Traces of transposable elements in genome dark matter co-opted by flowering gene regulation networks

Agnès Baud[1], Mariène Wan[1], Danielle Nouaud[2], Nicolas Francillonne[3], Dominique Anxolabéhère[2,4], Hadi Quesneville[1,3*]

[1]*Université Paris-Saclay, INRAE, URGI, 78026, Versailles, France.*
[2]*IJM, Institut Jacques Monod, CNRS UMR 7592, Université Paris-Diderot, Paris, France*
[3]*Université Paris-Saclay, INRAE, BioinfOmics, Plant bioinformatics facility, 78026, Versailles, France.*
[4]*Université Paris-Sorbonne, Paris, France*

* : Corresponding author

1 Abstract

Transposable elements (TEs) are mobile, repetitive DNA sequences that make the largest contribution to genome bulk. They thus contribute to the so-called "dark matter of the genome", the part of the genome in which nothing is immediately recognizable as biologically functional.

We developed a new method, based on *k-mers*, to identify degenerate TE sequences. With this new algorithm, we detect up to 10% of the *A. thaliana* genome as derived from as yet unidentified TEs, bringing the proportion of the genome known to be derived from TEs up to 50%. A significant proportion of these sequences overlapped conserved non-coding sequences identified in crucifers and rosids, and transcription factor binding sites. They are overrepresented in some gene regulation networks, such as the flowering gene network, suggesting a functional role for these sequences that have been conserved for more than 100 million years, since the spread of flowering plants in the Cretaceous.

2 Introduction

Transposable elements (TEs) are mobile, repetitive DNA sequences that make a major contribution to the bulk of the genome in many organisms. They can represent up to 85% of some genomes, as in wheat and maize [1–5].

TEs invade genomes, through their ability to amplify. However, they are also controlled by their host, through multiple pathways involving RNAi machinery. They invade genomes in a recurrent manner, through

bursts of transposition that are rapidly halted by host defense mechanisms. TEs remain quiescent in the genome for long periods of time, until they are reactivated by events such as genomic shocks. TE sequences also accumulate mutations, which may inactivate the sequence by rendering it too degenerate to be functional. The TE sequence thus gradually "blends into" the background genome sequence until it is no longer recognizable. It then contributes to the so-called "dark matter of the genome", the part of the genome in which nothing is immediately recognizable as biologically functional.

Little is known about the evolution and impact of TE sequences over long periods of time. We explored this question, by developing an innovative repeat annotation approach, which we call *cross-species TE annotation* because it uses closely related species to enhance detection sensitivity for ancient, degenerate repeated sequences [6]. We analyzed the genome of several relatives of *A. thaliana* that diverged about 5-40 million years (My) ago [7]. We generated a library of consensus repeat sequences that we appended to the *A. thaliana* TE reference library, to compile a "Brassicaceae" library. This compiled TE library was used to annotate the *A. thaliana* Col-0 genome to explore the effects of the long-term presence of TEs on genome evolution. Our Brassicaceae TE annotation, excluding annotations overlaping CDS, covers more than 31.8 Mb (26.7%) of the *A. thaliana* genome, and is highly sensitive for the detection of degenerate TE sequences, as it identified one third more TEs than the current official annotation [8]. The detection of many TE copies in *A. thaliana* on the basis of consensus sequences built from sequences in related species provides evidence in support of these *A. thaliana* repeats originating from the common ancestors of these species.

However, our ability to recognize the part of the dark matter derived from TEs remains limited by the sensitivity of current alignment algorithms. We present here a new tool that we developed to improve this strategy. Our new algorithm can find older and more degenerate TE sequences. Indeed, with this tool, we were able to detect up to 10% more of the *A. thaliana* genome as material derived from as yet unidentified TEs. By combining several strategies and tools, we were able to bring the proportion of the genome of this species known to be derived from TEs up to 50%. Interestingly, the new sequences detected were generally very short and located in the 500 bp immediately upstream from genes. Their epigenetic status, nucleotide composition, and long-term conservation in orthologous positions attest to their origination from an ancient TE. Moreover, they overlap with experimentally identified transcription factor binding sites (TFBS), suggesting that they have been co-opted for new functional roles. Interestingly, these sequences were found

to be overrepresented in the 5' sequences of flowering genes. A significant proportion of these TEs overlap with TFBSs able to bind transcription factors (TFs) known to be involved in flowering. Their overlaps with conserved non-coding sequences (CNS) suggest a long-term impact of TEs on flowering, since the initial global spread of flowering plants in the Cretaceous period.

3 Materials and Methods

Genome sequences

Genome sequences were obtained from the following sources: *A. thaliana* ecotype Col-0 (TAIR10 release) (http://www.phytozome.com/arabidopsis.php); *A. lyrata* (v1.0, http://www.phytozome.com/alyrata.php); *C. rubella* (initial release, http://www.phytozome.com/capsella.php); *A. alpina* (preliminary release, courtesy of Eva-Maria Willing, George Coupland, and Korbinian Schneeberger); *Schrenkiella parvulum* (formerly *Thellungiella parvula;* v2.0, http://thellungiella.org/data/); and *B. rapa* (v1.2, http://www.phytozome.com/napacabbage.php).

Genome annotation

TAIR10 gene and TE annotations were retrieved from the URGI website (https://urgi.versailles.inra.fr/gb2/gbrowse/tairv10_pub_TEs/).

The "Brassicaceae" TE annotation was developed in a previous published study [6]. Briefly, for all the genomes from the five *Arabidopsis thaliana* ecotypes that have been assembled (Col-0, Ler-1, Kro-0, Bur-0 and C24), *Arabidopsis lyrata*, *Capsella rubella*, *Arabis alpina*, *Brassica rapa*, *Thellungiella salsuginea*, and *Schrenkiella parvula*, the TEdenovo pipeline from the REPET package (v2.0) [9–11] was used with default parameters and combining the similarity and structural branches. Consensus sequences derived from the structural branches, which use LTR Harvest, were retained only when they presented pfam domains typical of LTR retrotransposons. Consensus sequences were classified with REPET, by checking for characteristic structural features and similarities to known TEs from Repbase Update (17.01)[12], and by scanning against the Pfam library (26.0)[13] with HMMER3 [14]. All the consensus repeat sequences generated were compiled into a "Brassicaceae" library, which we used to annotate the Col-0 genome with TEannot from the REPET package and default settings.

Brassicaceae TE copies

For all genomes from *Arabidopsis thaliana* Col-0 ecotype, *Arabidopsis lyrata*, *Capsella rubella*, *Arabis alpina*, *Brassica rapa* and *Schrenkiella parvula*, we used REPET package v2.5 with its two pipelines, TEdenovo and TEannot. We used the similarity branch of TEdenovo with default parameters on each genome, followed by TEannot with default parameters (sensitivity 2). From this first annotation, we selected consensus sequences containng at least one full-length copy (*i.e.* aligned over more than 95% of the length of the consensus sequence), on which which performed a second run of TEannot. This procedure has been shown to improve the quality of annotation [15]. Copies from the consensus annotated as 'PotentialHostGene' were removed.

Prediction accuracy

True positives (TP) are defined as predicted TE nucleotides that truly belong to a TE copy. False positives (FP) are the predicted TE nucleotides that do not really belong to a TE copy. True negatives (TN) are the nucleotides correctly predicted not to belong to a TE copy (correct rejection), and false negatives (FN) are the true TE copy nucleotides missed by the TE prediction process.

Sensitivity, the true positive rate, given by the formula TP/(TP+FP), is obtained by calculating the fraction of nucleotides in the predicted TE overlapping with the TE reference annotation.

Specificity, also refered to as the true negative rate, is less straightforward to calculate. It can be calculated according to the formula TN/(TN+FP), but TN and FP are difficult to determine for TEs, as they can only be known if we are sure that we have identified all the TE copies in the genome, which does not really seem possible. However, as a first approximation, we can consider that genes are not TEs, and are not derived from TEs, and use this information to obtain more accurate estimates for TN and FP. In this context, FP are predicted TE nucleotides that overlap a gene annotation, and TN are gene regions not predicted to be TEs.

Accuracy, given by ACC=(TP+TN)/(TP+TN+FN+FP), is the rate of correct predictions.

Epigenetic data

We used a small-RNA map from Lister et al. (2008) [16] corresponding to dataset GSM277608 from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). The original coordinates

were projected onto the TAIR10 assembly. The occurrences of multiply mapping reads were distributed evenly between genomic copies. This small-RNA dataset is derived from inflorescences of plants grown at 23°C with a 16 hours light period.

We used the 10 chromatin mark maps (H3K18ac, H3K27me1, H3K27me3, H3K36me2, H3K36me3,H3K4me2, H3K4me3, H3K9ac, H3K9me2 et H3) from Luo *et al.* [17].

Reads overlapping an annotation were counted with the CompareOverlapping.py script (option –O) of the S-Mart package [18].

We normalized counts by calculating the ratio of the mean number of reads overlapping an annotation to the number of overlapping reads from the input.

Hierarchical clustering was performed on the epigenetic marks, based on the normalized ratio, with the *seaborn* python library, the correlation metric and a standard-scale normalization for each mark.

TFBS and CNS data

We use TFBS data compiled by Heyndrickx *et al.* [19] from ChIP-seq experiments, which we downloaded from http://bioinformatics.psb.ugent.be/cig_data/RegNet/.

CNSs data were retrieved from the work of Van de Velde *et al.* [20] , from http://bioinformatics.psb.ugent.be/cig_data/Ath_CNS/Ath_CNS.php, and that of Haudry *et al.* [21] from http://mustang.biol.mcgill.ca:8885/download/A.thaliana/AT_CNS.bed.

Analysis of binding motifs

We searched for binding motifs with the MEME suite server [22] from http://meme-suite.org. We used MEME-ChIP [23] and JASPAR2018 CORE non-redundant databases.

Analysis of orthologous genes

We used OrthoMCL [24] version 2.0 to identify genes orthologous between *A. thaliana, A .lyrata, C.*

rubella, and *S. parvulum*. From the 21689 groups of orthologs obtained, we retained only 6921 for which four genes were identified, one from each species, to limit the detection of false-positive paralogs by this method.

Statistical analysis

We used the python libraries *pynum*, *scipy* for statistics, *matlibplot* for graphics and *panda* for data manipulation. *Jupyter notebooks* were used to monitor the analysis.

Sequence and coordinate manipulation

We obtained random sequences with *shuffle* from the SQUID 1.9g package [25] and *revseq* from the Emboss 6.1.0 [26] package.

Genome coordinates were manipulated with the S-Mart package [18]. In particular, we used `modifyGenomicCoordinates` (version 1.0.1) and `CompareOverlapping` (version 1.0.4) to extend coordinates within the 5' region of genes, and find overlaps, respectively.

4 Results

Duster: a new approach for analyzing old degenerate transposable elements

Following their divergence from a common ancestor, repeat families have different destinies in different genomes. A repeat family may stop multiplying in one species, but may continue to multiply in another closely related species. The burst of transposition in an autonomous repeat family is a highly selective process: only the copies that have accumulated limited numbers of mutations remain functional and are able to transpose during the burst. This selective burst of transposition drives multiplication of the best conserved copies, *i.e.* those most similar to the ancestral sequence. Therefore, the TE families that remain active in some genomes should conserve the ancestral sequence for longer than the decaying pool of related sequences in other genomes. Consequently, a repeat copy from one species may be considered to be relatively old if it closely resembles a sequence obtained from another species.

We previously showed [6] that identifying TEs in a species by comparison with reference sequences found in

the studied species but also in closely related species leads to the detection of older TE copies than searches exclusively with the reference sequence from the study species. Indeed, this approach detects old TE sequences that would not otherwise be recognized.

We developed a program called Duster that compares a genome sequence, here considered as a query sequence, to a large number of TE sequences, *i.e*. a sequence library. Its algorithm used *k-mers* to search for similar sequences without the need to generate nucleotide aligments. Hashed *k-mer* values can be used to speed up the search. Sensitivity is achieved by allowing one mismatch in *k*-mers every *n* consecutive nucleotides. Details of the algorithm are provided in Supplementary file 1, but it can be summarized as comparing *k*-mers between the genome and each sequence from the library, and reporting matches when at least two *k*-mers are found on the same alignment diagonal (i.e. the differences between the coordinates in the query and the sequence library are identical) with a maximal distance of *d k*-mers. The region bounded by the two-extreme *k*-mer position are reported as matching. Two matching regions on the genome separated by less than *x k*-mers are merged. At the end of this first pass, the region identified on the genome can be used as a new sequence library for a new search (the *-n* parameter). This procedure is repeated until genome coverage increases by less than 1% if *-n* is set to 0.

Supplementary file 1 presents performance assessment for Duster. It shows that Duster outperforms standard tools in term of speed. Duster has a higher sensitivity and a lower specificity, but its coverage is higher, suggesting that our tool may detect many more previously unknown potential TEs than existing methods. The false positive rate remained below 0.01.

Transposable elements account for up to 50% of the A. thaliana genome

Assuming that Duster would be able to detect interesting new TE sequences in the *A. thaliana* genome, we ran an analysis with all the Brassicaceae TE copies from *Arabidopsis thaliana*, *Arabidopsis lyrata*, *Capsella rubella*, *Schrenkiella parvulum*, *Arabis alpina*, and *Brassica rapa* that we had previously annotated (see Material and Methods). We used the parameter setting with *–d* 5 and *–S* 7, but changed *–n* to 0, allowing iteration of the algorithm until it reached a genome coverage difference between two successive iterations of less than 1%.

The TAIR10, Brassicaceae and Duster TE annotations together accounted for 49.75% of the genome

sequence. This figure is 29.72% higher than that for the TAIR10 TE reference annotation (20.03%), and 10.60% higher than that for the Brassicaceae TE annotation (39.15%).

Structural properties of Duster-specific copies

We characterized the new set of repeats identified by Duster, by using the annotations to extract copies that did not overlap with any Gene, TAIR10 TE, Brassicaceae, or *A. thaliana* REPET annotations (see Materials and Methods). We identified 19608 TE copies that were Duster-specific. We did the same for the TAIR10 and Brassicaceae annotations, thereby obtaining 177 TAIR10-specific and 5139 Brassicaceae-specific copies, by removing any copies with no overlap to another annotation.

We characterized these copies by comparing their length, chromosome distribution, and position relative to genes (figure 1). Duster-specific copies appeared to be significantly shorter than Brassicaceae-specific, TAIR10-specific, and TAIR10 copies (Figure 1A, chi-squared *p*-value respectively 3.09×10^{-192}, 2.70×10^{-8}, $<10^{-293}$). Figure 1B shows the distance to the closest 5' or 3' TE copy for each annotated gene. Duster-specific copies are more abundant close to genes than other copies (all chi-squared *p*-value $<10^{-293}$, versus *Brassicaceae*-specific, TAIR10-specific, and TAIR10 copies). Similarly, Brassicaceae-specific copies were more abundant than TAIR10-specific copies. They were more frequently found upstream from genes (Figure 1B, chi-squared *p*-value $<10^{-293}$), as were Brassicaceae-specific, and TAIR10 TE copies (all chi-squared *p*-value $<10^{-293}$). Figure 1C shows the distribution of TE copies over the chromosomes. It shows that Duster TE copies, and, to a lesser extent, Brassicaceae TE copies, follow the chromosomal distribution of genes (see the right panel of figure C), whereas TAIR10 TEs follow the opposite pattern. Duster and Brassicaceae TEs have a different chromosomal distribution from the annotated TEs from TAIR10.

Finally, we investigated the nucleotide composition of the sequences, including dinucleotides. The counts are presented as a radar plot in Figure 2A. The profile is similar for all TE copies (Duster-specific, Brassicaceae-specific, TAIR10-specific, and TAIR10 TEs). Interestingly, TAIR10-specific copies had the strongest bias towards TT, AA, AT, and TA dinucleotides, followed by Duster-specific copies. These biases, also shared by other TE copies but to a lesser extent, were thought to be a consequence of the process by which methylated cytosine is deaminated. The greater "A-T" richness of TAIR10-specific and Duster-specific copies may indicate that they have undergone a mutation over a longer period and are therefore more ancient.

Epigenetic profiles

We investigated the epigenetic status of the identified TE copies, considering small RNAs, and chromatin marks. The small RNAs studied were taken from Lister et al. [16], for which mapped data were available. There were 4.17%, 20.14%, 16.95%, and 60.44% of matching TE copies from the Duster-specific, Brassicaceae-specific, TAIR10-specific, and TAIR10 TE datasets, respectively, in the intersection between this dataset and our annotations, indicating a low targeting by small RNA of unknown TEs compared to known annotated TEs.

We analyzed nine epigenetic marks from Luo *et al.* [17], also available as mapped data. The hierarchical clustering algorithm identified distinctly different profiles for genes and for TAIR10 TEs (figure 2B). TAIR10 TEs were enriched in heterochromatin marks H3K27me1 and H3K9me2, and genes with euchromatin marks H3K36me2, H3K36me3, H3K4me2, H3K4me3, and H3K9ac as expected. The clustering algorithm associated Duster-specific, Brassicaceae-specific, and TAIR10-specific copies with the TAIR10 TE profile, indicating that their profiles were more similar to a typical TE profile than to a gene profile. However, Brassicaceae-specific, TAIR10-specific, and Duster-specific marks copies had very similar profiles which differ from TEs. Their copies appeared to have very few heterochromatic, however the active euchromatin marks H3K27me3 and H3K18Ac are predominant for method-specific TEs. Interestingly, H3K27me3 is known to be preferentially associated with genes expressed at low levels or in a tissue-specific manner [27–30].

TE conservation in flowering plants

As the Duster and Brassicaceae TE sequences appeared to be more ancient, we investigated the conservation of TE copies by searching for overlaps with known conserved non-coding sequences (CNSs) identified in previous studies. We compared the TE copies with CNSs identified in crucifers [21] and rosids [20]. For both datasets, a significant proportion of the TE copies overlapped with these CNSs (5.32 to 19.8%, see table 1). Some TE sequences overlapped with CNSs conserved in 12 rosid species: *Arabidopsis thaliana, Carica papaya, Glycine max, Malus domestica, Populustrichocarpa, Fragariavesca, Medicago truncatula, Lotus japonicus, Theobroma cacao, Ricinus communis, Manihot esculenta,* and *Vitis vinifera*. Fossil rosids dating back to the Cretaceous period, estimated by a molecular clock between 125 and 99.6 million years ago [31–

33], have been found. Our findings therefore reveal a remarkable conservation of 1521 and 1213 TE insertions identified by the Duster and Brassicaceae methods, respectively, over more than 100 million years, twice as many as can be detected with the traditional annotation approach available for the TAIR10 TE annotation. We also show here that the Duster approach can detect more TEs overlapping with CNSs than the Brassicaceae method.

Table 1: Number of CNSs and percentage overlap with TE annotations

	Crucifer CNSs	Rosid CNSs	Rosid CNSs – score 11
All Duster	14774 *14.1%*	20758 *19.8%*	1521 *1.45%*
All Brassicaceae	7798 *9.39%*	13702 *16.5%*	1213 *1.46%*
All TAIR10 TEs	1583 *5.32%*	4934 *16.6%*	527 *1.77%*
Duster-specific	3659 *18.7%*	2580 *13.2%*	194 *0.99%*
Brassicaceae-specific	1848 *36.0%*	2535 *49.3%*	246 *4.79%*
TAIR10-specific	27 *15.2%*	27 *15.2%*	3 *1.69%*
5’ Duster-specific	745 *15.5%*	968 *20.2%*	65 *1.36%*
5’ Brassicaceae-specific	270 *30.6%*	452 *51.3%*	26 *2.95%*
5’ TAIR10-specific	2 *6.67%*	4 *13.3%*	0 *0.0%*

The 65 rosid CNSs from the 12 rosid species associated with Duster-specific copies in the 500 bp upstream of genes included 58 Duster-specific sequences. A MEME-ChIP [22] analysis of these sequences identified a

significant 15-nucleotide TTTTTTTTT(G/T)TTT(G/T)(G/C) motif (E-value 3.4 x 10^{-6}) in 27 sites. This motif matched MA1281.1 (AT5G02460), MA1274.1 (OBP3), MA1278.1 (OBP1) MA1268.1 (AT1G69570), MA1267.1 (AT5G66940), MA1272.1 (AT2G28810), MA1371.1 (IDD4), MA1279.1 (COG1), MA1156.1 (JKD), MA1374.1 (IDD7), MA1160.1 (AT1G14580), MA1158.1 (MGP), MA1157.1 (NUC), MA1125.1 (ZNF384), MA1159.1 (SGR5), MA1277.1 (Adof1), and MA1370.1 (IDD5) with all q-values < 5 x 10^{-2}, described in the JASPAR database [36] as C2H2 zinc finger factors of the Dof-type. Interestingly, some of these motifs were found to be related to: (i) AT3G55370 (OBP3), which is known to encode a nuclear DOF domain-containing TF expressed primarly in roots that is responsive to salicylic acid in leaves and petals, (ii) AT1G69570 (CDF5), which is invoved in flower development and photo-periodism, (iii) AT1G29160 (COG1), which acts as a negative regulator in phytochrome-mediated light responses, (iv) AT3G50410 (OBP1), which acts as a positive regulation of transcription and play an important roles in plant growth and development and (v) AT5G02460.1, which is probably involved in early processes in vascular development. We then looked in detail at the conservation of Duster-specific, Brassicaceae-specific, and TAIR10-specific copies in the Brassicaceae. We considered only regions close to orthologous genes found with OrthoMCL (see Materials and Methods). We focused on *A. thaliana*, *A. lyrata*, *C. rubella*, and *S. parvulum*, as these species have divergence times of 5 to 40 My. Orthologous genes with 5 kb upstream regions were aligned with the *A. thaliana* region containing both the orthologous gene and a TE copy from a method-specific set. We obtained 11175, 5951, and 40TEs suitable for analysis for Duster-specific, Brassicaceae-specific, and TAIR10-specific TE copies. We considered a TE copy to be present if more than 50% of the *A. thaliana* annotated TE copy nucleotides were identical in the pairwise alignment. The TEs were oldest in the Duster-specific set, followed by the Brassicaceae-specific set, as shown by the height of the 111 bar of the histogram, which corresponds to the presence of a TE at orthologous positions in all four species (Figure 3). Interestingly, the 000 bar was also quite high. This bar corresponds to TEs found only in *A. thaliana*, but which belonged to method-specific sets and therefore escaped TE detection by the simple REPET *de novo* procedure limited to *A. thaliana*. They were therefore detectable only with TEs found in other species. These copies may result from horizontal transfer from these other species, or may simply have been identified in other genomes because they are better conserved in those genomes. This result illustrates the utility of our cross-species TE annotation approach and the greater efficiency of Duster than of the REPET annotation procedure.

Contribution of TEs to the architecture of gene regulatory networks

We investigated the functional role of these TE sequences, which may have been co-opted for some regulatory purpose. We chose to study two gene regulatory networks (GRNs) in which TEs might be suspected to play a role. The genes controlling flower development in *Arabidopsis thaliana* are good candidates, as some alleles have been reported to be controlled by a TE sequence in *A. thaliana*: the FLOWERING WAGENINGEN (FWA) locus [34], and FLOWERING LOCUS C (FLC) [35,36].

We considered the genes reported by Chen et al. 2018 [37] in their paper describing the architecture of GRNs controlling flower development in *Arabidopsis thaliana*. We searched the 500 bp immediately upstream from these genes for the presence of Duster-specific, Brassicaceae-specific, and TAIR10-specific TEs.

An enrichment in Duster-specific regions was observed in the 5' sequences of flowering genes: 33.1% of these sequences contained such regions, versus only 17.5% for all genes (chi-squared *p*-value=2.4 x 10^{-7}, table 2). Brassicaceae-specific regions and specific regions identified by TAIR10 displayed no particular enrichment, with 4.46% versus 3.21% (chi-squared *p*-value=0.37), and 0% *vs* 0.11% (chi-squared *p*-value=0.68), respectively of 5' sequences containing such regions.

Table 2: Method-specific TEs overlap with the 500 bp immediately upstream from genes

	Duster-specific	Brassicaceae-specific	TAIR10-specific
All genes	17.5%	3.21%	0.11%
All flowering genes	33.1%	4.46%	0.0%
Stress genes	16.5%	4.71%	0.0%

We futher explored the overrepresentation of Duster-specific and Brassicaceae-specific TEs in GRNs, by focusing on stress GRNs genes, which are also thought to be linked to TEs, as reported by several studies suggesting that transposition events may be triggered during plant stress responses including salt [38], wounding [39], bacteria [40], and viruses [41]. We focused on the genes expressed in various stress conditions described by Barah et al. [42]. We searched in the 500 bp immediately upstream from these genes for Duster-specific, Brassicaceae-specific, and TAIR10-specific TEs (table 2). We found no enrichment of these upstream regions in Duster-specific TE copies (chi-squared *p*-value=0.73) or TAIR10-specific TE

copies (chi-squared p-value=0.67). However, we found an enrichment for Brassicaceae-specific TEs (chi-squared p-value=5.1 x 10^{-7})

TEs and transcription factor binding sites

The conservation and overrepresentation described above suggest a probably functional role for these TEs. We then investigated their ability to regulate gene expression, by assessing their ability to bind TFs. We investigated the co-occurrence of the transcription factor binding sites (TFBSs) identified with 27 TFs in ChIP-seq experiments by Heyndrick et al. [19] and the various TE annotations studied here.

We found that TFBSs were more frequently present in Duster regions (29.0%) than in Brassicaceae and TAIR10 regions (19.5%, and 14.6%, respectively; Table 3). This pattern was even more marked for the analysis of method-specific regions: 53.9%, 29.7% and 24.9% of these regions, respectively, overlapped with TFBSs. This trend was even stronger for analyses limited to the 500 bp immediately upstream from genes (49.3% for Duster-specific and 38.2% for Brassicaceae-specific TEs, TAIR10-specific TEs being untestable due to the low counts). Interestingly, these regions contained 567 Duster-specific and 48 Brassicaceae-specific TFBS regions, associated with more than seven TFs, and referred to hereafter as *hot TFBS*s. The identification of these regions suggests that there may be a hub of target genes involved in the important function of crosstalk between different processes [43].

Table 3: Method-specific TEs overlap with TFBS identified by ChIPseq

	Hot TFBSs	TFBSs
All Duster	1.10%	29.0%
All Brassicaceae	0.51%	19.5%
All TAIR10 TEs	0.40%	14.6%
Duster-specific	2.89%	53.9%
Brassicaceae-specific	0.93%	29.7%
TAIR10-specific	1.13%	24.9%
5' Duster-specific	2.38%	49.3%
5' Brassicaceae-specific	0.79%	38.2%
5' TAIR10-specific	3.33%	6.67%

An analysis of the 5' sequences of flowering genes identified 6 key TFs known to be involved in flowering that could bind to both Duster-specific and Brassicaceae-specific regions (AGL-15, AP1, PI, AP3, SEP3, SOC1). Few sites for TFs involved in circadian rhythm and light response (PIF3, PRR5, PRR7) and development (GL3) were identified. Most were found in Duster-specific regions, with very few in Brassicaceae-specific regions, and none in TAIR10-specific regions. Interestingly, some Duster-specific regions were associated with several TFBSs.

We found 1757 and 1009 Duster-specific regions overlapping with crucifer and rosid CNS, respectively, and a TFBS. We found that 84 of these regions were highly conserved, as they overlapped with CNSs present in the 12 rosid species used for their identification, suggesting a presence in the common ancestors of the rosids more than 100 My ago. We also found that 9 of these highly conserved Duster-specific regions overlapped with a *hot TFBS*, suggesting the presence of a highly conserved hub of target genes involved in crosstalk between different processes. The top five highly conserved TFBSs from Duster-specific regions were AGL-15, AP1, SEP3, PRR5, and PIF4 (31, 23, 20, 14, 14 occurrences, respectively), all but one of which are

directly involved in flowering process, the exception being PRR5, which is more closely related to circadian rhythms and light responses.

The CNSs associated with Duster-specific copies in the 500 bp upstream of the gene present in the 12 rosid species included 58 Duster-specific sequences: 42 target genes of floral regulators according to Chen *et al.* [37], 16 for which Duster-specific regions were colocalized with a highly conserved CNS and a TFBS (Table 4).

Table 4: Sixteen floral regulators genes from Chen et al. [37] with a Duster-specific region in the 500 bp immediately upstream, colocalizing with highly conserved CNSs and TFBSs

Gene	TFBS	Profil from MEME-suite
AT1G20930	AGL-15	SOC1
AT1G49760	AGL-15	
AT1G75110	GL1	
AT2G05920	AGL-15; PIF4	SOC1
AT2G30400	AGL-15	
AT2G30970; AT2G30960	AGL-15; AP1; SEP3	
AT2G33750	SEP3	SOC1
AT2G41370	AGL-15; AP1; AP3; PI	
AT3G02040	AP3; AGL-15; PI	SOC1
AT3G14172	AGL-15	
AT3G19170	AGL-15; PIF4; PIF3; PI; AP3	SOC1
AT4G00810	PIF	SOC1
AT4G37260	SEP3	SOC1
AT4G39980	SEP3, AP2, AGL-15	
AT5G03680	AP1; SEP3; FLM; PIF4	
AT5G13990	PIF4	

Using the *MEME Suite,* we identified 7 of these genes as containing a sequence corresponding to a binding motif of the SOC1 TF, a MADS box factor active in flowering time control which may integrate signals from the photoperiod, vernalization and autonomous floral induction pathways. Thirteen of downstream genes were controlled by the floral regulation motifs of one or several type II TF-MADSs (AG, AP1, AP2, AP3, BLR, ETT, FLM, JAG, LFY, PI, RGA, SEP3, SVP).

5 Discussion

The need for a new dedicated repeat detection algorithm

RepeatMasker [44], Censor [45,46], and Blaster [19] are the tools most frequently used to annotate TE sequences in genomes. All these tools combine BLAST (or BLAST-like with seed and extend algorithms) calls with pre- and post-processing for the analysis of genomic sequences. They are all, therefore, subject to the intrinsic limitations of these algorithms, including a reliance on seeds to find alignments. These seeds in BLAST are *k*-mers with a default size of 11 nucleotides. BLAST requires two *k*-mers on the same diagnonal (i.e. alignment without gaps) to proceed with the analysis of an alignment to assess its relevance. An alignment score threshold determined with a probabilistic model is used for this assessment of relevance. These features may account for the lower sensitivity for this method than for Duster.

First, two *k*-mers are required to initiate an alignment. With the default BLAST parameters, this requires an exact match of at least 22 nucleotides between two sequences. This requirement can be decreased, as seed length is a parameter of BLAST that can be set, and it is decreased to 14 nucleotides for some implementations (seed size of 7 with WU-BLAST), but it still needs an exact match. For Duster, we allow mismatches in the *k*-mers, and the two k-mers may overlap. With the settings used for this analysis, we required a match of 21 nucleotides, but with some mismatches allowed.

Second, in the statistical test based on an alignment score threshold, even if the required exact match of 22 nucleotides is found, a gap-free alignment is produced for testing with the probabilistic model. The result depends on sequence length and on a model that is mathematically sophisticated, but too simple biologically,

in that it considers successive nucleotides to be independent and equally probable. We now know that neither of these assumptions hold true for real sequences. Consequently, the model is of debatable value and may reject some alignments differently according to sequence lengths. In Duster, we retain all regions that match two *k*-mers, and the empirically chosen parameters yielded very few false positives (0.001).

We can see here that BLAST is not the most appropriate algorithm for finding small degenerate TEs. It was developed for a different purpose: identification of the best match within databases to a sequence used as a query. Its use to identify TEs constitutes a major deviation from its initial purpose, for which it performs well.

Duster was designed for the express purpose of finding old and degenerate TE copies. In addition to having a different *k*-mer strategy, it is essentially an alignment-free algorithm. BLAST attempts many alignments before reporting a match. Duster does not really require an alignment, just boundary coordinates, accounting for the greater speed of this algorithm. Boundaries may be considered imprecise as they are based on *k*-mers and their precision is therefore limited by *k*-mer size and the shift in the coordinates of the *k*-mer on the genomic sequence. With the parameters used here, the precision is about seven nucleotides. We think that this is sufficient for the identification of regions, and it may not be appropriate to aim for greater precision in the identification of very old, degenerate TE copies.

The work presented here highlights the utility of specifically developed tools for addressing certain difficult biological questions. It highlights the need for a new generation of sequence-finding tools, tailored to the particular biological question posed and perhaps replacing BLAST with more adapted algorithms.

Long-term impact of TE copies

TEs are important sources of variation on which selection can operate in the evolution of species. Many examples of TEs generating new phenotypes have been reported in plants [47]. This phenomenon is known as “domestication” when the TE sequences are retrained in new genes, or “co-option” when TE insertions affect existing genes. TE sequences that become functional in the host are conserved by selection, which can be recognized over long periods of time. Other TE copies devoid of function in the host are progressively removed from the genome through the accumulation of point mutations and deletions. However, gene regulatory regions accumulate point mutations and deletions at a slower rate than other regions, because of

their function. Consequently, TE insertions in these regions, even if neutral, may be difficult to remove once established.

TFs control the transpositional activity of TEs by binding to them. The corresponding TFBS are, therefore, widespread throughout the genome. In some cases, TEs from the same family may be inserted close to several genes. This may lead to nearby genes being regulated by the same TF, potentially leading to their evolution into gene regulation networks. Genome-wide assessment revealed that hundreds of TEs have been co-opted into the regulatory regions of mammalian genes [48,49]. TEs have also been involved in both the creation of new regulatory networks [50,51] and the rewiring of preexisting ones [52]. Such networks are observed, for example, for the DAYSLEEPER gene in *A. thaliana* [53]. This gene has features in common with hAT transposases, suggesting that it may have been domesticated as a new TF. Another interesting example is provided by the retrotransposon ONSEN in *Arabidopsis* [54]. Thieme *et al.* [55] showed that, following the heat stress-dependent mobilization of ONSEN, the progenies of treated plants contain up to 75 new ONSEN insertions. Progenies with additional ONSEN copies display broad environment-dependent phenotypic diversity. This finding suggests that some of the new TE insertions affect the expression of genes in a temperature-dependent manner. It also suggests that TE sequences may have contributed to individual local adaptation through the mutations they induce during bursts of transposition. Some of these bursts of transposition may result from activation by environmental stresses, promoting environment-sensitive phenotypes.

However, little is known about the long-term impact of TEs. Our CNS data suggest that some of the TEs identified were inserted in their current positions more than 100 My ago, during the Cretaceous period. The most important evolutionary event during this period was, perhaps, the spread of flowering plants (Angiosperms) to colonize the entire planet. Flowering plants were particularly successful at colonizing new areas and replacing the older established flora, with which they competed. TEs undoubtedly played an important role in this process. Some of the TE insertions we detected may, indeed, have played this role. Duster-specific copies appear to be old, degenerate, short, and surprisingly close to genes, lying in the 5' flanking sequences known to correspond to gene regulatory regions. Their maintenance specifically in these zones suggests that they supply the host with a function, probably in the regulation of the neighboring gene.

The Duster-specific TEs identified here may have played an important role in building new pathways

allowing flowering plants to adapt to their environment. Indeed, we found that Duster-specific TE copies were overrepresented in the 5' regions of genes of the GRN for flowering. A significant proportion of these copies overlapped with TFBSs known to bind TFs involved in the control of flowering. Moreover, the histone H3K27me3 mark was identified predominantly in method-specific TEs (see figure 2). This histone mark has been reported to be associated with genes expressed at low levels of in a tissue-specific manner [30], such as those involved in flower development.

Our results suggest a possible link between the success of flowering plants during the Cretaceous period and the co-option of TEs in the flowering GRN. However, further analyses are required to demonstrate a causal role. This study is a first step in this direction, identifying previously unknown candidate TEs.

Flowering has been studied in considerable detail, generating a wealth of data. The data used here are, therefore, clearly biased towards flowering. However, other impacts on other GRNs may subsequently be discovered with our ancient TE annotation, as and when new data become available.

Interestingly, our results suggest that identifying very old TE copies could facilitate the identification of TE-based regulatory modules selected a long time ago. They support the detection of TFBS in ChipSeq experiment, but also suggest a TE-based origin for many TFBS.

6 Conclusions

In this study, we investigated the contribution of TEs to the bulk genome of *Arabidopsis* over a timescale that remains inaccessible to other approaches, through the use of a new tool that we developed, called Duster. Duster uses a new efficient algorithm, which identified an additional 10% of nucleotides as belonging to TEs. We have, thus, dug deeper into the dark matter than previous studies, leading to the recognition of old, degenerate TE sequences undetectable with other methodologies.

This study delivers a key result, improving our understanding of plant evolution and plant adaptation, by providing clues for identifying ancient TE remnants in gene regulatory regions underlying potential regulation modules. Some of the TE copies identified here may have been selected a long time ago, to drive adaption to changing environments.

7 Acknowledgment

We thank KlaasVandepoele for reading a first version of this manuscript and drawing our attention to interesting TFBS and CNS data that enabled us to improve the manuscript. We also thank Michaël Alaux and Johann Confais for their comments on the manuscript. This work was performed with the facilities of the Plant Bioinformatics Facilities (https://doi.org/10.15454/1.5572414581735654E12)

8 Author contributions

HQ: conceived the study, developed Duster, ran the software. AB: identified method-specific sets and described their general features. MW: performed conservation analysis on the Brassicaceae. DA, DN, HQ: performed CNS, TFBS and profile analyses. DA and HQ: drafted the manuscript. All the authors read and commented on the manuscript.

9 Materials & Correspondence

All requests for materials and information should be addressed to Hadi Quesneville at hadi.quesneville@inra.fr

10 Code availability

The Duster code is available on github, and is distributed as part of the TEfinder package at https://github.com/urgi-anagen/TE_finder

11 Data availability

The Brassicaceae TE copies from *Arabidopsis thaliana* Col-0 ecotype, *Arabidopsis lyrata*, *Capsella rubella*, *Arabis alpina*, *Brassica rapa* and *Schrenkiella parvula*, used for Duster annotation, and TAIR10 TE, Duster, and Brassicaceae annotations can be downloaded from

https://urgi.versailles.inra.fr/files/sequence/PubAthaDarkmatterData/PubAthaDarkmatterData.zip

The data are also available in a JBrowse genome browser at

https://urgi.versailles.inra.fr/jbrowse/gmod_jbrowse/?data=myData%2FAtha

12 Figure legends

Figure 1: **Structural characteristics of Duster-specific, Brassicaceae-specific, TAIR10-specific, and TAIR10TE copies.** (A) TE length distribution, (B) distance to the closest 5', or 3' TE copy for each annotated gene, (C) TE copy distribution on the chromosomes. Left panel: density plot, for 100 kb windows overlapping by 10 kb. Right panel: density differences, in 100 kb bins.

Figure 2: **Composition of Duster-specific, Brassicaceae-specific, and TAIR10-specific copies.** (A) Radar plot of the dinucleotide composition of the sequences. (B) Hierarchical clustering of TEs and genes with respect to heterochromatin marks (H3K27me1 and H3K9me2) and euchromatin marks (H3K18ac, H3K27me3, H3K36me2, H3K36me3, H3K4me2, H3K4me3, and H3K9ac).

Figure 3: **Numbers of sequences conserved in orthologous positions between species**. The three-digit code indicates the species in which the sequences were present. A "1" indicates presence and a "0" absence, in *A. lyrate* (position 1), *C. rubella* (position 2), and *S. parvulum* (position 3).

14 Literature cited

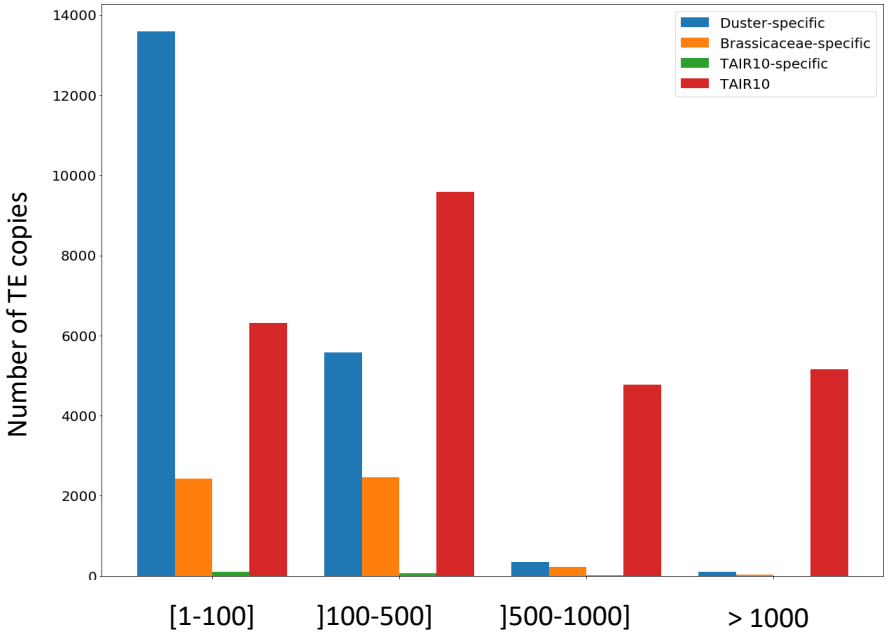
1. Kidwell MG. Transposable elements and the evolution of genome size in eukaryotes. Genetica. 2002;115: 49–63. doi:10.1023/A:1016072014259
2. Huang P, Studer AJ, Schnable JC, Kellogg EA, Brutnell TP. Cross species selection scans identify components of C4 photosynthesis in the grasses. J Exp Bot. 2017;68: 127–135. doi:10.1093/jxb/erw256
3. Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, et al. The B73 maize genome: complexity, diversity, and dynamics. Science. 2009;326: 1112–1115. doi:10.1126/science.1178534
4. Feschotte C, Jiang N, Wessler SR. Plant transposable elements: where genetics meets genomics. Nature Reviews Genetics. 2002;3: 329–341. doi:10.1038/nrg793
5. Wicker T, Gundlach H, Spannagl M, Uauy C, Borrill P, Ramírez-González RH, et al. Impact of transposable elements on genome structure and evolution in bread wheat. Genome Biology. 2018;19: 103. doi:10.1186/s13059-018-1479-0
6. Maumus F, Quesneville H. Ancestral repeats have shaped epigenome and genome composition for millions of years in Arabidopsis thaliana. Nat Commun. 2014;5: 4104. doi:10.1038/ncomms5104
7. Huang C-H, Sun R, Hu Y, Zeng L, Zhang N, Cai L, et al. Resolution of Brassicaceae Phylogeny Using Nuclear Genes Uncovers Nested Radiations and Supports Convergent Morphological Evolution. Mol Biol Evol. 2016;33: 394–412. doi:10.1093/molbev/msv226
8. Buisine N, Quesneville H, Colot V. Improved detection and annotation of transposable elements in sequenced genomes using multiple reference sequence sets. Genomics. 2008;91: 467–475. doi:10.1016/j.ygeno.2008.01.005
9. Quesneville H, Bergman CM, Andrieu O, Autard D, Nouaud D, Ashburner M, et al. Combined evidence annotation of transposable elements in genome sequences. PLoS Comput Biol. 2005;1: 166–175. doi:10.1371/journal.pcbi.0010022
10. Flutre T, Duprat E, Feuillet C, Quesneville H. Considering transposable element diversification in de novo annotation approaches. PLoS ONE. 2011;6: e16526. doi:10.1371/journal.pone.0016526
11. Hoede C, Arnoux S, Moisset M, Chaumier T, Inizan O, Jamilloux V, et al. PASTEC: an automatic transposable element classification tool. PLoS ONE. 2014;9: e91929. doi:10.1371/journal.pone.0091929
12. Bao W, Kojima KK, Kohany O. Repbase Update, a database of repetitive elements in eukaryotic genomes. Mob DNA. 2015;6: 11. doi:10.1186/s13100-015-0041-9
13. El-Gebali S, Mistry J, Bateman A, Eddy SR, Luciani A, Potter SC, et al. The Pfam protein families database in 2019. Nucleic Acids Res. 2018. doi:10.1093/nar/gky995
14. Accelerated Profile HMM Searches. [cited 25 Oct 2018]. Available: https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1002195
15. Jamilloux V, Daron J, Choulet F, Quesneville H. De Novo Annotation of Transposable Elements: Tackling the Fat Genome Issue. Proceedings of the IEEE. 2017;105: 474–481. doi:10.1109/JPROC.2016.2590833
16. Lister R, O'Malley RC, Tonti-Filippini J, Gregory BD, Berry CC, Millar AH, et al. Highly integrated single-base resolution maps of the epigenome in Arabidopsis. Cell. 2008;133: 523–536. doi:10.1016/j.cell.2008.03.029
17. Luo C, Sidote DJ, Zhang Y, Kerstetter RA, Michael TP, Lam E. Integrative analysis of chromatin states in Arabidopsis identified potential regulatory mechanisms for natural antisense transcript production. Plant J. 2013;73: 77–90. doi:10.1111/tpj.12017
18. Zytnicki M, Quesneville H. S-MART, a software toolbox to aid RNA-Seq data analysis. PLoS ONE. 2011;6: e25988. doi:10.1371/journal.pone.0025988
19. Heyndrickx KS, de Velde JV, Wang C, Weigel D, Vandepoele K. A Functional and

Evolutionary Perspective on Transcription Factor Binding in Arabidopsis thaliana. The Plant Cell. 2014;26: 3894–3910. doi:10.1105/tpc.114.130591
20. de Velde JV, Heyndrickx KS, Vandepoele K. Inference of Transcriptional Networks in *Arabidopsis* through Conserved Noncoding Sequence Analysis. The Plant Cell. 2014;26: 2729–2745. doi:10.1105/tpc.114.127001
21. Haudry A, Platts AE, Vello E, Hoen DR, Leclercq M, Williamson RJ, et al. An atlas of over 90,000 conserved noncoding sequences provides insight into crucifer regulatory regions. Nature Genetics. 2013;45: 891–898. doi:10.1038/ng.2684
22. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, et al. MEME Suite: tools for motif discovery and searching. Nucleic Acids Res. 2009;37: W202–W208. doi:10.1093/nar/gkp335
23. Machanick P, Bailey TL. MEME-ChIP: motif analysis of large DNA datasets. Bioinformatics. 2011;27: 1696–1697. doi:10.1093/bioinformatics/btr189
24. Li L, Stoeckert CJ, Roos DS. OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res. 2003;13: 2178–2189. doi:10.1101/gr.1224503
25. Eddy SR. SQUID - library of functions for biological sequence analysis Copyright (C) 1992-2002 Washington University School of Medicine. Available: http://eddylab.org/software/squid/squid-1.9g/
26. Rice P, Longden I, Bleasby A. EMBOSS: the European Molecular Biology Open Software Suite. Trends Genet. 2000;16: 276–277.
27. Turck F, Roudier F, Farrona S, Martin-Magniette M-L, Guillaume E, Buisine N, et al. Arabidopsis TFL2/LHP1 Specifically Associates with Genes Marked by Trimethylation of Histone H3 Lysine 27. PLOS Genetics. 2007;3: e86. doi:10.1371/journal.pgen.0030086
28. Zhang X, Clarenz O, Cokus S, Bernatavichute YV, Pellegrini M, Goodrich J, et al. Whole-Genome Analysis of Histone H3 Lysine 27 Trimethylation in Arabidopsis. PLoS Biol. 2007;5. doi:10.1371/journal.pbio.0050129
29. Oh S, Park S, Nocker S van. Genic and Global Functions for Paf1C in Chromatin Modification and Gene Expression in Arabidopsis. PLOS Genetics. 2008;4: e1000077. doi:10.1371/journal.pgen.1000077
30. Jacob Y, Stroud H, Leblanc C, Feng S, Zhuo L, Caro E, et al. Regulation of heterochromatic DNA replication by histone H3 lysine 27 methyltransferases. Nature. 2010;466: 987–991. doi:10.1038/nature09290
31. Davies TJ, Barraclough TG, Chase MW, Soltis PS, Soltis DE, Savolainen V. Darwin's abominable mystery: Insights from a supertree of the angiosperms. Proceedings of the National Academy of Sciences. 2004;101: 1904–1909. doi:10.1073/pnas.0308127100
32. Magallón S, Castillo A. Angiosperm diversification through time. American Journal of Botany. 2009;96: 349–365.
33. Hohmann N, Wolf EM, Lysak MA, Koch MA. A Time-Calibrated Road Map of Brassicaceae Species Radiation and Evolutionary History. The Plant Cell. 2015; tpc.15.00482. doi:10.1105/tpc.15.00482
34. Kinoshita Y, Saze H, Kinoshita T, Miura A, Soppe WJJ, Koornneef M, et al. Control of FWA gene silencing in Arabidopsis thaliana by SINE-related direct repeats. Plant J. 2007;49: 38–45. doi:10.1111/j.1365-313X.2006.02936.x
35. Liu J, He Y, Amasino R, Chen X. siRNAs targeting an intronic transposon in the regulation of natural flowering behavior in Arabidopsis. Genes Dev. 2004;18: 2873–2878. doi:10.1101/gad.1217304
36. Strange A, Li P, Lister C, Anderson J, Warthmann N, Shindo C, et al. Major-Effect Alleles at Relatively Few Loci Underlie Distinct Vernalization and Flowering Variation in Arabidopsis Accessions. PLOS ONE. 2011;6: e19949. doi:10.1371/journal.pone.0019949
37. Chen D, Yan W, Fu L-Y, Kaufmann K. Architecture of gene regulatory networks controlling flower development in Arabidopsis thaliana. Nature Communications. 2018;9. doi:10.1038/s41467-018-06772-3

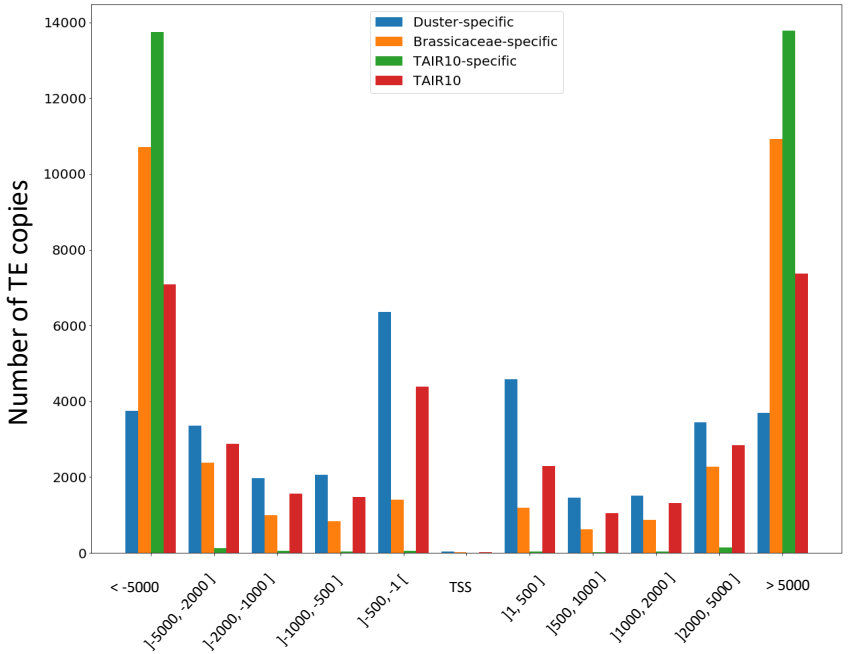
38. Naito K, Zhang F, Tsukiyama T, Saito H, Hancock CN, Richardson AO, et al. Unexpected consequences of a sudden and massive transposon amplification on rice gene expression. Nature. 2009;461: 1130–1134. doi:10.1038/nature08479
39. Mhiri C, Morel J-B, Vernhettes S, Casacuberta JM, Lucas H, Grandbastien M-A. The promoter of the tobacco Tnt1 retrotransposon is induced by wounding and by abiotic stress. Plant Mol Biol. 1997;33: 257–266. doi:10.1023/A:1005727132202
40. Grandbastien M-A, Audeon C, Bonnivard E, Casacuberta JM, Chalhoub B, Costa A-PP, et al. Stress activation and genomic impact of Tnt1 retrotransposons in Solanaceae. Cytogenet Genome Res. 2005;110: 229–241. doi:10.1159/000084957
41. Buchmann RC, Asad S, Wolf JN, Mohannath G, Bisaro DM. Geminivirus AL2 and L2 proteins suppress transcriptional gene silencing and cause genome-wide reductions in cytosine methylation. J Virol. 2009;83: 5005–5013. doi:10.1128/JVI.01771-08
42. Barah P, B N MN, Jayavelu ND, Sowdhamini R, Shameer K, Bones AM. Transcriptional regulatory networks in *Arabidopsis thaliana* during single and combined stresses. Nucleic Acids Research. 2016;44: 3147–3164. doi:10.1093/nar/gkv1463
43. Barabási A-L, Oltvai ZN. Network biology: understanding the cell's functional organization. Nat Rev Genet. 2004;5: 101–113. doi:10.1038/nrg1272
44. Smit A, Hubley R, Green P. RepeatMasker Open-4.0. 2013-2015.
45. Jurka J, Klonowski P, Dagman V, Pelton P. CENSOR--a program for identification and elimination of repetitive elements from DNA sequences. Comput Chem. 1996;20: 119–121.
46. Kohany O, Gentles AJ, Hankus L, Jurka J. Annotation, submission and screening of repetitive elements in Repbase: RepbaseSubmitter and Censor. BMC Bioinformatics. 2006;7: 474. doi:10.1186/1471-2105-7-474
47. Lisch D. How important are transposons for plant evolution? Nat Rev Genet. 2013;14: 49–61. doi:10.1038/nrg3374
48. Bejerano G, Lowe CB, Ahituv N, King B, Siepel A, Salama SR, et al. A distal enhancer and an ultraconserved exon are derived from a novel retroposon. Nature. 2006;441: 87–90. doi:10.1038/nature04696
49. Lowe CB, Bejerano G, Haussler D. Thousands of human mobile element fragments undergo strong purifying selection near developmental genes. Proc Natl Acad Sci USA. 2007;104: 8005–8010. doi:10.1073/pnas.0611223104
50. Wang J, Keightley PD, Halligan DL. Effect of divergence time and recombination rate on molecular evolution of Drosophila INE-1 transposable elements and other candidates for neutrally evolving sites. J Mol Evol. 2007;65: 627–639. doi:10.1007/s00239-007-9028-6
51. Cordaux R, Udit S, Batzer MA, Feschotte C. Birth of a chimeric primate gene by capture of the transposase gene from a mobile element. Proc Natl Acad Sci USA. 2006;103: 8101–8106. doi:10.1073/pnas.0601161103
52. Ackerman H, Udalova I, Hull J, Kwiatkowski D. Evolution of a polymorphic regulatory element in interferon-gamma through transposition and mutation. Mol Biol Evol. 2002;19: 884–890. doi:10.1093/oxfordjournals.molbev.a004145
53. Bundock P, Hooykaas P. An Arabidopsis hAT-like transposase is essential for plant development. Nature. 2005;436: 282–284. doi:10.1038/nature03667
54. Ito H, Gaubert H, Bucher E, Mirouze M, Vaillant I, Paszkowski J. An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. Nature. 2011;472: 115–119. doi:10.1038/nature09861
55. Thieme M, Lanciano S, Balzergue S, Daccord N, Mirouze M, Bucher E. Inhibition of RNA polymerase II allows controlled mobilisation of retrotransposons for plant breeding. Genome Biology. 2017;18. doi:10.1186/s13059-017-1265-4

Fig.1

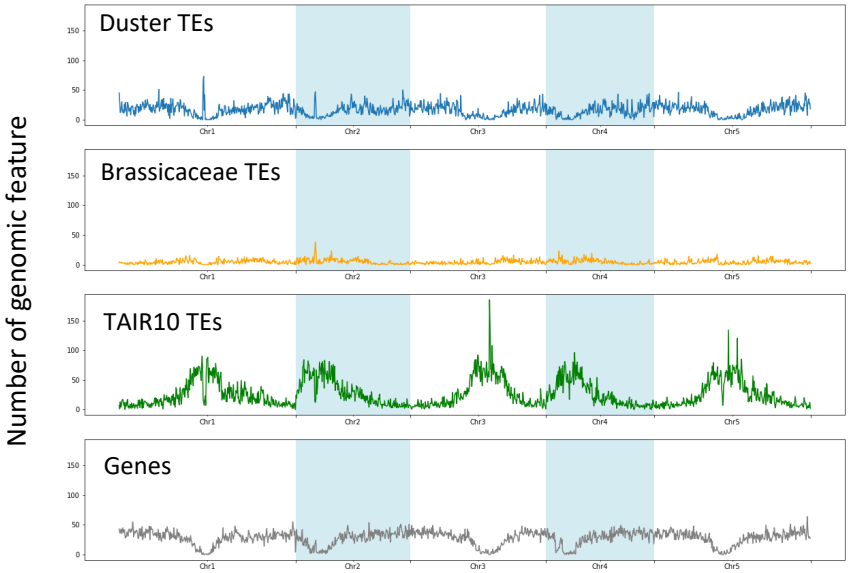
A)

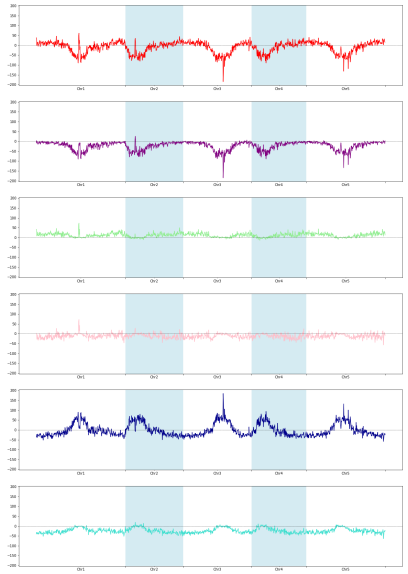


B)



C)





Duster – TAIR10 TEs

Brassicaceae TEs – TAIR10 TEs

Duster – Brassicaceae TEs

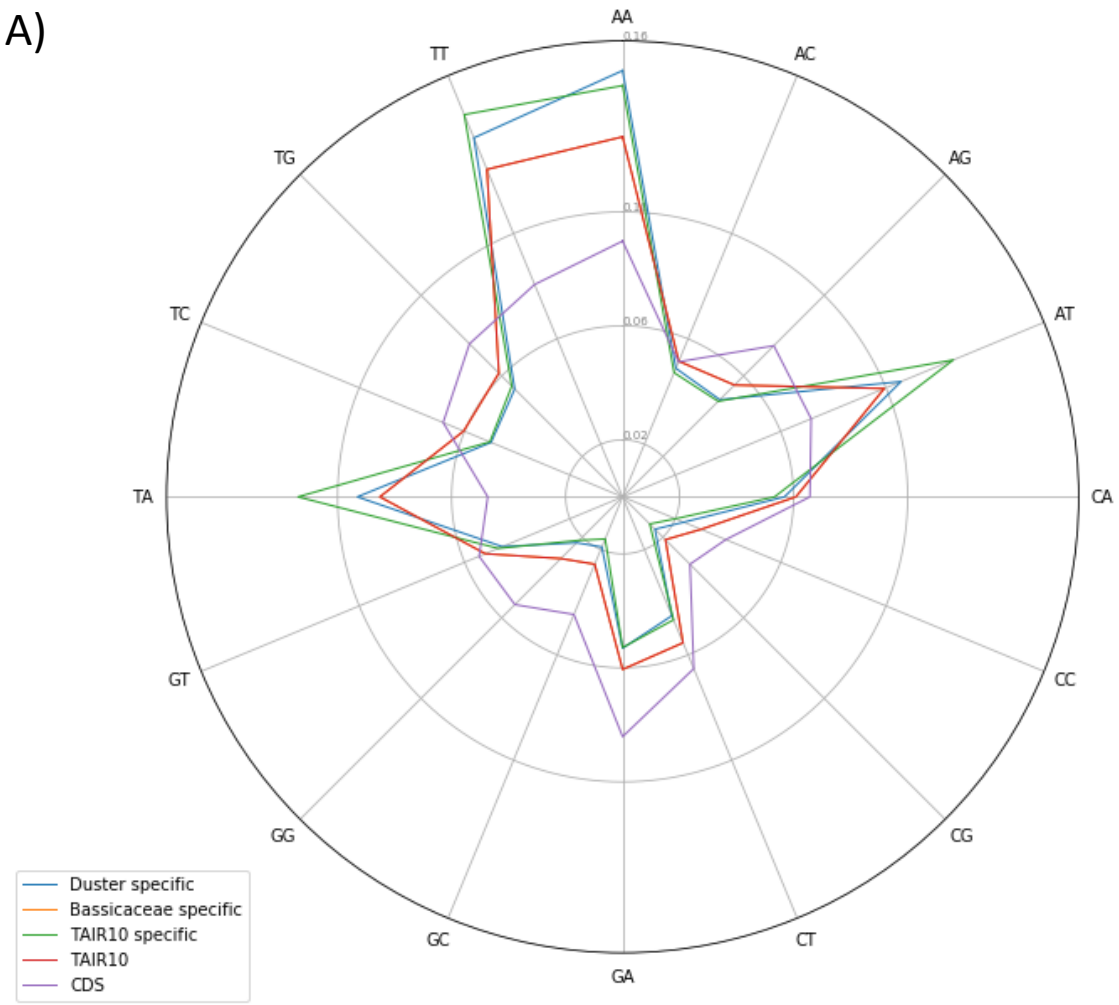
Duster – Genes

Brassicaceae TEs – Genes

TAIR10 TEs – Genes

Fig.2

A)


AA
AC
AG
AT
CA
CC
CG
CT
GA
GC
GG
GT
TA
TC
TG
TT
0.16
0.14
0.06
0.02
Duster specific
Bassicaceae specific
TAIR10 specific
TAIR10
CDS

B)

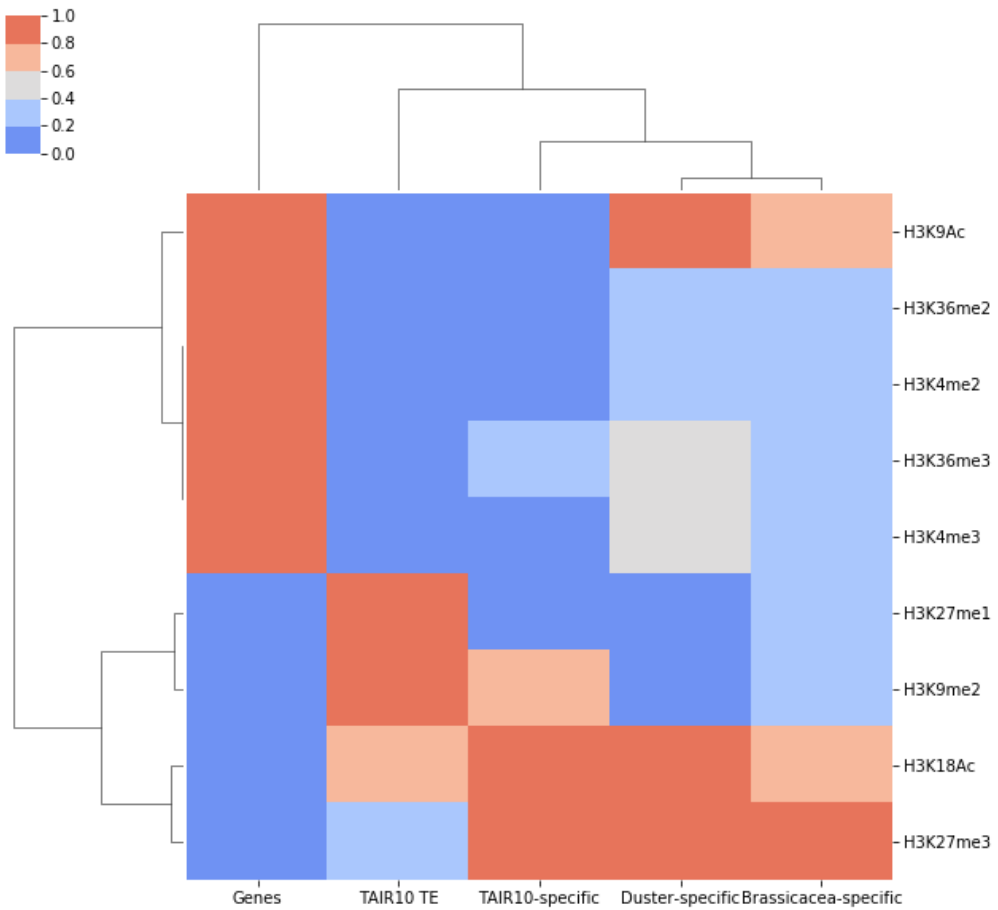
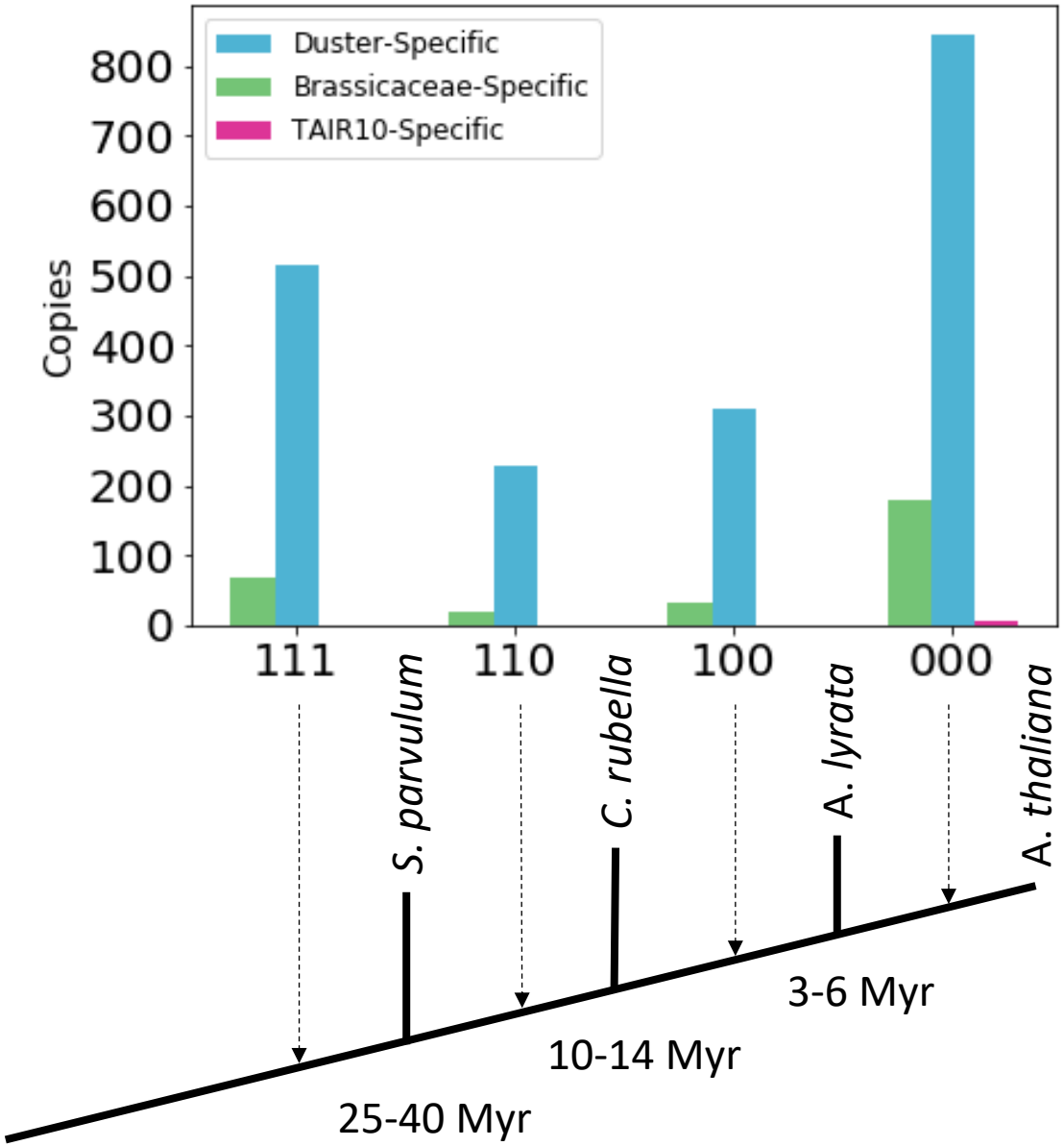

1.0
0.8
0.6
0.4
0.2
0.0
H3K9Ac
H3K36me2
H3K4me2
H3K36me3
H3K4me3
H3K27me1
H3K9me2
H3K18Ac
H3K27me3
Genes
TAIR10 TE
TAIR10-specific
Duster-specific
Brassicacea-specific

Fig.3


Duster-Specific
Brassicaceae-Specific
TAIR10-Specific
Copies
800
700
600
500
400
300
200
100
0
111
110
100
000
S. parvulum
C. rubella
A. lyrata
A. thaliana
3-6 Myr
10-14 Myr
25-40 Myr