

Data S1. Supplemental materials and methods

Transposon tagging of *narrow sheath* (*ns*) mutations

The eight independent alleles of *ns1* that were derived from *Mutator* transposon stocks were generated using a directed transposon-tagging strategy as described in Scanlon et al. (Scanlon et al., 2000). To summarize this strategy, *ns-R* mutant male plants homozygous for the reference alleles *ns1-R* and *ns2-R* were crossed to non-mutant female plants from *Mutator* active backgrounds into which the *ns2-R* allele had been introgressed. The genotypes of the *ns* mutant progeny were therefore *ns1-R/ns1-Mu;ns2-R/ns2-R*. The five independent alleles of *ns2* derived from *Mutator* transposon stocks were generated using a similar transposon-tagging strategy, in which male plants homozygous for the reference alleles *ns1-R* and *ns2R* were crossed to non-mutant female plants from *Mutator* active backgrounds homozygous for *ns1-R*. RFLP analyses, using *ns2*-linked maize clones contained within a paracentric inversion that includes *ns2-R*, indicated that these independently derived progenitor-tagging stocks were also homozygous for the non-mutant *Mutator* allele *Ns2-Mu*⁺. These parental genotypes were also inferred by the appearance of *ns* mutant plants in the M1 tagging generations in ratios of less than 1:5000 plants. Ultimately, analyses with *ns1* and *ns2* clones verified the genotypes of all *Mu*-tagging parental lines. Thus, all 13 independently derived *ns*-**Mu* alleles arose de novo in single gametes generated by the respective maternal, *Mu*-tagging parents.

Cloning of *ns* genomic DNA and cDNA

To amplify the homeodomain of the *ns1* gene (AC AJ536578), PCR was performed on genomic DNA of *Rscm2* maize using the primer pair ZmHD1 and ZmHD2 (see Table S1 at <http://dev.biologists.org/supplemental/>). Rapid amplification of 5'cDNA ends was then performed on RNA of *Rscm2* coleoptilar-stage embryos with primers ZmPRSa and ZmPRSb using the FirstChoice™ RLM-RACE Kit (Ambion), according to the manufacturer's protocol; 3'-RACE was performed with primers ZmPRSc and ZmPRSd. The complete genomic sequence of the *ns1* gene was obtained with multiple gene-specific primers (ZmPRS1-ZmPRS4; see Table S1) using the Universal Genome Walker™ Kit (Clontech). All PCR products were cloned into pCRII TOPO (Invitrogen) and sequenced. The ZmPRS clone was mapped to chromosome 2, between markers umc1003 (zpu1) and umc1065 (pbf1) using the DNA Kit of 94 IBM Lines (http://www.maizemap.org/dna_kits.htm), very close to *ns1* (Scanlon et al., 2000).

In order to clone *ns2* (GenBank AC AY472082) by homology to *ns1*, nested primers (see Table S1) were designed from the conserved homeobox region of *ns1* and used to identify homologous PCR products from a *DraI*-digested genomic DNA library prepared from *ns* mutant leaf using the Universal Genome Walker™ Kit (Clontech). The *ns* primers used were ns2F1 and ns2F2 (see Table S1), and two PCR products were obtained. One product was identical to the *ns1-R* allele and the second product was later mapped to *ns2* (see below and Results). Additional Genome Walker™ reactions were performed in order to obtain the complete 5' and 3' regions of the *ns2* alleles from inbred B73, the *ns-R* mutant and the *ns2*-**Mu1* mutant.

Full-length cDNA copies of *ns2* transcripts (GenBank it584557) were obtained by Rapid Amplification of cDNA Ends (RACE), performed according to recommended protocols (GeneRacer™ Kit, Invitrogen, Life technology) using four gene-specific primers (ns2R4, ns2R5, ns2R6, ns2R7; see Table S1). In these assays, total RNA was isolated from B73 and *ns-R* apical tissues (maize vegetative apices containing P5 and lower primordial, plus the shoot apical meristem). The results obtained with Genome Walker™ and GeneRacer™ were confirmed by standard genomic PCR and RT-PCR, using *ns2*-specific primers. All PCR products were cloned into pCRII or pCR4 vectors (Invitrogen, Life Technology) using standard procedures, and were sequenced with Bigdye Terminator version 3.1 Cycle Sequencing Kit™ (Applied Biosystems). Consensus sequences for the *ns2* full-length cDNA and genomic DNA clones were obtained using software found at Multalin™ (<http://prodes.toulouse.inra.fr/multalin/multalin.html>).