

Haploid plants produced by centromere-mediated genome elimination

Maruthachalam Ravi¹ & Simon W. L. Chan¹

Production of haploid plants that inherit chromosomes from only one parent can greatly accelerate plant breeding^{1–3}. Haploids generated from a heterozygous individual and converted to diploid create instant homozygous lines, bypassing generations of inbreeding. Two methods are generally used to produce haploids. First, cultured gametophyte cells may be regenerated into haploid plants⁴, but many species and genotypes are recalcitrant to this process^{2,5}. Second, haploids can be induced from rare interspecific crosses, in which one parental genome is eliminated after fertilization^{6–11}. The molecular basis for genome elimination is not understood, but one theory posits that centromeres from the two parent species interact unequally with the mitotic spindle, causing selective chromosome loss^{12–14}. Here we show that haploid *Arabidopsis thaliana* plants can be easily generated through seeds by manipulating a single centromere protein, the centromere-specific histone CENH3 (called CENP-A in human). When *cenh3* null mutants expressing altered CENH3 proteins are crossed to wild type, chromosomes from the mutant are eliminated, producing haploid progeny. Haploids are spontaneously converted into fertile diploids through meiotic non-reduction, allowing their genotype to be perpetuated. Maternal and paternal haploids can be generated through reciprocal crosses. We have also exploited centromere-mediated genome elimination to convert a natural tetraploid *Arabidopsis* into a diploid, reducing its ploidy to simplify breeding. As CENH3 is universal in eukaryotes, our method may be extended to produce haploids in any plant species.

Centromeres are the chromosomal loci that attach to spindle microtubules to mediate faithful inheritance of the genome during cell division. They are epigenetically specified by incorporation of CENH3 (CENP-A in humans; also called HTR12 in *A. thaliana*¹⁵), a histone H3 variant that replaces conventional H3 in centromeric nucleosomes¹⁶. We have isolated *cenh3-1*, an embryo-lethal null mutant in *A. thaliana* that allows us to completely replace native CENH3 with modified variants. *cenh3-1* plants complemented by transgenic green fluorescent protein-tagged CENH3 (*GFP-CENH3*, Fig. 1a) have a wild-type phenotype. The embryo-lethal phenotype of *cenh3-1* can also be rescued by *GFP-tailswap* (Fig. 1a), a transgene in which we replaced the hypervariable amino-terminal tail domain of CENH3 with the tail of conventional H3, using the H3.3 variant (encoded by At1g13370). *GFP-tailswap* plants (*cenh3-1* mutant plants rescued by a *GFP-tailswap* transgene) showed accurate mitosis, as we did not detect aneuploidy in somatic cells (data not shown). However, *GFP-tailswap* plants were sterile upon flowering, indicating that they may have a specific defect in meiosis. *GFP-tailswap* was mostly male sterile (data not shown), although it could be used as a pollen donor if many anthers were pooled. When crossed as the female to a wild-type male, *GFP-tailswap* plants were 60–70% as fertile as wild-type plants.

When *GFP-tailswap* plants were pollinated by wild-type plants, we observed several unusual phenotypes in the F₁ progeny. First, 80–95%

of fertilized ovules aborted early in development, yielding inviable seeds (Table 1). Second, although viable offspring were expected to be diploids heterozygous for *cenh3-1* and hemizygous for the *GFP-tailswap* transgene, we found that 10 out of 16 plants had only wild-type *CENH3* and lacked *GFP-tailswap*. Each of these plants was sterile despite having a wild-type genotype. Furthermore, crossing *GFP-tailswap* to a *quartet* mutant male also yielded sterile F₁ offspring (3 out of 5 plants) that showed the *quartet* mutant phenotype of fused pollen, despite the fact that *quartet* is recessive and the *GFP-tailswap* parent was expected to transmit a wild-type *QUARTET* allele. These striking observations indicated that sterile progeny had lost chromosomes from their *GFP-tailswap* female parent, and thus had fewer chromosomes than diploid *A. thaliana* ($2n = 10$). We examined the karyotype of these plants and found them to be haploids containing only five chromosomes (Fig. 1b–e).

As centromeres control chromosome inheritance, we reasoned that chromosomes that entered the zygote containing the *GFP-tailswap* variant of CENH3 would be mis-segregated and lost, creating haploid plants with chromosomes only from their wild-type parent (Supplementary Fig. 1). To confirm this, we crossed *GFP-tailswap* plants (in the Col-0 accession) to several polymorphic accessions and genotyped F₁ haploids for markers on all five *A. thaliana* chromosomes (Table 1 and Supplementary Fig. 2). Regardless of the wild-type parent used, haploid plants invariably contained only wild-type chromosomes (paternal haploids), indicating that the *GFP-tailswap* genome was eliminated (a total of 42 haploids were genotyped). Furthermore, our results show that the process of inducing haploids by centromere-mediated genome elimination is independent of the genotype of the wild-type parent.

Genome elimination induced by CENH3 alterations is not specific to the *GFP-tailswap* transgene. Crossing *cenh3-1* mutants complemented by *GFP-CENH3* to wild-type plants also yielded haploid plants, but at a lower frequency than *GFP-tailswap* (Table 1). We did not observe haploid progeny from self-fertilized *GFP-tailswap* or *GFP-CENH3* plants (Table 1). Our results indicate that general perturbations in centromere structure can impede chromosome segregation during zygotic mitosis, creating a haploid embryo when chromosomes containing mutant CENH3 compete with wild-type chromosomes on the same spindle.

Haploids are efficiently generated from a *GFP-tailswap* × wild-type cross, comprising 25–45% of viable offspring (Table 1). Remaining progeny were either diploid hybrids or aneuploid hybrids showing the developmental phenotypes typical of *A. thaliana* plants with more than 10 chromosomes¹⁷ (Table 1 and Supplementary Fig. 3). Aneuploidy might also account for the high level of seed abortion in a *GFP-tailswap* × wild-type cross, as some embryos with unbalanced karyotypes may be inviable.

Uniparental haploids may contain the genome of either their female or male parent. We also obtained haploids by crossing a

¹Department of Plant Biology, University of California, Davis, Davis, California 95616, USA.

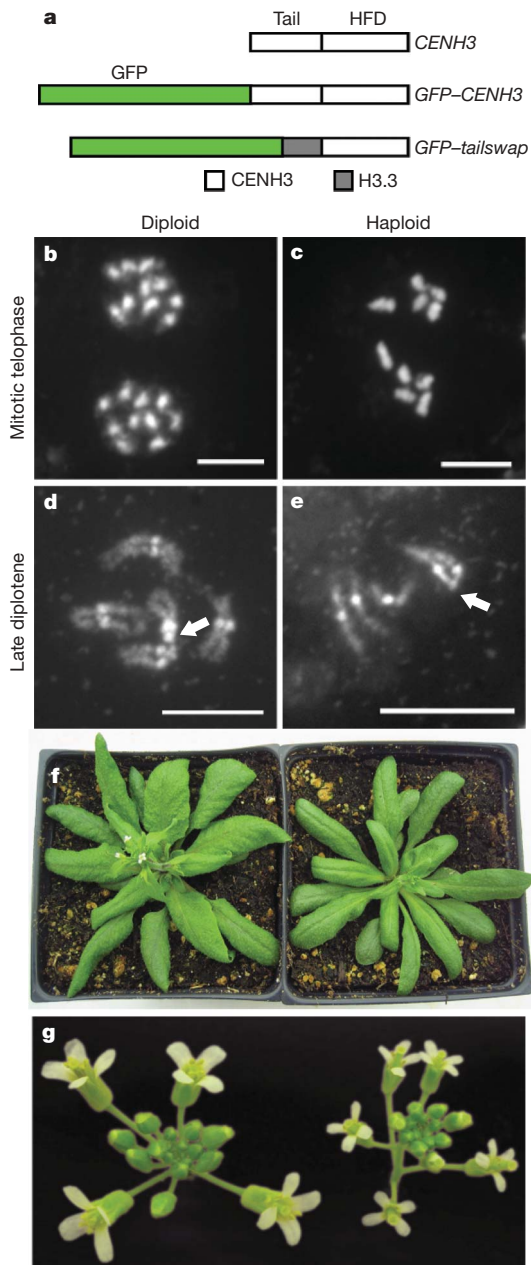


Figure 1 | Haploid *Arabidopsis thaliana* produced by crossing plants expressing altered CENH3 to wild type. **a**, *GFP-CENH3* and *GFP-tailswap* transgenes used in this study. Tail, N-terminal tail domain; HFD, C-terminal histone fold domain. **b**, **c**, Chromosome spreads from mitotic telophase in diploid and haploid *A. thaliana*, respectively. **d**, **e**, Chromosome spreads from late diplotene in diploid and haploid *A. thaliana*, respectively. In **d** and **e**, chromosomes 2 and 4 are joined at their nucleolar organizer regions independent of homologue pairing (arrows). **f**, Haploids (right) have narrower rosette leaves than diploids. **g**, Haploids (right) have smaller flowers than diploids. Scale bars: 0.5 μ m for **b** and **c**; 1 μ m for **d** and **e**.

wild-type female to *GFP-tailswap* as the pollen donor (Table 1). In this case, haploid progeny are purely maternal in origin. Genotyping of the plastid genome showed that both maternal and paternal haploids contained the cytoplasm of their maternal parent (Supplementary Fig. 4). This confirmed that we made either maternal or paternal haploids by using *GFP-tailswap* plants as the male or female parent, respectively, in a cross to wild type. The proportion of haploids and aneuploids was much lower if a wild-type female was crossed to a *GFP-tailswap* male (Table 1). We hypothesize that if *CENH3* is expressed earlier in development from the maternal (wild type) genome, wild-type *CENH3* could be incorporated into paternal

chromosomes derived from *GFP-tailswap*, preventing genome elimination in a wild-type \times *GFP-tailswap* cross.

Haploid *A. thaliana* plants are morphologically similar to diploids, but are comparatively smaller in size (Fig. 1f, g and Supplementary Fig. 5). Early in vegetative development, haploids have narrower rosette leaves (Fig. 1f). After bolting, haploids produce more leaves from secondary meristems. Haploid flowers are smaller than diploid flowers (Fig. 1g), which follows the general trend that flower size increases with ploidy in *A. thaliana*. Haploids are generally sterile. They contain a single copy of each chromosome and cannot undergo homologue pairing in meiosis, resulting in gametes that do not contain a full complement of chromosomes (Fig. 2 and Supplementary Fig. 6). Maternal and paternal haploid plants had similar adult morphology (data not shown). This is consistent with the fact that all documented imprinting in *A. thaliana* occurs in the short-lived endosperm, a structure confined to the seed.

To exploit the potential of haploids in crop improvement, their genome must be doubled to generate fertile diploids (doubled haploids)². A close inspection of *A. thaliana* haploids revealed that random siliques had one or two seeds. Each haploid plant yielded a total of 50–2,500 seeds depending on the wild-type parental accession (Supplementary Table 1). Most (95%) of these seeds appeared normal and gave rise to fertile diploids. To address how haploids gave rise to diploid seeds, we analysed chromosome segregation during haploid male meiosis (Fig. 2a–l). During prophase I the five chromosomes remained separate as univalents, which aligned properly in metaphase I (Supplementary Fig. 6). In anaphase I, most meocytes showed unbalanced reductional segregation (4-1, 3-2, etc.; Fig. 2e–h and Supplementary Fig. 6). Meiosis II in these cases gave rise to aneuploid tetrads. In a small minority of anaphase I cells, the five univalents migrated towards one pole (5-0 segregation) (Fig. 2i–k). In subsequent meiosis II, sister chromatids segregated equally, giving rise to haploid dyads (Fig. 2l) and viable gametes. Thus, we assume that occasional non-reduction during both male and female haploid meiosis yielded doubled haploids through self-fertilization, consistent with previous observations^{18,19}. In rare instances, we observed spontaneous chromosome doubling in somatic tissues of haploid *A. thaliana* plants; a side branch from the main inflorescence (2 out of 78 plants) or a random silique (6 out of 78 plants) showed a complete seed set (Fig. 2m and Supplementary Fig. 7). The microtubule polymerization inhibitor colchicine also induces somatic chromosome doubling in haploid *A. thaliana*, and diploid shoots that regenerate after treatment show a complete seed set (Supplementary Fig. 8). Although *A. thaliana* haploids have been produced through anther culture²⁰, spontaneous diploids recovered in these experiments were reportedly sterile²¹, and the method has not been widely adopted. The ease of generating haploids through seed by altering *CENH3*, and of converting haploids into diploids, allows large-scale generation of doubled haploids in *A. thaliana*.

Many commercial crops are polyploid²², but genetic analysis of polyploids is tedious. Reducing the ploidy of these crops will facilitate easy breeding, so we tested whether centromere-mediated genome elimination could scale down a tetraploid to diploid. *A. thaliana* is predominantly diploid, but tetraploid accessions exist¹⁷. We crossed *GFP-tailswap* to the natural tetraploid Warsaw-1 (Wa-1), and although over 98% of seeds were aborted, viable F_1 progeny included synthetic diploid plants containing only Wa-1 chromosomes (Fig. 3 and Table 1). Therefore, it is possible to extend centromere-mediated genome elimination to halve the ploidy of polyploids.

Centromere incompatibility was previously hypothesized to cause selective genome elimination in interspecies crosses^{12–14,23,24}, but it was not known how centromeres could be manipulated to achieve this. We have established a practical basis for engineering genome elimination by altering *CENH3*, a protein essential for centromere function in all eukaryotes. The fact that haploids were produced with both *GFP-tailswap* and *GFP-CENH3* transgenes indicates that multiple different alterations to the protein may induce genome elimination in other

Table 1 | Haploid plants contain only the nuclear genome of their wild-type parent

Cross	Seeds per siliqua	Normal seed (%)	Total plants analysed	Haploids (%)	Diploids (%)	Aneuploids (%)
WT Col-0 × WT Col-0	52 ± 6 (n = 23)	99.5	224	0 (0)	224 (100)	0 (0)
<i>GFP-tailswap</i> × <i>GFP-tailswap</i>	0.6 (n = 1,206)	80	213	0 (0)	197 (92)	16 (8)
<i>GFP-tailswap</i> × WT Col-0	32 ± 9 (n = 40)	12	67	23 (34)	23 (34)	21 (32)
WT Col-0 × <i>GFP-tailswap</i>	ND	ND	116	5 (4)	99 (85)	12 (11)
<i>GFP-tailswap</i> × WT Ler	30 ± 4 (n = 22)	23	127	32* (25)	32 (25)	63 (50)
<i>GFP-tailswap</i> × WT Ws-0	23 ± 5 (n = 14)	8	22	10* (45)	7 (32)	5 (28)
<i>GFP-tailswap</i> × WT C24/Ler	28 ± 5 (n = 13)	30	117	34† (29)	39 (33)	44 (38)
C24/Ler ms × <i>GFP-tailswap</i>	22 ± 14 (n = 18)	63	226	12† (5)	206 (91)	8 (4)
<i>GFP-CENH3</i> × <i>GFP-CENH3</i>	53 ± 4n = 21)	99	209	0 (0)	209 (100)	0 (0)
<i>GFP-CENH3</i> × WT	54 ± 7 (n = 18)	67	164	8 (5)	109 (66)	47 (29)
WT × <i>GFP-CENH3</i>	48 ± 6 (n = 13)	96	112	0 (0)	108 (96)	4 (4)

Cross	Seeds per siliqua	Normal seed (%)	Total plants analysed	Diploids (%)	Triploids (%)	Aneuploids (%)
<i>GFP-tailswap</i> × Wa-1 (tetraploid)	21 ± 6 (n = 96)	1.8	41	11 (27)	0	30 (73)

Frequency of haploid, diploid and aneuploid offspring in crosses featuring genome elimination. Sterile offspring with narrow leaves and small flowers were scored as haploid and further confirmed by chromosome counts. Fertile wild-type offspring were scored as diploid. Offspring with developmental defects were scored as aneuploid¹⁷. ms, male sterile; ND, not determined.

* Haploids from crosses to the Ler and Ws-0 accessions were genotyped for genetic markers described in Supplementary Fig. 2. They contained only nuclear genetic markers from their wild-type parent.

† The plastid genome in haploids from *GFP-tailswap* × C24/Ler and C24/Ler × *GFP-tailswap* crosses was genotyped, and found to be derived from the maternal parent (Supplementary Fig. 4).

plants. *A. thaliana* plants that co-express wild-type and *GFP-tailswap* or *GFP-CENH3* proteins do not act as a haploid inducer (data not shown). Therefore, our method currently relies on replacing native *CENH3* with an altered variant. A *cenh3* mutation or a gene silencing method such as RNA interference could be used to reduce or eliminate endogenous *CENH3* function in a novel species.

Haploid inducing lines have been described in the grasses^{25–27}, but their genetic basis is not known, except for maize *indeterminate gametophyte* (*ig*)²⁸. The effect of *ig* may be limited to maize, because mutations in the *A. thaliana* *ig* orthologue AS2 do not phenocopy its effect²⁹. Our process has key advantages over current methods for producing haploid plants. First, no tissue culture is needed, removing a major source of genotype dependence. Second, the same inducer produces maternal and paternal haploids. Third, crossing a *cenh3* mutant as the female transfers the nuclear genome of the male parent into a heterologous cytoplasm. This could accelerate production of cytoplasmic male sterile lines for making hybrid seed. Fourth, genome elimination occurs between parents that are isogenic except for *CENH3* alterations, avoiding fertility barriers inherent to wide crosses.

Genome elimination induced by changes in *CENH3* probably occurs during the first few zygotic mitoses, when centromeres from the two parents are loaded with different populations of *CENH3* proteins. Expression of both wild-type and mutant *CENH3* genes in subsequent cell cycles should rapidly equalize the amount of the two proteins in individual centromeres. Zygotic mitosis is normal in *GFP-tailswap* and in *GFP-CENH3* plants, because we did not see haploids from self-fertilized plants. Furthermore, *GFP-CENH3* plants have a completely wild-type phenotype. Subtle differences in centromere DNA binding, kinetochore assembly, or coupling to spindle microtubules may be sufficient to slow the segregation of chromosomes containing altered *CENH3*, resulting in genome elimination. Cell cycle checkpoints in plants must be relaxed enough to allow wild-type and mutant chromosomes to segregate differentially, and presumably to permit cytokinesis without complete chromosome segregation. The precise mechanism of genome elimination in our experiments remains unknown.

Centromere DNA sequences and the *CENH3* protein both evolve rapidly, and centromere differences have been proposed to create species barriers³⁰. Although our experiments used tagged proteins,

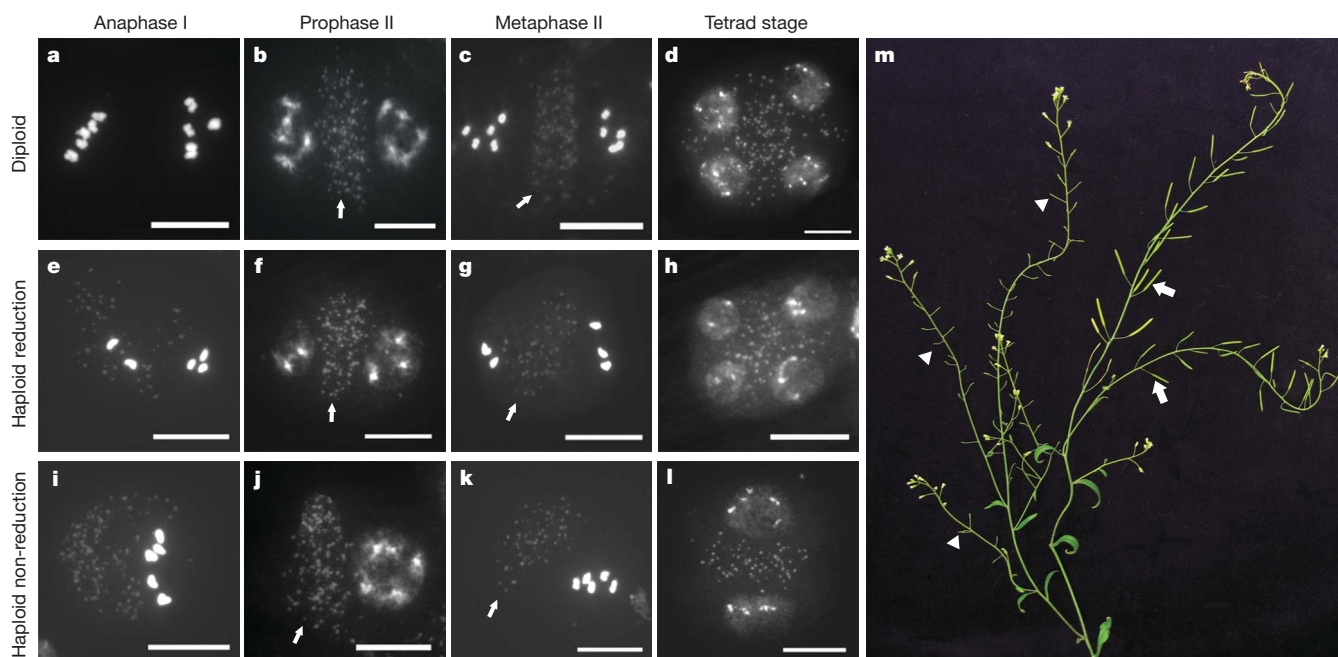


Figure 2 | Haploid *Arabidopsis thaliana* yield spontaneous diploid progeny. a–l, Meiosis in diploid (a–d) and haploid (e–l) *A. thaliana*. Meiosis II cells show a central organelle band (arrows), indicating that they have completed meiosis I. Panels e–h show unbalanced reductional segregation (3–2) in

haploid meiosis. Panels i–l show non-reductional segregation (5–0) in haploid meiosis, forming haploid dyads (l). m, Spontaneous chromosome doubling in somatic cells of haploid *A. thaliana* plants produces fertile diploid branches (arrows) on otherwise sterile haploid plants (arrowheads). Scale bars, 0.5 μm.

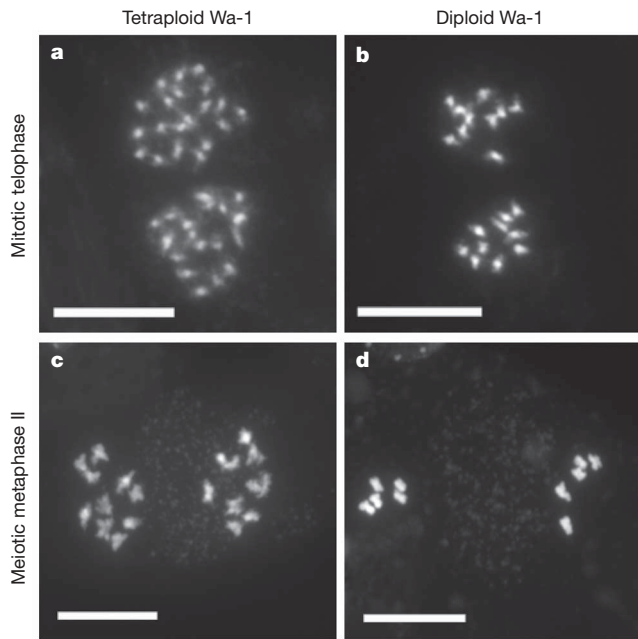


Figure 3 | A natural *Arabidopsis* tetraploid converted to diploid by centromere-mediated genome elimination. **a**, Mitotic telophase chromosome spread from tetraploid Wa-1 ($4n = 20$). **b**, Mitotic telophase from diploid Wa-1 ($2n = 10$) produced by centromere-mediated genome elimination. Diploid progeny from *GFP-tailswap* \times Wa-1 crosses contained only Wa-1 genetic markers (Table 1). **c**, Meiosis II from tetraploid Wa-1. **d**, Meiosis II from diploid Wa-1. Scale bars, 1 μ m.

they indicate that changes in CENH3 can induce specific chromosome loss in a hybrid zygote. Future experiments may indicate whether naturally occurring differences in CENH3 can affect chromosome segregation in zygotic mitosis of hybrids.

METHODS SUMMARY

Plant materials. *cenh3-1* is a G-to-A transition at nucleotide 161 relative to ATG = +1, and mutates a conserved splice acceptor in the second intron. *GFP-CENH3* and *GFP-tailswap* transgenes contained an N-terminal GFP, and used the endogenous *CENH3* promoter and terminator. Isolation and characterization of *cenh3-1*, cloning of the *GFP-CENH3* and *GFP-tailswap* transgenes, and construction of the complemented *cenh3-1 GFP-CENH3* and *cenh3-1 GFP-tailswap* lines are described elsewhere (see Methods). The location of the *GFP-tailswap* transgene was determined by thermal asymmetric interlaced PCR (TAIL-PCR), allowing us to determine whether the transgene was homozygous or hemizygous. The C24/*Ler* male sterile line was a gift from L. Comai. Male sterility was conferred by the A9-barnase transgene (Methods). Plants were grown under 16 h of light/8 h of dark at 20 °C.

Genomic DNA preparation and genotyping. Genomic DNA preparation and PCR genotyping were performed using standard methods. Polymorphic markers used to genotype the five nuclear chromosomes and the plastid genome are listed in the Methods and Supplementary Figs 3 and 4.

Cytogenetic analysis. To analyse meiotic progression and to determine ploidy, we prepared mitotic and meiotic chromosome spreads from anthers according to published protocols (Methods).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 29 November 2009; accepted 13 January 2010.

1. Dunwell, J. M. Haploids in flowering plants: origins and exploitation. *Plant Biotechnol. J.* (in the press).
2. Forster, B. P., Heberle-Bors, E., Kasha, K. J. & Touraev, A. The resurgence of haploids in higher plants. *Trends Plant Sci.* **12**, 368–375 (2007).
3. Forster, B. P. & Thomas, W. T. B. in *Plant Breeding Reviews* (ed. Janick, J.) 57–88 (John Wiley & Sons, 2005).

4. Guha, S. & Maheshwari, S. C. *In vitro* production of embryos from anthers of *Datura*. *Nature* **204**, 497 (1964).
5. Wedzony, M. et al. in *Advances in Haploid Production in Higher Plants* (eds Touraev, A., Forster, B. P. & Jain, S. M.) 1–33 (Springer, 2009).
6. Bains, G. S. & Howard, H. W. Haploid plants of *Solanum demissum*. *Nature* **166**, 795 (1950).
7. Barclay, I. R. High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. *Nature* **256**, 410–411 (1975).
8. Burk, L. G., Gerstel, D. U. & Wernsman, E. A. Maternal haploids of *Nicotiana tabacum* L. from seed. *Science* **206**, 585 (1979).
9. Clausen, R. E. & Mann, M. C. Inheritance of *Nicotiana tabacum*. V. The occurrence of haploid plants in interspecific progenies. *Proc. Natl Acad. Sci. USA* **10**, 121–124 (1924).
10. Hougas, H. W. & Peloquin, S. J. A haploid plant of the potato variety Katahdin. *Nature* **180**, 1209–1210 (1957).
11. Kasha, K. J. & Kao, K. N. High frequency haploid production in barley (*Hordeum vulgare* L.). *Nature* **225**, 874–876 (1970).
12. Bennett, M. D., Finch, R. A. & Barclay, I. R. The time rate and mechanism of chromosome elimination in *Hordeum* hybrids. *Chromosoma* **54**, 175–200 (1976).
13. Finch, R. A. Tissue-specific elimination of alternative whole parental genomes in one barley hybrid. *Chromosoma* **88**, 386–393 (1983).
14. Laurie, D. A. & Bennett, M. D. The timing of chromosome elimination in hexaploid wheat \times maize crosses. *Genome* **32**, 953–961 (1989).
15. Talbert, P. B., Masuelli, R., Tyagi, A. P., Comai, L. & Henikoff, S. Centromeric localization and adaptive evolution of an *Arabidopsis* histone H3 variant. *Plant Cell* **14**, 1053–1066 (2002).
16. Henikoff, S. & Dalal, Y. Centromeric chromatin: what makes it unique? *Curr. Opin. Genet. Dev.* **15**, 177–184 (2005).
17. Henry, I. M. et al. Aneuploidy and genetic variation in the *Arabidopsis thaliana* triploid response. *Genetics* **170**, 1979–1988 (2005).
18. Chase, S. S. Monoploids and monoploid-derivatives of maize (*Zea mays* L.). *Bot. Rev.* **35**, 117–168 (1969).
19. Jauhar, P. P., Dogramaci-Altuntepe, M., Peterson, T. S. & Almouslem, A. B. Seedset on synthetic haploids of durum wheat: cytological and molecular investigations. *Crop Sci.* **40**, 1742–1749 (2000).
20. Avetisov, V. A. Production of haploids during *in vitro* culturing of *Arabidopsis thaliana* (L.) Heynh anthers and isolated protoplasts. *Genetika* **12**, 17–25 (1976).
21. Scholl, R. & Amos, J. A. Isolation of doubled-haploid plants through anther culture. *Z. Pflanzenphysiol.* **96**, 407–414 (1980).
22. Udall, J. A. & Wendel, J. F. Polyploidy and crop improvement. *Crop Sci.* **46**, S3–S14 (2006).
23. Heppich, S., Tunner, H. G. & Greilhuber, J. Pre-meiotic chromosome doubling after genome elimination during spermatogenesis of the species hybrid *Rana esculenta*. *Theor. Appl. Genet.* **61**, 101–104 (1982).
24. Jin, W. et al. Maize centromeres: organization and functional adaptation in the genetic background of oat. *Plant Cell* **16**, 571–581 (2004).
25. Coe, E. H. A line of maize with high haploid frequency. *Am. Nat.* **93**, 381–382 (1959).
26. Hagberg, A. & Hagberg, G. High frequency of spontaneous haploids in the progeny of an induced mutation in barley. *Hereditas* **93**, 341–343 (1980).
27. Kermicle, J. L. Androgenesis conditioned by a mutation in maize. *Science* **166**, 1422–1424 (1969).
28. Evans, M. M. The *indeterminate gametophyte1* gene of maize encodes a LOB domain protein required for embryo sac and leaf development. *Plant Cell* **19**, 46–62 (2007).
29. Ori, N., Eshed, Y., Chuck, G., Bowman, J. L. & Hake, S. Mechanisms that control knob gene expression in the *Arabidopsis* shoot. *Development* **127**, 5523–5532 (2000).
30. Henikoff, S., Ahmad, K. & Malik, H. S. The centromere paradox: stable inheritance with rapidly evolving DNA. *Science* **293**, 1098–1102 (2001).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank L. Comai, D. Melters, J. Maloof and J. Ramahi for comments on the manuscript; and R. McNeilage, R. Clarke, P. Kwong and J. Stewart for technical assistance. This work was supported by a grant from the Hellman Family Foundation to S.W.L.C., and by the University of California, Davis.

Author Contributions M.R. and S.W.L.C. designed the study, performed the experiments, analysed the data and wrote the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/nature. Correspondence and requests for materials should be addressed to S.W.L.C. (srchan@ucdavis.edu).

METHODS

Plant materials. *cenh3-1* was isolated by the TILLING procedure in collaboration with V. Sundaresan and L. Comai at the University of California, Davis³¹. The TILLING population was created by mutagenizing *Arabidopsis thaliana* in the Col-0 accession with ethylmethane sulphonate, using standard protocols. We isolated *cenh3-1* by TILLING using the CEL1 heteroduplex cleavage assay, with PCR primers specific for the *CENH3/HTR12* gene.

cenh3-1 is predicted to disrupt normal splicing of *CENH3*, because it mutates a conserved splice acceptor site at the beginning of the second coding exon. Translation of an mRNA containing the first coding exon spliced to an incorrect location within *CENH3* is predicted to yield only 18 correct amino acids. As the histone-fold domain of *CENH3* begins at amino acid residue 82, we believe that *cenh3-1* is a null allele (this is supported by its embryo-lethal phenotype).

Cloning of the *GFP-CENH3* and *GFP-tailswap* transgenes, and construction of the complemented *cenh3-1 GFP-CENH3* and *cenh3-1 GFP-tailswap* lines, are described elsewhere (M.R. *et al.*, manuscript in preparation). Primer sequences and full details are available on request.

To cross wild type as the female to *GFP-tailswap* as the male, we used a dissecting microscope to observe directly pollen deposition on the stigma (*GFP-tailswap* is mostly male sterile). The amount of viable pollen in individual flowers of *GFP-tailswap* varies. We selected flowers that clearly showed higher amounts of pollen, and pollinated with more than 60 anthers (10 *GFP-tailswap* flowers) per wild-type stigma to achieve the seed set reported in Table 1. Using an optivisor (magnifying lens) and approximately 12 anthers (2 *GFP-tailswap* flowers) per wild-type stigma, we obtained a much lower seed set per silique.

The percentage of normal seeds was determined by visual inspection using a dissecting microscope. Seeds from *GFP-tailswap* × wild-type crosses were sown on 1 × MS plates containing 1% sucrose to maximize germination efficiency, particularly of seed that had an abnormal appearance. Late germinating seeds were frequently haploid.

The *quartet* mutant that we used was *qrt1-2* (ref. 32). Male sterility in the C24/*Ler* line was conferred by the A9-barnase transgene^{33,34}.

In the *GFP-tailswap* × Wa-1 experiment, progeny from the *GFP-tailswap* × Wa-1 cross that contained only Wa-1 chromosomes were confirmed as diploid using chromosome spreads. We scored plants that were heterozygous for some chromosomes (Col-0 and Wa-1 markers) and homozygous for other chromosomes (Wa-1 markers only) as aneuploid. We did not find triploid offspring (heterozygous for markers on all chromosomes). A subset of plants was further karyotyped by means of chromosome spreads to confirm aneuploidy.

Cytogenetic analysis. Mitotic and meiotic chromosome spreads from anthers were prepared according to published protocols³⁵.

Colchicine treatment. Colchicine treatment of developing haploid plants used a previously published protocol with minor modifications³⁶. A solution of 0.25% colchicine, 0.2% Silwet was prepared, and a 20 µl drop was placed on the meristem before bolting. Plants became transiently sick after colchicine treatment. Upon recovery, fertile inflorescences appeared from secondary meristems indicating successful chromosome doubling. Haploid plants can also be treated after bolting, although the rate of success is considerably lower.

31. Comai, L. & Henikoff, S. TILLING: practical single-nucleotide mutation discovery. *Plant J.* **45**, 684–694 (2006).
32. Francis, K. E., Lam, S. Y. & Copenhaver, G. P. Separation of *Arabidopsis* pollen tetrads is regulated by *QUARTET1*, a pectin methylesterase gene. *Plant Physiol.* **142**, 1004–1013 (2006).
33. Bushell, C., Spielman, M. & Scott, R. J. The basis of natural and artificial postzygotic hybridization barriers in *Arabidopsis* species. *Plant Cell* **15**, 1430–1442 (2003).
34. Paul, W., Hodge, R., Smartt, S., Draper, J. & Scott, R. The isolation and characterisation of the tapetum-specific *Arabidopsis thaliana* A9 gene. *Plant Mol. Biol.* **19**, 611–622 (1992).
35. Ross, K. J., Fransz, P. & Jones, G. H. A light microscopic atlas of meiosis in *Arabidopsis thaliana*. *Chromosome Res.* **4**, 507–516 (1996).
36. Josefsson, C., Dilkes, B. & Comai, L. Parent-dependent loss of gene silencing during interspecies hybridization. *Curr. Biol.* **16**, 1322–1328 (2006).