Linking genomic signatures of selection to expression variation and direct evidence of local adaptation

Nicholas Price,[a*] Jack L. Mullen,[a] Junjiang Lin,[a] Christina Boucher[b], John K. McKay[a]

[a]Department of Bioagricultural Sciences & Pest Management, Colorado State University, Fort Collins, CO 80523, USA
[b]Department of Computer and Information Science and Engineering, University of Florida, Gainesville, FL 32611, USA

*Corresponding author:
Name: Nicholas Price
email: price4890@gmail.com

Abstract

Understanding how genomic and expression variation is linked to adaptation of plants to local environments is fundamental to the fields of evolutionary biology and species conservation. Using locally adapted *Arabidopsis thaliana* Italy and Sweden populations, we examine how variation in gene expression under control and cold acclimation conditions, is linked to allele frequency differentiation (AFD); linkage disequilibrium (LD); selective constraint at nonsynonymous sites; and genetic-tradeoff quantitative trait loci (GT-QTL). Our results indicate that contrary to genes showing a main effect in environment (E), expression genotype by environment interactions (GxE) show significantly higher AFD along cis-regulatory and nonsynonymous sites than the neutral expectation; and interestingly, highly differentiated GxE genes show higher expression and inter-species selective constraint than the rest of the genes. When examining the association between genomic signatures of selection along GxE/E genes and GT-QTL, we find that GxE genes showing a high AFD and LD, display a significant and much higher enrichment along GT-QTL than the genome-wide/E set of genes. Nonetheless, E

genes show a higher enrichment than the genome-wide control. In summary, our results suggest, that these highly expressed and selectively constrained GxE genes, may have been part of a cold-responsive regulon of E genes that experienced recent selection when migrating to new environments. Candidate GxE genes underlying GT-QTL reveal interesting biological processes that may underlie local adaptation to temperature, including flowering time, light-dependent cold acclimation, freezing tolerance, and response to hypoxia. Finally, we find no evidence linking lower expression of the CBF-dependent freezing tolerance pathway to genetic-tradeoffs and adaptation to warmer climates.

Introduction

Populations may vary in genotype, phenotype, and fitness across geographical regions that differ in abiotic variables of the environment. Such variation maybe generated after populations become adapted to local climates, where local genotypes have higher fitness than foreign genotypes at home (Kawecki and Ebert 2004; Hereford 2009; Des Marais, et al. 2013). Abiotic stress responsive gene expression (i.e., gene expression plasticity) may play a pivotal role in local adaptation, since studies have linked it to increases in stress tolerance (Rockman, et al. 2003; López-Maury, et al. 2008; Thomashow 2010; Brown, et al. 2017), and the adjustment of an organism's life-cycle to favorable environmental conditions (Seo, et al. 2009; Chiang, et al. 2011). Understanding the link between genetic, expression, and fitness variation under different abiotic environments is central to the fields of evolutionary biology (Hoban, et al. 2016), conservation genomics (Razgour, et al. 2019), and plant breeding (Henry and Nevo 2014).

Gene expression responses under different environments, are often conserved between genotypes from different populations (Hannah, et al. 2006; Des Marais, et al. 2012), in which case, they will exhibit a main effect in environment ("E") when plotted in a norm of reaction plot (Baye, et al. 2011). On the contrary, they may exhibit genotype-by-environment interactions ("GxE"), in which case the ranks of genotypes (G) change or switch from one environment to another (Baye, et al. 2011). E-genes may underlie adaptations to common environmental changes, while GxE-genes may underlie adaptation to aspects of the environment that significantly differ between locations within a specie's range. For example, across the native range of a plant, all populations may face a slight deviation in temperature during winter/summer which engages a similar response among a set of genes of essential genes (i.e., E responses). On the other hand, parts of the native range may experience much harsher winters/summers than on average, thereby causing divergent selection between genotypes and the formation of GxE responses. If expression GxE interactions reflect fitness GxE interactions, then they may represent a important mechanisms of adaptation (López-Maury, et al. 2008; Franssen, et al. 2011; Morris, et al. 2014; Lovell, et al. 2016).

Genetic variation linked to expression and fitness GxE interactions can exhibit: (a) genetic tradeoffs, where the derived genotype is advantageous in one environment but deleterious in the other; and (b) conditional neutrality where the derived genotype is advantageous (conditionally advantageous) or deleterious (conditionally deleterious) in one environment and neutral in the other (Anderson, et al. 2011; Mee and Yeaman 2019). Despite the presence of fitness GxE interactions, instances of conditionally deleterious or more correctly non-locally maladaptive mutations, do not represent instances of 'adaptation' (Mee and Yeaman 2019). To identity

candidate genetic variation underlying local adaptation at the single nucleotide level, the two of the main approaches used are: (a) identifying single nucleotide polymorphism (SNP) that show significantly higher allele frequency differentiation between populations than expected under neutral models of evolution (Beaumont and Balding 2004; Foll, et al. 2014; de Villemereuil and Gaggiotti 2015) and (b) identifying alleles showing significant associations to environment while accounting for population/geographic structure (Lasky, et al. 2012; Zhou and Stephens 2012; Gunther and Coop 2013; Luu, et al. 2017; Caye, et al. 2019). Loci underlying genetic-tradeoffs are expected to exhibit significantly stronger population genomic evidence of local adaptation than the genome average and conditionally advantageous loci (Tiffin and Ross-Ibarra 2014; Yoder and Tiffin 2017; Mee and Yeaman 2019).

Some of the main difficulties in identifying SNPs underlying local adaptation is disentangling adaptive, from neutral or slightly deleterious variation generated by background/relaxed selection, and genetic drift (Zhen and Ungerer 2008b; Hoban, et al. 2016; Matthey-Doret and Whitlock 2019). Simulation studies comparing various methods used to identify genetic variation underlying local adaptation while accounting for the effects of population structure have shown that the power of each method can significantly change depending on the underlying evolutionary scenario, in addition to other factors (De Mita, et al. 2013; de Villemereuil, et al. 2014; Lotterhos and Whitlock 2015; Yoder and Tiffin 2017). Furthermore, in examining the link between GWA/population-genomic methods and empirical evidence of local adaptation, the strength of this link changed depending on the method(s) used in studies (Fournier-Level, et al. 2011; Lasky, et al. 2014; Yoder, et al. 2014; Exposito-Alonso, et al. 2018; Price, et al. 2018; Price, et al. 2020).

Despite these hurdles, there have been many studies examining the genetic basis of local adaptation (Savolainen, et al. 2013; Hoban, et al. 2016), but only a few linking genome-wide expression variation, sequence variation, and fitness variation across selective gradients (Kelly 2019). Among the few, a study by Lasky, et al. (2014) examined the link between genomic signatures of local adaptation, patterns of expression, and fitness variation in Arabidopsis. The main result of the study was that expression GxE genes showed a higher enrichment of climate-correlated SNPs than genes showing a main effect in environment (E); suggesting a role of expression GxE interactions in local adaptation (Lasky, et al. 2014). Nonetheless, the enrichment of fitness associations along GxE genes was not significant despite being higher than E genes. This discordance could be the result of differences in purifying selection between E and GxE genes leading to an enrichment of slightly deleterious climate-associated SNPs in the latter set (Mee and Yeaman 2019).

Arabidopsis wild populations offer a valuable resource to re-examine the interplay between genetic, expression, and fitness variation across climatic conditions. The native range of Arabidopsis includes parts of Northern and Southern Europe that experience significantly different climatic conditions. For example, populations in North Sweden, experience average soil temperatures below freezing for about a 1/3 of the year, while in North-Central Italy such temperatures are rarely recorded (Oakley, et al. 2014). Reciprocal transplant experiments have showed strong adaptive differentiation between these populations and evidence of genetic-tradeoffs (Ågren and Schemske 2012; Ågren, et al. 2013). Among the traits suggested to underlie these genetic-tradeoffs, is freezing tolerance (Oakley, et al. 2014); and more specifically freezing

121 tolerance variation associated with the CBF pathway (Thomashow 2010; Park, et al. 2015; Park,
122 et al. 2018). Studies have suggested that the lower freezing tolerance and expression of this
123 pathway (Cook, et al. 2004; Hannah, et al. 2006; McKhann, et al. 2008; Gehan, et al. 2015)
124 across Arabidopsis populations in warm climates (e.g., Italy), is an adaptive response that is
125 deleterious in cold climates (e.g., Sweden) (Oakley, et al. 2014). This adaptive response has been
126 linked to non-functionalization of the CBF-pathway (Oakley, et al. 2014; Gehan, et al. 2015;
127 Monroe, et al. 2016). Nonetheless this hypothesis has been disputed by studies showing that this
128 nonfunctionalization and decrease in freezing tolerance is due to relaxed selection in warmer
129 climates (Zhen and Ungerer 2008a, 2008b; Zhen, et al. 2011).

130

131 To re-examine the link between genetic, expression, and fitness variation of Arabidopsis
132 populations in different climates, and the role of the CBF-pathway in local adaptation to
133 temperature, the current study examines the following data: (a) re-sequenced genomes of locally
134 adapted (Ågren and Schemske 2012) South Italy and North Sweden (Price, et al. 2020); (b)
135 expression of Italy and Sweden genotypes under control and cold-acclimation conditions (Gehan,
136 et al. 2015); and (c) quantitative trait loci (QTL) explaining fitness variation of Italy and Sweden
137 recombinant inbred lines grown in a series of reciprocal transplant experiments (Ågren, et al.
138 2013). More specifically, we examine the link between allele frequency differentiation (AFD)
139 and linkage disequilibrium (LD) at cis-regulatory (sites found 1kb upstream from the
140 transcriptional start site) and nonsynonymous sites, to patterns of expression (E and GxE) and
141 genetic-tradeoff QTL, while taking into account the effects of selective constraint (or purifying
142 selection) at nonsynonymous sites. Among population genomic signatures of local adaptation we

chose AFD and LD, since these were previously found to be enriched along fitness QTL (Price, et al. 2020).

Materials and Methods

Extraction of RNA under cold conditions and sequencing

The Arabidopsis SW and IT accessions were collected from their native habitats in Sweden and Italy, respectively (Ågren and Schemske 2012). Plants were grown at 22°C on soil under a 12 h photoperiod for 18–26 days (control), or at 4°C under a 12 h photoperiod for 1 or 2 weeks (cold treatment). Rosette tissue was collected from plants exposed to low temperature (4°C) for 0, 1, and 2 weeks. Total RNA was isolated for each experimental replicate (three replicates). Nine replicates were collected for each accession, for a total of 18 biological replicates. Further details can be found in the study by Gehan et al. (2015).

RNA was submitted for RNAseq library prep and 100bp single-end RNAseq analysis to Michigan State University's Research Technology Support Facility (RTSF). Sample preparation was performed by MSU RTSF with standard protocols of the mRNA-Seq Sample Preparation Kit (Illumina). Sequencing was performed on an Illumina Genome Analyzer II (Illumina). Three samples were multiplexed in a lane for a total of 6 lanes. After quality trimming, RNAseq resulted in single-end reads ~75 bp in length with an average of 45,257,092 reads passing the Illumina purity filters for each sample. To map reads to the *Arabidopsis thaliana* genome we used Tophat (Trapnell, et al. 2009) and we estimated transcript abundance using Cufflinks (Trapnell, et al. 2010).

Identifying differentially expressed genes between Italy and Sweden accessions

To identify genes showing a main effect in environment (or condition) (E) and genotype by environment interactions (GxE) we used the we used the package DESeq2 (Love *et al*. 2014) and focused on expression after one week of cold. More specifically, using the function "DESeqDataSetFromMatrix" and a design to identify genotype by environment interactions ("genotype+condition+genotype*condition") we identified GxE genes that showed an adjusted p-value ("padj") of <0.01. Thereafter, using the "contrast" argument we extracted genes that showed a main effect in environment (E) using a padj <0.01. To ensure no main ffect in genotype among E genes we removed any genes showing a main effect in genotype (G) using a p-value of 0.05.

Comparing mean expression and selective constraint across E and GxE genes

To compare average expression of Italy and Sweden plants across E and GxE genes we first estimated the average "Fragments Per Kilobase of exon model per Million mapped fragments" ($\overline{\text{FPKM}}$) of the three samples under each pair of conditions ("control", "cold"). Using the average expression of each gene under control and cold conditions, we estimated the mean expression and 95% CI's of all genes in each category (i.e., E and GxE genes). To estimate 95% CI's we used10,000 bootstrap samples. In addition to average expression of genes across each condition, we also estimate average difference in expression between conditions ($\overline{\text{FPKM}_{cold}} - \overline{\text{FPKM}_{control}}$). Selective constraint/ purifying selection at nonsynonymous sites was examined using the ratio of nonsynonymous to synonymous rates of substitution (dN/dS). dN/dS ratios were downloaded from EnsemblPlants (Howe, et al. 2020) Biomart (Kinsella, et al. 2011), using *Arabidopsis thaliana* and *Arabidopsis halleri* orthologs. dN/dS ratios above 1 were ignored.

Population genomic signals of selection

As population genomic signatures of local adaptation we used a combination absolute allele frequency differentiation ($|f_{N.Sweden} - f_{S.Italy}|$) and linkage disequilibrium (LD) between a SNP and its neighboring SNPs with a 20kb window. LD was measured using the package 'PLINK' (Purcell, et al. 2007) and it was estimated as the mean square coefficient of correlation ($\overline{r^2}$). AFD and LD were estimated in a previous study (Price, et al. 2020).

Defining cis-regulatory and nonsynonymous variation

Cis-regulatory sites of genes were defined using a maximum length of 1 kb from the transcriptional start site unless there was overlap with the transcribed region of another gene in which case the promoter region was shorter. For sites that were associated to two genes, were assigned to the nearest gene. To call nonsynonymous variation among Italy and Sweden accessions we used bi-allelic sites, a publicly available python script (callSynNonSyn.py; archived at https://github.com/kern-lab/), and gene models downloaded from the TAIR database (TAR10 genome release) (Berardini, et al. 2015).

Circular permutation tests to examine evidence of local adaptation across groups of genes

To examine whether the proportion of (E/GxE) genes with cis-regulatory/nonsynonymous SNPs showing evidence of local adaptation (estimated using AFD and/or LD) is significantly higher than expected by chance we used a circular permutation test. This test has been previously explained in detail (Price, et al. 2020); but in brief, AFD's and/or LD's are shifted across the genome (not randomly shuffled) and according to certain criteria (e.g., AFD>0.60) we estimate the proportion of genes with high AFD and/or LD cis-regulatory/nonsynonymous SNPs. This

was repeated a thousand times and the resulting permutation distribution is compared to the observed proportion/number of genes with high AFD.

Assembling CBF-regulon genes and flowering time estimates

Genes predicted to be regulated by the three CBF transcription factors (CBF's 1-3) were retrieved from Park, et al. (2018) resulting in a set of 476 genes. Estimates of flowering time for 835 Eurasian *A. thaliana* accessions were downloaded from the study by Alonso-Blanco et. al (2016) (1001 Genomes Consortium 2016).

Results

Sweden but not Italy plants show significant upregulation of GxE genes under cold

To examine gene expression in Italy and Sweden plants under control (22 °C) and cold conditions (4 °C) we used DESeq2 (Love, et al. 2014). Using an FDR of <0.01, we identified 392 that showed genotype by environment interactions ("GxE"), and 2, 883 that showed a main effect in environment ("E") after removing genes that showed a main effect in genotype at a p-value <0.05. To compare mean expression of these genes between Italy and Sweden plants we estimated mean Fragments Per Kilobase Of Exon Per Million Fragments Mapped ($\overline{FPKM}$ x 10^{-3}) under control and cold conditions and determined the 95% CI's using a bootstrap approach (Figures 1a & 1b). As expected, mean expression of "E" genes under control and cold conditions was identical in Italy and Sweden plants (Figure 1a). On the other hand, mean expression of "GxE" genes was significantly different between Italy and Sweden plants under cold conditions (Figure 1b); with Sweden plants showing a significant upregulation of genes when compared to control conditions ($\overline{\text{FPKM}_{cold}} - \overline{\text{FPKM}_{control}} > 0$) (Figure 1c). On the contrary, Italy plants

showed a decrease in expression under cold conditions when compared to control conditions ($\overline{\text{FPKM}_{cold}} - \overline{\text{FPKM}_{control}}$<0) (Figure 1c).

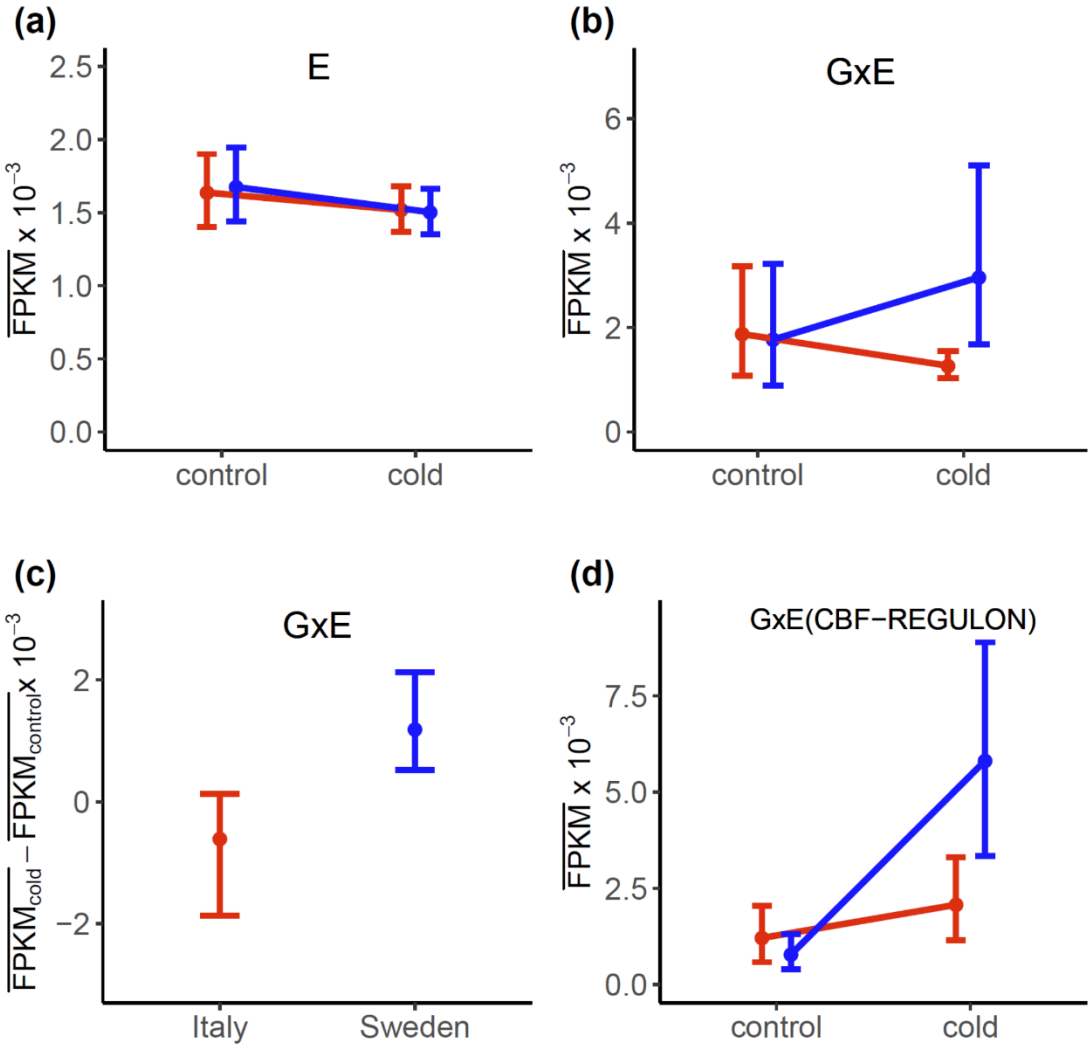


Figure 1. (a-b) Mean expression ($\overline{FPKM}$ x 10^{-3}) of E and GxE genes under control and cold conditions (FPKM: Fragments Per Kilobase Of Exon Per Million Fragments Mapped). Shown are the means and 95% confidence intervals estimated using a bootstrap approach. Mean expression of Sweden plants under cold conditions was significantly higher than in Italy plants (c) In comparison to control conditions ($\overline{\text{FPKM}_{cold}} - \overline{\text{FPKM}_{control}}$x 10^{-3}) Sweden plants showed a net upregulation (>>0) under cold, while Italy plants showed a net downregulation (<<0) (d) Despite the opposite trends (c), mean expression of CBF-regulon GxE genes in Italy and Sweden

plants, was higher under cold than control conditions; with Sweden plants showing the largest increase (>2 times higher).

To examine whether this decrease was also present among known freezing tolerance genes we examined mean expression of 53 CBF-regulon genes that showed GxE interactions. As shown in Figure 1d, expression of GxE CBF-regulon genes was significantly higher under cold than control conditions, in both Italy and Sweden plants. The increase in Sweden plants, however, was twice as high than in Italy plants. Among the set of 476 CBF-regulon genes (Park, et al. 2018), we identified 23 "E" genes and 53 "GxE" genes. In comparison to the total number of "E" and "GxE" genes (E: 2883, GxE: 392) CBF-regulon genes showed a significant enrichment (p-value<0.01) in GxE interactions according to single tail fisher's test ("fisher.test" implemented in R).

GxE but not E genes show significant allele frequency differentiation at cis-regulatory and nonsynonymous sites

Loci underlying local adaptation are expected to show significant allele frequency (Tiffin and Ross-Ibarra 2014) between populations. To examine such evidence along E and GxE genes, we looked at allele frequency differentiation at cis-regulatory and nonsynonymous SNPs. As a measure of allele frequency differentiation we used absolute allele frequency difference of the non-reference allele between Italy and Sweden populations (AFD: $|f_{N.Sweden} - f_{S.Italy}|$) (Berner 2019; Price, et al. 2020); which showed a strong correlation (R^2: 0.96, Figure S1) with a two population, two allele, F_{ST} measure (Bhatia, et al. 2013).

Along candidate cis-regulatory sites, the observed proportion of E genes (Figure 2a) with Cis-regulatory SNPs showing different levels of AFD's (AFD<0.40, AFD>0.60, 0.80) did not significantly differ than the expected proportion (Figure 2a). On the other hand, the proportion of GxE genes with cis-reg. SNPs showing low AFDs (<0.40) was significantly lower than the expectation, while the proportion associated with high AFD's (>0.60, 0.80) was significantly higher (Figure 2a). The difference in AFD's along cis-regulatory SNPs can also be seen when comparing the histograms of E and GxE genes. As shown in Figure 2b, GxE genes show a higher proportion of cis-reg. SNPs with high AFD's (>0.60) than E genes. The two histograms were found to be significantly different according to a X^2 test (X-squared = 25.177, df = 9, p-value = 0.0028).

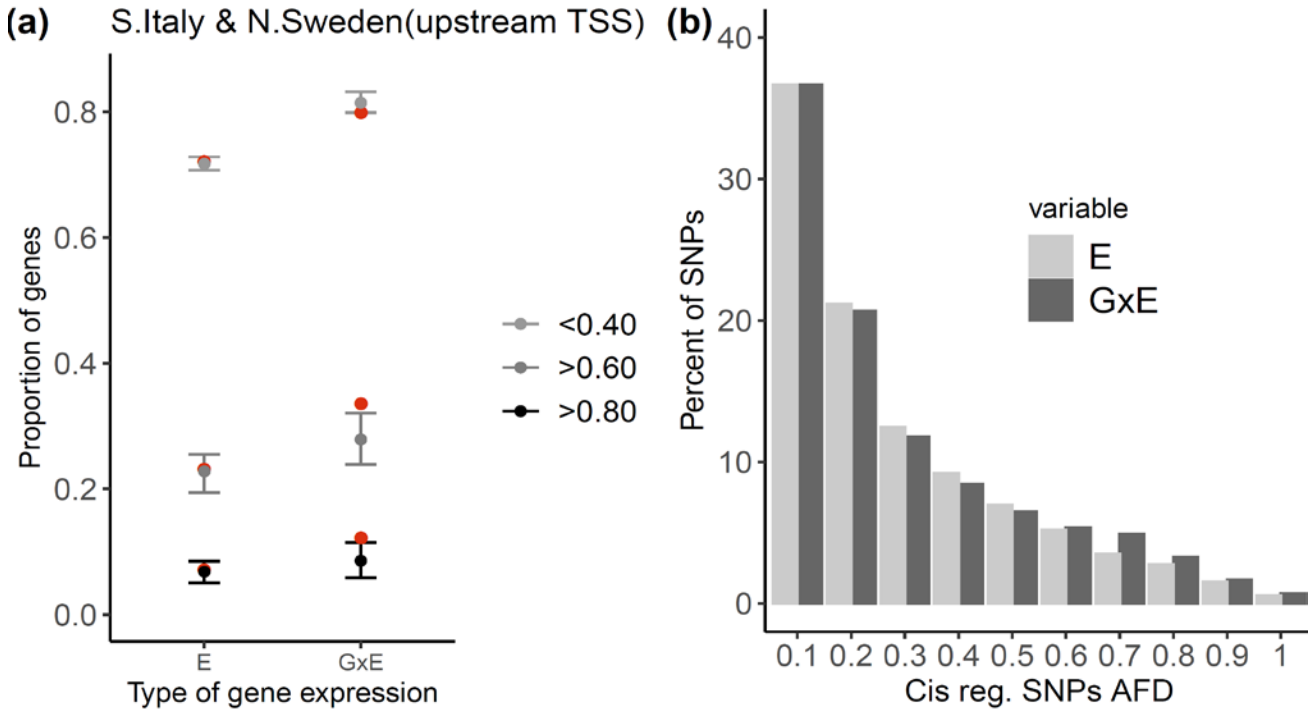
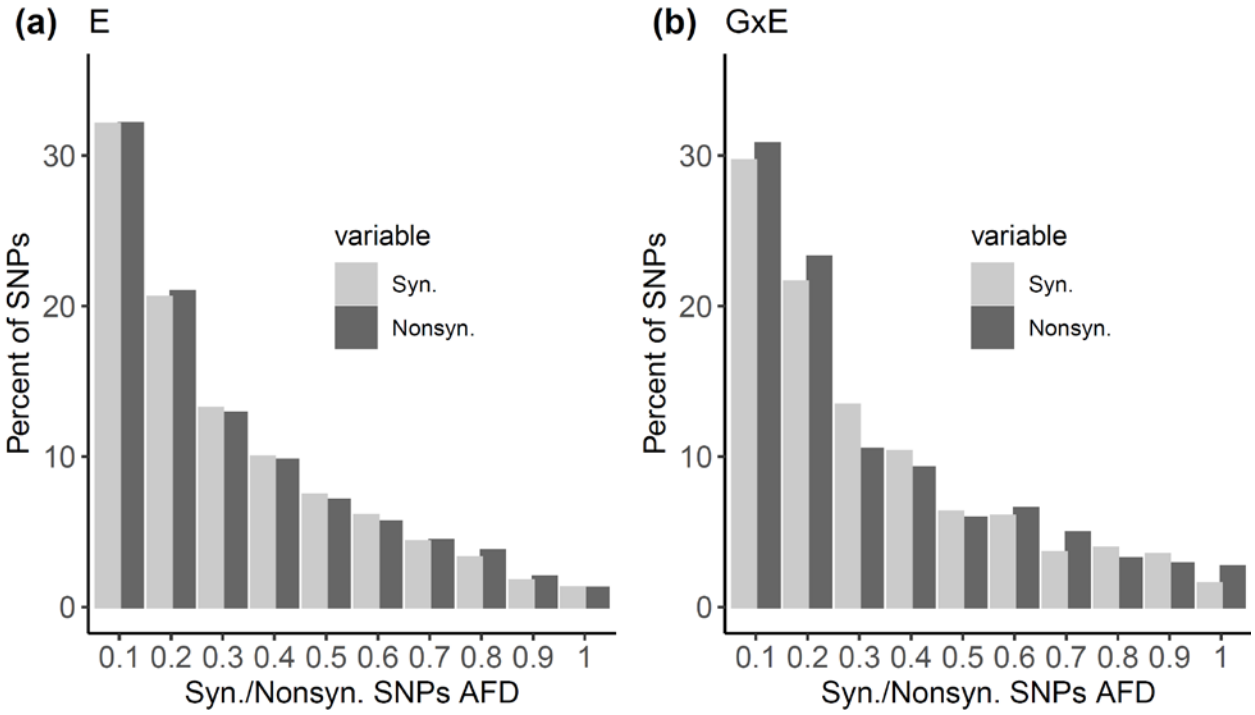


Figure 2. (a) The proportion of GxE genes showing high allele frequency divergence (AFD) at cis-regulatory sites was significantly higher than the expectation derived using circular permutations of genome wide SNPs. E genes on the other hand, showed no significant genetic differentiation along these sites. (b) The significantly higher differentiation is also observed

285 when comparing the AFD distributions along cis-regulatory sites of E and GxE genes (X-squared
286 = 25.177, df = 9, p-value = 0.0028). GxE genes show a higher proportion of cis-regulatory SNPs
287 with an AFD>0.60.
288

289 To test whether nonsynonymous variation across "E" and/or "GxE" genes showed evidence of
290 local adaptation we compared AFD distributions of nonsynonymous and synonymous variation.
291 As depicted in Figure 3a, the distribution of AFD's at nonsynonymous and synonymous sites of
292 "E" genes is not significantly different according to a X^2 test (X^2: 11.0, df:9, p-value: 0.28).
293 Contrary to "E" genes, the distribution of AFD's at nonsynonymous sites of "GxE" genes was
294 significantly different (X^2: 18.5, df: 9, p-value: 0.03) than synonymous sites (Figure 3b). More
295 specifically, nonsynonymous sites across "GxE" genes showed an enrichment in low AFD's and
296 high AFD's (Figure 3b) which can be caused by recent local adaptation and/or purifying
297 selection (Nielsen 2005).

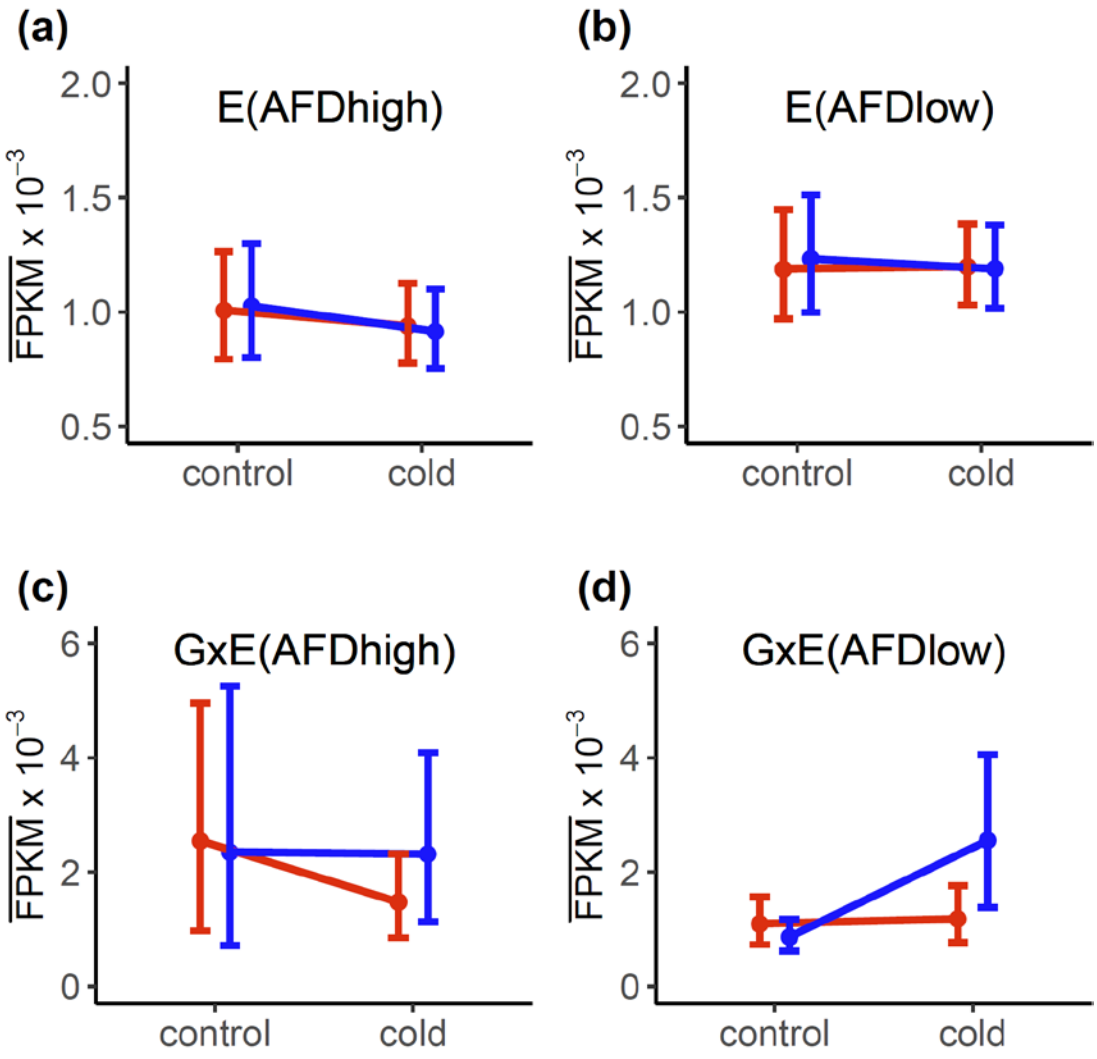


298

Figure 3. (a) Allele frequency divergence (AFD) between synonymous and nonsynonymous sites of E genes did not significantly differ according to a X2 test (X-squared = 11.002, df = 9, p-value = 0.28). (b) When comparing AFD between synonymous and nonsynonymous sites of GxE genes identifies a statistical significance (X: 18.546, df = 9, p-value = 0.029). The proportion of nonsynonymous SNPs with AFD's <0.2 and >0.6 was higher than synonymous sites. Such deviations could be caused by both recent selection and purifying selection.

Contrasting patterns of expression and purifying selection between GxE and E genes exhibiting low and high AFD

Variation in allele frequency differentiation between nonsynonymous sites of genes can result from differences in purifying selection, in addition to local adaptation. Furthermore, these differences could be associated with variation in gene expression among genes. To examine the link between AFD, purifying selection, and expression we first split E and GxE genes into ones that showed an AFD>0.60 at least one cis-regulatory/nonsynonymous site (AFDhigh) and ones that did not (AFDlow); furthermore, we narrowed down the sets of genes to those that we had estimates of dN/dS (dN: rate of nonsynonymous substitutions per site; dS: rate of synonymous substitutions per site); a measure of purifying selection at nonsynonymous sites.



316

317 **Figure 4**. (a-b) E genes with at least one nonsynonymous/cis-regulatory SNP showing an
318 AFD>0.6 (AFDhigh) were expressed at significantly lower levels when compared to the rest of
319 the E genes with SNPs at cis-regulatory/nonsynonymous sites (AFDlow). Mean expression
320 ($\overline{FPKM}$ x 10^{-3}) of AFDhigh E genes across ecotypes and conditions was ≈1, while that of
321 AFDlow E genes was >>1. (c-d) On the other hand, AFDhigh GxE genes showed a higher mean
322 expression (≈ 2.13 $\overline{FPKM}$ x 10^{-3}) across ecotypes and conditions than AFDlow GxE genes (≈
323 1.47 $\overline{FPKM}$ x 10^{-3}.

324

325 E genes with high AFD SNPs showed a mean $\overline{FPKM}$ x 10^{-3} of ≈1 across all conditions and
326 ecotypes, while E genes with low AFD showed an $\overline{FPKM}$ x 10^{-3} significantly higher than 1

(Figures 4a-4b). The opposite trend was seen across GxE genes. GxE genes with low AFD SNPs showed significantly lower expression (≈ 1.47 $\overline{FPKM}$ x 10^{-3}) across the two conditions and ecotypes, than GxE genes with high AFD SNPs (≈ 2.13 $\overline{FPKM}$ x 10^{-3}) (Figures 4c-4d). In association with the levels of expression we observed a change in patterns of selective constraint/purifying selection along protein coding genes. As shown in Figure 5, E genes that contained high AFD SNPs, and were expressed at lower levels than AFDlow E genes, also showed a higher dN/dS, which translates to lower levels of purifying selection. On the other hand, AFDhigh GxE genes showed higher expression than AFDlow GxE genes, and a lower dN/dS (Figure 5).

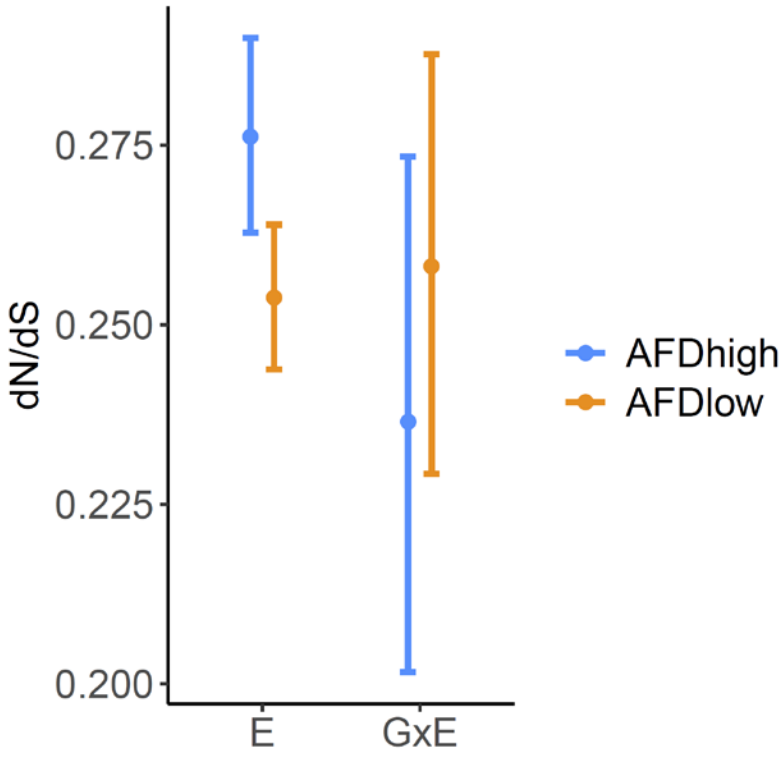


Figure 5. Patterns of purifying selection differed significantly between GxE and E genes that showed different levels of allele frequency divergence (AFD) at nonsynonymous sites/cis-

regulatory sites. E genes with high AFD SNPs (AFDhigh) showed significantly lower purifying selection (higher dN/dS) at nonsynonymous sites, than E genes with low AFD SNPs (AFDlow) and GxE genes with high AFD SNPs (AFDhigh). GxE genes with high AFD SNPs also showed a lower dN/dS than GxE genes with low AFD SNPs. These patterns of dN/dS seem to correlate with the level of expression associated with these genes (Figure 4). The ration dN/dS was estimated using pairs of *A. thaliana* and *A. halleri* orthologous genes.

Significant evidence linking GxE genes to recent selection and genetic tradeoffs

So far, we have showed that GxE genes showed significantly higher genetic differentiation than E genes at both cis-regulatory and nonsynonymous sites, in addition to higher levels of expression and purifying selection (Figures 2-5). To examine the association of E and GxE genes to fitness variation of Italy and Sweden genotypes at native sites, we used a set of 20 fitness QTL that were previously assembled into six genetic trade-off QTL that spanned ~18.6 Mbs of the genome (Ågren, et al. 2013). More specifically, we examined the distribution of genes showing high allele frequency differentiation (AFD) and linkage disequilibrium (LD) along genetic tradeoff QTL.

As shown in Table 1, in most instances the proportion of E genes withing genetic-tradeoff QTL was significantly higher than the genome-wide proportion; even when not filtering for high AFD and LD. On the other hand, GxE genes did not show any significant enrichment when not filtering for a high LD (Table 1). On the other hand, when we filtered for a high AFD (>0.60) and LD (>019, 0.32) (0.32 represents the 99th percentile of the genome wide distribution of LD) the proportion of GxE genes along genetic-tradeoff QTL was significantly higher than the genome-wide proportion and the proportion of E genes satisfying these criteria (Table 1). In

addition to examining the proportions along the six genetic tradeoff QTL, we also examined the proportions of these genes within 100kb of fitness QTL peaks (Table S1). The only significant enrichment was observed when examining the proportion of GxE genes with a high AFD (>0.60) and LD (>0.32) (Table S1). This proportion was significantly higher than the genome-wide set of genes, and E genes (Table S1).

TABLE 1. Comparing the proportion E and GxE genes exhibiting different signatures of local adaptation and selection, and found within six genetic tradeoff (GT) QTL peaks explaining fitness variation between Italy and Sweden populations (Ågren, et al. 2013). The table splits a set of genome-wide, E, and GxE genes according to their location along GT QTL and signatures of selection (AFD: Allele frequency divergence & LD: Linkage disequilibrium). The odd ratios depicted are derived by comparing the proportion of E/GxE genes to the genome-wide set of genes. A significantly higher proportion relative to the genome-wide set is indicated by a *, and significantly higher proportion when comparing E and GxE genes is indicated by a †. Comparison of proportions was done using a fisher's one-tail test (p-val<0.05) implemented in R ("fisher.test").

	NON-QTL			GT-QTL			E (Odds-ratio)	GxE (Odds ratio)
	Genome-wide	E	GxE	Genome-wide	E	GxE		
No filtering	24253	2166	305	5725	624	74	1.22*†	1.03
AFD>0.60	9082	734	128	2343	246	31	1.30*†	0.94
AFD>0.60 & LD>0.19	1209	105	11	419	49	11	1.35*	2.88*†
AFD>0.60 & LD>0.32	192	22	0	71	9	6	1.10	Inf*†

To identify potential candidates underlying genetic tradeoffs, we chose GxE genes with cis-regulatory/nonsynonymous SNPs with high AFD (>0.60) and LD (>0.19) (Table 2). As shown in Table 2, GxE genes within genetic tradeoff QTL showed twice the expression levels than GxE genes outside the QTL. Two of these genes (AT2G35050, *FLDH*) were previously identified as candidate genes (Price, et al. 2018; Price, et al. 2020) and four of the genes (AT2G35050, AT3G56408, AT4G33180, AT5G65860) have no known function. The rest of the genes have been associated with some very interesting biological processes, such as shade avoidance, light-dependent cold tolerance, drought and freezing tolerance, and response to hypoxia (Table 2). *COL7* which is located within genetic tradeoff QTL 1:3, is also located within a high confidence flowering time QTL (Chr1: 27.4-29.1 Mb) where the Sweden genotype showed significantly longer flowering time than the Italy genotype in Italy (Ågren, et al. 2017). As shown in the rooted *COL7* tree (Figure S2) Eurasian accessions with a similar sequence at the Swedish genotype flo wer significantly later, than accessions with sequences as the Italy genotype.

TABLE 2. Genes showing significant genetic differentiation (AFD>0.60) and linkage disequilibrium (LD>0.19,0.32) along genetic tradeoff (GT) QTL (ID's shown from Ågren, et al. 2013). Shown is also the mean expression ($\overline{FPKM}$ x 10^{-3}) of these genes under control (Ct. – 22 °C) and cold acclimation conditions (Cl.- 4 °C). † indicates genes with cis-reg./nonsyn. SNPs with an AFD>0.60 and LD>0.32). The Biological process were taiken from the TAIR database (Berardini, et al. 2015).

Gene ID's (common names)	GT QTL ID	Italy		Sweden		Biological processes
		Ct.	Cl.	Ct.	Cl.	

AT1G73870 (COL7)	1:3	0.85	1.42	0.88	0.63	positive regulation of transcription, secondary shoot formation, shade avoidance
AT2G35050 †	2:2	3.00	1.41	2.71	2.03	Unknown
AT2G36530 (LOS2)	2:2	6.22	23.55	8.40	18.84	Involved in light-dependent cold tolerance, glycolytic process, response to abscisic acid, cadmium ion, cold, light stimulus
AT2G36580	2:2	0.94	2.54	1.24	1.70	cellular response to hypoxia, glycolytic process
AT2G36830 (GAMMA-TIP) †	2:2	2.16	1.48	2.22	0.50	Response to salt stress, gibberellic acid mediated signaling pathway, hydrogen peroxide, urea, and water transport
AT3G56408	3:3	0.44	0.22	0.15	0.22	Unknown
AT4G33180 †	4:2	0.16	0.07	0.14	0.19	Unknown
AT4G33360 (FLDH) †	4:2	0.37	0.42	0.06	0.02	farnesol metabolic process, negative regulation of abscisic acid-activated signaling pathway
AT4G33470 (HDA14) †	4:2	0.86	0.60	0.48	0.55	negative responses to salinity stress, tubulin deacetylation
AT5G62530 (P5CDH) †	5:5	0.66	1.45	1.05	1.01	Drought and freezing tolerance, proline catabolic process to glutamate, reactive oxygen species metabolic process, response to salt stress
AT5G65860	5:5	0.15	0.42	0.22	0.31	Unknown
	QTL	1.44	3.05	1.60	2.36	
	NON-QTL	0.6	1.03	0.44	1.06	

402

403

404 *No evidence linking large decreases in mean expression of cold-induced and CBF pathway*

405 *genes do adaptation in warm climates*

406 Mean expression of high AFD and LD (AFD>0.60 & LD>0.19) GxE genes that showed

407 significant enrichment along genetic tradeoff QLT did not show significant difference in mean

408 expression (Table 1), as reflected in Figure 4c. The filtering for high LD maybe linked to

409 instances of genetic tradeoffs but removes instances conditional neutrality where linkage
410 disequilibrium is expected to be weaker but nonetheless higher than the neutral expectation. To
411 test whether conditional neutrality is linked to the lower expression of some GxE genes in Italy
412 (Figure 4c), we removed the genes in Table 2 from the set of high AFD GxE genes, and tested
413 whether there was a significant difference in LD between 89 genes where Italy plants showed
414 lower expression than Sweden plants under cold, and the rest of the 48 genes. Mean LD of these
415 sets of genes was approximately the same (LD ~0.09), and not significantly different than the
416 genome average (LD ~0.06), at cis-regulatory and nonsynonymous SNPs of LD<0.19. In
417 addition to just choosing genes with a lower expression in Italy, we also looked at LD across
418 genes where Italy plants showed lower or equal to half the expression of Sweden plants under
419 cold. This resulted in 21 (out of the 89) genes, that also showed approximately the same LD
420 (~0.09).

421

422 To further examine the possible role of the CBF pathway in causing genetic-tradeoffs we
423 examined the proportion of CBF-regulon genes with high AFD, or high AFD and LD, cis-
424 regulatory/nonsynonymous SNPs within genetic tradeoff QTL (Table S2). Relative to the
425 genome-wide set of genes none of the categories examined showed a significant enrichment
426 along the QTL (Table S2). In addition to CBF-regulon genes, we also examined whether the
427 genomic region that included the three CBF transcription factors (CBF1-3), showed any genetic
428 differentiation. Using a sliding-window approach we examined the proportion of high AFD
429 (>0.60) SNPs along these genes (Figure S3). The proportion of high AFD SNPs across these
430 genes was below the genome average (Figure S3). Under the assumption of genetic-tradeoffs we
431 would expect these regions to show a significant increase in allele frequency differentiation

(Tiffin and Ross-Ibarra 2014). Furthermore, we did not find any significant evidence for recent selection, since 19 cis-regulatory (no nonsynonymous SNPs) SNPs of the three CBF genes showed a very low mean LD (~0.05).

Discussion

The current study re-examines the link between genome-wide sequence and expression variation to fitness variation of Arabidopsis populations showing significant evidence of local adaptation in their native environments (Ågren and Schemske 2012; Ågren, et al. 2013). The enrichment of genes showing a main effect in environment (E) along the low-resolution fitness QTL, in combination with the even higher enrichment of GxE genes showing significant genetic differentiation and linkage disequilibrium, suggest that plastic responses play an important role in adaptation. More specifically, E genes may represent regulon of genes that are necessary for facing common environmental challenges, while GxE genes represent instances of loci that underwent divergent evolution to adapt to extreme environmental differences. Furthermore, our results suggest that local adaptation occurs through highly expressed and selectively constraint genes. Finally, we find no significant evidence linking significantly lower expression of the CBF-pathway, to adaptation to warmer climates.

Local adaptation is expected to cause allele frequency differentiation (AFD) between populations; especially in the case of genetic tradeoffs (Tiffin and Ross-Ibarra 2014). Furthermore, if local adaptation is recent, loci should also exhibit high linkage disequilibrium (LD) (Nosil, et al. 2009). The importance of these signatures were shown in a previous Arabidopsis study (Price, et al. 2020), where we found that high AFD and LD SNPs were

enriched along fitness QTL, and genes involved in life-history traits that are thought to play a major role in local adaptation; such as flowering time. One of these genes (*PIF3*) showed significant evidence of local adaptation in an evolutionary distant species of tree (*Populus balsamifera L.*) (Keller, et al. 2012). In the current study, we find that expression GxE genes with high AFD and LD SNPs are significantly enriched along individual fitness QTL and paired genetic tradeoff QTL, providing strong evidence for their possible role in local adaptation. Candidate GxE genes were tied to interesting biological processes such as: flowering time, light-dependent cold acclimation, freezing tolerance, and response to hypoxia.

Our final set of GxE genes with high AFD and LD did not show any significant differences in mean expression between Italy and Sweden (Table 2). On the other hand, high AFD GxE genes with lower LD (LD<0.19) showed large decreases in mean expression under cold in Italy plants (Figure 4C). These genes, however, did not show any enrichment along genetic-tradeoff QTL, and significant increases in LD. Some of the factors that may contribute to this observation, are that some of these expression interactions are neutral/nearly-neutral, or they involve adaptive mutations of small effect size (Yeaman 2015; Hoban, et al. 2016; Forester, et al. 2018; Mee and Yeaman 2019).

Among the set of genes showing no significant evidence of local adaptation were the tree freezing tolerance CBF transcription factors and CBF-regulon genes exhibiting GxE interactions. Among the GxE-CBF-regulon genes with cis-regulatory/nonsynonymous SNPs (51/53), only 20% contained high AFD SNPs (>0.60), which is similar to the proportion observed by E genes (Figure 2a). Furthermore, high AFD and/or LD GxE-CBF-regulon genes did not show significant

enrichment along genetic-tradeoff QTL (Table S2). Finally, we did not find any significant evidence of local adaptation (i.e., in AFD and/or LD) along the three CBF transcription factors, supporting evidence that lower expression of these genes in warmer climates is under relaxed (Zhen and Ungerer 2008b; Zhen, et al. 2011) and not positive selection (Monroe, et al. 2016). It will be quite surprising if there is a unique genomic signature of adaptation underlying the three CBF genes, since high AFD and LD SNPs were enriched along the same genetic tradeoff QTL (GT QTL 4:2) but in a different region (Price, et al. 2020).

Interestingly, we find that GxE genes showing evidence of adaptation, show high expression and selective constraint/purifying selection—a pattern observed in other species (Wollenberg Valero, et al. 2014; Maddamsetti, et al. 2017; Boissot, et al. 2020). An interesting hypothesis that could be tested in future studies using Crispr-Cas9 technology (Cong, et al. 2013), is whether the effect size of candidate adaptive variation is positively correlated with levels of expression and selective constraint. This is partially supported by our study, since genes underlying the small number of high effect size QTL (Table 2) show higher mean expression than other genes. Unfortunately, we could not compare selective constraint between genes in Table 2 and the genome average given the very small sample of genes (n=9) with orthologs in *A. halleri*.

In conclusion, our study shows how genomic signatures of local adaptation, recent selection, and selective constraint, are linked to expression and fitness variation between Italy and Sweden ecotypes. Temperature is one of the environmental variables that may underlie local adaptation underlying Arabidopsis populations, therefore, examining more variables (e.g., precipitation (Postma, et al. 2016; Exposito-Alonso, et al. 2018; Monroe, et al. 2018)) may help us further

501 understand the genetic architecture of local adaptation (Dittmar, et al. 2016) and the selection
502 forces underlying it.
503
504
505 **References**

506 1001 Genomes Consortium. 2016. 1,135 Genomes Reveal the Global Pattern of Polymorphism
507 in Arabidopsis thaliana. Cell 166:481-491.

508 Ågren J, Oakley CG, Lundemo S, Schemske DW. 2017. Adaptive divergence in flowering time
509 among natural populations of Arabidopsis thaliana: Estimates of selection and QTL mapping.
510 Evolution 71:550-564.

511 Ågren J, Oakley CG, McKay JK, Lovell JT, Schemske DW. 2013. Genetic mapping of
512 adaptation reveals fitness tradeoffs in Arabidopsis thaliana. Proc Natl Acad Sci U S A
513 110:21077-21082.

514 Ågren J, Schemske DW. 2012. Reciprocal transplants demonstrate strong adaptive
515 differentiation of the model organism Arabidopsis thaliana in its native range. New Phytologist
516 194:1112-1122.

517 Anderson JT, Willis JH, Mitchell-Olds T. 2011. Evolutionary genetics of plant adaptation.
518 Trends Genet 27:258-266.

519 Baye TM, Abebe T, Wilke RA. 2011. Genotype-environment interactions and their translational
520 implications. Per Med 8:59-70.

521 Beaumont MA, Balding DJ. 2004. Identifying adaptive genetic divergence among populations
522 from genome scans. Mol Ecol 13:969-980.

523 Berardini TZ, Reiser L, Li D, Mezheritsky Y, Muller R, Strait E, Huala E. 2015. The
524 Arabidopsis information resource: Making and mining the "gold standard" annotated reference
525 plant genome. Genesis 53:474-485.

526 Berner D. 2019. Allele Frequency Difference AFD(-)An Intuitive Alternative to FST for
527 Quantifying Genetic Population Differentiation. Genes (Basel) 10.

528 Bhatia G, Patterson N, Sankararaman S, Price AL. 2013. Estimating and interpreting FST: the
529 impact of rare variants. Genome Res 23:1514-1521.

Boissot N, Dutartre-Fricaux L, Beucher C, Vanlerberghe F. 2020. Highly conserved genes expressed in aphid saliva are candidates for host plant adaptation in Aphis gossypii. In: Research Square.

Brown SM, Heguy A, Zappile P, Chen H, Goradia A, Wang Y, Hao Y, Roy NK, Vitale K, Chambers RC, et al. 2017. A Dramatic Difference in Global Gene Expression between TCDD-Treated Atlantic Tomcod Larvae from the Resistant Hudson River and a Nearby Sensitive Population. Genome Biology and Evolution 9:2251-2264.

Caye K, Jumentier B, Lepeule J, Francois O. 2019. LFMM 2: Fast and Accurate Inference of Gene-Environment Associations in Genome-Wide Studies. Mol Biol Evol 36:852-860.

Chiang GC, Bartsch M, Barua D, Nakabayashi K, Debieu M, Kronholm I, Koornneef M, Soppe WJ, Donohue K, De Meaux J. 2011. DOG1 expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in Arabidopsis thaliana. Mol Ecol 20:3336-3349.

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, et al. 2013. Multiplex genome engineering using CRISPR/Cas systems. Science 339:819-823.

Cook D, Fowler S, Fiehn O, Thomashow MF. 2004. A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of Arabidopsis. Proc Natl Acad Sci U S A 101:15243-15248.

De Mita S, Thuillet AC, Gay L, Ahmadi N, Manel S, Ronfort J, Vigouroux Y. 2013. Detecting selection along environmental gradients: analysis of eight methods and their effectiveness for outbreeding and selfing populations. Mol Ecol 22:1383-1399.

de Villemereuil P, Frichot É, Bazin É, François O, Gaggiotti OE. 2014. Genome scan methods against more complex models: when and how much should we trust them? Molecular Ecology 23:2006-2019.

de Villemereuil P, Gaggiotti OE. 2015. A new FST-based method to uncover local adaptation using environmental variables. Methods in Ecology and Evolution 6:1248-1258.

Des Marais DL, Hernandez KM, Juenger TE. 2013. Genotype-by-Environment Interaction and Plasticity: Exploring Genomic Responses of Plants to the Abiotic Environment. Annual Review of Ecology, Evolution, and Systematics 44:5-29.

Des Marais DL, McKay JK, Richards JH, Sen S, Wayne T, Juenger TE. 2012. Physiological genomics of response to soil drying in diverse Arabidopsis accessions. Plant Cell 24:893-914.

561 Dittmar EL, Oakley CG, Conner JK, Gould BA, Schemske DW. 2016. Factors influencing the
562 effect size distribution of adaptive substitutions. Proc Biol Sci 283.

563 Exposito-Alonso M, Vasseur F, Ding W, Wang G, Burbano HA, Weigel D. 2018. Genomic basis
564 and evolutionary potential for extreme drought adaptation in Arabidopsis thaliana. Nat Ecol Evol
565 2:352-358.

566 Foll M, Gaggiotti OE, Daub JT, Vatsiou A, Excoffier L. 2014. Widespread signals of convergent
567 adaptation to high altitude in Asia and america. Am J Hum Genet 95:394-407.

568 Forester BR, Lasky JR, Wagner HH, Urban DL. 2018. Comparing methods for detecting
569 multilocus adaptation with multivariate genotype–environment associations. Molecular Ecology
570 27:2215-2233.

571 Fournier-Level A, Korte A, Cooper MD, Nordborg M, Schmitt J, Wilczek AM. 2011. A map of
572 local adaptation in Arabidopsis thaliana. Science 334:86-89.

573 Franssen SU, Gu J, Bergmann N, Winters G, Klostermeier UC, Rosenstiel P, Bornberg-Bauer E,
574 Reusch TB. 2011. Transcriptomic resilience to global warming in the seagrass Zostera marina, a
575 marine foundation species. Proc Natl Acad Sci U S A 108:19276-19281.

576 Gehan MA, Park S, Gilmour SJ, An C, Lee CM, Thomashow MF. 2015. Natural variation in the
577 C-repeat binding factor cold response pathway correlates with local adaptation of Arabidopsis
578 ecotypes. Plant J 84:682-693.

579 Gunther T, Coop G. 2013. Robust identification of local adaptation from allele frequencies.
580 Genetics 195:205-220.

581 Hannah MA, Wiese D, Freund S, Fiehn O, Heyer AG, Hincha DK. 2006. Natural genetic
582 variation of freezing tolerance in Arabidopsis. Plant Physiol 142:98-112.

583 Henry RJ, Nevo E. 2014. Exploring natural selection to guide breeding for agriculture. Plant
584 Biotechnol J 12:655-662.

585 Hereford J. 2009. A quantitative survey of local adaptation and fitness trade-offs. Am Nat
586 173:579-588.

587 Hoban S, Kelley JL, Lotterhos KE, Antolin MF, Bradburd G, Lowry DB, Poss ML, Reed LK,
588 Storfer A, Whitlock MC. 2016. Finding the Genomic Basis of Local Adaptation: Pitfalls,
589 Practical Solutions, and Future Directions. Am Nat 188:379-397.

590 Howe KL, Contreras-Moreira B, De Silva N, Maslen G, Akanni W, Allen J, Alvarez-Jarreta J,
591 Barba M, Bolser DM, Cambell L, et al. 2020. Ensembl Genomes 2020-enabling non-vertebrate
592 genomic research. Nucleic Acids Res 48:D689-D695.

593 Kawecki TJ, Ebert D. 2004. - Conceptual issues in local adaptation. - 7:- 1241.

594 Keller SR, Levsen N, Olson MS, Tiffin P. 2012. Local adaptation in the flowering-time gene
595 network of balsam poplar, Populus balsamifera L. Mol Biol Evol 29:3143-3152.

596 Kelly M. 2019. Adaptation to climate change through genetic accommodation and assimilation
597 of plastic phenotypes. Philos Trans R Soc Lond B Biol Sci 374:20180176.

598 Kinsella RJ, Kahari A, Haider S, Zamora J, Proctor G, Spudich G, Almeida-King J, Staines D,
599 Derwent P, Kerhornou A, et al. 2011. Ensembl BioMarts: a hub for data retrieval across
600 taxonomic space. Database (Oxford) 2011:bar030.

601 Lasky JR, Des Marais DL, Lowry DB, Povolotskaya I, McKay JK, Richards JH, Keitt TH,
602 Juenger TE. 2014. Natural variation in abiotic stress responsive gene expression and local
603 adaptation to climate in Arabidopsis thaliana. Mol Biol Evol 31:2283-2296.

604 Lasky JR, Des Marais DL, McKay JK, Richards JH, Juenger TE, Keitt TH. 2012. Characterizing
605 genomic variation of Arabidopsis thaliana: the roles of geography and climate. Molecular
606 Ecology 21:5512-5529.

607 López-Maury L, Marguerat S, Bähler J. 2008. Tuning gene expression to changing
608 environments: from rapid responses to evolutionary adaptation. Nat Rev Genet 9:583-593.

609 Lotterhos KE, Whitlock MC. 2015. The relative power of genome scans to detect local
610 adaptation depends on sampling design and statistical method. Mol Ecol 24:1031-1046.

611 Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for
612 RNA-seq data with DESeq2. Genome Biol 15:550.

613 Lovell JT, Schwartz S, Lowry DB, Shakirov EV, Bonnette JE, Weng X, Wang M, Johnson J,
614 Sreedasyam A, Plott C, et al. 2016. Drought responsive gene expression regulatory divergence
615 between upland and lowland ecotypes of a perennial C4 grass. Genome Res 26:510-518.

616 Luu K, Bazin E, Blum MG. 2017. pcadapt: an R package to perform genome scans for selection
617 based on principal component analysis. Mol Ecol Resour 17:67-77.

618 Maddamsetti R, Hatcher PJ, Green AG, Williams BL, Marks DS, Lenski RE. 2017. Core Genes
619 Evolve Rapidly in the Long-term Evolution Experiment with Escherichia coli. Genome Biol
620 Evol.

621 Matthey-Doret R, Whitlock MC. 2019. Background selection and FST : Consequences for
622 detecting local adaptation. Mol Ecol 28:3902-3914.

623 McKhann HI, Gery C, Berard A, Leveque S, Zuther E, Hincha DK, De Mita S, Brunel D, Teoule
624 E. 2008. Natural variation in CBF gene sequence, gene expression and freezing tolerance in the
625 Versailles core collection of Arabidopsis thaliana. BMC Plant Biol 8:105.

626 Mee JA, Yeaman S. 2019. Unpacking Conditional Neutrality: Genomic Signatures of Selection
627 on Conditionally Beneficial and Conditionally Deleterious Mutations. Am Nat 194:529-540.

628 Monroe JG, McGovern C, Lasky JR, Grogan K, Beck J, McKay JK. 2016. Adaptation to warmer
629 climates by parallel functional evolution of CBF genes in Arabidopsis thaliana. Mol Ecol
630 25:3632-3644.

631 Monroe JG, Powell T, Price N, Mullen JL, Howard A, Evans K, Lovell JT, McKay JK. 2018.
632 Drought adaptation in Arabidopsis thaliana by extensive genetic loss-of-function. Elife 7:e41038.

633 Morris MR, Richard R, Leder EH, Barrett RD, Aubin-Horth N, Rogers SM. 2014. Gene
634 expression plasticity evolves in response to colonization of freshwater lakes in threespine
635 stickleback. Mol Ecol 23:3226-3240.

636 Nielsen R. 2005. Molecular signatures of natural selection. Annu Rev Genet 39:197-218.

637 Nosil P, Funk DJ, Ortiz-Barrientos D. 2009. Divergent selection and heterogeneous genomic
638 divergence. Mol Ecol 18:375-402.

639 Oakley CG, Agren J, Atchison RA, Schemske DW. 2014. QTL mapping of freezing tolerance:
640 links to fitness and adaptive trade-offs. Mol Ecol 23:4304-4315.

641 Park S, Gilmour SJ, Grumet R, Thomashow MF. 2018. CBF-dependent and CBF-independent
642 regulatory pathways contribute to the differences in freezing tolerance and cold-regulated gene
643 expression of two Arabidopsis ecotypes locally adapted to sites in Sweden and Italy. PLoS One
644 13:e0207723.

645 Park S, Lee CM, Doherty CJ, Gilmour SJ, Kim Y, Thomashow MF. 2015. Regulation of the
646 Arabidopsis CBF regulon by a complex low-temperature regulatory network. Plant J 82:193-207.

647 Postma FM, Lundemo S, Agren J. 2016. Seed dormancy cycling and mortality differ between
648 two locally adapted populations of Arabidopsis thaliana. Ann Bot 117:249-256.

649 Price N, Lopez L, Platts AE, Lasky JR. 2020. In the presence of population structure: From
650 genomics to candidate genes underlying local adaptation. Ecology and Evolution 10:1889-1904.

651 Price N, Moyers BT, Lopez L, Lasky JR, Monroe JG, Mullen JL, Oakley CG, Lin J, Agren J,
652 Schrider DR, et al. 2018. Combining population genomics and fitness QTLs to identify the
653 genetics of local adaptation in Arabidopsis thaliana. Proc Natl Acad Sci U S A 115:5028-5033.

654 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de
655 Bakker PI, Daly MJ, et al. 2007. PLINK: a tool set for whole-genome association and
656 population-based linkage analyses. Am J Hum Genet 81:559-575.

657 Razgour O, Forester B, Taggart JB, Bekaert M, Juste J, Ibanez C, Puechmaille SJ, Novella-
658 Fernandez R, Alberdi A, Manel S. 2019. Considering adaptive genetic variation in climate
659 change vulnerability assessment reduces species range loss projections. Proc Natl Acad Sci U S
660 A 116:10418-10423.

661 Rockman MV, Hahn MW, Soranzo N, Goldstein DB, Wray GA. 2003. Positive selection on a
662 human-specific transcription factor binding site regulating IL4 expression. Curr Biol 13:2118-
663 2123.

664 Savolainen O, Lascoux M, Merila J. 2013. Ecological genomics of local adaptation. Nat Rev
665 Genet 14:807-820.

666 Seo E, Lee H, Jeon J, Park H, Kim J, Noh YS, Lee I. 2009. Crosstalk between cold response and
667 flowering in Arabidopsis is mediated through the flowering-time gene SOC1 and its upstream
668 negative regulator FLC. Plant Cell 21:3185-3197.

669 Thomashow MF. 2010. Molecular basis of plant cold acclimation: insights gained from studying
670 the CBF cold response pathway. Plant Physiol 154:571-577.

671 Tiffin P, Ross-Ibarra J. 2014. Advances and limits of using population genetics to understand
672 local adaptation. Trends Ecol Evol 29:673-680.

673 Trapnell C, Pachter L, Salzberg SL. 2009. TopHat: discovering splice junctions with RNA-Seq.
674 Bioinformatics 25:1105-1111.

675 Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold
676 BJ, Pachter L. 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated
677 transcripts and isoform switching during cell differentiation. Nat Biotechnol 28:511-515.

678 Wollenberg Valero KC, Pathak R, Prajapati I, Bankston S, Thompson A, Usher J, Isokpehi RD.
679 2014. A candidate multimodal functional genetic network for thermal adaptation. PeerJ 2:e578.

680 Yeaman S. 2015. Local Adaptation by Alleles of Small Effect. Am Nat 186 Suppl 1:S74-89.

Yoder JB, Stanton-Geddes J, Zhou P, Briskine R, Young ND, Tiffin P. 2014. Genomic signature of adaptation to climate in Medicago truncatula. Genetics 196:1263-1275.

Yoder JB, Tiffin P. 2017. Effects of Gene Action, Marker Density, and Timing of Selection on the Performance of Landscape Genomic Scans of Local Adaptation. J Hered 109:16-28.

Zhen Y, Dhakal P, Ungerer MC. 2011. Fitness benefits and costs of cold acclimation in Arabidopsis thaliana. Am Nat 178:44-52.

Zhen Y, Ungerer MC. 2008a. Clinal variation in freezing tolerance among natural accessions of Arabidopsis thaliana. New Phytol 177:419-427.

Zhen Y, Ungerer MC. 2008b. Relaxed selection on the CBF/DREB1 regulatory genes and reduced freezing tolerance in the southern range of Arabidopsis thaliana. Mol Biol Evol 25:2547-2555.

Zhou X, Stephens M. 2012. Genome-wide efficient mixed-model analysis for association studies. Nat Genet 44:821-824.