# **Supporting Information**

### Roux et al. 10.1073/pnas.1520687113

### SI Text

**Construction and Genotyping of Cytolines.** The construction of the 56 cytolines is illustrated in Fig. 1. The genetic composition of the plants throughout the cytoline construction is illustrated in Fig. S1. Three recurrent backcrosses with the nuclear donor parent were realized on 54 from the 56 possible F1s of the diallele cross. F1 seeds from reciprocal crosses between Bur-0 and Ita-0 did not germinate whatever the conditions tested. To obtain the two concerned cytolines, we started backcrosses with the nuclear donor on bridge genotypes. Specifically, a plant from the cross Bur-0 × [Jea × Ita-0] F1 was backcrossed with Ita-0 for the Bur-0<sup>cy</sup>Ita-0<sup>nuc</sup> combination; a plant from the cross [Ita-0 × Jea] F1 × Bur-0 was back-crossed with Bur-0 for the Ita-0<sup>cy</sup>Bur-0<sup>nuc</sup> combination. Consequently, the genotyping of these cytolines was designed to discriminate Bur-0, Ita-0 and Jea alleles.

For each combination, 29 plants from the third backcross were genotyped with a set of 384 SNP markers (55), among which 134 on average were informative, according to the considered combination. To keep the distance between two genotyped positions below 3 Mb, and the distance between the last and first genotyped positions and the telomeres below 1 Mb, microsatellites were used in those intervals too large between informative SNPs. Among the 56 cytonuclear combinations, 42 cytolines were obtained at this stage. For the 14 remaining combinations, 24 or 48 plants from the selfing descent of plants chosen on their genotype were grown and genotyped at the position(s) were the mother plant was heterozygous to select the corresponding cytoline among the progenies fixed with the nuclear donor allele. The rule was sometimes relaxed in the centromeric regions where polymorphic markers were difficult to find and meiotic recombination is known to be rare. The number of markers used for each cytoline is available in Table S1. The complete list of the markers, with their positions in the genome, are available on the Versailles Arabidopsis Stock Center website (publiclines.versailles.inra.fr/).

The cytoplasm of cytolines was verified by sequencing intergenic chloroplastic regions as previously described (25).

Seed Production of Cytolines. Seeds of the 56 cytolines and their eight parental accessions were produced in a large growth chamber (56 m<sup>2</sup>, light 16 h at 21 °C, dark 8 h at 18 °C). Sixteen plants of each genotype were grown. The plants were placed according to an experimental scheme designed to randomize the environmental heterogeneities in the chamber, known to be mainly due to border effects. One hundred twenty-eight plants were disposed on each of the eight tables in the chamber (Fig. S2). Plants were sown by groups of genotypes sharing the same nucleus. Seeds of the male sterile cytoline 39CV (Sha<sup>cy</sup>Cvi-0<sup>nuc</sup>) (47) were produced by hand pollination with pollen of the surrounding Cvi-0 plants. At the end of their life cycle (i.e., when siliques started to dry), plants were placed in a drying chamber and watering was stopped until complete drying of the plant. Plants were individually harvested and their seed production weighted. Bulks were made with equivalent amounts of seeds produced by each plant of a given genotype.

Four cytolines were still unsatisfactory at the start date of the field experiment, namely Blh-1<sup>cy</sup>Sha<sup>nuc</sup>, Bur-0<sup>cy</sup>Ita-0<sup>nuc</sup>, Ct-1<sup>cy</sup>Ita-0<sup>nuc</sup>, and Ct-1<sup>cy</sup>Jea<sup>nuc</sup>, and were therefore not included in this study.

**Field Experiment and Phenotypic Characterization.** An experiment of 2,700 *A. thaliana* plants was set up at the University of Lille 1 (North, France). The field experiment was organized in five

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blocks, each one being represented by nine arrays of 66 individual bottom-pierced wells (11 lines × 6 columns, Ø4 cm, vol. ~38 cm<sup>3</sup>) (TEKU, JP 3050/66) filled with damp standard culture soil (Huminsubstrat N3; Neuhaus). Each block corresponded to an independent randomization of 540 plants with nine replicates per cytoline (n = 52) and nine replicates per parental accession (n = 8). In each block, the remaining 54 wells were left empty.

Five seeds were sown in each well on 11 March 2013 to mimic the spring seasonal germination cohort observed in natural populations of A. thaliana in the North of France. Germination was promoted by stratifying seeds four days at 4 °C in a cold chamber. After the stratification treatment, arrays were preventively treated against dark-winged fungus gnats (Vectobac; 8 mL/L) and placed for 28 d in a frost-free greenhouse that mimics outdoor conditions (no additional light or heating) but protects seeds from rainfall. To reduce microenvironmental variations, arrays were rotated daily in the cold chamber and in the greenhouse. Germination date and germination rate were monitored in the frost-free greenhouse during 13 d after the stratification treatment (see below). Wells were thinned to two seedlings and one seedling 14 and 20 d after the stratification treatment, respectively. Thereafter, and in the main text, the time after sowing is meant counted from the end of the stratification treatment.

Twenty-eight days after sowing, arrays were transported outside to a field located at the University of Lille 1. For each block, the nine arrays were organized according to a grid of three columns and three lines. Soil was tilled to allow arrays to be slightly buried, thereby facilitating root development. Plants were protected from herbivory by vertebrates and slugs as described in ref. 56.

Each plant was scored for a total of 28 phenotypic traits related to germination (n = 5), phenology (n = 4), resource acquisition (n = 3), architecture and seed dispersal (n = 5), fecundity (n = 10), and survival (n = 1) (Dataset S1):

Germination: Germination time (GERM) was measured as the number of days between sowing and the emergence of the first seedling. Using a phenological model integrating both photoperiod length and temperature (56), GERM was scaled in photothermal units (PTUs). Germination percentage was estimated 4, 5, 6, and 13 d after sowing (PGERM4, PGERM5, PGERM6, and PGERM13).

Phenology: Bolting time (BT), flowering interval (INT), and the reproductive period (RP) were scored as the time intervals between germination date and bolting date, between bolting date and flowering date, and between flowering date and date of maturation of the last fruit, respectively. BT, INT, and RP were scaled in PTU. By summing these three phenological traits, we estimated the length of the life cycle (LCYCLE).

Resource acquisition: rosette surface area (AREA expressed in cm<sup>2</sup>) and rosette perimeter (PERIM expressed in cm) were measured using a nondestructive approach 28 d after sowing (Fig. S3). At the start of flowering, the maximum diameter of the rosette measured at the nearest millimeter was used as a proxy for plant size (DIAM).

Architecture and seed dispersal: After maturation of the last fruit, the above-ground portion was harvested and stored at room temperature until further phenotyping. Plants were phenotyped for the following architectural and seed dispersal related traits: height from soil to the first fruit on the main stem (H1F), maximum height (HMAX), number of primary branches on the main stem (NPB), number of basal branches (NBB), and total number of branches (TOTB = NPB + NBB).

Fecundity: Because the number of seeds in a fruit is highly correlated with fruit length (56, 57), total seed production was approximated by total fruit length (FITTOT). Seed production is a good proxy for fecundity in a highly selfing annual species like A. thaliana (51). FITTOT was obtained by adding the fruit length produced on the main stem (FITSTEM), the primary branches on the main stem (FITPB), and the basal branches (FITBB). These estimates of fruit length were obtained by counting the number of fertilized fruits produced on each type of branches (FRUITSTEM, FRUITPB, and FRUITBB) and multiplying these counts by an estimate of their corresponding fruit (or silique) length (SILSTEM, SILPB and SILBB), estimated as the average of three representative fruits. We also calculated three ratios corresponding to the percentage of seeds produced by one branch type as a function of the total amount of seed produced: RSTEM = FITSTEM/FITTOT, RPB = FITPB/FITTOT, and RBB = FITBB/FITTOT. We also estimated the rate of fruit abortion (STERILITY) as the number of aborted fruits divided by the total number of fruits.

Survival: All plants that germinated but did not survive were counted as dead (SURVIVAL = 0). Harvested plants were counted as alive (SURVIVAL = 1).

Because all plants carrying a nucleus from Ita-0 were late flowering in this study, they were not able to complete their life cycle before summer heat. Consequently, postflowering traits were not measured on the Ita-0 parental accession, as well as on the cytolines with the Ita-0 nucleus.

Due to the absence of basal branches in most cytolines (Dataset S1), the traits related to basal branches (FITBB, FRUITBB, SILBB, and RBB) were not statistically analyzed in this study.

### Data Analysis.

*Model.* Each trait was modeled separately using the following mixed model (1):

$$Y_{bncij} = \mu + \alpha_b + \beta_n + \gamma_c + (\alpha\beta)_{bn} + (\beta\gamma)_{nc} + L_{i(b)} + C_{j(b)} + E_{bncij},$$

where  $Y_{bncij}$  is the phenotype of the cytoline with nucleus *n* and cytoplasm *c*, measured on the *i*th line and *j*th column of block *b*. Parameters  $\alpha_b$ ,  $L_{i(b)}$  and  $C_{j(b)}$  correspond to the (fixed) effect of block *b* and the (random) effects of line *i* and column *j* within block *b*, respectively. These three effects account for the environmental variation within the experimental field. Parameters  $\beta_n$ ,  $\gamma_c$  and  $(\beta\gamma)_{nc}$  correspond to the main fixed effects of nucleus *n* and cytoplasm *c* and their interaction, respectively. A block × nucleus interaction parameter  $(\alpha\beta)_{bn}$  was also added to account for observed phenotypic differences between nuclei across blocks.  $E_{bncij}$  is the error term. Depending on traits, the error variance was chosen to be either homogeneous or nucleus (i.e., heterogeneous) dependent

$$E_{bncij} \hookrightarrow \mathcal{N}(0, \sigma^2), i. i. d. (\text{homogeneous})$$
$$E_{bncij} \hookrightarrow \mathcal{N}(0, \sigma_n^2), indpt (\text{heterogeneous}).$$

For each trait, selection between homogeneous or heterogeneous assumption for the variance of the error term was performed on the basis of the BIC criterion.

As illustrated in Fig. 3, accounting for heterogeneous error variance was relevant for many traits, and significantly improved

the accurate identification of parental pairs contributing to cytonuclear interactions. We also considered the inclusion of block  $\times$ cytoplasm and block  $\times$  cytoplasm  $\times$  nucleus interactions in the model. However, adding these extra terms precluded the heterogeneous error variance assumption due to numerical instability [nonconvergence of the restricted maximum likelihood (ReML) procedure, inconsistent estimated effects, and/or degrees of freedom for parental pair contrasts]. Nonetheless, fitting the model with the additional interaction terms and homogeneous error variances for each trait led to results similar to those obtained with the mixed model (1): the same cytoplasm  $\times$  nucleus interactions were detected across traits, except for the traits INT and FRUITPB (results not shown).

Because variation in rosette surface area and rosette perimeter may indirectly result from variation in germination time, the term GERM was also added as a covariate in the statistical model for the traits AREA and PERIM. All random effects were assumed to be Gaussian and independent, with a mean equal to 0. The line and column variances are  $\sigma_l^2$  and  $\sigma_c^2$ , respectively. Inference was performed using ReML estimation, using the

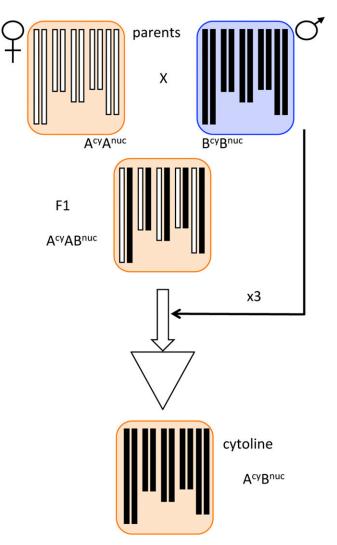
Inference was performed using ReML estimation, using the PROC MIXED procedure in SAS 9.1 (SAS Institute) for all traits with the exception of SURVIVAL, which was analyzed using the PROC GLIMMIX procedure in SAS 9.3. For all traits and genotypes, LSMs were computed.

*Test for interactions in specific pairs of parents.* To identify pairs of parents that contribute to the nucleus × cytoplasm interaction, the hypothesis H0 { $[(\beta\gamma)_{kl} - (\beta\gamma)_{kk}] - [(\beta\gamma)_{lk} - (\beta\gamma)_{ll}]$ } was tested for all pairs of parents (*k*,*l*) and all quantitative traits. A global correction for multiple testing was performed to control FDR (54) at a nominal level of 5%.

Cytoplasmic Diversity in a Local Metapopulation of A. thaliana. To evaluate cytoplasmic diversity in the local metapopulation TOU (37, 51), polymorphisms were analyzed (i) in four chloroplast intergenic regions (MatK-trnK, ndhC-trnV, rbcL-accD, ndhF-rpl32) for 24 individuals located in 11 stands (TOU-A1-41, TOU-C-2, TOU-D-5, TOU-E-7, TOU-F-1, TOU-I-2, TOU-P-6, TOU-Q-2, TOU-R-9, TOU-S-1, TOU-T-1, TOU-T-2, TOU-T-3, TOU-T-4, TOU-T-5, TOU-T-6, TOU-T-7, TOU-T-8, TOU-T-9, TOU-T-10, TOU-T-14, TOU-T-15, TOU-T-16, TOU-T-17) and (*ii*) in two mitochondrial regions (atp8-orf107c, ccmC) for 12 individuals (TOU-A1-41, TOU-C-2, TOU-D-5, TOU-E-7, TOU-F-1, TOU-I-2, TOU-P-6, TOU-Q-2, TOU-R-9, TOU-S-1, TOU-T-1, and TOU-T-8) (Dataset S2). Individuals TOU-T-2 to TOU-T-7, TOU-T-9, TOU-T-10, and TOU-T-14 to TOU-T-17 were assumed to carry the same cytoplasm as their sister plants with the same chlorotype. All chloroplast and mitochondrial polymorphisms were analyzed as described in ref. 25. The pairwise distances between the 11 stands range from 50 m to 1 km.

This analysis grouped TOU-A1-41, TOU-C-2, TOU-F-1, TOU-R-9, TOU-T-8, TOU-T-9, TOU-T-10, TOU-T-14, TOU-T-15, TOU-T-16, and TOU-T-17 in the previously described Z cytotype, whereas TOU-D-5 and TOU-S-1 were grouped in the previously described AA cytotype. These two cytotypes are very close to both the cytotype of the parental accession Jea and the cytotype Y, where fell TOU-P-6 (25). TOU-Q-2, TOU-T-1, TOU-T-2, TOU-T-3, TOU-T-4, TOU-T-5, TOU-T-6, TOU-T-7, and TOU-T-8 were grouped in the previously described BA cytotype. TOU-E-7 and TOU-I-2 correspond to cytoplasmic haplotypes that have not been described in a set of 95 worldwide accessions.

A cytoplasmic phylogenetic network of the TOU cytotypes was constructed using the same strategy as in (25) (Fig. S5).



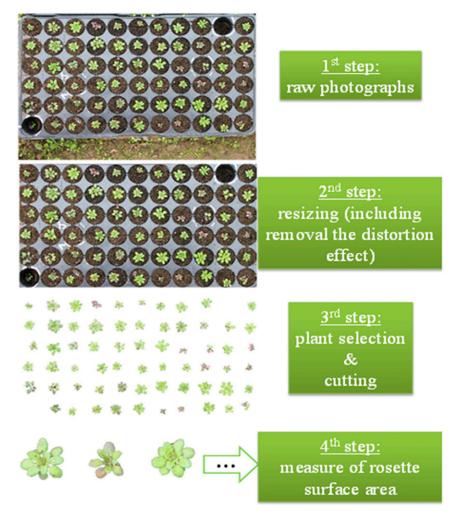
**Fig. S1.** Genetic composition of the plants throughout the cytoline construction. The 10 bars represent the five chromosome pairs of *A. thaliana*, with colors illustrating allelic origin (white: accession A, black: accession B). The background color of the rectangle stands for the origin of cytoplasm (orange: accession A, blue: accession B). After at least three backcrosses and genotyping, symbolized by the inversed triangle, the cytoline obtained possess the nuclear genome of parent B in the cytoplasmic background of parent A.

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1.1 1.2	2.1 2.2
180 B7C 172 79C 103 39C 52C 71C 25A 157 38C S0C S9C 642 102 52C 44C 100   AV AV V	71C 47C 101 86C 99C 157 88C 53C 95C 103 41C 95C 101 84C 95C 236 51C 82C 72C 99C 94C V CV V AV V V V V V V V V V V V V V V V
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99C 166 97C 224 76C 77C 43C 42C 39C 172 157 25A 66C 172 79C 93C 65C 52C 41C 90C 72C 82C V AV V AV V V V V V AV V V V V V V V V	180 41C 91C 56C 157 73C 162 93C 104 102 94C 89C 79C 166 50C 90C 46C 76C 99C 45C 52C AV V V V A V V AV V V V V V V V V V V
103 66C 73C 72C 105 94C CV V V V V V V V V V V V V V V CV V C	68C 45C 80C 39C 102 166 86C 236 85C 36C 55C 46C 224 42C 92C 103 72C 25A 66C 93C 236 157   v </td
47C 100 75C 85C 104 70C 25A 37C 98C 89C 36C 99C 53C 98C 44C 96C 162 224 71C 49C   V	95C 105 84C 53C 96C 103 42C 69C 64C 82C 105 97C 73C 83C 83C 83C 53C 71C 47C   V
55C 53C 95C 45C 90C 86C 69C 64C 51C 80C 36C 38C 42C 102 56C 89C 94C 50C 95C 64C	52C 99C 98C 172 38C 65C 100 87C 72C 47C 83C 69C 82C 86C 96C 80C 68C 36C 104 162 37C 172 V V V AV V V V V V V V V V V V V V V V
80C 236 92C 46C 41C 49C 91C 56C 82C 79C 96C 50C 84C 68C 43C 91C 45C 97C 77C 100 73C 78C 83C	76C 224 49C 70C 44C 66C 79C 75C 78C 94C 101 44C 100 78C 77C 84C 85C 91C 56C
71C 162 102 101 52C 180 44C 93C 38C 68C 78C 87C 86C 46C 47C 92C 104 85C 87C 236 75C V AV CV V AV V V V V V V V V V V V V V V	77C 37C 92C 90C 25A 97C 43C 71C 50C 51C 89C 101 51C 43C 39C 70C 55C 180 41C 49C 65C 0 V V V V V V V V V V V V V V V V V V
51 52	61 63
102 94C 89C 79C 166 50C 90C 46C 76C 99C 45C 52C 78C 66C 72C 236 93C 91C 45C 157 76C 89C 39C 102 CV V V V AV V V V V V V V V V V V V V V	53C 102 97C 51C 72C 41C 52C 101 98C 166 97C 224 76C 77C 43C 42C 39C 166 97C 224 76C 77C 43C 42C 39C 120 99C 166 97C 224 76C 77C 43C 42C 39C 120 90C 140
224 42C 92C 103 72C 25A 66C 98C 64C 93C 236 157 83C 41C 101 55C 84C 56C 77C 25A 99C	70C 75C 95C 96C 80C 66C 103 42C 94C 224 46C 25A 103 66C 73C 72C 105 94C 157 87C 65C 83C 84C
105 97C 75C 73C 83C 38C 95C 87C 53C 71C 47C 44C 97C 52C 43C 105 90C 49C 80C 69C 87C 162 71C	79C 105 104 69C 36C 50C 89C 87C 76C 44C 236 47C 100 75C 85C 104 70C 25A 37C 98C 89C 36C
69C 82C 86C 96C 80C 68C 36C 104 162 37C 172 73C 104 224 65C 64C 36C 94C 172 86C	99C 83C 77C 71C 68C 85C 65C 49C 43C 91C 92C 55C 53C 95C 45C 90C 86C 69C 64C 51C
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7.1 7.2	8.1 8.2
51C 82C 72C 99C 94C 41C 95C 101 84C 96C 236 88C 80C 89C 94C 56C 42C 102 52C 44C 100 V V V V V V V V V V V V V V V V V V V	71C 47C 101 36C 99C 157 68C 53C 95C 103 180 37C 172 79C 103 39C 92C 71C 25A 157 V V CV V V AV V V V V V V V AV V V V V V
53C 56C 52C 66C 42C 55C 98C 69C 36C 89C 44C 93C 55C 72C 45C 41C 65C 49C 236 172 87C 96C	166 98C 73C 82C 78C 77C 70C 84C 46C 69C 90C 43C 45C 90C 65C 75C 76C 85C 86C 38C 77C 70C 84C 45C 90C 43C 77C 70C 84C 77C 70C 70C 77C 70C 70C 77C 70C 70C 77C 70C 70
162 68C 87C 100 50C 166 43C 47C 93C 78C 102 80C 104 92C 66C 50C 39C 37C 162 79C 25A	180 83C 51C 85C 97C 91C 75C 224 76C 64C 86C 105 49C 70C 97C 83C 46C 105 224 73C 91C 64C 104 AV V V V V V V V V V V V V V V V V V V
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85C 86C 38C 77C 45C 90C 65C 75C 76C 70C 84C 46C 69C 90C 43C 166 98C 73C 82C 78C 77C	65C 49C 236 172 87C 96C 93C 55C 72C 45C 41C 53C 56C 52C 66C 42C 55C 98C 69C 36C 89C 44C
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Fig. 52. Experimental design for seed production. Each of the eight tables present in the growth chamber was divided in two parts. One plant per genotype was placed in each half of table. The positions were randomized in each block (colors). Genotypes are designed according to their accession name in the Versailles stock center (AV suffix for parental accessions, CV suffix for cytolines; Table S1). Empty positions were left empty or used to grow seeds from sister plants of some cytolines (not used in this study).

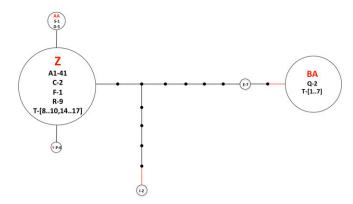
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**Fig. S3.** Measure of rosette surface area (AREA). Rosette surface area (AREA) was measured using a nondestructive approach, by imaging each tray 28 d after sowing, and using a Canon digital camera (model EOS 500D). Each image of an array was submitted to a four-step treatment. In the first step, a photograph of each array was taken in the field. In the second step, the raw photographs were centered, detrapezoided, and resized using the software Adobe Photoshop CS3 Extented (version 10.0) and the software ImageMagick (version 7:6.6.2.6–1ubuntu4.2; Available from www.imagemagick.org/script/index.php). In the third step, plants were then manually selected from background using the software GIMP 2.8 (Available from www.gimp.org/). Background-free images were then cutted using a custom Perl script to obtain an individual background-free image for each plant of the experiment. In the fourth step, rosette surface area and rosette perimeter of each plant were then estimated using the ImageJ software (version 4.01; Universal Imaging). Rosette surface area was automatically estimated for each plant on pretreated photographs using the Area Set Measurement command, which first estimates the number of pixels defined by an object and then converts this number into a metric value of surface (cm<sup>2</sup> in this study). Rosette perimeter was automatically estimated for each plant on pretreated photographs using the Perimeter Set Measurement command, which estimates the length of the outside boundary of the rosette image.



**Fig. S4.** Seed production of cytolines in a field located at the University of Lille. An experiment of 120 *A. thaliana* plants was organized in two blocks, each one being represented by 60 pots ( $9 \times 9 \times 9.5$  cm, vol. ~480 cm<sup>3</sup>; TEKU MQC) filled with damp standard culture soil (Huminsubstrat N3; Neuhaus). Each block is an independent randomization of 60 plants with one replicate per cytoline (n = 52) and one replicate per parental accession (n = 8). Experimental conditions in the frost-free greenhouse until the transport of pots outside to the field were similar to the conditions for the main experiment described in *S1 Text.* (*A*) Overview of the experiment. (*B*) Close view of a Sha<sup>Cy</sup>Cvi-0<sup>nuc</sup> plant protected by a plastic tube to avoid out pollination from neighboring conspecifics.



**Fig. S5.** Network of cytotypes found in the local metapopulation TOU. Each described cytotype is represented by a circle whose size is proportional to the number of individuals observed for this cytotype. Black dots represent hypothetical intermediates cytotypes that have not been observed in this study. The two red segments represent identical polymorphisms, whose distribution could lead to a reticulation of the network. Each segment between circles or dots represents one chloroplast or mitochondrial polymorphism. Capital red letters stand for cytotypes that were previously observed in ref. 2.

Cytoplasm donor	Sha	Sha	Sha	Sha	Sha	Sha	0y-0	0y-0	0y-0	0y-0	0y-0	0y-0	Jea	Jea	Jea	Jea	Jea	Jea	Cvi-0
Nucleus donor	Blh-1	Bur-0	Ct-1	Cvi-0	Jea	Oy-0	Sha	Blh-1	Bur-0	Ct-1	Cvi-0	Jea	Blh-1	Bur-0	Cţ-1	Cvi-0	Oy-0	Sha	Blh-1
Versailles Id	36CV	37CV	38CV	39CV	41CV	42CV	43CV	44CV	45CV	46CV	47CV	49CV	50CV	51CV	52CV	53CV	55CV	56CV	64CV
Inf. SNPs	139	143	123	112	136	130	131	144	153	139	134	132	148	130	123	121	133	127	159
MSAT	ß	9	10	14	8	10	10	11	7	48	15	8	9	6	80	6	7	6	80
Cytoplasm donor	Cvi-0	Cvi-0	Cvi-0	Cvi-0	Cvi-0	Ct-1	Ct-1	Ct-1	Ct-1	Ct-1	Ct-1	Bur-0	Bur-0	Bur-0	Bur-0	Bur-0	Bur-0	Blh-1	Blh-1
Nucleus donor	Bur-0	Ct-1	Jea	0y-0	Sha	Blh-1	Bur-0	Cvi-0	Jea	0y-0	Sha	Blh-1	Ct-1	Cvi-0	Jea	0y-0	Sha	Bur-0	Ct-1
Versailles Id	65CV	66CV	68CV	69CV	70CV	71CV	72CV	73CV	75CV	76CV	77CV	78CV	79CV	80CV	82CV	83CV	84CV	85CV	86CV
Inf. SNPs	139	125	127	136	138	140	140	128	118	144	118	174	138	133	134	123	139	159	143
MSAT	6	10	6	13	7	10	8	6	10	48	11	8	10	11	6	7	9	8	10
Cytoplasm donor	Blh-1	Blh-1	Blh-1	Blh-1	lta-0	lta-0	lta-0	lta-0	lta-0	lta-0	lta-0	Blh-1	Bur-0	Ct-1	Cvi-0	Jea	0y-0	Sha	
Nucleus donor	Cvi-0	Jea	0y-0	Sha	Blh-1	Bur-0	Ct-1	Cvi-0	Jea	0y-0	Sha	lta-0	lta-0	lta-0	lta-0	lta-0	lta-0	lta-0	
Versailles Id	87CV	89CV	90CV	91CV	92CV	93CV	94CV	95CV	96CV	97CV	98CV	99CV	100CV	101CV	102CV	103CV	104CV	105CV	
Inf. SNPs	154	154	142	118	148	89	140	77	139	146	140	146	116	121	90	134	144	141	
MSAT	10	5	12	6	7	16	11	15	7	7	10	7	12	13	16	7	∞	10	
The resource is available at the Versailles <i>Arabidopsis</i> Stock Center. Th given. Details are available on the Versailles <i>Arabidopsis</i> Stock Center we	ailable at ailable or	the Versa the Versa	illes Arabi silles Arab	dopsis Stc idopsis Stu	sck Center ock Cente	r. The nun r website	ther of int (publicline	formative ss.versaille	SNPs (Inf. ss.inra.fr/).	SNPs) anı	d microsa	tellite (MS	AT) marke	rs used to	assess fixat	tion of the	e number of informative SNPs (Inf. SNPs) and microsatellite (MSAT) markers used to assess fixation of the recurrent parent nucleus are ebsite (publiclines.versailles.inra.fr/).	barent nucl	eus are

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Table S2. Postharvest storage of seeds used in the phenoty	t storage of s	eeds used in t	the phenotyp	/ping experiment	ent																
Cytoplasm donor	Blh-1	Bur-0	Ct-1										ta-0			a					
Nucleus donor	Blh-1	Blh-1	Blh-1										ur-0 B			Q-					
Versailles Id	180AV	78CV	71CV										3CV 5			2.					
Nb of days of storage	$63.8 \pm 5.5$	$61.3 \pm 3.6$	62.4 ± 3.4	$63.9 \pm 3.7$	$63.9 \pm 7.3$	$62.8 \pm 4.1$	$64.3 \pm 4.8$ 6	$60.9 \pm 2.0$ 8	$83.5 \pm 3.0$ 82	$82.8 \pm 4.4$ 84	84.1 <u>±</u> 4.9 81.	$81.9 \pm 6.0$ 77.0	$77.0 \pm 4.7$ 85.1	85.1 ± 2.8 80.3	$80.3 \pm 4.2$ $84.1 \pm 3.2$	± 3.2					
(mean $\pm$ SD)																					
Cytoplasm donor	Blh-1	Bur-0	Ct-1																		
Nucleus donor	Ct-1	Ct-1	Ct-1																		
Versailles Id	86CV	79CV	162AV																		
Nb of days of storage	$90.9 \pm 1.7$	$89.3 \pm 2.5$	90.3 ± 1.8	$91.2 \pm 1.8$	$90.0 \pm 0.8$	89.8 ± 0.9	$90.8 \pm 1.4$	$90.1 \pm 1.3$ 6	$63.6 \pm 6.1$ $65$	$65.7 \pm 4.7$ 83	$83.4 \pm 2.7$ 83.	83.6 ± 2.7 80.5	80.5 ± 2.9 80.2	30.2 ± 3.1 63.1	$63.1 \pm 3.2$ $74.3 \pm 10.2$	$10.2$ 81.8 $\pm$ 5.5	$\pm$ 5.5 85.2 $\pm$ 3.2	$3.2$ $84.0 \pm 0.0$	$0.0  78.9 \pm 7.6$	$5$ 85.6 $\pm$ 3.4	$82.0 \pm 8.2$
(mean $\pm$ SD)																					
Cytoplasm donor	Blh-1	Bur-0	Cvi-0	lta-0		Oy-0	Sha	Blh-1			Cvi-0	lta-0 J	Jea (	Oy-0 Sh	Sha Bur	-0 Ct-1	-1 Cvi-0		Jea		
Nucleus donor	Jea	Jea	Jea	Jea		Jea	Jea						) 0-yc								
Versailles Id	89CV	82CV	68CV	96CV	25AV	49CV	41CV			76CV			5CV 22								
Nb of days of storage	$79.8 \pm 10.4$	$78.3 \pm 9.7$	86.5 ± 6.8	$79.9 \pm 9.8$		79.8 ± 10.0	79.6 ± 8.6		$89.4 \pm 1.9$ 90				$89.8 \pm 2.7$ 89.1			83.1 ± 1.8 85.0 ±		$-4.7$ 82.2 $\pm 3.2$		4 82.4 $\pm$ 2.6	
(mean ± SD)																					

Postharvest storage of seeds used in the phenotyping exper Table S2.

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Pair of accession	GERM	PGERM4	<b>PGERM5</b>	PGERM6	PGERM13	AREA	PERIM	DIAM	ВТ	INT	RP	LCYCLE	H1F	HMAX N	NBB	NPB TC	TOTB FITI	FITTOT FITSTEM	EM FRUITSTEM	EM SILSTEM	M FITPB	B FRUITPB	B SILPB	B RSTEM	M RPB	STERILITY	≿
Blh1/Bur0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS N	S	NS NS	NS	NS	SN	NS	NS	NS	NS	NS	
Blh1/Ct1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS N	VS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Blh1/Cvi0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	6	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Blh1/Ita0	Hom	Hom	NS	NS	NS	NS	NS	NS	NS	NS	NS	Het	ne	ne			a		ne	ne	ne	ne	ne	ne	ne	ne	
Blh1/Jea	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NSN	S	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Blh1/Oy0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS N	S	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Blh1/Sha	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	a	ne ne	ne	ne	ne	ne	ne	ne	ne	ne	
Bur0/Ct1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS N	S	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Bur0/Cvi0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS N	S	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Bur/0/Ita0	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne r	ne n	ne ne	ne	ne	ne	ne	ne	ne	ne	ne	
Bur0/Jea	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS N	S	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Bur0/Oy0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Bur0/Sha	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS N	S	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Ct1/Cvi0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Ct1/lta0	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne n	a	ne ne	ne	ne	ne	ne	ne	ne	ne	ne	
Ct1/Jea	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne		ne n	e		ne	ne	ne	ne	ne	ne	ne	ne	
Ct1/Oy0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NSN	S	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Ct1/Sha	NS	NS	NS	NS	NS	NS	NS	NS	Het	NS	NS	NS	Het	NS	NS	Het H	et	NS NS	NS	NS	Het	Het	NS	Hom	n Hom	NS	
Cvi0/Ita0	Hom	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	ne	ne	ne	ne	a	ne ne	ne	ne	ne	ne	ne	ne	ne	ne	
Cvi0/Jea	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS N	NS N	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Cvi0/Oy0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Cvi0/Sha	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	Het	Het	NS	Het	NS	NS N	s	NS NS	NS	Het	NS	NS	Het	NS	NS	Het	
lta0/Jea	Hom	Hom	Het	Hom	Het	NS	NS	NS	NS	NS	NS	NS	ne	ne	ne	ne r	ne n	ne ne	ne	ne	ne	ne	ne	ne	ne	ne	
Ita0/Oy0	Hom	Hom	Het	Hom	Het	NS	NS	NS	NS	NS	NS	NS	ne	ne	ne	ne r	ne n	ne ne	ne	ne	ne	ne	ne	ne	ne	ne	
lta0/Sha	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	ne	ne					ne	ne	ne	ne	ne		ne	ne	
Jea/Oy0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Jea/Sha	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS N	NS N	NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Oy0/Sha	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS NS	NS	NS	NS	NS	NS	NS	NS	NS	
Significant cytonuclear interactions in a specific pair of accessions are highlighted in red. Hom and Het stand for the error variance chosen to be either homogeneous or heterogeneous, respectively ( <i>Materials and Methods</i> and <i>SI Text</i> ). AREA, PERIM rosette surface area and perimeter 28 d after sowing; BT, bolting time; DIAM, rosette diameter at flow role of fruits, and mean fruit (silique) length, number of fruits, and mean fruit (silique) length, number of fruits, and mean fruit (silique) length on the main stem; FITTEM, FRUITSTEM, SILSTEM, total fruit length, number of fruits, and mean fruit (silique) length on the primary branches; FITSTEM, RUITSTEM, SILSTEM, total fruit length, number of fruit length, number of fruits, and mean fruit (silique) length on the main stem; FITTOT, total fruit length = proxy of total seed production; GERM, germination time; H1F, height from soil to the first fruit on the main stem; HIMAX, maximum height; INT, flowering interval; LCYCLE, length of the life cycle; NBB, number of basal branches; ne, not estimated due to ( <i>i</i> ) the missing of one of the following cytolines: Blh-1 <sup>orb</sup> Sha <sup>nuc</sup> , Ct-1 <sup>orb</sup> GHa-0 <sup>nuc</sup> , and Ct-1 <sup>orb</sup> GHa-0 <sup>nuc</sup> , and Ct-1 <sup>orb</sup> GERM4, PGERM4, PGERM5, PGERM13, germination percentage 4, 5, 6, and 13 d after sowing, respectively; RP, reproductive period; RSTEM, RPB, percentage of seeds produced on the main stem and on primary branches; STERM4, PGERM5, PGERM6, PGERM13, germination percentage 4, 5, 6, and 13 d after sowing, respectively; RP, reproductive period; RSTEM, RPB, percentage of seeds produced on the main stem and on primary branches; STERULTY, percentage 6, 5, 6, and 13 d after sowing, respectively; RP, reproductive period; RSTEM, RPB, percentage of seeds produced on the main stem and on primary branches; STERULTY, percentage of aborted fruits; SURVIVAL, completion of the life cycle; TOTB, total number of branches.	uclear intera UITPB, SILPB he main ste NPB, numb( fe cycle; TO <sup>-</sup>	ctions in a spe , total fruit ler m; HMAX, ma er of primary k IB, total numl	cific pair of acc ogth, number of ximum height;   yranches; NS, nc per of branches	essions are hig f fruits, and me INT, flowering ot significant; F	highlighted in red. Hom and Het stand for the error variance chosen to be either homogeneous or heterogeneous, respectively ( <i>Materials a</i> 1 mean fruit (silic dilicue) length, number of fruits, and mean fruit (silic ring interval; LCYCLE, length of the life cycle; NBB, number of basal branches; ne, not estimated due to (1) the missing of one of the followir ring interval; LCYCLE, length of the life cycle; NBB, number of basal branches; ne, not estimated due to (1) the missing of one of the followir ring interval; PGERMS, PGERMG, PGERM13, germination percentage 4, 5, 6, and 13 d after sowing, respectively; RP, reproductive period; RST	. Hom and H e) length on .E, length of .J5, PGERM6	let stand for the primary f the life cycl 5, PGERM13,	the error v branches; le; NBB, nul germinatic	/ariance ch FITSTEM, F mber of bi on percent	iosen to bi RUITSTEN asal brancl age 4, 5, 6	e either hi 1, SILSTEN hes; ne, ni 3, and 13 c	omogeneous 1, total fruit l ot estimated 1 after sowin	or hetero ength, nur due to ( <i>i</i> ) ig, respecti	geneous, res mber of fruit the missing vely; RP, rep	ipectively ( 5, and mea of one of 1 roductive	Materials a an fruit (sili :he followi period; RST	<i>nd Methods</i> que) length ng cytolines: EM, RPB, pe	and <i>SI Text</i> ). A on the main ste Blh-1 <sup>0/</sup> Sha <sup>nuc</sup> ,   rcentage of see	<i>id Methods</i> and <i>SI Text</i> ). AREA, PERIM rosette surface area and perimeter 28 d after sowing; BT, bolting time; DIAM, rosette diameter at lue) length on the main stem; FITTOT, total Fruit length = proxy of total seed production; GERM, germination time; H1F, height from soil g cytolines: BIh-1 <sup>or</sup> Sha <sup>muc</sup> , Bur-0 <sup>or</sup> ty, Ct-1 <sup>or</sup> Ita-0 <sup>muc</sup> , and Ct-1 <sup>cr</sup> Jea <sup>muc</sup> , or <i>(iii)</i> plants with a Ita-0 nucleus for which postflowering traits EM, RPB, percentage of seeds productions; GERU, germination time; H1F, height from soil g cytolines: BIh-1 <sup>or</sup> Sha <sup>muc</sup> , Bur-0 <sup>ort</sup> , Ct-1 <sup>or</sup> Ita-0 <sup>muc</sup> , and Ct-1 <sup>crJ</sup> Jea <sup>muc</sup> , or <i>(iii)</i> plants with a Ita-0 nucleus for which postflowering traits EM, RPB, percentage of seeds produced on the main stem and on primary branches; STERILITY, percentage of aborted fruits; SURVIVAL,	te surface area a fruit length = pr 1 <sup>cy</sup> lta-0 <sup>nuc</sup> , and the main stem a	and perimet oxy of total Ct-1 <sup>cy</sup> Jea <sup>nu</sup> nd on primä	er 28 d after so seed producti <sup>c</sup> , or <i>(ii</i> ) plants ary branches; S	owing; BT, k on; GERM, g with a lta-C iTERILITY, p	bolting time germinatioi 0 nucleus fo oercentage (	e; DIAM, rose n time; H1F, r which post of aborted fi	ette diameter height from s flowering tra uits; SURVIV/	`at soil AL,

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# Table S3. Cytonuclear interactions in specific pairs of parents

Phenotypic traits

## **Other Supporting Information Files**

Dataset S1 (XLS) Dataset S2 (XLS)

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