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Fluctuations in heteromorphic self-incompatibility systems

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Summary. The genetic determination of heterostyly is briefly reviewed and experimental data are presented on the inheritance of tristyly in *Oxalis compressa.* It is shown that the model appropriate to *Lythrum salicaria,* of two diallelic loci, is inadequate to explain segregation patterns found in *O. compressa,* especially the reversal of dominance of the short phenotype. An extended model having an additional allele at the short locus, and a separate modifier gene, is presented and its deterministic genotype frequency dynamics examined numerically. It is shown that fixation of these extra genes or alleles is unlikely. Problems of testing the extended model are considered.

Key words: Heterostyly – *Oxalis compressa* – Modifier gene – Self-incompatibility – Isoplethy

Introduction

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). Self-incompatibility systems may be classified as heteromorphic when differences in flower morphology characterize the incompatible types, or homomorphic where there are no such differences. A species is said to be distylic if it contains two sorts of flowers, one which bears long styles and short stamens and the other short styles and long stamens.

Bateson and Gregory (1905) showed that distyly in *Primula sinensis* was determined by two alleles (*S, s*) at a single locus such that plants with short styles, thrums, were of genotype *Ss* and those with long styles, pins, were *ss.* Pollen from thrums behaves in a uniform manner indicating sporophytic control. Thrum is dominant to pin in both pollen and style characters. Legitimate matings are either thrum × pin (*Ss* × *ss*) or pin × thrum (*ss* × *Ss*). The *S* gene consists of a number of tightly linked units which only very occasionally recombine to produce new forms of flowers such as homostyles.

Baker (1966) demonstrated a similar genetic basis for heterostyly in the Plumbaginacea, though in *Limonium* long is dominant. Ornduff (1979) has also shown that long is dominant in *Hypericum aegyptium* L. (Hypericaceae). This is unlike *Primula* and all other distylic species that have been studied (Charlesworth (1979) for lists).

There is evidence that the *S* locus in this system is subject to control and modification by genes at other loci. In *Primula sinensis* two genes, *a* and *m*, showing independent assortment, affect both the incompatibility system and the floral architecture (de Winton and Haldane 1933, Beale 1929, Mather 1950). The *S* locus is probably influenced by a complex network of polygenes (Mather and de Winton 1941) which respond to rupture of balance provoked by inbreeding (de Nettancourt 1977) or mutagenic treatment (Sharma and Boyes 1961).

In the more elaborate form of heterostyly, tristyly, three types of flowers are found, those with short styles and mid and long stamens (shorts); those with mid styles and long and short stamens (mids) and those with long styles and short and mid stamens (longs). Legitimate crosses are those involving pollen and styles from the same level. The inheritance of tristyly in *Lythrum* was established by Fisher and Mather (1943). Control is by two loci, with two alleles at each locus (*S,s* and *M,m*). In *Lythrum salicaria* and in some species of *Oxalis,* long is homozygous recessive at both loci, mid is dominant to long and short is epistatic to mid (Fisher and Martin 1948; Fyfe 1950). The two loci are unlinked in *Lythrum* but linked in those species of *Oxalis* which have been investigated (Fisher and Martin 1948; Weller 1976; Sved 1964, unpublished). In several species of *Oxalis* short is recessive (von Ubisch 1926; Fyfe 1956; Bennett 1982, personal communication). In these cases short is still epistatic to mid.

There are also examples of self-incompatibility mechanisms which are governed by two loci with similar epistatic relationships but no heteromorphism, e.g. *Capsella grandiflora* (Riley 1936); *Physalis* (Pandey 1957); and *Solanum* (Pandey 1962) and of heterostyly not associated with self-incompatibility e.g. in the Boraginaceae (Ray and Chisaki 1957), the Oxalidaceae (Ornduff 1964) and the Rubiaceae (Ornduff 1970).

The aim of the experiments described here was to determine the mode of inheritance of style length in *Oxalis compressa* and to determine whether any micro-evolutionary changes had occurred in the century since the species was introduced into South Australia from South Africa.

Materials and methods

O. compressa (2n = 14) has been maintained in this laboratory since about 1960. *O. compressa* sets seed readily from compatible crosses and the majority of seeds are fertile. Compatible crosses, made by rubbing anthers from the plant intended as male parent on each of the five styles of a flower from the female parent, yield 20–70 seed per capsule. Mid and short styled flowers used as females are emasculated. Leaf markers were used to monitor illegitimate crossing or contamination.

Results

In 1965 the current programme of crossing was initiated. In that year seed was grown from crosses involving four plants: a long L64.01, a mid M64.01 and a putative mid homostyle H64.01 (a plant with flowers in which the style and upper whorl of anthers were very close in the mid position, and the lower whorl of anthers was in the short position), all obtained from crosses made before 1964, and a short S64.01 collected from Port Lincoln which is several hundred kilometres from Adelaide.

The results of these initial crosses (Table 1) indicated that mid and short were dominant to long and a two-locus model, with short *v.* non-short controlled by one locus with two alleles *S,s* and mid *v.* long controlled by a second locus also with two alleles *M,m; S* being epistatic to *M* appeared adequate to account for the control of tristyly in *O. compressa.*

To assess the linkage relationship between the two loci crosses of short progeny from M64.01 × S64.01 to longs were made. The results of these crosses are shown in Table 2. Shorts S65.02 and S65.03 carry mid in repulsion and so would have the genotype *Sm/sM*. The progeny from these shorts are homogeneous and total 101 short : 107 mid. A progeny of this size leads to an estimate of the upper limit for the recombination fraction between the short and mid loci of 0.029.

Table 1. Style length segregation in the first series of crosses

	Short	Mid	Long
M64.01 × S64.01	24	5	5
L64.01 × S64.01	23	–	12
H64.01 × L64.01	–	24	41

A large number of shorts from S65.02, S65.03 and S65.04 were crossed to long. The progeny from all these were homogeneous and are shown in Table 3. The mids asterisked in Tables 2 and 3 were all thought to be attributable to contamination as they had leaf markers inconsistent with the parents of the crosses. Indeed, one has subsequently been shown to be *MM*. However, these progeny provide further evidence of the very tight linkage between the short and mid loci.

As all existing shorts were derived from S64.01 I decided to introduce new material into the programme. Plants S69.01, S69.02 and L74.01 were obtained from a location several hundred metres from the laboratory in a public park area known as Botanic Park.

The results of the first cross of S69.01 are presented in Table 4. Whilst indicating that S69.01 did not carry mid, the mode of inheritance of the short styled form appeared to be different from that previously found in *O. compressa.* Further crosses were made with S69.02 and L74.01. Table 5 is a summary of all crosses scored in 1980 involving these plants and other plants from crosses made before 1969.

The results from crosses with S69.02 as female parent suggested that this plant might be a pseudogamous apomict, but those from crosses with S69.02 as male parent provided further evidence for a mode of

Table 2. Style length segregation in the backcross progeny of shorts from M64.01 × S64.01

	Short	Mid	Long
L65.03 × S65.02	14	18	–
S65.03 × L65.03	14	18	–
S65.02 × L65.12	50	39	–
L65.12 × S65.02	23	32	–
S65.04 × L65.02	23	1[a]	25
L65.04 × S65.04	23	–	17
L65.01 × S65.06	31	–	18

[a] See text

Table 3. Style length segregation in the third series of short and long crosses

	Short	Mid	Long
S66.01 – S66.12 from S65.02	53	–	53
S66.13 – S66.26 from S65.03	199	2[a]	160
S66.27 – S66.32 from S65.04	42	–	52

[a] See text

Table 4. Style length segregation in the backcross progeny of the introduced short S69.01

	Short	Mid	Long
L66.24 × S69.01	83	–	236

Table 5. Crosses scored in 1980

	Short	Mid	Long		Short	Mid	Long
S69.02 × L74.01	30	–	–				
S69.02 × M66.14	1	–	–	M66.14 × S69.02	26	23	42
S69.02 × M66.89	21	2	1	M66.89 × S69.02	20	46	–
S69.02 × M67.10	12	–	–	M67.10 × S69.02	24	36	39
				M67.01 × S69.02	20	48	–

	Mid	Long	
M66.14 × L66.24 and L74.01 (reciprocally)	50	58	all data pooled were homogeneous
M66.89 × L66.24 and L74.01 (reciprocally)	127	0	
M67.10 × L66.24 and L74.01 (reciprocally)	70	62	

Table 6. Style length segregation in the reciprocal crosses to long of a short daughter of the introduced short S69.01

	Short	Mid	Long
S70.01 × L66.24	12	–	37
L66.24 × S70.01	3	–	20

inheritance of the short style form different from that found in the early crosses. The results from the set of mid × long crosses suggest that the mid and long plants used in crosses with S69.02 are not responsible for the curious segregation ratios observed. There is no evidence of spontaneous selfing or pseudogamous apomixis in these plants. Further evidence for the regular behaviour of L66.24 and L74.01 comes from crosses of these plants reciprocally to S65.04. The results of these crosses were all homogeneous and yielded a total of 55 short : 54 long.

Seed from crosses of a short daughter from L66.24 × S69.01 was also grown in 1980. (See Table 6.) These results are homogeneous and indicate that at least some of the short progeny of S69.01 behave in the same manner as their parent S69.01, and the other introduction, S69. 02.

These results for *O. compressa* are inconsistent with a simple two-locus model such as that which accounts for the inheritance of style length in *Lythrum salicaria* and the *Oxalis* species referred to previously. However, they are compatible with such a model if modifiers of the system are hypothesized to be segregating in South Australian populations.

Discussion

An extended model allowing for altered dominance relationships

The model is as follows: Assume that the material studied before 1969 was from a population in which there were two tightly linked loci, one determining the short *v.* non-short difference (*S,s*), epistatic to the other determining the mid *v.* long difference (*M,m*).

As the population from which S69.01 and S69.02 were drawn is geographically isolated from the source of all previously collected material, we may suppose that events have occurred in this population which did not occur in the established material.

It is postulated that a mutation S' occurred in the S gene so that $S's$ is non short. This S' allele does not allow self-fertilization, unlike homostyle mutations. A population in which only mid and long styled plants are present but in which short anthers are retained is extremely wasteful of pollen resources as only one quarter of all possible pollinations are compatible, viz. pollen from long anthers of mid plants on to long styles and from mid anthers of long plants on to mid styles. All short pollen is wasted as is long pollen on mid styles and mid pollen on long styles. Being phenotypically indistinguishable from *s*, the S' gene could be present in the population at a frequency high enough for a modifier *A*, such that AS' is phenotypically short to be present in the same individual. This would then be at an advantage and could spread readily through the population. Thus, given the $S \rightarrow S'$ change, the hypothetical modifier *A* would also increase in frequency.

This expanded model is of interest for we may see here an example of microevolution within the self-incompatibility system; a change of the kind which alters phenotypic frequencies quite markedly without disturbing incompatibility as such. That is, plants are still self-sterile but what was short with one set of genotypes at the *S* and *M* loci is no longer short. A number of workers from Finney (1952) onward (see Finney 1983 for review) have considered the genotypic equilibria for a variety of multilocus incompatibility systems, but to my knowledge no examples of systems undergoing changes have been presented. In the next section I relate my results to these analyses.

The testability of the model is considered in the final section.

Theoretical aspects of the extended model

The model proposed in the preceding section may be seen as having similarities with that proposed by von Ubisch (1926) for *Oxalis rosea*, but I can see no logical or functional genetical basis for her model. However, further evidence for the occurrence of modifier genes affecting heterostyly in *Oxalis* comes from Bennett (personal communication) who has found that an adequate explanation of data from *O. rosea* involves three loci in the inheritance of style length. The major difference between the model proposed here and the new model for *O. rosea* is that there are two alleles at each of three loci for *O. rosea* whereas for *O. compressa* three alleles occur at the short locus.

The first population of *O. compressa* studied seems to have the inheritance of three style forms controlled by two tightly linked loci and this should lead to an isoplethic equilibrium with genotype frequencies derived by Fisher (1941):

Short	Mid	Long
SsMM 0.024	*ssMM* 0.024	*ssmm* 0.333
SsMm 0.131	*ssMm* 0.309	
Ssmm 0.179		

with the addition that *SsMm* will be equally divided between coupling and repulsion double heterozygotes.

The mutation of $S \rightarrow S'$ is assumed to have been accompanied or followed by the loss of S and the subsequent mutation of $a \rightarrow A$ so that AS' is short. This leads to the following set of genotypes:

Short	Mid	Long
AA S'M/sM	*AA sM/sM*	AA sm/sm
AA S'M/sm or *S'm/sM*	*AA sM/sm*	*Aa sm/sm*
AA S'm/sm	*Aa sM/sM*	*aa S'm/S'm*
Aa S'M/S'M	*Aa sM/sm*	*aa S'm/sm*
Aa S'M/S'm	*aa S'M/S'M*	*aa sm/sm*
Aa S'm/S'm	*aa S'M/S'm*	
Aa S'M/sM	*aa S'M/sM*	
Aa S'M/sm or *S'm/sM*	*aa S'M/sm* or *S'm/sM*	
Aa S'm/sm	*aa sM/sM*	
	aa sM/sm	

Fixing A or S' will give the usual model of a dominant short in one case (A fixed) with short linked to mid and in the other (S' fixed) with short and mid assorting independently. Deterministic computer analysis of the recurrence relationships for genotype frequencies in a population in which the three genes postulated are segregating leads to genotypic stability after a variable but not small number of generations of random mating. All three style forms are retained in the population but the equilibrium may or may not be isoplethic.

When all genotypes were introduced into the population with equal frequencies among the style forms, each of these being present with a frequency of one third, equilibrium was reached after about 70 generations. In this case, the equilibrium was not isoplethic, yet the frequencies were maintained up to 300 generations. The population stabilized with short = 0.299, mid = 0.388 and long = 0.313. All genotypes were maintained in the population (Table 7) and the gene frequencies calculated from these are $A = 0.710$, $S' = 0.166$ and $M = 0.306$. The genotype proposed for shorts S69.01 and S69.02 (see next section) occurs with a frequency of 0.0658 among the shorts, i.e. 22% of shorts would have such a genotype so that selecting this type need not be regarded as an unlikely event.

Alternatively, starting with low frequencies of A and S' such that all short genotypes are equally likely and have a frequency of 0.01 (i.e. shorts are 0.09 of the population) and mids and longs are of variable frequency depending on whether they have A or S' or not, equilibrium was not reached until nearly 600 generations of random mating and in this case it was isoplethic: short = 0.336, mid = 0.332, long = 0.332. Once again all genotypes are maintained in the population (Table 8) but in this case the gene frequencies are $A = 0.175$, $S' =$

Table 7. Stable genotype frequencies of the three style forms after 70 generations of random mating

Genotype	Short	Mid	Long
1	0.0184	0.0142	0.1523
2	0.0677	0.1673	0.1217
3	0.0788	0.0097	0.0008
4	0.0004	0.1573	0.0134
5	0.0021	0.0001	0.0242
6	0.0025	0.0007	
7	0.0067	0.0018	
8	0.0566	0.0117	
9	0.0658	0.0015	
10		0.0240	

Table 8. Genotype frequencies of the three style forms after 600 generations of random mating

Genotype	Short	Mid	Long
1	0.0004	0.0001	0.0002
2	0.0011	0.0010	0.0051
3	0.0013	0.0006	0.2304
4	0.0172	0.0021	0.0076
5	0.0933	0.0169	0.0083
6	0.1268	0.2136	
7	0.0064	0.0062	
8	0.0387	0.0026	
9	0.0511	0.0006	
10		0.0078	

0.836 and $M=0.268$, i.e. there is a reversal of the relative frequencies of A and S'. The frequency of the short genotype proposed for S69.01 and S69.02 is again high, approximately 15%, making such a plant relatively common among shorts.

Although fixing A or S' leads to a pattern of segregation equivalent to the two locus dominant short case this does not occur here; instead all three loci are maintained segregating. Thus it would appear that modifiers which do not affect the incompatibility system per se could be segregating in populations of heterostylic plants and if so could be expected to be present at quite high frequencies.

Testability of the model

The genotype of a short such as S69.01 selected from this population would be *Aa S'm/sm* and when crossed to longs from the departmental stocks would give 1S : 3L as follows:

L66.24 × S69.01
aa sm/sm × *Aa S'm/sm*
↓
¼ *Aa S'm/sm* : ¼ *Aa sm/sm* : ¼ *aa S'm/sm* : ¼ *aa sm/sm*
¼ short : ¾ long

Short daughters, e.g. S70.01, would be expected to repeat this behaviour (Table 6).

Also

M66.14 × S69.02
aa sM/sm × *Aa S'm/sm*
↓

Gametes from	S69.02 / M66.14	¼ *A S'm*	¼ *A sm*	¼ *a S'm*	¼ *a sm*
	½ *a sM*	*S*	*M*	*M*	*M*
	½ *a sm*	*S*	*L*	*L*	*L*

i.e. ¼ *S* : ⅜ *M* : ⅜ *L*

Mid × long crosses among these progeny, e.g. *Aa sM/sm* × *aa S'm/sm* are expected to yield one quarter shorts. No such crosses were carried out for indeed among these progeny it would be necessary to carry out 12 mid × long crosses to be 95% sure of including a cross of the appropriate genotypic constitution. It is, however, a critical test of the model.

The results from the 1980 crosses (Table 9) did not clarify the problem at all. This was partly because when these crosses were made two alternative models for the alteration of the mode of inheritance of short in the Botanic Park population were under consideration. One model required a modifier *A,a* segregating such that *AS* was short and *aS*, *As*, or *as* non-short. The mid versus long differences are determined by the mid locus. Under the other model *aS* was short and *AS*, *As*, *as* non-short. The mid versus long differences are determined by the mid locus. Both of these models provide a satisfactory explanation of the data but they also require the *A* locus to be segregating in the departmental stock and there is no evidence of this. Critical evidence that such modifiers are not present in established stocks was provided by testing eight long daughters of S69.01 against random mids. No short progeny were produced.

Table 9. Style length segregation in crosses of short daughters of the second introduced short S69.02

	SHL[a]	Short	MHS[a]	Mid	Long
S80.19 × L80.11	6	9	–	23	–
L80.11 × S80.19	23	23	2	17	–
S80.10 × L80.05	3	5	–	5	–
L80.05 × S80.10	–	3	–	12	–
S80.11 × L80.35	2	1	–	4	–
L80.35 × S80.11	1	–	1	2	–
S80.16 × L80.25	3	–	1	3	–
L80.25 × S80.16	1	–	5	47	–
L80.06 × S80.16	11	3	10	34	–

[a] See text

The results from crossing short daughters of S69.02 are listed in Table 9.

In Table 9, SHL indicates that the plant had short styles with the lower whorl of anthers just above them and the upper whorl of anthers in the long position, MHS, mid styles, mid anthers and short anthers. Some of these plants appear to be spontaneously self-fertile but in fact the capsules set rarely contain fertile seed. Shorts S80.10 and S80.11 are from S69.02 × M66.89 and since they do carry mid indicate that M66.89 took part in fertilization, i.e. S69.02 is not a pseudogamous apomict.

S80.16 and S80.19 are from M66.89 × S69.02; L80.06 and L80.11 are from S70.01 × L66.24; L80.25 from M66.14 × L74.01 and L80.35 from L66.24 × S65.04. All these crosses have been referred to previously. These longs were chosen as it was argued that they could be of differing genetic constitution, but though this might well be the case the results are not explicable under any simple logical genetic model. It might be that the varied sources of the genetic material have led to a disturbance of meiotic behaviour or there could be other modifiers or lethals, but attempts to develop any such explanation would seem to be nugatory without extensive preliminary experimentation.

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