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Inheritance of floral and isozyme polymorphisms in *Turnera ulmifolia* L.

ABSTRACT: **The inheritance of four floral polymorphisms and 10 isozyme loci are investigated in diploid plants ($2n = 10$) of the distylous perennial weed, *Turnera ulmifolia* L. (Turneraceae). The four floral polymorphisms are each governed by a single gene locus that exhibits dominance. Three of the four floral polymorphisms are linked, including style form, petal spot, and anther line. Starch gel electrophoresis was undertaken and nine enzymes were assayed. A total of 10 isozyme loci segregating in three selfed families was investigated. All showed codominant expression of alleles. Linkage relationships among floral and isozyme loci were determined and three linkage groups were identified. Linkage group I consisted of petal spot, style form, anther line, *Pgm-c*, *Aco-1*, and *Pgd-c* loci. Linkage group II contained *Gpi-c*, *Lap-1*, and *Sdh-1*. A third linkage group consisted of *Gdh-1* and *Lap-2*. The study is the first examination of isozyme variation in the dicotyledonous family Turneraceae.**

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TURNERA ULMIFOLIA L. (Turneraceae) is a polymorphic polyploid complex of perennial weeds native to the Neotropics but introduced to various parts of tropical Asia and Africa[2]. As part of a study of the systematics and evolution of the complex, we have investigated the inheritance of four floral and 10 isozyme polymorphisms. Isozyme variation in the complex is being used to assess relationships among populations and taxa of contrasting ploidal level ($2x$, $4x$, $6x$) and to quantify the mating system of populations. Diploid and tetraploid populations are uniformly distylous and self-incompatible; hexaploid populations are homostylous and self-compatible[8,9]. Analysis of the inheritance of floral traits is associated with our investigations of distyly in the complex.

Materials and Methods

Plants used in this study were obtained from seed or stem cuttings collected from three sites in South America (population I1 from Barreirinhas, Brazil; I3 from Caracas, Venezuela; I32 from Arco Verde, Brazil). Further details of the reproductive biology of populations I1 and I3 are available[2]. All plants studied were diploid ($2n = 10$) and distylous. Methods employed for controlled pollinations and the culture of plants are detailed elsewhere[8,9]. The floral polymorphisms investigated were: style form (long-style versus short-style), petal spot (present versus absent), flower color (yellow versus white), and anther line (presence or absence of paired purple lateral lines on the abaxial surface of the anthers). Polymorphisms in the following enzymes were examined: glutamate oxaloacetate transaminase (GOT), esterase (EST), glutamate dehydrogenase (GDH), leucine aminopeptidase (LAP), phosphoglucomutase (PGM), aconitase (ACO), glucosephosphate isomerase (GPI), 6-phosphogluconate dehydrogenase (PGD), and shikimate dehydrogenase (SDH).

The inheritance of the four floral polymorphisms was assessed using several crosses. Linkage relationships among style form, flower color, and the petal spot polymorphisms were determined using an individual (I3I1-26) putatively heterozygous at all three loci, obtained by the cross I3-3 × I1-10 (see Shore and Barrett[9] for details of plant designation codes). This individual was backcrossed reciprocally to a plant putatively homozygous for all three genes (I3-4). To determine the linkage relationships among style form, petal spot, and anther line polymorphisms, three plants, B, G, and P, putatively heterozygous for the three genes were self-pollinated to produce F_2 families. These plants were originally obtained from the F_1 crosses I1-10 × I32-1, I1-1 × I32-2 and I32-

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2 × I1-1, respectively. These plants were specifically chosen as they carried genes determining self-compatibility[10] and this allowed seed to be obtained upon self-pollination, an unusual occurrence in this strongly self-incompatible variety[2,5].

All progenies were scored for floral phenotype. The anther line polymorphism was not detected until the progenies from the cross I3I1-26 × I4 (and reciprocal) were discarded and hence no information is available on the polymorphism from this cross. All ratios obtained were tested against single locus Mendelian expectations using the G statistic[11]. All possible pairs of floral polymorphisms were tested for independent assortment. Where deviations were detected, recombination frequencies were estimated. Maximum likelihood estimates of recombination frequencies, their standard errors, and heterogeneity tests were calculated following Allard[1].

The F_2 progenies of plants B, G, and P exhibited several enzyme polymorphisms and the inheritance and linkage relationships of these were determined using these families. Liquid extracts of the gynoecium and anthers of mature flowers were obtained by grinding the material on ice in 0.15 ml of 0.05M Na_2HPO_4 containing 0.15 mg dithiothreitol and 10 mg polyvinylpolypyrrolidone. These tissues were used as they yielded high activity for many enzymes. Extracts were absorbed in wicks cut from Whatman no. 3 chromatography paper. The wicks were placed in a cut 3.2 cm from the cathodal end of a 15.2 cm × 23.0 cm × 1.0 cm gel slab. Nine enzymes were assayed. Assays for GOT, EST, GDH, PGM, GPI, and PGD followed Cardy et al.[3]. The assay for LAP was obtained from Scandalios[6] and those for ACO and SDH were obtained from Soltis et al.[12]. To obtain information on the subcellular localization of isozymes, a procedure modified from Weeden and Gottlieb[13] was used. Gels were run that contained pollen soaked for four hours in the extraction buffer (above), pollen soaked for four hours in the extraction buffer including 1 percent Triton X-100, and crushed gynoecium tissue and anthers (above). Adjacent samples of each treatment obtained from the same plant were run.

The buffer system used for various assays are as follows: system 0 of Shields et al.[7] was used for GOT, GDH, and GPI; the system described by Scandalios[6] was used for EST and LAP; and system D of Cardy et al.[3] was used for PGM, ACO, PGD, and SDH. For all buffer systems, the starch concentration was 11 percent and gels were poured the day before they were run. Electrophoresis was undertaken in a refrigerator at 4°C. The first two gel systems were run at 55 ma for 5 hours and the third at 50 ma for 6 hours. Enzyme assays were performed in the dark at 37°C with the exception of the esterase assay that was stained at room temperature. Putative locus designations were made, with the fastest migrating zones (most anodal) assigned the lowest locus number. For example, *Got-1* represents the most anodal zone of activity for that enzyme. Where evidence of the subcellular localization of a particular isozyme was obtained, the suffix "p" is used to indicate plastid specific isozymes and "c" for cytosolic. With the exception of those loci evaluated in this study, all other loci implied by the nomenclature are tentative. Alleles coding for the allozymes were referred to as *F* for most anodal and *S* for most cathodal as not more than two homozygous classes were detected for any enzyme locus examined.

Loci from the three F_2 families were tested against 1:2:1 Mendelian expectations and, where data from more than one family were available, heterogeneity tests were performed. To examine linkage among the floral traits and enzyme polymorphisms, all possible locus pairs were tested for independent assortment. Where deviations occurred, recombination frequencies (*r*), standard errors and, where applicable, heterogeneity statistics were calculated as above. Linkage relationships among enzyme loci were evaluated in a similar manner after testing against a

Table I. Observed single-locus numbers of phenotypes for four floral traits and goodness-of-fit tests to Mendelian expectations

Locus	Cross	Phenotypes		Deviation (G)
		short	long	
	I32-1 self	109	33	0.24
	B self	32	12	0.12
	G self	23	8	0.01
	P self	30	5	2.42
Style form	I3-3 self	52	21	0.54
	I3-24 self	13	5	0.07
	I1-2 × I1-10	18	26	1.46
	I3-3 × I3-4	23	16	1.26
	I3I1-26 × I3-4	62	59	0.07
	I3-4 × I3I1-26	41	13	15.25***
		present	absent	
	I32-1 self	142	0	—
	B self	30	14	1.03
	G self	25	6	0.56
	P self	26	9	0.01
Petal spot	I3-3 self	0	73	—
	I3-24 self	0	18	—
	I1-2 × I1-10	28	16	3.31
	I3-3 × I3-4	0	39	—
	I3I1-26 × I3-4	60	61	0.01
	I3-4 × I3I1-26	13	41	15.25***
		present	absent	
	I32-1 self	0	142	—
	B self	33	11	0.00
	G self	23	8	0.01
	P self	31	4	4.05*
Anther line	I3-3 self	0	73	—
	I3-24 self	0	18	—
	I1-2 × I1-10	44	0	—
	I1-3 × I1-4	0	39	—
	I3I1-26 × I3-4	—	—	—
	I3-4 × I3I1-26	—	—	—
		yellow	white	
	I3-3 self	0	73	—
	I3-24 self	12	6	0.63
Flower color†	I3-3 × I3-4	0	39	—
	I3I1-26 × I3-4	54	67	1.40
	I3-4 × I3I1-26	19	35	4.81*

† All other families gave yellow flowers * $P < 0.05$ *** $P < 0.001$

1:2:1:2:4:2:1:2:1 ratio for independent assortment.

Results and Discussion

Inheritance of floral polymorphisms

Progeny could be scored unambiguously for all of the floral polymorphisms. The style length polymorphism is controlled by a single locus with the short-styled form heterozygous (*Ss*) and the long-styled form homozygous (*ss*) (Table I). A more detailed consideration of the inheritance of distyly in *T. ulmifolia* may be found in Shore and Barrett[9]. The petal spot is governed by a single locus with the allele determining the presence of a spot dominant to the unspotted condition. The anther line polymorphism is controlled by one locus with the dominant allele determining the presence of paired lateral lines on the anthers. The alternatives, yellow vs. white flower color, are determined by one locus with the allele determining yellow dominant to that determining white (Table I).

Most crosses met Mendelian expectations. However, a single F_2 family of individual P showed a deviation from the 3:1 expectation for the anther line polymorphism. This may represent sampling error. A more marked deviation is apparent in the backcross I3-4 × I3I1-26 for the three floral polymorphisms examined. The cross showing these marked deviations was excluded from linkage analyses. The hybrid individual I3I1-26 was generated to obtain an individual heterozygous for the three floral polymorphisms (Table II). The parents involved were taken from geographically distant localities (Brazil and Venezuela) and represent a wide intraspecific cross. Curiously, Mendelian ratios were obtained for the reciprocal cross that involved using the hybrid individual as the pistillate parent and applying nonsegregating pollen from individual I3-4. For the original cross (using I3I1-26 as a pollen parent and I3-4 as the pistillate parent), more progeny were obtained that carried alleles derived from population I3. Thus, an excess of alleles determining white flower color, absence of petal spot, and short style form were observed. The style of individual I3-4 may have selectively favored pollen grains carrying alleles derived from population I3, suggesting that gametophytic competition may have occurred, or zygotes not carrying these alleles are selectively aborted.

Tests for independent assortment revealed that the style form, petal spot, and anther line loci are all linked. Linkage estimates are provided in Table III. It is not clear why three of the four floral polymorphisms should be linked. However, since the style form polymorphism is maintained by disassortative mating, mutations of genes linked to the style form locus might be maintained by hitchhiking. Linkage estimates were homogeneous among progenies with the exception of the linkage estimate for the locus pair, style form-anther line. This was heterogeneous among the families with the repulsion phase family of plant B showing a higher recombination frequency than the other two families. Should recombination frequencies differ between pollen and megaspore mother

Table III. Observed two-locus numbers, recombination frequencies (*r*), standard errors (SE), and heterogeneity tests for floral traits showing deviation from independent assortment

Locus pair	Cross	Genotypes				$r \pm$ SE (hetero)
		S–, P–	*S–, pp*	*ss, P–*	*ss, pp*	
	B self	28	4	2	10	
Style,	G self	21	9	5	0	0.10 ± 0.02
spot	P self	17	6	8	0	(0.79)
	I3I1-26 × I3-4	6	56	54	5	
		S–, La–	*S–, lala*	*ss, La–*	*ss, lala*	
	B self	23	9	10	2	
Style,	G self	23	0	0	8	0.08 ± 0.03
anther	P self	30	0	1	4	(55.86)*
		P–, La–	*P–, lala*	*pp, La–*	*pp, lala*	
	B self	21	9	12	2	
Spot,	G self	17	8	6	0	0.29 ± 0.09
anther	P self	22	4	9	0	(1.29)

* $P < 0.001$

Table II. Multi-locus phenotypes and genotypes of parental and hybrid *Turnera ulmifolia* individuals used in the study

Plant	Style form	Petal spot	Anther line	Flower color	*Got1*	*Est1*	*Gdh1*	*Lap1*	*Lap2*	*Pgmc*	*Aco1*	*Gpic*	*Pgdc*	*Sdh1*
I1-1	short (*Ss*)	absent (*pp*)	present (*LaLa*)	yellow (*YY*)	*SS*	*SS*	*SS*	*FF*	*SS*	*FS*	*FF*	*FS*	*SS*	*SS*
I1-2	short (*Ss*)	absent (*pp*)	present (*LaLa*)	yellow (*YY*)	—	—	—	—	—	—	—	—	—	—
I1-10	long (*ss*)	present (*Pp*)	present (*LaLa*)	yellow (*YY*)	*SS*	*FS*	*FS*	*FF*	*FS*	*SS*	*FF*	*FF*	*SS*	*SS*
I32-1	short (*Ss*)	present (*PP*)	absent (*lala*)	yellow (*YY*)	*FS*	*SS*	*SS*	*FS*	*FF*	*FS*	*FS*	*FF*	*FS*	*FS*
I32-2*	long (*ss*)	present (*PP*)	absent (*lala*)	yellow (*YY*)	*FS*	*SS*	*SS*	*SS*	*FF*	*FS*	*FF*	*FF*	*SS*	*FS*
B†	short (*Ss*)	present (*Pp*)	present (*Lala*)	yellow (*YY*)	*SS*	*FS*	*FS*	*FF*	*FS*	*SS*	*FS*	*FF*	*FS*	*SS*
G‡	short (*Ss*)	present (*Pp*)	present (*Lala*)	yellow (*YY*)	*FS*	*SS*	*SS*	*FS*	*FS*	*FS*	*FF*	*FS*	*SS*	*FS*
P§	short (*Ss*)	present (*Pp*)	present (*Lala*)	yellow (*YY*)	*FS*	*SS*	*SS*	*FS*	*FS*	*FS*	*FF*	*FF*	*SS*	*FS*
I3-3	short (*Ss*)	absent (*pp*)	absent (*lala*)	white (*yy*)	—	—	—	—	—	—	—	—	—	—
I3-4	long (*ss*)	absent (*pp*)	absent (*lala*)	white (*yy*)	—	—	—	—	—	—	—	—	—	—
I3-24	short (*Ss*)	absent (*pp*)	absent (*lala*)	yellow (*Yy*)	—	—	—	—	—	—	—	—	—	—
I3I1-26††	short (*Ss*)	present (*Pp*)	present (*Lala*)	yellow (*Yy*)	—	—	—	—	—	—	—	—	—	—

* Derived from self of I32-1
† Derived from the cross I1-10 × I32-1
‡ Derived from the cross I1-1 × I32-2
§ Derived from the cross I32-2 × I1-1
†† Derived from the cross I3-3 × I1-10

Table IV. Observed single-locus numbers of genotypes for 10 enzyme loci, goodness-of-fit tests to a 1:2:1 ratio, and heterogeneity tests for selfed progeny arrays of parental heterozygotes

Enzyme locus	Self	Genotypes *FF*	*FS*	*SS*	Deviation (G)	Heterogeneity
Got-1	G	7	16	8	0.01	
	P	9	15	11	0.94	0.52
	Total	16	31	19	0.52	
Est-1	B	13	20	7	1.80	—
Gdh-1	B	16	23	5	5.59	—
Lap-1	G	10	16	5	1.65	
	P	10	17	8	0.26	0.48
	Total	20	33	13	1.48	
Lap-2	B	5	24	15	4.91	
	G	9	16	6	0.61	
	P	10	20	5	2.14	7.33
	Total	24	60	26	0.98	
Pgm-c	G	6	16	9	0.61	
	P	10	21	4	3.46	3.37
	Total	16	37	13	1.28	
Aco-1	B	11	23	6	2.15	—
Gpi-c	G	5	15	11	2.35	—
Pgd-c	B	4	15	14	6.33*	—
Sdh-1	G	5	18	8	1.39	
	P	6	19	10	1.17	0.10
	Total	11	37	18	2.68	

* $P < 0.05$

Table V. Observed two-locus numbers, recombination frequencies (*r*), standard errors (SE), and heterogeneity tests for morphological and isozyme locus pairs where a significant deviation from independent assortment was detected

Locus	Hybrid	*D**–, FF*	*D–, FS*	*D–, SS*	*dd, FF*	*dd, FS*	*dd, SS*	$r \pm$ SE (hetero)
Style, *Pgm-c*	G	6	16	1	0	0	8	0.03 ± 0.02
	P	10	20	0	0	1	4	(0.01)
Style, *Aco-1*	B	0	22	6	11	1	0	0.02 ± 0.02
Style, *Pgd-c*	B	4	15	4	0	0	10	0.10 ± 0.06
Spot, *Pgm-c*	G	0	16	9	6	0	0	0.01 ± 0.01
	P	1	21	4	9	0	0	(0.71)
Spot, *Aco-1*	B	2	18	6	9	5	0	0.16 ± 0.06
Spot, *Pgd-c*	B	4	15	0	0	0	14	0.02 ± 0.03
Anther, *Pgm-c*	G	6	16	1	0	0	8	0.01 ± 0.01
	P	10	21	0	0	0	12	(1.46)
Anther, *Aco-1*	B	9	21	1	2	2	5	0.20 ± 0.07
Anther, *Pgd-c*	B	1	15	12	3	0	2	0.21 ± 0.08

* *D* indicates the dominant phenotype and *dd* the recessive

cells, heterogeneous recombination frequencies are expected among coupling and repulsion progenies[4]. However, no such heterogeneity was observed for linkage of style form and petal spot, for which repulsion and coupling progenies were also available. Additionally, the F_2 family derived from plant B showed higher (although nonsignificant) recombination frequencies for all pairs of loci.

Inheritance of enzyme loci

Table II provides the multilocus genotypes of the parental and hybrid individuals involved in the analysis of the inheritance of isozymes. Tests for each of the 10 putative loci against expected 1:2:1 ratios are provided in Table IV. All expectations are met with the exception of *Pgd-c*, which shows a deficiency of *FF* homozygotes for the small sample evaluated. Thus, in total, 10 isozyme loci are segregating.

Linkage relationships among loci are provided in Tables III, V, and VI. While sample sizes are small, several loci exhibit tight linkages, showing only few recombinant individuals. There is evidence for three linkage groups. A linkage group containing the loci governing petal spot, style form, anther line, *Pgm-c*, *Aco-1*, and *Pgd-c* occurs. The order in which the loci should be arranged is not clear. Different arrangements are suggested from families of B versus G and P. A second linkage group, arranged as follows, *Gpi-c*, *Lap-1*, and *Sdh-1*, is apparent. A third linkage group consisting of *Gdh-1* and *Lap-2* also occurs. No linkage relationships among the loci *Got-1* and *Est-1* were detected.

This study reports on the inheritance of floral and isozyme loci in diploid ($2n = 10$) plants of the *Turnera ulmifolia* complex and provides the first electrophoretic results on a member of the Turneraceae. Although sample sizes were small, several tight linkage relationships were found among the loci examined. The species complex has a low base number[8], $x = 5$, and chiasma frequencies of

Table VI. Observed two-locus numbers, recombination frequencies (*r*), standard errors (SE), and heterogeneity tests for isozyme pairs showing a significant deviation from independent assortment

Locus pair	Family	Genotype *FF, FF*	*FF, FS*	*FF, SS*	*FS, FF*	*FS, FS*	*FS, SS*	*SS, FF*	*SS, FS*	*SS, SS*	$r \pm$ SE (hetero)
Lap-1, Sdh-1	G	0	0	10	0	15	1	5	0	0	0.05 ± 0.02
	P	0	1	9	1	15	1	5	3	0	(3.26)
Lap-1, Gpi-c	G	0	0	10	0	15	1	5	0	0	0.02 ± 0.02
Gpi-c, Sdh-1	G	5	0	0	0	14	1	0	4	7	0.08 ± 0.04
Aco-1, Pgd-c	B	0	0	9	1	15	5	3	0	0	0.10 ± 0.04
Gdh-1, Lap-2	B	0	1	15	0	23	0	5	0	0	0.01 ± 0.01

less than two chiasmata per bivalent (Shore and Barrett, unpub. data) increase the likelihood of detecting linkage among loci. The ease with which *T. ulmifolia* can be cultured and crossed[5,8] and the demonstrated presence of easily scored genetic markers make the plant suitable for experimental studies in systematics and genetics.

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