

host defences^{1,2}. Our results show that glucose oxidase, one of the principal components of *H. zea* saliva, is responsible for suppressing induced resistance in *N. tabacum*. This enzyme may prevent the induction of nicotine by directly inhibiting the wound-signalling molecule jasmonic acid and/or by antagonizing its interaction with other signalling pathways. As glucose oxidase is produced by a wide variety of caterpillar species^{1,6}, we may have discovered a new feature of the evolutionary arms race between plants and herbivores.

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COMMUNICATIONS ARISING

Biodiversity

Suspect evidence of transgenic contamination

Quist and Chapela¹ claim that transgenic DNA constructs have been introgressed into a traditional maize variety in Mexico, and furthermore suggest that these constructs have been reassorted and introduced into different genomic backgrounds. However, we show here that their evidence for such introgression is based on the artefactual results of a flawed assay; in addition, the authors misinterpret a key reference² to explain their results,

concluding that reassortment of integrated transgenic DNA occurs during transformation or recombination.

The discovery of transgenes fragmenting and promiscuously scattering throughout genomes would be unprecedented and is not supported by Quist and Chapela's data¹ — the incorrectly cited work² merely claims that multiple transgenes or transgene fragments can integrate into genetically linked regions of the genome during transformation, and not that they can move around the genome by recombination after integration (W. Pawlowski, personal communication).

The discovery of cauliflower mosaic virus promoter sequences, an element of

transgenic constructs, in the authors' samples is more consistent with F₁ hybridization than introgression. In introgression, a small, polymorphic genomic region is bred into a given variety through repeated backcrossing, so all progeny (or kernels) from an individual in which a (trans)gene has been introgressed will possess that gene. Quist and Chapela report, however, that on the basis of "low amplification" by the polymerase chain reaction (PCR), the transgene is evident only in a small percentage of kernels in each cob, citing a press release that reported a 3–10% abundance of transgenes in similar samples to support their claim.

The authors interpret their inverse PCR (i-PCR) results as evidence of a high frequency of transgene insertion into a range of genomic contexts, inferring from this that introgression events are relatively common and well maintained. However, their i-PCR products all seem to be artefacts of the methodology used. We examined the sequences of the reported i-PCR products (Fig. 1) and found that none contains a reasonable number of the features that would be expected in a legitimate product of amplified genomic DNA flanking the anchor sequence. An i-PCR product derived from the circularization of a single piece of DNA should contain the sequence

Editorial note

In our 29 November issue, we published the paper "Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico" by David Quist and Ignacio Chapela. Subsequently, we received several criticisms of the paper, to which we obtained responses from the authors and consulted referees over the exchanges. In the meantime, the authors agreed to obtain further data, on a timetable agreed with us, that might prove beyond reasonable doubt that transgenes have indeed become integrated into the maize genome. The authors have now obtained some additional data, but there is disagreement between them and a referee as to whether these results significantly bolster their argument.

In light of these discussions and the diverse advice received, *Nature* has concluded that the evidence available is not sufficient to justify the publication of the original paper. As the authors nevertheless wish to stand by the available evidence for their conclusions, we feel it best simply to make these circumstances clear, to publish the criticisms, the authors' response and new data, and to allow our readers to judge the science for themselves.
 Editor, *Nature*



Figure 1 Alignment of primers with cauliflower mosaic virus 35S promoter sequences and the ends of inverse polymerase chain reaction (i-PCR) products. The upstream and downstream orientations with respect to the 35S promoter were confused in the original publication¹ and are corrected here. In parentheses: primer name, or GenBank accession number and fragment size. Shaded regions represent the maize genomic sequences with the highest homology (BLAST e-value shown) to the preceding i-PCR fragment. The reverse complements of even-numbered primers are shown for alignment. The footprint of the *EcoRV* restriction enzyme is shown in bold and italicized if in alignment with the site on the 35S promoter. Capitals denote bases that match 35S sequences and are underlined in the regions of the primers; a dashed underline denotes these respective sequences in cases in which they appear at the wrong end and in the wrong orientation on the product. For the hypothetical legitimate i-PCR products, plus signs indicate areas where further transgene DNA would be found.

of the inward and outward primers on the anchor; a footprint of the restriction enzyme in both the anchor and the flanking genomic DNA (reconstituting a restriction site); and the intervening anchor 35S DNA between the primer site and the restriction site. The frequency at which the enzyme used cuts maize DNA indicates that products should average 2,000–4,000 bases.

It would also be expected that some, probably all, of the i-PCR products would contain further transgene sequences adjacent to the 35S promoter. We found that no product contained any transgene sequences. As proof that “introgressed DNA [has] retained its integrity”¹, two sequences are designated by Chapela and Quist as *adh1* sequences, “similar to synthetic constructs ... in transgenic maize ... such as Novartis Bt11”. These sequences show no similarity to the *adh1* intron sequences used in some synthetic constructs, and probably represent retrotransposon DNA.

Several mechanisms may have led to the production of i-PCR artefacts. The sequences of the authors’ primer pairs partially match several genomic sequences, which in turn show high sequence similarity to the amplified fragments (Fig. 1). Spurious products could have been generated depending on the (undisclosed) conditions of the i-PCR; for example, the 3’ ends of the primers used to amplify sequence AF434756 can directly base-pair with the genomic sequence that was amplified (Fig. 1). There was no negative control to address the possibility of i-PCR amplification from maize samples containing no transgenic DNA; furthermore, the restriction enzyme *EcoRV* generates blunt ends on digested DNA, providing no mechanism for preferential ligation of the anchor to digested DNA, rather than to random pieces of sheared DNA.

To consider any of the i-PCR products as legitimate flanking regions requires verification that the anchor is truly adjacent in the genome to the retrieved sequence. Confirmation entails a very simple experiment: PCR using a new outward primer on the anchor and a primer that is specific to the putative adjacent genomic DNA will amplify the same DNA as the i-PCR reaction, as long as the original was legitimate.

An empirical inference from Quist and Chapela’s results is that transgenic corn may be being grown illegally in Mexico, a situation that has already occurred with soybean in Brazil³ and cotton in India⁴. However, the approach used by the authors provides no mechanism for quantifying possible F₁ hybridization between traditional and transgenic varieties. The possibility that the 35S signal they detect by PCR in their five samples is due to

contamination cannot be ruled out. No indication is given of the number of repetitions in which each sample produced a positive or negative result, and results from the historical negative control sample are omitted as data not shown, with two lanes of data being excised from the gel in the authors’ Fig. 1.

Transgenic corn may or may not be hybridizing to traditional maize cultivars in Mexico. Whether these events will result in introgression of traits, and whether such introgression could have a negative effect on crop diversity, is pure speculation — so far, there is no evidence of transgenes fragmenting and scattering throughout genomes.

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Maize transgene results in Mexico are artefacts

Quist and Chapela’s conclusion¹ that the transgenes they claim to have detected in native maize in Oaxaca, Mexico, are predominantly reassorted and inserted into a “diversity of genomic contexts” seems to be based on an artefact arising from the inverse polymerase chain reaction (i-PCR) they used to amplify sequences flanking 35S transgenes from cauliflower mosaic virus (CaMV).

After i-PCR, the authors determined eight flanking sequences, two of which (K1 and A3) they claim contained transgenic sequences which they identified as homologous to *adh1*. The *adh1* region, one of the first large regions of the maize genome to be sequenced, is composed mainly of retrotransposons². *Adh1* introns are commonly used to increase expression of transgenes in maize^{3,4}.

Quist and Chapela may have confused a hit to the 160-kilobase (kb) *adh1* genomic region with a hit to an intron of the gene. The *adh1*-homologous regions that flank their amplification products K1 and A3 are located about 40 kb from the *adh1* gene. This sequence is a repetitive element that is also present in two other large maize genomic sequences, and is not an *adh1* intron or coding sequence. In fact, a search of GenBank reveals that K1 is more similar

to an element within the *bronze1* genomic sequence than to the *adh1* sequence.

The eight i-PCR products probably resulted from incorrect PCR priming, because 13 of the 15 base pairs of the authors’ primer iCMV2 can be found in the *bronze1* genomic region (GenBank accession no. AF391808.2) as well as the *adh1* genomic region (GenBank AF123535.1). In addition, the 10 base pairs at the 3’ end of the second primer used, iCMV1, are found within the same *adh1* genomic region. These represent the false priming sites amplified by Quist and Chapela as K1 and A3 (GenBank AF434754, AF434755).

Finally, the final seven base pairs of the primer iCMV2 are identical to a maize Opie retro-element (GenBank U68408.1), which is the third i-PCR sequence amplified, A2 (GenBank AF434756). These false priming sites are found at the boundaries between the primers used and the genomic sequences amplified, suggesting that mobile elements exist in the maize genome that have limited sequence similarity to CaMV. However, this does not show that transgenes are scattered throughout the genome.

Given the following facts — none of the flanking sequences contains an obvious transgene (or any expected CaMV sequence apart from the primers used), i-PCR is prone to generating artefacts⁵, and multiple false priming sites are present in the maize genome — it is likely that the i-PCR sequences are all artefacts and not genuine transgenes.

Southern blots of individual kernels could provide much more reliable information about introgression of transgenes into native populations. Transgenic corn may be being grown illegally in Mexico, but Quist and Chapela’s claim that these transgenes have pervaded the entire native maize genome is unfounded. It is important for information about genetically modified organisms to be reliable and accurate, as important policy decisions are at stake.

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