

RELATIVE FITNESS OF TRANSGENIC VS. NON-TRANSGENIC MAIZE × TEOSINTE HYBRIDS: A FIELD EVALUATION

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Abstract. Concern has been often expressed regarding the impact and persistence of transgenes that enter wild populations via gene flow. The impact of a transgene and its persistence are largely determined by the relative fitness of transgenic hybrids and hybrid derivatives compared to non-transgenic plants. Nevertheless, few studies have addressed this question experimentally in the field. Despite the economic importance of maize, and the fact that it naturally hybridizes with the teosinte taxon *Zea mays* ssp. *mexicana*, sometimes known as “chalco teosinte,” the question has received little experimental attention in this system.

Using a glyphosate-tolerant maize cultivar and chalco teosinte as parental lines, we carried out a field experiment testing (1) the relative fitness of maize × teosinte hybrids, compared to their parental taxa, as well as (2) the relative fitness of transgenic hybrids compared to non-transgenic hybrids created from the same parental stock. In order to evaluate the influence of the transgenic construct in different genetic backgrounds, our study included transgenic and non-transgenic pure maize progeny from the cultivar as well. We measured both vegetative and reproductive parameters.

Our results demonstrated that hybrids have greater vigor and produced more seeds than the wild parent. However, in the absence of selective pressure from glyphosate herbicide, we did not observe any direct positive or negative impact of the transgene on the fitness or vigor of either the hybrids or pure maize progeny. We discuss our results in terms of the potential for spontaneous transgene flow and introgression from transgenic maize into sympatric teosinte.

Key words: *biosafety; fitness; gene flow; hybrids; introgression; maize; risk assessment; teosinte; transgenic crops; Zea mays ssp. mays; Zea mays ssp. mexicana.*

INTRODUCTION

In the last decade crop-to-wild gene flow has attracted considerable attention from a wide variety of plant scientists (examples in Ellstrand 2003, den Nijs et al. 2004). This interest has been motivated primarily by concerns that engineered crop genes will enter and persist in wild populations and by the putative negative consequences of such persistence, such as increased weediness and increased risk of extinction of rare species (Ellstrand 2003). Morphological, molecular, and even transgenic markers have been used in descriptive and experimental studies investigating the potential of natural hybridization to act as a mechanism to deliver crop alleles to nearby populations of wild taxa. As a result of such studies, it is now known that most crops spontaneously hybridize with one or more wild relatives somewhere in the world (Ellstrand 2003).

While evidence for spontaneous hybridization between crops and their wild relatives is considerable, the likelihood of introgression (that is, the persistence and

spread) of crop alleles into wild populations has received much less attention. However, the relevant data set is growing, particularly with regard to the first step of the introgression process: the relative fitness of the hybrid under field conditions compared to that of the pure wild parent. If hybrid fitness is lower, introgression of crop alleles into the wild will be slowed; if that fitness is higher, introgression will be accelerated (Ellstrand 2003). Motivated by concerns regarding unintended transgene flow, studies have compared one or more fitness-related traits of conventional (non-transgenic) crop × wild hybrids to one or both parental species in sorghum (Arriola and Ellstrand 1997), rice (Song et al. 2004), carrot (Hauser 2002), sunflower (Snow et al. 1998), radish (Snow et al. 2001), and canola (Hauser et al. 1998a, b). The results vary with the system; in some cases, estimated hybrid fitness was lower; in others it was higher. Although these studies, among others, suggest that the introgression of crop traits into the genomes of many wild plants is rather likely, and, in some cases (e.g., Whitton et al. 1997) has been clearly demonstrated, they do not address fitness effects of transgenes themselves.

Clearly, a transgene should confer some selective advantage associated with its intended phenotype (e.g., herbicide tolerance if the relevant herbicide is applied).

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At the same time, transgenic constructs may prove to be detrimental to fitness in the field because they add a physiological load (e.g., Purrington and Bergelson 1997). In contrast, it is possible that a transgenic construct may have other pleiotropic effects (cf. Bergelson et al. 1998), which may actually enhance fitness. Therefore, in order to better predict the consequences of transgenic crop-to-wild gene flow, it is necessary to use transgenic plants in comparative studies. Such studies might not always be able to distinguish the fitness effects of the transgene itself from those associated with tightly linked alleles. However, they are still of utility because they demonstrate whether alleles in a transgenic hybrid are more or less likely to introgress into a wild population than those in a comparable non-transgenic hybrid.

Few studies have evaluated the fitness of transgenic vs. non-transgenic crop \times wild hybrids. Field studies on the consequences of crop-to-wild transgene flow are not yet common for many reasons: in many countries, government regulations prevent or restrict the outdoor cultivation of certain transgenic plants, the experiments themselves are discouragingly time-consuming, and creating the appropriate control plants for an accurate comparison is often difficult. To our knowledge, only a very few systems have yet been used for such comparative field experiments: rice (Oard et al. 2000), sunflower (Burke and Rieseberg 2003, Snow et al. 2003), canola (Gueritain et al. 2002), and squash (Fuchs et al. 2004).

Other crop systems have received little attention. For example, despite the economic importance of maize and the fact that it is one of the most important transgenic crops, studies comparing transgenic and non-transgenic hybrids have not yet been published. Spontaneous hybridization between maize (*Zea mays* ssp. *mays*) and most of the various wild taxa known as teosinte, especially *Zea mays* ssp. *mexicana*, has been well-documented (Doebley 1990, Wilkes 1977). Indeed, in addition to allozyme evidence (Doebley 1990, Blancas et al. 2002), there is now molecular evidence of introgression (Fukunaga et al. 2005). Sympatric populations of crop maize and *Zea mays* ssp. *mexicana* are common in the Chalco region (Valley of Mexico) and hybrids between the two subspecies, both possessing $2n = 20$ chromosomes, are often observed in these populations (Wilkes 1977, Doebley 1990). Yet the two taxa seem to have maintained distinct gene pools (Fukunaga et al. 2005). Clearly, experimental work would be useful for determining how easily maize transgenes would be integrated into wild populations after initial hybridization and whether those alleles would introgress at rates different from conventional crop alleles.

The primary goals of the present study are to (1) compare fitness of maize \times teosinte hybrids with that of their parental types and (2) compare the fitness of transgenic vs. nontransgenic maize \times teosinte hybrids and the fitness of transgenic vs. nontransgenic offspring of the maize cultivar. We used this approach because the

isogenic line (i.e., differing only in the presence or absence of the transgene) was not available (such lines are almost never available; cf. National Academy of Sciences 2002). In addition, we measured the fitness influence of the transgenic construct independent from intertaxon hybridity. The existence of a physiological cost of the transgene was investigated in the absence of the relevant selective pressure that would have favored the transgenic plants, i.e., no glyphosate was used. The results will help to evaluate the likelihood of spontaneous transgene flow and potential introgression from transgenic maize to its close relative *Z. mays* ssp. *mexicana* under field conditions.

MATERIAL AND METHODS

Plant material

The fitness of the following four types of plants was compared:

1) A genetically modified, glyphosate-tolerant maize hybrid, Roundup Ready corn 1 (GA-21; hereafter, MC), was provided by the Monsanto Company (St. Louis, Missouri, USA). This hybrid is hemizygous for the transgene, that is, it possesses only one copy of it and the alternate "allele" is null. In an experiment carried out simultaneously with the one described here (data not shown), we verified the expression of the glyphosate tolerance phenotype of the transgene by the MC. Like the vast majority of modern American cultivars, this transgenic cultivar is a "hybrid" between two inbred lines. In this case, one of those lines is homozygous for the transgene; the transgene was not present in the other.

2) Progeny of MC (hereafter, MP) were obtained by selfing MC plants. Segregation of the hemizygous transgene in MC should give an expected Mendelian ratio of 3:1 transgenic (glyphosate tolerant) : non-transgenic (glyphosate intolerant) in MP. The selfed progeny of elite F_1 varieties are expected to show inbreeding depression.

3) Teosinte (*Zea mays* ssp. *mexicana*; hereafter, T) individuals in the study were obtained by multiplying seeds from an original 1972 George Beadle collection from the Central Plateau and Valley of Mexico. About 100 seeds of the original collection were sown one year prior to our experiment and 85 germinated. During the flowering period, the male inflorescences of these plants were regularly shaken in order to maximize outcrossing. Among these plants, 45 set numerous seeds. We collected and pooled 10 seeds from 40 of these plants, 400 seeds in total, that we used for our experiment. Teosinte, like most plants, is glyphosate intolerant (N. C. Ellstrand et al., *unpublished data*).

4) The F_1 hybrids (see Plate 1) between MC and T (hereafter H) were produced using MC as the maternal parent, by growing several emasculated maize plants among numerous T plants in the field. Mature, pollen-shedding, teosinte male inflorescences were shaken over receptive maize female inflorescences daily. We attempted similar crosses in the other direction, with T as the



PLATE 1. Female ears of an F_1 hybrid between maize (*Zea mays* L.) and “mexicana” teosinte (*Zea mays* ssp. *mexicana* Iltis and Schrad). Photo credit: R. Guadagnuolo.

maternal parent. However, we were unable to obtain sufficient numbers of hybrid seed because thorough emasculating of T was extremely difficult. Consequently, for most T-produced seeds, paternity was too uncertain to proceed with planting. This category was thus excluded from our study. Segregation of the hemizygous transgene in H is expected to give a Mendelian ratio of 1:1 transgenic (glyphosate tolerant):non-transgenic (glyphosate intolerant) independent of the direction of the cross.

The field experiment

Field experiments were conducted at two University of California Field Stations: Agricultural Operations in Riverside and the Moreno Valley Station. With respect to initiation of flowering, teosinte is a short-day plant. In southern California, appropriate conditions occur in September. Therefore, seeds were sown in mid-August and cultivated until late December.

In order to compare a similar number of plants of each type in each plot, we limited the number of sown seeds, based on the least available seed, H in this case. Therefore, in each experimental plot we sowed 200 MC, 400 MP, 200 T, and 200 H (50% of H plants were assumed to be transgenic). Each plot was composed of 14 rows, 75 cm apart (8 of 71 plants and 6 of 72). Within the rows, seeds of the different types were planted in

randomly assigned locations. Spacing between plants was 20 cm. A corresponding color stake was used to identify the type of each plant. We followed standard practices for irrigation, soil preparation, and pesticide application of elite maize cultivars used in the United States, and these practices were monitored by the agricultural stations' personnel. In order to avoid artificial selective pressure that would directly favor the transgene, no herbicides, neither glyphosate nor others, were used in our experiment. Weeding was done manually; very few weeds were observed in the plots.

Identification of transgenic plants

For H and MP, discrimination between transgenic and non-transgenic plants was performed by polymerase chain reaction (PCR), using construct-specific primers (Sigma-Genosys, The Woodlands, Texas, USA) and following the protocol provided to us by the Monsanto Company. The DNA extraction was performed as described in Guadagnuolo et al. (2001) on ~ 2 cm² of leaf material, collected 45 d after sowing, i.e., when plants were almost mature, to minimize the effect of tissue removal on plant fitness.

Measurements

Measurements were made throughout the growing season. Germination rate was monitored daily for the first two weeks and weekly for two additional weeks. We then estimated plant survival and measured plant height 30 and 60 d after sowing. Finally, aboveground dry biomass was measured at the end of the experiment.

Female reproductive fitness was estimated for all the plants, based on seed production. Seed mass was measured for all the plants, and seed set was counted for 25 plants per type per plot. A correlation was then calculated between seed mass and seed number for each type and each plot and used to estimate seed set for each of the plants. All the correlations (Pearson's coefficient) were highly significant (i.e., $P \leq 0.0001$) and ranged between 0.92 and 0.98. To estimate male fitness, pollen viability was analyzed on a subset of 10 plants per type for each plot. Pollen viability was estimated based on the fluorescein diacetate stainability of 200 pollen grains/plant (Heslop-Harrison and Heslop-Harrison 1970). Finally, the number of days to flowering was measured for all the plants.

Data analysis

For each measured fitness parameter, differences between plant types, as well as differences between transgenic and non-transgenic plants within types, were evaluated using the nonparametric Mann-Whitney U test, corrected by the standard (i.e., nonsequential or step-down) Bonferroni method (Sankoh et al. 1997). A nonparametric test was chosen because of the non-normality of the distribution for most of the characters, as estimated using the Kolmogorof-Smirnoff test. Plot effect on the measured characters was estimated using a

nested ANOVA for data possessing an approximately normal distribution or that could be reliably transformed to have one. All data analysis was performed using the statistical software SPSS version 11.1 (SPSS, Chicago, Illinois, USA).

RESULTS

Comparison between plant types

Germination and survival.—There were few differences in germination rate among the plant types: H and MP germinated at a slightly lower rate (90% and 92%, respectively) than did T and MC (98% and 99%, respectively). However, based on plant morphology, we determined that 11% of the plants originally thought to be H were actually MP, indicating that some pollen contamination occurred during our forced crosses. Since it was impossible to determine whether the nongerminated seeds were H or MP, we cannot calculate the actual germination rate of H.

At Riverside, no survival differences were observed, since only two plants that germinated did not survive. In contrast, numerous plants of all types died in the Moreno Valley plot where plant survival was 88% for MC and H, 85.5% for T, and 78% for MP (not significant differences).

Vegetative vigor.—Despite the survival differences between sites noted above, no site \times plant type interactions were significant in nested ANOVAs for any of the other measured characters. Therefore the data of the two plots were pooled, and the plant types were compared using the whole data set.

Vegetative vigor was significantly greater in H than in T (Fig. 1a, b). Hybrids grew taller and produced more aboveground biomass than teosinte (Fig. 1a, b). Compared to MC, H had slightly higher mean values for almost all measured vegetative parameters, but only one difference was significant: plant height at day 60. MP was significantly less vigorous than the three other plant types.

Reproductive parameters.—Pollen stainability was highly variable within each subset of 20 individuals/plant type, but no significant differences were observed between types. In contrast, significant differences among plant types were observed for time to first flowering. H flowered significantly earlier than all the other types, including both parents. In terms of female fecundity, H produced 25% more seeds than the wild parent (T) and 14% more than its cultivated parent (MC), although only the H–T difference is significant (Fig. 1c).

Comparison between transgenic and non-transgenic plants

The PCR identification of the transgene in H confirmed the expected proportion of phenotypes: 48% ($N = 142$) transgenic:52% ($N = 153$) non-transgenic H (expected 50%:50%). In MP, the proportions, 70% ($N = 417$) transgenic:30% ($N = 185$) non-transgenic, were slightly but significantly different from the Mendelian expectation of 75%:25% (chi-square =

10.54, 1 df). Transgenic and non-transgenic plants within a type did not differ for any of the vegetative and reproductive parameters that we measured (Fig. 1). Indeed, we did not observe any significant difference between the performance of transgenic and non-transgenic H, nor between that of transgenic and non-transgenic MP.

DISCUSSION

Two observations are apparent from our data set. First, relative to their wild parent, hybrids between maize and teosinte show statistically significant heterosis for most of the characters measured. Moreover, the hybrids performed slightly better, although not significantly, than their cultivated maize parent. Second, none of the characters measured varied with the presence or absence of the transgene, neither in the hybrid genetic background nor in that of pure maize.

The only observed significant difference for the transgenics occurred with regard to their slight deviation from Mendelism in terms of fewer than expected transgenics in the MP group. This deviation is difficult to explain. Since the control gene, *adh 1*, amplified in all of our PCR-amplified samples (data not shown), it is unlikely that the significantly lower frequency of samples with an amplified transgene was due to problems with the quality of DNA in our samples. On the other hand, we cannot determine whether the transgene was present in the seeds that did not germinate. Therefore, it is impossible to judge whether deviations from Mendelism in MP are due to segregation distortion in their parents or simply differences in germination.

The similarity of our results obtained in two different plots also indicates that the transgene has no apparent physiological cost, at least for the genotypes and environments we studied. Similarly, in a growth chamber study, Snow et al. (1999) did not detect any physiological cost associated with a glufosinate resistance transgene introgressed into descendants of weedy *Brassica rapa* \times canola hybrids.

These observations indicate that, once successful hybridization has occurred, this particular transgene would likely start to introgress into this subspecies of teosinte at the rate and pattern expected of a neutral allele (cf. Ellstrand 2003). We would, of course, expect a different pattern in the presence of relevant selective pressure, that is, application of the herbicide glyphosate or if alleles that are closely linked to the transgene are masking positive or negative fitness effects.

Our conclusions are based on our specific experimental conditions and need to be interpreted in the context of the maize cultivation in Mexico, where the crop is sometimes sympatric with *Zea mays* ssp. *mexicana*. In areas of sympatry, it is not unusual to find hybrids between the two taxa (e.g., Sanchez González and Ruiz Corral 1997), likely including hybrids with both taxa as the female parent. Those areas are likely to receive less fertilizer and weed control than our experimental

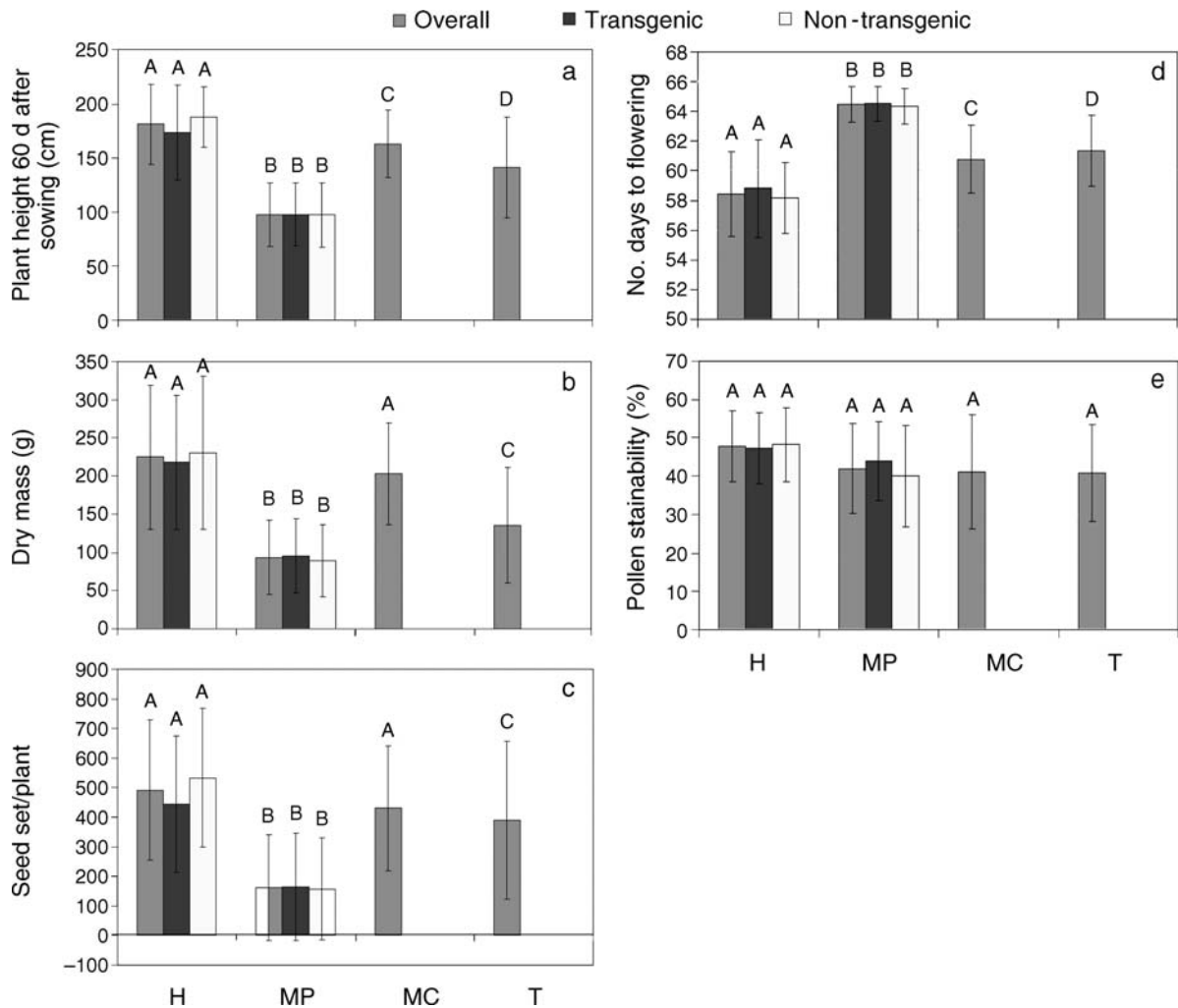


FIG. 1. Values for the measured vegetative and reproductive parameters (mean \pm sd): $N = 295$ for hybrids (H), $N = 602$ for maize progeny (MP), $N = 349$ for maize cultivar (MC), and $N = 336$ for teosinte (T), except for the pollen stainability, where $N = 20$ for each plant type. Statistics are based on Mann-Whitney U test after Bonferroni's correction. Different letters above the columns indicate significantly different values. Note that all differences between transgenic and non-transgenic H as well as between transgenic and non-transgenic MP are not significant.

conditions, but it is difficult to judge whether the difference in environmental conditions would increase or decrease the fitness differences we observed in our experiment. Still, where the two taxa are sympatric, farmers often grow maize landraces, sowing a portion of their harvested seeds (Blancas 2001). Therefore, those farmers probably occasionally end up sowing some maize \times teosinte hybrid seeds.

In our experiment we are limited to observations based on hybrids with maize as the maternal parent for the reasons explained in *Material and Methods*. Considering that the gene flow under scrutiny here is that from maize to teosinte, the lack of all possible hybrids as controls for the experiment is certainly a limitation to our study. However, the few hybrids sired by maize that we grew separately did not show any particular differences compared to plants from the opposite cross,

neither in terms of general performance nor regarding their reproductive morphology (data not shown).

Another difference between the conditions in our experimental study and those in Mexico is that our comparisons were obtained from plots where the frequency and density of hybrids were equal to that of teosinte. In Mexico, hybrids appearing to be F_1 s are never found in frequencies greater than or equal to the parental types. These hybrids are likely to occur at a much lower frequency than their wild parent, thus releasing a much lower density of pollen than that observed in our experiment. It is relevant that studies of hybrids between canola (*Brassica napus*) and *B. rapa* have shown the interaction between hybrid density and frequency to play an important role for both male and female fitness (Pertl et al. 2002, Hauser et al. 2003).

Moreover, because seed set was one of the measured parameters, we harvested all the seeds, and we were thus unable to quantify another extremely important parameter: seed dispersal. Seed dispersal varies dramatically among the plant types. While teosinte freely disperses seeds, domestic maize does not. The absence of seed dispersal is generally thought to be so disadvantageous in the wild that it might reduce the introgression of crop alleles to zero (e.g., Martínez-Soriano and Leal-Klevezas 2000). However, although the structure of hybrid cobs makes dispersal more difficult, in other studies (Wilkes 1977; R. Guadagnuolo and N. C. Ellstrand, *unpublished manuscript*) some hybrid cob breakage and seed dispersal have been observed, suggesting that some hybrids may disperse many of their seeds. Additionally, manual or mechanical harvesting of maize would result in some dispersal of seeds from hybrids also growing in the field, because hybrid cobs (see Plate 1) are not as compact as maize cobs. Moreover, F_2 plants show extreme variability in the shape and structure of their infructescences (data not shown), ranging from maize-like to teosinte-like, the latter effectively dispersing seeds. It is also worth noting that this variability has already been observed in some naturally occurring plants of hybrid ancestry in Mexico (Doebley 1990). Hence, although not quantified here, reduced seed dispersal may (or may not) influence the relative fitness of the F_1 hybrids, plants that are otherwise superior to the wild species for the characters that we measured. Still, it is not clear that such dispersal differences are sufficient to prevent the introgression of maize traits into teosinte.

Moreover, the F_1 hybrids are not only female fertile but also highly male fertile (Evans and Kermicle 2001). In another study, we found evidence that the F_1 s can easily and successfully pollinate teosinte and thus can act as a bridge for the transfer of maize-specific alleles, transgenic or not (R. Guadagnuolo and N. C. Ellstrand, *unpublished manuscript*). In addition, since the hybrids start flowering earlier than teosinte, their chance to hybridize with teosinte is increased when the latter starts to come into flowering.

As long as early-generation hybrids have moderate levels of fitness, intertaxon gene flow will not be prevented (Arnold et al. 1999). The survivorship and fertility of the maize \times teosinte hybrids we studied suggest that the process of neutral or beneficial maize allele introgression into a sympatric or nearby population of *Zea mays* ssp. *mexicana* would hardly be impeded. In the case of cultivated herbicide-tolerant maize, the common assumption is that the relevant herbicide would be used. The selective pressure would thus act in favor of the transgenic hybrids and increase the likelihood of the spread of the transgene in the wild subspecies or in the evolution of a new hybrid lineage.

What is more difficult to determine is whether an introgressed crop allele, transgenic or not, would be a threat to the environment or to teosinte itself. One of the

possible risks is that of increased weediness or invasiveness of teosinte in Mexico (Ellstrand 2003). However, although the invasiveness of a large number of plants is associated with intertaxon hybridization (Ellstrand and Schierenbeck 2000), those examples make up only a tiny fraction of the cases of natural and artificial hybridization. The likelihood of a native species becoming suddenly invasive due to hybridization seems relatively low, especially for a common weed that has been hybridizing with a crop for centuries, perhaps millennia (Wilkes 1977), and has maintained a distinct gene pool over time (Fukunaga et al. 2005). Still, the selective advantage that some alleles could confer, such as herbicide resistance if the relevant herbicide is applied, could reverse this pattern to the extent that another herbicide would be required to control the weed.

It is very unlikely that transgenic herbicide-tolerant maize varieties will be intentionally planted in the Chalco region, where this particular teosinte is found. Chalco is a mountainous region, with topography unsuitable for the mechanized “no-till” agriculture associated with herbicide-resistant crops. Although the possibility cannot be excluded, the likelihood of both extensive use of glyphosate and the unintentional planting of transgenic glyphosate-resistant maize, the joint conditions under which the transgene would be at its presumed selective advantage, are very unlikely for the Chalco region. If the allele did find its way into Chalco teosinte and use of glyphosate in the region increased dramatically, the allele would, of course, increase the fitness of transgenic teosinte.

The numerous examples of increased extinction risk due to interspecific hybridization and introgression (reviewed in Levin et al. 1996) suggest that this possibility should also be considered. The size and number of the various teosinte taxa, including the one that we studied, have generally dwindled over the last half century (Sanchez González and Ruiz Corral 1997). The vegetative and reproductive vigor observed in our first-generation hybrids, combined with an advantageous novel trait, could indeed allow an introgressed lineage to overcome the disadvantage from domestication traits expressed in the hybrids. If the introgressed hybrid-derived lineages maintain higher fitness and recover the seed-shattering trait, they could out-compete teosinte and finally result in its extinction, especially if the recipient teosinte population size is small (Wolf et al. 2001). In the specific case of herbicide tolerance, the use of the associated herbicide would reduce the size of the pure wild teosinte population and favor the hybrid derivatives. On the other hand, advanced generations may show breakdown of heterosis, as shown in a study of hybrids between the crop *Brassica napus* and weedy *B. rapa*, in which the first hybrid generation was very fit (Hauser et al. 1998a) while the second generation had a somewhat lowered fitness (Hauser et al. 1998b). At the moment, however, it seems that the primary threat to

teosinte is human disturbance, rather than hybridization (Sánchez González and Ruiz Corral 1997).

Most other studies on the relative performance of transgenic vs. non-transgenic crop \times wild hybrids have shown that presence of a transgene has no influence on the fitness or vigor of those plants in the field, especially in the absence of a selective pressure associated with the transgene itself. For example, a transgene conferring herbicide resistance had no effect on the fitness of crop \times wild rice hybrids (Oard et al. 2000), while the hybrid origin of those plants had a negative effect compared to the wild type. And the effect of the recurrent parent had also a much greater effect than the presence or absence of a transgene for herbicide tolerance for sixth generation backcrosses descended from hybrids between canola and wild radish (Guéritaine et al. 2002).

Not surprisingly, the results can be different when the study includes relevant selective pressure. Snow et al. (2003) showed a positive effect of a Bt transgene on the fitness of crop \times wild sunflower hybrids, due to an increased protection against herbivores. But no direct effect was observed for hybrids between wild and cultivated sunflower genetically modified for resistance to white mold in the presence of the pathogen (Burke and Rieseberg 2003). In the latter case, the protection against the pathogen did not necessarily increase the fitness of transgenic hybrids. In a study on crop \times wild squash hybrids, Fuchs et al. (2004) observed that only under high disease pressure did transgenic virus-resistant hybrids and two backcross generations have higher fitness than non-transgenic equivalent plants. Interestingly, the wild (non-transgenic) parent performed better than all the hybrids and backcross plants, transgenic or not, under low disease pressure.

In conclusion, our study provides evidence that if transgenic maize is introduced into areas where it will hybridize with *Zea mays* ssp. *mexicana*, the process of introgression is likely to begin for most alleles, transgenic or not. It is clear from this study and a growing number of others that transgenes themselves do not necessarily alter fitness due to physiological costs, except in those cases in which a selective pressure associated with the transgenic phenotype is present and not even in all of those cases.

ACKNOWLEDGMENTS

This research was funded by grants from the USDA (Biotechnology Risk Assessment Research Grants Program #2000-33210-9801 and #2002-33210-12769) and the National Science Foundation of the United States (DEB-0409984). Roberto Guadagnuolo's postdoctoral fellowship was funded by a grant from the Swiss National Science Foundation; the National Centre of Competence in Research (NCCR) Plant Survival research programme of the Swiss National Science Foundation has subsequently funded him. Monsanto's Ecological Technology Group provided access to the transgenic cultivar used in this study and methods used for the identification of the transgene. We are grateful to the staff at the University of California-Riverside Experiment Station for their preparation and care of our experimental plants. We especially express gratitude to the following for their aid in the project and/or

valuable comments offered on earlier versions of the manuscript: Subray Hegde, Lesley Blancas, François Felber, Kitty Formentin, Diana Pilson, and Sky Lee.

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