ily-specific probe. The subfamily-specific probe will detect all the bands from both genomic locations on a Southern blot, but only those bands belonging to the same location will co-segregate. We used the z1A and z1B-specific probes that were previously described (Song, R. and Messing, J, Plant Physiology 130:1626-1635, 2002) to check for sequence polymorphism of the parental lines of two maize recombinant inbred lines (Burr, B et al., Genetics 118:519-526, 1988). Based on a maximum of polymorphism detected by Southern blot analysis (number of bands and their polymorphism), we chose the Tx303xCo159 population cut with *Eco*RI for z1A subfamily. One band does not show polymorphism between the two parental lines. However, other bands could be sorted into two co-segregating groups (Fig. 1). One group contains most of the bands detected by Southern blot analysis and has the following mapping score:

212222121121112222121212111112112112222222

This gave us a map position of 40.90 on chromosome 4S according to the BNL map. At the same position, *uaz149(zp19)*, a 19-kDa zein gene has previously been mapped. Because this position contains most members of the z1A gene subfamily, it corresponds to the z1A-1 location as previously defined (Song, R. and Messing, J, Plant Physiology 130:1626-1635, 2002).

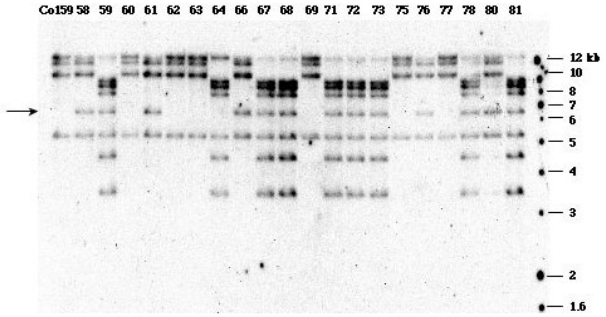

Figure 1. Co-segregation analysis of gene clusters of the z1A subfamily.

The other group contains a single band (Fig. 1, arrow indicated band) and has the following mapping score:

112122211121112121112111121222222122211221

This gave us a map position of 70.20 on chromosome 4S according to the BNL map. At the same position, *zpl3a*, a zein protein has previously been mapped. This position only contains a few zein gene copies and corresponds to z1A-2 location (Song, R. and Messing, J, Plant Physiology 130:1626-1635, 2002).

The same approach was taken for mapping the z1B subfamily. In this case, the Tx303xC0159 population cut with *Hin*dIII was selected for mapping. To our surprise, only one co-segregating group was detected by Southern blot analysis (Fig. 2), with the following score:

112222312211122211121211222212111211122222

This resulted in a map position of 52.30 on chromosome 7S. The same map position is also occupied by *uaz68a(zp19)* and *zpl2b*, two alpha zein genes. Even though we found two physically unlinked locations for the z1B subfamily in inbred line B73, they could still be tightly linked in terms of genetic distance because of the low resolution provided by the mapping population (only 41 samples).

A summary of all seven map positions is included in Table 1.

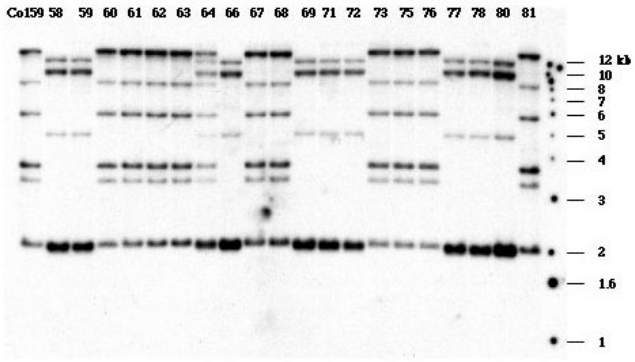

Figure 2. Co-segregation analysis of z1B subfamily.

Table 1. Genetic locations of the alpha zein gene family.

alpha zein subfamily	map position	mapping population	mapping method
z1A-1	chr 4, 40.90	C0159xTx303 RI	RFLP
z1A-2	chr 4, 72.20	C0159xTx303 RI	RFLP
z1B-1/z1B-2	chr 7, 52.30	C0159xTx303 RI	RFLP
z1C-1	chr 4, *php200725**	(Mo17xBSSS53)xMo17	RFLP
z1C-2	chr 4, *floury 2**	(Mo17xBSSS53)xMo17	SNP
z1D	chr 1, 122.4; 123.3**	N/A	RFLP

*please, refer to these two markers for relative positions in different genetic maps.
**segregating population not known (Woo, YM et al, Plant Cell 13:2297-2317, 2001).

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Paramutation in traditional varieties from Brazil

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Among the traditional varieties of maize, neither the occurrence of kernels showing different patterns of coloration nor the predominance of stippled phenotypes is unusual**.** During a germplasm survey on ethnovarieties from Paraná State, we detected some ears of the variety Carioca with variegated and full colored kernels, while ears of other varieties had only yellow kernels, among them Piranão, Cayano, Ferrinho and IAPAR 50.

Analysis of the variety Carioca showed that the sectors were localized in the aleurone. Different levels of pigmentation could be observed, and five classes were characterized as being full, near full, stippled, near colorless and colorless (Fig. 1). Comparing the phenotypes found in the Carioca original population with others described in the literature, we concluded that these phenotypes should be comparable to a paramutation event. All the other varieties are known to be *bb;rr*. In the summer of 1999-2000 we conducted a field experiment with all varieties without pollination control to analyze the occurrence of colored and stippled kernels.

Figure 1: Four phenotypic classes. (a) colorless, (b) near colorless, (c) stippled and (d) near colorfull. The colorfull phenotype has not been shown here.

The wind direction was favorable to lead the pollen grain of the Carioca onto other varieties. The results are presented in Table 1 with only three phenotypic classes. In the class Mot/Stt "near full", "stippled" and "near colorless" are included. The ear with paramutation means that at least one kernel was mottled. Interestingly, no kernel with a stippled phenotype was observed in the Ferrinho variety. Its position in the field was favorable to present at least one kernel with a stippled phenotype. Our hypothesis is that the alleles of color were completely null. These varieties have been analyzed carefully in order to verify why they did not show such a stippled phenotype. We have conducted controlled crosses between Ferrinho and Carioca as a complete diallel to check out possible differences when Ferrinho was used as female or male.

Table 1: Frequency of different color classes in the kernels of etnovarieties from Brazil

	Phenotypes				Ears		
	Color	Mot/Stt	Colorless	Total of kernels	Paramutation	Normals	Total
Carioca	118	304	10712	11134	30	29	59
Piranão	30	40	12844	12914	38	119	157
Ferrinho	-	-	-	-	0	34	34
Cayano	9	11	3725	3745	10	37	47
IAPAR 50	4	7	3401	3412	8	29	37
Piranão x Cusco	22	36	5999	6057	16	39	55
Sintético	5	23	2574	2602	10	21	31

Our results show that the allele was transmitted to other varieties, and the paramutation event was observed by the kernels showing different color levels or reduced expression of the dominant allele. The low level of introgression in some varieties such as IAPAR 50 and Cayano is a result of its position in the field. This low introgression reinforces the hypothesis of a null expression of the alleles, because these varieties were more distant from Carioca in the field than Ferrinho. The detailed analysis was not presented because we are conducting experiments with inbred lines and backcrosses derived from these varieties.

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TIGR Maize Gene Index

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Overview. The most comprehensive resource for the cataloging of transcribed genes is the vast body of data generated by the sequencing of Expressed Sequence Tags (ESTs). ESTs are single-pass, partial sequences of cDNA clones, and they have been used extensively for gene discovery and genomic mapping in various species. Similar to the other TIGR Gene Index databases, the Maize Gene Index uses assembly algorithms to first cluster, then assemble EST and gene sequences to produce tentative consensus (TC) sequences that represent the underlying mRNA transcripts. The resulting TCs can be used for eukaryotic genome sequence annotation, EST sequence annotation, integration of complex mapping data and identification of orthologous genes in other crop plants.

Building Maize Gene Index. Maize TCs were assembled by treating ESTs and Expressed Transcripts (ET) sequences as elements of a transcriptome shotgun sequencing project. Maize ESTs are downloaded daily from dbEST, cleaned to remove untrimmed vector, linker, ribosomal, mitochondrial, low quality, poly(A/T) and contaminating bacterial sequences. Maize ETs are extracted from annotated mRNA and CDS features in GenBank records. Cleaned ESTs and ETs are compared pair-wise to identify overlaps using megablast . Sequences sharing a minimum of 95% identity over a 40 nt or longer region with 20 bases or fewer of mismatched sequence at either end are grouped into a cluster. Each cluster is then assembled separately using CAP3, the resulting TC sequences are annotated to provide a provisional functional annotation, and the assemblies and their annotations are stored in a Sybase relational database that allows versioning and heritability to be maintained. All nonclustered, non-overlapping sequences remain as singletons. The resulting Gene Index is released through the TIGR maize gene index web site (www.tigr.org/tdb/tgi/zmgi/). Each TC reports (example TC160405, http://webtest.tigr.org/docs/tigr-scripts/tgi/tc_report.pl?species=maize&tc=TC160405&display=1) includes: the assembled TC sequence, the predicted open reading frames (ORFs) from ESTscan, DIANA and framefinder, coordinates of each EST and ET in the assembly, information about each EST and ET with links to a variety of databases, alternative splicing cluster, an expression summary by counting number of ESTs from different libraries, SNP detection, orientation determination, functional annotations including matches to a known protein and GO annotations, and tentative orthologous gene identifications in other species from the EGO database, and maps to rice and Arabidopsis genomes .

Using TIGR Maize Gene Index. There are a variety of means by which a user might gain entry to the Maize Gene Index database. Users can search the database using a variety of sequence identifiers, such as GenBank Accession or TC number, or by searching gene name or for TCs that are preferentially expressed in specific tissues.

However, the most common entry point for most users is the sequence search page (<http://tigrblast.tigr.org/tgi/>). Both BLASTN and TBLASTN versions of the WU-BLAST package have been implemented allowing DNA and protein queries to be used. Alignments to high scoring TCs and singleton ESTs are returned and users can view the individual target sequences by clicking on the TC number or EST_id. From the TC reports (see Figure 2), users can view the annotation provided for the sequence and its evidence, link to orthologues in EGO, or view genomic sequence alignments with rice and *Arabidopsis*.

In addition to the Web interface, the TIGR Maize Gene Index is available as a set of flat files. The TC consensus sequences are provided in a FASTA format file; the ESTs comprising each TC are specified in a separate file. Many users involved in the annotation of genomic sequence and in the analysis of cDNA microarray data have found these to be particularly useful. In addition, we provide a putative annotation of all the assembled and singleton ESTs in the database through the EST Annotator feature available through the main maize gene index page.

TIGR Maize Gene Index release data. The current release of the Maize Gene Index, ZmGI 11.0, was released on February 1, 2003, and contains 188,973 ESTS, 173,826 in TCs and 15,147 as singletons, as well as 3,463 expressed transcripts. New releases will be available every 120 days, provided a minimum 10% increase in the number of available ESTs.

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