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INVESTIGATION

Asymmetric Redundancy of *ZERZAUST* and *ZERZAUST HOMOLOG* in Different Accessions of *Arabidopsis thaliana*

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ABSTRACT Divergence among duplicate genes is one of the important sources of evolutionary innovation. But, the contribution of duplicate divergence to variation in Arabidopsis accessions is sparsely known. Recently, we studied the role of a cell wall localized protein, ZERZAUST (ZET), in Landsberg *erecta* (L*er*) accession, lack of which results in aberrant plant morphology. Here, we present the study of *ZET* in Columbia (Col) accession, which not only showed differential expression patterns in comparison to L*er*, but also revealed its close homolog, *ZERZAUST HOMOLOG (ZETH)*. Although, genetic analysis implied redundancy, expression analysis revealed divergence, with *ZETH* showing minimal expression in both Col and L*er*. In addition, *ZETH* shows relatively higher expression levels in Col compared to L*er*. Our data also reveal compensatory up-regulation of *ZETH* in Col, but not in L*er*, implying it is perhaps dispensable in L*er*. However, a novel CRISPR/Cas9-induced *zeth* allele confirmed that *ZETH* has residual activity in L*er*. Finally, the synergistic interaction of the receptor-like kinase gene, *ERECTA* with *ZET* in ameliorating morphological defects suggests crucial role of modifiers on plant phenotype. The results provide genetic evidence for accession-specific differences in compensation mechanism and asymmetric gene contribution. Thus, our work reveals a novel example for how weakly expressed homologs contribute to diversity among accessions.

KEYWORDS
Arabidopsis accessions
genetic redundancy
asymmetric gene expression
ZERZAUST
ZERZAUST HOMOLOG

How genetic variation translates into phenotypic variation is of immense scientific interest (Weigel 2012). Among others, gene duplication followed by functional divergence is an important source of evolutionary complexity and innovation in multicellular organisms (Ohno 1970; Lynch and Conery 2000; Lynch and Katju 2004). The Arabidopsis genome underwent several duplication events which resulted in large number of homologous genes and regions across the genome (Blanc and Wolfe 2004; Ambrosino *et al.* 2016; Panchy *et al.* 2016). The functional importance of homologs has been demonstrated in various aspects of plant signaling and metabolism (Briggs *et al.* 2006). But, whether and how the differentiation in duplicate gene expression contributes to accession variation in Arabidopsis is not known.

Studies have shown that divergence of many duplicate genes occurs by expression divergence among and within species (Gu *et al.* 2004; Li *et al.* 2005). This phenomenon expands gene regulatory networks and contributes to physiological and morphological diversity (Carroll 2000; Lynch and Conery 2000; Gu *et al.* 2004; Rensing 2014). In Arabidopsis, about two-thirds of duplicates were shown to exhibit expression divergence (Haberer *et al.* 2004). An evolutionary study on gene duplication revealed that duplicate genes show a high degree of variance in expression within species and suggested that this variation partly depends upon the biological function of the gene involved (Kliebenstein 2008). Another study also found high variance of duplicated gene expression between closely related *A. thaliana* and *A. arenosa* (Ha *et al.* 2009).

doi: https://doi.org/10.1534/g3.119.400211
Manuscript received March 25, 2019; accepted for publication May 9, 2019; published Early Online May 21, 2019.

Supplemental material available at FigShare: https://doi.org/10.25387/g3.8152808.
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Functional redundancy among homologs is widespread in Arabidopsis, since several single loss-of-function mutants lack phenotype (Briggs *et al.* 2006). Homologous genes can be either fully or partially redundant. But, when two homologous genes show unequal genetic redundancy, a mutation in one of them causes a phenotype and the phenotype is enhanced when the other homolog is mutated as well. Interestingly, the defect in the other homolog doesn't result in any phenotype on its own. For example, the receptor-like kinase gene *BRASSINOSTEROID INSENSITIVE 1 (BRI1)* is accompanied by its close homolog *BRI1-LIKE1 (BRL1)*. Although *brl1* lacks a mutant phenotype it enhances the severe dwarf phenotype of *bri1* mutants (Caño-Delgado *et al.* 2004). This kind of unequal functional redundancy can be explained by divergence in duplicate expression, however, their perseverance in plant genome is under debate given the dispensable nature of the duplicate.

Genetic factors involved in plant morphogenesis will have crucial role in the differentiation of various Arabidopsis accessions. Tissue morphogenesis in Arabidopsis requires the cell wall-localized GPI-anchored β-1,3 glucanase ZERZAUST (ZET) (Vaddepalli *et al.* 2017). *ZET* was initially identified as a genetic component of the *STRUBBELIG (SUB)* signaling pathway along with QUIRKY, a C2 domain containing protein (Fulton *et al.* 2009). Absence of *ZET* results in a so-called *strubbelig-like mutant* (*slm*) phenotype characterized by abnormal integument initiation and outgrowth, aberrant floral organ and stem morphology, reduced plant height and irregular leaf shape.

Our previous studies have shown that mutations in *SUB* and *QKY* in Col background result in obvious *slm* mutant phenotypes similar to their respective mutants in L*er* background (Fulton *et al.* 2009; Vaddepalli *et al.* 2011, 2014). But in the current work, we discovered that *ZET* acts differently in Col accession due to the presence of the close homolog *ZERZAUST HOMOLOG* (*ZETH*). Using genetic and gene expression tools, we show how *ZET* and *ZETH* diverged between the two common laboratory accessions Col and L*er* in terms of expression and function. Furthermore, we try to understand the contribution of the weakly expressing redundant homolog on the morphological diversity of accessions.

MATERIALS AND METHODS

Plant work, Plant Genetics and Plant Transformation

Arabidopsis thaliana (L.) Heynh. var. Columbia (Col-0) and var. Landsberg (*erecta* mutant) (L*er*) were used as wild-type strains. Plants were grown as described earlier (Fulton *et al.* 2009). The *zet-1* mutant was described previously (Vaddepalli *et al.* 2017). T-DNA insertion lines were received from the GABI-KAT (*zet-3*, GABI-KAT-460G06) (Kleinboelting *et al.* 2012) and Wisconsin collections (*zet-4*, WiscDsLoxHs057_03H; *zeth-1*, WiscDsLoxHs066_12G) (Sussman *et al.* 2000). Plants were transformed with different constructs using Agrobacterium strain GV3101/pMP90 (Koncz and Schell 1986) and the floral dip method (Clough and Bent 1998). Transgenic T1 plants were selected on Hygromycin (20 μg/ml) or Glufosinate (Basta) (10 μg/ml) plates and transferred to soil for further inspection. The figures represent the phenotyping analysis performed on at least 4 to 6 plants for each genotype.

Recombinant DNA work

For DNA and RNA work standard molecular biology techniques were used. PCR-fragments used for cloning were obtained using Phusion high-fidelity DNA polymerase (New England Biolabs, Frankfurt, Germany) or TaKaRa PrimeSTAR HS DNA polymerase (Lonza, Basel, Switzerland). PCR fragments were subcloned into pLitmus 28i (NEB). All PCR-based constructs were sequenced. Primer sequences used for cloning and qRT PCR in this work are listed in S1 Table.

Cloning

Genomic fragments of *ZETH* were amplified from Col-0 and L*er* backgrounds using primers ZETHCol_F/ZETHCol_R and ZETHL*er*_F/ZETHL*er*_R and sub cloned into pZET::TS:ZET (Vaddepalli *et al.* 2017) using *Xma*I/*Bam*HI restriction sites to obtain pZET::TS:ZETHCol and pZET::TS:ZETHL*er* respectively. For CRISPR/cas9 ZETH construct, the egg cell-specific promoter-controlled CRISPR/Cas9 system was used as described (Wang *et al.* 2015). *zet-1* plants were transformed with the CRISPR/cas9 ZETH construct by floral dip method. T1 plants were screened for exaggerated phenotype and *ZETH* locus was sequenced for identifying the mutation.

Quantitative RT-PCR analysis

Tissue for quantitative real-time PCR (qPCR) was harvested from plants grown in long day conditions. With minor changes, tissue collection, RNA extraction and quality control were performed as described previously (Box *et al.* 2011). RT-PCR was performed on Biorad CFX96 by using iQ SYBR Green Supermix (Bio-Rad) according to the manufacturer's recommendations. All expression data were normalized against reference genes At5g25760, At4g33380, and At2g28390 by using the ΔΔ-Ct method (Czechowski 2007). Experiments were performed in biological and technical triplicates.

Data availability

Strains and plasmids are available upon request. The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article. Supplemental material available at FigShare: https://doi.org/10.25387/g3.8152808.

RESULTS

Molecular identification of ZETH

In L*er* background, *zet-1* carrying a loss-of-function mutation in *ZET* locus (At1g64760) (Figure 1A,B), shows a strong *slm* mutant phenotype (Vaddepalli *et al.* 2017) (Figure 1D,F). Except one amino acid in the signal peptide, ZET shows no difference between Col and L*er*. We investigated the functionality of *ZET* in Columbia accession by analyzing two available T-DNA insertion lines (*zet-3* and *zet-4*) (Figure 1A). We expected the T-DNA insertion in *zet*-4 to cause a mutant phenotype as it is predicted to result in a truncated ZET protein (Figure 1A,B). But, the plants surprisingly failed to display the twisted morphology, characteristic of *slm* mutants (Fig. S1B). We asked, if the observed accession-specific phenotypic differences could relate to a close homolog of *ZET*. An NCBI BLAST search with the *ZET* coding sequence revealed that *ZET* is most closely related to At2g19440 with 89% identity at the amino acid level (Fig. S1A). We named this gene *ZERZAUST HOMOLOG* (*ZETH*). *ZET* and *ZETH* form a subclade within the larger β clade of β-1,3 glucanase (BG) genes, which comprises 11 members (Doxey *et al.* 2007; Gaudioso-Pedraza and Benitez-Alfonso 2014). Sequence-based analysis of the evolutionary history revealed that ZET and ZETH duplication is specific to species within the Arabidopsis lineage (Fig. S2).

To assess ZETH activity in Col, we investigated a T-DNA line (*zeth-1*), which is presumed to carry a truncated protein (Figure 1A,B). But, like *zet-4*, the *zeth-1* insertion line also failed to show a mutant phenotype (Fig. S1C). However, the *zet-4* and *zeth-1* double mutant exhibited a strong phenotype (Figure 1G-I, S1D) suggesting that the two genes act redundantly. The double mutant resembled *zet-1*

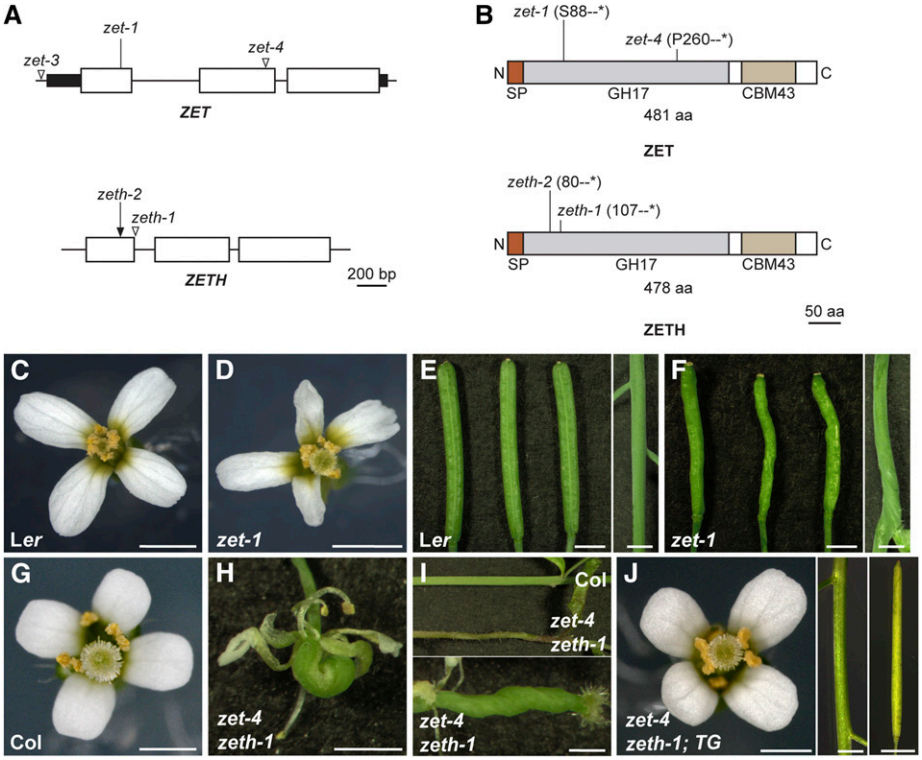

Figure 1 Molecular and genetic characterization of *ZET* and *ZETH*. (A) Cartoon depicting the genomic organization of *ZET* and *ZETH*. Horizontal lines represent introns. Filled rectangles mark untranslated regions. The positions of EMS-, CRISPR-, and T-DNA-induced mutations are denoted by lines, arrow, and open triangles, respectively. (B) Schematic view of the predicted ZET protein. The signal peptide (SP), GH17 and CBM43/X8 domains are highlighted. The position and effects of the *zet* mutations are indicated. Phenotypic analysis (C-J). Comparison of (C, E) L*er*. (D, F) *zet-1*, (G) Col and (H, I) *zet-4 zeth-1*. Note the aberrant flower (D, H), stem and silique (F, I) morphology of mutants. Mutant phenotype is strong in *zet-4 zeth-1* double mutant (H, I). (J) *zet-4 zeth-1* double mutant phenotype is complemented by *pZET::TS:ZET* (*TG*). Scale bars:1 mm.

plants except for appearing less bushy but with exaggerated twisting of flowers and increased sterility. Nevertheless, we could complement the double mutant plants by introducing a construct encoding a translational fusion of ZET to the GFP variant T-Sapphire driven by the endogenous *ZET* promoter (pZET::TS:ZET: *zet-4 zeth-1* 4/4 T1 plants), ruling out the contribution of any other background mutation (Figure 1J). This construct was used in a previous study to complement *zet-1* mutants in L*er* background (Vaddepalli *et al.* 2017).

Accession-specific regulation of ZET and ZETH expression

Next, we analyzed the expression pattern of *ZET* and *ZETH* in Col and L*er* accessions to assess the cause for the mutant phenotype disparities between the two accessions. Our qPCR data from various tissues revealed that these genes are co-expressed (Figure 2A). Surprisingly, we found much lower levels of *ZETH* transcripts in comparison to *ZET*. Moreover, *ZETH* expression was even further reduced in L*er* where it was barely detectable in rosette leaves, stems, or flowers. Additional qPCR tests revealed that *ZET* and *ZETH* expression levels in seedlings and flowers undergo compensatory regulation in the *zeth-1* and *zet-4* mutants in Col, respectively (Figure 2B). This result provides evidence for redundant functions of *ZET* and *ZETH* in the Col background and thus offers a convenient explanation for the lack of phenotype in single mutants in this accession. Interestingly, this compensatory regulation appears to be absent in flowers of L*er* accession since *ZETH* expression was not detectably upregulated in *zet-1* mutant.

Ler carries a functional ZETH gene

Despite minimal *ZETH* expression profiles in both accessions, appearance of prominent phenotypes only in L*er*, when *zet* is mutated, can be attributed to any or all of the following reasons. It could be because of the low expression of *ZETH*, the lack of compensatory mechanism, or the L*er* version of ZETH exhibiting a different amino acid composition when compared to Col which might affect its activity. The L*er*/Col variants of ZET differ by only one amino acid at position 3 in the predicted signal peptide (change from an asparagine to a lysine) but there are nine amino acid differences between the L*er*/Col variants of ZETH (Figure 3A). We wanted to test if these changes affect the activity of ZETH in L*er*. For this purpose, we replaced the *ZET* coding sequence 3′ to the predicted signal peptide with the equivalent L*er* or Col variants of *ZETH* sequence in our complementing *pZET::TS:ZET* reporter (Vaddepalli *et al.* 2017). This resulted in *zet-1* plants transgenic for the L*er* or Col variants of *ZETH*, under the control of the native *ZET* promoter (*pZET::TS:ZETHL/C zet-1*). Interestingly, the T1 plants of the transgenic *zet-1* lines exhibited a wild-type phenotype with both variants (Figures 3B-G) (80/80 T1 plants (*TS:ZETHL*), 167/172 T1 plants (*TS:ZETHC*)) indicating that the accession-specific amino acid alterations do not affect ZETH function.

Although *ZETH* of L*er* is functional, its expression is quite weak indicating its functional contribution is perhaps insignificant. But, *zet1 zeth-1* double mutants in Col accession exhibit a stronger phenotype compared to *zet-1* (L*er*) (Figure 1G-J). These interesting observations prompted us to check whether *ZETH* has some residual activity in L*er* even though its expression is very low. Using CRISPR\Cas9 technique (Wang *et al.* 2015) we generated a mutation in the first exon of *ZETH* in the *zet-1* background. The novel allele *zeth-2* is predicted to result in a truncated ZETH protein consisting of only the first 80 amino acids (Figure 1A,B). Surprisingly, *zet-1 zeth-2* double mutant plants in L*er* showed an exaggerated *zet-1* phenotype and appeared closer to *zet-4 zeth-1* double mutants in the Columbia background (Figure 3H-J). The finding indicates that the weakly expressed *ZETH* in L*er* exhibits residual activity, which is insufficient to fully substitute for the lack of *ZET*.

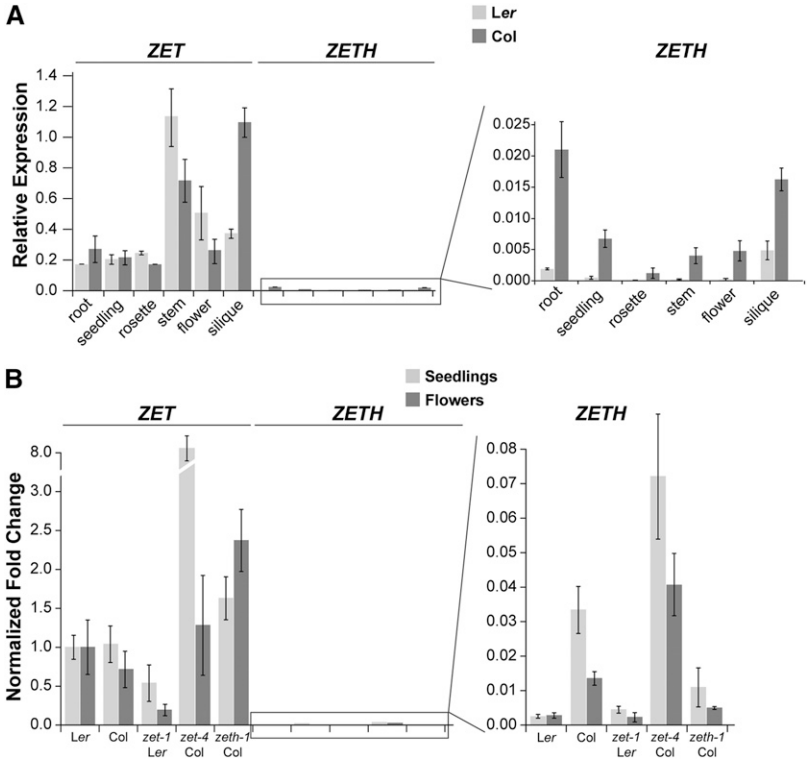

Figure 2 Expression analysis of *ZET* and *ZETH*. (A) Tissue distribution of *ZET* and *ZETH* transcript expression levels by qPCR. (n= 3 biological replicates). Means ± SEMs are indicated. Age of plants: roots and seedlings, 10 days; rosette, 3 weeks; stem, flowers (stages 1-12) and siliques (stage 17), 5 weeks. (B) Comparison of *ZET* and *ZETH* mRNA levels in seedlings and stage 1-12 flowers of indicated mutants by qPCR. (n= 3 biological replicates). Means ± SEMs are indicated.

ZETH acts in a dose dependent manner

Thus far, our results have established the functional role for the weakly expressed *ZETH* in both Col and L*er* accessions, implying small amount of ZETH protein can have a noticeable impact. Next, we asked if this gene is acting in a dose-dependent manner. For this purpose, we checked the effect of *ZETH* gene copy number on *zet* mutant phenotype (Figure 4). Interestingly in the L*er* background, *zet-1 zeth-2*/+ displayed an intermediate phenotype between the single *zet-1* mutant and the double *zet-1 zeth-2* mutant. The leaf petioles are somewhat elongated in *zet-1* with narrow blades. This phenotype got slightly enhanced in *zet-1 zeth-2*/+ background, whereas *zet-1 zeth-2* double mutants show further worsening of the phenotype. The phenomenon of dosage-dependent enhancement of mutant phenotype was also observed for floral organs and was particularly obvious for siliques depending on the *ZETH* copy number. Surprisingly, in Columbia background the *zet-4 zeth-1*/+ mutant showed wild type morphology in all the organs tested except siliques which displayed shortening of length with no twisting. Despite these peculiarities between accessions, the observations indicate that mutation in *zeth* contributes to the overall exaggerated morphologies of double mutants in a dose dependent manner. Our results also imply that the extent of the *ZETH* effect on plant morphology is accession dependent.

ERECTA influences the zet-1 phenotype

Col and L*er* accessions display obvious discrepancies in their phenotypic appearance. For example, L*er* exhibits shorter stems and more compact inflorescences (Passardi *et al.* 2007). The phenotypic differences could be ascribed to genetic variability between the accessions and the contribution of accession-specific modifiers needs to be addressed. To further understand the accession specific effects on *zet* mutant phenotype, we crossed *zet-1* (L*er*) with *zet-4 zeth-1*/+ (Col). In the resulting F1 population plants with *zet-1* (L*er*) /*zet-4* (Col) displayed WT phenotype as expected (Figure 5A and C), whereas *zet-1 ZETH* (L*er*) /*zet-4 zeth-1* (Col) displayed twisted flowers and siliques, but interestingly the stem twisting was absent and plant height was normal (Figure 5B and C). Major genetic difference between the Col and L*er* accessions is the lack of *ERECTA* (*ER*) in the L*er* background, which is present in the *zet-1 ZETH* (L*er*) /*zet-4 zeth-1* (Col) genotype. Previous work revealed that *SUB* and *QKY* show a synergistic interaction with *ERECTA* (*ER*) with respect to the control of plant height (Vaddepalli *et al.* 2011, 2014). We investigated the phenotype of *zet-1* (L*er*) plants transformed with pKUT196, a plasmid carrying 9.3 kb of Col-0 DNA spanning the entire genomic *ER* locus (Torii *et al.* 1996; Godiard *et al.* 2003). Like *zet-1 ZETH* (L*er*) /*zet-4 zeth-1* (Col) genotype, *zet-1 ER* transgenic plants also exhibited ameliorated plant height and stem twisting whereas aberrant floral organ phenotype appeared unaffected by the *ER* transgene (Figure 5). Our result exemplifies the influence of accession-specific modifiers on plant morphology.

DISCUSSION

Numerous studies have shown the compensation of gene loss by duplicate genes implying that close homologs give robustness to the plants against mutations (Hanada *et al.* 2009). Studies have also shown stronger reduction in duplicate expression and this expression divergence was noted as an important innovation for conservation of the duplicate gene (Ganko *et al.* 2007; Panchy *et al.* 2016). Despite acknowledging this interesting pattern, examples are missing that show functional relevance of gene duplicates, since retention of almost identical duplicates goes against the evolutionary instability of genetic redundancy (Lynch and Conery 2000). Here, our work reveals an unexpected variation of a weakly expressed gene and its homolog between accessions. Our results imply that divergence in duplicate expression may play a crucial role in accession-specific genetic variations.

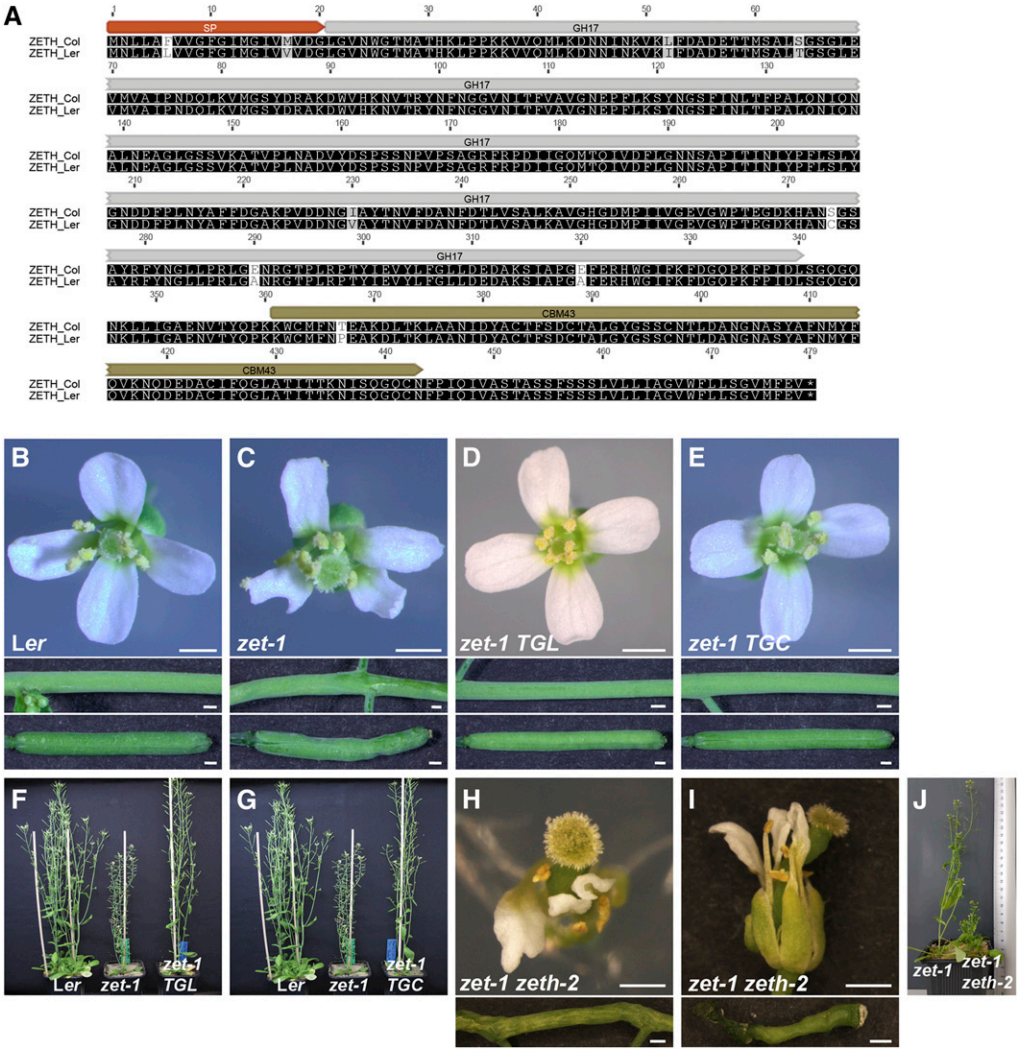

Figure 3 *ZETH* in L*er* has residual function. (A) Alignment of ZETH amino acid sequences of Col and L*er*. (B-E) Complementation of *zet-1* phenotype by two TS:ZET reporter constructs. Upper panels: Stage 13 flower. Middle panel: siliques. Bottom panel: Stem. Genotypes are indicated. (C) Note aberrant floral morphology. Siliques and stems are twisted. (D, E) Note normal phenotype of a *zet-1* plant carrying either the L*er* or Col variant of *ZETH* under the control of the endogenous *ZET* (L*er*) promoter (TGL: *pZET*::*TS:ZETH*/L*er*, TGC: *pZET*::*TS:ZETH*/Col). (F, G) Whole-plant appearance. Genotypes are indicated. (H-J) Phenotype of *zet-1 zeth-2* (L*er*) mutant. Mutant phenotype is exaggerated. Scale bars: 0.5 mm.

Redundancy between duplicate genes by a compensation mechanism via feedback responsive circuit serves as an advantage for biological systems to deal with stochastic fluctuations in signaling pathways (Kafri *et al.* 2006, 2009). In the Col background, an overall higher expression level of *ZETH*, in combination with a compensatory upregulation in *zet-4* mutants seems to account for their wild-type appearance. Although this phenomenon is absent in L*er*, the residual expression of *ZETH* in L*er* seems to be above the threshold, otherwise the *zet-1 zeth-2* double mutant would have resembled the single mutant *zet-1*. Furthermore, understated changes in expression pattern may further enhanced by tissue level sampling. Although segregating mutants in Col background appear to be fine morphologically, we may have missed subtler cellular phenotypes in our analysis. All the experiments were performed in controlled lab conditions. There is also a possibility that *ZETH* may express at higher levels under different conditions and reveal a novel function. Indeed, the role of SUB in coordinating cell proliferation and differentiation during leaf development was revealed only at high ambient temperature of 30° (Lin *et al.* 2012).

Initially, we assumed *ZETH* in L*er* background as a pseudogene, since a previous RNA-seq analysis has found that *ZETH* transcripts were undetectable in young L*er* flowers (Jiao and Meyerowitz 2010). Although our qRT-PCR results showed *ZETH* expression, the functionality was still in question given the very low expression pattern in all the tissues tested. But, surprisingly our results indicate that weakly expressed *ZETH* is functionally very relevant. Our results also highlight the importance of gene specific analysis, since in large scale studies differentiating between identical duplicate sequences is challenging. Thus, duplicate genes with low expression levels are often overlooked, as the focus goes inadvertently on highly expressed genes.

If minimal *ZETH* expression in Col was enough for wild type appearance of *zet-4* mutants, then the high *ZET* expression implies that it may have additional roles apart from development. Transcriptome analysis performed on *slms* showed that several *ZET* responsive genes are related to biotic and abiotic stress responses (Fulton *et al.* 2009). Since *ZET* is localized to cell wall, it is intriguing to speculate that the high expression of *ZET* may have a role in dealing with imminent stress situations which demand instant response. Future experiments could test this possibility by assessing if *zet* is more susceptible to stress compared to *zeth* or vice versa. Interestingly, *ZET* transcript level was shown to be altered in Arabidopsis plants infected with *Fusarium oxysporum* (Fallath *et al.* 2017). Thus, further analysis of *ZET* may reveal its potential role in adaptation, apart from morphogenesis.

Duplicate genes provide mutational robustness to living organisms. In yeast and *Caenorhabditis elegans*, functional compensation by the

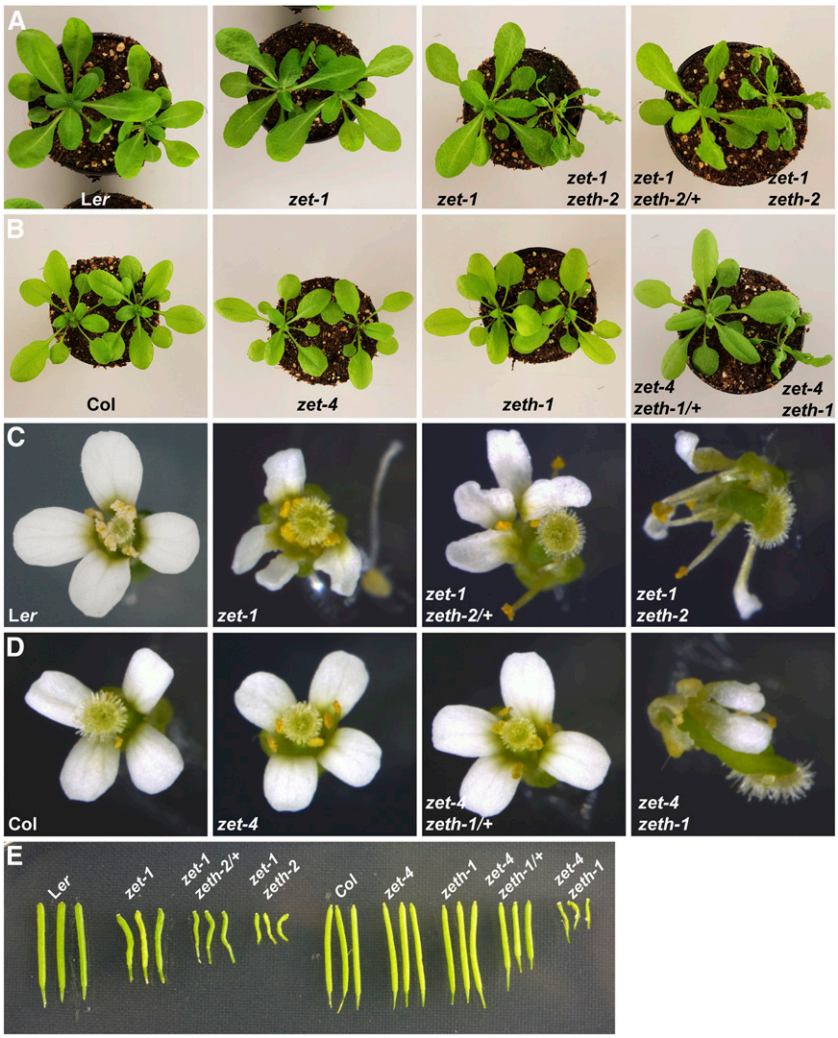

Figure 4 *ZETH* acts in dosage dependent manner. Genotypes are indicated. (A, B) Rosette leaves of three-week-old plants. (A) Notice slightly elongated petiole and narrow leaf blade phenotype in *zet-1*, which gets enhanced in *zet-1 zeth-2/+* and *zet-1 zeth-2* mutants. (B) In Col background aberrant morphology is apparent only in double mutants *zet-4 zeth-1*. (C, D) Flowers. (E) Siliques. Similar to leaves, twisting morphology of flowers and siliques is exaggerated progressively in L*er* mutants, but in Col only double mutant shows phenotype with the exception of siliques. Siliques of *zet-4 zeth-1/+* in Col are shorter.

duplicated gene displayed higher robustness to gene perturbation than singletons (Gu *et al.* 2003; Conant and Wagner 2004). But, *ZETH* showed a highly reduced expression pattern compared to *ZET*. Such a reduction in expression was proposed to facilitate the retention of duplicates and the conservation of their ancestral functions (Qian *et al.* 2010). In this scenario, loss of either of the duplicate genes renders the total expression level lower than normal which would hamper the function. This also inhibits functional divergence of duplicated genes and helps in rebalancing gene dosage after duplication. Interestingly, with *ZET* this phenomenon was observed only in L*er*, where mutating *ZET* was enough for the manifestation of phenotype, but not in the Col background. These results indicate that the divergent behavior of duplicates may vary depending on the accession and the specific gene pair under study. Since the *ER* locus was able to partially alleviate *zet-1* mutant phenotype, the influence of accession-specific differences on gene contribution needs to be considered. Further, it would be interesting to know if there exists a correlation between accession-specific modifiers and expression divergence of certain duplicates.

A study on homologs revealed significant diversity in expression pattern among different accessions of Arabidopsis (Kliebenstein 2008). For instance, background specific regulation and unequal genetic redundancy has been observed for *BRI1* (Caño-Delgado *et al.* 2004; Zhou *et al.* 2004). In another example, the sucrose transporter AtSUC1 was shown to have differential tissue expression pattern depending on the accession it was tested (Feuerstein *et al.* 2010). The observed accession-specific disparities in *ZETH* expression levels may relate to differences in its *cis*-regulatory region, either caused by DNA polymorphisms, as was found for the tomato *fw2.2*, rice *qSH1*, or Arabidopsis *FLOWERING LOCUS* (*FT*) loci (Konishi *et al.* 2006; Cong *et al.* 2008; Schwartz *et al.* 2009; Liu *et al.* 2014), or by epigenetic variation (Durand *et al.* 2012). Thus, our work provides an interesting example for the diversification of cis and/or trans regulatory elements between two Arabidopsis accessions. A correlation between duplicate divergence and evolution of *cis*-regulatory elements and networks was observed (Arsovski *et al.* 2015). But, how the transcriptional networks regulate the levels of the duplicated genes and their role in the context of evolution is largely unexplored. Whole genome analysis and comparison of L*er* with reference sequence of Col revealed immense sequence differences between the two accessions with hundreds of diversified regions and unknown accession specific genes (Zapata *et al.* 2016). But how these differences contribute to natural variation in terms of phenotype and adaptation is a challenging task and requires novel approaches. Thus, our study gives an example of how variation in duplicates involved in morphogenesis could serve as a great tool to understand their contribution in natural variation among difference accessions of Arabidopsis.

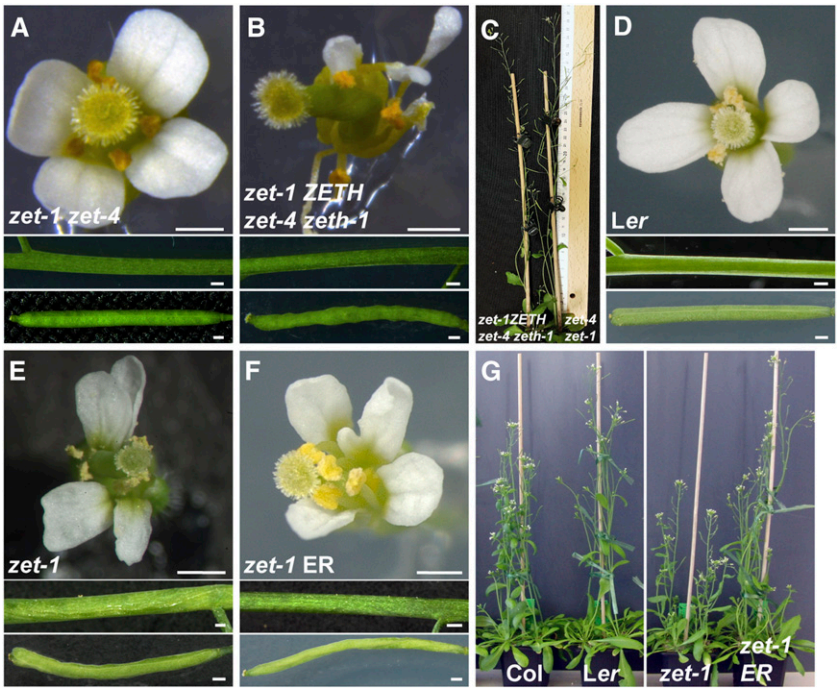

Figure 5 The *zet-1* phenotype in the presence of functional *ERECTA* (*ER*). (A, B, D-F) Morphology of flowers (upper panel), stems (central panel) and siliques (bottom panel). (A) *zet-1* (*Ler*)/*zet-4* (Col). Morphology looks essentially wild type. (B) *zet-1 ZETH* (*Ler*)/*zet-4 zeth-1* (Col). Note the irregular flower and silique morphology. Stem morphology is essentially normal. (C) Plant height comparisons of six-week-old *zet-1 ZETH* (*Ler*)/*zet-4 zeth-1* (Col) and *zet-1* (*Ler*)/*zet-4* (Col) respectively. (D) Wild-type *Ler*. (E) *zet-1*. (F) Transgenic *zet-1 ER*. Flower and silique morphology is irregular. Stem shows wild type morphology. (G) Plant height comparisons of six-week-old *zet-1 ER* transgenic plants in comparison to wild type and mutant reference lines. Note the rescue of plant height in *zet-1 ER* plants. Scale bars: (A, B and D-F) 0.5 mm.

ACKNOWLEDGMENTS

We acknowledge Maxi Oelschner for technical assistance. We also thank Dolf Weijers and Sumanth Mutte for their constructive feedback on the manuscript. This work was funded by the German Research Council (DFG) through grants SCHN 723/6-1 and SFB924 (TP A2) to KS and VA 1156/1-1 to PV.

LITERATURE CITED

Ambrosino, L., H. Bostan, P. di Salle, M. Sangiovanni, A. Vigilante *et al.*, 2016 pATsi: Paralogs and Singleton Genes from Arabidopsis thaliana. Evol. Bioinform. Online 12: 1–7. https://doi.org/10.4137/EBO.S32536

Arsovski, A. A., J. Pradinuk, X. Guo, S. Wang, and K. L. Adams, 2015 Evolution of Cis-Regulatory Elements and Regulatory Networks in Duplicated Genes of Arabidopsis. Plant Physiol. 169: 2982–2991.

Blanc, G., and K. H. Wolfe, 2004 Widespread Paleopolyploidy in Model Plant Species Inferred from Age Distributions of Duplicate Genes. Plant Cell Online 16: 1667–1678. https://doi.org/10.1105/tpc.021345

Box, M. S., V. Coustham, C. Dean, and J. S. Mylne, 2011 Protocol: A simple phenol-based method for 96-well extraction of high quality RNA from Arabidopsis. Plant Methods 7: 7. https://doi.org/10.1186/1746-4811-7-7

Briggs, G. C., K. S. Osmont, C. Shindo, R. Sibout, and C. S. Hardtke, 2006 Unequal genetic redundancies in Arabidopsis – a neglected phenomenon? Trends Plant Sci. 11: 492–498. https://doi.org/10.1016/j.tplants.2006.08.005

Caño-Delgado, A., Y. Yin, C. Yu, D. Vafeados, S. Mora-García *et al.*, 2004 BRL1 and BRL3 are novel brassinosteroid receptors that function in vascular differentiation in Arabidopsis. Development 131: 5341–5351. https://doi.org/10.1242/dev.01403

Carroll, S. B., 2000 Endless Forms: The Evolution of Gene Regulation and Morphological Diversity. Cell 101: 577–580. https://doi.org/10.1016/S0092-8674(00)80868-5

Clough, S. J., and A. F. Bent, 1998 Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16: 735–743. https://doi.org/10.1046/j.1365-313x.1998.00343.x

Conant, G. C., and A. Wagner, 2004 Duplicate genes and robustness to transient gene knock-downs in Caenorhabditis elegans. Proc Royal Soc Lond Ser B Biological Sci 271: 89–96. https://doi.org/10.1098/rspb.2003.2560

Cong, B., L. S. Barrero, and S. D. Tanksley, 2008 Regulatory change in YABBY-like transcription factor led to evolution of extreme fruit size during tomato domestication. Nat. Genet. 40: 800–804. https://doi.org/10.1038/ng.144

Czechowski, T., 2007 Genome-Wide Identification and Testing of Superior Reference Genes for Transcript Normalization in Arabidopsis. Plant Physiol. 139: 5–17. https://doi.org/10.1104/pp.105.063743

Doxey, A. C., M. W. Yaish, B. A. Moffatt, M. Griffith, and B. J. McConkey, 2007 Functional divergence in the Arabidopsis β-1, 3-glucanase gene family inferred by phylogenetic reconstruction of expression states. Mol. Biol. Evol. 24: 1045–1055. https://doi.org/10.1093/molbev/msm024

Durand, S., N. Bouché, E. Strand, O. Loudet, and C. Camilleri, 2012 Rapid Establishment of Genetic Incompatibility through Natural Epigenetic Variation. Curr. Biol. 22: 326–331. https://doi.org/10.1016/j.cub.2011.12.054

Fallath, T., B. N. Kidd, J. Stiller, C. Davoine, S. Björklund *et al.*, 2017 MEDIATOR18 and MEDIATOR20 confer susceptibility to Fusarium oxysporum in Arabidopsis thaliana. PLoS One 12: e0176022. https://doi.org/10.1371/journal.pone.0176022

Feuerstein, A., M. Niedermeier, K. Bauer, S. Engelmann, S. Hoth *et al.*, 2010 Expression of the AtSUC1 gene in the female gametophyte, and ecotype-specific expression differences in male reproductive organs. Plant Biol. 12: 105–114. https://doi.org/10.1111/j.1438-8677.2010.00389.x

Fulton, L., M. Batoux, P. Vaddepalli, R. Yadav, W. Busch *et al.*, 2009 DETORQUEO, QUIRKY, and ZERZAUST represent novel components involved in organ development mediated by the receptor-like kinase STRUBBELIG in Arabidopsis thaliana. PLoS Genet. 5: e1000355. https://doi.org/10.1371/journal.pgen.1000355

Ganko, E. W., B. C. Meyers, and T. J. Vision, 2007 Divergence in Expression between Duplicated Genes in Arabidopsis. Mol. Biol. Evol. 24: 2298–2309. https://doi.org/10.1093/molbev/msm158

Gaudioso-Pedraza, R., and Y. Benitez-Alfonso, 2014 A phylogenetic approach to study the origin and evolution of plasmodesmata-localized glycosyl hydrolases family 17. Front. Plant Sci. 5: 212. https://doi.org/10.3389/fpls.2014.00212

Godiard, L., L. Sauviac, K. U. Torii, O. Grenon, B. Mangin *et al.*, 2003 ERECTA, an LRR receptor-like kinase protein controlling development pleiotropically affects resistance to bacterial wilt. Plant J. 36: 353–365. https://doi.org/10.1046/j.1365-313X.2003.01877.x

Gu, Z., S. A. Rifkin, K. P. White, and W.-H. Li, 2004 Duplicate genes increase gene expression diversity within and between species. Nat. Genet. 36: 577–579. https://doi.org/10.1038/ng1355

Gu, Z., L. M. Steinmetz, X. Gu, C. Scharfe, R. W. Davis *et al.*, 2003 Role of duplicate genes in genetic robustness against null mutations. Nature 421: nature01198. https://doi.org/10.1038/nature01198

Haberer, G., T. Hindemitt, B. C. Meyers, and K. Mayer, 2004 Transcriptional Similarities, Dissimilarities, and Conservation of cis-Elements in Duplicated Genes of Arabidopsis. Plant Physiol. 136: 3009–3022. https://doi.org/10.1104/pp.104.046466

Ha, M., E.-D. Kim, and J. Z. Chen, 2009 Duplicate genes increase expression diversity in closely related species and allopolyploids. Proc. Natl. Acad. Sci. USA 106: 2295–2300. https://doi.org/10.1073/pnas.0807350106

Hanada, K., T. Kuromori, F. Myouga, T. Toyoda, W.-H. Li *et al.*, 2009 Evolutionary Persistence of Functional Compensation by Duplicate Genes in Arabidopsis. Genome Biol. Evol. 1: 409–414. https://doi.org/10.1093/gbe/evp043

Jiao, Y., and E. M. Meyerowitz, 2010 Cell-type specific analysis of translating RNAs in developing flowers reveals new levels of control. Mol. Syst. Biol. 6: 419. https://doi.org/10.1038/msb.2010.76

Kafri, R., M. Levy, and Y. Pilpel, 2006 The regulatory utilization of genetic redundancy through responsive backup circuits. Proc. Natl. Acad. Sci. USA 103: 11653–11658. https://doi.org/10.1073/pnas.0604883103

Kafri, R., M. Springer, and Y. Pilpel, 2009 Genetic Redundancy: New Tricks for Old Genes. Cell 136: 389–392. https://doi.org/10.1016/j.cell.2009.01.027

Kleinboelting, N., G. Huep, A. Kloetgen, P. Viehoever, and B. Weisshaar, 2012 GABI-Kat SimpleSearch: new features of the Arabidopsis thaliana T-DNA mutant database. Nucleic Acids Res. 40: D1211–D1215. https://doi.org/10.1093/nar/gkr1047

Kliebenstein, D. J., 2008 A Role for Gene Duplication and Natural Variation of Gene Expression in the Evolution of Metabolism. PLoS One 3: e1838. https://doi.org/10.1371/journal.pone.0001838

Koncz, C., and J. Schell, 1986 The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of Agrobacterium binary vector. MGG Molecular & General Genetics 204: 383–396. https://doi.org/10.1007/BF00331014

Konishi, S., T. Izawa, S. Lin, K. Ebana, Y. Fukuta *et al.*, 2006 An SNP Caused Loss of Seed Shattering During Rice Domestication. Science 312: 1392–1396. https://doi.org/10.1126/science.1126410

Lin, L., S.-H. Zhong, X.-F. Cui, J. Li, and Z.-H. He, 2012 Characterization of temperature-sensitive mutants reveals a role for receptor-like kinase SCRAMBLED/STRUBBELIG in coordinating cell proliferation and differentiation during Arabidopsis leaf development. Plant J. 72: 707–720. https://doi.org/10.1111/j.1365-313X.2012.05109.x

Liu, L., J. Adrian, A. Pankin, J. Hu, X. Dong *et al.*, 2014 Induced and natural variation of promoter length modulates the photoperiodic response of FLOWERING LOCUS T. Nat. Commun. 5: 4558. https://doi.org/10.1038/ncomms5558

Li, W.-H., J. Yang, and X. Gu, 2005 Expression divergence between duplicate genes. Trends Genet. 21: 602–607. https://doi.org/10.1016/j.tig.2005.08.006

Lynch, M., and J. S. Conery, 2000 The Evolutionary Fate and Consequences of Duplicate Genes. Science 290: 1151–1155. https://doi.org/10.1126/science.290.5494.1151

Lynch, M., and V. Katju, 2004 The altered evolutionary trajectories of gene duplicates. Trends Genet. 20: 544–549. https://doi.org/10.1016/j.tig.2004.09.001

Ohno, S., 1970 *Evolution by Gene Duplication*, Springer, New York. https://doi.org/10.1007/978-3-642-86659-3

Panchy, N., M. Lehti-Shiu, and S.-H. Shiu, 2016 Evolution of Gene Duplication in Plants. Plant Physiol. 171: 2294–2316.

Passardi, F., J. Dobias, L. Valério, S. Guimil, C. Penel *et al.*, 2007 Morphological and physiological traits of three major Arabidopsis thaliana accessions. J. Plant Physiol. 164: 980–992. https://doi.org/10.1016/j.jplph.2006.06.008

Qian, W., B.-Y. Liao, A. Chang, and J. Zhang, 2010 Maintenance of duplicate genes and their functional redundancy by reduced expression. Trends Genet. 26: 425–430. https://doi.org/10.1016/j.tig.2010.07.002

Rensing, S. A., 2014 Gene duplication as a driver of plant morphogenetic evolution. Curr. Opin. Plant Biol. 17: 43–48. https://doi.org/10.1016/j.pbi.2013.11.002

Schwartz, C., S. Balasubramanian, N. Warthmann, T. P. Michael, J. Lempe *et al.*, 2009 Cis-regulatory Changes at FLOWERING LOCUS T Mediate Natural Variation in Flowering Responses of Arabidopsis thaliana. Genetics 183: 723–732. https://doi.org/10.1534/genetics.109.104984

Sussman, M. R., R. M. Amasino, J. C. Young, P. J. Krysan, and S. Austin-Phillips, 2000 The Arabidopsis Knockout Facility at the University of Wisconsin–Madison. Plant Physiol. 124: 1465–1467. https://doi.org/10.1104/pp.124.4.1465

Torii, K. U., N. Mitsukawa, T. Oosumi, Y. Matsuura, R. Yokoyama *et al.*, 1996 The Arabidopsis ERECTA gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats. The Plant Cell Online 8: 735–746. https://doi.org/10.1105/tpc.8.4.735

Vaddepalli, P., L. Fulton, M. Batoux, R. Yadav, and K. Schneitz, 2011 Structure-Function Analysis of STRUBBELIG, an Arabidopsis Atypical Receptor-Like Kinase Involved in Tissue Morphogenesis. PLoS One 6: e19730. https://doi.org/10.1371/journal.pone.0019730

Vaddepalli, P., L. Fulton, J. Wieland, K. Wassmer, M. Schaeffer *et al.*, 2017 The cell wall-localized atypical β-1,3 glucanase ZERZAUST controls tissue morphogenesis in Arabidopsis thaliana. Development 144: 2259–2269.

Vaddepalli, P., A. Herrmann, L. Fulton, M. Oelschner, S. Hillmer *et al.*, 2014 The C2-domain protein QUIRKY and the receptor-like kinase STRUBBELIG localize to plasmodesmata and mediate tissue morphogenesis in Arabidopsis thaliana. Development 141: 4139–4148. https://doi.org/10.1242/dev.113878

Wang, Z.-P., H.-L. Xing, L. Dong, H.-Y. Zhang, C.-Y. Han *et al.*, 2015 Egg cell-specific promoter-controlled CRISPR/Cas9 efficiently generates homozygous mutants for multiple target genes in Arabidopsis in a single generation. Genome Biol. 16: 144. https://doi.org/10.1186/s13059-015-0715-0

Weigel, D., 2012 Natural Variation in Arabidopsis: From Molecular Genetics to Ecological Genomics. Plant Physiol. 158: 2–22. https://doi.org/10.1104/pp.111.189845

Zapata, L., J. Ding, E.-M. Willing, B. Hartwig, D. Bezdan *et al.*, 2016 Chromosome-level assembly of Arabidopsis thaliana Ler reveals the extent of translocation and inversion polymorphisms. Proc. Natl. Acad. Sci. USA 113: E4052–E4060. https://doi.org/10.1073/pnas.1607532113

Zhou, A., H. Wang, J. C. Walker, and J. Li, 2004 BRL1, a leucine-rich repeat receptor-like protein kinase, is functionally redundant with BRI1 in regulating Arabidopsis brassinosteroid signaling. Plant J. 40: 399–409. https://doi.org/10.1111/j.1365-313X.2004.02214.x

Communicating editor: P. Morrell