

Research review

Breeding system variation, genetics and evolution in the Turneraceae

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Summary

Key words: heterostyly, distyly, homostyly, *Turnera*, *Piriqueta*, Turneraceae, self-incompatibility, polyploidy.

We review the genetics and evolution of breeding systems in the Turneraceae. Distyly occurs in seven of 10 genera and 81% of species. The remaining species are homostylous. Polyploid evolution has been significant in *Turnera*. Approximately 60% of species are polyploid ranging from diploid through decaploid. No relationship between breeding system and polyploidy is evident. The genetics of distyly involves a one-locus two-allele system (*S* and *s*). Evidence from crosses with homostylous species and mutants is consistent with the possibility that a '*Primula*-type' supergene underlies distyly but does not prove this to be the case. A polygalacturonase, and an α -dioxygenase specific to the transmitting tissue of short-styled plants both exhibit morph-limited expression in concert with predictions from an evolutionary model. The function of the proteins in distyly, if any, is unknown. We have begun constructing a fine-scale genetic map of *Turnera*. Two genetic markers lie within 0.2 cm of the distyly locus. This should provide a starting point for positional cloning of the distyly locus and reveal the genetic architecture and molecular basis of distyly.

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Introduction

Distyly has provided an important model system in plant genetics and breeding system evolution since Darwin's investigations (Darwin, 1877; Barrett, 1992; Ornduff, 1992). Hypotheses on the genetic architecture, evolution and breakdown of distyly have emerged from theoretical studies and empirical work on various distylous taxa, and in particular, from *Primula* species. In this paper we describe breeding system variation in the Turneraceae and explore some of these hypotheses. Specifically, we will provide the first modern comprehensive review of breeding systems in the Turneraceae, describing breeding system

variation, its inheritance and evolution. We re-examine whether there is a relationship between breeding system and polyploidy. We review the genetic basis of distyly in *Turnera*, and evidence for and against the hypothesis that a gene complex or 'supergene' determines distyly in *Turnera*. Finally, we review our progress in determining the molecular genetic basis of distyly.

Turneraceae and the evolution of distyly

The Turneraceae are in the order Malpighiales (APG, 2003). Among the 40 or so families in the Malpighiales (APG, 2003), four (Erythroxylaceae, Hypericaceae, Linaceae and Turneraceae)

possess distylous species. Molecular phylogenetic analyses indicate that the Turneraceae are, however, sister to the Passifloraceae, and together, both are sister to the Malesherbiaceae (Davis & Chase, 2004). Neither the Passifloraceae nor Malesherbiaceae possess any heterostylous species. Based upon this recent molecular phylogenetic evidence, distyly must have arisen independently at least once within the Turneraceae.

The Turneraceae comprise 10 genera and 226 species and subspecific taxa (Table 1) most widely distributed in the Neotropics which possesses approximately 170 species in four genera. The bulk of the species occur in just two genera: *Piriqueta* with 45 and *Turnera* with c. 128 species. *Adenoa* Arbo is a monotypic genus endemic to Cuba, *Mathurina penduliflora* Balf. f. is endemic to Rodrigues island, *Erblichia* has one species in America and four in Madagascar, and the remaining genera/species occur in Africa (Urban, 1883; Arbo, 1979, 1995b, 1997, 2000, 2004, 2005).

Turnera is divided into nine series (Urban, 1883). The genus has a centre of high species diversity in the Brazilian states of Bahia, Goiás, and Minas Gerais, and a secondary centre in Paraguay (Arbo, 1987, 2004). *Turnera* species extend north into the southern USA and south into central Argentina. There are two *Turnera* species native to Africa (*Turnera oculata* Story and *Turnera thomasii* (Urb.) Story) and two species (*Turnera ulmifolia* L. and *Turnera subulata* Sm.) have been introduced into and have become weeds in various tropical regions of the world (Arbo, 2005).

Breeding systems

The taxonomic distribution of breeding systems in the Turneraceae has not been reviewed comprehensively since Urban's (1883) monograph, yet numerous species have been described over the past century. We review the distribution of breeding systems categorizing them into those that are distylous vs. homostylous. Distylous species show reciprocal herkogamy, and *Turnera* species that have been investigated also show pollen size and production dimorphisms, and are commonly strongly self-incompatible (Barrett, 1978; Barrett & Shore, 1985). There is somewhat of an asymmetry in the lengths to which incompatible pollen tubes grow (Tamari *et al.*, 2001) as in many distylous taxa (Wedderburn & Richards, 1990; Dulberger, 1992; Barrett & Cruzan, 1994; Wong *et al.*, 1994b). For the purpose of consistency with our previous work, we refer to species/populations exhibiting floral monomorphism as possessing a homostylous breeding system. Homostylous species commonly have anthers and stigmas in close proximity within a flower and are self-compatible, although some (e.g. *Turnera campaniflora* and *Turnera velutina*) show approach herkogamy (Barrett & Shore, 1987).

Seven of the 10 genera within the Turneraceae possess distylous species (Tables 1 and 2). The three genera lacking distylous species are either monotypic (*Adenoa* and *Mathurina*) or possess few species (*Erblichia*; Table 1). *Hyalocalyx* is a monotypic distylous genus. In *Tricliceras* and *Streptopetalum* most species are distylous, while in *Loewia*, two of three species are distylous (see later). *Stapfiella* has six species with

very small flowers (2–4 mm long). The reproductive system has not been described and the available material is very scarce. *Stapfiella lucida* var. *lucida* may be homostylous and *Stapfiella usambarica* is likely to be distylous. We have categorized the breeding systems of *Stapfiella* with a degree of uncertainty (Table 1). Interestingly, the flowers of species of four African genera (*Hyalocalyx*, *Loewia*, *Streptopetalum* & *Tricliceras*) possess stamens of different lengths, mostly two shorter and three longer within a flower, for both homostylous and distylous species (Table 1; Urban, 1883; Arbo, 2006).

Distyly is widespread in *Turnera* and *Piriqueta*. In *Turnera*, distyly is the predominant breeding system (possessed by c. 80% of species) but there is variation in the proportion of distylous species across the taxonomic series (Table 2). Series *Turnera* possesses the lowest percentage of distylous species (59%) while four series possess only distylous species (Table 2). Interestingly, five species of *Turnera* possess both distylous and homostylous populations.

In the genus *Piriqueta* with 48 taxa (species, subspecies and taxonomic varieties), 38 (79%) are distylous, six are homostylous and four species are composed of populations, some of which are distylous and the others homostylous (Tables 1 and 2). *Piriqueta assuruensis* Urb., *Piriqueta racemosa* (Jacq.) Sw. and *Piriqueta capensis* (Harvey) Urb. (the last being the only African species of the genus) all possess both distylous and homostylous populations (Arbo, 1995b). *Piriqueta viscosa* Griseb. is a small-flowered diploid homostylous species sister to *Piriqueta morongii* Rolfe, the latter of which possesses both distylous and homostylous populations (Arbo, 1995b; Truyens *et al.*, 2005). Plants in distylous populations of *P. morongii* are self-fertile (J. S. Shore, pers. obs.). No crosses between or within these species have been undertaken so the nature and inheritance of homostyly is unknown. *Piriqueta cistoides* (L.) Griseb. ssp. *cistoides* is a diploid homostylous subspecies, while *Piriqueta cistoides* ssp. *caroliniana* is the distylous subspecies that has likely given rise to the homostyle (Ornduff, 1970). Interestingly, there is considerable morphological and molecular variation among distylous populations of *Piriqueta cistoides* ssp. *caroliniana sensu lato*, in Florida. Cruzan (2005) has explored patterns of hybridization among them.

The breeding systems of the other genera of Turneraceae have not been studied to any great extent, and we have classified their breeding systems based largely upon surveys of herbarium material and/or literature surveys. Further work will be required to characterize their breeding systems.

The distribution of breeding systems among species should provide insights into the origins of distyly and homostyly in the family. This might allow the recognition of species that are primitive or 'primary homostyles' (should they occur) as opposed to those that are derived or 'secondarily' homostylous (*sensu* Ernst, 1955). Recent molecular phylogenetic analyses (Mast *et al.*, 2006) indicate that there are apparently no primary homostyles within *Primula*. A comprehensive phylogenetic analysis will be required to assess breeding system evolution in

Table 1 List of all 226 species/subspecific taxa of Turneraceae and their breeding system, chromosome numbers and ploidy levels (where known)

Species	Breeding system ^{a,e}	Chromosome numbers	Ploidy levels ^e
Turnera (135 taxa)			
Series <i>Turnera</i> (29 taxa)			
Subseries <i>Turnera</i> (21 taxa)			
<i>T. aurelia</i>	Hom ¹	SC	2n = 40 ²
<i>T. campaniflora</i>	Hom ¹	SC	2n = 30 ³
<i>T. candida</i>	Hom ¹	SC	2n = 10 ⁴
<i>T. coerulea</i> var. <i>coerulea</i>	Dis ¹	SI	2n = 10, 20 ⁴
<i>T. coerulea</i> var. <i>surinamensis</i>	Dis ¹	SI	2n = 10 ⁴
<i>T. concinna</i>	Dis ¹	SI	2n = 10 ²
<i>T. cuneiformis</i>	Hom ¹	SC	2n = 40 ⁵
<i>T. fernandezii</i>	Dis ¹	SI	2n = 40 ^{1,6}
<i>T. grandidentata</i>	Dis ¹	SI	2n = 20 ²
<i>T. grandiflora</i>	Dis ¹	SI	2n = 10 ⁶
<i>T. krapovickasii</i>	Dis ¹	SI	2n = 10, 20 ⁶
<i>T. lucida</i>	Hom ¹		
<i>T. occidentalis</i>	Hom ¹	SC	2n = 30 ¹
<i>T. oculata</i>	Hom ¹		
<i>T. orientalis</i>	Hom ¹	SC	2n = 30 ²
<i>T. scabra</i>	Dis ¹	SI	2n = 10, 20 ⁶
<i>T. subulata</i>	Dis ¹	SI	2n = 10, 20 ⁷
<i>T. thomasii</i>	Hom ¹		
<i>T. ulmifolia</i> var. <i>acuta</i>	Hom ¹	SC	2n = 30 ⁸
<i>T. ulmifolia</i> var. <i>ulmifolia</i>	Hom ¹	SC	2n = 30 ⁹
<i>T. velutina</i>	Hom ¹	SC	2n = 30 ¹⁰
Subseries Umbilicatae (8 taxa)			
<i>T. arcuata</i>	Dis ¹		Autotetraploid
<i>T. coriacea</i>	Dis ¹		Octoploid
<i>T. hermannioides</i>	Dis ¹	SI	Diploid
<i>T. joelii</i>	Dis ¹	SI	Diploid
<i>T. leptosperma</i>	Dis ¹		
<i>T. purpurascens</i>	Dis ¹		
<i>T. simulans</i>	Dis ¹		
<i>T. stenophylla</i>	Dis ¹		
Series Annulares (4 taxa)			
<i>T. annularis</i>	Dis ¹²		
<i>T. aromatica</i>	Dis ¹²		
<i>T. breviflora</i>	Dis ¹²		
<i>T. odorata</i>	Dis ¹²		
Series Anomalae (14 taxa)			
<i>T. amazoniana</i>	Hom? ¹		
<i>T. bahiensis</i>	Dis ¹		
<i>T. blanchetiana</i>	Dis ¹		
<i>T. cearensis</i>	Dis ¹		
<i>T. chrysoccephala</i>	Dis ¹		
<i>T. discors</i>	Dis ¹		
<i>T. gardneriana</i>	Hom? ¹		
<i>T. involucrata</i>	Dist ¹		
<i>T. kuhlmanniana</i>	Dist ¹		
<i>T. laciñata</i>	Dis ¹		
<i>T. reginae</i>	Dis ¹		
<i>T. sancta</i>	Dis ¹		
<i>T. stipularis</i>	Dis ¹		
<i>T. tapajoensis</i>	Hom? ¹		
Series Capitatae (10 taxa)			
<i>T. albicans</i>	Dis ¹²		
<i>T. capitata</i>	Dis ¹²		
<i>T. dasystyla</i>	Hom ¹²		
<i>T. hatschbachii</i>	Dis ¹²		
<i>T. maracasana</i>	Dis ¹²		
<i>T. marmorata</i>	Dis ¹²		
<i>T. pernambucensis</i>	Dis? ¹²		
<i>T. princeps</i>	Dis ¹²		
<i>T. schomburgkiana</i>	Dis ¹²		
<i>T. waltherioides</i>	Dis ¹²		
Series Leiocarpace (50 taxa)			
<i>T. acaulis</i>	Hom ¹³		
<i>T. argentea</i>	Dis ¹⁴		

Table 1 continued

Species	Breeding system ^{a,e}	Chromosome numbers	Ploidy levels ^e
<i>T. callosa</i>	Dis ¹³		
<i>T. cipoensis</i>	Dis ¹⁵		
<i>T. crulsii</i>	Hom ¹⁶	2n = 14 ¹¹	Diploid
<i>T. curassavica</i>	Dis ¹³		
<i>T. dasytricha</i>	?		
<i>T. dichotoma</i>	Dis ¹³		
<i>T. discolor</i>	Dis ¹⁷		
<i>T. dolichostigma</i>	Dis ¹⁸		
<i>T. elliptica</i>	Dis ¹³		
<i>T. foliosa</i>	Dis ¹⁶		
<i>T. genistoides</i>	Dis ¹³		
<i>T. goyazensis</i>	Dis ¹⁶		
<i>T. guianensis</i>	Dis ¹³		
<i>T. harleyi</i>	Dis ¹⁹		
<i>T. hassleriana</i>	Dis ¹⁸	SI	2n = 14, 28 ⁶
<i>T. hilaireana</i>	Dis ¹³		Diploid and autotetraploid
<i>T. huberi</i>	Dis ¹⁴		
<i>T. incana</i>	Dis ¹³		
<i>T. lamiifolia</i>	Dis ¹³	2n = 28 ⁶	Tetraploid
<i>T. lanceolata</i>	Dis ¹³		
<i>T. lineata</i>	Dis?		
<i>T. longiflora</i>	Dis ¹³		
<i>T. luetzelburgii</i>	Dis ²⁰		
<i>T. melanorhiza</i>	Dis ¹⁶		
<i>T. melochia</i>	?		
<i>T. melochioides</i>	Dis ¹³	SC	2n = 14 ⁶
<i>T. nana</i>	Dis ¹³		Diploid
<i>T. nervosa</i>	Dis ¹⁸	SI	2n = 14 ⁶
<i>T. oblongifolia</i>	Dis ¹³		Diploid
<i>T. opifera</i>	Dis ¹³	SI	Decaploid
<i>T. paruana</i>	Dis ¹⁴		
<i>T. pinifolia</i>	Hom ¹³		
<i>T. pohliana</i>	Dis ¹³		
<i>T. prancei</i>	Dis ¹⁵		
<i>T. pumilea</i> var. <i>pumilea</i>	Hom ¹⁸	SC	2n = 14 ⁶
<i>T. pumilea</i> var. <i>piauhensis</i>	Dis ^{21,42}	SC	2n = 14 ⁴²
<i>T. revoluta</i>	Dis ¹⁷		Diploid
<i>T. riedeliana</i>	Dis ¹³		
<i>T. sidoides</i> ssp. <i>sidoides</i>	Dis and Hom ²²	SI	2n = 28 ^{6,23}
<i>T. sidoides</i> ssp. <i>carnaea</i>	Dis and Hom ²²	SI	2n = 14, 28, 42 ^{6,23}
<i>T. sidoides</i> ssp. <i>holosericea</i>	Dis and Hom ²²	SI	2n = 28, 42 ^{6,23}
<i>T. sidoides</i> ssp. <i>integrifolia</i>	Dis ²²	SI	2n = 14, 28, 42, 56 ^{6,23}
<i>T. sidoides</i> ssp. <i>pinnatifida</i>	Dis ²²	SI	2n = 14, 28, 42 ^{6,23}
<i>T. stachydifolia</i>	Dis ¹³		Diploid and autopolyploid
<i>T. subnuda</i>	Dis ¹⁶		Autotetraploid
<i>T. tenuicaulis</i>	Dis ¹⁶		Diploid and autopolyplid
<i>T. trigona</i>	Dis ¹³	2n = 14 ⁸	Autotetraploid and autohexaploid
<i>T. uleana</i>	Dis ²⁰		Diploid and autopolyploid
Series Microphyllae (5 taxa)			Diploid and autopolyplid
<i>T. asymmetrica</i>	Dis ⁴³		Diploid and autopolyploid
<i>T. calyptrocarpa</i>	Dis ¹²		Autotetraploid and autohexaploid
<i>T. collotricha</i>	Dis ¹⁹		Diploid and autopolyploid
<i>T. diffusa</i>	Dis ¹²		Diploid and autopolyploid
<i>T. hebetepetala</i>	Dis ¹²		Diploid and autopolyploid
Series Papilliferae (2 taxa)			Diploid and autopolyploid
<i>T. caatingana</i>	Dis ¹²		
<i>T. chamaedrifolia</i>	Dis and Hom ¹²	SC	2n = 26 ⁶
Series Salicifoliae (12 taxa)			Diploid
<i>T. amapaensis</i>	?		
<i>T. brasiliensis</i>	Dis ²⁴		
<i>T. clauseniana</i>	Dis ²⁴		
<i>T. glaziovii</i>	Dis ²⁴		
<i>T. hindsiana</i>	Dis ²⁴		
<i>T. ignota</i>	Dis ⁴³		
<i>T. panamensis</i>	Dis ²⁴		
<i>T. rupestris</i>	Dis ²⁴		
<i>T. serrata</i>	Dis ²⁴		

Table 1 continued

Species	Breeding system ^{a,e}		Chromosome numbers	Ploidy levels ^e
<i>T. steyermarkii</i>	Dis ²⁴			
<i>T. venosa</i>	Dis ²⁴			
<i>T. weddelliana</i>	Dis ²⁴	SI	2n = 14 ⁶	Diploid
Series Stenodictyae (9 taxa)				
<i>T. acuta</i>	Dis ²⁴			
<i>T. annectens</i>	Dis ²⁴			
<i>T. aurantiaca</i>	Dis ²⁴			
<i>T. benthamiana</i>	Dis ²⁴			
<i>T. castilloi</i>	Dis ²⁴			
<i>T. cicatricosa</i>	Dis ²⁴			
<i>T. longipes</i>	Dis ²⁴			
<i>T. macrophylla</i>	Dis and Hom ²⁴		2n = 14 ⁶	Diploid
<i>T. urbanii</i>	Hom ²⁴			
Series uncertain (1 species)				
<i>T. rubrobracteata</i>	Dis ¹⁵			
Piriáqueta (48 taxa)				
<i>P. abairana</i>	Dis ²⁵			
<i>P. araguaiana</i>	Dis ²⁶			
<i>P. asperifolia</i>	Dis ²⁶			
<i>P. assuruensis</i>	Dis and Hom ²⁶			
<i>P. aurea</i>	Dis ²⁶			
<i>P. breviseminata</i>	Dis ²⁶			
<i>P. caiapoensis</i>	Dis ²⁶			
<i>P. capensis</i>	Dis and Hom ^{27,28}			
<i>P. carneae</i>	Dis ²⁶			
<i>P. cistoides</i> ssp. <i>cistoides</i>	Hom ²⁶	SC	2n = 14 ⁶	Diploid
<i>P. cistoides</i> ssp. <i>caroliniana</i>	Dis ²⁶	SI	2n = 14 ^{11,29}	Diploid
<i>P. constellata</i>	Dis ²⁶			
<i>P. corumbensis</i>	Dis ²⁶			
<i>P. cristobaliae</i>	Dis ²⁶			
<i>P. dentata</i>	Dis ²⁶			
<i>P. densiflora</i>	Dis ²⁶			
<i>P. douradinha</i>	Dis ²⁶			
<i>P. duarteana</i>	Dis ²⁶		2n = 14 ⁶	Diploid
<i>P. emasensis</i>	Dis ³⁰			
<i>P. flammee</i>	Dis ²⁶			
<i>P. grandifolia</i>	Dis ²⁶			
<i>P. guianensis</i>	Dis ²⁶			
<i>P. hapala</i>	Hom ²⁶			
<i>P. lourteigiae</i>	Dis ²⁶			
<i>P. mesoamericana</i>	Dis ²⁶			
<i>P. mexicana</i>	Hom ²⁶			
<i>P. morongii</i>	Dis and Hom ²⁶	SC	2n = 14 ^{6,31}	Diploid
<i>P. mortonii</i>	Hom ²⁶			
<i>P. nanuzae</i>	Dis ²⁶			
<i>P. nitida</i>	Dis ²⁶			
<i>P. ochroleuca</i>	Dis ²⁶		2n = 14 ³¹	Diploid
<i>P. plicata</i>	Dis ²⁶			
<i>P. racemosa</i>	Dis and Hom ²⁶	SC	2n = 14 ⁶	Diploid
<i>P. revoluta</i>	Dis ²⁵			
<i>P. rosea</i>	Dis ²⁶		2n = 14 and 28 ⁶	Diploid and autotetraploid
<i>P. sarae</i>	Dis ²⁶			
<i>P. scabrida</i>	Dis ²⁶			
<i>P. sidifolia</i> var. <i>sidifolia</i>	Dis ²⁶			
<i>P. sidifolia</i> var. <i>multiflora</i>	Dis ²⁶		2n = 14 ³¹	Diploid
<i>P. suborbicularis</i>	Dis ²⁶	SI	2n = 28 ⁶	Tetraploid
<i>P. subsessilis</i>	Dis ²⁶			
<i>P. sulfurea</i>	Dis ²⁶			
<i>P. tamberlikii</i> ssp. <i>rotundifolia</i>	Dis ²⁶		2n = 28 ²⁶	Tetraploid
<i>P. tamberlikii</i> ssp. <i>tamberlikii</i>	Dis ²⁶			
<i>P. taubatensis</i>	Dis ²⁶	SI	2n = 42 ^{6,31}	Segmental allohexaploid
<i>P. undulata</i>	Dis ²⁶			
<i>P. venezuelana</i>	Hom? ²⁶			
<i>P. viscosa</i>	Hom ²⁶			
Adenoa (1 species)				
<i>Adenoa cubensis</i>	Hom ³²			

Table 1 continued

Species	Breeding system ^{a,e}	Chromosome numbers	Ploidy levels ^e
<i>Erblichia</i> (5 species)			
<i>E. antsingyae</i>	Hom ³³		
<i>E. bernieriana</i>	Hom ³³		
<i>E. integrifolia</i>	Hom ³³		
<i>E. madagascariensis</i>	Hom ³³		
<i>E. odorata</i>	Hom ³³		
<i>Hyalocalyx</i> (1 species)			
<i>H. setiferus</i>	Dis ^{b,27} and Hom ³⁴		
<i>Loewia</i> (3 species)			
<i>L. glutinosa</i>	Dis ³⁵		
<i>L. microphylla</i>	Hom ^{c,36}		
<i>L. tanaensis</i>	Dis? ^{b,37}		
<i>Mathurina</i> (1 species)			
<i>M. penduliflora</i>	Hom ³⁸		
<i>Stapfiella</i> (7 taxa)			
<i>S. claoxyloides</i>	?		
<i>S. lucida</i> var. <i>lucida</i>	Hom ³⁹		
<i>S. lucida</i> var. <i>pubescens</i>	?		
<i>S. muricata</i>	?		
<i>S. ulugurica</i>	?		
<i>S. usambarica</i>	Dis? ³⁷		
<i>S. zambesiensis</i>	Hom ²⁷		
<i>Streptopetalum</i> (6 taxa)			
<i>S. arenarium</i>	Dis ^{c,35}		
<i>S. graminifolium</i>	Dis? ^{c,40}		
<i>S. hildebrandtii</i>	Dis ^{c,13}		
<i>S. luteoglandulosum</i>	Dis ²⁷		
<i>S. serratum</i>	Hom ^{b,c,d,13}		
<i>S. wittei</i>	Dis ^{c,27,39}		
<i>Tricliceras</i> (19 taxa)			
<i>T. auriculatum</i>	? ^{b,27}		
<i>T. bivinianum</i>	Dis ^{b,37}		
<i>T. brevicaule</i> var. <i>brevicaule</i>	Dis ^{b,27}		
<i>T. brevicaule</i> var. <i>rosulatum</i>	? ^{b,27}		
<i>T. elatum</i>	Dis ^{b,27}		
<i>T. glanduliferum</i>	Dis and Hom ^{b,27,28}		
<i>T. hirsutum</i>	Dis ^{b,27}		
<i>T. lacerata</i>	Dis ^{b,c,28}		
<i>T. lanceolatum</i>	Dis ^{b,27}		
<i>T. lobatum</i>	Hom ^{b,13,34}		
<i>T. longepedunculatum</i>	Dis ^{b,c,13,37}		
<i>T. longepedunculatum</i> var. <i>eratense</i>	?		
<i>T. mossambicense</i>	Dis ^{b,c,27,28}		
<i>T. pilosum</i>	Hom ^{b,13,34}		
<i>T. prittwitzii</i>	Dis? ^{c,41}		
<i>T. schinzii</i> var. <i>schinzii</i>	?		
<i>T. schinzii</i> var. <i>juttae</i>	Dis ^{b,d,27,41}		
<i>T. tanacetifolium</i>	Dis ^{b,27,28}		
<i>T. xylorhizum</i>	?		

^aBreeding systems have been classified as distyly (Dis) or homostyly (Hom). For convenience, we refer to all monomorphic breeding systems as homostyly. For some species, both distyly and homostyly have been observed. A question mark (?) indicates that the breeding system is either unknown or uncertain, where incomplete information is available for a species. We also indicate whether plants are self-compatible (SC) or self-incompatible (SI).

^bStamens variable in length within a flower, having two shorter and three longer stamens, or four shorter and one longer, in both morphs and/or 'homostyles'.

^cStamens equal in length, although other species in the genus possess variable stamen lengths within a flower.

^dStyles of varying length within a flower.

^eBreeding systems, chromosome counts and cytogenetic data were published in the following: 1, Arbo (2005); 2, Fernández & Arbo (1993a); 3, Baker & Shore (1995); 4, Fernández & Arbo (1996); 5, Fernández & Arbo (2000a); 6, Fernández (1987); 7, Arbo & Fernández (1983); 8, Fernández & Solís Neffa (2004); 9, Fernández & Arbo (2000b); 10, Solís Neffa & Fernández (1993); 11, Fernández *et al.* (1994); 12, Arbo (2000); 13, Urban (1883); 14, Arbo (1990a); 15, Arbo (1993); 16, Urban (1898); 17, Urban (1893); 18, Arbo (1987); 19, Arbo (1981); 20, Arbo (1995a); 21, Urban (1907); 22, Arbo (1985); 23, Solís Neffa & Fernández (2001); 24, Arbo (1997); 25, Arbo (1999); 26, Arbo (1995b); 27, Fernandes (1978); 28, Obermeyer (1976); 29, Lewis *et al.* (1962); 30, Arbo (2002); 31, Lavia & Fernández (1993); 32, Arbo (1977); 33, Arbo (1979); 34, Fernandes & Fernandes (1962); 35, Thulin (1993); 36, Rotti Michelozzi (1969); 37, Lewis (1954); 38, Arbo (1990b); 39, Robyns (1964); 40, Urban (1895); 41, Urban (1914); 42, Lopez (1998); 43, Arbo, M.M. (pers. obs.).

Table 2 Summary of numbers of species/subspecific taxa with distyly, homostyly or both breeding systems^a in various genera of Turneraceae and in the series of the genus *Turnera*

Genus or Series	Distyly	Homostyly ^b	Distyly and homostyly
Turnera			
Series Turnera	17	12	0
Series Annulares	4	0	0
Series Anomalae	11	3	0
Series Capitatae	9	1	0
Series Leiocarpe	43	4	3
Series Microphyllae	5	0	0
Series Papilliferae	2	0	1
Series Salicifoliae	11	0	0
Series Stenodictyae	7	1	1
Turnera (totals)	109	21	5
<i>Piriqueta</i>	38	6	4
<i>Adenoa</i>	0	1	0
<i>Erblichia</i>	0	4	0
<i>Hyalocalyx</i>	1	0	0
<i>Loewia</i>	2	1	0
<i>Mathurina</i>	0	1	0
<i>Stapfiella</i>	1	2	0
<i>Streptopetalum</i>	5	3	0
<i>Tricliceras</i>	12	3	1
Totals	168	40	10

^aWe ignored the uncertainty in categorization of breeding systems in Table 1.

^bNote that some species/populations listed as homostylos, show varying degrees of approach herkogamy.

the Turneraceae. In an initial phylogenetic analysis of c. 40 species, Truyens *et al.* (2005) indicated that homostyly appears to have evolved independently at least three times in *Turnera*.

Polyplody and breeding systems

In a study of the *T. ulmifolia* complex, Barrett & Shore (1987) reported that diploids and autotetraploids were distylos and self-incompatible while allohexaploids were homostylos and self-compatible. Barrett & Shore (1987) speculated that reduced inbreeding depression resulting from allohexaploidy might have been responsible for allowing the spread of selfing homostyles through hexaploid populations.

There is a tendency towards the evolution of homostyly in polyplloid *Primula* species (Kelso, 1992; Richards, 2003; Guggisberg *et al.*, 2006). Richards (2003) indicated that chromosome numbers of 29 homostyle species of *Primula* are known and that 16 of these species are polyploid. The polyploids range from tetraploid through tetrakaidecaploid. A majority of the 13 diploid homostyles were at one time thought to be primitively monomorphic (Richards, 2003), but it now seems that distyly is the ancestral breeding system in *Primula* and so homostyly must be derived in these diploids (Mast *et al.*, 2006). By comparison, only five distylos species

are polyploid (4% of species), outside of two sections of the genus (*Parryi* and *Auricula*) where all species are either tetraploid or hexaploid (Richards, 2003).

In a recent molecular phylogenetic analysis of species in *Primula* sect. *Aleuritia* subsect. *Aleuritia* using cpDNA, Guggisberg *et al.* (2006) demonstrated multiple origins of polyploidy coupled with the evolution of homostyly. In this subsection of *Primula*, only a single polyploid (tetraploid) is distylos, all others are homostylos. Most of the polyploids in subsection *Aleuritia* are believed to be allopolyploids. In *Damnacanthus* (Rubiaceae), there is a complete association between diploidy and distyly vs. tetraploidy and monomorphism. The monomorphic species are phenotypically long-styled but their pollen is like that of short-styled plants (Naiki & Nagamasu, 2004). By contrast, molecular phylogenetic analyses of *Amsinckia* (Boraginaceae) do not reveal any strong associations between polyploidy and breeding system (Schoen *et al.*, 1997).

Here we re-examine the pattern reported in Barrett & Shore (1987) reviewing a broader range of species for which cytological and breeding system data are now available. Polyploid evolution has been very significant in *Turnera* as c. 60% of the species/populations studied have chromosome numbers in the tetraploid through decaploid range. Evidence indicates that c. 35% of the polyploids are autoployploids (Solís Neffa & Fernández, 2002). The mechanism of polyploidization is likely to be through unreduced gamete formation (Fernández & Arbo, 1990).

Polyplloid distylos species occur in both *Turnera* and *Piriqueta* ranging from tetraploid through decaploid, or tetraploid through hexaploid, respectively (Table 1). It is possible that the distylos polyploids are restricted to those having had autoployploid origins. This certainly appears to be the case for species in series *Turnera* ($x=5$) including *Turnera subulata*, *Turnera scabra* Millsp., *Turnera krapovickasii* Arbo, and *Turnera coerulea* DC. var. *coerulea*, all of which occur at both diploid and autotetraploid levels (Fernández, 1987; Shore, 1991a,b; Truyens *et al.*, 2005) while distylos *Turnera fernandezii* is an auto-octaploid (Fernández, 1987; Arbo, 2005; Table 1). Five subspecies occur within the *T. sidoides* L. complex (series *Leiocarpe*, $x=7$). Two of the subspecies possess only distylos populations, while the other three subspecies possess both distylos and homostylos populations (Table 1). Ploidy levels range from diploid through octaploid, and all the polyploids appear to be autoployploids (Arbo, 1985; Fernández, 1987; Solís Neffa & Fernández, 2000, 2002).

Exceptions to this pattern of autoployploid distylos species occur for tetraploid *Turnera grandidentata* (Urb.) Arbo, which is a segmental allotetraploid (Fernández, 1987; Fernández & Arbo, 1990, 1993b), and *Turnera chamaedrifolia* Cambess. which is a 'diploid' having a base chromosome number of $x=13$, as opposed to $x=5$ or $x=7$ for all other *Turnera* species (Fernández, 1987). Cytogenetic and phylogenetic evidence suggests that the increased base chromosome number of *T. chamaedrifolia* is the result of polyploid evolution and

subsequent aneuploid reduction (Solís Neffa & Fernández, 2000; Truyens *et al.*, 2005). Homostylous collections of this latter species have also been made. Interestingly, the distylos specimens cultivated in Corrientes Argentina were somewhat self-compatible and set a few autogamous seeds although the ovaries possess c. 90 ovules (Arbo, 2000).

Overall, there appears to be no impediment to the occurrence of distyly in polyploids. Is there, however, a tendency for homostyly to evolve preferentially in polyploids? Within series *Turnera* there are 29 species and/or subspecific taxa (Arbo, 2005). Eight of these are homostylous polyploid species (six hexaploids and two octaploids). The polyploids all appear to be allopolyploids based upon examination of meiosis in these species, although Fernández & Arbo (2000b) have suggested that octaploids *Turnera aureliae* and *Turnera cuneiformis*, and hexaploids *Turnera orientalis*, *T. ulmifolia* var. *ulmifolia* and *T. velutina* may be segmental allopolyploids based upon studies of meiosis in hybrids (Fernández & Arbo, 1993a, 2000a,b; Fernández, 1997). *Turnera candida* Arbo is the only known self-compatible diploid homostylous species in this series (Fernández & Arbo, 1996). The nature of homostyly is unknown in this species since compatibility relationships (Fig. 1) and inheritance studies with related distylos species have not been carried out. A single long-styled diploid hybrid (pollen fertility 19.7%) was obtained in a cross with *Turnera grandiflora* (Urb.) Arbo. Crosses of *T. candida* with short-styled hybrids of *T. grandiflora* × *Turnera coerulea* var. *coerulea* were unsuccessful (Fernández & Arbo, 1996).

While data from series *Turnera* appear to support a propensity for the evolution of homostyly in allopolyploids/segmental allopolyploids, in fact, it is unclear whether the homostylous polyploids have had independent origins. Molecular phylogenetic analysis of internal transcribed spacer (ITS) sequence

data have not provided sufficient resolution to determine whether homostyly in these eight polyploids was the result of a single or multiple origins (Truyens *et al.*, 2005). The two homostylous octaploids *T. aureliae* and *T. cuneiformis* certainly appear to share a hexaploid homostylous progenitor, *T. orientalis*, based upon studies of meiosis in hybrids and molecular phylogenetic analysis (Fernández & Arbo, 2000a; Truyens *et al.*, 2005). Thus, homostyly did not arise independently in these octaploids.

In other series of the genus *Turnera* investigated, homostyly occurs in diploid *T. pumilea* L. The nature of homostyly is unknown in this species (Truyens *et al.*, 2005) which also possesses a distylos variety, *T. pumilea* var. *piauhyensis* Urb.

All the polyploids known in *Piriqueta* are distylos (Table 1). *Piriqueta suborbicularis* and *Piriqueta tamberlikii* var. *rotundifolia* are tetraploids, *Piriqueta rosea* has diploid and autotetraploid populations, and finally *Piriqueta taubatensis* appears to be a segmental allohexaploid (Fernández, 1987; Lavia & Fernández, 1993; Arbo, 1995b).

In contrast to the observations of Barrett & Shore (1987), at present, it appears to be premature to conclude that there is a causal relationship between polyploidy and the evolution of homostyly in the Turneraceae. The investigation of further species in a phylogenetic context will be required to address this question rigorously. It will be necessary to determine the progenitors of the allopolyploid species to understand the contributions of reticulate evolution to breeding systems of allopolyploids.

Inheritance of distyly: genetic architecture in *Turnera*

Distyly is inherited by what appears to be a single Mendelian locus with two alleles (Lewis & Jones, 1992). Short-styled (thrum) plants are commonly heterozygous, *Ss*, while long-styled (pin) plants are recessive homozygotes, *ss*. In autotetraploid *Primula obconica*, Dowrick (1956) showed that the same dominance relationships hold, however, tetrasomic inheritance occurs at the locus. We have studied the inheritance of distyly in *T. scabra* and *T. subulata* (formerly *T. ulmifolia* var. *intermedia* and *T. ulmifolia* var. *elegans*, respectively) at both the diploid and autotetraploid levels (Shore & Barrett, 1985). We found a common pattern of inheritance. Short-styled plants are *Ss* and long-styled plants are *ss* in diploids, and we have demonstrated tetrasomic inheritance in the autotetraploids.

Detailed studies, particularly by Ernst (Ernst, 1955), indicate that distyly in *Primula* is determined by a series of tightly linked loci comprising a supergene. Ernst's data suggest that three loci may be separable by recombination and others have postulated the existence of, and provided evidence for additional linked genes (Dowrick, 1956; Kurian & Richards, 1997). Population genetic models indicate that the distylos polymorphism is able to establish if the genes for various traits are linked forming a supergene (Charlesworth & Charlesworth,

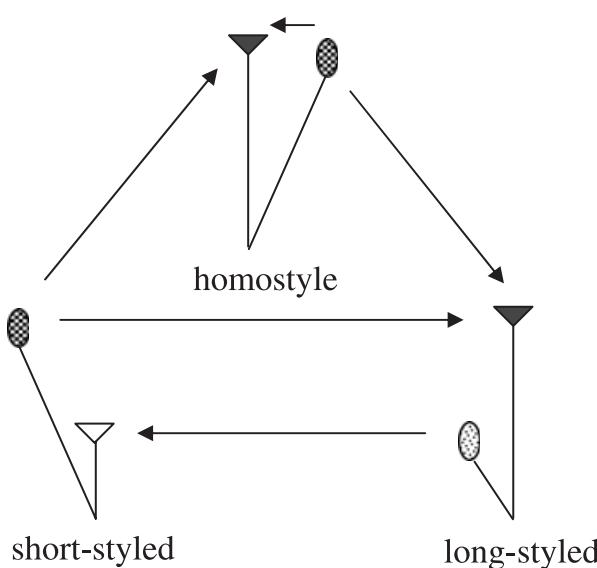


Fig. 1 Compatibility relationships among long-styled, short-styled and homostyled plants.

1979). In contrast, the phenotypic models of Lloyd & Webb, 1992) allow a broader range of genetic bases including the possibility of major linked genes determining androecial and gynoecial characters, as well as modifiers that are not necessarily linked. The expression of modifiers may be morph-limited and coordinated with the activities of the supergene.

Because of its historical precedence, we begin by reviewing the evidence for a supergene in *Turnera* and subsequently consider the model of Lloyd & Webb (1992). Under perhaps the simplest supergene model, three tightly linked loci are responsible for distyly (Lewis & Jones, 1992). The *G/g* locus determines style length and its incompatibility, the *P/p* locus determines pollen size and its incompatibility, while the *A/a* locus determines anther height. Under this model, short-styled plants are heterozygous at all three loci and are genotypically *GPA/gpa* while long-styled plants are homozygous, *gpa/gpa*.

There are three sources of experimental data bearing upon the question of whether there is a supergene in *Turnera*. The first approach is to cross (reciprocally) distylous species with homostylous species to explore compatibility relationships between long-styled, short-styled and homostyled plants. We have carried out these experiments for a number of homostylous species in series *Turnera* examining seed set and pollen tube growth (Barrett & Shore, 1987; Tamari *et al.*, 2001). If the homostylous species of *Turnera* have origins analogous to the recombinant long-homostyles of *Primula*, then we can predict a particular pattern of compatibility among pollen from various sources (Fig. 1). Our data show such a pattern for six homostylous species investigated. Interestingly, when homostyles reject pollen from long-styled plants, the incompatibility response appears similar to that observed when long-styled plants are selfed. That is, pollen tubes germinate, penetrate the stigma and grow into the style where they are inhibited. Pollen plugs are visible in the pollen tubes (Tamari *et al.*, 2001). When short-styled plants reject pollen from homostyles, pollen germinates, but is inhibited in the stigma and no callose plugs are apparent. The incompatibility reaction of short-styled plants to pollen from homostyles resembles their rejection response to self pollen (Tamari *et al.*, 2001).

If the homostyles in *Turnera* have arisen via recombination within a supergene controlling distyly (following the *Primula* model), there is a predicted pattern of dominance relationships that should occur in the F_1 progeny from crosses between distylous and homostylous species. That is, homostyles should appear to be determined by an allele, S^H , of the distyly locus and the dominance hierarchy, $S > S^H > s$, should occur. This is, in fact, the pattern of progeny observed for three homostylous species investigated including, *T. ulmifolia*, *T. velutina* and *T. orientalis* in crosses with distylous *T. subulata* or *T. scabra* (Shore & Barrett, 1985). Unfortunately, we have had little opportunity to explore the other homostylous species of *Turnera* in this way. The occurrence of a single long-styled plant from a cross between homostylous *T. candida* and distylous *T. grandiflora* (Fernández & Arbo, 1996), suggests that the

origins of *T. candida* may not be the result of recombination within a supergene (should it exist), but this warrants further investigation.

Perhaps the best evidence for a supergene would be the direct observation of recombinants at the 'locus'. This would require extremely large sample sizes and flanking genetic markers to confirm that phenotypically novel progeny are the result of recombination and not mutation. While we have not detected directly any such recombinants, we have studied the inheritance of two homostylous mutants. The first mutant clearly did not arise as a result of meiotic recombination, but rather arose as a somatic mutant branch on an otherwise short-styled plant. The homostyle mutant is inherited as if determined by an allele of the distyly locus, and exhibits the dominance hierarchy above (Tamari *et al.*, 2005). While not the result of meiotic recombination, the occurrence and inheritance of this mutant is consistent with the supergene hypothesis. The mutant also exhibits compatibility relationships and pollen size expected of a recombinant long-homostyle (Fig. 1).

We observed and studied a second homostyle 'mutant' in autotetraploid *T. scabra*. The long-homostyled plant was first observed as a seedling growing out of the pot of an adult plant. Studies of its inheritance also revealed that it is determined by an allele of the distyly locus showing the dominance hierarchy above. In this instance, tetrasomic inheritance occurs at the locus, and oddly, the original 'mutant' has the genotype $S^H S^H ss$ (Tamari *et al.*, 2005). This homostyle also exhibits compatibility relationships and pollen size expected of a recombinant long homostyle (Fig. 1).

If a supergene occurs in *Turnera*, recombination frequencies within it might be extremely low. As another means of exploring whether a supergene might be present we have initiated a mutagenesis experiment. We generated short-styled plants of *T. subulata* that were genetically *SS*. The *S*-allele is marked by alleles of two isozyme loci that lie on either side of the distyly locus (Athanasios & Shore, 1997) so that we can confirm and follow the fate of mutated chromosomes. In a pilot study, we irradiated pollen of the *SS* short-styled plants and pollinated a long-styled plant homozygous for alternative alleles of the two linked isozyme loci. Out of approximately 1000 progeny, the vast majority of which were short-styled, we recovered two mutants. One is a long-homostyle and the other a long-styled plant. Unfortunately, both plants are female sterile, and we have been unable to transmit the putative mutant allele of distyly to progeny through the mutant's pollen. At present, we do not know the genetic basis of the mutant phenotypes. The occurrence of the mutants is certainly consistent with the hypothesis that a supergene determines distyly as we appear to have been able to independently mutate (or possibly delete the gene(s) or a portion of it, as X-rays commonly cause deletions) the putative *G* allele (which determines style length and its incompatibility) for the homostyle, and the putative '*G*', '*P*' and '*A*' alleles for the long-styled mutant. An alternative explanation for these mutant phenotypes is that

they are determined by new mutant alleles of a single gene that resides at the *S*-locus. At present we cannot distinguish between these possibilities.

In *Primula*, it has been suggested that it is difficult to obtain *SS* genotypes because a recessive lethal embedded in the distyly supergene may cause nonviability of progeny carrying this genotype (Kurian & Richards, 1997; Richards, 1997). Richards (2003) model for the evolution of distyly postulates the occurrence of a supergene which has embedded within it such a linked recessive lethal gene. In diploid *Turnera* spp. there is no evidence for such a linked recessive lethal. Data from selfing (or bud-selfing) short-styled plants has shown no departure from the expected three short-styled to one long-styled ratio, for plants of *T. scabra* and *T. subulata* (Shore & Barrett, 1985; Athanasiou & Shore, 1997). Furthermore, we have produced short-styled plants that are homozygous, *SS*, by exploiting a self-compatible short-styled plant of *T. subulata*. We have shown in test-crosses of these plants to long-styled plants, that only short-styled progeny are produced, confirming the homozygosity of the short-styled parents (J.S. Shore, unpublished).

The evolutionary model of Lloyd & Webb (1992) postulates the possible involvement of both linked genes (i.e. a supergene) as well as unlinked modifiers with morph-limited expression, in the genetic makeup of distyly. We reviewed (above) evidence that is consistent with, but certainly does not prove the existence of, a supergene in *Turnera*. Our work to discover morph-specific proteins, and their corresponding genes, has, however, provided the first evidence for genes with morph-limited expression (see later). We have demonstrated that both a polygalacturonase and an α -dioxygenase are expressed in the transmitting tissue of only short-styled plants (Athanasiou *et al.*, 2003; Khosravi *et al.*, 2004). Recently, McCubbin *et al.* (2006) provided evidence for morph-limited and/or differential expression of genes in *Primula vulgaris*.

Molecular genetic basis of distyly

One approach to discovering both the genetic architecture and molecular basis of distyly is to search directly for proteins specific to one or the other morph. This approach had been taken by Golynskaya *et al.* (1976), and Shivanna *et al.* (1981) for *Primula*, as well as for other distylous species, including *Averrhoa carambola* (Wong *et al.*, 1994a) and *Fagopyrum esculentum* (Miljuš-Đukić *et al.* 2004). McCubbin *et al.* (2006) have used subtractive hybridization to discover genes differentially expressed between the morphs of *Primula vulgaris*. They identified a number of different classes of genes involved potentially as downstream components of floral heteromorphism. None of the genes, however, appear to be at the *S*-locus (McCubbin *et al.*, 2006).

Athanasiou & Shore (1997) identified protein expression differences between the morphs of *Turnera subulata* and Athanasiou *et al.* (2003) subsequently identified the proteins

and their corresponding genes. One protein is a polygalacturonase specific to the transmitting tissue of the short-styled morph of species in series *Turnera* (Khosravi *et al.* 2003; Tamari & Shore, 2004). While linked to the distyly locus, the polygalacturonase gene is 4.6 cm distal to it (Athanasiou *et al.*, 2003). Athanasiou *et al.* (2003) also showed that the pollen protein discovered by Athanasiou & Shore (1997) was also a polygalacturonase belonging to a clade (clade C) of pollen expressed plant polygalacturonases. In a survey of additional species of series *Turnera*, Tamari & Shore (2004) were unable to confirm the short morph specificity of the pollen polygalacturonase.

Khosravi *et al.* (2004) discovered a 68 kDa protein specific to short styles. They showed, based upon sequence similarity, immunocytochemistry, and phylogenetic analysis, that the protein is an α -dioxygenase specific to the transmitting tissue of short styles of species in series *Turnera*. The gene encoding the α -dioxygenase is not closely linked to the distyly locus. α -Dioxygenases have only been recently discovered and they appear to play a role in signalling in response to plant pathogens (Sanz *et al.*, 1998). The roles of both the polygalacturonase and α -dioxygenase in distyly, if any, remain to be determined (Tamari & Shore, 2006). We have continued to search for proteins distinguishing the morphs using two-dimensional gel electrophoresis and tandem mass spectrometry to identify the proteins. Two additional candidate proteins have been tentatively identified and others await identification and verification of morph-specificity (Khosravi *et al.*, 2006).

Studies by Athanasiou *et al.* (2003) and Khosravi *et al.* (2004) clearly demonstrate that the *S*-allele of the distyly locus has the capacity to regulate the expression of two genes that do not reside at this locus. The proteins encoded by the style polygalacturonase gene and the α -dioxygenase gene both show morph-limited expression and are expressed only in the transmitting tissue of short-styled plants. These studies are in concert with predictions of the model of Lloyd & Webb (1992) which postulates that some of the genes involved in distyly might exhibit morph-limited expression. Unfortunately we do not yet know what role these two proteins play in distyly. This remains an important avenue for future research.

Genetic localization

Genetic localization is currently being used as a means to positionally clone the locus or loci determining distyly. Manfield *et al.* (2005) identified a random amplified polymorphic DNA (RAPD) marker linked to the *S*-locus (thrum allele) in *P. vulgaris*. They subsequently sequenced an 8.8 kb region corresponding to this marker, and used homostyles to further define the region encompassing the *S*-locus. A similar approach has been initiated in *F. esculentum* (Aii *et al.*, 1998; Nagano *et al.*, 2001a,b) where linked molecular markers and candidate BAC clones containing the *S*-locus have apparently been obtained.

We have recently initiated a mapped-based approach in *Turnera* (J. Labonne and J.S. Shore, unpublished). We did so by

exploiting a mutant homostyle discovered by Tamari *et al.* (2005). The mutant allele was backcrossed into *T. subulata* and a backcross mapping population of approximately 700 progeny was generated. We used a range of molecular markers including isozymes, RAPD, intersimple sequence repeat (ISSR) and randomly amplified microsatellite polymorphism (RAMP) markers. We have now identified a number of closely linked markers, one of which is within approximately 0.2 cm of the distyly locus. We will continue mapping and construct a BAC library in an effort to clone the gene(s) at the distyly locus. This work should ultimately yield a clear understanding of the molecular genetic basis of distyly and a test of whether a supergene is involved.

Future prospects

While breeding systems in the Turneraceae are perhaps among the best studied for families possessing distylous species, considerable efforts will be required to resolve important outstanding questions. The origins of distyly in the family, including the number of origins, and the recognition of primitively homostylous or monomorphic species (should they occur) will necessitate that a broad phylogenetic analysis is undertaken. Coupled with this phylogeny will be the more basic need to characterize the breeding systems of a number of species, particularly in the African genera.

Polyplody is rampant in *Turnera*. Continued chromosome number surveys and cytogenetic analyses within the Turneraceae will be required to explore the relationship between breeding system and polyploid evolution. The process of reticulate evolution will require that the progenitors of allopolyploid species are identified to fully understand breeding system evolution. More recent methods such as genomic *in situ* hybridization (GISH) are currently being used to explore the origins of some of the polyploids.

The inheritance of distyly and homostyly has been explored in a restricted number of species in series *Turnera*. Further study of species possessing both distylous and homostylous populations (e.g. *Piriqueta morongii*), and of sister species that vary in breeding system (e.g. homostylous *Turnera candida* and distylous *T. grandiflora*), will be important to understand the modes of origin of homostyly and/or provide further insight into whether a supergene underlies distyly in the Turneraceae. These species are also important candidates for ecological genetic investigations to explore the selective forces acting on breeding systems.

Finally, we require further investigations to elucidate the molecular genetic basis of distyly. Ideally, the development of transgenic or RNA interference methods in *Turnera*, would allow us to knock out genes for morph-specific proteins previously identified (e.g. style polygalacturonase) to explore their function. Similarly, the function of candidate genes identified through positional cloning can be explored in this manner. Once the locus or loci are identified, a clear picture of the genetic architecture of distyly should emerge.

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