

## NEWS AND VIEWS

### PERSPECTIVE

## Unwanted Transgenes Re-Discovered in Oaxacan Maize

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*Received 7 November 2008; revision received 26 November 2008; accepted 27 November 2008*

Transgenic [GM (genetically modified)] maize is shipped all over the world, and its ability to germinate, grow, and hybridize with local landraces of the crop has generated tremendous scientific, social, and political controversy. The great genetic diversity of farmer-produced landraces represents a vital resource for subsistence farmers, future crop breeding, and cultural heritage preservation. Although the Mexican government banned GM maize cultivation in 1998, vast quantities of living GM grain are imported from the USA and seeds can easily enter the country by other routes. In 2000, Quist & Chapela (2001) discovered transgenes in four ears of landrace maize and in seeds from a government-sponsored Distribution Conasupo Sociedad Anonima (DICONSA) grain distribution centre in Oaxaca. Their controversial paper in *Nature* set off an explosion of publicity and speculation about how widely these novel genetic elements had proliferated, and what the consequences of ubiquitous gene flow might be. However, a more extensive survey of this region failed to detect transgenes in 2003 and 2004 (Ortiz-García *et al.* 2005a), suggesting that transgenic plants were rare or absent in the sampled fields. Here, Pineyro-Nelson *et al.* (2008) provide a valuable counterpoint to that survey, resolving apparent contradictions in the literature and raising the bar for subsequent studies of immigrating transgenes. They show that transgenes were present in Oaxaca in both 2001 and 2004. Their paper explains how sampling methods, statistical analyses, and problems with analytical techniques can lead to inconsistent estimates of transgene frequencies in maize populations. This is a must-read paper for those who follow genetically modified organisms (GMO) biosafety research.

Even with non-GM crops, researchers have worried about whether the diverse and locally adapted gene pools of landraces could be overwhelmed by abundant gene flow from modern cultivars. Indeed, another study in this issue of *Molecular Ecology* reports a significant amount of introgression from modern hybrid maize into 'flint' landraces that are preferred for making polenta in Italy (Bitocchi *et al.* 2008). Despite several decades of gene flow into Italian landraces, no evidence for

genetic erosion has been found to date. A similar process could be taking place in Mexico, depending on the dynamics of gene flow via pollen and seeds, yet a more sensitive topic is the unintended spread of transgenes that are banned for cultivation. Several types of GM maize are grown in other countries, but the key role of landraces in Mexico, which is the centre of origin for this staple crop, has prompted caution for allowing GM varieties to be cultivated. Some environmental groups, in particular, view the spread of transgenes as genetic contamination, and many people are dismayed by the apparent futility of efforts to keep transgenes out of landrace gene pools.

Monitoring for the presence of transgenes in the Mexican countryside is a daunting task that requires teams of experts from several disciplines (e.g. Serratos-Hernández *et al.* 2007; Mercer & Wainwright 2008). Furthermore, the politically sensitive nature of this information has made it difficult for researchers to publish their findings. Here, Pineyro-Nelson *et al.* (2008) have carried out a masterful job of assembling a large data set that will help advance the scientific quality of future efforts to monitor the dispersal of GMOs in the environment. Nearly all GM maize varieties have the 35S promoter from the cauliflower mosaic virus, and most have a nopaline synthase (NOS) terminator from *Agrobacterium tumefaciens*. Thus, these elements are useful markers for other transgenes, and polymerase chain reaction (PCR)-based methods can be used to detect a single transgenic seed in a homogenized sample of several thousand seeds. However, the exquisite sensitivity of PCR amplification is both a benefit and a liability, as trace amounts of contamination can yield false-positives. Pineyro-Nelson *et al.* used duplicate or triplicate, blind-coded samples to address this problem, and positive PCR results from their 2001 samples were confirmed with Southern analyses and sequencing.

Pineyro-Nelson *et al.* (2008) found evidence for transgenes in three out of 20 different communities that were sampled in 2001 and/or 2002 (Table 1). When two of the three communities with positive results were sampled more intensively in 2004, transgenic plants were detected in 3 of 30 fields in Santiago Xiacui, and 8 of 30 fields in Santa Maria Jaltianguis. Based on interviews with farmers about seed exchange, the authors concluded that transgenes probably persisted in these communities after 2001, rather than having been re-introduced. They also present a simulation model showing that transgenes are likely to be highly aggregated geographically when pollen and seed mixing are limited, as appears to be the case in these communities. This finding challenges statistical methods for calculating the probability of detecting rare events based on assumptions of random or uniform geographical distributions (see Cleveland *et al.* 2005; Ortiz-García *et al.* 2005b). Indeed, the patchiness of transgenic plants could explain why none were detected by Ortiz-García *et al.* (2005a), even though they sampled several

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**Table 1** Finding transgenic maize plants: a comparison of two surveys in Oaxaca, Mexico

| Year                                           | Number of sampled communities* |                             | Number of sampled fields or households |                             |                                      |
|------------------------------------------------|--------------------------------|-----------------------------|----------------------------------------|-----------------------------|--------------------------------------|
|                                                | Total                          | No. with transgenes present | Total                                  | No. with transgenes present | Total no. of maternal plants sampled |
| Pineyro-Nelson <i>et al.</i> 2008 (this study) |                                |                             |                                        |                             |                                      |
| 2001                                           | 20                             | 3†                          | 21                                     | 3                           | seeds from 1–5 ears per household    |
| 2002                                           | 5‡                             | 0                           | 117                                    | 0                           | 682 plants (17 114 seeds)            |
| 2004                                           | 2§                             | 2                           | 60                                     | 11                          | 18 000 plants (not seeds)            |
| Ortiz-García <i>et al.</i> 2005a               |                                |                             |                                        |                             |                                      |
| 2003                                           | 16¶                            | 0                           | 43                                     | 0                           | 164 plants (50 126 seeds)            |
| 2004                                           | 16¶                            | 0                           | 81                                     | 0                           | 706 plants (103 620 seeds)           |

\*No. of different communities sampled in each year, not including samples obtained from seed markets. Many communities were sampled in more than one year. No transgenes were found in grain from two seed markets sampled in 2001 or one sampled in 2002.

†In preliminary analyses, 10 communities had transgenic samples in 2001, but this was not confirmed by further analyses and therefore might have been due to false-positives. The presence of transgenic plants was confirmed with Southern blot analyses in three communities.

‡None of these communities had confirmed transgenic samples in 2001.

§Both of these communities had transgenic maize plants in 2001 and were sampled more intensively for this reason.

¶These communities include the two that had transgenic plants in 2001 and 2004: Santa Maria Jaltianguis and Santiago Xiacui (Pineyro-Nelson *et al.* 2008). Ortiz-García *et al.* (2005a) collected seeds from a total of seven fields in Santa Maria Jaltianguis and six fields in Santiago Xiacui in 2003–2004.

fields in Santiago Xiacui and Santa Maria Jaltianguis. If transgenic plants are aggregated within and among communities, future sampling efforts should include as many widely spaced fields and communities as possible (Pineyro-Nelson *et al.* 2008). Also, if aggregated distributions are common, estimates of the maximum frequencies at which undetected transgenic plants would be overlooked should be interpreted with strong caveats about the assumptions underlying these calculations.

Another important aspect of the study is the authors' evaluation of various methods for reliably detecting transgenes within and among laboratories. A commercial seed-testing company reported negative results for the 35S promoter for three leaf samples that the authors confirmed as positive for 35S. Other findings from the company were supported by the authors' analyses, including a blind sample with one transgenic seed in a sample of 1000 and negative results from all of the 2002 seed samples (> 17 000 seeds). However, the authors note that the company's standardized methods for scoring gel photos should be examined closely, as was carried out by Ortiz-García *et al.* (2005a), to avoid reporting false-negatives from faint banding patterns.

In summary, this study confirms what many people have long suspected — that transgenes have entered landrace maize populations in Oaxaca. Based on the scant published literature and the fact that Pineyro-Nelson *et al.* found some transgenic plants in three communities, we do not have enough information to estimate the frequencies of transgenic plants in this region or others, or to know whether introductions are still taking place. Mexican farmers are known to plant seeds of modern varieties of maize alongside their local landraces (Perales *et al.* 2003), thereby allowing genes from imported grain to introgress into landraces, perhaps irreversibly. Apparently, DICONSA no longer distributes GM maize, but

Mexico still imports yellow GM maize from the USA for food and animal feed.

Looking towards the future, containment of commercial maize transgenes will be impossible and eradication from landraces will be difficult (CEC 2004), but preventative measures and confinement of introduced transgenes are viable goals (Bitocchi *et al.* 2008). Further surveys will be helpful for identifying centres of introduction and preserving essentially GM-free zones of maize landraces in Mexico. Meanwhile, the uneven acceptance of GM crops around the globe will continue to spawn disputes over the sovereignty of national gene pools.

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doi: 10.1111/j.1365-294X.2008.04063.x