# Background-dependent effects of polyglutamine variation in the *Arabidopsis thaliana* gene *ELF3*

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**Tandem repeats (TRs) have extremely high mutation rates and are often considered to be neutrally evolving DNA. However, in coding regions, TR copy number mutations can significantly affect phenotype and may facilitate rapid adaptation to new environments. In several human genes, TR copy number mutations that expand polyglutamine (polyQ) tracts beyond a certain threshold cause incurable neurodegenerative diseases. PolyQ-containing proteins exist at a considerable frequency in eukaryotes, yet the phenotypic consequences of natural variation in polyQ tracts that are not associated with disease remain largely unknown. Here, we use *Arabidopsis thaliana* to dissect the phenotypic consequences of natural variation in the polyQ tract encoded by *EARLY FLOWERING 3* (*ELF3*), a key developmental gene. Changing ELF3 polyQ tract length affected complex ELF3-dependent phenotypes in a striking and nonlinear manner. Some natural *ELF3* polyQ variants phenocopied *elf3* loss-of-function mutants in a common reference background, although they are functional in their native genetic backgrounds. To test the existence of background-specific modifiers, we compared the phenotypic effects of *ELF3* polyQ variants between two divergent backgrounds, Col and Ws, and found dramatic differences. In fact, the Col-*ELF3* allele, encoding the shortest known ELF3 polyQ tract, was haploinsufficient in Ws × Col $F_1$ hybrids. Our data support a model in which variable polyQ tracts drive adaptation to internal genetic environments.**

circadian clock | hybrid incompatibility | microsatellite

In coding regions, tandem repeat (TR) copy number variation can have profound phenotypic effects (1). For example, TR copy number mutations that expand polyglutamine (polyQ) tracts past a threshold number of glutamines can cause incurable neurodegenerative diseases, such as Huntington disease and Spinocerebellar Ataxias (2, 3). PolyQ tract length correlates with onset and severity of polyQ expansion disorders, but for intermediate polyQ tracts this correlation is far weaker (4–8), suggesting that genetic and environmental modifiers exist (9–12). Despite their potential for pathogenicity, variable polyQ tracts occur frequently in eukaryotic proteins, many of them functioning in development and transcription (1, 13–15). Model organism studies have suggested that coding TRs are an important source of quantitative genetic variation that facilitates evolutionary adaptation (1, 16–19). For example, TR copy number variation in the yeast gene *FLO1* correlates linearly with flocculation (20), a phenotype that is important for stress survival (17). As polyQ tracts often mediate protein interactions (2, 3, 21), polyQ-encoding TR copy number mutations could produce large and possibly adaptive phenotypic shifts.

To determine the phenotypic impact of naturally occurring polyQ variation (18, 22, 23) in a genetically tractable model, we focused on the gene *EARLY FLOWERING 3* (*ELF3*), which encodes a polyQ tract that is highly variable across divergent *Arabidopsis thaliana* strains (accessions) (19, 24). ELF3 is a core component of the circadian clock and a potent repressor of flowering, and is considered a "hub protein" for its many interactions with various proteins (24–31). Consequently, *elf3* loss-of-function mutants show pleiotropic phenotypes: they flower early, show poor circadian function, and grow long embryonic stems (hypocotyls) in light (25–27, 29, 30, 32). SNPs in *ELF3* affect shade avoidance, a fitness-relevant plant trait (24, 33). ELF3 polyQ variation has been suggested to correlate with two parameters of the circadian clock: period and phase (19). The ELF3 polyQ tract may mediate ELF3 membership in protein complexes, although thus far no ELF3-binding protein is known to bind it (26, 28–30). We discovered that altering polyQ tract length has dramatic effects on ELF3-dependent phenotypes and that these effects are dependent on genetic background.

## Results

***ELF3-TR* Variation Affects ELF3-Dependent Phenotypes.** Among 181 natural *A. thaliana* accessions, the *ELF3-TR* encoded between 7 and 29Q (Fig. S1*A* and Table S1). For comparison, polyQ expansions over 20Q are associated with disease in the context of the *SCA6* gene, although most other disease-associated polyQ expansions are longer (2, 19, 24). The most frequent *ELF3-TR* encoded 16Q, whereas the shortest TR (7Q) was found in the reference strain Col-0. We set out to test whether naturally occurring *ELF3-TR* alleles affect ELF3-dependent phenotypes, and whether they do so in a linear manner as suggested by association studies (19) and found for coding TR variation in other genes (16, 20). We generated expression-matched transgenic lines for most natural *ELF3-TR* alleles in the loss-of-function *elf3-4* mutant (Ws background, Fig. S1*C* and Tables S2 and S3) (32) and measured their flowering time and circadian clock-related phenotypes (Fig. 1 and Fig. S2 *A–G*). *ELF3-TR* variation significantly affected ELF3-dependent phenotypes, but there was no evidence of a linear relationship. The different *ELF3-TR* alleles resulted in phenotypes ranging from nearly full complementation of *elf3-4* to nearly phenocopying the loss-of-function mutant. We used principal components analysis (PCA) to describe the complex effects of *ELF3-TR* alleles on all tested ELF3-dependent phenotypes

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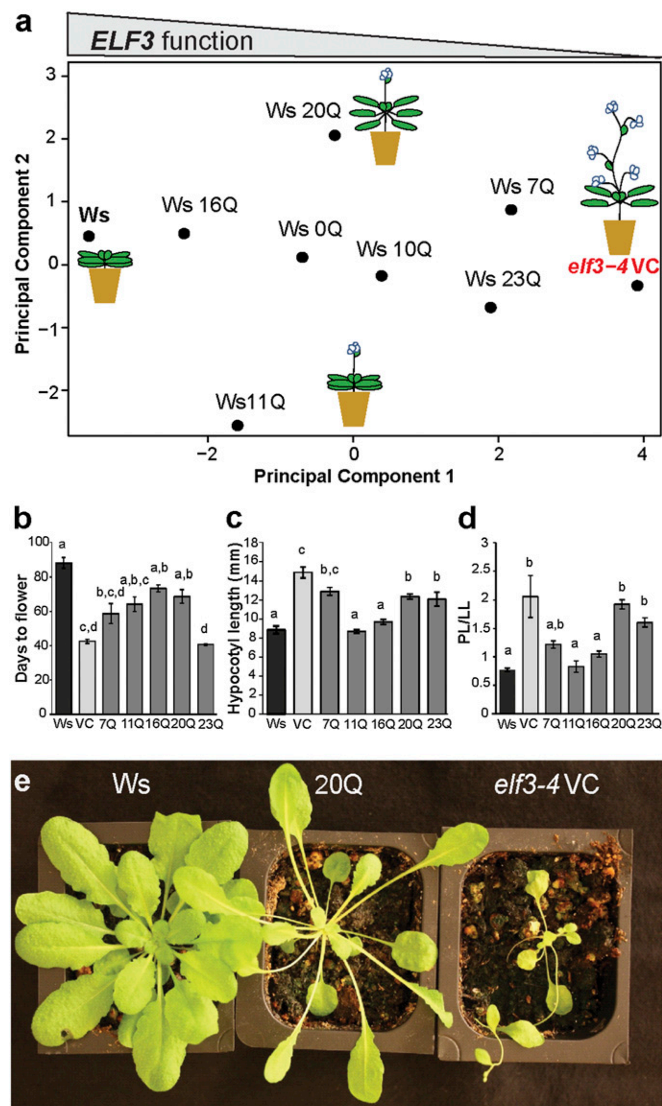


**Fig. 1.** *ELF3-TR* variation has nonlinear phenotypic effects. (*A*) PCA of developmental traits of all *ELF3-TR* copy number variants. *A. thaliana* images illustrate *ELF3-TR* effects on the traits days to flower and hypocotyl length under SD and LD, petiole-length/leaf-length ratio (PL/LL) under SD only, and rosette leaf number under LD only. The contributions of specific phenotypes to PCs are in Fig. S2*J*. Representative TR copy number alleles are shown from an analysis including all alleles (for all alleles see Fig. S2 *H* and *I*). (*B*) Days to flower under SD conditions for selected lines. *n* = 6 plants per transgenic line. (*C*) Hypocotyl length at 15 d under SD for selected lines. *n* = 20–30 seedlings per transgenic line. (*D*) PL/LL of the fourth leaf for selected lines. Data are from the same plants as in *B*. (*E*) Plants carrying the *ELF3-20Q* allele (*Center*) are specific hypomorphs under SD with the elongated petioles of the *elf3-4* mutant (vector control, VC, *Right*) and a wild-type flowering phenotype (Ws, *Left*). *ELF3-TR* alleles are indicated with the number of Qs encoded, Ws is wild-type, VC is the e*lf3-4* vector control. Error bars are SEs of means. Genotypes labeled with different letters differed significantly in phenotype by Tukey-HSD test with $\alpha = 0.05$. For all Ws-background phenotype data, see Fig. S2 *A–G*. Data are from multiple independently generated expression-matched (Fig. S1*C*) T3 and T4 lines for each TR copy number allele (Table S2). These experiments were repeated at least once with similar results. The tested *ELF3-20Q* lines contained unique insertions that did not affect genes with known function (Table S3).

(Fig. 1*A* and Fig. S2 *H–J*). Principal component 1 (PC1) corresponds to general functionality of ELF3 in all measured phenotypes, with wild-type Ws and mutant *elf3-4* defining the extremes. Separation along PC1 is driven by the tendency of plants with functional ELF3 to show short hypocotyls, late flowering, increased rosette leaf number, and short petioles (Fig. 1 *B–D* and Fig. S2). The endogenous *ELF3-16Q* allele complemented both the early-flowering and long-hypocotyl phenotypes of *elf3-4* (Fig. 1 *B–D* and Fig. S2). In contrast, both the long *ELF3-23Q* and the short *ELF3-7Q* allele (endogenous TR alleles in Br-0/Bur-0 and Col-0, respectively) behaved similarly to the *elf3-4* loss-of-function allele (Figs. 1 *B–D* and Fig. S2), although they are functional in their native backgrounds. Neither Col-0 nor Br-0 and Bur-0 show the phenotypic characteristics of *elf3*-mutants [early flowering (34), long hypocotyls (35), and long petioles (36)], suggesting that *ELF3-TR* alleles may interact with background-specific modifiers. *ELF3-0Q*, an artificial *ELF3* allele lacking the TR, partially complemented *elf3-4* (Fig. 1*A* and Fig. S2). Hence, the polyQ-encoding TR is not necessary for all ELF3 function, but changes in TR copy number are sufficient to enhance or ablate ELF3 function.

PC2 separated *ELF3-20Q* and *ELF3-11Q*, which behaved as hypomorphs in certain phenotypes but not others (Fig. 1*A*). For example, *ELF3-20Q* plants had significantly longer hypocotyls than wild-type and its petioles phenocopied the extremely long petioles of the *elf3-4* mutant (Fig. 1 *C–E*), but they did not differ from wild-type in flowering time (days to flower, Fig. 1*B*). The existence of both general and specific hypomorphs suggests that polyQ variation affects the multiple ELF3 functions separately.

As part of a protein complex, ELF3 affects expression of *Phytochrome-interacting factor 5* (*PIF5*) and *Pseudoresponse regulator 9* (*PRR9*) (28, 37, 38). *PIF5* and *PRR9* expression were strongly affected by ELF3 polyQ variation (Fig. S3). *ELF3-16Q* phenocopied wild-type *PRR9* and *PIF5* expression, and the hypomorphic *ELF3-23Q* phenocopied *elf3-4* (28, 37, 38), mirroring their developmental phenotypes. Consistent with their divergence along PC2 (Fig. 1*A*), *ELF3-11Q* and *ELF3-20Q* differed in their effect on *PRR9* expression, but not on *PIF5* expression (Fig. S3 *A* and *B*), demonstrating that ELF3 polyQ variation differentially affects the regulation of downstream genes.

***ELF3-TR*** **Variation Modulates the Precision of the Circadian Clock.** To directly assess the role of ELF3 polyQ variation in the circadian clock, we used the *CCR2::LUC* reporter system (25, 39). We observed little difference in circadian period among wild-type Ws and tested *ELF3-TR* alleles (Fig. S4*A*), contradicting a previously observed association of TR copy number with period in natural accessions (19). However, we found that the relative amplitude error (RAE) of oscillation varies substantially across *ELF3-TR* genotypes (Fig. 2*A* and Fig. S4*B*). RAE measures the precision of a circadian period (40): high RAE values (>0.4) indicate poor oscillation and clock dysfunction (41). The endogenous Ws *ELF3-16Q* nearly complemented the *elf3-4* RAE defect, whereas the TR alleles *ELF3-7Q*, *ELF3-10Q*, and *ELF3-23Q* showed higher RAE, approaching arrhythmic *elf3-4* levels (Fig. 2 *A* and *B*), consistent with their hypomorphic performance in other ELF3 traits (close to *elf3-4* in PC1) (Fig. 1*A*). Taken together, these results suggest that ELF3 polyQ tract length is a critical determinant of circadian clock precision—but not period length—in *A. thaliana*.

***ELF3-TR*** **Variation Interacts with Genetic Background.** To test our hypothesis that *ELF3-TR* variation interacts with genetic background, we regenerated all *ELF3-TR* transgenic lines in the *elf3-200* loss-of-function mutant with matched transgene expression (Col background; Fig. S1*D* and Table S4) (42). We used PCA to compare *ELF3-TR* effects between Ws and Col backgrounds (Fig. 3*A* and Fig. S5).

The Col-specific *ELF3-7Q* allele complemented *elf3-200* in some traits, such as flowering time (in short days, SD) and hypocotyl length (in long days, LD), but not others (Fig. 3 *A* and *B*, and Figs. S5 and S6). This result may be because of the absence

Fig. 2. *ELF3-TR* variation modulates the precision of the circadian clock. (*A*) RAE of *CCR2::LUC* circadian oscillation in seedlings with indicated *ELF3-TR* alleles. Bars represent 99% confidence intervals. (*B*) Mean values of circadian period and RAE (points) were measured in seedlings with indicated *ELF3-TR* alleles. Dotted ellipses represent SEMs for both period and RAE. Note that plants with high RAE have extremely unreliable estimates of circadian period. Bioluminescence rhythms from the *CCR2::LUC* reporter in *ELF3-TR* transgenic lines were used to measure circadian parameters under LL after 5 d of entrainment in 12-h light:12-h dark cycles. $n$ = >100 seedlings for all genotypes. Aggregate data from four independent experiments are shown. See Fig. S4 for RAE and period data for all alleles.

of the small 5′ intron from the *ELF3* construct used in this study. However, there was still a dramatic spread of phenotypes: all longer *ELF3-TR* alleles (>20 Qs) nearly complemented *elf3-200*, delaying flowering and shortening hypocotyls, whereas few of the shorter alleles did (Fig. 3, and Figs. S5 and S6). Results were similar when the Col data were analyzed alone (Fig. S6). Thus, in contrast to our results in the Ws background, *ELF3-TRs* appeared to show a threshold effect for TR copy number in the Col background. We speculate that the intensive laboratory propagation of the Col-0 accession may have altered selection on the *ELF3-TR*, resulting in an extremely short "hypomorphic" allele, whereas under natural conditions a longer TR might be more functional.

Comparing TR allele effects between the two backgrounds revealed striking differences. For example, the *ELF3-23Q* allele was a general hypomorph in the Ws background (*elf3-4*), whereas it produced highly functional ELF3 in the Col background (*elf3-200*) (Fig. 3). In turn, the *ELF3-16Q* allele produced highly functional ELF3 in the Ws background (*elf3-4*), but was a general hypomorph in the Col background (*elf3-200*). The consistent performance of the artificial *ELF3-0Q* allele across backgrounds suggests that the background effect is TR-dependent (Fig. 3*A* and Fig. S5). Collectively, our results suggest that *ELF3-TR* alleles interact with background-specific modifiers.

**Col *ELF3* Allele Is Haploinsufficient in Col × Ws Hybrids.** To address whether Ws and Col-specific background effects are sufficient for altered hybrid phenotypes, we generated $F_1$ populations between wild-type and *elf3*-null plants in the Ws and Col backgrounds and measured ELF3 function by assessing hypocotyl length. Ws × Col $F_1$ hybrids resembled their wild-type parents (Fig. 4). $F_1$ hybrids containing both loss-of-function alleles had significantly longer hypocotyls than either parent (Fig. 4). Both *ELF3* alleles were haplosufficient in $F_1$ crosses within their native backgrounds, as expected for recessive mutants (Fig. 4). In stark contrast, we observe that *ELF3-Col,* but not *ELF3-Ws*, phenocopied the extreme hypocotyl length of the double loss-of-function mutant (Fig. 4). Consistent with the results from our transgenic lines, our $F_1$ hybrid data suggest that full ELF3 function depends on a permissive genetic background.

## Discussion

Our results demonstrate that natural ELF3 polyQ variation that is not associated with disease has dramatic phenotypic consequences,

Fig. 3. The phenotypic effects of *ELF3-TR* variation are strongly background-dependent. (*A*) PCA of developmental traits of all *ELF3-TR* alleles in Ws and Col genetic backgrounds. Shared color indicates a given *ELF3-TR* allele in both genetic backgrounds. *A. thaliana* images are as in Fig. 1*A*. The contributions of phenotypes to principal components are similar to Fig. 1*A*, except that PC2 is inverted (no effect on interpretation, loadings in Fig. S5*C*). Representative TR copy number alleles are shown from an analysis including all alleles (for all alleles see Fig. S5; for Col-background specific PCA, see Fig. S6). (*B*) Days to flower under SD and hypocotyl length under LD differ for particular TR alleles between Ws (*Upper*) and Col (*Lower*) backgrounds. *ELF3-TR* alleles are indicated with the number of Qs encoded, Ws and Col-0 are wild-type, *elf3-4* and *elf3-200* are respective vector controls (VC). Error bars represent SEM. Genotypes labeled with different letters differed significantly in phenotype by Tukey-HSD test with $\alpha = 0.05$. For all Col-background phenotype data, see Fig. S6 *A–G*. Data are from multiple independently generated expression-matched (Fig. S1 *C* and *D*) T3 and T4 lines for each TR copy number allele (Table S4). These experiments were repeated at least once with similar results.

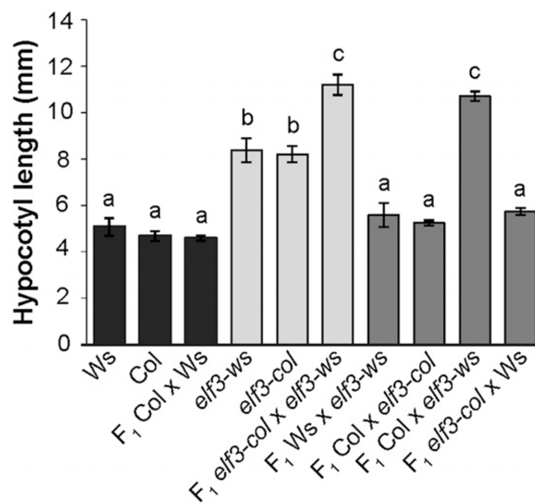


**Fig. 4.** *ELF3-Col* is haploinsufficient in a hybrid Col × Ws genetic background. Hypocotyl length under SD was measured in seedlings from parental and $F_1$ lines. *elf3-ws* is the *elf3-4* loss-of-function mutant in the Ws background; *elf3-col* is the *elf3-200* loss-of-function mutant in the Col background. Reciprocal crosses for each $F_1$ showed similar results. $n$ = 15–20 for each genotype, except for $F_1$Ws x *elf3-ws* ($n$ = 5). Error bars are SEM. Genotypes labeled with different letters differed significantly by Tukey-HSD test with $\alpha$ = 0.05.

and that these consequences depend on genetic background. For *ELF3*, in at least the Ws background, the relationship between TR copy number and phenotype does not follow a linear or threshold pattern as observed for other coding TR and polyQ disorders (1, 2, 16, 17, 20). Studies correlating TR variation with phenotype often apply linear models, treating TR copy number as a quantitative variable (19, 22, 23). Our data show that this approach is not appropriate for all TRs.

Instead, *ELF3-TR* alleles seem "matched" to specific genetic backgrounds in which they are functional, whereas they are incompatible with other backgrounds. The haploinsufficiency of the *elf3-col* allele in Ws × Col hybrids supports this interpretation. In contrast, the *ELF3-Ws* allele is haplosufficient in hybrids, indicating that the *ELF3*-Col × Ws incompatibility is asymmetric. This observation agrees with Orr's assertion that incompatibility between recently diverged populations is usually asymmetrical, because it tends to arise from the derived allele (i.e., *ELF3-Col*) (43). Variable TRs, and the *ELF3-TR* in particular, have been previously suggested as agents of adaptation to new external environments (1, 16, 17, 20, 24, 44). Our results suggest that polyQ-encoding TRs are also agents of coadaptation within genomes.

We speculate that the observed background effects arise from background-specific polymorphisms in genes encoding physically interacting proteins (26, 28–30). TRs have a far higher mutation rate than nonrepeated regions ($10^{-4}$ per site per generation for TR vs. $10^{-8}$ for SNPs) (45, 46) and, as we show, their expansion or contraction can have dramatic phenotypic impact. ELF3's partner proteins may have acquired compensatory mutations to accommodate new ELF3-TR variants and vice versa. Alternative explanations for the background effects are compensatory mutations in *ELF3* (intragenic suppressors), or ELF3 interactions that are unique to a given background. Intragenic variation and protein modification can play an important role in polyQ-mediated phenotypes (47, 48). At least for the *ELF3-Col* allele, however, our $F_1$ data are not consistent with intragenic suppressors.

Consistent with polyQ-mediated background effects, in at least one case a modifier mutation has been shown to delay onset of Huntington disease (11). Hypothetically, population genetic approaches could identify incompatible alleles that may contribute to variable disease onset in patients with polyQ expansions and to ELF3-dependent background effects in *A. thaliana*. However, the great diversity of TR alleles compared with SNP alleles and the small number of individuals carrying specific TR alleles render a population genetics approach infeasible. Extensive genetic mapping or other experimental approaches will be needed to identify the determinants of *ELF3-TR*–dependent background effects.

As TRs are rapidly evolving, we speculate that polyQ-mediated incompatibilities and the resulting fitness loss in hybrids and their offspring may contribute to disruption of gene flow between closely related populations. This speciation mechanism would be of particular importance for organisms with many polyQ-encoding TRs, thousands of offspring, and an inbreeding lifestyle. Even in humans, however, about 1% of proteins contain polyQ tracts (13, 14, 45). Our results identify TR copy number variation, and in particular polyQ variation, as a phenotypically important class of genetic variation that warrants genome-wide assessment in model organisms, crops, and humans alike.

## Materials and Methods

Information on the genotypes and experimental growth conditions, recombinant DNA techniques and generation of *ELF3* transgenic plants, and RNA extraction, processing and quantitative real-time PCR used in this study is provided in *SI Materials and Methods*. See Tables S5–S7 for primer information.

**Developmental Phenotype Assays.** Phenotypic assays were performed with expression-matched, homozygous T3 or T4 transgenic plants (Fig. S1 *C* and *D* and Tables S2–S4). For hypocotyl length and flowering-time experiments, plants were grown and measured in a pseudorandomized design under SD and LD conditions. More details are provided in *SI Materials and Methods*.

**Luciferase Imaging and Period Analysis.** Luciferase assays were performed with lines harboring the *CCR2::LUC* reporter. Further details are provided in *SI Materials and Methods*.

**Principal Components Analysis.** PCA on transformed phenotype data was performed using the R function *prcomp* (R Foundation for Statistical Computing, 2011, http://www.r-project.org/). More details are provided in *SI Materials and Methods*.

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