Absence of detectable transgenes in local landraces of maize in Oaxaca, Mexico (2003–2004)

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In 2000, transgenes were detected in local maize varieties (landraces) in the mountains of Oaxaca, Mexico [Quist, D. & Chapela, I. H. (2001) *Nature* 414, 541–543]. This region is part of the Mesoamerican center of origin for maize (*Zea mays* L.), and the genetic diversity that is maintained in open-pollinated landraces is recognized as an important genetic resource of great cultural value. The presence of transgenes in landraces was significant because transgenic maize has never been approved for cultivation in Mexico. Here we provide a systematic survey of the frequency of transgenes in currently grown landraces. We sampled maize seeds from 870 plants in 125 fields and 18 localities in the state of Oaxaca during 2003 and 2004. We then screened 153,746 sampled seeds for the presence of two transgene elements from the 35S promoter of the cauliflower mosaic virus and the nopaline synthase gene (nopaline synthase terminator) from *Agrobacterium tumefaciens*. One or both of these transgene elements are present in all transgenic commercial varieties of maize. No transgenic sequences were detected with highly sensitive PCR-based markers, appropriate positive and negative controls, and duplicate samples for DNA extraction. We conclude that transgenic maize seeds were absent or extremely rare in the sampled fields. This study provides a much-needed preliminary baseline for understanding the biological, socioeconomic, and ethical implications of the inadvertent dispersal of transgenes from the United States and elsewhere to local landraces of maize in Mexico.

corn | gene flow | biosafety | germplasm | introgression

The potential dispersal of transgenes from genetically modified (GM) maize into relatively isolated landraces of maize in Mexico raises important scientific and policy issues. Worldwide, regulatory approval of transgenic crops has proceeded at different rates in different nations, sometimes resulting in trade and stewardship disputes. Also, accidental or illegal transport of transgenic crops may occur independently of domestic regulatory systems, and it is expected that transgenic crops such as maize (corn), soybean, cotton, rice, and canola will not be completely contained after they are released commercially in a given country. Seeds of transgenic crops are easily carried across international borders, intentionally or not, and in some cases local farmers can be expected to selectively propagate plants with useful transgenic traits (e.g., maize and cotton with insect resistance or soybean with herbicide resistance; ref. 1). The inevitable international dispersal of transgenic crops may take these products to areas where their possible health, environmental, or socioeconomic effects are different from those in the country of origin.

Transgenic maize has been cultivated commercially in the United States since 1996, and by 2000 ≈25% of American maize had transgenic resistance to certain insects and/or herbicides (this proportion was ≈40% by 2003, http://ers.usda.gov/Data/BiotechCrops/ExtentofAdoptionTable1.htm). Other governments that had approved environmental releases of GM maize by 1996 include Argentina and Canada (www.agbios.com/dbase.php?action = ShowProd&data = 176). In contrast, Mexico imposed a de facto moratorium on all environmental releases of GM maize starting in 1998 and continuing through 2004, as a policy of the Secretary of Agriculture, Livestock, Rural Development, Fisheries, and Food. Before 1998, a few GM maize varieties were used in controlled, small-scale field experiments in Mexico, but under these conditions no pollen or seed escape was likely because of the biosafety measures applied. All transgenes in maize plants that were tested before the 1998 de facto moratorium had the 35S cauliflower mosaic virus (CaMV) promoter, the nopaline synthase (NOS) terminator sequence, or both.

In 2001 and 2002, David Quist and Ignacio Chapela (2, 3) published evidence for the presence of transgenic DNA constructs in native maize landraces that were sampled from northern Oaxaca in the autumn of 2000. They detected the 35S promoter sequence in pooled kernel samples from four of six cobs of maize by using PCR-based methods and a dot-blot DNA hybridization technique. The 35S sequence was also present in a bulk sample of maize grain from local stores of the Mexican governmental agency Diconsa, which distributes subsidized food throughout Mexico. It was assumed that this transgenic grain had been imported from the United States and may have been planted unknowingly by farmers, although other routes of gene flow were also possible (4). Quist and Chapela (2) also used PCR-based methods to detect the NOS terminator sequence in two of the four transgenic landrace cobs, and a Bt *cry1Ab* endotoxin sequence from *Bacillus thuringiensis* was detected in one of these cobs. Further studies by the Mexican government provided corroborating evidence for the presence of transgenes in Oaxacan landraces, as described below. Other findings that were reported by Quist and Chapela (2, 3) were criticized because they were based on results from inverse PCR, which is especially prone to artifacts (5–7).

To confirm the possible presence of transgenic maize in Oaxaca, the National Institute of Ecology and the National Commission for the Knowledge and Use of Biodiversity sampled grain from landraces grown in Oaxaca and harvested in 2000 and commissioned molecular analyses from two independent national laboratories in Mexico. Both laboratories used standard PCR-based methods and reported the presence of the 35S CaMV promoter sequence in some of the sampled material (8).†† A third agency, the Interministerial Commission on Biosafety

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and Genetically Modified Organisms, also confirmed the presence of transgenic DNA in native Mexican maize that was grown in Oaxaca in 2000 and 2001.[††]

Although no peer-reviewed reports of the Mexican government studies have been published in scientific journals, the presence of transgenes in Oaxaca was widely acknowledged (e.g., refs. 4, 9, and 10). Mexico imports several million tons of maize from the United States each year (e.g., 6.5 million tons in 2001 and 5.4 million tons in 2002; www.infoaserca.gob.mx/boletineszip/boletines.shtml), representing mixtures of grain from conventional and GM hybrid varieties, and rural farmers are known to plant these seeds in some cases (11, 12). Also, it would be relatively easy for hybrid GM maize seeds to reach Mexico by being carried or shipped from the United States unofficially. Therefore, many people concluded that transgenes from modern hybrids could be introduced repeatedly into native Mexican landraces. This conclusion led to debate over possible biological, economic, and cultural implications of transgenes that might introgress into maize landraces in the Mexican countryside, prompting a review of maize biodiversity by the trinational Commission for Environmental Cooperation under Article 13 of North America Free Trade Agreement in 2002–2004 (4). Meanwhile, the National Institute of Ecology initiated a campaign in Oaxaca to inform small-scale traditional farmers about biotechnology and biosafety issues and advise them against accidentally planting transgenic maize during the government's de facto moratorium. Thus, one of several possible sources of transgenic maize seed that was available to rural farmers may have been diminished.

A key recommendation of Quist and Chapela (2) and the Commission for Environmental Cooperation report (4) was to gain a better understanding of transgene introgression into indigenous landraces of maize in Mexico and, if present, to identify which types of transgenes occur and how common they are. In agreement with these recommendations, our study, which was carried out in 2003 and 2004, is a peer-reviewed systematic survey of transgenic elements in Mexican landraces. This study included the same region that was examined by Quist and Chapela (2, 3) and the three government agencies. Although our results should not be extrapolated to other regions of Mexico, or to previous years in the same region, they provide a useful frame of reference and an example of how additional monitoring for transgenes could be carried out in the future.

Materials and Methods

Sample Collection. The goal of this study was to sample large numbers of seeds from many unrelated maize plants to estimate local frequencies of transgenes in Oaxaca. Maize is an outcrossing species; each kernel represents an independent pollination event. Many kernels on the same cob are likely to be sired by different paternal plants, depending on the number of plants per field, proximity to other fields that flower at the same time, and how pollen dispersal is affected by local humidity and wind speed (e.g., refs. 13 and 14). Our strategy for determining frequencies of seeds with at least one transgenic parent was to analyze samples of pooled seeds to be able to detect rare, transgenic seeds at a reasonable cost. Because we expected to find transgenes in at least a portion of the samples, we ground the seeds in groups that could be analyzed in combination (for initial screening) or separately (if a combined sample included transgenes).

In November and December of 2003 and 2004, we collected seeds from landrace crops in the Sierra de Juárez region of Oaxaca, Mexico (Fig. 1). These included plants with kernels that were yellow (different intensities), white, black, or pinto (speckled with different color combinations). In nearly all cases, we collected cobs directly from their maternal plants. In two cases

when this was not possible, farmers gave us representative grain samples from their fields, as indicated in Table 1. In 2003, we sampled one to five fields in each of 16 localities (Table 1). One cob from each of four or five maternal plants was sampled from each field. The total number of kernels analyzed from the 2003 growing season was 50,126, collected from a total of 164 maternal plants, with an average of 306 seeds per maternal plant (Table 1).

Samples were collected in a similar manner in 2004, when we also collected seeds from "stressed" plants in most of the farmers' fields and sampled an average of 10 maternal plants per field (five normal and five stressed plants, Table 1). Based on our results from 2003, we hypothesized that early-generation hybrids between the progeny of modern maize varieties and local landraces might appear to be less healthy than landrace plants because modern varieties have not been selected to tolerate local growing conditions. Therefore, we reasoned that stressed plants (i.e., plants that were smaller or less vigorous than their neighbors) might be more likely to possess transgenes from modern transgenic varieties than more vigorous, normal plants from the same fields. Seeds from stressed and normal plants in each locality were grouped separately to test for possible differences in the presence of transgenic elements. Two of the localities that were sampled in 2003 could not be revisited in 2004, so we added two new localities to the 2004 survey (Table 1). We analyzed DNA from a total of 103,020 seeds from >706 maternal plants in 2004, with an average of 147 seeds per maternal plant (Table 1).

Choice of Seed-Testing Laboratories and Transgene Sequences. Samples were sent in double plastic bags to Genetic ID (www.genetic-id.com) (2003 and 2004) and GeneScan (www.gmotesting.com) in Metairie, LA (2004), as indicated in Table 1. Both of these commercial companies are certified for compliance with international standards set by the International Organization for Standardization. Their services are widely used by private industry, government agencies, and nongovernmental organizations, and they routinely test for very low concentrations of transgenic material in maize grain (e.g., ref. 15). Initial testing for the presence of transgenic maize typically involves the CaMV 35S promoter, which is found in all commercialized transgenic maize except Monsanto's glyphosate-tolerant GA21 variety (deregulated in the United States in 1997; refs. 15 and 16). A second useful marker is the NOS terminator sequence, which is present in several commercialized transgenic maize varieties, including the GA21 variety (www.agbios.com/dbase.php?action = ShowForm; ref. 16).

In 2003, all molecular analyses were carried out by Genetic ID. In 2004, one-third of the seeds from each locality was sent to Genetic ID, one-third was sent to GeneScan, and the remaining third was archived in the laboratory at the Instituto Nacional de Ecología. We have archived ground homogenates from all of the analyzed seeds at Ohio State University, and they are available for further testing.

Numbers of Seeds in Ground Samples for Analyses. We used extra precaution to minimize the possibility of failing to detect transgenic seeds in the ground samples used for DNA analyses. Genetic ID and GeneScan routinely quantify 0.01% transgenic material, i.e., one transgenic seed in a sample of 10,000, with a degree of accuracy that is close to 100%, regardless of whether the seed is hemizygous or homozygous for the transgene elements. In 2003, when we suspected that frequencies of transgenic seeds might be >5% in some fields, we analyzed samples from each field separately and each sample of ground homogenate represented no more than 300 seeds. Two replicate 0.5-g samples of homogenate from each field were used for DNA extraction. In 2004, after we had not detected transgenes in the previous year, we doubled the numbers of
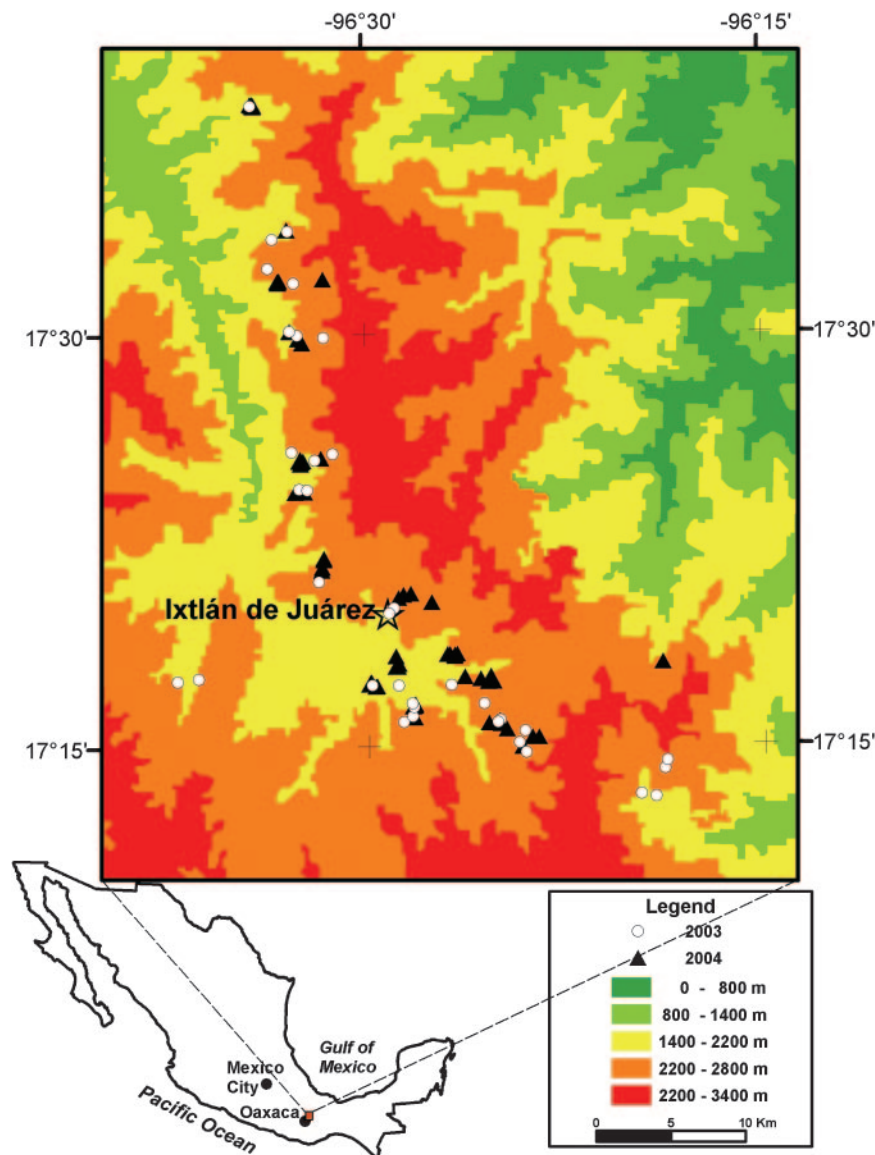
Fig. 1. Map of fields in Oaxaca, Mexico, where seeds were collected from maize landraces in 2003 and 2004. Some symbols overlap where fields were close to each other. Global Positioning System coordinates of the localities (villages) in which the fields were located are listed in Table 1.

seeds that were sampled and used larger numbers of seeds per homogenate, ranging from 810 to 5,630 (Table 1). At Genetic ID, each sample of seeds from normal or stressed plants in a given field was ground, homogenized, and subsampled to provide equal aliquots of at least 0.5 g each. These aliquots were combined to obtain duplicate composite samples of normal vs. stressed seeds from each locality. At GeneScan, seeds from normal and stressed plants at each locality were ground separately, and equal aliquots of the ground homogenate from these two groups were combined to create duplicate composite samples. All composites represented sample sizes that were well below the limit of 10,000 seeds needed to ensure reliable detection of a single transgenic seed.

Laboratory Analyses. Genetic ID and GeneScan used similar analytical methods for DNA extraction, amplification, and electrophoresis. DNA was purified by using DNA extraction kits, which use RNase digestion followed by chloroform extraction. The aqueous phase was further cleaned by using silica membrane technology with microspin columns. Purified DNA

was quantified with UV spectroscopy at 260 nm (at Genetic ID), or a sample of extracted DNA was run on an agarose gel to assess DNA yield and quality (at GeneScan).

Standard primers were used to amplify the 35S CaMV and NOS sequences in subsamples from each extraction sample, using sufficient amplification cycles to detect at least 0.01% transgenic material. The duplicate extraction samples, two buffer blank controls, and two or more positive controls with known amounts of 35S or NOS target sequences, each 10 μl per reaction, were subjected to PCR (positive controls included 0.01% transgenic material; Fig. 2). The amount of sample DNA per reaction was 200 ng. At both Genetic ID and GeneScan a known maize gene, *adh1*, also was amplified from each extraction sample as a further positive control to guarantee that the sample included good-quality DNA.

DNA from each amplification product was analyzed by using qualitative (end point) and/or quantitative (real time) PCR. For qualitative PCR, amplification products were separated by gel electrophoresis and stained with ethidium bromide. Gels were scored only if the positive and negative controls resulted

Table 1. Localities and sample sizes for molecular analyses of landrace seeds collected from farmers' fields in Oaxaca Mexico in 2003 and 2004

Locality	ID	North	West	Group ID	No. of fields per locality	No. of maternal plants sampled	No. of seeds analyzed by Genetic ID	No. of fields per locality	No. of maternal plants sampled	Stressed	Normal	Stressed plus normal
		Global Positioning System location			2003			2004		No. of seeds analyzed by Genetic ID		No. of seeds analyzed by GeneScan
Ixtlán	1	96°29'14"	17°19'50"	1	2	7	2,398	4	52	1,450	1,600	3,050
Sn Andrésyatuni	2	96°24'06"	17°14'57"	2	2	9	2,069	4	35	800	1,425	2,225
Santiago Comaltepec	3	96°32'54"	17°33'54"	5	2	6	1,833	5	>21*	1,010	1,150	2,160
San Pablo Macuiltianguis	4	96°33'12"	17°32'04"	6	2	8	2,928	6	47	1,360	2,400	3,760
San Juan Luvina	5	96°32'55"	17°30'14"	7	4	16	5,845	5	52	1,195	1,975	3,170
San Juan Bautista Atepec	6	96°32'18"	17°25'36"	8	3	9	2,673	5	56	1,850	1,950	3,800
Trinidad	7	96°25'05"	17°15'65"	3	2	9	2,095	4	41	1,020	1,600	2,620
Sn. Fco. La Reforma	8	96°34'11"	17°38'21"	4	5	24	9,166	8	80	3,230	2,400	5,630
San Juan Ev. Analco	9	96°32'20"	17°24'24"	9	2	4	1,383	5	51	1,675	2,000	3,675
Santa Maria Jaltianguis	10	96°31'40"	17°21'43"	10	2	5	1,238	5	53	1,200	2,000	3,200
Capulalpan de Mendez	11	96°26'46"	17°18'22"	11	0	0	0	5	51	1,005	1,900	2,995
Santiago Xiacui	12	96°25'55"	17°17'33"	12	1	4	1,091	5	42	810	2,000	2,810
Santa Maria Yahuiche	13	96°28'52"	17°17'57"	13	0	0	0	5	>38*	840	1,950	2,790
San Miguel Amatlan	14	96°28'16"	17°16'42"	14	2	8	1,773	4	38	1,025	1,200	2,225
San Juan Chicomezuchitl	15	96°29'42"	17°17'15"	15	5	16	4,275	6	51	1,700	2,100	3,800
Santa Catarina Lachatao	16	96°28'19"	17°16'05"	16	2	10	2,882	5	57	1,900	2,000	3,900
Nuevo Zoquiapan	17	96°37'12"	17°17'26"	17	3	12	3,989	0	0	0	0	0
Santiago Laxopa	18	96°18'40"	17°13'01"	18	4	17	4,488	0	0	0	0	0
Totals					43	164	50,126	81	706	51,810		51,810
Mean no. seeds per field							1,166			1,279		
Mean no. seeds per locality							3,133			6,476		
Total no. of seeds analyzed												153,746

The numbers of ground seeds represented in each DNA extraction are shown for 2004 (maximum of 5,630). In 2003, < 300 seeds were used in each DNA extraction (see text).

*These are the minimum number of maternal plants sampled. Farmers had already harvested and separated the kernels from the maternal plants in some of the sampled fields.

in the presence or absence of known DNA fragments of the expected size (Fig. 2). A negative score indicates that no band was detected by visual inspection, including faint bands that might indicate a transgene frequency of <0.005%. In all cases, and for both PCR methods, replicates from the same composite sample gave the same result, which was negative for both the 35S and NOS sequences.
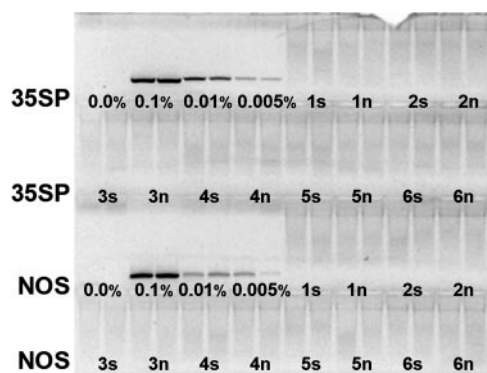

Fig. 2. PCR amplification products for the 35S CaMV marker (195 bp) and the NOS marker (102 bp) from known dilutions and representative landrace samples. Two replicate lanes for each sample on an agarose gel are shown. Dilutions of 0.0%, 0.1%, 0.01%, and 0.005% were prepared by using purified DNA from certified GM maize. Samples 1–9 were obtained from landrace localities 1–9 in 2004 (Table 1). s, Stressed plants; n, normal plants.

Statistical Analyses. We used two approaches to calculate the probabilities of failing to sample transgenic seeds by chance alone in each locality and year. For the first set of calculations, seeds taken from the same cobs were considered to be independent samples because their silks were likely to receive pollen from many different paternal plants. For the second set of calculations, we considered seeds from the same cob to be nonindependent, given that their paternity was not known, and we used maternal plants (cobs) as the unit of observation. Although this second approach is undoubtedly far too conservative, it provides a minimum estimate of the number of independent observations in the study. Maternal plants were considered to be independent because they were selected haphazardly, with the stipulation that half of the plants in 2004 were in the stressed group.

First, for each sample of seeds i collected from a given locality in a given year, we calculated the binomial probability of failing to include even a single transgenic seed if such seeds occurred with an underlying frequency q of 0.01%, as P_i (0 inclusions|q = 0.0001). Multiplying across i localities, we then calculated the joint probability of failing to detect even a single transgenic seed, $P_{overall}$ (0 inclusions|q = 0.0001), for all localities in a given year. In addition, we calculated the frequency ($q_{0.95}$) at which we would have included at least one transgenic seed with 95% certainty across all localities in each year. We also performed these calculations with maternal plants as the unit of observation. A Bayesian analysis of the data gave similar results (David Andow, personal communication).

Results and Discussion

We found no evidence of either the 35S CaMV or NOS transgene sequences in this survey, which indicates that the frequency of transgenic seeds from maize grown in the sampled region was near zero in 2003 and 2004. To evaluate how close to zero this frequency may be, we consider two possible limitations of this study: first, potential limitations of our analytical methods and, second, recognized limits caused by the numbers of seeds that were analyzed.

PCR-based methods are more prone to give false-positive results, because of minute levels of contamination, than false negatives (17). Nonetheless, there is a small chance that we obtained false negatives despite the fact that we used two ubiquitous transgene elements and two independent laboratories, each of which analyzed duplicate composite samples from each locality (i.e., four replicates per locality in 2004). False negatives could occur because of human error and/or random chance at any stage of the analytical process. However, these sources of error seem extremely unlikely given the fact that these companies have stringent operating procedures that are routinely checked for compliance with international certification standards. Most of our ground samples for DNA extraction represented <3,000 seeds and all were derived from <10,000 seeds (Table 1), which is the maximum sample size in which a single transgenic seed is detected reliably. Moreover, even if a transgenic seed lacked the 35S promoter, as is the case for Monsanto's GA21 variety, it would have had the NOS terminator sequence. Thus, we conclude that the likelihood of observing false negatives in our molecular analyses was negligible.

Another source of uncertainty is the thoroughness with which seeds were sampled relative to the total population of seeds that represent the local maize crop at each field, locality, and all localities combined. We analyzed an average of 3,133 and 6,476 seeds from each locality in 2003 and 2004, respectively (Table 1). When all of the sampled seeds were considered as independent observations, the joint probability of failing to detect a single transgenic seed at any of the localities was 0.00665 in 2003 and 0.00003 in 2004, assuming that the true frequency of transgenic seeds was at least 0.01% [$P_{overall}$ (0 inclusions|q = 0.0001)]. In other words, these joint probabilities were miniscule when considered across localities in the same year. Likewise, we can conclude with 95% certainty that if transgenic seeds occurred in the study region, their frequency ($q_{0.95}$) was no greater than 0.00006 in 2003 and 0.00003 in 2004. In contrast, when maternal plants (cobs) were used as the unit of observation rather than seeds, we estimated with 95% certainty that the frequency of cobs with at least one transgenic seed was <0.0043 in 2004 and <0.0032 for both years combined. The maximum frequency of transgenic seeds in our study should fall somewhere between these seed-based and plant-based estimates. This value is likely to be closer to 0.0001 (0.01%) because we expect that many seeds from the same cob were sired by different paternal plants.

Now, it is possible to discuss the potential consequences of current transgene introgression in landraces in the context of quantitative data. These consequences include possible effects on human health and the environment, effects on the genetic diversity that is maintained in landrace populations, and effects on the perceptions of local farmers about the cultural integrity of maize (4, 12). Our results suggest that many concerns about unwanted or unknown effects of this process can be discounted at present, at least within the sampled region. Despite the fact that Mexico imports transgenic maize grain for food, feed, and processing, and despite the expectation that some of this grain may be planted in farmers' fields, the introgression of transgenes in the sampled area appeared to be negligible in 2003 and 2004.

Assuming that transgenes were present before, several mechanisms may have prevented them from persisting at detectable frequencies in the sampled seeds. First, the influx of transgenic seeds may have declined after farmers became aware of this issue. Meanwhile, transgenes that were present in 2000 or 2001 may have backcrossed into landrace populations such that their current frequencies are extremely low. Transgenes could also be lost because of genetic drift if they were rare enough to become locally extinct. Furthermore, transgene frequencies could decline if plants that are grown from imported commercial seeds produce less pollen or seed than other plants in the same population. This process could lead to natural selection as well as farmers' selection against the transgenic plants and their immediate progeny. Imported grain is derived from open pollination of modern commercial F_1 hybrid varieties, and these F_2 seeds may produce plants that are less vigorous than their parents because of reduced hybrid vigor. Also, seeds from commercial varieties have not been selected to perform well under the abiotic and biotic stresses that prevail where landraces are grown.

Nonetheless, transgenic F_2 plants (and any F_1 hybrid plants that may have been planted inadvertently or intentionally) could cross-pollinate with nearby landrace plants, even if these plants are not as vigorous as their neighbors. The transgenes that they possess may or may not become more common, depending on whether the novel gene confers a net selective advantage in early and advanced generations of backcrossing with the landraces (4, 18). If these transgenes are tightly linked to deleterious genes of modern cultivars, farmers and local environmental conditions could select against them. In contrast, some transgenes, including Bt genes (e.g., ref. 19), may provide a selective advantage to recipient populations. These types of transgenes have the potential to increase in frequency within landrace populations if they are not tightly linked to other genes that would be discriminated against by farmers' breeding practices or other selective pressures in the environment, particularly if they confer resistance to local insect pests.

One question that has received a great deal of publicity is whether the presence of transgenes might compromise the genetic diversity that is maintained in locally adapted genotypes of maize in Mesoamerica (4, 12, 20). A portion of this landrace diversity is housed in seed collections, such as the germplasm collection at the International Maize and Wheat Improvement Center (www.cimmyt.org). However, a much larger and more dynamic source of genetic diversity resides in the evolving populations of maize that are repeatedly mixed and selected by individual farmers (11, 12, 21, 22). Although it is unlikely that the presence of a few transgenes would reduce the genetic diversity of these populations to a greater extent than gene flow from non-GM modern cultivars, transgenes have been perceived as a threat to the crop's cultural identity by local farmers (4, 12). In that sense, cultural perceptions and the pride of local farmers in the value of their traditional crop lineages is a fundamental tool for the conservation of germplasm diversity (4, 12). Our findings help address farmers' concerns about the possible presence of transgenes in landraces.

Evidence that transgenes are rare or absent in the sampled area should not be extrapolated to other regions of Mexico without quantitative data, nor is the current situation likely to remain static. Although the Instituto Nacional de Ecología sampled other localities in Guerrero (2002) and Michoacán (2003) with similar negative results from Genetic ID (see *Supporting Text* and Table 2, which are published as supporting information on the PNAS web site), transgenes in Mexican maize could be present in other locations and years. The Mexican government recently enacted new legislation for evaluating GM crops, including special protection for maize and other plants for which Mexico is a center of origin. Depending on how this law is interpreted and enforced, commercial cultivation of modern GM maize in Mexico may or may not take

place. In the future, however, we expect that the prevalence and variety of transgenic traits in maize will increase because other countries are actively promoting new GM varieties and the global area of GM maize cultivation is increasing rapidly. Also, several biotechnology companies are developing transgenic pharmaceutical-producing maize varieties that would require strict containment measures (e.g., refs. 23 and 24). Whether new types of transgenic traits will enter Mexico and be propagated in landraces, intentionally or not, is not clear.

In conclusion, we found no current evidence for transgene introgression into maize landraces in the Sierra de Juarez of Oaxaca. Our data provide a baseline for further research and an important frame of reference for local farmers, government agencies, nongovernmental organizations, and others who are concerned about the biological, socioeconomic, and ethical implications of transgenic crops that disperse across international borders. Maize plays a central role in the diet and culture of Mexican society, and approximately two-thirds of the maize grown in Mexico consists of landraces (4, 11, 12). Further

monitoring of transgene introgression is warranted because there are many routes by which novel transgenes could potentially spread and multiply within and among maize populations (4, 11, 12, 21, 22). Worldwide, the ability of transgenes to disperse among countries merits closer scientific monitoring, especially when the receiving country has not approved them for environmental release, and in cases when the genetic modification eliminates or impairs the use of a particular crop as food.

1. Aldhous, P. (2002) *Nature* **420,** 730–731.
2. Quist, D. & Chapela, I. H. (2001) *Nature* **414,** 541–543.
3. Quist, D. & Chapela, I. H. (2002) *Nature* **416,** 602–603.
4. Commission for Environmental Cooperation of North America (2004) *Maize and Biodiversity–The Effects of Transgenic Maize in Mexico: Key Findings and Recommendations*, Article 13 Report (North American Commission for Environmental Cooperation, Montreal).
5. Christou, P. (2002) *Transgenic Res.* **11,** iii–v.
6. Metz, M. & Fütterer, J. (2002) *Nature* **416,** 601–602.
7. Kaplinski, N., Braun, D., Lisch, D., Hay, A., Hake, S. & Freeling, M. (2002) *Nature* **416,** 602.
8. Ezcurra, E., Ortiz, S. & Soberón Mainero, J. (2001) in *LMOs and the Environment: Proceedings of an International Conference*, ed. Roseland, C. R. (Organization for Economic Cooperation and Development, Paris), pp. 286–329.
9. Wisniewski, J.-P., Frangne, N., Massonneau, A. & Dumas, C. (2002) *Biochimie* **84,** 1095–1103.
10. Butler, D. (2002) *Nature* **415,** 948–949.
11. Perales, R. H., Brush, S. B. & Qualset, C. O. (2003) *Econ. Bot.* **57,** 21–34.
12. Bellon, M. R. & Berthaud, J. (2004) *Plant Physiol.* **134,** 883–888.
13. Luna, V. S., Figueroa, M. J., Baltazar, M. B., Gomez, L. R., Townsend, R. & Schoper J. B. (2001) *Crop Sci.* **41,** 1551–1557.
14. Brown, A. H. & Allard, R. W. (1970) *Genetics* **66,** 133–145.
15. Lipp, M., Shillito, R., Giroux, R., Spiegelhalter, F., Charlton, S., Pinero, D. & Song, P. (2005) *J. AOAC Int.* **88,** 136–155.
16. Matsuoka, T., Kuribara, H., Takubo, K., Akiyama, H., Miura, H., Goda, Y., Kusakabe, Y., Issiki, K., Toyoda, M. & Hino, A. (2002) *J. Agric. Food Chem.* **50,** 2100–2109.
17. Wolf, C., Scherzinger, M., Wurz, A., Pauli, U. Hübner, P. & Lüthy, J. (2000) *Eur. Food Res. Technol.* **210, 367,** 372.
18. Ellstrand, N. C. (2003) *Dangerous Liaisons? When Cultivated Plants Mate with Their Wild Relatives* (Johns Hopkins Univ. Press, Baltimore).
19. Snow, A. A., Pilson, D., Rieseberg, L. H., Paulsen, M., Pleskac, N., Reagon, M. R., Wolf, D. E. & Selbo, S. M. (2003) *Ecol. Appl.* **13,** 279–286.
20. Gepts, P. & Papa, R. (2003) *Environ. Biosafety Res.* **2,** 89–103.
21. Pressoir, G. & Berthaud, J. (2004) *Heredity* **92,** 88–94.
22. Bellon, M. R., Berthaud, J., Smale, M., Aguirre, J. A., Taba, S., Aragon, F., Diaz, J. & Humberto, C. (2003) *Genet. Resources Crop Evol.* **50,** 401–416.
23. Daniell, H., Streafield, S. J. & Wycoff, K. (2001) *Trends Plant Sci.* **6,** 219–226.
24. Howard, J. A. & Donnelly, K. C. (2004) *J. Agric. Environ. Ethics* **17,** 545–558.