Cytonuclear interactions affect adaptive traits of the annual plant *Arabidopsis thaliana* in the field

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Although the contribution of cytonuclear interactions to plant fitness variation is relatively well documented at the interspecific level, the prevalence of cytonuclear interactions at the intraspecific level remains poorly investigated. In this study, we set up a field experiment to explore the range of effects that cytonuclear interactions have on fitness-related traits in *Arabidopsis thaliana*. To do so, we created a unique series of 56 cytolines resulting from cytoplasmic substitutions among eight natural accessions reflecting within-species genetic diversity. An assessment of these cytolines and their parental lines scored for 28 adaptive whole-organism phenotypes showed that a large proportion of phenotypic traits (23 of 28) were affected by cytonuclear interactions. The effects of these interactions varied from slight but frequent across cytolines to strong in some specific parental pairs. Two parental pairs accounted for half of the significant pairwise interactions. In one parental pair, Ct-1/Sha, we observed symmetrical phenotypic responses between the two nuclear backgrounds when combined with specific cytoplasms, suggesting nuclear differentiation at loci involved in cytonuclear epistasis. In contrast, asymmetrical phenotypic responses were observed in another parental pair, Cvi-0/Sha. In the Cvi-0 nuclear background, fecundity and phenology-related traits were strongly affected by the Sha cytoplasm, leading to a modified reproductive strategy without penalizing total seed production. These results indicate that natural variation in cytoplasmic and nuclear genomes interact to shape integrative traits that contribute to adaptation, thereby suggesting that cytonuclear interactions can play a major role in the evolutionary dynamics of *A. thaliana*.

cytolines | cytoplasm × nucleus interactions | fitness-related traits | plant adaptation | organelles

The genomes of eukaryotes originate from ancient endosymbiotic associations that eventually led to energy-harnessing organelles: mitochondria, common to all eukaryotes, and chloroplasts in the "green" lineage. The evolution of endosymbionts into cellular organelles was accompanied by massive gene loss, with a large proportion being transferred to the nucleus (1, 2). Nevertheless, mitochondria and chloroplasts retained a few (30–80) protein-encoding genes that play crucial roles in energy metabolism (respiration and photosynthesis). Mitochondrion and chloroplast metabolisms rely on the proper interaction of nuclear-encoded proteins and their counterparts encoded in the organelle genome. Consequently, the genes in nuclear and organellar compartments are expected to be coadapted (3).

Cytonuclear coadaptation has been demonstrated by altered phenotypes observed on interspecific exchanges of cytoplasm between related species in mammals (4), yeast (5), arthropods (6), and plants, whose interspecific crosses are frequently successful (7). These alterations affect organelle function and even the organism phenotype, indicating epistasis between nuclear and cytoplasmic genes. Although cytonuclear coadaptation is generally studied at the interspecific level, the existence of intraspecific genetic diversity in organelle genomes suggests a potential for genomic coadaptation within species. A few studies have reported phenotypic effects of intraspecific cytonuclear epistasis in nonplant species (8–11). In plants, many studies have focused on cytoplasmic male sterility (CMS), an impairment of pollen production governed by nucleo-mitochondrial interactions in some hermaphroditic species (12), in particular in crops and their relatives (13). The phenotypic effects of intraspecific cytonuclear epistasis other than CMS have been reported in only a limited number of plant systems (14–17), with evidence that cytoplasmic variation contributes to local adaptation (18, 19).

In recent years, several studies using reciprocal segregating populations of the model plant *Arabidopsis thaliana* have investigated the effect of cytonuclear epistasis on a number of laboratory-measured phenotypes such as the metabolome, defense chemistry and growth (17, 20, 21), water-use efficiency (22, 23), and seed germination (24, 25). Although some studies have reported significant effects of cytonuclear epistasis (17, 20, 21, 23, 25), others have found additive cytoplasmic effects but with weak or no cytonuclear epistasis (22). Each of these studies (with the exception of ref. 25) was, however, based on a single reciprocal cross between two natural accessions, thereby preventing the estimation of the prevalence of

Significance

As the centers of photosynthesis and respiration, chloroplasts and mitochondria play a crucial role in energy metabolism. Nuclear and cytoplasmic genomes are known to be coadapted at the species level, because organelle metabolism relies on the proper interaction of organelle-encoded and nuclear-encoded proteins. We explored the extent of cytonuclear coadaptation at the intraspecific level in the classic model plant *Arabidopsis thaliana*: we measured in a field experiment 28 adaptive whole-organism traits on cytolines developed by substituting cytoplasmic genomes among natural strains. Our results indicate that interactions between nuclear and cytoplasmic genomes shape natural variation for most of the traits we studied, suggesting that these interactions can affect the evolutionary dynamics of natural populations of *A. thaliana*.

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Data deposition: Genotyping data of the 56 cytolines are available on the Versailles Arabidopsis Stock Center website, publiclines.versailles.inra.fr/.

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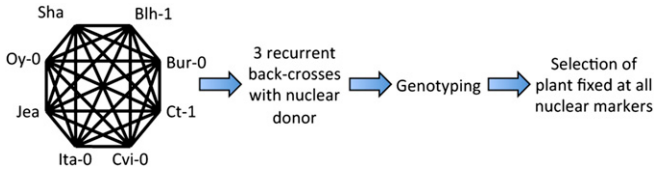

Fig. 1. General strategy for the production of the cytolines. Diallele crosses were performed between eight natural accessions, and three backcrosses were performed with the male parent (nuclear donor). After dense genotyping, a plant with a fixed allele from the recurrent male parent at all markers was used as the cytoline founding mother. Details on the production and genotyping of cytolines are given in *SI Text*.

cytonuclear epistasis in this species. In addition, although these reports involve adaptive traits (26–30), the investigation of the effect of cytonuclear epistasis on adaptive phenotypes in field conditions is, at best, scarce in *A. thaliana*.

Here, following the modern standards of ecological genomics (31), we explored the prevalence of cytonuclear interactions on adaptive whole-organism traits in the model plant *A. thaliana* in a field experiment. To do so, based on eight natural accessions of a core collection that covers a significant part of the species' cytoplasmic and nuclear genetic diversity in *A. thaliana* (25, 32), we created eight series of seven cytolines. Cytolines are genotypes that combine the nuclear genome from one parent with the organelle genomes of another (33). We examined the cytolines and their parental accessions for effects of cytonuclear interactions on 28 field-measured traits related to germination, phenology, resource acquisition, plant architecture and seed dispersal, fecundity, and survival.

Results

To test the prevalence of cytonuclear interactions on fitness-relevant traits, we constructed relevant genetic resources in *A. thaliana*, hereafter named cytolines, by exchanging nuclear and cytoplasmic genomes between eight natural accessions, namely Blh-1, Bur-0, Ct-1,

Table 1. Global effects of nucleus, cytoplasm, and cytonuclear interactions on phenotype

	Model terms										
	Block		Nucleus		Cytoplasm		Cytoplasm × nucleus		Block × nucleus		Variance structure[†]
Phenotypic class	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	
Germination											
Germination time	18.05	***	96.46	***	0.55	NS	2.94	***	5.36	***	hmg
Germination percentage 4 das	37.24	***	221.52	***	0.86	NS	1.72	**	4.02	***	hmg
Germination percentage 5 das	5.67	***	127.64	***	2.11	NS	2.63	***	2.25	***	htg
Germination percentage 6 das	3.04	*	125.78	***	1.3	NS	2.69	***	3.69	***	hmg
Germination percentage 13 das	15.46	***	52.55	***	4.65	***	1.7	**	2.53	***	htg
Resource acquisition											
Rosette surface area 28 das	11.45	***	259.35	***	5.29	***	2.8	***	1.82	*	hmg
Rosette perimeter 28 das	8.75	***	111.98	***	3.54	**	1.62	**	2.34	***	htg
Rosette diameter at flowering	3.57	*	33.93	***	2.59	*	1.62	**	1.83	*	hmg
Phenology											
Bolting time	1.75	NS	2648.29	***	3.1	*	2.53	***	2.8	***	htg
Flowering interval	2.38	NS	57.59	***	0.96	NS	1.55	*	2.98	***	htg
Reproductive period	5.65	**	32.1	***	1.09	NS	2.75	***	1.88	*	htg
Length of life cycle	12.7	***	273.23	***	5.21	***	5.43	***	5.02	***	htg
Architecture and seed dispersal											
Height from soil to the first fruit on the main stem[‡]	14.29	***	227.55	***	8.22	***	3.94	***	3.07	*	htg
Maximum height[‡]	11.95	***	101.59	***	7.08	***	4.11	***	2.38	*	htg
Number of basal branches[‡]	0.28	NS	9.01	***	1.11	NS	0.67	NS	0.56	NS	hmg
Number of primary branches[‡]	0.78	NS	681.29	***	1.68	NS	1.87	**	1.88	NS	htg
Total number of branches[‡]	0.98	NS	491.58	***	1.71	NS	1.74	**	1.77	NS	htg
Fecundity											
Total fruit length = proxy of total seed production[‡]	4.36	**	54.04	***	0.54	NS	0.77	NS	1.04	NS	htg
Total fruit length on the main stem[‡]	0.7	NS	124.16	***	1.22	NS	0.92	NS	0.95	NS	htg
Fruit number on the main stem[‡]	1.6	NS	124.64	***	1.32	NS	0.96	NS	1.02	NS	htg
Mean fruit length on the main stem[‡]	0.41	NS	210.03	***	1.28	NS	4.34	***	2.07	NS	htg
Total fruit length on primary branches[‡]	3.27	*	29.29	***	0.51	NS	0.99	NS	1.25	NS	htg
Fruit number on primary branches[‡]	13.14	***	70.08	***	1.79	NS	1.87	**	2.42	***	htg
Mean fruit length on primary branches[‡]	3.8	**	95.28	***	0.85	NS	3.76	***	1.56	NS	htg
Ratio of seeds produced on the main stem[‡]	3.84	**	31.76	***	2.33	NS	1.6	*	1.8	*	hmg
Ratio of seeds produced on primary branches[‡]	3.62	*	29.91	***	2.26	NS	1.85	**	1.61	*	hmg
Percentage of aborted of fruit[‡]	4.63	NS	85.51	***	1.64	NS	10.36	***	1.61	NS	htg
Survival	91.55	***	69.12	***	20.95	***	29.51	***	10.03	***	hmg

All traits were measured quantitatively with the exception of survival which is a binary trait. das, days after sowing; NS, not significant.
*$0.05 > P > 0.01$, **$0.01 > P > 0.001$, ***$P < 0.001$.
[†]Each trait was modeled separately using a mixed model. Depending on the trait, the error variance was chosen to be either homogeneous (hmg) or nucleus (i.e., htg) dependent. Therefore, the terms of block × cytoplasm and block × cytoplasm × nucleus interactions were not included in the model to allow proper convergence of the estimation of parameters (for details, see *SI Text*). A correction for the number of tests was performed for each modeled effect (i.e., per column) to control the FDR at a nominal level of 5%.
[‡]Because all plants carrying a nucleus from Ita-0 were unable to complete their life cycle before the onset of summer heat, postflowering traits were not measured on the Ita-0 parental accession or on the cytolines with the Ita-0 nucleus.

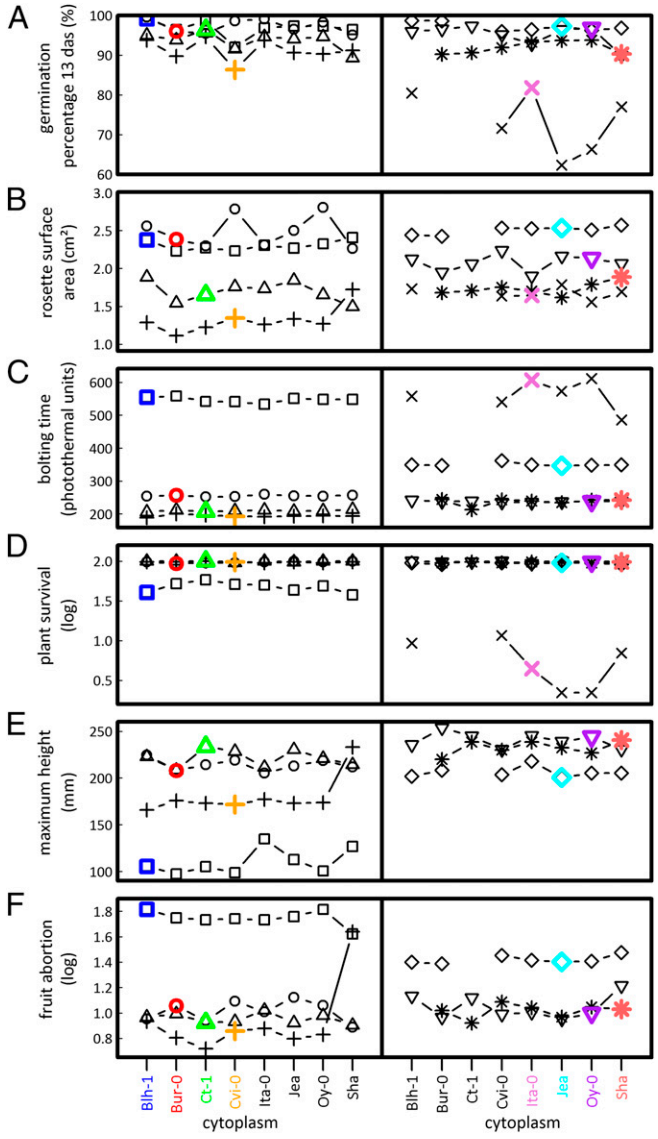

Fig. 2. Cytoplasm origin has a variable influence according to the considered trait and nuclear host genome. For each trait, least-squares means (LSMs) are plotted by cytoplasm parent (horizontal axis). LSM values of cytolines with the same nucleus share the same symbol and are connected across cytoplasms. Values for the parental accessions are indicated in color. For better visual distinction, lines with a Blh-1, Bur-0, Ct-1, or Cvi-0 nucleus are plotted in the left panels, whereas lines with a Ita-0, Jea, Oy-0, or Sha nucleus are plotted in the right panels. (*A*) Percentage of germination 13 d after sowing (das). (*B*) Rosette surface area 28 d after sowing. (*C*) Bolting time. (*D*) Percentage of plants that completed their life cycle. Values were log-transformed for clearer visualization of the results. (*E*) Maximum height of the plant. (*F*) Percentage of aborted fruits. Values were log-transformed for a clearer visualization of the results. Because many plants of the lines with the Ita-0 nucleus died prematurely, postflowering traits were not analyzed for these lines.

Cvi-0, Ita-0, Jea, Oy-0, and Sha (Fig. 1, *SI Text*, Fig. S1, and Table S1). These eight accessions amply cover the nuclear and cytoplasmic diversity found in *A. thaliana* (25, 32). Hereafter, a cytoline possessing the cytoplasm of accession A and the nucleus of accession B is designated as $A^{cy}B^{nuc}$. The genetic composition of the 56 cytolines was verified by genotyping (*SI Text*). Given the dense genotyping strategy used, the genomic regions affected by residual heterozygosity, if any, should be very limited in size. Bulks of seeds of each cytoline and parental accession were produced in controlled conditions to reduce maternal effects (*SI Text*, Fig. S2, and Table S2), and then used for conducting a phenotyping experiment in a field in northern France (*Materials and Methods* and *SI Text*), where adaptation based on nuclear genetic variation has been previously detected (28, 30). Each line was scored for a total of 28 phenotypic traits described as adaptive in *A. thaliana* (34–37) in a randomized complete block design (*SI Text*, Fig. S3, and Dataset S1). These phenotypic traits belong to six phenotype classes: germination, phenology, resource acquisition, architecture and seed dispersal, fecundity, and survival. Each trait was modeled separately using a mixed model. Depending on traits, the error variance was chosen to be either homogeneous or nucleus (i.e., heterogeneous) dependent (Table 1 and *SI Text*).

In agreement with the genetic diversity captured by the core collection of *A. thaliana* used in this study (32), all measured traits varied significantly across nuclear backgrounds (Table 1). The mean effect of the cytoplasm was significant for 9 of the 28 traits and was detected in each phenotype class, with the exception of fecundity (Table 1). More importantly, significant cytonuclear interactions were observed for most phenotypic traits (i.e., 23 of 28 traits) and for each phenotype class (Table 1). These observations are illustrated in Fig. 2 for each phenotype class with a representative trait showing significant nucleus, cytoplasm, and cytonuclear interaction effects (Table 1).

Although no significant cytonuclear interaction was detected for total seed production, other individual traits related to fecundity (such as the number of seeds per fruit or the percentage of seeds produced on different types of branches) were significantly affected by cytonuclear interactions (Table 1). This observation suggests that cytolines of a given nuclear background produced the same number of seeds, but with contrasting reproductive strategies.

After testing for pairwise cytonuclear interactions in each parental pair for all quantitative traits (Table S3), several main features were discerned. First, we observed a large range of phenotypic effects among the 22 quantitative traits globally influenced by cytonuclear interactions. Although a significant global effect for cytonuclear interactions was detected for the three traits related to resource acquisition (surface area and perimeter of the rosette 28 d after sowing and diameter of the rosette at flowering) and for flowering interval (Table 1), no significant effect for pairwise cytonuclear interaction was detected for these traits in any specific parental pair (Table S3). Therefore, for these traits, cytoplasm substitution commonly led to phenotypic effects of minor intensity. In contrast, the significant global cytonuclear interactions detected for the remaining traits were mainly driven by pairwise cytonuclear interactions in one to four parental pairs, with the parental pairs Ct-1/Sha and Cvi-0/Sha accounting for 14 of the 28 observed significant pairwise cytonuclear interactions (Table S3). Second, significant asymmetrical phenotypic effects were observed between reciprocal cytoplasmic substitutions. For instance, the cytoline $Ct\text{-}1^{cy}Sha^{nuc}$ bolted earlier than its parental accession Sha, whereas the cytoline $Sha^{cy}Ct\text{-}1^{nuc}$ bolted at the same time as its parental accession Ct-1 (Fig. 3*A*). Asymmetrical effects of cytoplasm exchange were also observed for the pair Cvi-0/Sha. No differences were observed for any phenotypic trait scored in this study between the cytoline $Cvi\text{-}0^{cy}Sha^{nuc}$ and its parental accession Sha. In contrast, in comparison with the parental accession Cvi-0, plants of the cytoline $Sha^{cy}Cvi\text{-}0^{nuc}$ showed low fertility with a fruit abortion rate of 43.6% (Figs. 2*F* and 3*B*) and reduced fruit length in nonaborted fruits on the main stem (Fig. 3*C*), along with a longer reproductive period (Fig. 3*D*) and a greater maximum height (Fig. 3*E*). Finally, in some traits and for specific parental pairs, nuclear genotypes had contrasting effects when combined with the same cytoplasm (Fig. 4). For instance, Sha and Ct-1 nuclei had opposite and symmetrical effects on the percentage of seeds produced on the main stem (Fig. 4*A*) and on the height from the soil to the first fruit on the main stem (Fig. 4*B*) in their reciprocal cytoplasms (as shown by significant contrast for these traits; Table S3), but also in other cytoplasms, such as Ita-0.

Discussion

We constructed a series of 56 intraspecific cytolines that substitute cytoplasms among eight accessions of the model species *A. thaliana*.

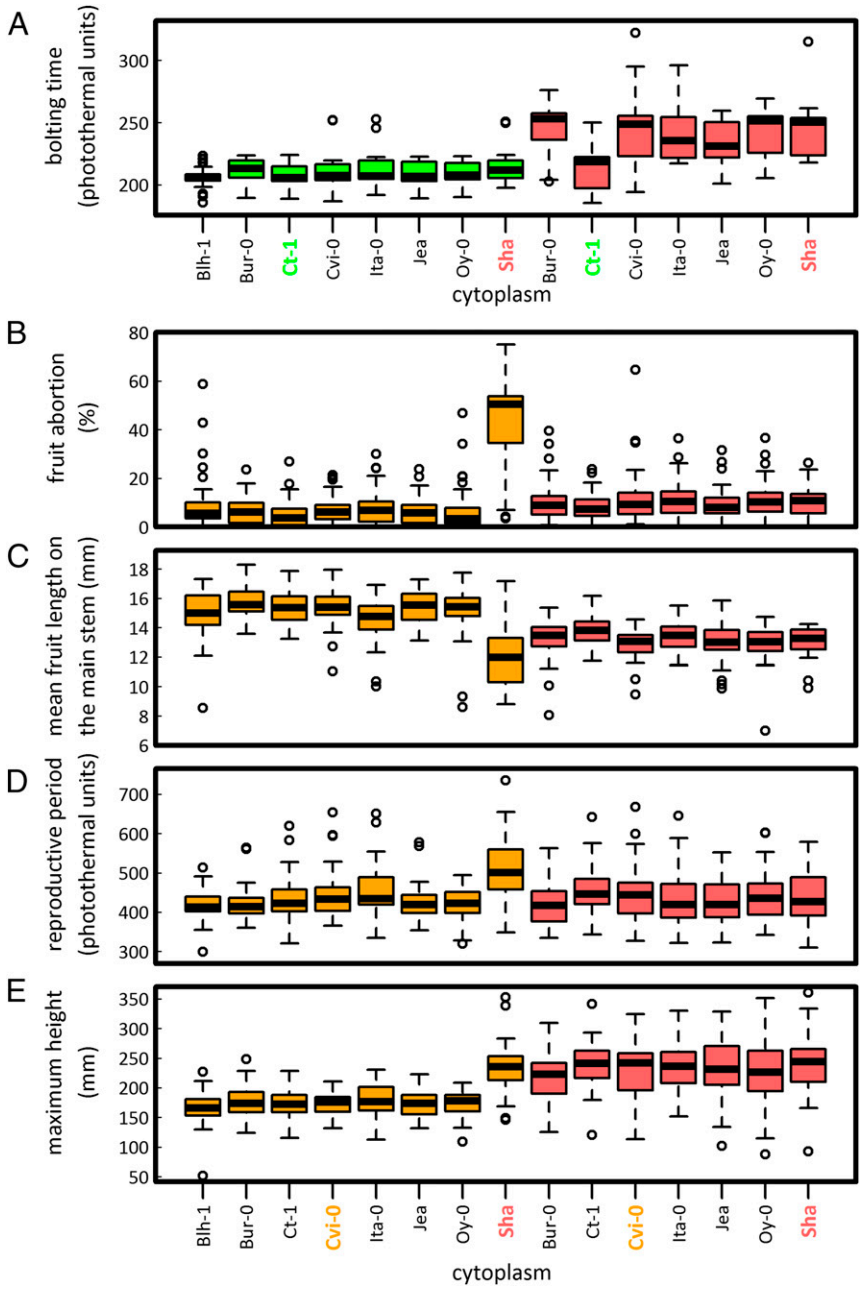

Fig. 3. Asymmetrical cytonuclear interactions observed in the Ct-1/Sha and Cvi-0/Sha pairs. Box plots of fitted values are presented for all genotypes with the two nuclei involved in the significant cytonuclear interaction. Values are plotted by cytoplasm donor in each group of genotypes sharing the same nucleus, color coded according to the nuclear donor. The color code is identical for the cytoplasm and the nucleus. (*A*) Bolting time for the Ct-1/Sha pair. (*B*) Percentage of aborted fruits for the Cvi-0/Sha pair. (*C*) Mean fruit length of fertilized fruits on the main stem for the Cvi-0/Sha pair. (*D*) Reproductive period for the Cvi-0/Sha pair. (*E*) Maximum height of the plant for the Cvi-0/Sha pair.

This genetic material constitutes a valuable resource for further exploration of the effects and the prevalence of natural organelle genome variation and cytonuclear interactions on phenotypic adaptive traits in *A. thaliana*.

Alloplasmic lines resulting from cytoplasm exchanges at the interspecific level often demonstrate severely affected phenotypes due to incompatibilities accumulated in the genetic compartments of species of long-diverged lineages (3, 7). Our intraspecific cytolines allow exploration of both (*i*) the nonneutral cytoplasmic diversity at the intraspecific level and (*ii*) the proportion of intraspecific genetic diversity that is involved in the interaction between the organelle and nuclear genomes. This genetic resource is available to the scientific community through the Versailles *Arabidopsis* Stock Center (publiclines.versailles.inra.fr/), thereby facilitating its widespread use for analysis of cytonuclear interactions at all possible phenotypic scales. The diallele design underlying the cytolines described in this study provides access to the cytonuclear effects between all possible pairs of parents and potentially to their third-order interactions with the environment (11, 38). Cytonuclear interactions in *A. thaliana* have been also reported using reciprocal segregating populations (17, 39). Both types of genetic resources are complementary and valuable for testing the cytonuclear epistasis underlying the studied traits. Although segregating populations can help detect cytonuclear interactions that involve two or more nuclear loci in opposition to each other (15) (a feature that is clearly overlooked in cytolines), subtle phenotypic effects resulting from cytonuclear interactions can be masked due to transmission ratio distortion and interparental allelic epistasis (15), both of which are absent in cytolines.

In this study, in agreement with previous studies based on intraspecific cytolines in insects and yeast (9, 11), a greater proportion of phenotypic variance is likely explained by cytonuclear interactions than cytoplasmic effects alone. In addition, as previously observed in laboratory/greenhouse conditions (17, 20, 21, 23, 25), cytonuclear interactions in *A. thaliana* can affect a large proportion of adaptive whole-organism traits (>80%; Table 1) in field conditions. This

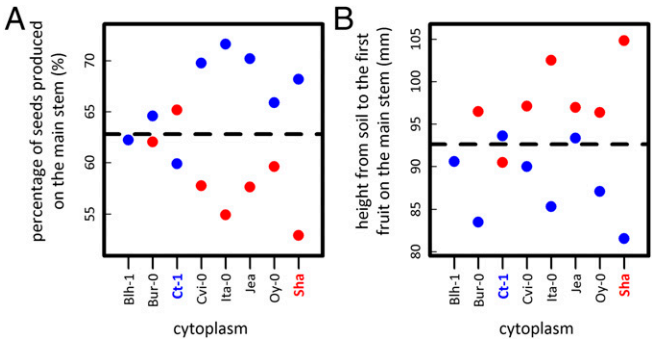

Fig. 4. Symmetrical and opposite effects of Ct-1/Sha nuclear alleles depend on the cytoplasm they are combined with. LSMs are plotted for genotypes carrying either a Ct-1 (blue) or a Sha (red) nucleus, according to their cytoplasm parent (horizontal axis). (*A*) Percentage of seeds produced on the main stem. (*B*) Height from soil to the first fruit on the main stem. Both traits influence seed dispersal and both are significantly affected by the cytonuclear interactions in the Ct-1/Sha pair.

observation suggests that variation in organelle function affects a large range of integrative traits, not only in controlled conditions, but also in more complex and ecologically realistic environments.

In other plant species, phenotypic evaluations of intraspecific cytolines are, at best, scarce. In maize, widespread phenotype effects of cytonuclear interactions have been observed in intrageneric, interspecific alloplasmic lines, but intraspecific cytolines based on cytoplasm exchange between subspecies of *Zea mays* are generally phenotypically indistinguishable from the parental cultivar (15). In that study, the absence of observable phenotypic effects of cytonuclear interactions at the intraspecific level may originate from the crossing design and the limited number of tested nuclei (one nucleus–seven *Z. mays* cytoplasm donors). In the present study, the number of parental lines, their coverage of natural genetic diversity, and the diallele crossing design not only revealed extensive effects of cytonuclear interactions in *A. thaliana,* but also led to observations that would have been overlooked in a specific, unique parental pair.

For instance, among the significant pairwise cytonuclear interactions observed for specific parental pairs, both asymmetrical and symmetrical responses were observed. Asymmetrical responses are observed when one cytoline has a clear differentiated phenotype, whereas the reciprocal cytoline behaves similarly to its nuclear parent. In addition to being a characteristic feature of CMS, asymmetrical responses to reciprocal cytonuclear exchange have been previously reported in plants, including at the interspecific level (7). Symmetrical responses have also been observed in the reciprocal exchange of genetic compartments, with nonparental combinations showing impaired phenotypes compared with the parental lines, e.g., longevity in intraspecific seed beetle (*Acanthoscelides obtectus*) cytolines (40) and fitness breakdown in reciprocal F2s of a copepod (*Tigriopus californicus*) (41, 42). In this latter case, nuclear-encoded cytochrome *c* and mitochondria-encoded subunits of cytochrome *c* oxidase have diverged between the two parental populations such that the interaction between the mismatched partners impairs complex IV activity (43). This example illustrates that allelic differences in nuclear genes coding for organellar proteins may have different outputs when their product interacts with organelle partners of different origin. Interestingly, the Ct-1/Sha parental pair illustrates both types of phenotypic responses (Figs. 3 and 4) and this combination affected more measured traits (8 of 28) than any other parental pair. In addition to bolting time, the other affected traits (Table S3) are all assumed to contribute to seed dispersal (35). A clear asymmetrical response was identified for bolting time (Fig. 3). In contrast, opposite symmetrical effects of the Sha and Ct-1 nuclear genomes were observed for the height from the soil to the first fruit on the main stem and for the percentage of seeds produced on the main stem: in a given cytoplasm, the phenotype of plants with the Ct-1 nucleus mirror those with the Sha nucleus (Fig. 4). This pattern may reflect nuclear polymorphisms that affect interactions between nuclear and organellar gene products. The next challenges will be to identify the genetic factors involved in these interactions and decipher the pathway from their molecular interaction to the whole-organism integrative traits (44, 45). This line of research will undoubtedly benefit from the vast information available on genetic polymorphisms for all but one (Ita-0) of the parental accessions used (1001 Genomes Project) (46).

The relatively high number of parental lines used in this study also allowed the identification of pairs of parents showing remarkable effects of disrupted cytonuclear coadaptation. For instance, the Cvi-0/Sha pair accounted for 6 of the 28 significant specific interactions (Table S3), all due to Sha^{cy}Cvi-0^{nuc} cytoline behavior. The Sha cytoplasm can induce CMS in other natural accessions (47); likewise, the Sha^{cy}Cvi-0^{nuc} cytoline is male-sterile, producing no or very few seeds in laboratory conditions (i.e., greenhouse and growth chamber), so that hand-pollination was necessary to produce the seeds used in this work (*SI Text*). Surprisingly, in our field experiment, the Sha^{cy}Cvi-0^{nuc} plants produced the same amount of seeds as Cvi-0 plants. Cross-pollination by neighboring plants is unlikely to account for their seed production because plants of the Sha^{cy}Cvi-0^{nuc} cytoline grown in the same common garden at the same period, but protected from foreign pollen by plastic tubes, also set seeds (Fig. S4). Although the total seed production of Sha^{cy}Cvi-0^{nuc} and Cvi-0 plants was comparable, the former had reduced numbers of seed per fruit and a higher percentage of fruit abortion, consistent with what is observed in laboratory growth conditions. However, they produced more flowers than Cvi-0 plants, due to their greater plant height and longer reproductive period, which compensated for their poor fertility. Hence, we predict that a cytoplasmic variant with a phenotype similar to that observed in the Sha^{cy}Cvi-0^{nuc} line has a much higher potential to produce progeny by outcrossing, without completely relying on cross-pollination for seed set. This compensatory system may have a significant impact on population adaptive response by transiently modifying the outcrossing rate (48) before the selection and eventual fixation of nuclear restorer(s) of fertility (49, 50). Consequently, the relative contribution of CMS to variation in the outcrossing rate among natural populations (e.g., from 0% to 20% in *A. thaliana*) (51) may be underestimated in highly selfing species.

The next step to address the adaptive significance of cytonuclear interactions will involve the phenotyping of the cytolines in the native habitat of their parental accessions. In addition, because local populations of *A. thaliana* have shown substantial cytoplasmic polymorphism with the occurrence in the same locality of several cytotypes distributed across Eurasia (*SI Text*, Dataset S2, and Fig. S5) (52, 53), studying the evolutionary dynamics of natural populations of *A. thaliana* will benefit from considering the contribution of cytonuclear interactions.

Materials and Methods

Plant Material: Creation and Seed Production of Cytolines. A complete diallele cross was carried out between the eight selected natural accessions, followed by three backcrosses with the male parent and dense genotyping (Fig. 1 and Fig. S1). A detailed procedure of the production and genotyping of cytolines is given in *SI Text*. The genetic resources and the complete list of the genotyped markers for each cytoline are available on the Versailles *Arabidopsis* Stock Center website (publiclines.versailles.inra.fr/).

Field Experiment, Phenotype Characterization, and Data Analysis. A field experiment of 2,700 *A. thaliana* plants was set up at the University of Lille 1 (northern France) following a randomized complete block design. 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). All traits were measured quantitatively with the exception of survival, which is a binary trait. Details of the field experiment and phenotype characterization are provided in *SI Text*.

Each trait was modeled using a mixed model described in detail in *SI Text*. In a first step, we assessed the impact of each factor in the model. To account for multiple testing, a Benjamini–Hochberg procedure (54) was

performed within each term of the model, across the tested phenotypes to control false discovery rate (FDR) at nominal level 5%. In a second step, we conducted pairwise comparisons within the mixed model to identify pairs of parents that significantly contribute to cytonuclear interactions for each quantitative trait (*SI Text*). A global Benjamini–Hochberg adjustment of the *P* values was performed across pairs and traits to control FDR (nominal level: 5%).

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