

Chromosomal evidence for autotetraploidy in the *Turnera ulmifolia* complex (Turneraceae)

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The hypothesis that tetraploids of two taxonomic varieties of the *Turnera ulmifolia* complex, vars. *elegans* and *intermedia*, have had autopolyploid origins, was tested. Chromosome counts within each variety show that two cytotypes occur with somatic numbers of $2n = 10$ and $2n = 20$. Tetraploids of both var. *intermedia* and var. *elegans* have pollen fertility approximately 13% less than that of diploids. Synthetic tetraploids produced by colchicine doubling exhibit pollen fertilities virtually identical to those of the natural tetraploids. While diploids exhibited only bivalent formation, tetraploids showed varying frequencies of univalents, bivalents, trivalents, and quadrivalents. The chromosome pairing model of R. C. Jackson and D. P. Hauber (1982. *Am. J. Bot.* **69**: 644–646) and a minor modification of the goodness-of-fit test for that model, were used to test the hypothesis of an autopolyploid origin. For four of the six populations studied meiotically, the data fit the model. The data indicate that the tetraploid cytotypes of *T. ulmifolia* vars. *elegans* and *intermedia* have had autopolyploid origins.

Key words: *Turnera ulmifolia*, autotetraploid, chromosome pairing model.

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L'auteur a vérifié l'hypothèse que les tétraploïdes de deux variétés taxonomiques du complexe *Turnera ulmifolia* vars. *elegans* et *intermedia* auraient eu des origines autopolyploïdes. Le décompte chromosomique montre que, pour chaque variété, il existe deux cytotypes possédant des nombres somatiques de $2n = 10$ et $2n = 20$. La fertilité des pollens des deux variétés tétraploïdes, var. *elegans* et var. *intermedia*, est inférieure à celle des pollens des diploïdes par environ 13%. Les tétraploïdes synthétiques obtenus par doublage avec la colchicine montrent des fertilités polliniques virtuellement identiques à celles des tétraploïdes naturels. Alors que les diploïdes ne montrent que des formations bivalentes, les tétraploïdes montrent des fréquences variables d'univalents, de bivalents, de trivalents et de tétravalents. Le modèle de pairage chromosomique de R. C. Jackson et D. P. Hauber (1982. *Am. J. Bot.* **69**: 644–646) ainsi qu'une modification mineure de l'essai de validité de la similitude (goodness-of-fit) pour ce modèle, ont été utilisés pour vérifier cette hypothèse de l'origine autopolyploïde. Pour quatre des six populations où la méiose a été étudiée, les données correspondent au modèle. Les données indiquent que les cytotypes tétraploïdes du *T. ulmifolia* vars. *elegans* et *intermedia* ont des origines autopolyploïdes.

Mots clés: *Turnera ulmifolia*, autotétraploïde, modèle de pairage chromosomique.

[Traduit par la rédaction]

Introduction

Polyloidization is an important speciation process in the angiosperms and has received considerable attention (e.g., Stebbins 1971; Lewis 1980). While a number of investigations have been carried out on allopolyploids, autopolyploids have received less attention. Autopolyploidy apparently occurs less frequently than allopolyploidy, but some workers suggest that the occurrence of autopolyploidy may have been underestimated (Levin 1983; Soltis and Rieseberg 1986). Recently, additional examples of autopolyploidy have come to light through chromosomal and genetic analyses (Hauber 1986; Soltis and Rieseberg 1986; Fernandez 1987; Soltis and Soltis 1988; Krebs and Hancock 1989; Bayer 1989; Ness *et al.* 1989; Wolf *et al.* 1989).

Autopolyploidy was originally defined using cytogenetic criteria. As such, the demonstration of autopolyploidy requires either a direct analysis of meiotic configurations or alternatively, a test of deductions that can be drawn when autopolyploid chromosomal pairing occurs. In the former instance, pairing models have been proposed to predict the expected behaviour of chromosomes at diakinesis or first metaphase of meiosis (Jackson and Casey 1982; Jackson and Hauber 1982; Crane and Sleper 1989b). For the latter, it is possible to deduce segregation patterns at individual gene loci. Autopolyploids should show polysomic segregation. Further deductions may

be drawn when putative diploid progenitors are available for analysis. Morphological and chemosystematic characters may provide additional information on the origin of an autopolyploid. Indeed, a multifaceted approach to demonstrating an autopolyploid origin is often espoused (Stebbins 1950; Soltis and Rieseberg 1986). It is, however, important to point out that tests of hypotheses of autopolyploid origins require that no genetic divergence of characters examined has occurred since the time of origin of the putative autopolyploid, and this may not be a reasonable expectation in all cases.

Turnera ulmifolia L. is a woody perennial native to the Neotropics with some adventive populations in other tropical regions (Barrett 1978). Distylous and homostylous breeding systems occur within this species complex (Barrett 1978; Barrett and Shore 1987) that contains an assemblage of 12 taxonomic varieties. A number of varieties represent distinct biological species (Shore and Barrett 1985a; Arbo and Fernandez 1987; Barrett and Shore 1987). Some varieties have been elevated to specific rank (Lock 1904; Backer 1951; Fernandez and Arbo 1989). Polyploidy has played a prominent role in speciation within the genus as a whole as well as within the species complex, which contains diploid, tetraploid, hexaploid, and octoploid cytotypes and a base number of $x = 5$ (Raman and Kesavan 1964; Barrett 1978; Arbo and Fernandez 1983; Barrett and Shore 1985a; Barrett and Shore 1987; Fernandez 1987; Fernandez and Arbo 1989).

TABLE 1. Varietal status, chromosome number, and locality of populations for which new chromosome counts have been obtained as well as populations used for meiotic analyses

Variety	Pop. code	Chromosome no. (2n)	Locality	Collection No.
<i>intermedia</i> *†	I2	10	Calabozo, Venezuela	Barrett 1126
<i>intermedia</i> *†	I3	10	Caracas, Venezuela	Barrett 1125
<i>intermedia</i>	I26	10	Santa Rosa, Costa Rica	Barrett and Shore 1375
<i>intermedia</i>	I27	10	Comayagua, Honduras	Barrett and Shore 1374
<i>intermedia</i>	I29	10	La Pacifica, Costa Rica	Barrett and Shore 1377
<i>elegans</i>	I30	10	Sao Luis, Brazil	Barrett and Shore 1366
<i>intermedia</i> *†	I4	20	Dagua, Colombia	Barrett 689
<i>intermedia</i>	I8	20	Rio Piedras, Puerto Rico	Barrett and Shore 1348
<i>intermedia</i>	I9	20	Guayama, Puerto Rico	Barrett and Shore 1349
<i>intermedia</i>	I10	20	Parbueyon, Puerto Rico	Barrett and Shore 1350
<i>intermedia</i>	I11	20	Joyuna, Puerto Rico	Barrett and Shore 1351
<i>intermedia</i>	I12	20	Dorado, Puerto Rico	Barrett and Shore 1352
<i>intermedia</i>	I13	20	Manati, Puerto Rico	Barrett and Shore 1353
<i>intermedia</i>	I16	20	Tortuguera, Puerto Rico	Barrett and Shore 1356
<i>intermedia</i>	I17	20	Jarabacoa, Dominican Republic	Barrett and Shore 1357
<i>intermedia</i>	I19	20	Cabrete, Dominican Republic	Barrett and Shore 1359
<i>intermedia</i>	I20	20	San FCO de Macoris, Dominican Republic	Barrett and Shore 1360
<i>intermeida</i>	I21	20	Fantino, Dominican Republic	Barrett and Shore 1361
<i>intermedia</i>	I23	20	Bani at Playa, Dominican Republic	Barrett and Shore 1363
<i>intermedia</i> *	I24	20	St. Cristobal, Dominican Republic	Barrett and Shore 1364
<i>intermedia</i> *	I25	20	Santo Domingo, Dominican Republic	Barrett and Shore 1365
<i>elegans</i> *†	E2	20	Manaus, Brazil	Barrett 1130
<i>elegans</i> *†	E3	20	Belém, Brazil	Barrett 693
<i>elegans</i>	E5	20	Leticia, Colombia	Barrett and Shore 1378
<i>elegans</i>	E7	20	Recife, Brazil	Barrett and Shore 1380
<i>elegans</i>	E8	20	Aracaju, Brazil	Barrett and Shore 1369
<i>elegans</i> *	E9	20	Maceio, Brazil	Barrett and Shore 1370
<i>elegans</i>	E10	20	Vitória de S. Antão	Barrett and Shore 1371
<i>elegans</i>	E12	20	Mara, Malaysia	Barrett and Shore 1381
<i>elegans</i>	E13	20	Abidin, Malaysia	Barrett and Shore 1382

*Populations used in meiotic studies.

†Chromosome counts previously published (Shore and Barrett 1985a).

Here I provide chromosome numbers of populations of two taxonomic varieties of *T. ulmifolia*, vars. *elegans* Urb. and *intermedia* Urb. I examine pollen fertility of plants obtained from natural populations and artificial hybrids. Finally, using chromosome pairing models of Jackson and Hauber (1982), an analysis of meiotic configurations is performed to test the hypothesis of an autopolyploid origin for tetraploid *T. ulmifolia* vars. *elegans* and *intermedia*.

Materials and methods

Pollen fertility and mitotic and meiotic squashes were obtained from plants grown under glasshouse conditions following methods detailed in Shore and Barrett (1985a). Plants were originally collected as seed samples in the field and hybrids were generated by controlled crosses. Triploid hybrids were obtained by intravarietal (tetraploid *intermedia* × diploid *intermedia*) or intervarietal crosses (tetraploid *elegans* × diploid *intermedia*). Synthetic tetraploids, produced by Shore and Barrett (1986) with colchicine, were obtained through somatic chromosome doubling of diploid seedlings. Pollen fertility was assessed by staining freshly collected pollen with cotton blue in lactophenol. Two hundred pollen grains from each of two flowers per plant and at least two plants per population were scored as either stained (cytoplasm present, taking up the stain) or unstained (empty grains). To determine whether there were statistically significant differences among ploidal levels and varieties, an analysis of variance (ANOVA) was performed on arcsine – square root transformed population mean proportions of stainable pollen, followed by Scheffé's a posteriori test. No significance test was used for artificial hybrids and synthetic tetraploids because of the small number of plants available.

Mitotic squashes were made by excising root tips from plants and incubating these at room temperature for 3–4 h in a 0.05% solution of colchicine. Root tips were then fixed in acetic alcohol (ethanol – glacial acetic acid, 3:1). Root tips were hydrolyzed in 1 M HCl at 60°C for 10 min and chromosomes subsequently stained by immersing root tips in leucobasic fuchsin for 1–2 h. Chromosome squashes were then prepared.

Meiotic preparations were made by fixing flower buds in acetic alcohol for at least 2 days. After three rinses with 70% ethanol, buds were immersed in alcoholic carmine and stained following the procedure of Snow (1963). Anthers were subsequently dissected and squashes of pollen mother cells prepared. Meiotic configurations were scored for cells at diakinesis or first metaphase if they were sufficiently flattened to avoid ambiguous interpretation of configurations. Two to five plants were examined per population.

Observed frequencies of meiotic configurations in tetraploids were tested for goodness-of-fit to the autotetraploids pairing model developed by Jackson and Hauber (1982), allowing a maximum of two chiasmata per bivalent. In addition, a modification of the goodness-of-fit test was also applied. The modification simply involves scoring the frequency of pairs of bivalents and is otherwise identical to the goodness-of-fit tests used by Jackson and Hauber (1982). This modification corrects for a small difference that otherwise occurs between the sum of the observed and expected frequency distributions (see Discussion).

Results

Chromosome numbers obtained by counts of meiotic or mitotic preparations are presented for populations of *T. ulmi-*

TABLE 2. Mean (and SD) percent stainable pollen from natural populations of *Turnera ulmifolia*

Variety	Ploidy level	No. of populations	Mean (SD) stainable pollen (%)
<i>intermedia</i>	2x	6	92 (6) ^a
<i>elegans</i>	2x	2	97 (2) ^a
<i>intermedia</i>	4x	8	80 (4) ^b
<i>elegans</i>	4x	8	83 (6) ^b

NOTE: Means sharing the same letter are not significantly different as assessed by ANOVA ($F = 13.3$, $P < 0.001$) on aresine - square-root transformed proportions, followed by Scheffé's test.

folia vars. *elegans* and *intermedia* including 25 populations not previously reported (Table 1). Populations of *T. ulmifolia* vars. *intermedia* and *elegans* are either diploid ($2n = 10$) or tetraploid ($2n = 20$). Diploids of both varieties are morphologically similar and highly interfertile (Shore and Barrett 1985a; Arbo and Fernandez 1987) but may be most easily distinguished by the presence of a purple petal spot, which is, however, known to be inherited by a single dominant gene (Shore and Barrett 1987).

All chromosomes in diploids and tetraploids of both varieties are approximately equal in size and metacentric to submetacentric in morphology, although a single larger pair of metacentrics occurs (Fig. 1). A pair of chromosomes bearing a small terminal satellite was observed in preparations in which the chromosomes were not highly contracted. In some meiotic preparations a single chromosome pair was associated with the nucleolus.

Pollen fertility was assessed for 8 diploid populations, 16 tetraploid populations (8 of each variety) as well as for triploid and tetraploid hybrids and synthetic tetraploids. Diploids of both varieties do not differ significantly and have a greater mean percentage of stainable pollen (94.5%) than tetraploids (81.5% stainable pollen, Table 2). Pollen stainability of the tetraploid varieties was not significantly different (Table 2). Hybrid tetraploids produced by the crosses of tetraploid var. *elegans* (E2) \times tetraploid var. *intermedia* (I4) and tetraploid var. *intermedia* (I16) \times tetraploid var. *intermedia* (I4) gave mean percent pollen stainabilities similar to those of the natural tetraploids (79% and 71%, respectively; Table 3). Although no statistical analysis was performed, both means are less than 1 SD from the mean of the natural tetraploid populations (Tables 2 and 3). Interestingly, tetraploids synthesized using colchicine had a mean pollen stainability of 82%, virtually identical to that of the natural tetraploids (Tables 2 and 3). As expected, triploids had uniformly low levels of stainable pollen (12%).

As a result of the similarity in size and morphology of chromosomes (Fig. 1), it was not possible to distinguish among nonhomologous chromosomes during diakinesis or first metaphase and thus results from all chromosomes were pooled (Table 4). Meiotic observations of diploids at diakinesis and first metaphase revealed a maximum of two chiasmata per bivalent and chiasmata tend to occur distal to the centromere (Fig. 2). Some pollen mother cells showed a maximum of five ring bivalents (oII), indicating the presence of two chiasmata per bivalent (one in each arm), while others showed varying numbers of ring (oII) and chain bivalents (cII, one chiasma per bivalent) (Fig. 2). A relative measure of chiasma frequency, the chiasma coefficient (P value, Jackson and Hauber 1982), was calculated for diploids and tetraploids. This coef-

TABLE 3. Mean (and SD) percent stainable pollen from artificial hybrids and synthetic tetraploids of *Turnera ulmifolia*

Cross	Ploidy level	No. of crosses	Mean (SD) stainable pollen (%)
<i>elegans</i> \times <i>intermedia</i> [*]	4x	1	79 (13)
<i>intermedia</i> \times <i>intermedia</i> [†]	4x	1	71 (18)
<i>intermedia</i> \times <i>intermedia</i> [‡]	3x	2	12 (5)
<i>intermedia</i> [§]	4x	1	82 (9)

^{*}Cross of tetraploid var. *elegans* \times tetraploid *intermedia* from Colombia.

[†]Cross of Puerto Rican tetraploid \times Colombian tetraploid.

[‡]Crosses of tetraploid var. *intermedia* from the Dominican Republic and Colombia with diploid var. *intermedia*.

[§]Synthesized from 2x var. *intermedia* using colchicine.

ficient measures the average number of chiasmata per bivalent relative to the maximum of two chiasmata per bivalent. Diploids show chiasma coefficients of 0.85 and 0.89 (Table 4). Tetraploids exhibit similar chiasma coefficients of 0.79 to 0.87 (Table 4).

For tetraploids of both varieties, numbers of various meiotic configurations were scored for each pollen mother cell and frequencies are detailed in Table 4. In addition to ring and chain bivalents, a large proportion of chromosomes were associated in ring quadrivalents (oIV, Fig. 3), having four chiasmata (one in each pair of arms) and chain quadrivalents (cIV, having three chiasmata, Fig. 3). A small proportion of trivalents and univalents (III, I) also occurred (Table 4). For a single plant of population I25, occasional unpaired univalents (Is), not associated with trivalents, also occurred.

A contingency test using the G -statistic (Sokal and Rohlf 1981) was carried out to determine whether proportions of various meiotic configurations were homogeneous across populations and varieties. The analysis was carried out using only cII, oII, cIV, and oIV classes, as other classes occurred only in low frequencies and would likely cause an upward bias in the magnitude of the G -statistic. The analysis gave a G -value of 39.2 ($P < 0.001$, $df = 15$), indicating that significant differences occur among populations for various proportions of meiotic configurations. A similar analysis within each variety also revealed heterogeneity of proportions of meiotic configurations for var. *intermedia* ($G = 14.9$, $P < 0.05$, $df = 6$), but not for var. *elegans* ($G = 10.8$, $P > 0.05$, $df = 6$).

The data fit the autotetraploid pairing model (Jackson and Hauber 1982) for four of the six populations (Table 4). Note that the goodness-of-fit tests of Jackson and Hauber (1982) and those obtained by scoring the frequency of pairs of bivalents yielded qualitatively identical results. One population of var. *intermedia* (I25) and one of var. *elegans* (E3) gave statistically significant deviations from the pairing models. Where significant deviation from expectations occurred, there was a deficiency of cIV and an excess of bivalents.

For populations I25 and E3, separate analyses were done for each plant scored in each population. Frequencies of meiotic configurations were scored for five plants of population I25. Only one of the five showed a significant deviation ($X^2 = 16.7$, $P < 0.001$, $df = 2$) from the autotetraploid pairing model of Jackson and Hauber (1982). Three plants were scored for population E3. Again, only one plant showed a marginally nonsignificant deviation ($X^2 = 5.95$, $P = 0.051$, $df = 2$) from the autotetraploid pairing model.

Configurations not predicted by the model also occur. Trivalents and univalents were seen in some preparations as were

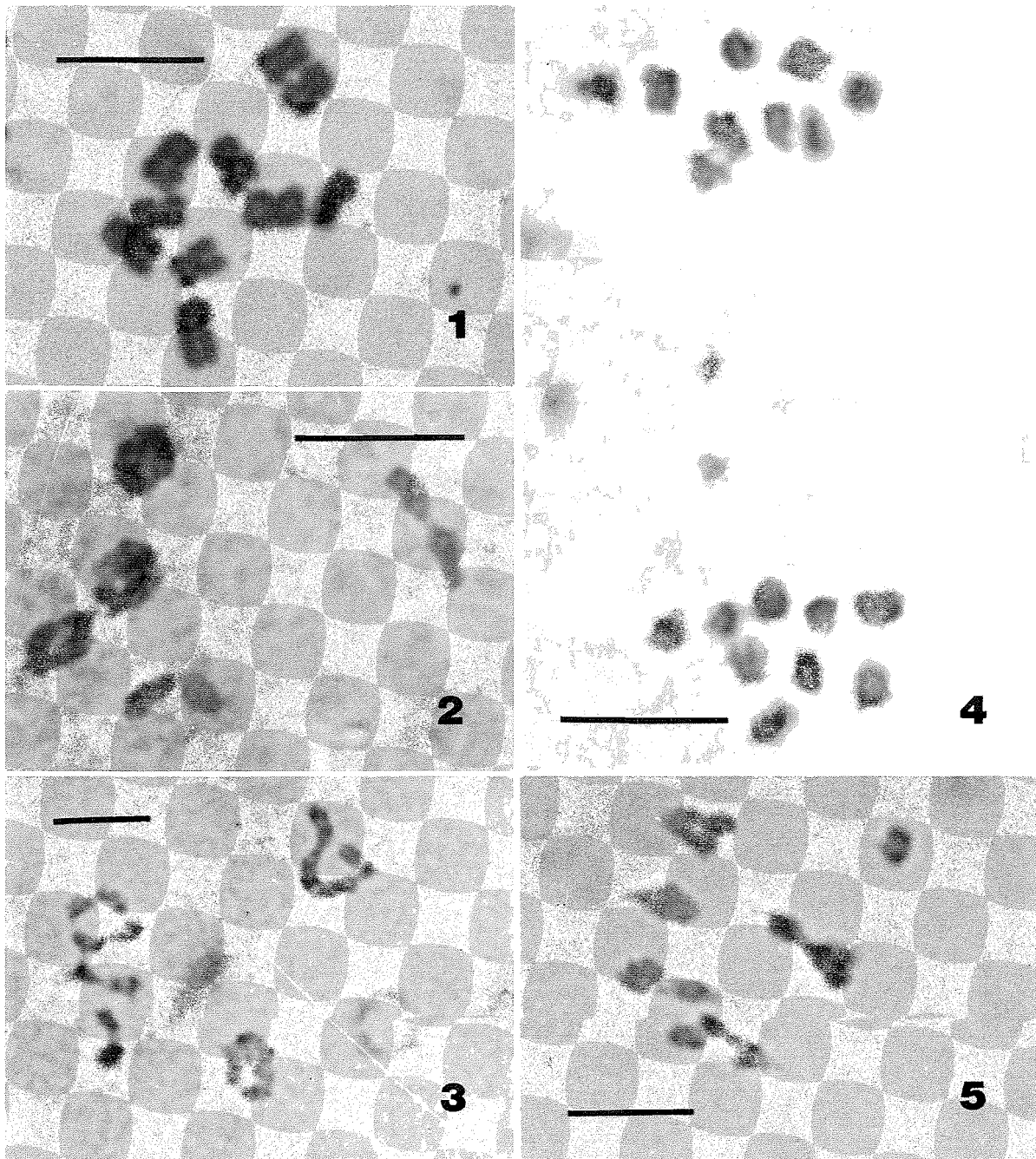


FIG. 1. Mitotic metaphase in diploid *T. ulmifolia* var. *intermedia* showing 10 metacentric to submetacentric chromosomes of approximately equal length. Scale bar $\approx 5 \mu\text{m}$. FIG. 2. Diakinesis in a pollen mother cell of diploid *T. ulmifolia* var. *intermedia* showing three oII and two cII. Scale bar $\approx 5 \mu\text{m}$. FIG. 3. First metaphase of meiosis in a pollen mother cell of tetraploid *T. ulmifolia* var. *elegans* showing three oIV, one cIV and two cII. Scale bar $\approx 5 \mu\text{m}$. FIG. 4. Late anaphase I of meiosis in a pollen mother cell of tetraploid *T. ulmifolia* var. *intermedia*. A single lagging chromosome is undergoing a precocious division. An additional chromosome did not migrate to either nucleus (not shown in photograph). Scale bar $\approx 5 \mu\text{m}$. FIG. 5. First metaphase of meiosis in a pollen mother cell of a triploid hybrid produced by the cross of tetraploid var. *elegans* \times diploid var. *intermedia*, showing four cIII, one oII, and one I. Scale bar $\approx 5 \mu\text{m}$.

univalents not associated with any trivalents. To test the adequacy of the models, the frequency of the anomalous class (III,I) was doubled and pooled with the cII class, as suggested by Jackson and Hauber (1982) and a goodness-of-fit statistic was calculated. For the population (i.e., 125) where unpaired univalents occurred, these were not included in the analysis.

Observations of first and second anaphase in diploids revealed normal segregation of chromosomes to poles. Tetra-

ploids of both varieties, however, showed a low frequency of chromosomes lagging on the anaphase plate (Fig. 4).

Observations of diakinesis and first metaphase of meiosis in triploids obtained via intravarietal and intervarietal crosses revealed that univalents, bivalents (both cII and oII) as well as chain trivalents occur at varying frequencies (Fig. 5). Although autotriploid pairing models are available in the literature (Jackson and Casey 1982; Jackson and Hauber 1982;

TABLE 4. Observed numbers of meiotic configurations (Obs1 and Obs2) for diploid and tetraploid populations of *Turnera ulmifolia*, expected (Exp1 and Exp2) numbers of configurations and goodness-of-fit tests to pairing models for tetraploids

Pop. and ploidy level	Configurations						No. of cells	P	χ^2
	I	cII	oII	III,I	cIV	oIV			
I2 2x	0	92	328	0	0	0	84	0.89	
I3 2x	0	130	290	0	0	0	84	0.85	
I4 4x									
Obs1	0	30	28	1	20	25	15	0.83	
Exp1	0	32.7	32.7	0	19.3	23.0			0.9
Obs2	0	30			20	25			
Exp2	0	32.7			19.3	23.0			0.2
I24 4x									
Obs1	0	15	13	0	9	22	9	0.87	
Exp1	0	13.6	22.0	0	10.4	16.8			5.6
Obs2	0	14			9	22			
Exp2	0	17.8			10.4	16.8			2.0
I25 4x									
Obs1	8	171	233	11	74	136	85	0.84	
Exp1	0	161.8	193.0	0	105.2	140.4			23.7**
Obs2	8	213			74	136			
Exp2	0	177.4			105.2	140.4			16.0**
E2 4x									
Obs1	0	120	72	2	42	60	40	0.79	
Exp1	0	112.4	78.0	0	53.6	51.2			5.7
Obs2	0	98			42	60			
Exp2	0	95.2			53.6	51.2			3.7
E3 4x									
Obs1	0	38	38	1	11	20	14	0.82	
Exp1	0	32.7	29.7	0	18.3	20.5			6.9*
Obs2	0	39			11	20			
Exp2	0	31.2			18.3	20.5			4.2*
E9 4x									
Obs1	0	17	23	0	7	18	9	0.87	
Exp1	0	13.6	22.0	0	10.4	16.8			2.0
Obs2	0	20			7	18			
Exp2	0	17.8			10.4	16.8			1.0

NOTE: P is the chiasma coefficient. I, univalents; cII, chain bivalents; oII, ring bivalents; III,I, univalent and trivalent; cIV, chain quadrivalent; oIV, ring quadrivalent. Obs1 and Exp1 are observed and expected frequencies based on the pairing model of Jackson and Hauber (1982). Exp1 was tested with 2 df. Obs2 and Exp2 are observed and expected frequencies obtained by determining the frequency of pairs of bivalents. Exp2 was tested with 1df using Yate's correction. *, $p < 0.05$; **, $p < 0.001$.

Crane and Slepser 1989a), insufficient numbers of cells were scored to allow the application of these models. Meiotic studies of triploid hybrids of the cross tetraploid var. *elegans* × diploid var. *intermedia* (Fig. 5) suggest that chromosomes of the two varieties are homologous, a conclusion also made by Fernandez and Arbo (1989) based on crosses among diploids.

Discussion

Pollen fertility data (Table 2) show that both tetraploid varieties have lower pollen fertility than diploids, and this observation is in accord with expectations of reduced pollen fertility in an autotetraploid. Presumably, the occasional occurrence of univalents and trivalents (Table 4) and of lagging chromosomes (Fig. 4) leads to the production of unbalanced gametes that may be inviable. Raman and Kesavan (1964), Barrett (1978), and Arbo and Fernandez (1983) have also observed

lagging chromosomes at anaphase I in tetraploid *T. ulmifolia* var. *elegans*. Fernandez (1987) observed pollen fertilities of 74% and 87% for tetraploid *T. ulmifolia* vars. *intermedia* and *elegans*, respectively. These values are similar to those obtained here, but they cannot be compared statistically, as only ranges are given for the data of Fernandez (1987).

Chromosome pairing models developed by Jackson and Hauber (1982), which predict frequencies of meiotic configurations expected in an autotetraploid having a particular chiasma frequency, allow a test of the hypothesis that the plant under examination is an autotetraploid. Chromosome pairing analyses conducted here are consistent with an autotetraploid origin for *T. ulmifolia* vars. *elegans* and *intermedia*. Pairing models applied to the data, however, did not fit for two of six populations. For the two aberrant populations only a single plant in each deviated from the model predictions. One possible explanation for the deviations is that mutations have

occurred at loci influencing chromosome pairing behaviour. To test this hypothesis it would be necessary to undertake controlled crosses and examine chromosome pairing in a number of progeny.

The data presented here are further supported by genetic data (Shore and Barrett 1985b; Shore 1991). The occurrence of tetrasomic inheritance at three isozyme loci (Shore 1991) and the inheritance of distyly in tetraploid var. *elegans* (Shore and Barrett 1985b) are consistent with the hypothesis of an autopolyploid origin for these varieties. Meiotic studies by Raman and Kesavan (1964), Arbo and Fernandez (1983), and Fernandez (1987) also support an autotetraploid origin for these varieties. The data of these authors were not collected in a manner that allows the application of the pairing models applied here. Taken together, however, these data provide evidence of natural autotetraploidy, thus extending the list of natural autopolyploid species. Interestingly, Fernandez (1987) observed quadrivalents in other tetraploid species of *Turnera*, suggesting that although no pairing models have been applied, these species are also autopolyploid. Further investigation of these species would be of interest.

The pairing model of Jackson and Hauber (1982) allows a test of the hypothesis of an autotetraploid origin and has been applied to tetraploid *Haplopappus spinulosus* (Jackson and Hauber 1982; Hauber 1986) and to tetraploid *Heuchera grossulariifolia* (Wolf *et al.* 1989). A slight discrepancy, however, occurs between the sum of the observed frequencies and expected frequencies of meiotic configurations in both these studies. This leads to a small bias in the magnitude of the X^2 goodness-of-fit statistic and to a much larger bias if the G -statistic is used to assess goodness-of-fit. The discrepancy arises because the model of Jackson and Hauber (1982) actually predicts the simultaneous disposition of all four homologous chromosomes, not the disposition of chromosomes into various numbers of meiotic configurations (i.e., oIV, cIV, oII, and cII). Thus, if a single set of four homologous chromosomes can be distinguished and analyzed, there are in fact five possible ways (not four as in Jackson and Hauber 1982) in which four homologous chromosomes can be disposed: oIV, cIV, 2 oII, 1 oII and 1 cII, and 2 cII (ignoring the cIII, I or unpaired Is for simplicity). Thus, where it is possible to distinguish a single homologous set of four chromosomes, it is possible to observe all five classes above and a goodness-of-fit test can be performed. This also results in an increase in the number of degrees of freedom by one. It is also necessary to derive a model that explicitly predicts the proportions of these three bivalent classes.

When it is not possible to distinguish among nonhomologous chromosomes, it is also not possible to correctly classify bivalents into the three categories above. A solution to this difficulty is to pool all bivalents into a single class containing the number of pairs of bivalents, as was done here (Table 4). This overcomes the discrepancy between the sum of the observed and expected frequency distributions but results in the loss of one degree of freedom.

Another alternative is to use the meicyte as the unit of observation rather than a single set of four chromosomes and extend the model of Jackson and Hauber (1982) to predict the frequency of meicytes containing various frequencies of meiotic configurations. The expected frequencies can be obtained by first predicting the frequencies of the classes oIV, cIV, 2 oII, 1 oII and 1 cII, and 2 cII, for a single homologous chromosome set and then multiplying the set of frequencies

by itself x times, where x is the number of nonhomologous chromosome sets scored (e.g., for this study all five were scored so that $x = 5$). The number of possible meicyte types rises very rapidly as x increases and thus requires a large sample size. For example, if we consider the case where $x = 2$ and allow only bivalents and quadrivalents to occur, then the number of possible types of meicytes, some of which might have 2 oIV, or 1 oIV and 1 cIV, or 4 cII, etc., is 14. Under the same conditions, if $x = 3$, there are 30 possible types of meicyte and if $x = 5$ there are 91 possible types of meicyte. Thus, for the present study too few cells were scored to allow the application of this method, but an investigation is underway to test its usefulness. This extension of the model also requires the assumption that the number and distribution of chiasmata for each set of four homologous chromosomes is independent of that for other nonhomologous chromosomes.

The occurrence of recently described diploid species of *Turnera* that have homologous chromosome sets may complicate the phylogenetic inferences that can be drawn from meiotic analyses alone. Fernandez and Arbo (1989) examined first meiotic metaphase of meiosis for all possible diploid hybrids among *T. ulmifolia* vars. *elegans* and *intermedia*, *T. concinna*, and *T. Krapovickasii* and suggest that all the species possess the same basic genome.

The occurrence of autotetraploidy in two taxonomic varieties of *Turnera ulmifolia*, var. *elegans* that is widespread in Brazil and var. *intermedia* found in the Dominican Republic and Puerto Rico, raises the issue of the number of times autotetraploidy has arisen within the species complex. In addition, Barrett (1978) was uncertain of the varietal status of the Columbian tetraploid (population I4) that differs in a number of vegetative characters from var. *elegans* yet is polymorphic for the purple petal spot characteristic of that variety. The geographic distribution and morphological data support the possibility that there have been at least three independent origins of autotetraploidy in the species complex. The occurrence of putatively autotetraploid *T. Krapovickasii* and *T. sidoides* suggests that the genus may be amenable to frequent shifts to the autopolyploid condition. Molecular markers might aid in elucidating the number of times autotetraploidy has arisen independently in *T. ulmifolia* following the approach to Soltis *et al.* (1989) where multiple origins of autotetraploid *Heuchera micrantha* were demonstrated.

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