Theor Appl Genet (1984) 68: 139–144

An unusual heteromorphic incompatibility system

3. On the genetic control of distyly and self-incompatibility in *Anchusa officinalis* L. (Boraginaceae)

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Received December 12, 1983; Accepted January 9, 1984
Communicated by H. F. Linskens

Summary. By means of intra- and intermorph crosses it has been shown that the distyly in *Anchusa officinalis* L. is governed by a single diallelic locus with dominance. Presence of the dominant allele is reflected in the short-styled morph, while homozygous recessives become long-styled. Most of the short-styled plants are heterozygous, but homozygotes have been found among the raised progeny resulting from crossings. Diallel crosses have revealed that at least two incompatibility loci must exist. These and the morphological locus segregate independently. The type of incompatibility system is as yet unknown, though the great frequency of non-reciprocal incompatibility points to a sporophytic system.

Key words: *Anchusa officinalis* – Boraginaceae – Distyly – Genetics of self-incompatibility – Heteromorphic incompatibility

Introduction

Distyly, i.e. the presence within a plant species of two morphs differing from each other by a reciprocal position of the stigma and anthers, is known from some twenty angiosperm families (Ganders 1979).

Despite its obvious polyphyletic origin, distyly appears as a syndrome of characters which can be found in most of the involved species: besides being dimorphic in stamen and style height, the plants are dimorphic in size and form of pollen grains and stigmatic papillae, and in the number of pollen grains per anther. A self-incompatibility (SI) system is usually associated with the dimorphism, rendering self- and intramorph pollinations incompatible, leaving only intermorph pollinations compatible. As the incompatibility type is morph specific, the genetical control of this kind of heteromorphic incompatibility system is explained by a single super-gene with two alleles, one recessive and one dominant. Crossing experiments with a number of species have revealed that, as a rule, the short-styled morph (thrum) is heterozygous, while the long-styled (pin) is homozygous recessive (Ornduff 1979; Ganders 1979). Totally intramorph incompatible species are therefore expected to have an isoplethic distribution of pins and thrums in natural populations, and investigations show that this is generally true. An excess of pins in the progeny of artificially selfed thrums (Ray and Chisaki 1957; Mather and de Winton 1941; Weller and Ornduff 1977), and in the progeny of naturally pollinated heterozygous thrums (Ganders 1979) suggests that being a homozygous dominant thrum is a selective disadvantage.

The evolution of distyly has been dealt with by comparative studies (Baker 1966) and in a more speculative way involving computer runs (Charlesworth and Charlesworth 1979). Theories of the exact pathway from a homomorphic self-compatible ancestor to plants with a fully developed heteromorphic SI-system may vary, though all authors do agree about two things. Firstly, that a diallelic SI system must be established before the evolution of the morphological dimorphisms, and secondly, that if the system is to remain established, the incompatibility and the morphological genes have to be closely linked.

In a previous paper it was stated that *Anchusa officinalis* L. is distylous and in possession of several of the dimorphic characters typical to distyly (Philipp and Schou 1981). However, some striking deviances from the normal heteromorphic SI system were found. Pin plants were found to be in great excess in every natural population sampled, and though the plants definitely are self-incompatible, intramorph pollinations are as successful as intermorphic. These compatibility relations were sustained in an extended crossing programme, and hence it was postulated that the morphological and the incompatibility loci segregate independently (Schou and Philipp 1983). Moreover it was shown that even following self-pollinations, the pollen tubes reach the ovary and enter the ovules. This is highly surprising inasmuch as the heteromorphic SI system is sporophytic by definition, meaning that the incompatibility type of the pollen grains is determined by the diploid genome of the pollen mother plant and is supposedly imprinted the pollen grains as specific proteins in its exine (Heslop-Harrison et al. 1975). Consequently the incompatibility reaction should take place shortly after the contact between the stigma and pollen grains, and not between the gametes in the ovary.

The present paper submits the results from a genetical analysis of the heteromorphic SI system in *A. officinalis.* It is stated that the morphological traits are governed by single locus with two alleles. Pin plants are homozygous recessive, and most frequently thrum plants are heterozygous, though homozygous dominants are found. It is also stated that there are at least two incompatibility loci, which are unlinked to the morphological locus. The type of SI system is not revealed, but it seems to involve a combination of sporophytic and gametophytic control. The exceptional status of *A. officinalis* as related to the normal distylous system outlined above is discussed.

Materials and methods

Seeds (nutlets) were originally obtained in 1979 from a natural population at a site called Harløse (UTM-coordinates: 33UUB294932) north of Copenhagen, Denmark. They were sown in the experimental field belonging to the University Botanical Garden in Copenhagen. During the summers of 1980 and 1981 crossings were made between the plants raised in the experimental field in order to illucidate the genetics of the morphological traits. (The flower morphology, flowering phenology, and the pollination and isolation procedures have been described (Philipp and Schou 1981).) Seeds from the pollinated flowers were harvested at maturity and sown in October in flat pots, which wintered in garden frames, simulating natural conditions. After germination the following spring, the seedlings were pricked out singly in pots, and later planted in the field when their leaf rosette was about 15 cm in diameter. On flowering the morph type of the F_1 plants was recorded.

For a further study of the SI system an F_1 family was chosen at random. Its ten plants were transferred to an insect-proof greenhouse. Here they were selfed and reciprocally crossed in the summer of 1983. In every combination, four to five flowers were pollinated, and the compatibility was determined from the development of seeds. The results are given as a percentage of full seed set.

Results

Segregation into morph types

The results from the intra- and intermorph crossings are seen in Table 1. From the score of individuals of each morph in the progeny it is found that intermorph crossings yield almost equal numbers of pins and thrums, and when pins are crossed with pins all but one of the offspring are pins. These morph frequencies are consistent with the hypothesis that the pin plants are recessive homozygotes, while thrum plants are heterozygotes, as judged from the chi-square and *P* values given in Table 1. It is seen from the table that the hypothesis is not substantiated with the intramorphic thrum crossings, if one assumes that the homozygous dominant is not found among the offspring. The consequence of this assumption is a 2 : 1 offspring ratio of thrums to pins, and when testing this, the obtained chi-square value is well beyond the 0.001 significance level due to an excess of thrums. If, however, the homozygous dominant *is* viable but present among the offspring only, one would expect a 3 : 1 ratio and thus find a chi-square value of 3.536, which is just too small to indicate significance at the 0.05 level. It thus appears that the homozygous thrums are present in the progeny resulting from the thrum × thrum crosses in numbers close to those expected from Mendelian segregation following a cross between two heterozygous plants. A reasonable conclusion drawn from the results is that distyly in *A. officinalis* is governed by a single diallelic locus with dominance. Carriers of the dominant allele are thrums, most of which are heterozygotes, but an unknown, relatively small number of homozygous dominants excist among the F_1 plants.

In order to illustrate the existence of homozygous dominant thrum plants, some of the offspring from the thrum × thrum crosses were crossed with plants from three other offspring groups. Table 2 shows the results. It is seen that about half of the plants in this group must have been homozygous dominants, because they yield only thrum offspring irrespective of the genotype of the pollen donor. To get an impression of the frequency of homozygous dominants in natural populations, seed samples were taken from eight naturally pollinated thrums in the Harløse population. Both pin as well as thrum plants developed from six of the samples, showing the parents of these to have been heterozygotes. The remaining two plants yielded only thrum offspring, though in numbers (one and two, respectively) far too small to make reliable statements concerning the parent genotypes.

Diallel crosses

The chosen family consists of seven thrum and three pin plants. It is seen from Table 3 that every plant is completely self-incompatible, and that in eleven crosses pollination is totally unavailing. Of the eleven, seven are compatible in the reciprocal cross, with mean fertilities ranging from 10 to 50%. Generally, reciprocal differences are found in most of the crosses, and the compatibility as expressed in seed set is highly variable. It appears from the frequency distribution for the percentage of seed set (Fig. 1) that it is impossible to draw a sharp delimitation between incompatible and compatible crosses. This implies that compatibility between any two plants is not a question of either/or, but rather is expressed as a specific degree varying with the crosses. Thus, in *A. officinalis,* incompatibility seems to be a quantitative, not a qualitative effect.

It can be argued that recordings of reciprocal differences are owed to misclassifications due to chance

Table 1. Segregation into morph types of progeny from inter- and intramorph crosses of pins and thrums

Crosses ♀×♂	No. of parent pairs	Total no. of pollinated flowers	Total no. of seeds	% seed set	Observed/expected no. of each morph type in progeny[a]		χ^2 [b]	P
					Pins	Thrums		
Pin × thrum	47	446	301	16.9	48/47	46/47	0.0106	0.92
Pin × pin	31	211	172	20.4	47/48	1/ 0	0.0052	0.94
Thrum × pin	26	178	260	36.5	22/29	36/29	2.9138	0.09
Thrum × thrum	52	451	412	22.8	17/34.3	86/68.7	12.3756[c]	0.0004

[a] The expected numbers are obtained by assuming that the genotypes of pin and thrum are ss and Ss respectively

[b] Yates correction factor is used in the determination of the chi-squares

[c] Assuming that the morph frequency is 2 : 1. If it were to be 3 : 1, the value of χ^2 becomes 3.5358, and $P = 0.06$

Table 2. Evidence for homozygous dominants (thrums) among progeny from crossings in the experimental field. A-plants, thrum progeny from thrum × thrum; B-plants, progeny from thrum × pin; C-plants, progeny from pin × thrum; hom rec, homozygous recessive; het, heterozygous; hom dom, homozygous dominant

Crosses A-plant × B-plant or C-plant	Observed no. of each morph type in progeny		Concluded genotype of the A-plant
	Pin	Thrum	
A1 × B1 (hom rec)	0	1	A1 : hom dom
A1 × B2 (het)	0	5	
A1 × B3 (hom rec)	0	1	
A2 × B2 (het)	0	6	A2 : hom dom
A3 × B4 (hom rec)	0	2	A3 : hom dom
A3 × B5 (het)	0	2	
A3 × B6 (het)	0	1	
A4 × B3 (het)	0	11	A4 : hom dom
A4 × C1 (het)	0	6	
A5 × B2 (het)	1	7	A5 : het
A6 × B7 (het)	1	5	A6 : het
A7 × C2 (hom rec)	4	0	A7 : het
A8 × C3 (het)	2	1	A8 : het
A8 × B8 (het)	0	2	
A8 × C4 (het)	1	1	

Table 3. Data on intrafamilial pollinations in the F_1 family 13,938. The numbers reveal the percentage of maximum seed set. T, thrum; P, pin. Underlined numbers indicate the seven cases, where a difference in compatibility is manifested in a complete absence of seeds in one cross

♂ ♀	T 1	T 2	T 3	P 4	P 5	T 6	T 7	P 8	T 9	T 10
T 1	0	6	38	0	5	31	6	19	25	0
T 2	10	0	10	13	0	25	13	50	0	19
T 3	5	0	0	38	20	44	19	50	25	25
P 4	38	44	63	0	31	38	31	6	31	50
P 5	31	13	19	31	0	13	56	50	50	19
T 6	45	6	6	25	25	0	19	75	31	25
T 7	35	25	0	38	25	25	0	19	63	25
P 8	20	0	25	10	56	50	63	0	44	44
T 9	0	0	6	19	15	6	25	38	0	13
T 10	0	25	25	0	15	25	13	6	63	0

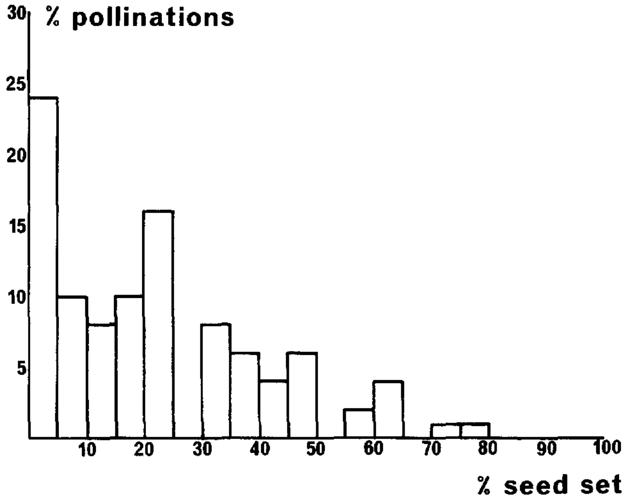

Fig. 1. Frequency histogram of percent seed set following diallel crosses in the F_1 family 13938

events. However, a completely negative result in four or five replicate pollinations are hardly achieved by chance alone, and neither are the conspicuous differences between several of the reciprocal crosses.

Any attempt to group the plants according to their compatibility reactions has failed. Each individual in the tested family has a specific compatibility pattern (Table 3), which is unique inside the family, and consequently we are led to conclude that as to incompatibilities they are genotypically different from each other.

How to get further

More work is needed to understand fully the genetics of the SI system of *A. officinalis.* We would like to establish some larger F_1 families for diallel crosses, partly in order to confirm the above results, and partly because that with a larger number of plants in a family it might become possible to reveal the number of incompatibility loci. Furthermore, backcrossing of progeny with parental plants is an important tool when evaluating the control of pollen compatibility, i.e. whether it is sporophytic or gametophytic. At present we do not feel that the pain fits the profit, mainly because of the low seed set and the considerable loss of potential progeny during the germination phase. It can be calculated from Table 1 that the mean germination percentage is as low as 24. The genetical study will be resumed only if we can control germination better.

Discussion

Self-incompatibility is notorious in *A. officinalis*, a conclusion valid to plants in natural populations, to their raised progeny, and to F_1 plants. Following selfing, the pollen tubes reach the ovary and enter the micropyles, suggesting that the recognition reaction resides in the ovules (Schou and Philipp 1983). Whether the rejection reaction is pre- or postzygotic is as yet unknown, but if it is found to be postzygotic it might be questioned whether it is due to an SI system, or to selfing depression instead, with reference to the definition held by Lundqvist (1964), cited and approved by de Nettancourt (1977).

According to this definition, self-incompatibility is "the inability of a fertile hermaphrodite seed plant to produce zygotes after self-fertilization", in other words a purely prezygotic event. However, selfing depression can hardly account for the total lack of seeds in selfed flowers, even of plants in the wild, and mean seed set following crossings of the F_1 family is not smaller relative to that of the parental plants. Therefore, we still support the presence of an SI system. Furthermore, in *Borago officinalis*, a homomorphic borage, the rejection reaction is also delayed until after fertilization (Crowe 1971), as it is reported to be in *Narcissus tazetta* (Dulberger 1964), *Malus* (Cooper 1929), *Medicago* (Brink and Cooper 1939), *Beta* (Savitsky 1950), *Theobroma* (Cope 1962), *Liquidambar* (Schmitt and Perry 1964), *Ribes* (Arasu 1970), and *Lotus* (Giles 1949). Dobrofsky and Grant (1980) have recently investigated the ovary protein complement in *Lotus* by means of electrophoresis, and differences were found between selfed and crossed flowers. The authors considered that this implies a biochemically mediated self-recognition in the ovary, and they suggested it to be prezygotic. We believe, that a prezygotic selfrecognition might not necessarily contradict Giles' (1949) observation, because the rejection reaction could be postponed relative to the recognition, and thus become a postfertilization event. Then it turns out to be rather academic to question the presence of an SI system.

We have proved from this and our previous studies that the SI system in *A. officinalis* is genetically unlinked to the distyly, but its type, and the exact number of loci governing it, are not revealed. If only one single locus was present, the intrafamilial crosses would have elicited a maximum of four groups of sibs, each group being characterized by a specific compatibility pattern. Each of the ten sibs had its own specific pattern, i.e. the self-incompatibility is based on more than one locus. When evaluating the system, this and other characteristics have to be considered. Two or more loci are known mainly from species with the gametophytic type of SI system (Lundqvist 1956; Pandey 1957; Lundqvist et al. 1973), and from a single species with the sporophytic type (Verma et al. 1977; Lewis 1977). Non-reciprocal incompatible matings are frequent with sporophytic systems, and their percentage in a diallel crossing scheme can be very high. On the contrary, with gametophytic systems non-reciprocity is rare, and with more than one locus involved, only a very few percent of the diallel crosses would show non-reciprocity (Lewis 1977). These facts, and the results from Table 3, point to a sporophytic SI system in *A. officinalis*, as does its 3-celled pollen grains (Brewbaker 1957). Opposed to this stands the marked variability in cross-compatibility as expressed in seed sets. This finding, and the actual variability in seed set, correspond well with that which has been found in an F_1 family of *Borago officinalis* (Crowe 1971). As pointed out by Crowe, with sporophytic SI systems all gametes from a plant have the same incompatibility type, which should lead to an all-or-nothing effect in crosses. Influenced by the reciprocal differences in compatibility, which are also prominent in *B. officinalis*, Crowe suggested two different modes of action: either a sporophytic control in the female tissue and a gametophytic in the male, or a system with epistasis between the loci. Both systems can apply to *A. officinalis* as well, and conclusively its SI system cannot at the moment be characterized with certainty as either gametophytic or sporophytic.

It has become evident that the distyly of *A. officinalis* is governed by a single diallelic locus, operating such that the presence of the dominant allele is reflected in the thrum phenotpye, while homozygous recessive plants are of the pin type. As explained in the introduction, distyly is commonly based on this kind of genetics when considering the analyses previously made on some ten species (Ganders 1979). Homozygous dominants are not normally expected, because the plants, due to the close linkage of the morphological locus with the incompatibility locus and the existence of only two alleles of the latter, are self- and intramorph incompatible. Such homozygotes have only been found in cases where the self-incompatibility is incomplete (Dahlgren 1922; Ernst 1928; Mather and de Winton 1941), and where the SI system has become totally eroded (Ray and Chisaki 1957). As mentioned previously, the very low frequency of homozygous dominants even in these species, points to serious deleterious effects of this genetical constitution. Among the parental generation of the raised *A. officinalis* plants, most of the thrums were heterozygous as shown by the statistically significant 1 : 1 morph ratio in the progeny resulting from intermorph crosses. However, it is significantly shown that homozygous dominants can be bred, and moreover, such individuals have proved fertile. Investigations have been initiated which will enable us to confirm or reject their existence in natural populations. From the above mentioned 1 : 1 morph ratio, and from the great excess of pins in natural populations despite of intramorph compatibility, it is reasonable to believe that homozygous dominants will prove to be very rare.

Among the distylous species so far described, *A. officinalis* can be grouped with three others, all of which are unusual with regard to their genetical system. These are *A. undulata, Veronica gentianoides,* and *Narcissus tazetta.* For *A. undulata* ssp. *hybrida,* a self-incompatible species, Dulberger (1970) hypothesized that the morphological and the incompatibility loci segregate independently. Dulberger based the proposal on the finding of intramorph compatibility, but she did not study these traits further. Similar results have been achieved with *Veronica gentianoides,* which was studied by Correns (1924). Although the paper is an outstanding example of a thorough investigation and its conclusions unchallenged, it has been overlooked by most authors concerned with heteromorphic incompatibility. Correns showed that *V. gentianoides* is distylous, but lacks the other dimorphisms typical to distylous species, including that of stamen height. The latter is not exceptional, as such absence is also found in *Linum grandiflorum* (Darwin 1877; Lewis 1943). The pin morph was found to be controlled by a single recessive factor, and the SI system was suggested to be of the gametophytic type, with multiple alleles. Interesting in the present context is that pins were always in great excess in natural populations, just as is the case with *A. officinalis,* and to some degree with *A. undulata* (Dulberger 1970). The distyly in *Narcissus tazetta* is also obvious, and here too the stamens are situated at the same level in both morphs (Dulberger 1964). It is included within the group because it is completely self-incompatible but as intra- as intermorph compatible, and because it has the common genetical government of distyly. Unfortunately population morph ratios are not available.

Charlesworth and Charlesworth (1979) have presented different models for the evolution of distyly. An assumption for the models is that stamen and style height differences evolved after the establishment of the incompatibility system. Only in one model (Case 1), a heterostylous system evolves. In what they refer to as Case 2 the model leads to populations with two complementary morphologies as in distyly, but uncorrelated with the incompatibility type. This is considered as not being true heterostyly, and the Charlesworths conclude that no apparently heterostyled species have proved to be of this type (p. 486). This seems to us to be in contradiction with what can be read a few pages ahead, where they mention *Mirabilis froebelii,* which is tristylous, *Narcissus tazetta,* and *Veronica gentianoides* as examples of species with polymorphism of the stigma position (p. 474). Concomitantly they mention that these species possess a system where the stigma position and incompatibility reaction type are independant of each other. We are not fully aware why these species are not examples of Case 2. One of the results of the present investigation is that one species, *A. officinalis,* has been added to the above mentioned group, which, with *A. undulata* ssp. *hybrida,* now is a group of five.

Acknowledgements. We wish to thank Lise Groth, Ruth Bruus Jakobsen and Cherry Nielsen for their excellent technical assistance.

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