Supplemental Information

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5 Natural variation in abiotic stress responsive gene expression and local adaptation
6 to climate in Arabidopsis thaliana

to climate in *Arabidopsis thaliana*

 $7\over 8$

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SUPPLEMENTAL METHODS

 Hijmans et al. (2005) created WorldClim data by spatially interpolating 1950- 2000 weather station data and resolving it to 30" grid squares. The authors (Hijmans et al. 2005) estimated mean monthly minimum, mean, and maximum temperatures and mean monthly precipitation averaged across years of the time period. Furthermore, Hijmans et al. (2005) derived variables of climatic extremes 21 and variability. We used Climate Research Unit (CRU) humidity and temperature data to

 approximate vapor pressure deficit (VPD), which is the difference between water vapor partial pressure and maximum potential pressure at a given air temperature, and indicates evaporative demand on plants (Johnson and Ferrell 1983). CRU data come from 1961-1990 weather station data that were subsequently interpolated to 27 10' resolution (New et al. 2002).

We used a third database to estimate inter-annual variability in

precipitation. The National Centers for Environmental Prediction (NCEP)

generated Reanalysis data on a T62 grid (resolution ~ 210 km) for the years 1948-

2009 (data provided by NOAA/OAR/ESRL PSD, http://www.esrl.noaa.gov/psd/)

- (Kalnay et al. 1996). We used monthly surface precipitation rates to calculate
- each calendar month's coefficient of variation (CV) across years (Lasky et al.
- 2012).
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diurnal temperature range / annual range), 3) standard deviation (SD) of monthly

where *y is* the *n* x 1 vector of observed climate data for each accession (total of *n*

 accessions). For association mapping with fitness, *y* was a vector of accession fitness data. *X* is an *n* x *q* matrix of data for *q* fixed effects, consisting of intercept and SNP effects. *β* is a *q* x 1 vector giving the slope of the fixed effects. The *u* term gives the random error due to kinship

101 $Var(u) = \sigma_g^2 K$ (eqn. 2)

 with *K* being the *n* x *n* kinship matrix. The *e* term gives the random error of each accession

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Var(e) = \sigma_e^2 I
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 (eqn. 3).

 The kinship matrix was constructed using identity in-state of SNPs (Kang et al. 2008). The significance of SNP-climate or SNP-fitness relationships, *β1*, was assessed using t-tests, the *p*-values of which indicate the significance of SNP associations to climate or fitness (Kang et al. 2008). All climate variables were scaled to have a mean of 0 and a standard deviation of 1. Survival data were arcsin square-root transformed in order to

improve normality. Only SNPs with a minor allele frequency greater than 0.1

 among tested accessions were analyzed in order to avoid spurious significant associations (Atwell et al. 2010).

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- **GWAS stratified by flowering time –** The study of Des Marais *et al*. (2012),
- which provided our drought stress gene expression data, split microarray

 experiments into early and late-flowering groups of accessions (in the absence of vernalization) because flowering time groups may have varied phenology and life-history that affects response to abiotic stress (McKay et al. 2003; Donohue 2005; Korves et al. 2007). We followed Des Marais *et al*. (2012) and our previous study (Lasky et al. 2012), conducting a subset of GWAS on putative early and late-flowering groups.

 Because flowering time data were only available for 476 of the 1,307 accessions with genomic data, in the previous study we used data from the 476 to predict flowering time variation in the remaining accessions (Lasky et al. 2012) . Methods are described in greater detail in Lasky *et al*. (2012). We used data from 13 common garden experiments in different environments, all without vernalization (Table S10, Figure S4) (Shindo et al. 2005; Zhao et al. 2007; Atwell et al. 2010; Li et al. 2010; Kenney 2012) because vernalization accelerates the flowering of late-flowering putative winter annuals, which would have limited our ability to distinguish life history variation (Stinchcombe et al. 2004). We then used these empirical flowering time data to categorize accessions using a SNP- based model of flowering-time category for accessions lacking data. For the flowering time model, we used candidate SNPs identified in the original flowering time association studies (Atwell et al. 2010; Li et al. 2010) and SNPs within 100 kb of *FRI* and *FLC,* two interacting genes in the vernalization-sensitivity pathway (Michaels and Amasino 1999; Stinchcombe et al. 2004; Zhao

- selection statistics as did SNPs from randomly chosen genes. We used a
- permutation enrichment test based on that of Segrè et al. (2010). The test

each gene in the candidate list (eSR or eGEI genes). If a single SNP had the

 We then created a null distribution by permuting gene classifications as eSR, eGEI or neither circularly around the genome 10,000 times in order to maintain LD patterns and the number of genes in each category (excluding the same genes that were excluded in analyses of microarrays described above). For each random gene set we calculated a test statistic in the same way as the observed test statistic. We then compared observed test statistics to permuted test statistics. Finally, we conducted a two-tailed permutation-based hypothesis test because gene lists might be biased toward many or few strong SNP associations to climate and fitness and selection statistics. To conduct the two-tailed test we determined the proportion of random sets with a test statistic in the tail beyond our observed statistic and doubled this proportion to get a two-tailed permutation *p*-value. This was the *p*-value for the null hypothesis that the stress responsive

candidate genes co-occurred randomly with respect to strong SNP associations

(Segrè et al. 2010).

Promoter motif polymorphisms from resequencing data

We quantified ABRE and DRE/CBF motifs across the 1000 bp promoters of the

first 80 genomes of the 1001 Arabidopsis genomes project (Cao et al. 2011).

Quantification of the number of motifs was conducted on the transcribed strand

with custom perl scripts. Based on past studies, we counted ABRE type motifs

only in the 5' to 3' orientation with the transcribed gene (Zhang et al. 2005;

Maruyama et al. 2012; Fujita et al. 2013). In contrast, DRE/CBF type motifs have

been show to be functional in both the 5' to 3' and 3' to 5' orientations (Geisler et

al. 2006) and so we counted them in both directions.

 For each gene set, we tested whether each motif showed differences in frequency among accessions or in variance of frequency compared to random genes. We tested the aggregated enrichment of gene lists for all motif variants of the same core using the test statistic of O'Brien (1984). The O'Brien (1984) method allows calculation of a non-parametric statistic for multivariate responses

(here the multiple variants of each core motif). For each gene, we calculated the

mean frequency and variance in frequency of the motif among of accessions (out

- of 80). We then ranked the mean frequency and variance in frequency of each
- gene and averaged across motif variants to get a statistic for each gene. For

Climate association enrichments for combined flowering time categories

Among accessions of both flowering time categories combined, drought eGEI

genes were most strongly associated with intra-annual CV of monthly

- 351 **Tables**
- 352 **Table S1.** Results for enrichment tests with selection statistics. Enrichments
- 353 (frequency of genes in list having SNPs in the $5th$ percentile of test selection
- 354 statistics) are calculated as a *z*-score using the distribution from null permutations.
- 355 Gene lists with significant enrichment of selection statistics are shown in bold.
- 356 Tests were not stratified by flowering time because selection statistics were
- 357 calculated on a panel with both types [2].

358 **Table S2.** Results for enrichment tests with climate associations. The enrichment

359 of candidate gene sets with associations to cold-related climate variables.

- Enrichments (frequency of genes in list having SNPs in the $5th$ percentile for p -
- 361 values) are calculated as a *z*-score using the distribution from null permutations.

362 Gene lists with significant enrichment of climate variables are shown in bold.

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 Table S3. Results for enrichment tests with fitness associations. Enrichments 367 (frequency of genes in list having SNPs in the $5th$ percentile for *p*-values) are calculated as a *z*-score using the distribution from null permutations. Gene lists with significant enrichment of fitness variables are shown in bold. Tests were not stratified by flowering time because of limited sample size in fitness experiments.

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 Table S4. Results for enrichment tests with selection statistics based on resequencing of 80 accessions. The mean statistic for genes in each gene list, standardized to the null permutations, is shown as "Mean (z-score)." Gene lists with significant enrichment of selection statistics are shown in bold. Tests were conducted without stratifying by flowering time because resequencing data were from too few accessions.

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 Table S5. [attached .xls file] Table of accessions included in the various data sets analyzed in this study.

 Table S6. Enrichment of gene lists for mean rank of motif counts (O'Brien 1984). The mean statistic for genes in each gene list, standardized to the null permutations, is shown as a z-score. For example, a z-score > 0 indicates the motif is more common in the gene list compared to random genes. Gene lists with a significant motif frequency are shown in bold. DRE/CBFs are read both forward and reverse (2d) or only one direction at a time (1d).

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405 direction at a time (F-forward or R-reverse).

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440 **Table S10.** Flowering time experiments used to fit a SNP-based model of

441 flowering time category.

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- **Figure S1.** Coding regions of eSR (blue) and eGEI (red) genes from a cold-
- acclimation experiment (Hannah et al. 2006) mapped across the genome. Genes
- that are both eSR and eGEI are shown in purple. A small border is added to each
- gene to improve visibility.

- **Figure S2.** Coding regions of eSR (blue) and eGEI (red) genes from a drought
- experiment (Des Marais et al. 2012) mapped across the genome. Genes that are
- both eSR and eGEI are shown in purple. A small border is added to each gene to
- improve visibility.

Enrichment

 Figure S4. Reaction norm of flowering time across 13 published experiments (Table S10). Lines connect accessions and open circles represent accessions not present in the adjoining experiments in the figure. Green accessions are those classified as late-flowering and blue are early-flowering. Closed black circles represent cluster means used to define early versus late-flowering groups in k-means clustering conducted on accessions present in all experiments.

 Figure S5. The enrichment of candidate gene sets with associations to fitness variables. Observed enrichments are calculated as a z-score using the distribution from null permutations. Enrichment of eSR genes is shown in blue, while eGEI 478 genes are shown in red. Null permutations are shown as small gray dots $(^{0}$ [,] p < 479 0.1, '*' $p < 0.05$, '**' $p < 0.01$, '***' $p < 0.005$).

eGEI genes

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Spain

- **Figure S6**. Quantile-quantile plots for climate association *t*-statistics for early-
- flowering accessions. Observed distributions (y-axis) are compared with a
- theoretical normal distribution (x-axis).

- **Figure S7**. Quantile-quantile plots for climate association *t*-statistics for late-
- flowering accessions. Observed distributions (y-axis) are compared with a
- theoretical normal distribution (x-axis).

- **Figure S8**. Quantile-quantile plots for climate association *t*-statistics for all
- accessions combined. Observed distributions (y-axis) are compared with a
- theoretical normal distribution (x-axis).

Figure S9. Quantile-quantile plots for fitness association *t*-statistics. Observed

distributions (y-axis) are compared with a theoretical normal distribution (x-axis).

Climate outliers removed

- Two high-altitude outlier accessions were removed, Kas-2 and Pi-2. The plots
- below show the distribution of climate data for the remaining accessions. Red
- lines indicate the boundary between accessions used in association mapping and
- outliers excluded.
- *Among all accessions*
- CV monthly precipitation, accessions removed: Shahdara, Kondara, Sorbo
- SD monthly temperature, accessions removed: Kz-1, Kz-9, Per-1, Rubezhnoe-1,
- Stw-0
- Minimum temperature coldest month, accessions removed: Kz-1, Kz-9, Per-1
- Temperature annual range, accessions removed: Kz-1, Kz-9
- Mean growing season precipitation, accessions removed: Ka-0, Oy-0, Ty-0, Ty-1,
- UKID115, UKID120
- CV growing season precipitation, accessions removed: Kondara
- Mean growing season VPD, accessions removed: Lag-1-6
- Interannual CV growing season precipitation, accessions removed: Ayu-Dag-3,
- Kondara, Kz-1, Kz-9, Shahdara, Sorbo
- All other climate variables had no outliers
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- **Figure S10.** Distributions of climate variables among panels with both early and
- late-flowering accessions combined. Red lines show outlier thresholds.

Among early-flowering accessions

- CV monthly precipitation, accessions removed: Shahdara, Kondara, Sorbo
- SD monthly temperature, accessions removed: Kz-1, Kz-9, Per-1, Rubezhnoe-1,
- Stw-0
- Minimum temperature coldest month, accessions removed: Kz-1, Kz-9, Per-1
- Temperature annual range, accessions removed: Kz-1, Kz-9, Per-1
- Mean growing season precipitation, accessions removed: Ka-0, Oy-0, Ty-0
- CV growing season precipitation, accessions removed: Kondara
- Mean growing season temperature, accessions removed: Lag-1-6
- Mean monthly minimum growing season temperature, accessions removed: Alc-
- 0, Ka-0, Kz-1, Kz-9
- Mean growing season VPD, accessions removed: Lag-1-6
- Interannual CV growing season precipitation, accessions removed: Ayu-Dag-3,
- Kondara, Kz-1, Kz-9, Shahdara, Sorbo
- All other climate variables had no outliers
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- **Figure S11.** Distributions of climate variables among panels with early-flowering
- accessions. Red lines show outlier thresholds.

- *Among late-flowering accessions*
- Mean growing season precipitation, accessions removed: Ty-1, UKID115,
- UKID120
- Mean growing season temperature, accessions removed: Blh-1, Blh-2, Mc-0,
- UKID115, UKID120
- Mean monthly minimum growing season temperature, accessions removed: Mc-0
- Mean growing season VPD, accessions removed: Blh-1 Blh-2
- All other climate variables had no outliers
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- **Figure S12.** Distributions of climate variables among panels with late-flowering
- accessions. Red lines show outlier thresholds.

