Genetic modifications of dimorphic incompatibility in the *Turnera ulmifolia* L. complex (Turneraceae)

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Diploid and tetraploid populations of *Turnera ulmifolia* are distylous and exhibit a strong self-incompatibility system. Distyly is governed by a single locus with two alleles. Several self-compatible variants were, however, obtained and the nature and genetic control of self-compatibility was assessed using controlled crosses. The study documented the occurrence of self-compatible variants in four contrasting situations. These included the following. (i) Self-compatibility in a diploid short-styled variant. The gene(s) governing self-compatibility interact with the distyly locus and are expressed only in short-styled plants. When tetraploids carrying the genes were synthesized, self-incompatibility reappeared. (ii) Self-compatibility occurred in a cross between geographically separate diploid populations. Self-compatibility appeared sporadically in the F₁. Crosses revealed that self-compatibility is likely under polygenic control. (iii) Low levels of self-compatibility occurred in a tetraploid population. Crosses revealed that self-compatibility was under polygenic control. A small response to selection for increased self-compatibility was observed. (iv) Hexaploids were synthesized from crosses between distylous diploids and tetraploids. All hexaploids obtained were long- or short-styled indicating that hexaploidy per se does not cause homostyly. A single long-styled plant showed aberrant pollen behaviour, resulting in a moderate degree of self-compatibility. All of the variants studied exhibited either aberrant pollen or stalar incompatibility responses. In no instance was the genetic control of self-compatibility solely the result of segregation at the distyly locus.

**Key words:** Turnera, dimorphic incompatibility, polyploidy, self-compatibility, distyly.


Des populations diploïdes et tétraploïdes de *Turnera ulmifolia* possèdent deux styles et un système hautement auto-incompatible. La distylie est gouvernée par un seul locus avec deux allèles. Cependant plusieurs variants auto-compatibles ont été obtenus et la nature de cette auto-compatibilité, de même que son contrôle génétique, ont été évalués à l’aide de croisements contrôlés. Pour cette étude, une documentation a été obtenue sur l’occurrence des variants auto-compatibles en fonction de quatre situations contrastantes. Celles-ci incluent: (i) l’auto-compatibilité survenant chez un variant diploïde brévistyle. Le ou les gènes qui gouvernent l’auto-compatibilité ont réagi avec le locus de la distylie et ne se sont exprimés que chez les plantes brévistylistes. Lorsque des tétraploïdes porteuses des gènes furent synthétisées, l’auto-incompatibilité est réapparue. (ii) L’auto-compatibilité survenant entre des populations diploïdes géographiquement séparées. L’auto-compatibilité s’est manifestée sporadiquement chez les F₁. Les croisements ont révélé que l’auto-compatibilité est probablement sous contrôle polygénique. (iii) De faibles niveaux d’auto-compatibilité survenant chez une population tétraploïde. Les croisements ont révélé que l’auto-compatibilité est sous contrôle polygénique. Une faible réponse à la sélection en vue d’augmenter l’auto-compatibilité a été observée. (iv) Des hexaploïdes ont été synthétisés par des croisements entre des diploïdes et des tétraploïdes distylistiques. Toutes les hexaploïdes obtenues se sont avérées brevii- ou longii-styles, ce qui indique que l’hexaploïdie, comme telle, ne cause pas l’homostylie. Une seule plante longistyle a présenté un comportement aberrant, se traduisant par un degré modéré d’auto-compatibilité. Tous les variants à l’étude ont présenté soit un pollen aberrant, soit des réponses d’incompatibilité stylaire. Le contrôle génétique de l’auto-compatibilité n’a pas été, dans aucun cas, le seul résultat de la ségrégation au locus de la distylie.

**Mots clés:** Turnera, incompatibilité dimorphique, polyplioïdie, auto-compatibilité, distylique.

[Traduit par la revue]

**Introduction**

Dimorphic incompatibility (distyly) is a genetic polymorphism in which populations consist of two mating groups that usually differ in style length, stamen length, and incompatibility relationships. Several other floral traits are commonly dimorphic; these include pollen size, pollen production, pollen exine sculpturing, and stigma morphology (see Ganders (1979) for a review). The incompatibility system in distylous species enforces disassortative mating between long- and short-styled plants. In the 12 genera from 10 families where the inheritance of distyly has been investigated (Ornduff 1979), the polymorphism has been shown to be governed by segregation of two alleles at a single locus. The

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short-styled morph is most commonly determined by the dominant allele, $S$, although exceptions are known (Baker 1966; Ornduff 1979). Distyly has undoubtedly had several independent origins as it occurs in a wide range of plant taxa including both dicotyledonous and monocotyledonous families (Charlesworth and Charlesworth 1979a).

Genetic studies of natural variants in *Primula* spp. (Ernst 1933, 1936a, 1936b, 1953, 1955; Dowrick 1956) provide evidence that distyly is controlled by at least three tightly linked loci and as such represents one of the classic examples of a supergene. The supergene model of the inheritance of distyly in *Primula* may be applicable to all distyly taxa since it is difficult to envision a single gene controlling all of the complementary features that characterize the floral morphs. An additional feature of the supergene model is it predicts that certain crossovers within the supergene will generate self-compatible homostylous variants with styles and stamens of equal length. Styles of homostylous plants exhibit the incompatibility behaviour of one of the distyly morphs while the pollen has the incompatibility reaction of the alternate morph. Homostyles are found at low frequencies in distyly populations (Ernst 1936a; Crosby 1949; Charlesworth and Charlesworth 1979b; Piper et al. 1984; Curtis and Curtis 1985; Cahalan and Gliddon 1985), or more commonly as close relatives of distyly taxa (Ganders 1979). Although the supergene model adequately explains several features of the distyly syndrome and its breakdown in *Primula*, little evidence is available from other taxa with dimorphic incompatibility (although see Baker 1958, 1966; Shore and Barrett 1985a; Barrett and Shore 1987).

Homostyly variants can also arise by genetic mechanisms other than by recombination within the distyly supergene. Nonrecombinant homostyles are known in *Amsinckia spectabilis* (Ganders 1979) and nonallelic major genes as well as polygenes are known to influence stamen and style length in *Primula sinensis* (Mather and de Winton 1941; Mather 1950). To determine the mode of origin of homostyly from distyly it is necessary to have information on the genetic basis of both distyly and homostyly in the species under investigation. In addition, studies of the compatibility relationships of distyly and homostyly forms are particularly useful in distinguishing between the various genetic mechanisms responsible for homostyle formation since the crossover model predicts a specific pattern of residual incompatibility in homostyles (see Fig. 1 in Dowrick 1956).

In the *Turnera ulmifolia* L. complex (Turneraceae), dimorphic incompatibility occurs in at least four taxonomic varieties at both diploid and tetraploid levels (Barrett 1978; Shore and Barrett 1985b). Distyly is controlled by a single gene locus with two alleles (Shore and Barrett 1985a). In diploids, short-styled plants are $Ss$ and long-styled plants $ss$. Tetraploids are $Sss$ and $sss$, respectively. The incompatibility system in *T. ulmifolia* is strongly developed and few seeds are produced by selfing or intramorph crosses (Martin 1965; Barrett 1978; Bentley 1979). In addition to the distyly variants within the complex, at least three self-compatible homostylous varieties have been described (*T. ulmifolia* vars. angustifolia Wild., orientalis Urb., and velutina Urb.). These are hexaploid (Shore and Barrett 1985a, 1985b; Arbo 1985). The homostyly variants of *T. ulmifolia* are atypical in showing wide variation in the relative positions of reproductive organs and hence the facility for autogamy (Barrett and Shore 1987). While all styles are “long,” anther height ranges from “short” to “long.” As a result, some populations exhibit the typical long-homostyle phenotype, with long styles and stamens, while others (particularly in var. velutina) have phenotypes similar to long-styled plants from dimorphic populations. This variation raises the possibility that the origin of self-compatible homostyles in the *T. ulmifolia* complex occurs by at least two distinct pathways. Homostyles may arise via recombination within the distyly supergene or alternatively, through loss of self-incompatibility in long-styled plants. The occurrence of rare self-compatible variants within distyly populations of *T. ulmifolia* lends support to the latter alternative. These variants form the basis of the present study.

In this paper the modes of origin of self-compatibility within the *T. ulmifolia* complex are explored by examining the nature and genetic basis of self-compatibility of variants from distyly populations or that have been synthesized from interpopulation crosses. We investigate the compatibility relationships and genetic basis of self-compatibility in four contrasting situations: (i) a diploid self-compatible variant and its synthetic tetraploid derivatives; (ii) a diploid self-compatible variant derived from an interpopulation cross; (iii) naturally occurring tetraploid self-compatible variants; and (iv) hexaploids synthesized from diploid and tetraploid distyly plants. We discuss the significance of the self-compatible variants for the evolution of breeding systems in the species complex, consider the genetic bases of self-compatibility, and implications for the genetical architecture and functioning of heterostyly.

**Materials and methods**

All experimental work was performed under uniform conditions in the glasshouse facility at the Department of Botany, University of Toronto. Plants were established from bulk seed collections with the exception of plant 132-15 which was obtained from field-collected stem cuttings. Plants are referred to by a code specified in Shore and Barrett (1985a); the first letter indicates the varietal status, followed by population number, the individual’s number, and its style form (L, long styled; S, short styled). Detailed localities may be found in Shore and Barrett (1985a, 1985b) with the exception...
of population I22 which was collected along a roadside outside
the town of Lucas Diaz in the Dominican Republic (Barrett and
Shore 1362 TRT). See Barrett and Shore (1985a, 1985b) for
cultural and pollination methods used in this study.

Self-compatibility in a diploid variant

The short-styled variant I32-1S was the sole representative of
T. ulmifolia var. intermedia in a roadside population of T.
ulmifolia var. elegans at Arco Verde, N.E. Brazil. The variant
has a diploid chromosome number of 2n = 10, whereas var.
elegans is tetraploid (2n = 20) in this area (Barrett and Shore
1987). Because the variant is phenotypically most similar to
plants of population I1 from Barreirinhas, N.E. Brazil (2n =
10), plants from this population were used in crosses with
I32-1S to determine the compatibility relationships and genetic
basis of self-compatibility.

Compatibility relationships of the variant I32-1S were
determined by selfing and crossing reciprocally to long- and
short-styled plants during the summer of 1984. Capsules were
harvested just prior to dehiscence and the number of seeds per
pollination and the percentage fruit set recorded. To determine
the genetic basis of self-compatibility, several crosses were
performed as follows: the variant was selfed to generate an
S1 family and a single long-styled plant (I32-2L) was selected
from the progeny for further crosses. Four F1 families were
produced by the crosses I32-1S x 11-10L, I32-2L x 11-1S, and
reciprocals. Three F2 families were generated by selfing the
short-styled plants, B, G, and P, selected from the F1
crosses, 11-10L x I32-1S, 11-1S x I32-2L, and I32-2L x
11-1S, respectively. Progeny were screened for self-compatibility
in the following manner: replicate self-pollinations were
performed on each progeny individual and fruit and seed
set recorded, with the exception of the four F1 families where
only percent fruit set was recorded. Screening was undertaken
for the selfed family using five replicate self-pollinations per
plant (autumn of 1982), for the four crosses (F1’s) (summer of
1983), the three F2 families using three replicate pollinations
for the latter families (summer of 1984).

To investigate the influence of ploidal level on the compati-
bility behaviour of the variant, several seedlings of the cross
I32-1S x 11-10L were treated with 1% colchicine by suspend-
ing a drop of aqueous solution on the apical meristem of
2-day-old seedlings. The treatment was repeated for 2 or 3
days. Several tetraploid plants were obtained, some of which
were diploid–tetraploid mosaics, with some branches possessing
the diploid chromosome number, 2n = 10, and others
tetraploid, 2n = 20 (as determined from chromosome counts
of meiotic material). An analysis of the compatibility be-
behaviour of the mosaics was performed by selfing and crossing
diploid and tetraploid flowers of the mosaics to long- and
short-styled plants of equivalent ploidal level. In addition,
interploidal crosses were also undertaken (summer of 1985).

Self-compatibility in an interpopulation cross

To investigate the influence of interpopulation crosses on
the expression of self-incompatibility in T. ulmifolia, a wide
cross involving diploid material of var. intermedia from Brazil
and Venezuela was undertaken. The parental individuals were
strongly self-incompatible. An initial screening of several F1
progeny from the cross I3-3S x 11-10L revealed weakening of
the self-incompatibility system (summer of 1981). A single
highly self-compatible long-styled plant (II13-3L) among the
F1 progeny was discovered. The plant was selfed to determine
the genetic basis of self-compatibility. Its progeny (F2) and
the original F1 were screened for self-compatibility using five
replicate self-pollinations for each plant (summer of 1982). In
addition, the compatibility behaviour of the self-compatible
plant and three of its selfed progeny were determined (summer
of 1985) by selfing and crossing individuals with plants from
the parental populations from which they were derived (popu-
lations I3 and I1).

Self-compatibility in tetraploid variants

Surveys of self-incompatibility in a tetraploid population of
T. ulmifolia var. elegans (E5) revealed the presence of one
moderately self-compatible long-styled plant (E5-1L) and a
short-styled plant (E5-10S) which set a small number of seeds
upon self-pollination. Plant E5-1L was originally discovered
by Barrett (1978) and has been maintained clonally for 10
years, during which time it has consistently displayed a
moderate degree of self-compatibility. The compatibility
behaviour of both plants was determined by crossing the
variants to self-incompatible plants obtained from the same
population.

To examine the inheritance of self-compatibility, several
crosses were performed as follows: the self-compatible plants
were selfed and crossed reciprocally to generate two S1
families and an F1 family, respectively. Two highly self-
compatible individuals were selected from each of the three
families and selfed to generate four S2 families and two F2
families. The plants selected were LL61L and LL51L from the
S1 family of E5-1L, SS3S and SS77S from the S1 family of
E5-10S, and SL36L and SL34S from the F1 family of the cross
E5-1L x E5-10S. All families and the parental population
were screened for self-compatibility (as above). The parental,
F1, and S1 families were screened using 10 replicate self-
pollinations per plant (summer of 1981) and the F2 and S2
families were screened using five replicate pollinations (sum-
mer of 1982).

Self-compatibility in synthetic hexaploids

Because an association between hexaploidy and homostyly
in T. ulmifolia occurs (Shore and Barrett 1985a), hexaploids
were synthesized in the following manner to determine if
hexaploidy causes homostyle formation. Triploid seed was
produced from the tetraploid x diploid crosses I24-2S x
I6-12L and I22-4L x I6-31S. Seedlings were then raised and
treated with colchicine, as indicated above, to produce 18
hexaploid plants, (five long- and 13 short-styled plants).
Ploidal levels were verified by chromosome counts of meiotic
material. Synthetic hexaploids were easily distinguished by
high pollen fertility and large pollen size. In contrast, triploid
plants were virtually pollen sterile. The synthetic hexaploids
were screened for self-compatibility by replicate self-pollina-
tions. A single self-compatible long-styled plant was obtained,
it was selfed, and progeny were screened for self-compatibility
(summer of 1985). Additionally, the hexaploid synthetic
variant and its selfed progeny were crossed to diploid long- and
short-styled plants of population I1 to determine their com-
patibility behaviour.
Results

Self-compatibility in a diploid variant

Compatibility behaviour of the short-styled variant 132-1S is presented in Table 1. The plant is highly self-compatible, setting an average of 30 seeds/fruit when self-pollinated, and a full complement of seed when used as the female parent in crosses with long- and short-styled plants. However, the plant is only successful as a pollen parent in crosses with long-styled individuals. These data indicate that the variant possesses aberrant stylar behaviour. Its style will not reject pollen from short-styled plants, while its pollen exhibits the normal incompatibility response.

The genetic basis of self-compatibility in the stylar variant was evaluated in several crosses (Table 2). A large number of progeny from the S1 family of 132-1S (136) were screened for self-compatibility. A striking pattern emerged with all long-styled progeny showing virtually complete self-incompatibility, while short-styled plants were self-compatible to varying degrees (Table 2 and Fig. 1). A continuous distribution of selfed seed set was exhibited among short-styled progeny. These data are consistent with a single-locus model of the control of self-compatibility. A modified allele at the distyly locus (Sc), which is dominant to s, confers self-compatibility upon short-styled plants. The continuous distribution of selfed seed set among short-styled progeny may result from segregation of genes with minor effects on the expression of self-incompatibility as well as environmental effects.

To test the hypothesis of a single allele, Sc, governing self-compatibility in the stylar variant, the following crosses were performed: (i) the variant 132-1S was crossed reciprocally with I1-10L to test for maternal effects; (ii) a single long-styled plant (132-2L) obtained from the selfed progeny of the variant 132-1S was crossed reciprocally with a self-incompatible short-styled plant from population I1 (I1-1S). If the hypothesis is correct, all progeny from the latter cross (and reciprocal) should be self-incompatible, as neither 132-2L or I1-1S would carry the Sc allele. Data from the cross I32-1S × I1-10L and reciprocal are consistent with the hypothesis of a single dominant Sc allele governing self-compatibility in the variant (Table 2). However, data from the cross I32-2L × I1-1S falsify the hypothesis since all long-styled progeny exhibit self-incompatibility, while short-styled plants are largely self-compatible (Table 2). Neither cross shows reciprocal effects. Thus, self-compatibility in the stylar variant is not determined by a single dominant allele segregating at the distyly locus, nor is a cytoplasmic factor involved.

An alternative hypothesis consistent with these data is that self-compatibility in the diploid stylar variant is controlled by a nonallelic dominant gene possibly linked to the distyly locus. The gene exhibits an epistatic interaction with the distyly locus with self-compatibility only expressed in the presence of the S allele. Three short-styled individuals, B, G, and P (obtained from the F1 crosses I110L × I32-1S, I11S × I32-2L, and I32-2L × I11S, respectively), heterozygous for the putative gene were selfed and their F2 progeny screened for self-compatibility. Under the above hypothesis (and in the absence of linkage) we would expect three-quarters of the short-styled progeny to be self-compatible, while the remaining quarter should be self-incompatible; all long-styled progeny should be self-incompatible. In the presence of linkage there are different expectations for the selfed families of B versus G and P and these will vary with the recombination frequency. For the family of B, we expect all short-styled plants to exhibit

Table 1. Compatibility behaviour of 132-1S in crosses with plants from population I1. All plants are from diploid (2n = 10) populations of Turnera ulmifolia var. intermedia. The number of plants of population I1 used is indicated.

<table>
<thead>
<tr>
<th>Cross</th>
<th>No. of plants</th>
<th>No. of pollinations</th>
<th>Mean seeds/pollination ± SD</th>
<th>% fruit set</th>
</tr>
</thead>
<tbody>
<tr>
<td>I32-1S × short</td>
<td>7</td>
<td>35</td>
<td>36.0±5.7</td>
<td>100.0</td>
</tr>
<tr>
<td>I32-1S × long</td>
<td>5</td>
<td>14</td>
<td>36.5±6.2</td>
<td>100.0</td>
</tr>
<tr>
<td>Short × I32-1S</td>
<td>7</td>
<td>35</td>
<td>0.1±0.2</td>
<td>8.6</td>
</tr>
<tr>
<td>Long × I32-1S</td>
<td>5</td>
<td>14</td>
<td>28.9±6.6</td>
<td>100.0</td>
</tr>
<tr>
<td>I32-1S selfed</td>
<td>—</td>
<td>5</td>
<td>30.0±8.3</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 2. Comparison of selfed fruit set among eight families derived from crosses involving plant 132-1S. Results are based on three replicate pollinations per plant with the exception of the 132-1S selfed family for which five replicate pollinations were performed. 132-2L is a long-styled plant from the selfed progeny of 132-1S

<table>
<thead>
<tr>
<th>Cross</th>
<th>No. of plants</th>
<th>% fruit set</th>
</tr>
</thead>
<tbody>
<tr>
<td>I32-1S self (S1)</td>
<td>31</td>
<td>1.9</td>
</tr>
<tr>
<td>I32-1S × I1-10L</td>
<td>18</td>
<td>0.0</td>
</tr>
<tr>
<td>I1-10L × I32-1S</td>
<td>18</td>
<td>0.0</td>
</tr>
<tr>
<td>I32-2L × I1-1S</td>
<td>20</td>
<td>0.0</td>
</tr>
<tr>
<td>I1-1S × I32-2L</td>
<td>34</td>
<td>0.0</td>
</tr>
<tr>
<td>B selfed (F2)a</td>
<td>6</td>
<td>5.6</td>
</tr>
<tr>
<td>G selfed (F2)b</td>
<td>11</td>
<td>3.0</td>
</tr>
<tr>
<td>P selfed (F2)c</td>
<td>1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

a Derived from cross I110L × I32-1S.
b Derived from cross I11S × I32-2L.
c Derived from cross I32-2L × I11-1S.
self-compatibility in the absence of recombination and for families of G and P, we expect two-thirds of the three short-styled progeny to be self-compatible.

Data from the three selfed families (F2) indicate that long-styled progeny are largely self-incompatible exhibiting only 3% fruit set from self-pollination in all families (Table 2). Among the short-styled progeny 84, 88, and 71% of the progeny from the three selfed families of B, G, and P, respectively, set fruit. The similar levels of fruit set among the selfed families of B and G indicate that the single unlinked gene model may be more appropriate. However, a continuous distribution of selfed seed set is observed among the short-styled progeny from all families in which it was evaluated (Fig. 1). Thus, although the data are consistent with a single gene model, the model does not readily explain the continuous distribution of selfed seed set among short-styled progeny. It is possible that more than one gene is involved in determining self-compatibility in the stylar variant and that these interact with the S locus so that self-compatibility is only exhibited by short-styled plants.

To investigate the effect of polyploidization on the compatibility behaviour of the variant, tetraploids were synthesized. Eight diploid–tetraploid mosaics were obtained by colchicine doubling, of which six were short-styled and two were long-styled. Polyploid shoots could be readily distinguished from diploid shoots by pollen size. In the short-styled mosaics, diploid shoots had a mean pollen length of 77.3 ± 0.7 μm and tetraploid shoots 95.2 ± 2.8 μm. Diploid shoots of long-styled mosaics had a mean pollen length of 65.1 ± 0.6 μm and tetraploid shoots 83.8 ± 2.6 μm. Meiotic counts from shoots classified as diploid or tetraploid based on pollen size confirmed the ploidal level of shoots in the five instances where it was examined. Pollen fertility (assessed by scoring the stainability of 100 pollen grains from each ploidal level on each of six mosaics) was lower for tetraploid shoots than diploid (75.7 ± 9.2 vs. 95.6 ± 5.5 mean % pollen stainability). Other "gigas" features shown by tetraploid shoots included, greater floral diameter, stamen length, and style length (about 1 mm larger).

The seven mosaic plants and three synthetic nonmosaic tetraploids, all derived from the same family, were tested for their compatibility behaviour (Tables 3 and 4). Remarkably, tetraploid sectors of short-styled plants are largely self-incompatible, while diploid shoots on the same plant are highly self-compatible. Both diploid and tetraploid shoots of long-styled plants remained self-incompatible. Legitimate crosses of tetraploid short-styled shoots exhibit a lower seed set than diploids, however, the reduction is insufficient to account for the low degree of selfed seed set in tetraploids. Thus self-compatibility seems to have reappeared in the synthetic tetraploids. Interestingly, crosses between diploid and tetraploid short-styled plants reveal that the stylar modification remains in the synthetic tetraploids. Ample seed is set when the tetraploid short-styled plants are crossed with pollen of diploid short-styled plants; the reverse pattern does not occur (Tables 3 and 4).

The synthetic tetraploid short-styled plants are of genotype SSss as a result of somatic chromosome doubling. To examine whether dosage effects involving the S allele are responsible for reemergence of self-incompatibility, several short-styled progeny obtained from the cross of a synthetic tetraploid short- and short-styled plant (LNIL × SN1S and reciprocal) were screened for self-compatibility. Short-styled progeny from this cross will be of genotype SSSS. A total of 106 plants was screened for self-compatibility using three replicate pollinations per plant. The combined selfed
seed set data yielded a mean of 0.3 ± 0.7 seeds/self-pollination. Of the 16 long-styled plants in the progeny, none set seed. Further, the compatibility behaviour of 10 short-styled progeny were assessed in crosses with diploid short-styled plants. When used as females in crosses to diploid short-styled males, these plants set 12.9 ± 9.0 seeds/ pollination while the reciprocal cross yielded 2.9 ± 1.7. Hence, SSSS and Ssss short-styled synthetic tetraploids exhibit similar compatibility behaviour indicating no dosage effects of the S allele on self-incompatibility.

**Self-compatibility in an interpopulation cross**

To examine the influence of interpopulation crosses on the expression of self-incompatibility, an F1 was produced by crossing plants from geographically separate populations. An initial screening of the F1 revealed the presence of a plant (1113-3L) which set an average of 13 seeds upon self-pollination (Table 5). An F2 family was produced by selfing this plant; all progeny, as expected, were long styled. The compatibility relationships of individual 1113-3L and three of its selfed progeny indicate that they are pollen variants. Pollen from the plants effects fertilization on both long- and short-styled maternal plants. The styles, however, retain the expected incompatibility reactions of long-styled morphs in typical distylosous populations (Table 5). The distributions of self-compatibility in the original F1 population and among the F2 generated by selfing 1113-3L are presented in Fig. 2. A single generation of selection yielded a small positive response in the family means. The mean degree of self-compatibility, as
TABLE 4. Summary of compatibility behaviour among diploids (2n = 10) and synthetic tetraploids (2n = 20) derived from the cross 132-1S × 11-10L of T. ulmifolia var. intermedia. Mean seeds/pollination ± SD (no. of male parents) are presented

<table>
<thead>
<tr>
<th>Female parent</th>
<th>Style morph</th>
<th>Ploidal level</th>
<th>Male parent</th>
<th>Mean seeds/pollination ± SD (no. of male parents)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Short</td>
<td>4x</td>
<td>Short (4x)</td>
<td>2.0±2.1(7)</td>
</tr>
<tr>
<td></td>
<td>Short</td>
<td>2x</td>
<td>Short (2x)</td>
<td>21.3±9.2(7)</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>4x</td>
<td>Long (4x)</td>
<td>22.7±11.6(3)</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>2x</td>
<td>Long (2x)</td>
<td>26.2±8.1(3)</td>
</tr>
<tr>
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<td>20.9±6.5(4)</td>
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<td>17.8±10.3(2)</td>
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<td>24.4±1.6(2)</td>
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<td>0.3±0.3(3)</td>
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*Number of pollinations.

TABLE 5. Compatibility behaviour of I311-3L (derived from the cross 13-3S × 11-10L) and three of its selfed progeny (F2) in crosses with plants from the parental populations I1 and 13 of T. ulmifolia var. intermedia (2n = 10)

<table>
<thead>
<tr>
<th>Cross</th>
<th>No. of pollinations</th>
<th>Mean seeds/pollination ± SD</th>
<th>% fruit set</th>
</tr>
</thead>
<tbody>
<tr>
<td>I311-3L selfed</td>
<td>8</td>
<td>13.3±4.7</td>
<td>100.0</td>
</tr>
<tr>
<td>I311-3L × short</td>
<td>5</td>
<td>11.0±7.1</td>
<td>100.0</td>
</tr>
<tr>
<td>I311-3L × long</td>
<td>7</td>
<td>0.0±0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Short × I311-3L</td>
<td>3</td>
<td>20.3±5.1</td>
<td>100.0</td>
</tr>
<tr>
<td>Long × I311-3L</td>
<td>7</td>
<td>4.1±4.9</td>
<td>57.1</td>
</tr>
<tr>
<td>F2 selfed</td>
<td>8</td>
<td>6.6±5.3</td>
<td>87.5</td>
</tr>
<tr>
<td>F2 × short</td>
<td>3</td>
<td>15.3±1.1</td>
<td>100.0</td>
</tr>
<tr>
<td>F2 × long</td>
<td>9</td>
<td>0.0±0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Short × F2</td>
<td>6</td>
<td>21.0±5.1</td>
<td>100.0</td>
</tr>
<tr>
<td>Long × F2</td>
<td>10</td>
<td>9.3±12.7</td>
<td>60.0</td>
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</tbody>
</table>

measured by the number of seeds per self-pollination, increased from 0 in the parental populations (see data in Barrett 1978) to 1.1 in the F1, and 6.1 in the selected F2.

The genetic basis of self-compatibility in this material is not readily interpretable. The F2 shows a continuous distribution of seed set on self-pollination suggesting polygenic control. The limited appearance of self-compatibility in the F1 and its retention in the F2 suggests that some unique combination of genes may have been brought together in the F1, resulting in the sporadic occurrence of self-compatibility.

Self-compatibility in tetraploid pollen variants

Occasional individuals in tetraploid T. ulmifolia var. elegans possess a small degree of self-compatibility (Barrett 1978). The compatibility relationships of two

Fig. 2. The distributions of mean seed set per self-pollination among progeny of F1 and F2 families of an interpopulation cross of T. ulmifolia var. intermedia (2n = 10). The F1 family was derived by the interpopulation cross I3-3S × I1-10L. The F2 family was obtained by selfing the most highly self-compatible F1 plant, I311-3L, indicated by the arrow. Five replicate pollinations were used for each plant screened. The mean selfed seed set, mean percent fruit set, and number of plants used are indicated.
variants studied here revealed that self-compatibility is the result of aberrant pollen behaviour. Intramorph pollinations as well as intermorph pollinations yield the result of aberrant pollen behaviour. Intramorph variants studied here revealed that self-compatibility is of the pollen variants exhibit normal incompatibility polygenic. Several crosses were conducted and the pollination. However, the long-styled variant shows a both plants is lower than that obtained by legitimate plants from population E5 of T. ulmifolia var. elegans (2n = 20), the number of self-incompatible plants used is indicated results are presented in Fig. 3. Results for the two variant.

The inheritance of self-compatibility in tetraploid individuals of T. ulmifolia var. elegans is likely to be polygenic. Several crosses were conducted and the results are presented in Fig. 3. Results for the two families have been pooled for each generation since they were not significantly different. Additionally, long- and short-styled progeny in segregating families exhibited little variation in the degree of self-compatibility and accordingly only pooled results are shown. Low germination percentage of S2 families derived from E5-1L resulted in limited numbers of progeny for this cross.

The most striking feature of the data obtained from selfs and crosses of the pollen variants is the occurrence of a large number of self-incompatible progeny that have arisen from selfing plants that exhibit moderate levels of self-compatibility (e.g., E5-1L selfed). However, an increase in the mean seed set per self-pollination and percent fruit set occurred in successive generations (Fig. 3). The mean seed set in the parental population was 0.7 (11% fruit set) and increased to a maximum of 2.5 seeds/pollination (41% fruit set) in the S2 family with the highest mean level of self-compatibility.

Plant E5-1L was the most self-compatible individual in the parental population. None of its selfed progeny exhibited as large a degree of self-compatibility. In contrast, selfed progeny from E5-10S were more highly self-compatible than the original parent, with one plant setting a mean of 27 seeds/self-pollination. The data suggest that several genes are segregating in the population and these confer varying degrees of self-compatibility upon both style forms, via aberrant pollen compatibility behaviour.

Self-compatibility in synthetic hexaploids

All hexaploid varieties of T. ulmifolia that have been studied are self-compatible. To evaluate whether hexaploidy per se might result in a weakening of the incompatibility system, synthetic hexaploids were generated from self-incompatible distyloous plants. Five long- and 13 short-styled hexaploids were synthesized. All individuals were strongly self-incompatible with the exception of a single long-styled plant which produced moderate amounts of seed upon self-pollination (Table 7).

The compatibility relationships of the long-styled variant and four of its selfed progeny were assessed (Table 7). All progeny were moderately self-compatible. Pollen from these plants was capable of fertilizing either style form. However, their styles exhibited the usual incompatibility response. Thus the plant and its progeny exhibit aberrant pollen behaviour similar to that observed in the natural tetraploid variants and the diploid variant derived from an interpopulation cross, both discussed above.

Discussion

The present study documents the occurrence of self-compatible distyloous variants in diploid and tetraploid populations of the Turnera ulmifolia complex. All plants are either long- or short-styled. None of the
Fig. 3. The distributions of mean seed set per self-pollination among the parental population (P), F₁, F₂, S₁, and S₂ families of *T. ulmifolia* var. *elegans* (2n = 20) obtained from population E5. The arrows indicate the plants used in generating subsequent populations. For the parental distribution (P) the arrow at the right indicates the mean selfed seed set of E5-1L which was selfed to generate the S₁L family. Two plants from this family (S₁L), indicated by the arrows, were selfed (LL51L and LL61L) to generate the S₂L family. Similarly, the most self-compatible short-styled plant in the parental population was selfed (indicated by the arrow on the far left in P) to generate the S₁S family. Two individuals from this family (S₁S), indicated by the arrows, were selfed (SS77S and SS3S) to generate the S₂S families. The two parental individuals were crossed to generate the F₁. Two plants in the F₁, indicated by the arrows (SL36L and SL34S), were selfed to generate the F₂. The mean selfed seed set, mean percent fruit set, and number of plants used are provided.
variants display the compatibility relationships expected of self-compatible recombinants generated by crossing-over within the supergene controlling distyly. Instead, they behave as though either their pollen or stigmatic incompatibility function has been abolished or weakened in strength. Recombinants of the distyly supergene are expected to show both pollen and stigmatic incompatibility reactions. The variants observed in the present study are perhaps best described as genetically pseudo-compatible (East 1927; Pandey 1959; Mulcahy 1984), although the term encompasses a range of different compatibility phenomena and several different genetic mechanisms. In this discussion, the implications of the occurrence of self-compatible variants for the evolution of breeding systems within the *T. ulmifolia* complex is considered. Specifically, we address the question of whether similar variants could have given rise to the self-compatible varieties that occur within the species complex (Urban 1883; Barrett 1978; Shore and Barrett 1985a, 1985b). Additionally, the genetic basis of the observed patterns of self-compatibility are discussed and the possible implications for the genetical architecture and function of distyly are also considered.

In this study an unusual diploid short-styled stigmatic variant was identified. The plant cannot be interpreted as having arisen via recombination within the distyly supergene as its style exhibits null activity, accepting pollen from either long- or short-styled plants. Crosses were performed to see if a modified *S* allele was responsible for self-compatibility. The results indicate that the gene(s) responsible for self-compatibility are not allelic to the distyly locus. Self-compatibility may be the result of a single dominant gene, or more likely several genes, which interact with the distyly locus and result in self-compatibility in the presence of at least one *S* allele. Shore and Barrett (1985a) demonstrated the occurrence of four self-compatible *SS* short-styled plants among 19 short-styled progeny examined from the self of this variant. The compatibility behaviour of the variant indicates that genes at other loci may modify the incompatibility response, as has been documented in *Primula sinensis* (Beale 1938; Mather and de Winton 1941; Mather 1950).

Synthetic diploid--tetraploid mosaics were produced from the stylist variant. The plants carry the gene(s) conferring self-compatibility. Surprisingly, tetraploid short-styled shoots exhibited a high degree of self-incompatibility, while diploid shoots on the same plants were highly self-compatible. The phenomenon is not the result of a reduced fertility of the tetraploids as legitimate crosses set ample seed and pollen fertility is only 20% lower than that of the diploids. This is possibly the first report of a transition from a self-compatible to a self-incompatible condition via polyploidy. It is the exact reversal of the situation in dicotyledonous species with gametophytic incompatibility. In these plants polygenic results in loss of self-incompatibility (Stout and Chandler 1942; Lewis 1947; Brewbaker 1954, 1958; Annerstedt and Lundqvist 1967; de Nettancourt 1977).

Further crosses involving the synthetic tetraploids, with pollen derived from diploids, revealed that the stigmatic modification remained. Pollen obtained from short-styled diploid shoots gave abundant seed. The significance of these findings to the operation of the incompatibility system is not immediately apparent. As pollen size in synthetic tetraploids is larger than in diploids, it is possible that the variant style only allows pollen that is below some threshold size to achieve fertilization. Alternatively, some correlate of pollen size may differ between the ploidal levels and may be responsible for the observed effect.

All remaining self-compatible variants of *T. ulmifolia* that we examined show aberrant pollen behaviour, with pollen exhibiting a weakened incompatibility response in self- and own-form pollinations. The appearance of self-compatibility in the *F*1 progeny of a cross between two geographically separated diploid populations and the continuous distribution of self-compatibility in the *F*2 suggests that polygenic modification of the self-incompatibility system is involved. Self-compatibility may result from disruption of the genetic background in which the *S* alleles normally function. That other loci or polygenes can modify the strength of self-compatibility in distyly has been demonstrated (see above), and ample evidence of such effects are available for homomorphic gametophytic and sporophytic systems (Mather 1943; Nasrallah and Wallace 1968; Richards and Thurling 1973; Henny and Ascher 1976; Sharma and Murty 1979; Litzow and Ascher 1983). The interpopulation cross performed here is likely to have given rise to unique combinations of genes. In this background the *S* alleles may not function with complete efficiency resulting in varying levels of self-compatibility. The occurrence of natural and artificial self-compatible polyploids may also result from similar processes. Polyploidy may lead to the origin and establishment of self-compatible taxa that arise by hybridization between differentiated population systems through disruption of genetic backgrounds.

The occurrence of dimorphic incompatibility in synthetic hexaploids demonstrates that hexaploidy, at its inception, does not disrupt the genes governing distyly. However, it should be noted that it was possible to establish a self-compatible line from a single long-styled synthetic hexaploid pollen variant. The self-compatible plants do not, however, exhibit the compatibility relationships shown in natural hexaploids (Barrett and Shore 1987).

Some progress was made in selecting for increased
self-compatibility in pollen variants from a tetraploid population. However, the increase in strength of self-compatibility was not marked, and the most striking feature of the data was the high proportion of self-incompatible plants in the selfed progeny of highly self-compatible parental plants. It is important to note that the $F_1$ and $S_1$ populations were screened in a different year than the $F_2$ and $S_2$ populations. Thus, while every effort was made to ensure that screening was carried out under similar environmental conditions, the possibility exists that the observed response was partly environmental in origin. Additionally, if inbred progeny show a reduction in seed set as a result of inbreeding depression, then the measure of self-compatibility used here will underestimate the true value. The bias, if such an effect occurs, is towards reducing the possibility of detecting a selective response. Mather and de Winton (1941) have suggested that selection for increased self-compatibility has occurred in horticultural strains of Primula sinensis. This is presumably the result of polygenic modification of the strength of self-incompatibility.

The evolutionary significance of the low levels of self-compatibility observed in natural populations is not clear. Genes determining self-compatibility may rarely be exposed to selection owing to the prevalence of disassortative mating in populations. However, on rare occasions, as a result of colonizing episodes, the small amount of seed that can be produced on self-fertilization may lead to the establishment of self-compatible populations (Baker 1955; Jain 1976). The data further suggest that if two previously isolated populations hybridize, genetic variation for self-compatibility could be exposed to selection.

Natural hexaploid populations of $T. ulmifolia$ are self-compatible and commonly homostylos although some populations are phenotypically similar to the long-styled morphs of distyloous populations. The incompatibility behaviour of these plants is not like that exhibited by long-styled self-compatible variants described in this study. Instead they show the incompatibility response of recombinant homostyles derived by crossing-over within the distyly supergene (Barrett and Shore 1987). Thus, while the self-compatible variants described here provide an alternate pathway for the evolution of self-compatibility within the $T. ulmifolia$ complex, this seems not to have been the route followed by homostylos taxa. That modifiers can lead to the evolution of self-compatibility in distyloous species has been treated theoretically by Charlesworth and Charlesworth (1979b), and Lande and Schemske (1985) have modelled the evolution of selfing based on polygenic variation.

The demonstration of self-compatible variants of two types, pollen and sternal, both of which exhibit null or reduced activity, may shed light on the genetic architecture and functioning of dimorphic incompatibility. Charlesworth and Charlesworth (1979a) and Ganders (1979) argue that the incompatibility system in distyloous species is not homologous with the $S$ loci found in taxa with homomorphic incompatibility systems (although see Muenchow (1981) for an alternative view). An important issue in this debate is whether null style or pollen activity mutants are controlled by the distylo locus (Charlesworth 1982). While variants of both types were identified in this study, neither appeared to be controlled by the distylo locus. Self-compatibility in the variants varies quantitatively, and genetic background is important in regulating the expression of the self-incompatibility system.

**Acknowledgements**

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CHARLESWORTH, B., and D. CHARLESWORTH. 1979a. The


