Haploid induction by a maize cenh3 null mutant

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The production of haploids is an important first step in creating many new plant varieties. One approach used in Arabidopsis involves crossing plants expressing different forms of centromeric histone H3 (CENP-A/CENH3) and subsequent loss of genome with weaker centromeres. However, the method has been ineffective in crop plants. Here, we describe a greatly simplified method based on crossing maize lines that are heterozygous for a cenh3 null mutation. Crossing +/cenh3 to wild-type plants in both directions yielded haploid progeny. Genome elimination was determined by the cenh3 genotype of the gametophyte, suggesting that centromere failure is caused by CENH3 dilution during the postmeiotic cell divisions that precede gamete formation. The cenh3 haploid inducer works as a vigorous hybrid and can be transferred to other lines in a single cross, making it versatile for a variety of applications.

INTRODUCTION

Tens of thousands of maize haploid lines are generated by breeding companies around the world each year as a prerequisite for creating new inbreds, which are ultimately used to produce hybrids for sale. The induced haploids are doubled by colchicine and immediately tested for agronomic performance. The traditional technology is based on an inbred called Stock 6 that induces haploids when crossed as a male (1) and, in modern lines, has been reported to induce haploids at frequencies as high as ~15% (2). The key underlying gene, called Matrilineal (MATL/ZMPLA1/NLD), is a patatin-like phospholipase expressed primarily in pollen (3–5). Its mechanism of action is not understood but may involve a change in membrane properties during fertilization that leads to a loss of the paternal chromosomes. Mutations in matrilineal also induce haploids in rice and wheat (6–8). The Matrilineal gene is not conserved in dicotyledonous plants, although recent work has revealed a conserved enhancer of matrilineal that induces haploids at low levels in Arabidopsis (9).

A potentially superior and broadly useful method of inducing haploids was pioneered by Ravi and Chan (10), who showed that crossing Arabidopsis lines with a structurally altered centromeric histone H3 (CENP-A/CENH3) protein yielded haploids and aneuploids at frequencies as high as 25 to 45%. CENP-A, called CENH3 in plants, is a histone variant that recruits constitutive centromere proteins that subsequently recruit overlying kinetochore proteins (11–14). The original study involved a construct called GFP-tailswap where the N-terminal tail of CENH3 was replaced with sequence from another H3 variant and modified with a green fluorescent protein (GFP) tag. Recent data demonstrate that point mutations and small deletions of CENH3 can also induce haploids at similar frequencies (15–17). However, outside of Arabidopsis, centromere-mediated haploid induction has proven to be less effective, generally producing <1% haploids (18).

In an earlier report, we proposed that the mechanism of centromere-mediated haploid induction is based on differences in effective centromere size between haploid inducers and their wild-type crossing partners (19). CENH3 point mutations reduce CENH3 loading in somatic cells (15), and GFP-tailswap lines show impaired CENH3 loading in meiosis (20), suggesting that haploid inducers transmit small or weak centromeres to gametophytes. When crossed to wild-type plants, the progeny have a centromere size imbalance, which we argued leads to targeted destruction of the smaller centromeres by natural clearing mechanisms that remove misplaced CENH3 and spurious small centromeres (19). The centromere size model predicts that cenh3 mutants with the most severe loss of function will be the best haploid inducers. However, severe cenh3 mutants also compromise plant growth, making it difficult to breed a healthy haploid inducer line. This contradiction in goals likely explains the poor success record of centromere-mediated haploid induction in species other than Arabidopsis.

RESULTS

The current study was designed to test the centromere size model in maize, initially using the GFP-tailswap method. However, this approach is complicated by the fact that it requires both a mutant of native cenh3 and a functional GFP-tailswap transgene that complements the mutant. Another group had already shown some success using an existing maize mutant (cenh3-mu1015598) caused by a Robertson’s Mutator (Mu) insertion in the 5′ untranslated region (5′UTR) of the gene (21, 22). They crossed GFP-tailswap into the cenh3-mu1015598 background and observed an average of 0.86% haploids when crossed as a male and no haploids when crossed as a female (21). We also obtained cenh3-mu1015598 and self-crossed three heterozygous plants. Genotyping revealed that two ears segregated a low frequency of homozygous mutants that grew to various states of maturity (table S1). The recovery of homozygous mutants indicates that cenh3-mu1015598 is not a null and that prior results may have been confounded by a low level of wild-type CENH3 expression. The variable penetrance of the cenh3-mu1015598 allele can be explained by the fact that Mu elements can promote low levels of expression when inserted into 5′UTR regions (23).

To overcome the selection against true null cenh3 alleles, we opted to create a cenh3 null using a two-construct CRISPR-Cas9 approach. One line was transformed with a simple construct expressing Cas9 driven by an ubiquitin promoter. A second line was transformed with a construct expressing a guide RNA (gRNA) targeting the native CENH3 gene and an “ImmuneCENH3” gene that contains a full-length native CENH3 gene with five silent nucleotide changes in the gRNA target area (Fig. 1, A and B). After we crossed the two lines together, Cas9 generated mutations in the native CENH3 gene

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but left the transgene unaffected. We chose a cenh3 allele with a single-nucleotide deletion that causes a stop codon in the αN helix that connects the N-terminal tail to the inner nucleosome core particle (Fig. 1C). In the presence of ImmuneCENH3, the cenh3 muta-
tion segregates as a simple Mendelian recessive trait (Table 1). We then created transgenics with TailswapCENH3, a close replica of the Arabidopsis GFP-tailswap construct, and crossed it to the cenh3 muta-
tion. We were unable to obtain any plants that contained TailswapCENH3 and were homozygous for cenh3 (Table 1), suggesting that the trans-
gene does not complement a true null. This is likely because the GFP tag interferes with CENH3 loading or recruitment of other centromere proteins. Arabidopsis GFP-tailswap plants are severely stunted (24) and nearly sterile (20), indicating a similar negative impact. However, this may not prove to be true in all cases since, in animals, the function of CENP-A seems less affected by GFP or other large tags [e.g., (25, 26)].

During the course of these studies, we noted that cenh3 was occasion-
ally transmitted in the absence of ImmuneCENH3. By crossing to wild-type lines, we were able to obtain a simple segregating cenh3 line that lacked both of the original transgenes. Among selfed progeny from a +/cenh3 line, there were 163 +/+ wild-type individ-
uals, 55 +/+ cenh3 heterozygotes, and 0 cenh3/cenh3 homozygotes, indicating that the mutant is homozygous, lethal and poorly trans-
mittted through gametophytes. We also carried out reciprocal crosses between +/+ cenh3 heterozygotes and wild-type plants. A Mendelian trait is normally transmitted to 50% of testcross progeny; however, we observed that only 12.1% of the progeny received cenh3 when crossed through the male and 25% when crossed through the fe-
male (Table 2). The reduction in transmission is expected because sperm and eggs are carried within multicellular haploid gameto-
physes. Two haploid cell divisions precede the formation of sperm, and three haploid cell divisions precede the formation of an egg. Those gametophytes with the cenh3 allele must use CENH3 carried over from the sporophytic phase while it is natu-
urally diluted at each cell cycle (27). A general expectation is that cenh3 sperm would have about one-quarter of the normal amount of CENH3 and an egg carrying cenh3 would have about one-eighth relative to the cenh3 heterozygous parent. Assuming no dosage compensation, those values would be reduced by an additional one-half relative to a normal homozygous wild-type parent. As a result, sperm and eggs carrying cenh3 presumably have smaller centromeres.

To test whether +/+ cenh3 heterozygous mutants are able to induce haploids, we crossed cenh3 heterozygotes with tester lines in both directions. In the first test, we crossed wild-type and +/+ cenh3 plants to a line that is homozygous for a recessive glossy8 (gl8) mutation on

### Table 1. Segregation of cenh3 in ImmuneCENH3 and TailswapCENH3 backgrounds.
The number of plants of each genotype are shown in parentheses.

| Cross               | Transgene genotypes                                                                 | cenh3 genotypes |
|---------------------|-------------------------------------------------------------------------------------|-----------------|
|                     | +/ImmuneCENH3 or ImmuneCENH3/ImmuneCENH3                                         | +/+ (54)        |
|                     | (172)                                                                               |                 |
|                     | +/+                                                                            | +/+ (33)        |
|                     | +/+ (16)                                                                              |                 |
| +/ImmuneCENH3, +/cenh3 | +/ImmuneCENH3 or ImmuneCENH3/ImmuneCENH3                                         | +/+ (54)        |
|                     | (172)                                                                               |                 |
|                     | +/+                                                                            | +/+ (33)        |
|                     | +/+ (16)                                                                              |                 |
|                     | +/+ (16)                                                                              |                 |
| +/TailswapCENH3, +/cenh3 | +/TailswapCENH3 or TailsCENH3/TailsCENH3                                         | +/+ (47)        |
|                     | (53)                                                                                |                 |
|                     | +/+                                                                            | +/+ (47)        |
|                     | +/+ (47)                                                                              |                 |

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chromosome 5 that causes seedling leaves to have a shiny appearance. We observed that 0.5% of the progeny were glossy when +/cenh3 heterozygotes were crossed as male and 5.0% of the progeny were glossy when +/cenh3 plants were crossed as female (Table 3). Flow cytometry analysis revealed that all 45 of the glossy plants were haploids, an interpretation that was confirmed by counting chromosomes in root tip cells of three plants (Fig. 2, A and B). When grown to maturity, the haploid plants were short and sterile as expected (Fig. 2, C and D) (28). We also observed two nonglossy plants with stunted phenotypes that we hypothesized might be aneuploids. These two plants were skim sequenced along with six haploids. While the haploids showed uniform sequence coverage, the stunted plants did not; one was trisomic for chromosome 3, and the other was monosomal for chromosomes 2 and 4 and trisomic for chromosome 10 (Fig. S1).

We then carried out a second set of tests using gl8, which has a similar phenotype but the mutation is on chromosome 7. In these crosses, we also scored the germination rate, which is an indirect measure of karyotypic abnormality commonly used to estimate the efficacy of Arabidopsis haploid inducers (16, 29). In crosses where +/cenh3 heterozygotes were the female, 5.2% of the progeny were glossy and haploid by flow cytometry measurements. Another 3.3% of the progeny showed the glossy phenotype but had a higher DNA content than expected for haploids and were scored as aneuploids (Table 3 and table S2). Different crosses differed considerably in the germination rate (65 to 91%), frequencies of haploids (1.2 to 8.9%), and aneuploids (2.1 to 5.1%) (table S2). Sequence data from five aneuploid plants confirmed that all except one were missing chromosomes. One glossy plant that appeared to have two complete copies of chromosome 7, sometimes in conjunction with the loss of other chromosomes. One glossy plant that appeared to have two complete copies of chromosome 7 may have had a small interstitial deletion that was not detectable by skim sequencing [segmental aneuploids are common in Arabidopsis GFP-tailswap crosses (30)]. The results from the gl8 tests are more in line with what has been observed in Arabidopsis, where any given cross with GFP-tailswap generally yields haploids and aneuploids in similar proportions (10, 24, 29).

If CENH3 dilution is the underlying mechanism for haploid induction, then only gametes carrying the cenh3 mutation from the +/cenh3 parent should induce haploids. Unfortunately, it is not possible to score seedlings for the presence of the cenh3 allele because the genome of the haploid inducer is lost. However, data from Arabidopsis GFP-tailswap crosses show that endosperm rarely displays complete uniparental genome elimination when the seedling is haploid (29). If true in maize as well, then the genotype of the endosperm could be used to determine the original genotype of the seedling. We genotyped the remnant endosperm from a set of 11 haploid plants produced from a +/cenh3 × gl8 cross. All 11 were heterozygous for the cenh3 allele, indicating that haploid induction is caused by gametes carrying cenh3. Since only 25% of the progeny from a female cross receive cenh3 (Table 2), yet our haploid frequencies are calculated on the basis of total seed counts, the effective haploid induction frequency is on the order of 20%. Bringing this level of haploid induction into practice will require screening for kernels that received cenh3. There are multiple publicly available endosperm-expressed GFP transgenes within 100 kb of the CENH3 gene that could be linked to the null for this purpose (31) (www.acdsinsertions.org/).

### DISCUSSION

We have described a highly simplified haploid induction system that is based on crossing plants heterozygous for a cenh3 null mutation. The data suggest that CENH3 is diluted to critically low levels during the cell divisions of cenh3 gametophytes and that the comparatively small centromeres are selectively degraded, leaving only the chromosomes from the crossing partner. Our work builds on the classic GFP-tailswap studies and recent improvements (10, 15, 17, 24, 32), as well as earlier work describing crosses between divergent plant species (often differing in centromere size) (19, 32) that result in

### Table 2. Transmission of cenh3 through male and female crosses. WT, wild type; het, +/+cenh3 heterozygote; hom, cenh3/cenh3 homozygote.

| Cross               | No. seedlings | Expected (WT:het:hom) | Observed (WT:het:hom) | Observed het frequency |
|---------------------|---------------|------------------------|------------------------|------------------------|
| +/cenh3 ♂          | 218           | 55:108:55              | 163:55:0               | 25.2%                  |
| +/cenh3 ♀ × B73 ♂  | 184           | 92:92:0                | 138:46:0               | 25.0%                  |
| B73 ♀ × +/cenh3 ♂  | 140           | 70:70:0                | 123:17:0               | 12.1%                  |

### Table 3. Haploid and aneuploid induction by +/cenh3 heterozygotes.

| Cross               | No. seedlings | No. glossy plants | No. haploid | Haploid ratio | No. aneuploid | Aneuploid ratio |
|---------------------|---------------|-------------------|-------------|---------------|---------------|----------------|
| +/cenh3 ♀ × gl8 ♂   | 838           | 42                | 42          | 5.0%          | 2*            | 0.2%           |
| WT ♀ × gl8 ♂        | 1000          | 0                 | –           | –             | –             | –              |
| gl8 ♀ × +/cenh3 ♂   | 597           | 3                 | 3           | 0.5%          | 0             | 0              |
| gl8 ♀ × WT ♂        | 826           | 0                 | –           | –             | –             | –              |
| +/cenh3 ♀ × gl1 ♂   | 844           | 75                | 44          | 5.2%          | 28            | 3.3%           |
| WT ♀ × gl1 ♂        | 1114          | 0                 | –           | –             | –             | –              |

*The two aneuploid plants were nonglossy plants with stunted phenotypes.
centromere failure, haploidization, and reproductive isolation. Similar principles have emerged from the animal literature, where variation in centromere repeats can affect the quantity of CENP-A and skew meiotic transmission (33, 34) in a process known as centromere drive (35). Centromere drive is a form of genetic conflict that is hypothesized to result in rapid evolution of both centromere repeats and associated centromere proteins (35, 36) and, in principle, can lead to centromere-based incompatibilities of the type observed with crosses involving different plant species. A central theme is that modifications to the quantity and/or quality of CENP-A/CENH3 can lead to postzygotic incompatibility, which, in plants, often results in haploid formation.

All prior literature on centromere-mediated haploid induction in plants describes the complementation of a null allele with structural variants of CENH3 or alleles that produce altered or partially deleted forms of CENH3 (10, 15, 17, 24, 32). These data have served to sustain the original interpretation that haploid induction is caused by a competition between two structurally different forms of CENH3 and ultimate rejection of the altered centromeres by a surveillance mechanism for improper assembly (10, 17, 18, 37). In contrast, we have achieved high levels of haploid induction using a cenh3 mutation with a premature stop codon that is predicted to remove the sequence of the protein that interacts with other histones. The results suggest that quantitative reductions in CENH3 alone can induce centromere-mediated haploid induction, as predicted by the centromere size model (19). It is also possible that hypomorphic mutant alleles such as cenh3-mu1015598 may induce haploids when crossed as heterozygotes, but this has yet to be tested.

One of the notable elements of centromere-mediated haploid induction is that it is effective in only a subset of progeny. In some individuals, both the chromosomes from the haploid-inducer parent are lost, and in another much larger subset, no chromosome loss occurs. The relatively small aneuploid class represents “partial haploid induction” events, where some chromosomes were lost but others survived. The fact that the gl8 crosses yielded more true haploids than the gl1 crosses may be related to the fact that the former were

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**Fig. 2. Confirmation that plants are haploid.** (A) Flow cytometric analysis of haploids. Diploid plants show peaks at 2N and 4N, where 4N is the result of endoreduplication in differentiated tissues. Haploid plants have 1N and 2N peaks. (B) Chromosome spreads. Maize diploids have 20 chromosomes, whereas haploids have 10. Scale bar, 20 µm. (C) Haploids plants have a shorter stature. (D) Haploid plants are sterile without exerted anthers. Photo credit: Na Wang, University of Georgia.

**Fig. 3. Comparison of the GFP-tailswap–based method to the cenh3 null method.** (A) The GFP-tailswap method and its improved forms. In most applications, a transgene expressing a structurally altered CENH3, or other mutant form, is used to complement a loss-of-function mutation (10, 15, 17, 24). An EMS (ethyl methanesulfonate)–induced point mutation of native CENH3 has also been used (16). In all cases, the plant must grow to maturity with a partially disfunctional CENH3 gene, which affects plant performance. The most effective haploid inducers are weak plants with poor fertility. (B) The cenh3 null method. The plant is heterozygous and can be used as a vigorous hybrid. Haploid induction occurs at the gametophyte level. The female gametophyte is shown, where three mitotic cell divisions dilute CENH3 to low levels in the egg cell.
carried out in the summer, while the latter were carried out in winter. It is also possible that the selection scheme played a role. Studies using the maize r-X1 deletion line, which generates monosomics at high frequency, have demonstrated that some chromosomes are recovered as monosomics at higher frequency than others (38). Monosomics for chromosome 5 (with gl8) are rarely recovered, whereas monosomics for chromosome 7 (with gl1) are far more common [17 times more common (38)]. Two of five sequenced aneuploids from gl1 crosses were monosomic for chromosome 7 only (fig. S1). These data suggest that the gl8 tester favors the recovery of haploids, while the gl1 tester recovers both haploids and aneuploids.

It is likely that the frequency of haploid induction can be improved under standard breeding practices, similar to how the original ~3% haploid frequency observed with Stock 6 (1) was improved to ~15% in multiple breeding programs around the world (2). The major advantages of the cenlh3 approach are that it can be used to create either paternal or maternal haploids, that it does not require a transgene, and that the plants are phenotypically wild type and can be used as vigorous hybrids (Fig. 3). Since the inducer works as a heterozygote, cenlh3 can be crossed to any line, and the F1 will become a haploid inducer. This feature should make it particularly useful when combined with other technologies that are built upon haploids, such as genotype-independent gene editing (39) and synthetic apomixis (40).

METHODS

Plant materials

The gl1, gl8, and cenlh3-mu1015598 transposon insertion lines were obtained from the Maize Genetics Cooperation Stock Center, Urbana, IL. The cenlh3-mu1015598 allele is one of several mutations in the UFMU-01386 stock line. All plants were grown in the University of Georgia Plant Biology greenhouses. The gl1 crosses were grown in August of 2019, and the gl8 crosses were carried out in December of 2019.

Construct preparation and transformation

The Ubi-Cas9 construct contains 1991 base pairs (bp) of the maize polyubiquitin promoter (GenBank, S94464.1) driving a maize codon-optimized version of Cas9 terminated by the NOS terminator. The gRNA-ImmuneCENH3 construct contains two components, a gRNA module and the ImmuneCENH3 gene. The gRNA portion contains the maize U6 promoter (41) driving a gRNA (TCCCGCAGGGCTA-CAGTCCC) terminated by the Pol III terminator TTTTTT TT. The ImmuneCENH3 portion contains 6454 bp of the native CENH3 gene (coordinates Chr6:166705239-166711693 on Zm-B73-REFERENCE-42), but has five silent codon changes in the gRNA target area (CCAGGTACCGTGCCTGCAGCA). The promoter includes 2184 bp of sequence upstream of the ATG.

To create the gRNA-TailswapCENH3 construct, the natural 5′ UTR of CENH3 was retained, and a codon-optimized GFP sequence was inserted at the ATG of ImmuneCENH3. This was followed by a linker sequence ATGGATGAACTATACAAGGGCGGAGGCGGTG-ACTTTTTTTT and a tail sequence of the maize H3.3 gene (GenBank NM_001294303.2) including its intron, fused to the native CENH3 gene 3′-upstream of the gRNA target area. Our construct was based on the sequence of Arabidopsis GFP-tailswap obtained from the Comai laboratory.

Sequences of all three constructs are provided in the Supplementary Materials. The constructs were synthesized by GenScript (www.genscript.com) and cloned into the binary vector pTF101.1 (42). The constructs were transformed into the maize Hill line at the Iowa State University Plant Transformation Facility (Ames, IA) and grown in the University of Georgia greenhouses. To generate the cenlh3 mutation, transgenic lines carrying Ubi-Cas9 were crossed with lines carrying gRNA-ImmuneCENH3.

DNA extraction, genotyping, and sequence analysis

For standard leaf genotyping, genomic DNA was prepared using a CTAB (cetyl trimethylammonium bromide) protocol (43). Endosperm tissue was collected after the kernels had germinated and the glossy phenotype could be distinguished. Embryos and pericarps were removed with forceps, and the endosperm was ground to a powder with a mortar and pestle. The endosperm DNA was extracted with the IBI Plant Genomic DNA Mini Kit (IBI Scientific, IB47231).

To identify the presence of ImmuneCENH3 and Cas9 in transgenic lines, primers CENH3-F2 and CENH3-R3 were used to amplify ImmuneCENH3, and primers Cas9-F1 and Cas9-R1 were used to amplify Cas9 (table S3). To identify the original cenlh3 mutation in Cas9 plants, polymerase chain reaction (PCR) was carried out using the Phusion High-Fidelity PCR Kit (New England Biolabs, Ipswich, MA) with primers CENH3-F1 and CENH3-R1 in table S3. The PCR products were either directly Sanger sequenced or cloned using a TOPO TA cloning kit (Thermo Fisher Scientific, #K457501) and then Sanger sequenced.

In lines that lack ImmuneCENH3, the cenlh3 null allele was differentiated from the native CENH3 allele by PCR and restriction enzyme digestion. PCR amplifies a 496-bp PCR product using primers CENH3-F2 and CENH3-R2. When this product is digested with the restriction endonuclease AlwNI (New England Biolabs), the wild-type allele is cleaved into two pieces of size 284 and 212 bp, while the mutant cenlh3 allele is not cleaved.

The cenlh3-mu1015598 allele was scored using the primers CENH3-F4, CENH3-R4, and Mumix (a 1:1 mix of the two primers EoMu1 and EoMu2 in table S3). The wild-type allele is amplified with CENH3-F4 and CENH3-R4, while the Mu allele is amplified with CENH3-F4 and Mumix.

Ploidy evaluation

Progeny from +/cenlh3 crosses were grown indoors under grow lights for 10 to 13 days and water sprayed on the seedlings to identify the glossy phenotype. All glossy plants were subsequently assayed by flow cytometry. For each individual, about 1 g of flash-frozen leaves or roots were collected and finely chopped with a razor blade in 1.5 ml of prechilled nuclei extraction buffer [2 mM EDTA, 15 mM tris-HCl (pH 7.5), 20 mM NaCl, 80 mM KCl, 0.5 mM spermine, 15 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, and 0.1% Triton X-100]. After chopping, the mixture was filtered twice through a 40-μm cell strainer (pluriSelect, 43-10040-60). The nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI) and loaded into flow cytometers hosted by the CTEGD Cytometry Shared Resource Lab at the University of Georgia.

Chromosome spreads

Chromosome analysis was carried out as described in (44). Briefly, root tips were collected from the haploid and diploid plants, incubated in a chamber with nitrous oxide for 3 hours, and fixed with 90% acetic acid. Root tips were cut with a razor blade and digested in an enzyme solution (1% pectolyase Y-23 and 2% cellulase Onozuka) and then Sanger sequenced.
R-10) at 37°C for 50 min. The root section was washed in ethanol and then immersed in 90% acetic acid. A metal pick was used to crush the roots tips, and 10 µl of the cell suspension was dropped onto microscope slides. Slides were dried and mounted with a glass coverslip using ProLong Gold with DAPI (Thermo Fisher Scientific, catalog no. P36931). Slides were imaged on a Zeiss Axios Imager M1 fluorescence microscope with a 63× Plan-Apochromat oil objective, and SlideBook software (Intelligent Imaging Innovations, Denver, CO, USA) was used to analyze the data.

**Skim sequencing of haploids and aneuploids**

For each sample, DNA (12 ng/µl) was sonicated in a 100-µl volume with a Diagenode Bioruptor for 7 min on high setting with 30-s on-off intervals, yielding fragments averaging about 500 bp in length. DNA sequencing libraries were prepared using the KAPA HyperPrep Kit (KK8502) with KAPA single-indexed adapters (KK8700). Six hundred nanograms of sonicated DNA was used as input for each sample, and three cycles of PCR were used to amplify libraries. One hundred fifty–nucleotide Illumina sequencing reads were adapter-trimmed and quality-filtered using Cutadapt version 1.9.1 (45) with parameters as follows: “-q 20 -a AGATCGGAAGAGC -e .05 -O 1 -m 50.” Raw coverage was visualized using IGVTools version 2.3.98 (47) with coverage calculated on 25-Mb intervals. Raw sequence data are available in the National Center for Biotechnology Information (NCBI) BioProject database under accession number PRJNA646652.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/4/eabe2299/DC1

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Acknowledgments: We thank M. Tindall Smith, F. Fu, and S. Greenlaw for help with genotyping, W. A. Ricci for help with the flow cytometry analysis, and J. Liu for critically reading the manuscript. We appreciate the support of the Georgia Genomics and Bioinformatics Core facility, the UGA Cytometry Shared Resource Laboratory, and the Georgia Advanced Computing Resource Center. Funding: This work was funded by grant 1444514 from the NSF. Author contributions: R.K.D. supervised the research. N.W. and J.J.G. performed the experiments. N.W. and R.K.D. prepared the figures. N.W., J.J.G., and R.K.D. wrote the paper. Competing interests: R.K.D. is an inventor on provisional patents related to this work filed by the University of Georgia (numbers 63/036,902 and 63/036,910, filed 9 June 2020). The other authors declare that they have no competing interests. Data and materials availability: Sequence data are available in the NCBI BioProject database under accession number PRJNA646652. Seeds carrying the celn3 null mutation can be obtained from the corresponding author under a University of Georgia material transfer agreement. All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 7 August 2020
Accepted 5 November 2020
Published 20 January 2021
10.1126/sciadv.abe2299

Citation: N. Wang, J. I. Gent, R. K. Dawe, Haploid induction by a maize celn3 null mutant. Sci. Adv. 7, eabe2299 (2021).

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*Sci Adv* 7 (4), eabe2299.
DOI: 10.1126/sciadv.abe2299