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VARIATION AND EVOLUTION OF BREEDING SYSTEMS IN THE *TURNERA ULMIFOLIA* L. COMPLEX (TURNERACEAE)

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Abstract.—The evolutionary and functional relationships among breeding systems and floral morphology were investigated in the *Turnera ulmifolia* complex. Predictions of a model of breeding system evolution among distylous and homostylous varieties were tested. Chromosome counts of 73 accessions revealed an association between breeding system and chromosome number. Diploid and tetraploid populations of five taxonomic varieties are distylous and self-incompatible, whereas hexaploid populations of three varieties are homostylous and self-compatible. The latter occur at different margins of the geographical range of the complex. Crossing studies and analyses of pollen and ovule fertility in F_1's revealed that the three homostylous varieties are intersterile.

To test the prediction that homostylous varieties are long homostyles that have originated by crossing over within the distyly supergene, a crossing program was undertaken among distylous and homostylous plants. Residual incompatibility was observed in styles and pollen of each homostylous variety with patterns consistent with predictions of the cross-over model. The intersterility of hexaploid varieties suggests that long homostyly has arisen on at least three occasions in the complex by recombination within the supergene controlling distyly. Deviation from expected compatibility behavior occurs in populations of var. *angustifolia* that have the longest styles. These phenotypes displayed the greatest separation between anthers and stigmas (herkogamy) and set little seed in crosses with long- or short-styled plants. This suggests that they are derived from long homostyles with shorter length styles. It is proposed that selection for increased outcrossing has favored the evolution of herkogamy in long homostyles.

Estimates of outcrossing rate in a distylous population using allozyme markers confirmed that dimorphic incompatibility enforces complete outcrossing. Significant genetic variation for floral traits likely to influence the mating system, such as stigma-anther separation, occurs within and among homostylous populations of var. *angustifolia* on Jamaica. Estimates of the mating system of families from a population with varying degrees of stigma-anther separation, using five isozyme loci, were heterogeneous and ranged from $t = 0.04$–0.79. Families exhibiting the largest mean stigma-anther separation have higher outcrossing rates than those with little separation.

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The change from obligate outbreeding, enforced by physiological self-incompatibility, to predominant self-fertilization is one of the major pathways of breeding system evolution in flowering plants (Stebbins, 1974; Grant, 1975; Jain, 1976; de Nettancourt, 1977). Comparative studies in several herbaceous families (e.g., Onagraceae, Oxalidaceae, Polemoniaceae, Gramineae) have provided evidence that the shift to selfing has evolved repeatedly in association with ecological radiation into temporary, pioneer habitats (Raven, 1979; Ornduff, 1972; Grant and Grant, 1965; Stebbins, 1957). As the acquisition of autogamy is usually associated with a loss of outcrossing adaptations (Lloyd, 1965; Ornduff, 1969) and reduction in the genetic variation of populations (Brown, 1979; Hamrick et al., 1979), self-fertilization has been considered an evolutionary blind alley with selfing species rarely contributing to major evolutionary trends. Since the evolution of predominant selfing is usually viewed as unidirectional, it is often assumed that among closely related selfers and outcrossers, the former are derived (Lewis, 1954; Stebbins, 1957). Evidence in support of this interpretation can, however, be difficult to obtain since changes in the breeding systems of plants are frequently rapid and often associated with the development of reproductive isolation and speciation (Baker, 1961).

Families that possess heteromorphic incompatibility are of interest to the general problem of the evolutionary relationships among plant breeding systems. Taxa can exhibit either floral heteromorphism, with two (distyly) or three (tristyly) morphologically distinct mating groups, or floral monomor-

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phism. Difficulties of evolutionary interpretation arise since taxa that are monomorphic for floral traits may be either ancestral or derived from heterostylous relatives. Taxa that are monomorphic and derived were termed homostylous by Darwin (1877). They are often small-flowered and self-compatible, possess anthers and stigmas at the same relative position within a flower, and are usually highly autogamous (Ganders, 1979). In contrast, taxa that are primitively monomorphic, although self-compatible, frequently possess large flowers and outcrossing adaptations, and most probably exhibit mixed mating systems. In some families these differences are, however, not clear-cut, resulting in difficulties of interpretation (Ernst, 1955; Baker, 1966; Mosquin, 1971; Sobrevila et al., 1983; Nicholls, 1985; Ornduff, 1986). If homostyly arises through crossing over within the heterostyly supergene, controlled crosses between heteromorphic and homomorphic taxa can reveal the presence of residual self-incompatibility (Dowrick, 1956; Baker, 1975). Since incompatibility is unexpected in primitively monomorphic taxa (Charlesworth and Charlesworth, 1979*b*), crossing data provide a means of distinguishing between the two evolutionary interpretations (Ernst, 1952).

Here we examine the evolutionary relationships among dimorphic and monomorphic breeding systems in the *Turnera ulmifolia* complex (Turneraceae), a polyploid complex of perennial weeds. A series of general questions raised by Jain (1976) in his review of the evolution of inbreeding in plants is addressed: 1) Do selfers arise from cross-pollinated ancestors; 2) Are selfers more frequently found towards the geographical margins of the range of outbreeders; 3) Is the evolution of selfing associated with speciation; and 4) Does inbreeding represent an evolutionary blind alley? We begin by describing our observations on geographical patterns of floral variation within the complex. These are then used to formulate a working hypothesis of breeding system evolution which is evaluated using evidence from chromosome studies, controlled crosses, and isozyme analysis of mating systems.

Variation in the Turnera ulmifolia *Complex*

Turnera ulmifolia is a polymorphic polyploid complex of herbaceous, perennial weeds native throughout much of the New World tropics and adventive in parts of Africa, India, and southeast Asia. Populations are conspicuous as weeds of roadsides and open waste ground but rarely colonize arable land (Barrett, 1978). In a monograph of the genus Urban (1983) recognized 12 intergrading varieties within the *T. ulmifolia* complex. Subsequent regional treatments have elevated some varieties to specific rank, whereas others are of dubious taxonomic status. Details of the morphological variation and crossability of varieties are presented in Shore and Barrett (1985*a*).

Two contrasting patterns of floral morphology occur in *T. ulmifolia* populations (Fig. 1). Populations are either dimorphic or monomorphic for a range of floral traits (e.g., style length, stamen height, pollen size) associated with the breeding system. Dimorphic populations exhibit typical features of the distylous genetic polymorphism with strong self-incompatibility and a 1:1 ratio of the long- and short-styled morphs (Barrett, 1978). Dimorphism is controlled by a single gene locus with long-styled plants of genotype *ss* and short-styled plants of genotype *Ss* (Shore and Barrett, 1985*b*). Monomorphic populations are self-compatible with long styles and a range of stamen heights. Populations with long styles and long-level stamens have phenotypes that correspond to the long-homostyle phenotype in *Primula* (Ernst, 1955; Dowrick, 1956; Charlesworth and Charlesworth, 1979*a*). Plants of this phenotype have the capacity for automatic self-pollination because of the close proximity of their sexual organs. At the other extreme are monomorphic populations with long styles and short-level stamens. They are non-autogamous and resemble the typical long-styled morph of distylous populations.

Individual varieties of *T. ulmifolia* are either dimorphic or monomorphic. The three varieties *angustifolia, orientalis,* and *velutina* are monomorphic, each possessing a characteristic range of floral variation.

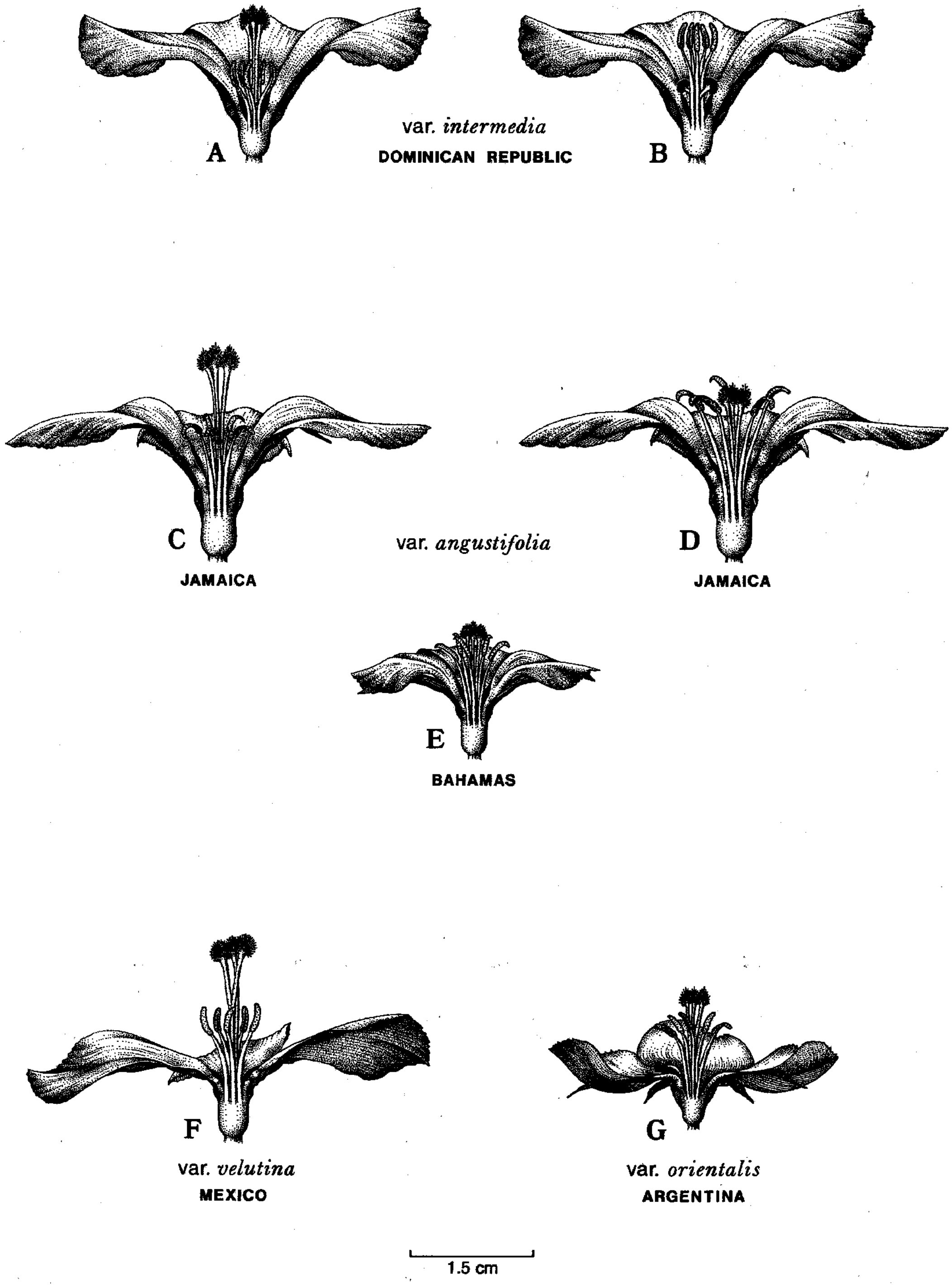

FIG. 1. Floral variation in the *Turnera ulmifolia* complex. A, B) Long- and short-styled morphs, respectively, of var. *intermedia* (4*x*) from a distylous population from the Dominican Republic. C, D) Homostylous populations of var. *angustifolia* (6*x*) from Jamaica with contrasting stigma-anther separations. E) Small-flowered, autogamous population of var. *angustifolia* from the Bahamas. F) var. *velutina* (6*x*) from Mexico. G) var. *orientalis* (6*x*) from Argentina.

Populations of var. *velutina* exhibit a large separation of stigmas and anthers. In var. *orientalis,* stamens are intermediate in length and flowers are markedly protogynous; and in var. *angustifolia,* there is the entire range of style and stamen positions from long homostyle to long-styled phenotype (Fig. 1). In var. *angustifolia* this variation is associated with a diverse array of floral syndromes from small-flowered self-pollinating populations on Grand Bahama Island, Bahamas, to large-flowered herkogamous populations serviced by hummingbirds and bees on Jamaica (du Quesnay, 1971; Percival, 1974; S. C. H. Barrett, pers. observ.).

The range of floral variation in *T. ulmifolia* raises several questions concerning the function and evolutionary relationships among breeding systems in the complex. 1) Are monomorphic populations ancestral or derived from dimorphic populations? 2) What are the evolutionary relationships among monomorphic varieties as well as among populations with contrasting floral syndromes? 3) How does floral variation influence the mating systems of populations? 4) Are dimorphic populations primarily outcrossing? 5) Do monomorphic populations experience a high level of self-fertilization, the magnitude of which depends on the relative positions of reproductive organs?

To address these issues, a general working hypothesis of breeding system evolution in the complex was formulated (Fig. 2). The hypothesis predicts that: 1) distylous populations are outcrossing and ancestral to monomorphic populations; 2) monomorphic populations are long-homostyles derived by crossing over within the distyly supergene; and 3) differential selection pressures on the mating system of monomorphic populations account for the observed variation in floral traits. Our investigation of floral monomorphism has focused primarily on var. *angustifolia* since this taxon displays the greatest range of reproductive variation.

MATERIALS AND METHODS

Chromosome Number and Breeding System

To examine the relationship between ploidal level and breeding system, chromosome counts were obtained from a total of 73 accessions comprising seven varieties of *T. ulmifolia.* Sampling was throughout much of the Neotropical range of the complex and also included five accessions from Asia. A list of populations and their localities is presented in Shore (1986). Chromosome counts were made following methods presented in Shore and Barrett (1985*a*). A majority of counts was obtained from squashes of anthers of glasshouse-grown plants; fewer were obtained from colchicine-pretreated root tips of the same. Artificial self- and cross-pollinations of each accession were undertaken to determine the presence of self-incompatibility.

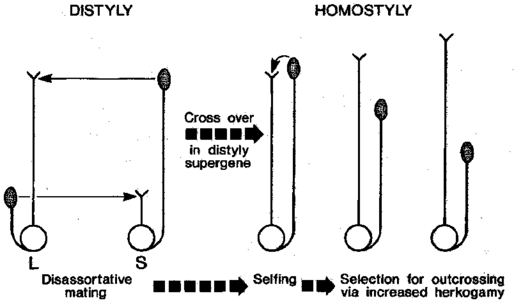

FIG. 2. Model of evolutionary relationships among breeding systems within the *Turnera ulmifolia* complex.

Controlled Pollination Studies

To test the hypothesis that monomorphic variants are derived from distylous ancestors, controlled pollinations among populations with the two breeding systems were conducted in a pollinator-free glasshouse. If floral monomorphism in *T. ulmifolia* has arisen by crossing over within the distyly supergene, residual self-incompatibility behavior may be evident in the pistils and pollen of plants from monomorphic populations. Specifically, the hypothesis predicts that plants with long styles and long-level stamens will yield seed when crossed to short-styled plants as egg parents and long-styled plants as pollen parents. The reciprocal combinations should, however, result in little seed set. Since some monomorphic populations exhibit floral phenotypes with long styles and short-level stamens, their behavior in crosses is more difficult to predict and depends on their origin and evolutionary history. If these phenotypes are descended from long homostyles then we may anticipate that pollen from short-level stamens will exhibit the incompatibility behavior of long-level stamens. In contrast, if they are long-styled plants that have developed self-compatibility through mutation, their behavior in crosses with distylous morphs should not resemble the pattern observed for long-homostyles, but instead should depend on the nature of the mutation(s) modifying self-incompatibility (see Shore and Barrett, 1986).

Long- and short-styled plants were crossed in all pollen-carpel combinations to plants from monomorphic populations. Ten plants (five of each style morph) were used from each of three distylous populations. The distylous populations were *T. ulmifolia* var. *intermedia,* population I1 ($2n = 10$); var. *intermedia,* population I24 ($2n = 20$); and var. *elegans,* population E5 ($2n = 20$). The monomorphic populations were 10 populations of var. *angustifolia* from Jamaica and one each of vars. *orientalis* and *velutina*; they included nine of the populations used in assessing interpopulation variation in floral traits (see below). The four possible crossing combinations between distylous and monomorphic plants were performed on each of three genotypes per population with each treatment replicated 15 times. Pollinated flowers were marked and seed was harvested at or just prior to capsule dehiscence.

The degree of interfertility among the three monomorphic varieties of *T. ulmifolia* was examined by generating all possible hybrid combinations. These were obtained by crossing at least two pairs of parental individuals from each population of each variety (populations A12, O1 and V1). Hybrid seed were germinated, and pollen stainability and ovule fertility assessed. Pollen from five hybrid plants of each type was stained with cotton blue in lactophenol, and 100 pollen grains from each of two flowers per plant were scored. Ovule fertility was determined by backcrossing as egg parents at least five hybrid plants of each type to both parents. Several replicate pollinations were performed and seed set per pollination determined.

Floral Variation in Monomorphic Populations

To assess the extent of interpopulation variation for floral traits that might influence the mating system, seed collections from 10 Jamaican populations of *T. ulmifolia* var. *angustifolia* were made. Fourteen plants from each population were grown in 10-cm pots in a completely randomized design under uniform glasshouse conditions during spring 1980. Floral traits were measured to the nearest 0.1 mm with a pair of calipers, on five flowers per plant. Traits measured were style length, stamen length (both measured from the base of the ovary to their maximum length), and flower diameter. Additionally, five flowers were self-pollinated and five were left unmanipulated to assess the degree of autogamous seed set under pollinator-free glasshouse conditions. The three treatments (i.e., floral measurement, self-pollination, and autogamous seed set) were applied alternately to successive flowers on each plant. Data were analyzed as a two-level nested analysis of variance with all effects considered random. Prior to analysis, data were examined for normality and homogeneity of variances. Self and autogamous seed set per flower were log (seed set + 1) transformed. Variance components were calculated for each level in the analysis (Sokal and Rohlf, 1981).

The magnitude of intrapopulation variation of floral traits was examined by sampling 25 progeny from each of 10 families obtained from a single Jamaican population (A20). The population exhibited considerable phenotypic variation in floral traits. Families were grown in a design similar to that described above during summer 1985 with all floral traits measured as above. Two replicate flowers were measured per plant and four flowers were left unmanipulated to assess the degree of autogamy. Analyses described above were also performed on these data.

Mating System Estimates

To verify that distylous populations are predominantly outbreeding whereas monomorphic populations display considerable self-fertilization, electrophoretic markers were used to determine the rate of outcross-

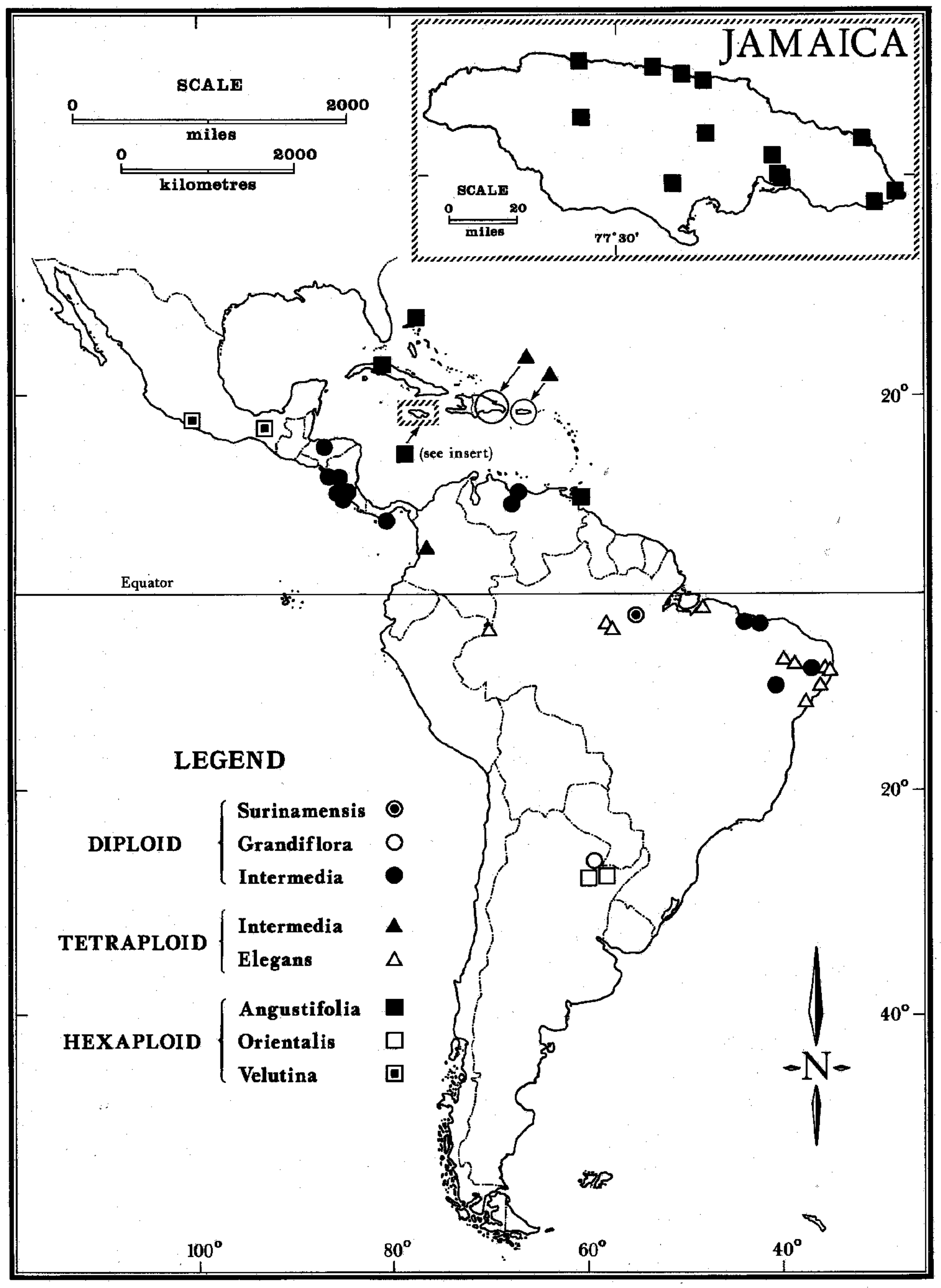

FIG. 3. Geographical location, ploidal level, and varietal status of populations in the *Turnera ulmifolia* complex from which chromosome counts were obtained. Many populations from the Dominican Republic and Puerto Rico were sampled; all were var. *intermedia* (4*x*). Old World and garden accessions not included.

ing in a tetraploid distylous population of *T. ulmifolia* var. *elegans* from Brazil (population E11) and a monomorphic hexaploid population of *T. ulmifolia* var. *angustifolia* from Jamaica (population A20). For the Brazilian population, a single marker locus corresponding to *Lap-1* in diploid *T. ulmifolia* var. *intermedia* was used. Approx-

TABLE 1. The relationships between ploidal level and breeding system in the *Turnera ulmifolia* complex. SI = self-incompatible, SC = self-compatible, $x = 5$. * Data from Arbo (1985).

Variety	Number of populations	$2x$	$4x$	$6x$	Compatibility behavior	Stamen-style polymorphism
Intermedia	32	13	19	—	SI	Dimorphic
Grandiflora	1	1	—	—	SI	Dimorphic
Surinamensis	1	1	—	—	SI	Dimorphic
Elegans	13	—	13	—	SI	Dimorphic
*Grandidentata**	1	—	1	—	SI	Dimorphic
Angustifolia	21	—	—	21	SC	Monomorphic
Orientalis	2	—	—	2	SC	Monomorphic
Velutina	2	—	—	2	SC	Monomorphic

imately eight progeny were assayed from each of nine open-pollinated long-styled plants and 11 short-styled plants. Separate estimates of the rate of outcrossing, t ($t = 1 - s$, where s is the selfing rate), were obtained for each morph using a modification of the procedure described in appendix A of Clegg et al. (1978). To accommodate tetrasomic inheritance exhibited by this quadrivalent-forming tetraploid, the procedure was modified (see Appendix). To obtain outcrossing estimates for the monomorphic Jamaican population (A20), 25 progeny were assayed from each of 10 open-pollinated families. These were the same progeny that were used to assess the extent of floral variation within the population (see above). Five isozyme loci were scored, *Est-1a, Aco-2a, Pgd-ca, Gpi-ca,* and *Pgm-ca.* The isozyme nomenclature is more complex than for diploids and tetraploids (see Shore and Barrett, 1987) as the bivalent-forming hexaploids exhibit multiple isozyme loci, presumably the result of genome triplication. However, disomic inheritance is exhibited for these loci in *T. ulmifolia* var. *angustifolia.* A five-locus estimate of outcrossing rate was obtained for the population using the method of Ritland and Jain (1981), and outcrossing rates and pollen allele frequencies were determined for each family separately using the procedures specified in Ritland and El-Kassaby (1986). Approximate variance estimates were obtained by inversion of the information matrix. For families exhibiting a single polymorphic locus, only the outcrossing rate was estimated; the pollen pool was taken as that estimated for the entire population. A heterogeneity test was performed among outcrossing rates using the method of Bailey (1961).

RESULTS

Geographical Distribution, Ploidal Level, and Breeding System

Chromosome counts for the 73 accessions of *T. ulmifolia* are summarized in Table 1, and the geographical distribution of Neotropical populations is illustrated in Figure 3. The data indicate an association between ploidal level and breeding system. Diploid and tetraploid populations are uniformly distylous and self-incompatible; hexaploid populations are monomorphic and self-compatible. Several geographical patterns are evident from the counts and surveys of herbarium specimens. Monomorphic populations predominate at the geographical margins of the range (vars. *angustifolia,* Caribbean; *orientalis,* s. Brazil and n. Argentina; *velutina,* Mexico) and are absent from most of the range occupied by distylous populations. The most widespread variety in the complex, var. *intermedia,* is the only taxon examined that has more than one cytotype ($2x$, $4x$). All populations of var. *intermedia* sampled from the Dominican Republic and Puerto Rico are tetraploid; diploid populations occur in Central America, Venezuela, and Brazil.

Residual Incompatibility in Monomorphic Populations

Results of the controlled pollination study provide unequivocal evidence that the three monomorphic varieties of *T. ulmifolia* are derived from distylous ancestors (Table 2). In each of the 12 monomorphic populations the pattern of seed set obtained in crosses with distylous morphs was as predicted for the long-homostylous phenotype. Plants from monomorphic populations yielded

TABLE 2. Percentage fruit set and mean number of seeds per fruit following controlled pollinations of distylous and homostylous populations of *Turnera ulmifolia*. Each pollination treatment was replicated 45 times. * Homostylous populations are ranked in order of increasing style length.

	Homostyle × Long		Homostyle × Short		Long × Homostyle		Short × Homostyle	
Population*	Percentage fruit set	Mean no. seeds (SD)	Percentage fruit set	Mean no. seed (SD)	Percentage fruit set	Mean no. seeds (SD)	Percentage fruit set	Mean no. seeds (SD)
O1	13.3	2.2 (1.0)	93.3	31.4 (10.4)	55.5	17.0 (11.3)	2.2	2.0 —
A11	17.9	1.0 —	84.4	32.6 (24.1)	17.8	9.0 (6.3)	0	— —
A17	0	— —	88.9	13.7 (6.0)	48.9	10.5 (6.8)	0	— —
V1	4.4	11.0 (7.1)	46.7	16.0 (11.6)	95.6	19.6 (8.8)	0	— —
A5	0	— —	57.8	20.5 (11.4)	13.3	3.3 (2.0)	0	— —
A6	0	— —	51.1	17.6 (10.6)	0	— —	2.2	2.0 —
A7	0	— —	86.7	32.7 (17.9)	4.4	4.5 (2.1)	2.2	1.0 —
A4	0	— —	55.6	18.2 (13.5)	2.2	3.0 —	0	— —
A9	0	— —	24.4	9.1 (7.6)	44.4	5.4 (4.4)	2.2	1.0 —
A12	0	— —	8.9	8.5 (7.8)	37.8	4.7 (3.8)	0	— —
A10	0	— —	2.2	34.0 —	11.1	5.2 (3.8)	0	— —
A8	0	— —	2.2	11.0 —	8.8	3.3 (3.8)	0	2.0 —

fruit and seed when crossed as egg parents to the short-styled morph (monomorphic × short-styled: mean fruit set = 50.2%, $N =$ 540 pollinations) and as pollen parents to the long-styled morph (long-styled × monomorphic: mean fruit set = 28.3%, $N =$ 540 pollinations), whereas reciprocal crosses were incompatible resulting in little or no seed (monomorphic × long-styled: mean fruit set = 3.0%, $N = 540$ and short-styled × monomorphic: mean fruit set = 0.9%, $N =$ 540 pollinations). The monomorphic varieties *angustifolia, orientalis,* and *velutina* of the *T. ulmifolia* complex are long homostyles since they display the residual self-incompatibility relationships expected under the cross-over model for the origin of this phenotype.

TABLE 3. Fertility relationships among three monomorphic hexaploid varieties of *Turnera ulmifolia*. Hybrids were generated by crossing at least two pairs of parental individuals from each variety. A = var. *angustifolia*, O = var. *orientalis*, V = var. *velutina*.

A) Pollen stainability

Hybrid	Number of individuals	Mean percent of stainable grains	Standard deviation
A × O	5	0	0
A × V	5	3.8	3.4
O × V	5	0	0

B) Ovule fertility

Hybrid (egg parent)		Pollen parent	Number of hybrids	Number of pollinations	Number of fruits set
AO	×	A	7	22	0
AO	×	O	7	17	0
AV	×	A	5	19	0
AV	×	V	5	20	0
OV	×	O	5	12	0
OV	×	V	5	18	0

A noteworthy feature of the crossing data is the variation of fruit set in different population combinations. The variation is doubtless in part the result of ploidal effects and varying degrees of genetic divergence among populations used. When monomorphic populations were employed as female parents in crosses with the short-styled morph, a statistically significant relationship was evident between maternal style length and fruit set ($r = -0.84$, $P < 0.01$). Monomorphic populations with longer styles set less fruit than populations with shorter styles.

Interfertility of Monomorphic Varieties

The three monomorphic varieties of *T. ulmifolia* are morphologically differentiated and occur at different margins of the geographical range of the complex (Fig. 3). This suggests that the breakdown of distyly to homostyly has occurred independently on several occasions. One line of evidence in support of this hypothesis results from investigating the interfertility of monomorphic varieties. Previous crossing work has indicated that populations of the same ploidal level are usually interfertile (Shore and

TABLE 4. Measurements of floral traits (in mm) for 10 Jamaican populations of *Turnera ulmifolia* var. *angustifolia* grown under uniform glasshouse conditions. Populations are ranked by increasing stigma-anther separation. Means (SD) and variance components are from a nested ANOVA.

Population	Style length	Stamen length	Stigma-anther separation	Autogamous seed set	Selfed seed set	Flower diameter
A5	19.1	19.5	−0.4	31.6	54.0	43.3
	(1.4)	(1.9)	(1.0)	(19.9)	(23.8)	(5.2)
A6	19.4	19.3	0.1	36.5	47.1	44.5
	(1.5)	(1.8)	(0.9)	(20.6)	(19.9)	(6.2)
A4	20.7	20.5	0.2	28.5	47.3	40.1
	(1.5)	(1.8)	(1.1)	(20.1)	(17.8)	(6.1)
A7	19.9	18.3	1.6	29.8	56.1	42.2
	(1.3)	(1.2)	(1.1)	(22.1)	(19.3)	(5.9)
A13	20.6	19.0	1.6	13.5	41.9	45.6
	(2.1)	(2.1)	(1.7)	(16.2)	(16.9)	(7.2)
A11	17.9	15.8	2.1	6.3	45.9	37.3
	(1.6)	(1.7)	(1.1)	(12.3)	(15.5)	(4.5)
A12	22.2	17.5	4.7	0.4	35.9	38.8
	(1.2)	(1.1)	(1.1)	(1.6)	(17.6)	(4.3)
A10	23.5	18.7	4.8	0.2	45.1	45.5
	(1.9)	(1.7)	(1.5)	(1.4)	(23.4)	(5.7)
A8	24.6	19.6	5.0	3.2	64.7	47.4
	(2.1)	(1.7)	(1.2)	(12.6)	(28.0)	(10.5)
A9	22.0	14.2	7.8	1.0	31.2	44.5
	(1.4)	(0.9)	(0.9)	(6.0)	(8.4)	(5.3)
Population	60.4**	57.2**	83.4**	60.4**	3.0*	19.0**
Plants	12.3**	10.5**	6.8**	4.6**	16.1**	19.6**
Error	27.3	32.3	9.8	35.0	80.9	61.4

* $P < 0.02$, ** $P < 0.001$.

Barrett, 1985*a*). However, results of intercrossing the three monomorphic hexaploid varieties indicate a markedly different pattern (Table 3). All hybrid combinations are completely sterile. Few pollen grains are stainable and no seeds are produced following pollination of hybrids with fertile parental pollen. The intersterility of the three hexaploid varieties is consistent with the hypothesis that they are products of separate speciation events.

Floral Variation among Monomorphic Populations

Field observations of Jamaican populations of var. *angustifolia* indicated complex patterns of phenotypic variation within and between populations. The uniform glasshouse study revealed significant interpopulation genetic differentiation for all floral traits that were examined among the 10 populations (Table 4). Between population variance components were large (greater than 50%) for style length, stamen length, stigma-anther separation, and autogamous seed set. Smaller, although significant, between-population components occurred for the remaining traits. Stigma-anther separation ranged from a slightly negative value (−0.4 mm in population A5) with anthers slightly above the stigma, to a separation of 7.8 mm in population A9. Mean autogamous seed set ranged from 0.2 to 36 seeds per flower among populations. Within-population variance components were significant although generally smaller than between-population components for all floral traits.

Floral Variation and Mating System

Monomorphic populations with a small stigma-anther separation may be expected to exhibit a greater facility for autogamous seed set than those with a large separation. This was verified by interpopulation measures of seed set in undisturbed flowers under glasshouse conditions (Fig. 4A). Population A20 exhibited large differences among families for all floral traits measured (see below) and was therefore used to assess the

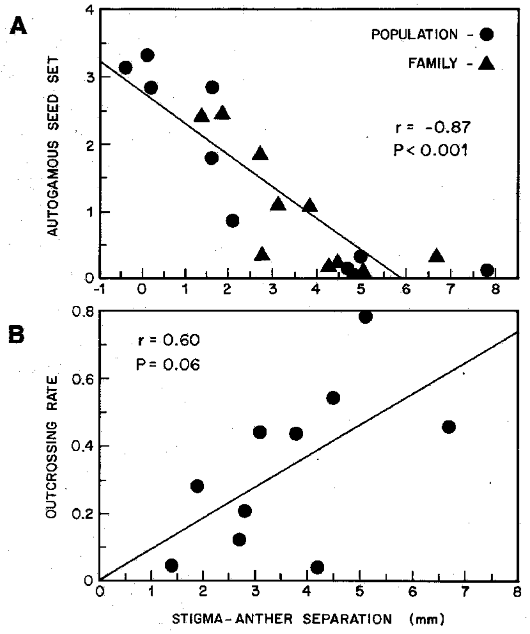

FIG. 4. A) The relationship between the degree of autogamy and stigma-anther separation (style length − stamen length) within and between populations of *Turnera ulmifolia* var. *angustifolia* from Jamaica. The degree of autogamy for each population or family was calculated as the mean of the log(autogamous seed set per flower + 1). Stigma-anther separation is the mean for each family or population. B) The relationship between the rate of outcrossing (t) and stigma-anther separation (style length − stamen length) for 10 families from Jamaican population (A20).

TABLE 5. Measurements of floral traits (in mm), mean (SD), and outcrossing rates, mean (SE), in 10 families from a Jamaican population of *Turnera ulmifolia* var. *angustifolia*. Families are ranked by increasing stigma-anther separation.

Family	Style length	Stamen length	Stigma-anther separation	Autogamous seed set	Outcrossing rate	Flower diameter
H	22.1 (0.9)	20.7 (1.0)	1.4 (1.2)	21.8 (22.7)	0.04 (0.16)	55.4 (3.0)
I	22.7 (1.3)	20.8 (1.0)	1.9 (1.2)	23.1 (22.3)	0.28 (0.12)	55.7 (2.8)
J	25.4 (1.4)	22.7 (1.4)	2.7 (1.5)	17.8 (22.5)	0.12 (0.08)	60.1 (3.7)
G	23.5 (1.0)	20.7 (1.4)	2.8 (1.2)	3.3 (12.2)	0.21 (0.13)	47.8 (2.4)
O	23.1 (1.4)	20.0 (1.9)	3.1 (1.5)	9.5 (18.0)	0.44 (0.13)	52.9 (4.9)
A	23.1 (1.2)	19.3 (1.4)	3.8 (1.2)	8.7 (15.9)	0.44 (0.16)	55.7 (3.1)
N	24.5 (1.3)	20.3 (1.3)	4.2 (1.2)	2.1 (10.6)	0.04 (0.12)	51.8 (3.5)
E	24.2 (1.4)	19.7 (1.3)	4.5 (1.1)	2.2 (8.5)	0.54 (0.14)	50.8 (3.3)
B	24.9 (2.1)	19.8 (1.4)	5.1 (1.8)	0.8 (6.4)	0.79 (0.14)	52.5 (4.9)
C	26.2 (1.9)	19.5 (1.5)	6.7 (1.6)	3.3 (12.1)	0.46 (0.14)*	53.5 (4.6)

* Heterogeneity $X^2 = 32.1$, $P < 0.001$.

relationship between autogamous seed set and stigma-anther separation within a population (Fig. 4A). Correlations for both data sets were not statistically different and only the pooled result is provided. The data demonstrate a large negative correlation between stigma-anther separation and autogamous seed set ($r = -0.87$, $P < 0.001$).

A direct test of the hypothesis that stigma-anther separation influences the mating system was undertaken by estimating the outcrossing rate of individual maternal parents in population A20. The population displayed considerable genetically based variation for reproductive traits including style length and stamen height (Table 5). Outcrossing rates among families are significantly heterogeneous ($X^2 = 32.1$, $P < 0.001$) ranging from 0.04 to 0.79 (Table 5) with a pooled t of 0.19 (SE = 0.03). Outcrossing rates for each family are plotted against the family means for stigma-anther separation (Fig. 4B). There is a positive nonsignificant correlation ($r = 0.60$; $P = 0.06$) between the rate of outcrossing and stigma-anther separation. Families that exhibit a large stigma-anther separation show higher rates of outcrossing than those with small stigma-anther separation.

As expected, the distylous population of *T. ulmifolia* var. *elegans* displayed high outcrossing rates for both floral morphs (long-styled morph $t = 1.00$, SE = 0.16, $N = 9$ families, 75 progeny; short-styled morph $t = 1.14$, SE = 0.16, $N = 11$ families, 91 progeny) indicating that strong self-incompatibility enforces outbreeding.

DISCUSSION

In the *Turnera ulmifolia* complex self-fertilization is a derived condition resulting from the evolutionary breakdown of distyly to homostyly. Many monomorphic populations in the complex are atypical for homostylous forms since they possess showy flowers that are larger in size than those of distylous populations. In addition, well developed herkogamy precludes autogamous seed set. Earlier work on these forms led Barrett (1978) to question whether they were strictly homostylous in the Darwinian sense. Results of the present study indicate that the three monomorphic varieties examined are best interpreted as recombinant long homostyles notwithstanding the evolutionary modifications that have taken place since their origin.

The evolution of homostyly in *T. ulmifolia* is associated with several independent speciation events. Each of the three homostylous varieties is hexaploid and thus reproductively isolated from the remainder of the complex. The basis of the association between homostyly and hexaploidy is therefore important for understanding the genetic processes responsible for the evolution of breeding systems in the complex. To examine whether hexaploidy per se may promote recombination within the distyly supergene (see Dowrick, 1956), synthesized hexaploids have been produced using colchicine, by doubling triploid seedlings from crosses between diploid and tetraploid populations of var. *intermedia* (Shore and Barrett, 1986). The resulting hexaploids were uniformly distylous with the majority of plants exhibiting strong self-incompatibility. Thus, at its inception, hexaploidy does not appear to initiate homostyle formation, at least in the experimental material we have studied.

It is important to note that the breakdown of distyly to homostyly in *T. ulmifolia* is not a necessary outcome of polyploidy since all tetraploid populations in the complex are distylous. Cytological and electrophoretic studies indicate that while all tetraploids form quadrivalents (up to the maximum of five per pollen mother cell) and exhibit tetrasomic inheritance, hexaploids form only bivalents and display considerable fixed heterozygosity at isozyme loci (J. S. Shore and S. C. H. Barrett, unpubl.). These findings suggest that tetraploid populations are autopolyploid whereas hexaploid populations may be allohexaploid, or at least the respective polyploids lie toward different ends of the auto-allopolyploid continuum. This difference may be relevant to homostyle evolution. The establishment and rate of spread of selfing variants in outcrossing populations depend on the equilibrium genetic load and resultant level of inbreeding depression. Lande and Schemske (1985) have shown theoretically that the magnitude of inbreeding depression resulting from deleterious recessive genes is reduced by tetraploidy. By analogous argument, allohexaploidy may provide further reduction in the level of inbreeding depression. If this is true in *T. ulmifolia* the equilibrium level of genetic load in hexaploid populations may be sufficiently reduced in comparison with diploid and tetraploid populations to favor the spread of recombinant homostyles. Both empirical studies and theoretical analysis on the influence of the type and level of polyploidy on the magnitude of inbreeding depression are required to substantiate these ideas.

Many heterostylous genera contain homostylous taxa that are small-flowered, highly self-pollinating, genetically depauperate in isozyme variation, and adapted to pioneer environments (Baker, 1953; Ray and Chisaki, 1957*a*, 1957*b*; Ornduff, 1972; Ganders, 1975; Barrett, 1985; Glover and Barrett, 1986; J. Piper, unpubl.). In these instances it is possible that high selfing rates and reduced levels of genetic variation may restrict the redevelopment of outcrossing mechanisms. Lande and Schemske (1985) have shown that populations with a history of predominant selfing should display relatively low inbreeding depression and as a result cannot accumulate sufficient deleterious recessive mutations to enable selection for increased rates of outcrossing even with large population size. However, their models do not support the notion that selfing is necessarily an evolutionary dead end since substantial quantitative genetic variation can still be maintained by polygenic mutation in highly selfing populations.

The acquisition of homostyly in *T. ulmifolia,* while associated with self-compatibility, has not resulted in the adoption of predominant selfing as the primary reproductive mode. In vars. *velutina* and *orientalis,* the floral syndromes are relatively uniform and involve traits normally associated with outcrossing (e.g., large flowers, herkogamy, and protogyny). The apparent absence of highly autogamous forms in these continental taxa may be significant and suggests that outcrossing may be of selective value under the ecological conditions found at the extremities of the native range. In contrast, the high diversity of floral syndromes that is evident in var. *angustifolia* is most likely associated with repeated colonizing episodes and adaptive radiation in the Caribbean region. The variation suggests that the evolution of selfing via homostyle formation has not impeded opportunities for adaptive evolution. The allohexaploid nature of homostyles in *T. ulmifolia* may provide sufficient hybridity that populations contain a store of hidden genetic variation on which selection can act. This variation could become exposed by intergenomic recombination (Chapman et al., 1979; Brown and Marshall, 1981) or through mutation of duplicated genes (Ohno, 1970).

Although the mating system of only a single distylous population was quantified, it seems reasonable to assume, based on strong self-incompatibility, that populations are largely outcrossing. Mating patterns in homostylous populations, on the other hand, are likely to vary considerably, depending on the floral characteristics of populations and local demographic and environmental conditions. The occurrence of genetic variation in the facility for autogamous seed set both within and among populations suggests that variable outcrossing rates are a feature of homostylous populations, particularly in var. *angustifolia.* Further progeny testing of families and the use of electrophoretic markers are necessary to confirm this expectation.

Finally, the model of breeding system evolution presented in Figure 2 is addressed. The assumption that distylous populations are outcrossing and ancestral to monomorphic populations has been verified. This pattern is commonly inferred in distylous taxa although there have been relatively few quantitative estimates of mating systems (Ganders et al., 1985; Piper et al., 1984) or experimental tests to establish the derived nature of floral monomorphism (Ernst, 1952; Dowrick, 1956; Baker, 1975). Results from the pollination study as well as formal genetic analysis (Shore and Barrett, 1985*b*) demonstrate that the three homostylous varieties of *T. ulmifolia* are long homostyles that originated by crossing over within the distyly supergene. This finding is consistent with Charlesworth and Charlesworth's (1979*a*) theoretical model of homostyle evolution. They have shown that if the allele determining the short-styled morph is dominant, as in *T. ulmifolia* (Shore and Barrett, 1985*b*), long homostyles are likely to spread to fixation with greater probability than other self-compatible phenotypes (see also Dowrick, 1956).

Following each independent origin of homostyly in the *T. ulmifolia* complex, it appears that homostyles migrated into habitats not occupied by their distylous progenitors. This pattern has been observed in other heterostylous groups (Baker, 1953; Ray and Chisaki, 1957*a,* 1957*b*; Ganders, 1975; Barrett, 1985) and may be associated with the ability of homostyles to reproduce under conditions of low pollinator availability as a result of their autogamous nature. During their spread, it is hypothesized that selection pressures favoring outcrossing resulted in the reestablishment of herkogamy in some populations. Evidence to support the hypothesis that monomorphic herkogamy is derived from the long homostylous phenotype comes from the pollination study. Crosses of different homostylous phenotypes gave variable results. While long homostyles exhibited the expected patterns of compatibility for recombinants, populations with longer styles (and well developed herkogamy) deviated from this behavior, producing little seed when pollinated with compatible pollen from short-styled plants. The development of intersterility is correlated with increased style length. Herkogamous populations have the longest styles and exhibit the greatest levels of intersterility in crosses with distylous plants. This suggests that they are more genetically differentiated from the ancestral distylous condition than long homostylous forms and is consistent with the proposal that monomorphic herkogamy is derived from the long homostylous phenotype. To determine whether outbreeding advantage is the major selective force responsible for the evolution of herkogamy in homostyles requires additional study.

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APPENDIX

Conditional progeny genotype distributions given maternal genotype and assuming the mixed mating model (see Clegg, 1980) for a diallelic tetrasomic locus not exhibiting double reduction (see Shore and Barrett, 1985*b*) are derived in Table 6. The alleles A_1 and A_2 are co-dominant. The conditional probability of observing a particular progeny genotype given the maternal genotype is specified in terms of the selfing rate, s, the outcrossing rate, t (where $t + s = 1$), and the diploid gametic frequencies in the pollen pool, where p, q, and r ($p + q + r = 1$) are the frequencies of the diploid genotypes A_1A_1, A_1A_2, and A_2A_2, respectively. Five maternal and progeny genotypes are possible in contrast to three in the case of a diallelic disomic locus. Estimation of the three independent parameters, t, p, and q, is performed using the two-step procedure of Clegg et al. (1978). The first step estimates the maternal genotype frequencies using the gene counting method of Ceppellini et al. (1955) based on provisional estimates of s, t, p, q, and r, and then new estimates of these parameters are obtained by the method of maximum likelihood using Fisher's scoring method. The steps are repeated until convergence is achieved for all parameter estimates at both steps. Variance estimates are obtained by inversion of the resulting information matrix.

TABLE 6. Conditional genotype distributions for a diallelic tetrasomic locus.

	Progeny genotype				
Maternal genotype	$A_1A_1A_1A_1$	$A_1A_1A_1A_2$	$A_1A_1A_2A_2$	$A_1A_2A_2A_2$	$A_2A_2A_2A_2$
$A_1A_1A_1A_1$	$s + tp$	tq	tr	0	0
$A_1A_1A_1A_2$	$s/4 + tp/2$	$s/2 + t(p + q)/2$	$s/4 + t(q + r)/2$	$tr/2$	0
$A_1A_1A_2A_2$	$s/36 + tp/6$	$2s/9 + t(4p + q)/6$	$s/2 + t(p + 4q + r)/6$	$2s/9 + t(q + 4r)/6$	$s/36 + tr/6$
$A_1A_2A_2A_2$	0	$tp/2$	$s/4 + t(p + q)/2$	$s/2 + t(q + r)/2$	$s/4 + tr/2$
$A_2A_2A_2A_2$	0	0	tp	tq	$s + tr$