

Spontaneous Hybridization between Maize and Teosinte

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The closest wild relatives of maize, *Zea mays* ssp. *mays* are various *Zea* taxa known as "teosinte." Hybrids between maize and the teosinte taxon, *Zea mays* ssp. *mexicana*, often occur when the 2 are sympatric in Mexico. Measuring the spontaneous hybridization rate of the 2 taxa would shed light on the mechanisms contributing to the evolution and persistence of these hybrid swarms. We conducted a series of field experiments in Riverside, CA, to measure the natural hybridization rates between maize and 2 teosinte taxa, *Z. m. ssp. mexicana* and *Zea mays* ssp. *parviglumis*. We planted teosinte within and near maize plantations. Hybrids were identified by progeny testing for a maize-specific herbicide resistance allele and a teosinte-specific allozyme allele. Hybridity was confirmed by growing putative hybrid progeny to maturity to evaluate whether they had the characteristic morphology of maize \times teosinte hybrids. We found that maize and *Z. m. ssp. mexicana* naturally hybridize at a low rate ($<1\%$), whereas *Z. m. ssp. parviglumis* hybridizes with the crop at a high rate ($\gg 50\%$).

Hybrid swarms can be sites of natural evolutionary experimentation and diversification. Long-term hybrid swarms can persist in different ways (Anderson 1949; Arnold 1997). They might be maintained largely by recurrent gene flow; that is, spontaneous hybridization between the parental taxa might occur at a sufficiently high frequency to counterbalance selection against the hybrids and hybrid derivatives. Alternatively, hybridization might be very rare, but individuals of hybrid ancestry might be maintained largely by selective advantage.

Hybrid swarms involving the crop maize (*Zea mays* ssp. *mays* L.) and the wild *Zea mays* ssp. *mexicana* L. (Schrud.) Iltis, one of the taxa known collectively as "teosintes" (Sánchez González and Ruiz Corral 1997), have been the object of considerable descriptive study for decades. The hybrid swarms are commonly found in and near Mexican maize fields when the wild taxon is abundant (Wilkes 1977). A population genetic study (Blancas et al. 2002) provided molecular

support for the hybrid ancestry of many of the plants in these populations.

They collected plant material from intermingled maize and *Z. m. ssp. mexicana* populations in central Mexico, carefully segregating the morphologically intermediate individuals from morphologically "pure" individuals for separate analysis. Allopatric populations of both taxa were also collected for comparison. The collections were genetically analyzed for 18 allozyme loci. When genetic distances were calculated, pairs of locally sympatric populations of teosinte and maize were more similar to each other than to any of the other populations, including members of the same taxon. The molecular confirmation of the hybrid origin of these plants leads to the question of how these populations of plants are maintained. After all, these annuals often occur year after year in the same location (Wilkes 1977).

Continual replenishment by hybridization counterbalanced by opposing selection is one possibility. However, in contrast to most teosinte taxa, such as *Zea mays* ssp. *parviglumis* (Goodman 1995), hand-crossing studies demonstrate that *Z. m. ssp. mexicana* and maize exhibit genetically based, cross-incompatibility (Kermicle 1997; Baltazar et al. 2005). The incompatibility is asymmetric, being very strong when maize is the pollen parent, but weaker when *Z. m. ssp. mexicana* is the pollen parent (Baltazar et al. 2005; Kermicle and Evans 2005). In addition to genetically based incompatibility, data on phenological differences and other biological factors suggest that hybridization rates in the field are probably very low (Baltazar et al. 2005). Nonetheless, hand-crossing is not necessarily an adequate surrogate for natural cross-pollination under field conditions (Ellstrand 2003); furthermore, the order of magnitude of natural hybridization cannot be estimated from such studies.

If natural hybridization rates are considerably higher than those suggested by hand-pollination, gene flow pressure alone could explain the high frequency of hybrid-derived plants in areas of sympatry. But if hand-crosses are a good

predictor of a very low spontaneous hybridization rate, then other mechanisms must be sought to explain the persistence of hybrid swarms. These questions gain added significance in the current science policy discussions of whether intertaxon hybridization would be expected to occur at a sufficient rate to introduce maize transgenes into teosinte populations (e.g., Serratos et al. 1997; CEC 2004). Therefore, we conducted a series of field experiments using genetic markers to measure the spontaneous mating rate of maize and *Z. m. ssp. mexicana* under field conditions.

Materials and Methods

Plant Material

The maize (*Z. m. ssp. mays*) variety used for this experiment was obtained with the cooperation of the Monsanto Company. The genotype chosen was hemizygous for a transgene that conferred tolerance to the herbicide glyphosate (Roundup®). Therefore, a cross between this genotype and a nontolerant plant should yield equal fractions of glyphosate-tolerant progeny and glyphosate-intolerant progeny. This pattern of Mendelian inheritance for the transgene was confirmed by polymerase chain reaction analysis in a prior study (Guadagnuolo et al. 2006).

Mexicana teosinte (*Z. m. ssp. mexicana*) individuals in the study were obtained by multiplying seeds from a 1972 collection by George Beadle, provided by J. Giles Waines. In order to create a fresh seed supply, 100 seeds from that collection were sown in the greenhouse in late 1998, and 85 germinated. When they flowered in early 1999, the male inflorescences of individual plants were regularly shaken over the female inflorescences of other plants in order to maximize outcrossing. Forty-five of these plants set abundant seed. We collected 10 seeds from each of 40 of these plants, for a total of 400 seeds.

Creating Hybrids by Hand-Crossing

Maize–teosinte hybrids were produced to compare their morphology with that of putative spontaneous hybrids. Hand-crosses of greenhouse grown maize and teosinte were performed. Maize plants were detasseled, and newly shedding teosinte male inflorescences were shaken over receptive maize silks. The resulting seeds were germinated in pots and grown to maturity in the greenhouse.

The Field Experiments

Field experiments were conducted in late summer to early fall at the University of California Riverside Agricultural Experiment Station in Riverside, CA. We acknowledge that this field site is not necessarily equivalent to the physical environments where *Zea* taxa co-occur in Mexico. However, the location has the advantage that it is distant from both maize and teosinte populations that could serve as unwanted sources of pollen contamination: hundreds of kilometers from the nearest commercial maize plantation and thousands of kilometers from the nearest teosinte.

In each of the years, 2000, 2001, and 2002, a 2023 m² (half-acre) block of maize was planted, using standard commercial maize cultivation practices, including regular irrigation. Five rows of teosinte were planted parallel to the maize rows on both the eastern and western sides of the maize block. The 3 rows nearest the maize were planted at the rate of 40 plants per row (with 1 m spacing between plants). The 2 rows most distant from the maize contained 20 plants per row. In 2001 and 2002, 20 teosinte plants were planted within each of the maize rows adjacent to the pure teosinte rows. These planting schemes roughly approximate the conditions of a small Mexican maize field in which the density of teosinte is likely to be highest at the immediate edges of the field and lower within the maize field itself. In 2002, we greenhouse germinated a small sample of *parviglumis* teosinte (*Z. m. ssp. parviglumis* L. Iltis and Doebley) seeds from the Beadle collection and hand-planted these into the field.

We timed our planting to match the same temporal pattern of maize and teosinte germination in Mexico. Those times also optimize phenological overlap. Maize and teosinte were planted in early August. When the maize began to flower in September, the *mexicana* teosinte had also just begun to flower. However, we found that *parviglumis* teosinte takes substantially longer to begin blooming in California. Only 2 *parviglumis* teosinte plants flowered simultaneously with maize, and the others did not flower at all during the experiment. Plants were irrigated for 10 weeks after the date of first flowering to assure proper seed development. Maize harvests were conducted 4–5 weeks after the cessation of maize flowering.

Seeds from teosinte plants were collected every year. In 2000, 45 teosinte tillers with receptive silks—each on a different plant—were marked at the time of maize pollen release. Seeds were collected only from the marked tillers for greenhouse screening. In 2001, one or more tillers were harvested from each plant. All mature seeds produced by these tillers were collected. A sample of 20–50 seeds from each plant was screened in the greenhouse. In 2002, teosinte plants were unusually small; whole plants were harvested, and 20 seeds per plant were screened. In the first 2 years, uncollected seeds were allowed to fall into the field for subsequent field screening.

Progeny Testing

Every year a sample of up to 50 seeds per teosinte maternal plant were germinated in Petri dishes. The seedlings were transferred to flats and grown to the 3-leaf stage (about 3 weeks) in the greenhouse. They were then sprayed with 0.7% Roundup® (0.004% glyphosate, the active ingredient) twice (the second spray 7–10 days from the first). This rate was experimentally determined (Clegg J, unpublished data) to optimize the treatment such that only plants containing the transgene survived. The total number of seedlings screened per year is detailed in Table 1.

Teosinte, like most plants, is glyphosate intolerant (Guadagnuolo et al. 2006). Therefore, progeny surviving that treatment were deemed to be putative hybrids. To confirm

Table I. Spontaneous hybridization between *Zea mays* ssp. *mays* and 2 teosinte taxa, *Zea mays* ssp. *mexicana* and *Zea mays* ssp. *parviglumis*

Year	Pollen parent	Seed parent	No. seedlings tested	Screening method (details in text)	No. hybrids detected (estimated)	Estimated hybridization rate (%) (see text)	95% confidence limits ^a
2000	<i>Z. m. ssp. mays</i>	<i>Z. m. ssp. mexicana</i>	2825	Greenhouse herbicide	3 (6)	0.2	0.08–0.43%
2000	<i>Z. m. ssp. mays</i>	<i>Z. m. ssp. mexicana</i>	ca. 500 000	Field herbicide	1 (2)	0.0004	0.0001–0.0014%
2001	<i>Z. m. ssp. mays</i>	<i>Z. m. ssp. mexicana</i>	14 590	Greenhouse herbicide	0	0	0–0.02%
2001	<i>Z. m. ssp. mays</i>	<i>Z. m. ssp. mexicana</i>	ca. 350 000	Field herbicide	8 (16)	0.005	0.003–0.007%
2001	<i>Z. m. ssp. mexicana</i>	<i>Z. m. ssp. mays</i>	1500	Lab allozyme	0	0	0–0.20%
2002	<i>Z. m. ssp. mays</i>	<i>Z. m. ssp. mexicana</i>	1530	Greenhouse herbicide	2 (4)	0.2	0.08–0.62%
2002	<i>Z. m. ssp. mays</i>	<i>Z. m. ssp. parviglumis</i>	25	Greenhouse herbicide	13 (26)	100	89–100%

^a Confidence intervals were constructed based on Bayesian statistics (Jaynes 1976).

hybridity, each of these plants was grown to reproductive maturity in the greenhouse to compare the morphological characteristics of the infructescences with those of the hybrids we created by hand (see Figure 1). Maize geneticists have also observed intermediate infructescences in synthetic maize–teosinte hybrids (e.g., Collins 1919; Doebley 2004).

Teosinte seeds disperse easily. Thus, it was not surprising that thousands of seedlings appeared spontaneously at the field site the following spring. The rate of hybridity of adventitious seedlings that germinated after the 2000 and 2001 field seasons was determined. The number of volunteer seedlings was estimated by counting plants in 20 randomly assigned 1 m × m plots at each end of the former maize field (40 total plots) and extrapolating based on total field area. The seedlings were sprayed twice with Roundup® by Agriculture Experiment Station personnel using standard application procedures. After spraying, all surviving seedlings were transplanted to the greenhouse and grown to maturity.

To estimate the spontaneous hybridization rate involving maize as the maternal parent and teosinte as the paternal par-

ent, we progeny tested maize seeds. In 2001, we selected 1500 maize seeds from 150 randomly collected cobs, each from a different maternal plant in our experimental field. We germinated these seeds and screened the seedlings for an aspartate aminotransferase allozyme allele that is fixed in teosinte and absent in maize, as described in Blancas (2001).

Results

Hybrid Morphology

Every hybrid resulting from the hand-crosses between maize and teosinte had the characteristic infructescence and fruit morphology that is intermediate to and discrete from either parent (Figure 1). The mature infructescence of teosinte is a “cob-less” single row of 4 or more disarticulated grains enclosed in hard black fruitcases, a combination of hardened glume and cupule (White and Doebley 1998). Maize’s mature infructescence comprised many rows of grains not enclosed in fruitcases but firmly attached to the characteristic central cob, a thickened nonshattering rachis. Hybrids observed in this experiment had 4 rows of grains partially expanded beyond their fruitcases and attached to a cob (Figure 1). The hybrid morphology was the same as other maize–teosinte F₁s produced by other researchers (e.g., Wilkes 1977).

Spontaneous Hybridization: 2000

The 45 teosinte tillers harvested in 2000 produced 3581 seeds of which 2825 (76%) germinated and were screened for herbicide tolerance. Three individuals survived herbicide treatment. The census of teosinte seedlings resulting from seed dispersed into the field site after harvesting in fall 2000 resulted in approximately 500 000 volunteers. Only one of these individuals survived herbicide treatment. All 4 survivors exhibited characteristic hybrid phenotypes when grown to maturity.

Spontaneous Hybridization: 2001

Of the seed collected in 2001, 14 590 seedlings were screened. Not a single individual survived herbicide treatment. Approximately 350 000 volunteers were screened in the field. Eight individuals survived. All survivors exhibited characteristic hybrid phenotypes when grown to maturity.



Figure 1. Infructescences and fruits of teosinte (*Zea mays* ssp. *mexicana*), maize (*Zea mays* ssp. *mays*), and their F₁ hybrid. From left to right: Teosinte fruits, teosinte infructescence, hybrid infructescence, and maize infructescence.

None of the 1500 maize seeds screened for the teosinte-specific aspartate aminotransferase allozyme allele were found to be heterozygous for the marker.

Spontaneous Hybridization: 2002

Of the 1530 seedlings germinated from *mexicana* teosinte seed collected in 2002, only 2 individuals survived herbicide treatment. Of the 25 seedlings germinated from *parviglumis* teosinte seed, 13 survived. All survivors exhibited characteristic hybrid phenotypes when grown to maturity.

Discussion

The results of our experiment demonstrate that maize spontaneously hybridizes with both *mexicana* teosinte and *parviglumis* teosinte under field conditions. We estimated hybridization rates from both progeny testing experiments and field screening of teosinte volunteers. The fraction of hybrid progeny was multiplied by 2 to account for the fact that the maize parents are hemizygous for the dominant herbicide tolerance marker (assuming all gametes have equal transmission success). The results are displayed in Table 1. We detected hybrids sired by maize on teosinte for 4 of 5 experiments.

The very low hybridization rates we observed for maize and *mexicana* teosinte are consistent with the incompatibility barrier known to exist between them (e.g., Kermicle 1997; Baltazar et al. 2005). Those rates ($\ll 1\%$) are much lower than what has typically been observed for experimental studies of spontaneous hybridization between other crop-wild pairs (Ellstrand 2003). The lower hybridization rates calculated from the volunteers is not unexpected because those seedlings represent seed set throughout the life of the teosinte parents, whereas the progeny testing was only done on seeds from tillers that flowered simultaneously with maize.

In contrast, the hybridization rate between maize and *parviglumis* teosinte, where incompatibility is absent, was much higher. Apparently, Riverside is on the margin of the range where *parviglumis* teosinte can flower, and only 2 plants did so. But more than half the seedlings tested bore the marker allele. Assuming Mendelian transmission, the Bayesian correction suggests that every seedling tested was a hybrid; that is, the hybridization rate was estimated to be 100%.

If the rate of hybridization between *mexicana* teosinte and maize is representative of their rate of hybridization in sympatry in Mexico, how, then, does one account for their persistent hybrid swarms? First, even a little gene flow can be evolutionarily significant. Population genetic theory predicts that a single successful hybridization event per generation should eventually homogenize the neutral allele frequencies of populations (Wright 1951), but reaching equilibrium might take 100 or more generations (Varvio et al. 1986). Given that maize and *mexicana* teosinte have occurred in local sympatry for thousands of years, recurrent maize-to-teosinte gene flow at the low level measured is sufficient to permit the flow of neutral or beneficial alleles into the wild. But the rate is so low that it should serve as a substantial barrier against alleles that would be detrimental in the recipient populations (Slatkin 1987).

Restricted, but nonzero, hybridization will not limit subsequent introgression if other factors do not impede it. Hybrids between maize and *mexicana* teosinte are compatible with both parents (Roberto Guadagnuolo, Janet Clegg, and Norman Ellstrand in preparation). Furthermore, F_1 s have a higher maternal fitness than their wild parents under field conditions (Guadagnuolo et al. 2006). Thus, the few hybrids that are created can serve as a local genetic bridge (sensu Reagon and Snow 2006) if they survive to flower because they can backcross with either parent. Genetic bridges provide the opportunity for “introgressive hybridization” to be “an important evolutionary force even when the initial formation of F_1 hybrids in natural populations is rare” (Broyles 2002). The molecular data suggest neutral allele introgression, but the persistence of individuals generation after generation that share a combination of characters from both parents suggests that selection—perhaps evolution of crop mimicry—maintains them.

In this system, natural selection will be the primary determinant of the spread of immigrant alleles (Chapman and Burke 2006). With regard to the question of whether hybridization will permit maize transgenes to enter and persist in sympatric natural populations of teosinte in Mexico, it appears that enough recurrent hybridization occurs so that neutral or beneficial maize alleles, transgenic or not, have ample opportunity to move into the wild. If certain maize alleles are expected to create unacceptably undesirable consequences in teosinte populations, steps must be taken to prevent hybridization from occurring.

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