

## BREEDING SYSTEM EVOLUTION IN *TARASA* (MALVACEAE) AND SELECTION FOR REDUCED POLLEN GRAIN SIZE IN THE POLYPLOID SPECIES<sup>1</sup>

JENNIFER A. TATE<sup>2</sup> AND BERYL B. SIMPSON

Section of Integrative Biology and Plant Resources Center, The University of Texas at Austin, Austin, Texas 78712 USA

Polyploidy, primarily allopolyploidy, has played a major role throughout flowering plant evolution with an estimated 30–80% of all extant angiosperms carrying traces of ancient or recent polyploidy. One immediate and seemingly invariant phenotypic consequence of genome doubling is larger cell size in polyploids relative to their diploid progenitors. In plants, increases in pollen grain and guard cell sizes exemplify this rule and are often used as surrogate evidence for polyploidy. *Tarasa* (Malvaceae), a genus of 27 species primarily distributed in the high (>3000 m) Andes, has numerous independently generated tetraploid species, most of which have pollen grains smaller than their putative diploid parents. The tetraploids are also unusual because they are annual, rather than perennial, in habit. Data correlate these apparent anomalies to a change in the breeding system within the genus from xenogamy (outcrossing) in the diploid species to autogamy (inbreeding) in the tetraploids, leading to a convergence in reduced floral morphology. The harsh environment of the high-elevation Andean habitats in which all the tetraploid annuals are found is implicated as a critical factor in shaping the evolution of these unusual polyploids.

**Key words:** autogamy; breeding system; Malvaceae; pollen size; polyploidy; *Tarasa*.

In plants, the union of unreduced gametes or hybridization between compatible entities followed by genome doubling (polyploidy) has been an important avenue of speciation. Estimates of polyploidy in angiosperms alone range from 30 to 80% (Stebbins, 1950; Gottschalk, 1976; Masterson, 1994). Although established polyploid species are ubiquitous in the plant kingdom, a newly formed polyploid is immediately challenged by the lack of potential mates because it is reproductively isolated from its progenitors (Fowler and Levin, 1984). Thus, selection favors those traits that contribute to the reproductive success of the neopolyploid. Consequently, most plant polyploids are perennial in habit and/or possess a means of asexual reproduction, which may confer the ability to delay reproductive efforts in the absence of a suitable mate (Stebbins, 1940; Gustafsson, 1948). Likewise, a breakdown of self-incompatibility mechanisms frequently accompanies polyploidization, which precludes the need for a mate (Stebbins, 1957; de Nettancourt, 1977; Grant, 1981; Chawla et al., 1997; Richards, 1997; Ramsey and Schemske, 1998). Stebbins (1950) noted that polyploidy in annual plants, which is uncommon, would invariably be restricted to those taxa that were self-compatible. A few autogamous annual polyploids were identified by Grant (1956), including members of *Clarkia* (Onagraceae), *Gilia* (Polemoniaceae), *Mentzelia* (Loasaceae), and *Microseris* (Asteraceae). Several studies have shown that pre-

dominantly selfing plant species, particularly autogamous species, typically have flowers that are drab in color and reduced in size relative to their xenogamous counterparts (Richards, 1997). Like autogamous diploid species, primarily autogamous polyploids may exhibit reduced flower sizes (Lewis and Lewis, 1955; Grant, 1956; Brochmann, 1993). However, this reduction in flower size appears to contradict one of the central tenets of polyploidy, namely, that polyploids are “bigger” than their diploid relatives. Genome doubling typically results in an increase in cell size in polyploids relative to the diploid progenitors (Stebbins, 1950). In plants, the sizes of two nonendoreduplicating cell types, guard cells and pollen grains (Nagl, 1978), are commonly used as proxies for ploidal level in closely related diploid and polyploid species. Occasionally, pollen grain sizes in diploids and polyploids may overlap, making accurate inferences of ploidal levels difficult in some cases (e.g., Lewis, 1980; Small, 1983; Butterfass, 1987; Brochmann, 1992).

Here we examine the potential interplay between cell size (pollen grains) and breeding system in the tetraploid species of *Tarasa* (Malvaceae), which represent another group of annual polyploids. In a recent molecular phylogenetic analysis using both chloroplast and nuclear sequence data, we inferred multiple origins of the polyploid species of *Tarasa* (Tate and Simpson, 2003). Molecular and geographic data indicated that the *Tarasa* polyploids (at least eight species) are allopolyploids likely derived from different pairs of sympatric diploid annual species, although exact parentage for all tetraploids could not be assigned (Tate and Simpson, 2003). Previous phylogenetic investigations have demonstrated that multiple origins are common for a single polyploid species (Doyle et al., 1990, 1999; Brochmann et al., 1992; Cook et al., 1998; Seagraves et al., 1999; Soltis and Soltis, 1999; Sharbel and Mitchell-Olds, 2001). However, the polyphyly of the *Tarasa* tetraploids was particularly unexpected because of their unusual habit and strikingly similar floral morphologies. All are sprawling to erect annuals (up to 0.5 m in height) with white or pale lavender flowers, petals 1–3 mm in length, anthers

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<sup>2</sup> Current address: Florida Museum of Natural History, University of Florida, P.O. Box 117800, Gainesville, Florida 32611 USA; E-mail: jtate@flmnh.ufl.edu.

TABLE 1. Floral and breeding system characteristics of *Taraxacum* species. *N* is the number of populations sampled. Habit is abbreviated for the species: "A" denotes annuals and "P" perennials. Means and standard deviations (in parentheses) are shown for the quantitative characters measured. Breeding system abbreviations: X = xenogamous, FX = facultatively xenogamous, FA = facultatively autogamous, OA = obligately autogamous. The species are sorted first by habit and second by chromosome number.

Species	<i>N</i>	<i>2n</i>	Habit	Petal color	Petal length (mm)	Grains per anther	Polar diameter of pollen grain (µm)	Equatorial diameter of pollen grain (µm)	Anthers per flower	Ovules per flower	P/O ratio	Log P/O ratio	Breeding system
<i>T. albertii</i> Phil.	2	10	P	Magenta	10-15	309.83 (47.85)	37.93 (0.24)	40.65 (1.27)	55 (4)	10 (0)	1704.08	3.23	X
<i>T. capitata</i> (Cav.) Bates	3	10	P	Lilac	5-15	350.00 (111.66)	39.90 (1.61)	42.13 (2.18)	47 (13)	13 (3)	1265.38	3.10	X
<i>T. humilis</i> (Gillies ex Hook. et Arn.) Krapov.	2	10	P	Magenta	8-10	322.50 (18.15)	39.61 (0.97)	42.96 (1.00)	69 (2)	12 (0)	1840.94	3.27	X
<i>T. operculata</i> (Cav.) Krapov.	2	10	P	White	8-10	376.17 (156.27)	42.49 (4.52)	45.21 (4.21)	76 (8)	11 (1)	2722.73	3.44	X
<i>T. thyrsoides</i> Krapov.	2	10	P	Lilac	10	441.50 (20.98)	39.12 (6.44)	41.65 (7.52)	102 (9)	13 (1)	3584.98	3.55	X
<i>T. rhombifolia</i> Krapov.	1	?	P	Lilac	9-10	265.33	38.15 (2.21)	40.67 (2.00)	40	10	1061.33	3.03	X
<i>T. cardenasii</i> Krapov.	2	10	A	Lilac	2-3	143.50 (8.25)	41.55 (0.01)	44.73 (1.51)	24 (2)	10 (1)	337.23	2.53	FX
<i>T. heterophylla</i> (Gris.) Krapov.	2	10	A	Lilac	5-7	305.33 (60.81)	36.15 (0.64)	39.04 (0.27)	38 (2)	9 (1)	1272.22	3.10	X
<i>T. meyeri</i> Krapov.	2	10	A	Lilac	8-10	258.17 (5.42)	40.22 (1.21)	42.88 (1.01)	30 (0)	12 (1)	673.48	2.83	FX
<i>T. irsecta</i> (Gris.) Krapov.	2	10	A	Lilac	5-7	382.50 (20.03)	38.43 (1.03)	41.91 (2.70)	60 (0)	9 (1)	2086.36	3.32	X
<i>T. antefegastana</i> (Phil.) Krapov.	5	20	A	White	3-4	102.20 (43.29)	40.19 (3.10)	43.37 (3.51)	15 (4)	10 (2)	155.43	2.19	OA
<i>T. cerrateae</i> Krapov.	2	20	A	White	1.5-2	159.00 (37.24)	39.90 (1.67)	43.13 (2.85)	10 (0)	10 (3)	159.00	2.20	OA
<i>T. geranioides</i> (Cham. et Schl.) Krapov.	1	20	A	White	1.5-2	32.00	34.34 (1.67)	37.16 (2.67)	5	9	17.78	1.25	OA
<i>T. nototrichooides</i> Krapov.	2	20	A	White	1-2	227.33 (51.38)	38.06 (0.43)	40.17 (1.37)	5 (0)	9 (1)	126.30	2.10	OA
<i>T. odonellii</i> Krapov.	2	20	A	White	2-3	59.50 (9.19)	35.26 (1.58)	38.88 (2.06)	5 (0)	10 (2)	31.32	1.50	OA
<i>T. tarapacana</i> (Phil.) Krapov.	3	20	A	White	1.5-2	136.33 (37.55)	32.44 (2.88)	36.52 (1.95)	9 (1)	8 (0)	159.06	2.20	OA
<i>T. tenella</i> (Cav.) Krapov.	2	20	A	White	1.5-2	110.17 (47.38)	35.16 (2.10)	38.42 (2.49)	8 (4)	10 (1)	86.97	1.94	OA
<i>T. tenuis</i> Krapov.	2	20	A	Lilac	6-7	198.83 (100.17)	34.46 (5.32)	37.11 (5.11)	19 (5)	8 (1)	490.46	2.69	FA/FX
<i>T. ur-baniana</i> (Ulbr.) Krapov.	2	20	A	White	1.5-2	92.50 (86.03)	37.99 (0.50)	40.51 (0.06)	7 (3)	8 (1)	86.33	1.94	OA
<i>T. congestiflora</i> (L.M. Johnston) Krapov.	2	?	A	White	2.5-3	70.50 (14.38)	33.31 (2.11)	36.00	10 (0)	8 (0)	88.13	1.95	OA
<i>T. corrugata</i> Krapov.	2	?	A	Pink	2.5-3	123.83 (69.55)	38.57 (1.20)	40.76 (1.78)	17 (5)	7 (1)	291.89	2.47	FA/FX
<i>T. latearistata</i> Krapov.	1	?	A	Lilac	7-8	348.00	38.88 (2.01)	40.89 (1.76)	40	10	1392.00	3.14	X
<i>T. marinii</i> Krapov.	1	?	A	White	6-7	245.67	32.99 (1.07)	36.30 (0.83)	20	8	614.17	2.79	FA/FX
<i>T. pediculata</i> Krapov.	2	?	A	White	2-3	62.50 (6.84)	40.08 (1.32)	44.00 (2.83)	10 (1)	9 (1)	69.85	1.84	OA

numbering 5–20, and highly lobed leaves. The diploid species, in contrast, are morphologically distinct from one another. They are annuals (up to 1 m) or perennials (up to 1.5 m) typically with lavender or magenta flowers, petals 5–10 mm in length, anthers numbering 30–100, and shallowly divided leaves. The *Tarasa* polyploids are also unusual because some were found to have smaller pollen grains than the diploid species sampled in a palynological study conducted by Tressens (1970). Thus, the *Tarasa* tetraploids contradict two main tenets of polyploids: they are annuals and have smaller pollen grains than their diploid relatives.

For this study, our objectives were to determine if pollen size was consistent across the ploidal levels (as suggested by the earlier work of Tressens, 1970) and if pollen size was correlated with breeding system and associated floral characteristics.

## MATERIALS AND METHODS

**Pollen grain measurements**—Using light microscopy, pollen grains were measured for 24 of 27 species of *Tarasa*, including four documented diploid ( $2n = 10$ ) annuals, five diploid perennials, and eight tetraploid ( $2n = 20$ ) annuals (Krapovickas, 1954, 1960; Fernandez, 1974; Tate, 2002) (Table 1). Measurements were also taken for six other annual species and one perennial species for which chromosome numbers are unknown (Table 1). Herbarium specimens were utilized for all pollen studies. For each species, at least one individual was sampled and when possible, two or more individuals from separate populations were sampled to account for geographic variability. The pollen grains from a single anther were washed for 15 min in 800  $\mu$ L acetone to remove any residual pollenkitt, centrifuged, and allowed to oven dry after decanting the acetone. Grains were stored in 80% ethanol. A drop of pollen/ethanol mixture was placed on a slide and the ethanol evaporated. The grains were mounted in acid fuchsin-stained glycerin jelly. Twenty-five grains per individual were evaluated for size, shape, and gross morphological features. The measurements of the grains excluded the spines and were recorded for both polar and equatorial diameters. Pollen diameters (polar axis) were used to calculate surface area to allow comparison with other studies.

A subset of taxa was selected for a scanning electron microscopy (SEM) study to represent the morphological diversity among the species. The SEM preparation consisted of placing a droplet of pollen/ethanol suspension on a conductive carbon tab (Electron Microscopy Sciences, Fort Washington, Pennsylvania, USA) mounted on an SEM stub and allowing the ethanol to evaporate (at 60°C). The samples were sputter coated on a Ladd Benchtop Sputter Coater (Ladd Research, Winston, Vermont, USA) with 60/40 Ag/Pd target at 2.5 kV (20 mA) for 75 s (coating thickness of 15 nm). Grains were examined on a Philips 515 scanning electron microscope (FEI Company, Eindhoven, The Netherlands) at 13 kV, bias set to 2, spot size of 40 nm, and a working distance of 30 mm (tilt approximately 15°). Digital images were captured with a SEMICAPS (San Jose, California, USA) 2000 system (Microscopy Laboratory, the Institute for Cellular and Molecular Biology, The University of Texas at Austin).

**Determination of breeding systems**—For the same species studied for pollen size, we also inferred breeding system indirectly from pollen/ovule ratios (Cruden, 1977), and for some, we directly studied the breeding system with greenhouse manipulations. Pollen/ovule (p/o) ratios have traditionally been used as a conservative indicator of the relative degree of outcrossing vs. selfing in plants (Cruden, 1977). In many unrelated plant taxa, pollen/ovule ratios have been shown to be negatively correlated with the amount of self-fertilization, pollen size, and the ratio of the stigmatic area to the pollen-bearing area on the pollinator (Cruden, 1977, 2000; Cruden and Miller-Ward, 1981; but see Barrett et al., 1996). To determine the number of pollen grains per flower, the most mature, unopened bud on an inflorescence was selected. For each individual, the numbers of anthers and styles (equal to the number of ovules in *Tarasa*, Krapovickas, 1954) in a single flower were recorded.

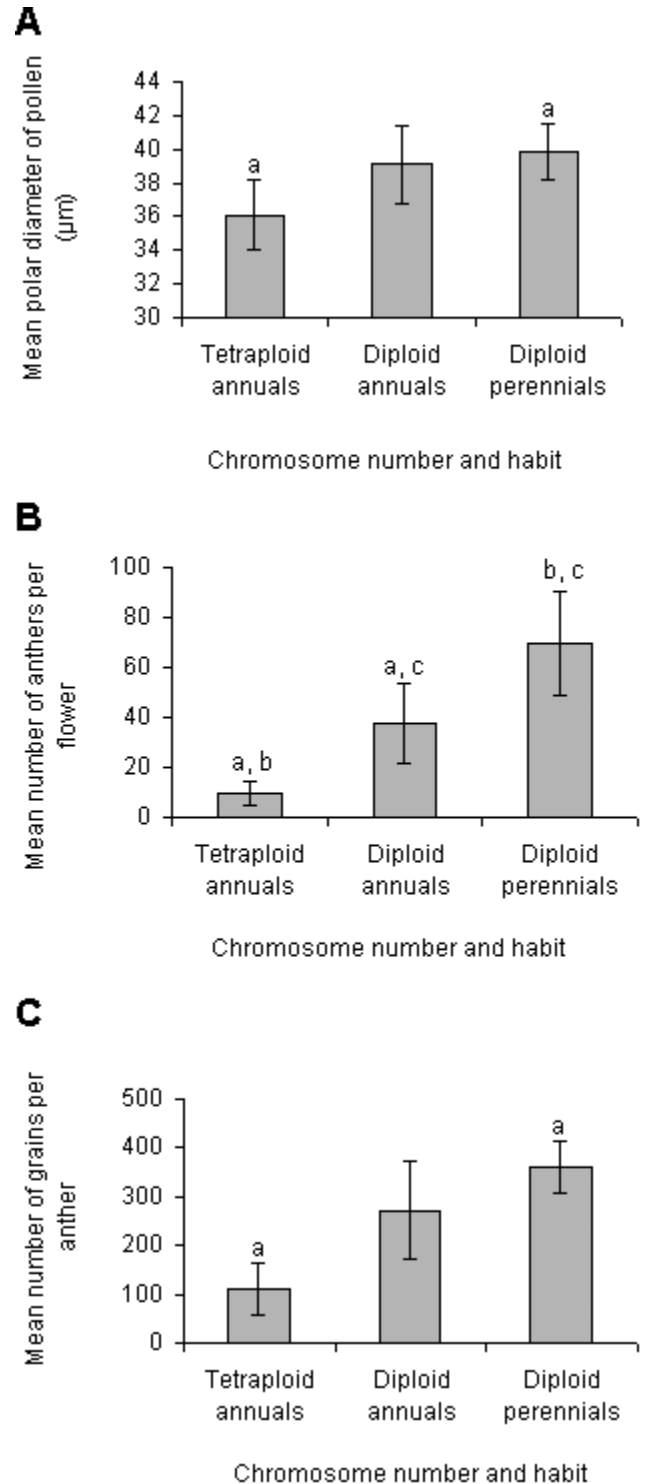


Fig. 1. Histogram of pollen measurements and breeding system variables for species of *Tarasa* (known chromosome number only). Species were categorized according to ploidal level and habit. A superscript letter indicates statistically significantly different pairs (see Table 3). (A) Pollen grain measurements (polar axis). (B) Mean number of anthers per flower. (C) Mean number of grains per anther.

TABLE 2. Means (and standard deviations) for pollen grain size and breeding system variables in *Tarasa* species when grouped according to ploidal level and habit (known chromosome number only). Significance values (ANOVA) are shown for each category except Log P/O ratio when the species were categorized by ploidal level or ploidal level and habit.

Category	Polar diameter of pollen grains ( $\mu\text{m}$ )		Equatorial diameter of pollen grains ( $\mu\text{m}$ )		Anthers per flower		Pollen grains per anther		P/O ratio		Log P/O ratio
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Diploid annuals	39.087	(2.34)	42.14	(2.38)	38	(16)	272.37	(100.04)	1092.32	(767.26)	3.04
Diploid perennials	39.81	(1.68)	42.52	(1.72)	70	(21)	360.00	(52.27)	2223.62	(926.96)	3.35
Tetraploid annuals	36.56	(1.66)	39.44	(1.35)	10	(5)	111.32	(53.42)	148.29	(149.13)	2.17
ANOVA	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	
Ploidal level (df = 2)	7.157	0.0060	8.611	0.0029	17.926	<0.0001	18.076	<0.0001	15.071	0.0002	
Ploidal level and habit (df = 3)	4.707	0.0165	5.462	0.0097	24.490	<0.0001	14.441	0.0001	14.588	0.0001	

The total number of pollen grains in an anther was counted for three anthers per flower. Because the time to anther maturity varies along the staminal column, one anther was selected from the top, middle, and bottom of the column. Each anther was macerated in 10  $\mu\text{L}$  of lactophenol-aniline blue (Kearns and Inouye, 1993) in a 0.5- $\mu\text{L}$  tube and then vortexed for 30 s. The pollen/lactophenol mixture was placed on a microscope slide, and all grains in that anther were counted at 200 $\times$  magnification on a Leitz microscope.

To calculate p/o ratios, the number of grains per anther was averaged for the three anthers. The mean number of grains per anther was then multiplied by the total number of anthers in the flower examined, and this was divided by the number of ovules per flower. Pollen/ovule ratios were log transformed in order to compare them to Cruden's (1977) extensive breeding system data set and to infer breeding system for each species. Although Cruden (1977) did not specifically state why he log transformed the p/o ratios, presumably this was to facilitate comparisons among the breeding system categories and among species from diverse angiosperm families. We also calculated the Pearson product-moment correlation coefficient to determine the strength of association between pollen grain size and p/o ratio.

Greenhouse studies were conducted to directly study breeding system for some species of *Tarasa*. Plants were grown in a greenhouse at The University of Texas at Austin from seed collected in the field or sampled from herbarium specimens. The study included five diploid perennials (*Tarasa albertii*, *T. capitata*, *T. humilis*, *T. operculata*, and *T. thyrsoidea*), one diploid annual (*T. trisecta*), and six tetraploid annuals (*T. antofagastana*, *T. geranioides*, *T. odonellii*, *T. tarapacana*, *T. tenella*, and *T. urbaniana*). Plants were allowed to self-pollinate without any mechanical manipulation. If the plants did not produce selfed seeds, the flowers were manually self-pollinated. In order to test for apomixis, diploid flowers were emasculated before the buds opened, but attempts to emasculate buds of the tetraploids proved unsuccessful because the minute flowers did not mature after handling. Thus, for the tetraploid annuals we were unable to determine if seed set was the result of selfing or apomixis. The number of fully mature seeds per schizocarp was counted for 50 fruits on a single plant. Seed set was calculated as the proportion of mature seeds produced relative to the total possible.

**Chromosome numbers and statistical analyses**—For statistical analyses, species of *Tarasa* were grouped according to ploidal level or ploidal level and habit. Previously published chromosome counts were available for 17 of the 27 species of *Tarasa* (Krapovickas, 1954, 1960; Fernandez, 1974; Tate, 2002). The data presented here include only those species for which chromosome number is known, although additional analyses were conducted with

all species included (chromosome number inferred based on morphology) (Tate, 2002). Single-factor ANOVA was first conducted to test significance of mean pollen size, anther number, grains per anther, and pollen/ovule ratio. Bonferroni/Dunn post-hoc ANOVA was used to determine the source of significance.

## RESULTS

**Pollen grain size in diploid and tetraploid species of *Tarasa***—Within a species, the pollen grain size typically varied only a few micrometers (Table 1). The mean polar diameter in the diploid annual taxa ranged from 36.15 to 41.55  $\mu\text{m}$ , the diploid perennials from 37.93 to 42.49  $\mu\text{m}$ , and the tetraploid annual species from 32.44 to 38.57  $\mu\text{m}$  (Fig. 1A). ANOVA detected significant differences for the categories of ploidal level (polar diameter  $F = 7.157$ ,  $df = 2$ ,  $P = 0.006$ ; equatorial diameter  $F = 8.611$ ,  $P = 0.003$ ) and ploidal level and habit (polar diameter  $F = 4.707$ ,  $df = 3$ ,  $P = 0.016$ ; equatorial diameter  $F = 5.462$ ,  $P = 0.010$ ) (Table 2). Diploid perennials and tetraploid annuals differed significantly in pollen grain size under Bonferroni/Dunn post-hoc ANOVA (polar diameter  $P = 0.0057$ , equatorial diameter  $P = 0.0054$ ) (Table 3). While smaller, the tetraploid annuals were not significantly different in pollen size from the diploid annuals, nor were the diploid annuals significantly different from the diploid perennials (Table 3, Fig. 2).

**Breeding systems in *Tarasa***—Within *Tarasa*, the diploid perennials exhibited the highest p/o ratios with the mean of the species ranging from 1061 to 3584 (indicating xenogamy when compared to Cruden's [1977] data set). The diploid annual species ranged in mean p/o ratio from 673 to 2086 (suggesting xenogamy to facultative xenogamy). The tetraploid annuals had the lowest p/o ratios, 18–490, indicating autogamy or facultative xenogamy (Table 1). Contributing to this lower p/o ratio in the tetraploids is not only the presence of fewer anthers per flower, but also fewer grains produced per anther than the diploids (Fig. 1B, C). Anther number differed significantly between the diploid annuals and perennials ( $P =$

TABLE 3. Results of Bonferroni/Dunn post-hoc ANOVA. An asterisk (\*) indicates  $P < 0.05$  level.

Comparison	Mean polar diameter	Mean equatorial diameter	Anther number per flower	Number of grains per anther	P/O ratio
Diploid annuals, diploid perennials	0.5521	0.7382	0.0032*	0.1271	0.0349
Diploid annuals, tetraploid annuals	0.0339	0.0183	0.0039*	0.0117	0.0511
Diploid perennials, tetraploid annuals	0.0057*	0.0054*	<0.0001*	0.0002*	0.0002*

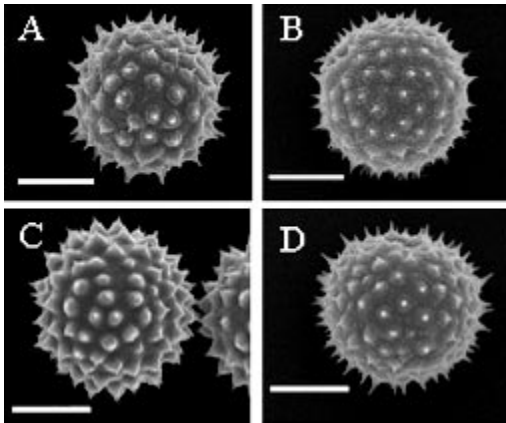


Fig. 2. (A–D) Representative scanning electron micrographs of diploid and tetraploid pollen grains (scale bar equals 20  $\mu\text{m}$ ). (A) *Tarasa geranioides* (tetraploid annual). (B) *T. urbaniana* (tetraploid annual). (C) *T. meyeri* (diploid annual). (D) *T. albertii* (diploid perennial).

0.0032), the diploid annuals and tetraploids ( $P = 0.0039$ ), as well as the diploid perennials and tetraploids ( $P < 0.0001$ ) under Bonferroni/Dunn (Table 3). The number of grains per anther was significantly different only between the documented diploid perennials and tetraploids ( $P = 0.0002$ ) (Table 3). A significant positive correlation between pollen size (as surface area) and p/o ratio was found for all species examined (Pearson product-moment correlation coefficient  $r = 0.663$ ,  $df = 22$ ,  $P < 0.01$ ; Fig. 3).

The inferred breeding system based on p/o ratios is consistent with our greenhouse studies. In the greenhouse, none of the diploid perennials (five species studied) were self-compatible. Only one diploid annual species was available for study; it was autogamous toward the end of its life cycle, but the resulting progeny were weak. All six tetraploid species studied were autogamous and produced vigorous offspring. In the tetraploids, autogamous seed set ranged from 0.42 (SD = 0.20) to 0.87 (SD = 0.14) per flower.

## DISCUSSION

**Breeding system evolution in *Tarasa***—Following polyploidization, a shift from an outcrossing to a selfing breeding system has been shown empirically in many taxa (Stebbins, 1950; de Nettancourt, 1977; Grant, 1981; Richards, 1997). This switch occurs in species with a single-locus gametophytic self-incompatibility (GSI) system and is not known to occur in species with multi-genic GSI or sporophytic self-incompatibility (SSI) systems (de Nettancourt, 2001). The genetic SI system in the Malvaceae has not been characterized and appears to be ambiguous based on traditional stigmatic (dry vs. wet) and pollen (binucleate vs. trinucleate) characteristics (Heslop-Harrison and Shivanna, 1977). Many genera, including *Gossypium*, *Malva*, and *Sida*, have primarily self-compatible species, while other genera may contain self-compatible and self-incompatible species (East, 1940). One of the few Malvaceae genera that has been investigated in detail, *Theobroma cacao* (subfamily Sterculioideae), apparently has a mixed GSI-SSI system (Knight and Rogers, 1955; Cope, 1962). Based on our present findings of a change from outcrossing to selfing that is associated with polyploidy, we hypothesize that a GSI system is operating in the *Tarasa* diploid species.

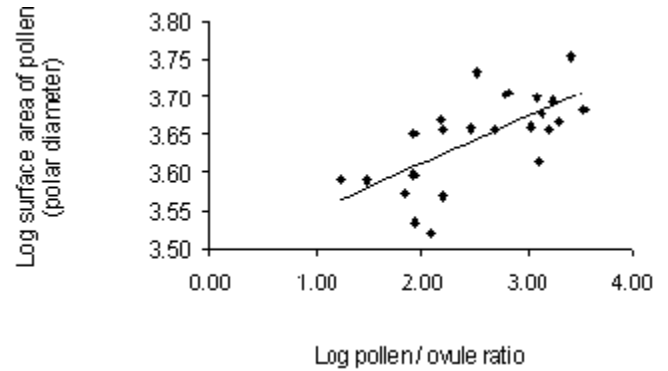


Fig. 3. Correlation between pollen grain size (as surface area) and pollen/ovule ratio in all species of *Tarasa* studied. A significant positive correlation exists between the two variables (Pearson product-moment correlation coefficient [ $r$ ] = 0.663,  $df = 22$ ,  $P < 0.01$ ).

Keeping in mind that a correlation is not equivalent to a causation, we propose a model for breeding system evolution in *Tarasa* and suggest possible influences for the evolution of smaller pollen in the polyploid species. In the evolutionary history of the *Tarasa* species, a shift from a perennial to an annual habit likely occurred and was followed (or accompanied) by increased self-compatibility in the diploid annuals. This trend is supported by the results presented here, as well as previous molecular phylogenetic data (the perennial species occupy a basal position within the genus and the annuals are derived) (Tate and Simpson, 2003). The diploid perennial species possess floral characteristics typical of outcrossing species, including relatively large, showy flowers and high p/o ratios. The diploid annual species also display showy flowers, often with nectar guides and the styles exerted well beyond the anther mass. Although predominantly outcrossing, the diploid annual species may possess a “leaky” self-incompatibility system (Levin, 1996) that allows for occasional selfing. After the formation of the tetraploid species (from the diploid annuals), autogamy probably became the predominant breeding system, and those morphological features typical of autogamous taxa (inconspicuous flowers, few anthers, and few grains produced per anther) likely evolved in parallel.

Detailed information on pollinators for *Tarasa* species is lacking. However, males of *Colletes* sp. (Colletidae) have been observed on *Tarasa thyrsoides* (diploid perennial), males of *Diadasia* sp. on *T. albertii* (diploid perennial), and males of *Callonychium* sp. on *T. tenella* (tetraploid annual) (J. Neff, Central Texas Mellitological Institute, personal communication). Males and females of an *Anthrenoides* sp. were also seen visiting *T. tenella* and *T. antofagastana*, and the females were collecting pollen (J. Neff, personal communication).

**Evolution of small pollen size in *Tarasa* polyploids**—Pollen grain size is not strictly correlated with ploidal level in *Tarasa* species (Fig. 2). Instead, our data show a significant positive correlation between pollen size (as surface area) and pollen/ovule ratio for all species examined (Fig. 3). This result suggests that selection for an overall reduced morphology, including pollen grain size, may have occurred and that the unusual small cell size in the polyploids may be a by-product of selection for an autogamous breeding system. Like other polyploids investigated, *Tarasa* tetraploids have guard cells statistically significantly larger than their diploid relatives (J.



Fig. 4. Greenhouse-grown plants (scale bar equals 1 cm). (a) *Tarasa antofagastana*, a tetraploid annual, with (b) a putative diploid annual parent, *T. meyeri* (right).

McDill, J. Tate, and B. Simpson, unpublished data). We therefore suggest that in *Tarasa*, a complex of selective forces has led to a remarkable convergence on reduced floral morphology across the independently generated polyploids (Fig. 4). One of these factors was likely the movement from lower elevation habitats into the cold, dry, and highly irradiated environment of the high central Andes. It is well known that plants at very high elevations have reduced morphological features (Clausen et al., 1940; Hedberg, 1964; Cabrera, 1968). In *Tarasa*, novel genetic combinations resulting from allopolyploidization may have allowed the tetraploids to colonize the high Andean habitats that were not previously occupied by the diploid species. Because the highest elevation habitats have only been available for colonization since the end of the last glaciation (Simpson, 1979; Clapperton, 1993), this migration must have been relatively recent. A switch from xenogamy to autogamy might have ensured reproductive success for the tetraploid annuals in the new environments where pollinators are comparatively rare (Arroyo et al., 1983, 1985) and the growing season short. This change in breeding system was probably also accompanied by the traditional loss of petal color, reduction in pollen production, and, as a result, a lower pollen/ovule ratio (Table 1).

Our findings suggest that the combination of severe environmental conditions at elevations up to 4000 m in the central Andes and a shift in breeding system overcame the increase in pollen cell size recorded in most instances of polyploidy, but not the increase in guard cell size. Polyploids found in similar selective regimes should be investigated to determine if other polyploids break the pollen cell size "rule."

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