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Pollination between maize and teosinte: an important determinant of gene flow in Mexico

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Abstract Gene flow between maize [*Zea mays* (L.)] and its wild relatives does occur, but at very low frequencies. Experiments were undertaken in Tapachula, Nayarit, Mexico to investigate gene flow between a hybrid maize, landraces of maize and teosinte (*Z. mays* ssp. *mexicana*, races Chalco and Central Plateau). Hybridization, flowering synchrony, pollen size and longevity, silk elongation rates, silk and trichome lengths and tassel diameter and morphology were measured. Hybrid and open-pollinated maize ears produced a mean of 8 and 11 seeds per ear, respectively, when hand-pollinated with teosinte pollen, which is approximately 1–2% of the ovules normally produced on a hybrid maize ear. Teosinte ears produced a mean of 0.2–0.3 seeds per ear when pollinated with maize pollen, which is more than one-fold fewer seeds than produced on a maize ear pollinated with teosinte pollen. The pollination rate on a per plant basis was similar in the context of a maize plant with 400–500 seeds and a teosinte plant with 30–40 inflorescences and 9–12 fruitcases per inflorescence. A number of other factors also influenced gene-flow direction: (1) between 90% and 95% of the fruitcases produced on teosinte that was fertilized by maize pollen were sterile; (2) teosinte collections were made in an area where incompatibility systems that limit fertilization

are present; (3) silk longevity was much shorter for teosinte than for maize (approx. 4 days vs. approx. 11 days); (4) teosinte produced more pollen on a per plant basis than the landraces and commercial hybrid maize; (5) teosinte frequently produced lateral branches with silks close to a terminal tassel producing pollen. Collectively these factors tend to favor crossing in the direction of teosinte to maize. Our results support the hypothesis that gene flow and the subsequent introgression of maize genes into teosinte populations most probably results from crosses where teosinte first pollinates maize. The resultant hybrids then backcross with teosinte to introgress the maize genes into the teosinte genome. This approach would slow introgression and may help explain why teosinte continues to co-exist as a separate entity even though it normally grows in the vicinity of much larger populations of maize.

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Introduction

Gene flow between maize (*Zea mays* ssp. *mays*) and teosinte (*Zea* spp.) occurs in Mexico and Central America, and maize × teosinte hybrids are recorded in archaeological archives (Wilkes 1977; Eubanks 2001). Models suggest that gene flow from a large maize population carrying genes with a selective advantage would ultimately swamp the teosinte genome (Rhymer and Simberloff 1996; Stewart et al. 2003). However, maize and teosinte have continuously co-existed for thousands of years, thereby raising questions about the degree and the direction of gene flow and factors that affect pollination and introgression (Kato 1997; Kato and Sanchez 2002).

There are seven recognized species of teosinte (Eubanks 2001). While gene-flow studies have been reported in *Zea mays* ssp. *mexicana* (Baltazar and Schoper 2001 and 2002), little information is available on sympatric maize and other non-studied teosinte populations from different regions. Such information would be invaluable

as well as essential in order to better understand the potential for gene flow from maize to teosinte in Mexico and Central America.

Gene flow between maize and teosinte (ssp. *mexicana*) is strongly influenced by genetics (Kermicle 1996). Evans and Kermicle (2001) demonstrated that when teosinte pollen is applied to maize silks hybrids are produced between the two sub-species. However, when teosinte was pollinated by maize, plants of *Zea mays* ssp. *mexicana* (Chalco and Central Plateau races) set seed very inconsistently or not at all. These researchers determined that incompatibility between teosinte and maize is under control of the teosinte crossing barrier 1 (*Tcb1*) gene located on the short arm of chromosome 4. Because of the absence of reciprocal pollination, Evans and Kermicle (2001) suggested that *Tcb1* “could play a significant role in reproductively isolating teosinte from maize” in areas of Mexico and Guatemala where teosinte and maize grow sympatrically. Kermicle and his colleagues noted that it is not known whether such incompatibility systems function reciprocally in situ.

Cross-incompatibility factors known as *gametophyte factors* (*ga*) are numerous in maize (Nelson 1996) and can limit gene flow. The impact of cross-incompatibility on gene flow in traditional maize cropping systems in Mexico, Central and South America is still unclear. Cervantes (1998) demonstrated that incompatibility factors play an important role in conserving landrace germplasm by preventing gene flow from other varieties that are grown in close proximity. Current knowledge indicates that most of the popcorns in the USA and many Mexican and Central American races carry *Gal*, while most North American dents and flints are *gal/gal* (Kermicle 1996).

Gene flow between maize and teosinte is also influenced by the biology of the pollen and silks, the environmental conditions and the physical isolation between the pollen source and potential receptor plants. Luna et al. (2001) studied isolation distance between maize plants in Tapachula, Nayarit and found that very limited cross-pollination occurred at 100 m and that no pollination occurred at distances beyond 200 m. Wind and atmospheric conditions at the time of pollen release into the atmosphere are critical factors affecting pollen movement and longevity (Baltazar and Schoper 2001 and 2002; Luna et al. 2001). Other researchers have shown that pollination could be as high as 60% between contiguous rows but that from more remote rows it decreased with increasing distance such that no outcrosses were found 32 m away from the pollen source (Castillo and Goodman 1997; Louette 1996; Cervantes 1998). Wind direction, turbulence and velocity affect pollen movement (Di-Giovanni and Kevan 1991; Di-Giovanni et al. 1995), and the physical features of the pollen itself influences pollen transport and deposition (Di-Giovanni et al. 1995).

The objective of the investigation reported here was to measure outcrossing rates and to estimate the basis for gene flow among commercial hybrid maize, Mexican

maize landraces and teosinte. Spatial and biological factors that could influence pollination, flowering synchrony, duration of the silk elongation rates, length and diameter of the silk and trichome, pollen longevity and tassel characteristics of the different species and varieties were also investigated. Collectively, these biological factors as well as environmental, physical and genetic factors all need to be understood to properly understand and manage gene flow.

Materials and methods

The study site

Experiments were conducted in Tapachula, Nayarit, Mexico, which is situated 20°49' N, 105°55' W at an elevation 26 m.a.s.l. This valley has fertile soils and available water and produces high-yielding maize (*Zea mays* ssp. *mays*). Experiments were conducted during the 1999–2003 winter cycles, and all plants were grown using standard, optimal cultural practices for maize production. Details on crop management are given by Luna et al. (2001). Teosinte (*Zea* spp.) has not been reported in this region (Sanchez et al. 1998). The maize and teosinte accessions used are listed in Tables 1 and 2. A typical teosinte plant showing multiple branches, tassels and ears is presented in Fig. 1.

Pollination between hybrid maize and teosinte

The experiment was organized as a randomized complete block design with three replications. Replications were blocked in time with one complete replication of treatments being completed per day. The experiment was planted on November 3, 1999 and on March 7, 2001. In both years, treatments consisted of 50 hand-made reciprocal crosses between a Pioneer commercial hybrid, P36D14, and two teosinte populations (*Z. mays* ssp. *mexicana*, races Chalco and Central Plateau). Both teosinte populations were collected and provided by Dr. Dulce Maria Arias, University of Morelos. Race Chalco teosinte was collected near Amecameca, Estado de Mexico, and the Central Plateau teosinte was collected near San Agustin del Maiz, Estado de Michoacan. Teosinte was selected for this study because it represents one of the closest relatives of *Z. mays*. These races were selected because maize and teosinte grow sympatrically in Amecameca and San Agustin del Maiz and because maize × teosinte hybrids occur in these regions.

Pollination between landraces and teosinte

A second experiment was conducted in the 2000–2001 season to measure the level of hybridization between each of the two teosinte populations and a commercial hybrid, a non-proprietary inbred and three open-pollinated

Table 1 Silk and pollen characteristics of teosinte (*Zea mays* ssp. *mexicana*) and maize (*Z. mays* ssp. *mays*)

Region	Number of accessions	Species	Race	Silk length (cm) ^a	Pollen diameter (μm) ^b	Collapsed pollen grains (%) ^b	
						10:00 a.m.	11:00 a.m.
Toluca	2	ssp. <i>mexicana</i>	Chalco	9	74	73	86
Puebla	7	ssp. <i>mexicana</i>	Chalco	11	79	62	91
Opopeo (Michoacan)	1	ssp. <i>mexicana</i>	Central Plateau	10	74	38	85
Puruandiro (Michoacan)	3	ssp. <i>mexicana</i>	Central Plateau	13	77	72	90
Cuitzeo (Michoacan-Guanajuato)	7	ssp. <i>mexicana</i>	Central Plateau	11	78	54	88
Churintzio (Michoacan)	7	ssp. <i>mexicana</i>	Central Plateau	9	75	68	86
La Barca-Poncitlan-San Jeronimo (Jalisco)	4	ssp. <i>mexicana</i>	Central Plateau	11	72	57	92
Durango	3	ssp. <i>mexicana</i>	Central Plateau	10	76	39	89
Nabogame (Chihuahua)	2	ssp. <i>mexicana</i>	Central Plateau	6	72	61	87
Hybrids (two temperate and one tropical)	2	ssp. <i>mays</i>	Maize	30	103	54	84
Improved open-pollinated varieties	3	ssp. <i>mays</i>	Maize	28	100	58	68
Landraces	11	ssp. <i>mays</i>	Maize	24	94	38	80
Overall mean	78	<i>Zea mays</i>		14	81	56	86
Mean by species and race	16	ssp. <i>mays</i>	Maize	27	99	50	77
	9	ssp. <i>mexicana</i>	Chalco	10	76	67	88
	27	ssp. <i>mexicana</i>	Central Plateau	10	75	55	88

^aFresh silks were collected in the morning hours to do the measurements, and the values presented are the mean of three replications

^bPollen was collected at 9:30 a.m. and exposed to atmospheric conditions for two different time periods. Pictures were taken and

the percentage of collapsed grains counted under the microscopy. Pollen samples collected at 9:30 were used to measure pollen size, and the values presented are the mean of three replications per accession

maize varieties, using a completely randomized block design with three replications. Treatments consisted of 50 handmade crosses in which the silks of the maize genotypes were pollinated with pollen of the two teosinte populations of *Z. mays* ssp. *mexicana*, races Chalco and Central Plateau. The open-pollinated populations POP 21, POP 502 and POP 902 were obtained from the International Maize and Wheat Improvement Center, CIMMYT, El Batan, Mexico. A Pioneer hybrid (P36D14) and a non-proprietary inbred genetic marker

stock containing the genetic markers R-r, C1, C2, A1, A2, Bz1, Bz2 and Pr were also included in the experiment as controls.

Crossability between a hybrid and landraces of maize

Two blocks consisting of white-seeded landraces and a yellow-seeded commercial maize hybrid, P3394, were used to estimate the level of cross-pollination. One block

Table 2 Tassel characteristics and flowering dates of hybrid, landraces and teosinte

Genotype	Number of branches per tassel ^a	Number of tassels per plant ^a	Number of spikelets per tassel ^a	Tassel weight (g) ^a	Tassel size (cm) ^a	Number of days to flowering ^b	
						Silking (50%)	Anthesis (50%)
P- 3394	6	1	769	5	32	64	66
Tuxpeño	32	1	2,762	20	32	84	82
Tabloncillo	12	1	817	8	28	59	57
Zapalote Chico	17	1	1,034	8	23	54	52
Bolita	14	1	786	9	31	58	56
Celaya	22	1	1,823	13	31	72	70
Palomero Toluqueño	10	1	1,639	11	30	70	68
Jala	37	1	2,688	22	30	82	79
Conico	5	1	1,026	10	26	61	59
POP 21	21	1	1,443	11	29	74	74
POP 502	16	1	1,263	10	28	72	71
POP 902	11	1	836	8	27	58	56
Teosinte	11	48	3,921	0.5–5	23	54	55
Standard error	0.85		68.2	0.47	0.51		

^aTen fresh randomly collected tassels per genotype were dried in an oven to determine tassel weight. After drying, tassel size, number of branches and number of spikelets per tassel were determined

^bFlowering dates were calculated when 50% of the population were at the silking or anthesis stage. The experiment was planted in 1999–2000 and 2000–2001



Fig. 1 Teosinte (*Zea mays* ssp. *mexicana*) plant showing multiple branches and tassels

contained nine landraces (Tuxpeño, Tabloncillo, Zapalote Chico, Bolita, Celaya, Chalqueño, Palomero Toluqueño, Jala, Conico) and the three improved open-pollinated populations (POP 21, POP 502, and POP 902). These landraces represent the most important racial groups in Mexico (Wellhausen et al. 1952). The second block contained P3394. Both blocks were sown on December 14, 2001. Each landrace was planted in plots of five 5-m-long rows, with a between-row spacing of 0.8 m. P3394 was planted in plots of 80 5-m-long rows, with a between-row spacing of 0.8 m. The plots were isolated in time from other pollen sources. Yellow-seeded P3394 wind-pollinated silks of the open-pollinated varieties and landraces. At harvest, ten randomly selected ears were harvested from each of the white-seeded landraces. The ears were shucked and the number of white and yellow seeds determined. Plots were monitored for synchrony between silking of the landraces and pollen shed of the hybrids.

Experiments investigating silk elongation rates, silk and trichome length and silk diameter

Silk elongation was measured as an estimate of ovule viability in an experiment sown on December 14, 2001 as a randomized complete block design with ten replications.

The replications were blocked in time with one complete replication of treatments being completed per day. Silk elongation per day and total growth were measured over a 10-day period for ten randomly chosen individual plants from a hybrid, each landrace and a teosinte (ssp. *mexicana*, race Chalco). Ear shoots were covered with shoot bags prior to silk emergence to prevent pollination. Twenty to thirty silks per plant were tied with a thread, and silk elongation was measured daily from the apex of the husk sheath to the apex of the silks. Silk growth was recorded in the morning and afternoon to determine silk growth during the day versus the night.

The length and diameter of the silks and trichomes were measured on eight plants of each of three Chalco and four Central Plateau teosinte populations. One to two silks were collected from each plant, and the measurements were carried out using a Zeiss Axiostar Plus microscope (Carl Zeiss, Germany) and the pictures taken with a Zeiss Axiocam MRc (1300×1030) camera and Zeiss AXIOVISION 4.1 software.

Pollen size and longevity and tassel characteristics

Experiments to determine pollen longevity and pollen size were planted on December 14, 2001 and February 1, 2003. In the first season, pollen size and longevity were determined for 12 landraces and two teosinte populations. In the second season, experiments included 12 landraces and 36 teosinte populations. A Pioneer hybrid, P3394, was utilized as a control in both experiments. Pollen was collected at 9:30 a.m. and allowed to air-dry under natural conditions. The visual appearance of the pollen was recorded at 10:00 a.m. and at 11:00 a.m. Occasionally, the pollen was also observed at 12:30 p.m. to record changes in color and shape as an indication of pollen viability loss (Luna et al. 2001). Pollen size was measured using a Leica DME microscope (Leica Microsystems, Buffalo, N.Y.) fitted with a 40× magnification lens and a digital camera (Leica model ICCA) with Leica software [IMAGE MANAGER 500 (IM 500)]. The number of pollen measurements for each population ranged from 3 to 21.

Tassel densities at anthesis were calculated as follows: ten tassels per genotype were randomly collected within each plot, weighed and then placed in an oven at 40°C until the tassels were completely dry. Tassel dry weight, tassel size, number of primary branches per tassel, number of tassels per plant and number of spikelets per tassel were recorded for each tassel. One-way ANOVAs were calculated for each trait.

Results

Pollination between hybrid maize and teosinte

Analysis of variance of reciprocal crosses between maize × teosinte showed significant differences for seed

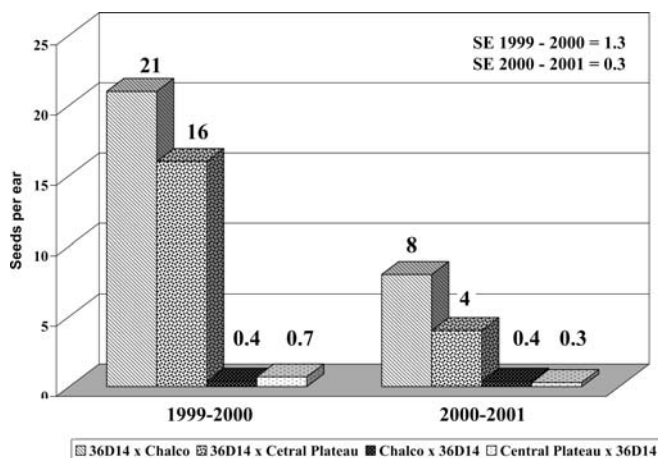


Fig. 2 Gene flow between a yellow-seeded maize hybrid (36D14) and two teosinte (*Z. mays* ssp. *mexicana*) races, Chalco and Central Plateau, as indicated by the formation of seeds in reciprocal crosses. Individual plant data were used for the analysis. The number of seeds is the average of four replications, and the experiments were conducted during the 1999–2000 and 2000–2001 crop cycles. Standard errors (*SE*) of the mean kernel number per plant were 1.3 for 1999–2000 and 0.3 for 2000–2001

number in both seasons. In general, seed production was very limited in both years, with more seeds being produced in 1999–2000 than in 2000–2001 (Fig. 2). The mean number of seeds per plant in each season was 13 and 3, respectively. A mean of 12 seeds per ear were produced from crosses between P36D14 as the female parent with pollen from teosinte; this contrasted with the 0.5 seeds per ear obtained when teosinte silks were pollinated by maize. When the silks of teosinte races Chalco and Central Plateau were pollinated with pollen of hybrid P36D14, 90–95% of the fruitcases that were produced were sterile.

Pollination between landraces and teosinte

When the commercial hybrid 36D14, an inbred carrying purple marker genes, landraces and improved open-pollinated varieties were pollinated with teosinte pollen, the resulting hybrids produced significantly different numbers of seeds per ear. The mean for individual ears was only 7.6 seeds per ear. The hybrids of POP 21, POP 502 and POP 902 pollinated with teosinte pollen race Chalco and POP 502 pollinated with teosinte race Central Plateau were not statistically different from each other with respect to seed number, with 10.4, 10.5, 10.3, and 10.5 seeds per ear, respectively (Fig. 3). Significantly lower seed numbers (5.1 and 2.8 seeds per ear), resulted when POP 21 and POP 902 were pollinated with teosinte race Central Plateau. The average number of seeds per ear in crosses involving silks of the purple marker inbred pollinated with teosinte race Chalco and race Central Plateau pollen were 6.0 and 9.4, respectively. When silks of 36D14 were pollinated with teosinte pollen race Chalco and Central Plateau, the resulting hybrid

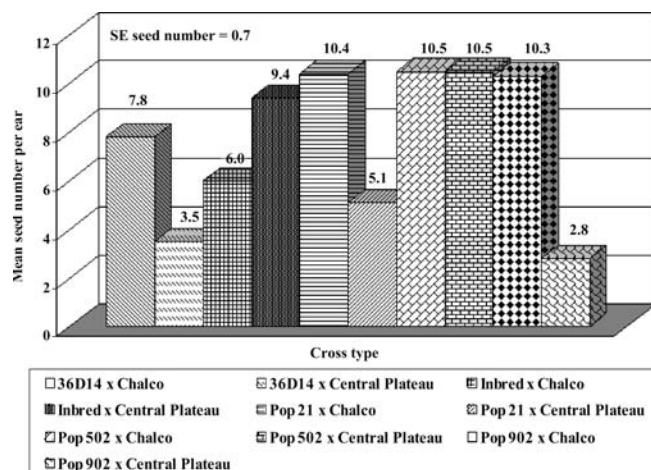


Fig. 3 Gene flow among a yellow-seeded maize hybrid (36D14), an inbred, landraces and two teosinte (*Z. mays* ssp. *mexicana*) races, Chalco and Central Plateau, as indicated by the formation of seeds. Silks of the hybrid, inbred and landraces were pollinated with pollen from each teosinte race separately. The number of seeds represents the average of four replications, and the experiments were conducted during 2001. The standard error (*SE*) for the mean kernel number was 0.7

produced, on average, 7.8 and 3.5 seeds per ear, respectively.

Pollination between hybrid maize and landraces

The number of yellow seeds produced on the white-seeded landraces and the open-pollinated varieties due to outcrossing from P3394 are shown in Fig. 4. Overall

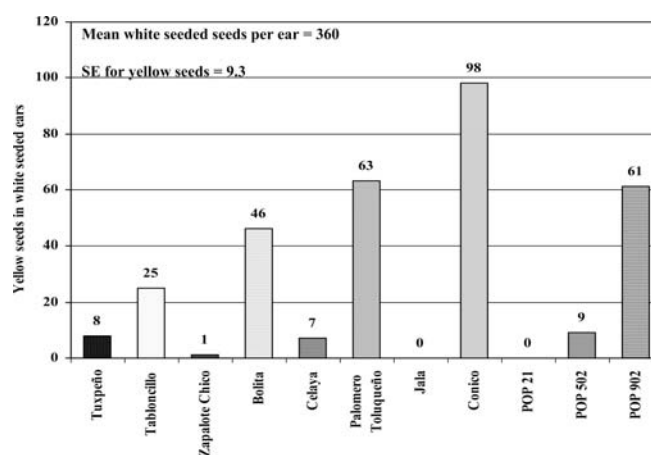


Fig. 4 Cross-pollination frequency between pollen of a yellow-seeded hybrid, P3394, and silks of 12 white-seeded Mexican landraces. A block containing all 12 landraces was planted directly alongside a block containing the P3394 hybrid in the 2001–2002 experiments. The landraces and the yellow-seeded hybrid were allowed to pollinate freely, and the appearance of yellow seeds on white-seeded ears indicated cross-pollination. The mean number of white seeds was 360 per ear. Plots were monitored for synchrony between silking of the landraces and pollen shed of the hybrids. Standard error (*SE*) of the mean for number of seeds is 9.3

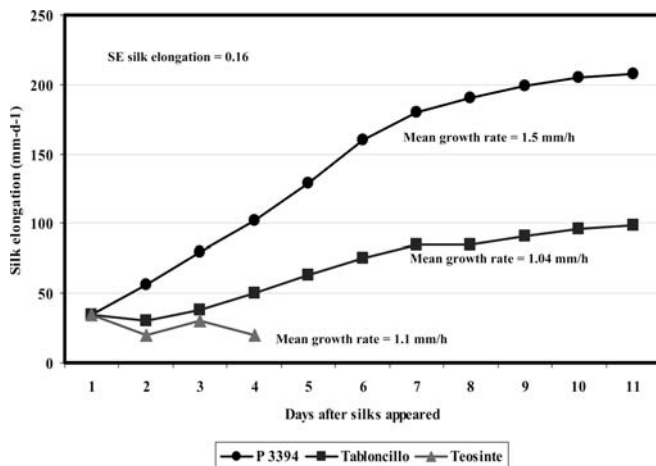


Fig. 5 Silk elongation rates of a yellow-seeded maize hybrid (P3394), a landrace (Tabloncillo) and teosinte (*Z. mays* ssp. *mexicana*, race Chalco). Measurements of silk elongation rates were made on ten individual plants selected at random from each landrace, hybrid or teosinte population. Silk elongation was measured daily as the growth of the point where silks were tied to the top of the outer leaf of the husks in the 2001–2002 experiment. Mean silk elongation rates were 1.04 mm/h for Tabloncillo, 1.1 mm/h for teosinte and 1.5 mm/h for P3394. Standard error (SE) for mean silk elongation rate was 0.16 mm/h

seed set, including both white and yellow seeds, in the experiment averaged 360 seeds per ear. Landraces varied in the number of yellow seeds that were produced. Conico had the largest number of yellow seeds, 98, followed by Palomero Toluqueño (63), POP 902 (61), Chalqueño (50), Bolita (46) and Tabloncillo (25). Landraces POP 502, Tuxpeño, Celaya and Zapalote Chico had 9, 8, 7 and 1 yellow seeds, respectively. POP 21 and Jala each had no yellow seeds. A seed production of more than ten seeds per ear occurred primarily only in those landraces that flowered within a window that extended from 8 days before to 4 days after 50% pollen shed by the yellow-seeded P3394. Low levels of outcrossing and fewer than nine seeds per ear were found for those populations that deviated to a larger extent in the timing of silk emergence relative to pollen shed by P3394.

Elongation rate and length of the silks, pollen size and longevity and tassel characteristics

Silk elongation data for P3394, Tabloncillo and teosinte are presented in Fig. 5. Teosinte silks elongated at a rate of 1.1 mm/h, which is comparable to that of Tabloncillo (1.04 mm/h), while the elongation rate of P3394 was 1.5 mm/h. For the teosinte silks, the average silk length at the termination of silk elongation was 10 cm and 9 cm for races Chalco and Central Plateau, respectively (Table 1). Landrace silks reached an ultimate length of 24 cm, with Chalqueño having the longest silks –30 cm. The average silk length for the hybrid P3394 was 30 cm. The silks of both landraces and the commercial hybrid were still elongating when the experiment was termi-

nated. Teosinte silks were approximately 50–60% shorter than those of maize. There were more trichomes per unit of length of silk in teosinte than in maize, and the teosinte trichomes were 30% wider (570 μm) and 50% longer (440 μm) than those of hybrid maize (410 μm and 240 μm , respectively).

Pollen diameter data are presented in Table 1. Pollen diameter of teosinte (ssp. *mexicana*) race Chalco was 76 μm and of race Central Plateau, 75 μm . The average pollen grain diameter for the landraces was 94 μm , with landrace Chalqueño having the largest pollen grains, 106 μm , and Tabloncillo, the smallest pollen grains, 80 μm . The average pollen size of CIMMYT's improved open-pollinated varieties was 100 μm , and that of the hybrid maize, 103 μm .

Teosinte pollen longevity, as indicated by the percentage of collapsed pollen grains, was between 30 min and 60 min (Table 1). Teosinte pollen was often partially dehydrated when collected (9:30 a.m.), and for eight of the teosinte populations, 90% or more of the pollen grains collapsed within 1 h of collection. All pollen from the hybrid, improved open-pollinated varieties and landraces appeared to be viable when first collected. After a 0.5-h exposure to atmospheric conditions, 50–60% of the pollen of the hybrid and the improved open-pollinated varieties was dehydrated. After a 1-h exposure to atmospheric conditions, pollen dehydration in the improved open-pollinated varieties, hybrids and landraces varied from 68% to 84%.

There were noticeable differences with respect to tassel weight and size among P3394, the landraces and teosinte (Table 2). In general, tassels of the landraces were heavier with a larger number of branches and spikelets than those of hybrid P3394. Landraces Tabloncillo, Bolita and the improved open-pollinated variety POP 902 were similar to P3394 for number of spikelets per tassel, with 817, 786 and 836, respectively. For the teosinte populations, the number of tassels per plant ranged from 9 to 48 compared to only one for each of the landraces and for the hybrid P3394 (Table 2). Lateral branches, exhibited by teosinte, with silks close to a terminal tassel were also counted as tassels. When all of the tassels and spikelets per plant were totaled for the hybrid, the landraces and teosinte, teosinte had the most tassels (range: 9 to 48) and the most spikelets (3,921).

Discussion

Crosses involving the pollination and subsequent fertilization of maize silks by teosinte pollen resulted in a consistent low level of seed set. Both teosinte populations, ssp. *mexicana* races Chalco and Central Plateau, were effective at producing seed. Plants with an obvious teosinte phenotype developed from seeds harvested from the maize \times teosinte hybrids. A typical maize ear may have as many as 1,000 silks (Kiesselbach 1999). It is highly probable that not all of the material had this

number of silks due to the significant genetic diversity in the accessions we used, but even with a conservative assumption of 500 silks per ear being available for pollination, only approximately 1–2% of the ovules successfully produced seeds when pollinated by teosinte.

Hand crosses of maize pollen onto teosinte silks were generally unsuccessful, with an average seed production that was one-tenth that of the reciprocal cross; many teosinte inflorescences remained barren. Teosinte typically developed an average of only 8–15 silks per inflorescence, which is over an order of magnitude lower than the number of silks produced by maize. The pollination rate on a per plant basis was similar, when we consider a maize plant with 400–500 seeds and a teosinte plant with 30–40 inflorescences and 10–14 fruitcases per inflorescence. However, at least 90–95% of the fruitcases produced on teosinte were sterile, and only 5–10% of the plants within a population were able to produce fertile seed.

The successful pollinations from crosses involving teosinte silks and maize pollen may have been influenced by the teosinte seed source. The teosinte seed utilized in this study was harvested in bulk from two locations, San Agustin del Maiz, Michoacan, and Amecameca, Estado de Mexico, where incompatibility systems for maize pollen on teosinte silks are known to be present (Evans and Kermicle 2001). It is also common to observe maize × teosinte hybrids in these locations. Since the seed was harvested in bulk, some of these seeds may have originated from teosinte plants with some introgression from maize. On average, 2.5% of the original bulked teosinte seed utilized in this study produced hybrid maize × teosinte plants. It is highly likely that the high number of barren ears was influenced by the presence of the genetic barriers *Tcb1* or *Gal-s*, as described by Evans and Kermicle (2001).

The physical properties of the pollen and silks of maize and teosinte also may account for the limited fertilization of teosinte silks by maize pollen. The floral structures on maize and teosinte are different. Teosinte pollen is about 33% smaller in diameter than maize pollen, and the teosinte silks are about 50–60% shorter than those of maize. Teosinte pollen desiccated more rapidly than maize pollen. There are more trichomes per unit length of silk in teosinte than in maize. The teosinte trichomes are 30% wider and 50% longer than those of hybrid maize. These large differences may account for the lack of fertilization of teosintes by maize. However, further studies on silk channel and pollen germ-tube size are required before we can gain any understanding of whether these factors are important determinants of seed set.

Several features of teosinte plants may discourage pollination by other taxa. Teosinte produces a very large volume of pollen and has a very modest number of silks relative to maize. Teosinte typically sheds pollen with a large number of tassels emerging asynchronously over approximately 15 days as compared to the 5 days for a commercial single-cross hybrid. Teosinte frequently

produces lateral branches with the silks in close proximity to a terminal tassel. These factors substantially increase the probability of teosinte pollen fertilizing teosinte silks and decrease the probability that teosinte would be pollinated by maize. Other factors such as low seed production, sterile seeds and seed dormancy would also limit gene flow.

The results reported here complement those of other investigations in adding to our understanding of gene flow in the genus *Zea*. A number of factors come together to limit gene flow, including the very large amount of pollen and low number of silks produced by teosinte relative to maize, the limited duration of teosinte silk viability, the relative proximity of staminate to pistillate inflorescences in teosinte and the existence of multiple genetic incompatibility systems. These factors will tend to result in gene flow being greater from teosinte to maize than from maize to teosinte. Any maize × teosinte hybrids would still then have to be involved in further backcrossing for introgression to occur. Additionally, crop management, local ecology, physical distance, flowering synchrony and human selection will influence the level of gene flow under a farmer's field conditions. Collectively, these factors could slow introgression considerably. The present results may help explain why teosinte and maize have been able to co-exist as genetically separate entities even under conditions where they grow in proximity in Mexican farmers' fields.

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