a diallel cross obtained from progeny from an initial compatible cross, a plot of the male CC against the female CC should, according to Anderson et al., show clusters of observations at (0,0) and (1,1), whereas other types of barriers should give varied responses. Furthermore, for a known self-incompatibility system, values other than (0,0) and (1,1) should reveal the segregation of deleterious genes (Lundqvist 1990c), inbreeding depression etc.

Figure 1 shows the plots for four diallels carried out in borage, *Borago officinalis* L., by Leach et al. (1990). There is no evidence of self-incompatibility, in that there is no clustering of values close to (0,0) and (1,1) in any of the diallels. For cross 2, the observed coefficient of correlation between FCC and MCC is significantly different from that of cross 1. Leach et al. (1993) had already concluded that borage is not self-incompatible. [Analyses used included generalised linear models, as al-

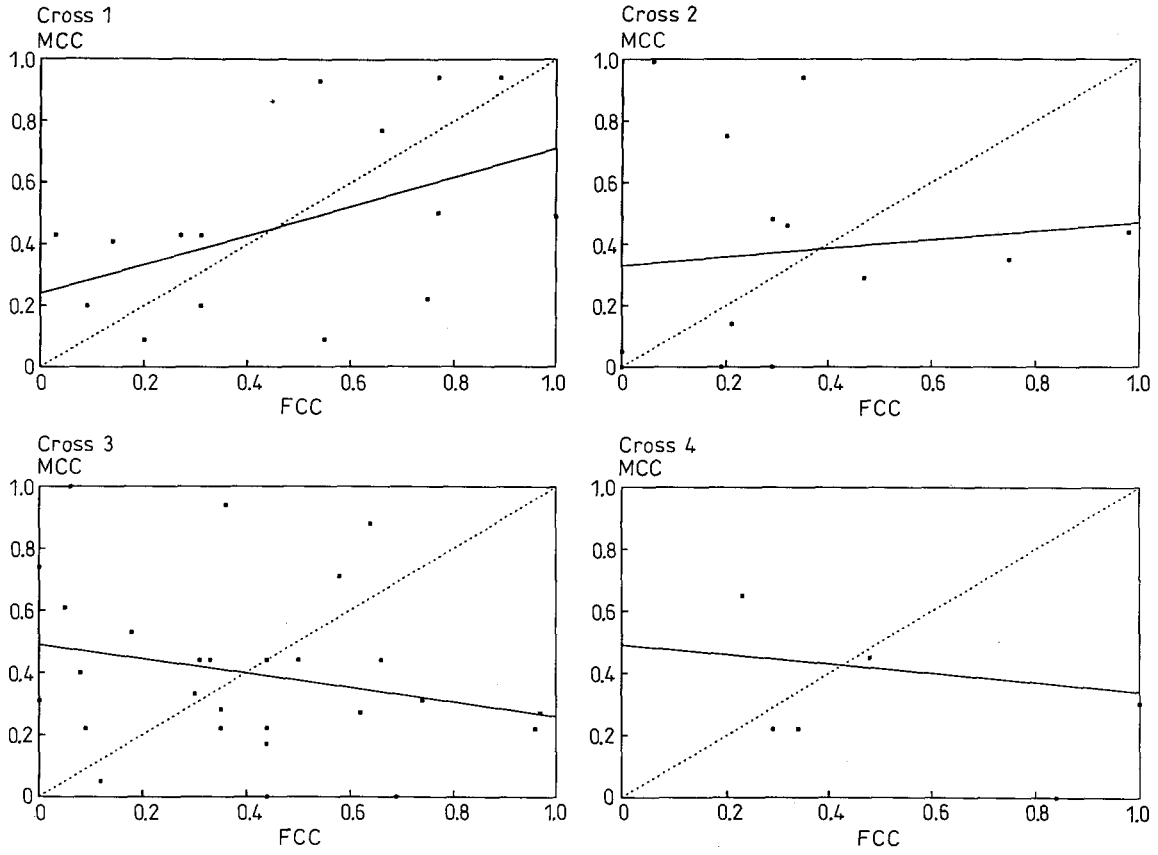

Fig. 1. Plots of male crossability coefficient against female crossability coefficient for four diallel crosses of Leach et al. (1990). *Solid line* is simple regression of MCC on FFC; *dotted line* MCC = FCC. *Cross 1* 6 × 6 diallel of sibling plants from two reciprocal crosses of field-collected plants, *Cross 2* 7 × 7 diallel of sibling plants from three reciprocal crosses of field-collected plants, *Cross 3* 9 × 9 diallel of sibling plants from different progeny of one of the crosses that established cross 2, *cross 4* 4 × 4 diallel of two plants raised from seed from two different commercial suppliers

so proposed by James et al. (1992); the labour is contained in the crossing, not in the statistical methods.]

The essence of Anderson et al.'s argument is that a given cross and its reciprocal should give identical results with respect to compatibility. This is true for the single-locus gametophytic system, but not in general for multi-locus gametophytic systems, when assessed by the pattern of seed set. Consider the two-locus system, for simplicity. There are three possible types of fully compatible cross:

double heterozygote × double heterozygote, e.g. $S_{1\cdot2}\,Z_{1\cdot2} \times S_{3\cdot4}\,Z_{3\cdot4}$

double heterozygote × single heterozygote, e.g. $S_{1\cdot2}\,Z_{1\cdot2} \times S_{3\cdot3}\,Z_{3\cdot4}$

single heterozygote × single heterozygote, e.g. $S_{1\cdot1}\,Z_{1\cdot2} \times S_{3\cdot3}\,Z_{3\cdot4}$

These crosses are also reciprocally fully compatible. There are, however, other possibilities:

double heterozygote × double heterozygote, e.g. $S_{1\cdot2}\,Z_{1\cdot2} \times S_{1\cdot2}\,Z_{1\cdot4}$

double heterozygote × single heterozygote, e.g. $S_{1\cdot2}\,Z_{1\cdot2} \times S_{1\cdot1}\,Z_{1\cdot2}$

The double × double case is reciprocally compatible and if pollen is freely available should set as much seed as any of the fully compatible crosses. However, if pollen-tube growth be examined, only one-half of all pollen grains should prove capable of growth down the style. The double × single case is incompatible as shown (female parent first), but its reciprocal is compatible to the same extent as the double × double case, i.e. one-half of gametes can effect fertilization.

The use of pollen-tube growth as a test provides conclusive discrimination in the cases described, at the cost of a great deal of labour. Seed-set data are inadequate or misleading.

Although double homozygotes are impossible to obtain, the frequency of single homozygotes is not expected to be low, since each of the two self-incompatibility loci can be in Hardy-Weinberg equilibrium; therefore, for k equally frequent alleles at each locus, the expectation that a plant would be homozygous at one and heterozygous

at the other would be $2(k-1)/k^2$. [Weber et al. (1982) in a more exact examination of the problem show that with five alleles at each locus almost 30% of the genotypes are of this kind, and with 20 alleles at each locus the frequency is still over 9%.] The frequencies of the alleles will not be identical; this will increase the overall frequency of homozygotes and decrease that of heterozygotes, other things being equal. Hence, reciprocally different crosses are unlikely to be rare in a two-locus system.

Linkage analysis

Leach (1988) presented a method for linkage detection and estimation through the degree of disturbance of segregation in a cross involving a co-dominant set of alleles at one locus hypothetically linked to a gametophytic self-incompatibility locus. It should be noted in passing that such disturbed segregations are not possible with any of the known sporophytic systems, so that this provides a method of discrimination, though rather a crude one, between systems.

For a single locus, pollination can be 0%, 50% or 100% compatible, and only that which is 50% compatible yields a disturbed segregation ratio, as in

$$\frac{S_1\,A_1}{S_2\,A_1} \times \frac{S_1\,A_1}{S_3\,A_2}$$

which will yield $A_1\,A_1$ and $A_1\,A_2$ in the ratio $r:1-r$, when r is the rate of recombination between the self-incompatibility locus S and the unrelated locus A.

For two loci, pollination can be 0%, 50%, 75%, or 100% compatible. For 75% compatible, as illustrated by two loci, we have

$$\frac{S_1\,A_1}{S_2\,A_1}\,\frac{Z_1\,B_1}{Z_2\,B_2} \times \frac{S_2\,A_1}{S_3\,A_2}\,\frac{Z_2\,B_1}{Z_3\,B_2}$$

	Exp.	A_1A_2	A_1A_1
		$\frac{1+r_A}{3}$	$\frac{2-r_A}{3}$
	Obs.	y_1	y_2

For any locus linked to one of the self-incompatibility loci, an equivalent relation holds. This gives

$$\tilde{r}=\frac{2y_1-y_2}{y_1+y_2};\quad Var(\tilde{r})=\frac{9\,y_1\,y_2}{(y_1+y_2)^3}$$

For three loci, pollination can be 0%, 50%, 75%, 87.5% or 100% compatible. In general, for n segregating loci we have $100(1-(\frac{1}{2})^n)\%$ compatible for the maximal partial compatibility, yielding

Expected	$A_1\,A_1$	$A_1\,A_2$
	$\frac{2^{n-1}-1+r_A}{2^n-1}$	$\frac{2^{n-1}-r_A}{2^n-1}$
Observed	y_1	y_2

$$\tilde{r}=\frac{2^{n-1}\,y_1-(2^{n-1}-1)\,y_2}{y_1+y_2};\quad Var(\tilde{r})=\frac{(2^n-1)^2\,y_1\,y_2}{(y_1+y_2)^{2^n-1}}$$

It is difficult in principle to distinguish the different systems by the detection of classes with different levels of compatibility (which then yield different linkage results), but even if it were simpler, the problem remains that establishment of the putative genotypes is immensely laborious; somes simplification of the methodology is needed, but it will not be provided by linkage analysis.

Bioassay

Jackson and Linskens (1990) have recently reviewed the use of bioassays for detection and determination of the properties of self-incompatibility and have concluded that bioassays are more likely to be useful for sporophytic than for gametophytic systems because of the nature of the different incompatibility reactions. The problems they discuss highlight the lack of a satisfactory substitute for extensive crossing of the diallel type with progeny testing, to determine patterns of cross-compatibility, reciprocal differences (usually characterisic of sporophytic systems) and numbers of mating types arising from a single cross (more than four usually indicating multiple loci in a diploid).

Conclusions

All of the methods discussed, apart from the traditional genetical techniques, lack discriminatory power. There is at present no other method for distinguishing between different systems. Rapid, straightforward elucidation of a given self-incompatibility system depends on the availability of molecular methods. These methods would be based on the identification of multiple sites occupied by representative sequences from self-incompatibility genes that have been characterised using in situ hybridisation, Southern hybridisation or quantitative analysis of polymerase chain reaction (PCR) amplified products.

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