Running title: Botrytis eGWA of co-transcriptome

**Pathogen genetic control of transcriptome variation in the *Arabidopsis thaliana* – *Botrytis cinerea* pathosystem**

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**ABSTRACT**

Little is known about the genetic mechanisms of interaction between generalist pathogens and their plant hosts. To elucidate directional interactions which contribute to pathogen virulence, we examined the co-transcriptome of *Botrytis cinerea* on *Arabidopsis thaliana*, across a genotyped and genetically diverse collection of 96 *B. cinerea* isolates. We performed genome-wide association (GWA) in Botrytis cinerea for each of NUMBER variable transcript expression profiles in the host, and NUMBER transcripts in the pathogen. We identified mostly trans-eQTL in the pathogen and found eQTL hotspots dispersed across the pathogen genome. These hotspots, and their genetic targets, suggest links to several known and many novel mechanisms of virulence in the plant-pathogen interaction. Genes annotated to these hotspots provide potential targets for silencing manipulation of the host response by this ubiquitous generalist pathogen.

**INTRODUCTION**

Infectious disease is an interaction between host and pathogen, determined by the genetics of both and their interactions. Plant-pathogen interactions are often parsed as qualitative, in which few genetic variants interact to determine binary disease outcomes, or quantitative, in which a spectrum of outcomes may occur due to genetic variation between the host and pathogen. The past decade has revealed the molecular basis of large-effect loci on either the host side or the pathogen side that control qualitative interactions. However, the majority of plant-symbiont interactions are quantitative. Studies have begun to elucidate the host genetic basis of quantitative plant-pathogen interactions, where there is a lack of evidence for qualitative virulence/ resistance genes underlying quantitative disease outcomes in plant-pathogen interactions. Rather, the genetic basis of plant resistance in these interactions is highly polygenic (Glazebrook 2005, Nomura, Melotto et al. 2005, Goss and Bergelson 2006, Rowe and Kliebenstein 2008, Barrett, Kniskern et al. 2009, Corwin, Copeland et al. 2016, Fordyce, Soltis et al. 2018). The pathogen genetic basis is less clearly defined and is being elucidated through the study of phenotypic variation across genetically diverse hosts and pathogens, finding links to genetic variation (Bartoli and Roux 2017).

On the pathogen side, recent studies have accumulated evidence for a polygenic basis of virulence as well (Corwin, Copeland et al. 2016, Bartoli and Roux 2017, Wu, Sakthikumar et al. 2017, Zhang, Corwin et al. 2017, Atwell, Corwin et al. 2018, Soltis, Atwell et al. 2019). These studies provide many candidate loci for pathogen resistance, with diverse functional annotations. Some of these loci appear to modulate virulence across multiple virulence phenotypes, including lesion size across multiple hosts and independent phenotypes of lesion growth (Corwin, Copeland et al. 2016, Fordyce, Soltis et al. 2018, Soltis, Atwell et al. 2019). However, thus far we know little about the molecular mechanism of action by which these genes affect virulence outcomes, or how the host and pathogen genetics interact.

Several methods are available to untangle the genomics underlying quantitative disease interactions. A genome-wide association (GWA) approach can be applied to the plant and pathogen genomes simultaneously, as well as their genetic interactions (Wang, Roux et al. 2018). In these co-genomic approaches, most of the variation in disease resistance appears to be dominated by genomics on the pathogen side of the interaction (Bartha, McLaren et al. 2017, Wang, Roux et al. 2018). Further, applying GWA to co-transcriptome data can clarify the directionality, and ultimately causality, of plant-pathogen molecular crosstalk.

Expression quantitative trait loci (eQTL) are the markers correlated with, and hypothetically controlling, variation in transcript expression profiles as determined by recombinant inbred lines (RIL) or GWA. Locally acting (*cis*) eQTL may indicate regulatory variation within the expressed gene itself, or nearby. Additional markers distant from the responding gene are classified as *trans*-eQTL. *trans*-eQTL hotspots (loci linked to expression variation across many transcripts) may point to master regulators, with extensive pleiotropy across many genes. eQTL analysis focusing on one side of the plant-pathogen interaction can identify pathogen loci controlling pathogen gene expression in planta, or plant loci controlling plant gene expression over the course of infection (Chen, Hackett et al. 2010, Christie, Myburg et al. 2017). Previous studies have examined eGWA (expression genome-wide association) in human disease (Hsu and Smith 2012, Zou, Chai et al. 2012, Allen, Carrasquillo et al. 2016). However, few studies have conducted genome-wide association for any infectious disease traits on the pathogen side, let alone expression traits for eQTL or eGWA (Bartoli and Roux 2017).

Studies encompassing transcriptomic variation in both the host and pathogen, and genomic variation within one of the interacting organisms, can look for signs of interspecific *trans*-eQTL; loci in the pathogen that modulate expression in the infected host, or loci in the host that modulate expression in the infecting pathogen. A few studies have examined variation in host-pathogen interactions in this way, validating the ability of this approach to identify pathogen loci modulating host expression levels, and thus candidate loci for interspecific signals (Wu, Cai et al. 2015, Guo, Fudali et al. 2017). These cross-species *trans*-eQTL can be used to hypothesize causal relationships between individual genes in the interspecific interaction (Wu, Cai et al. 2015, Guo, Fudali et al. 2017).

Previous studies have identified hundreds of plant host genes with one or more eQTL in the pathogen modulating their expression (Wu, Cai et al. 2015, Guo, Fudali et al. 2017), and one small study identified 3 bacterial parasite genes modulated by human host eQTL (Guo, Fudali et al. 2017). Hundreds of pathogen eQTL linked to host expression changes (Wu, Cai et al. 2015), and a single eQTL may modulate expression of over 60 host genes (Guo, Fudali et al. 2017). Thus, the majority of the disease interactions appear to be modulated by very few loci within the pathogen genome.

In this study, we focus on the co-transcriptome between an extreme generalist pathogen with high genetic diversity, *B. cinerea*, and the model plant host, *A. thaliana*. The genetic interactions within this host-pathogen system are not dominated by large effects, which allows for investigation of more complex small-effect points of interaction (Denby, Kumar et al. 2004, Rowe and Kliebenstein 2008, Zhang, Corwin et al. 2017, Atwell, Corwin et al. 2018). Previous studies have proven that we can successfully measure the co-transcriptome through simultaneous RNA-Seq in both the host and pathogen (Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018). Previous analysis showed that the vast majority of transcripts, on both the host side and on the pathogen side, are affected by variation in the *B. cinerea* genome (Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018). We have also previously successfully conducted GWA in this pathogen population for the phenotype of lesion size (Atwell, Corwin et al. 2018, Soltis, Atwell et al. 2019).

The genomes of both the host and the pathogen harbor extensive diversity, with potential for identifying interactions between the two. *B. cinerea* exhibits highly quantitative virulence, facilitated through natural genetic variation (Rowe, Walley et al. 2010, Corwin, Copeland et al. 2016, Zhang, Corwin et al. 2018). *A. thaliana* contains natural variation for immune pathways with large effects on defense against *B. cinerea* (Zhang, Corwin et al. 2017). Interactions between these species are well-characterized phenotypically, and we have previous information on some of the potentially relevant genetic factors on both the pathogen and host sides of the interaction (Denby, Kumar et al. 2004, Rowe and Kliebenstein 2008, Zhang, Corwin et al. 2017, Atwell, Corwin et al. 2018, Soltis, Atwell et al. 2019).

As such, we conducted genome-wide association (GWA) to look for evidence of loci in *B. cinerea* that may be modulating this heritable expression variation. In this study, we ask how genetics within the pathogen may modulate expression variation over the course of infection. We work with the gene expression data from Zhang *et al*., performing genome-wide association (GWA) of variation in individual transcript expression profiles with SNP level variation within the *B. cinerea* genome when infecting the wildtype host Col-0 *A. thaliana*. This gives us a hypothesis of directionality; any locus in *B. cinerea* linked to expression variation in the host or pathogen is directly or indirectly modulating expression. With numerous traits in this analysis, we focused on general patterns of eQTL distribution across the genome, and identification of major hotspots of eQTL. Our analysis found mostly small-effect polymorphisms dispersed throughout the *B. cinerea* genome, with several hotspots of *trans*-eQTL activity. These hotspot loci were largely linked to variation in transcripts in only the host or the pathogen, suggesting a fairly independent basis of transcriptional regulation of host and pathogen by the *B. cinerea* genome. Among these hotspot loci, all appeared to tag novel genes not previously identified as controlling plant-pathogen virulence interactions. Expression of several of these hotspot genes positively correlates with lesion size, and their downstream targets include many genetic functions, including some virulence traits. Overall, we identify a mix of novel loci potentially controlling the interaction of *A. thaliana* and *B. cinerea* via modulation of gene expression, with evidence for connections to virulence.

**RESULTS**

**eQTL indicate polygenic transcriptome modulation**

To better understand how natural genetic variation in the pathogen influences both the host and pathogen transcriptomes, we performed eGWA across all genes expressed in both species within the *B. cinerea* - *A. thaliana* pathosystem. This incorporated the expression profiles of 9,267 *B. cinerea* genes and 23,947 *A. thaliana* genes,each as individual traits. For each trait, we used a used Genome-wide Efficient Mixed Model Association (GEMMA) mode with a previous genome-wide SNP dataset of 237,878 SNPs with a minimum minor allele frequency of 0.20 (Zhou and Stephens 2012, Atwell, Corwin et al. 2018). GEMMA estimates the significance of effects of each SNP on the focal trait as a p-value after accounting for potential effects of population structure within the *B. cinerea* isolates. In total, GEMMA was able to identify *B. cinerea* SNPs linked to transcriptional variation in 5,213 *A. thaliana* genes and 1,616 *B. cinerea* genes. For these genes with significant SNPs, there was a median of XX SNPs per transcript (Range 1 to 16,818 SNPs) for *B. cinerea*, and a median of XX SNPs per transcript (Range 1 to 24,623 SNPs) for *A. thaliana* transcripts (Figure N1). Further, the distribution of p-values for significant SNP found little evidence for large effect polymorphisms suggesting a highly polygenic basis of loci modulating transcriptome variation.

Given the scale of this dataset, it was not viable to estimate empirical significance thresholds for each and every transcript using 1,000 or more permutations. However, we permuted the whole dataset across all of the tens of thousands of traits five times and repeated the GEMMA to get a feel for the potential for dominant patterns that may exist randomly (individual expression profiles in *B. cinerea* and *A. thaliana*). We then compared the permuted minimum p-value per transcript across all SNPs to the data obtained from real traits. This showed that the top SNP per trait for most genes show a stronger association in our observed data than across any of the 5 permutations. In *B. cinerea*, the observed p-value is lower for 69% of genes, and in *A. thaliana* the observed p-value is lower for 58% of genes. Thus, to develop genomic images of the results, we focused on the top SNP per transcript for the remaining analysis.

**Absence of transcriptome cis-effect dominance**

A hallmark of eQTL mapping studies using either GWA or structured mapping populations in a wide range of species is the occurrence of large-effect loci that map to the gene itself, i.e. *cis-*eQTL or *cis*-SNPs (Brem, Yvert et al. 2002, Schadt, Monks et al. 2003, Monks, Leonardson et al. 2004, Keurentjes, Fu et al. 2007, West, Kim et al. 2007, Zou, Chai et al. 2012). However, we did not identify a large number of outlier p-values as would be expected if there were numerous large-effect *cis*-eQTL. To test if the *B. cinerea* transcriptome shows a similar cis-eQTL pattern, we plotted the position of the transcript’s genomic position against the top GWA SNP for all the *B. cinerea* transcripts. We first focused on the single top SNP hit per transcript, with the highest probability (lowest p-value) of significant effect on expression in the gene of interest. If control of gene expression is localized to the gene itself or toproximate loci, we would expect a strong linear (*cis*-diagonal) association between the center of each gene and the genomic location of its top SNP hit. However, there was no evidence of any cis-diagonal (Figure N2). This pattern holds whether we examine the top 1 SNP per transcript (Figure N2a) or the top 10 SNPs per transcript (FigureN2b). In contrast, there was evidence for *trans*-eQTL hotspots; loci which modulate expression variation across many of the pathogen genes (Figure N2).

To test if there might be a bias towards *cis*-effects that may function at a close distance, we calculated the distance between the center of each transcript and the top associated SNP. If *cis*- acting loci contribute the bulk of genetic control of expression variation, we would expect to see a high frequency of short-distance associations, and a rapid decline to a plateau moving away from the gene of interest. However, we observe that distances between transcript center and top SNP as far as 2 Mb are common (Figure N3). These distances are similar to what would happen if the causal SNPs had no *cis-* association and were instead scattered across the genome (Figure SX1). As such, we do not see evidence for *cis*-effect loci overrepresented in the top candidates for control of expression variation. Rather, most of the loci that we can associate with potentially influencing gene expression variation in *B. cinerea* on *A. thaliana* is *trans*-acting.

**Search for *cis*-effects through focus on gene networks with presence-absence polymorphism**

The absence of a dominant *cis*- pattern in the genome-wide transcript-to-SNP associations could be caused by a relative absence of *cis-* variation. Alternatively, haplotype heterogeneity or allele frequency may complicate the ability to accurately identify *cis*-polymorphisms (Chan, Rowe et al. 2010). To test between these possibilities, we conducted a more focused analysis on three biosynthetic pathways that exist as gene clusters. These biosynthetic pathways contribute to *B. cinerea* virulence and the botcinic acid biosynthetic pathway (13 genes, 55.8 kb), botrydial biosynthetic pathway (7 genes, 26 kb), and a putative cyclic peptide pathway (10 genes, 46.5 kb) (Deighton, Muckenschnabel et al. 2001, Colmenares, Aleu et al. 2002, Porquier, Morgant et al. 2016, Zhang, Corwin et al. 2018). These pathways have known presence-absence polymorphisms and should have *cis*-eQTL but none were detected (Siewers, Viaud et al. 2005, Pinedo, Wang et al. 2008, Zhang, Corwin et al. 2018). Critically, the transcripts within each of these pathways are highly correlated across the isolates, suggesting that their expression variation is controlled by pathway-specific variation (Zhang, Corwin et al. 2018). Thus, these loci may have false-negative issues that prevented the detection of real *cis*-eQTL.

To test if these pathways have undetected *cis*-eQTL we used all of the SNPs for each biosynthetic cluster to align the *B. cinerea* isolates and investigate haplotype diversity. We first investigated the botcinic acid cluster which identified a number of distinct haplotypes with a few individual outlier isolates (e.g. B05.10, Fd1) (Figure N4a). We then utilized the haplotypes to test for specific effects on transcript expression for the biosynthetic pathway. This identified a single clade of isolates with a distinctly lower level of expression than the other clusters (Figure N4b). Investigating the short-reads and SNP calls showed that these 12 isolates share a 53.5 kb deletion that removes the entire biosynthetic cluster (Figure N4c). After removing the major deletion, we found no remaining significant effect of cluster membership in the remaining 3 major clusters on expression profile (F(1,74)=0.36, p=0.55). However, within each of these clusters there are independent isolates with very low pathway expression, suggesting loss-of-expression polymorphisms (Noble Rot, 01.04.03, Apple 517, 02.04.09) (Figure N4b). While these isolates each contain smaller deletions that are independent of each other, it is not clear what is functionally leading to the loss of botcinic acid biosynthetic pathway expression (Figure N4c). This suggests that for this clustered pathway, there are undetected *cis*-effect polymorphisms, a large common deletion and rarer additional events.

We then investigated the other two biosynthetic pathways for additional evidence of missed *cis*-acting genetic variation. The botrydial biosynthetic network, and the cyclic peptide pathway, exhibit a similar lack of dominant *cis*-effect SNP patterns to the botcinic acid biosynthetic network. Hierarchical clustering within each of these networks by genic SNP variation divides the isolate population into two groups that are not associated with mean pathway expression (Figure S1, Figure S2). However, within the cyclic peptide pathway, minor deletions within the intergenic regions correlate with low expression, and two isolates with partial deletions within the genes early in the pathway exhibit very low pathway expression (1.05.16, 1.05.22) (Figure S4). In contrast, there was no evidence for *cis-*effects, although this pathway did not harbor any obvious loss-of-expression events (Figure S3). As such, we can detect cis-acting variation in the form of deletions for two of the biosynthetic pathways. This suggests that there are missing *cis*-effects within the *B. cinerea* GWA, likely missed due to SNP data not incorporating structural variation, as well as the frequency of structural variants often falling below the minor allele cutoffs. Testing whether insertion and deletion events account for the majority of localized control of expression variation would require long-read sequencing to accurately identify these structural variants and computational approaches that can blend SNP and indel information (Wang, Roux et al. 2018).

**Detection of *trans*-eQTL hotspots**

While *cis*-effects are difficult to identify, there was a strong signature of SNPs that appeared to affect more transcripts than expected by chance (Figure N2). These are considered positions where there is a causal polymorphism that influences the regulation of numerous genes in *trans*, i.e. a *trans*-eQTL hotspot. In this dataset, we can extend this analysis to look for *trans*-eQTL hotspots that extend beyond *B. cinerea* and influence the expression of genes in the host, *A. thaliana*. We queried for hotspots in both the *B. cinerea* and *A. thaliana* transcriptome by using the top SNP per transcript (Figure N6). By permuting the SNP positions, we identified maximum permuted hotspots as SNP associated with 11 *B. cinerea* transcripts or 80 *A. thaliana* transcripts. For further analysis of hotspots, we utilized a conservative threshold of 20 linked transcripts for *B. cinerea* and 150 transcripts for *A. thaliana*. This analysis identified 13 SNPs as potential *trans*-eQTL hotspots for the *B. cinerea* transcriptome and 12 SNPs as potential cross-species *trans*-eQTL influencing *the A. thaliana* transcriptome (Figure N6, Figure N7). The *trans*-eQTL hotspots are spread throughout the genome (Figure N6, Table N1).

The benefit of a co-transcriptome is that it should be possible to map how polymorphisms cause effects in the pathogen and how these effects transmit to an altered transcriptome in the host. This would suggest that a *trans*-eQTL hotspot for *B. cinerea* transcripts may control virulence pathways and thus cause an associated *trans*-eQTL hotspot in the *A. thaliana* response. However, we found no overlap in eQTL hotspots across the two transcriptomes; hotspots targeting *B. cinerea* gene expression linked to 0 to 56 transcripts in *A. thaliana*, and hotspots targeting *A. thaliana* gene expression linked to 0 to 3 *B. cinerea* transcripts. All of these are values that are below the permutation threshold. To test if this is caused by using solely the top SNP per transcript, we repeated the full analysis by selecting the top 10 SNPs per transcript. This again identified a limited number of *trans*-eQTL hotspots with little overlap between the two species’ transcriptomes (Table SX1; Figure SX3). This suggests that the pathogen’s influence on the host’s transcriptome is not solely limited to major interactions between *tran*s-eQTL hotspots but can involve narrower changes in the pathogen that are magnified in the host’s response.

**Annotation of eQTL hotspots and targets**

We annotated these hotspots with gene and functional information, to understand possible mechanisms of expression modulation in the host and pathogen transcriptomes. The 12 *A. thaliana* hotspots, annotated to 11 genes, included 4 enzymes and 2 genes associated with isolate compatibility (Table N1). From *B. cinerea* expression profiles, the 13 hotspots annotated to 11 genes included 4 enzymes (Table N1).

To look for connections to virulence, we examined links to co-expression networks and lesion size variation from previous RNA-Seq analysis of these transcripts and GWA analysis of these isolates (Zhang, Corwin et al. 2017, Atwell, Corwin et al. 2018, Zhang, Corwin et al. 2018) (Table X1). eQTL hotspots linked to these co-expression networks could indicate regulatory points for modules of expression variation.

Among the 22 eQTL hotspot genes, three of the *B. cinerea* hotspot genes and two of the *A. thaliana* hotspot genes are strongly positively correlated to lesion size variation across all tested *A. thaliana* genotypes, including immune pathway mutants (Table N1) (Zhang, Corwin et al. 2018). A fourth *B. cinerea* hotspot gene is consistently identified as a top GWA hit controlling lesion size across host genotypes and association methods (Table N1) (Atwell, Corwin et al. 2018).

Nine of the 11 *B. cinerea* eQTL hotspot genes targeted genes in one or more of four major *B. cinerea* co-expression networks on *A. thaliana* (Figure N8). In particular, two of these were host-specific networks functionally associated with virulence, with 7 of the 11 *B. cinerea* hotspot genes associated with one of these virulence co-expression networks. As such, we hypothesize that these major points of *B. cinerea* gene expression modulation may also exhibit regulation of virulence strategies on *A. thaliana*. Nine of the *A. thaliana* eQTL hotspots targeted genes from two major genotype-dependent *A. thaliana* co-expression networks when infected with *B. cinerea* (Figure N8). These networks contain genes pointing to network function in jasmonate and salicylic acid signaling processes and camalexin biosynthesis (Network I), or photosynthesis in the host (Network IV). We observe particularly strong links between 4 of these hotspots and immune networks in *A. thaliana* (Figure N8). These frequent links suggest that our eQTL hotspots may exhibit regulatory control over co-expressed modules of genes active in virulence interactions between *B. cinerea* and its host. If these eQTL hotspots are modulating expression of many genes, and affecting lesion size, they may be major *B. cinerea* control points in the plant-pathogen interaction.

We examined annotations of the genes linked to our eQTL hotspots, to hypothesize mechanisms of regulation by these hotspots. We annotated the genes targeted by these hotspots with functional information. Many of the *B. cinerea* genes targeted by the hotspots are enzymatic (34% of our list of annotated hotspot target genes) (Table N2). These genes also include 11 transcription factors and two genes associated with virulence. While we find annotation information suggestive of metabolic interactions between host and pathogen, and known virulence mechanisms, 32% of our hotspot genes and 21% of the downstream targets do not yet have gene ontology (GO) information (Table N1, Table N2). Thus, this study identifies a large number of loci potentially involved in novel virulence mechanisms of *B. cinerea*.

We looked for functional overrepresentation among the *A. thaliana* genes targeted by hotspots (Table N1, Table N3). Many of the hotspots target genes active in metabolism, particularly primary metabolism including carbohydrate metabolism, nucleic acid metabolism, and amino acid biosynthesis. Three of the hotspots, including hotspots annotated as two enzymes and a heterokaryon incompatibility locus, show an overrepresentation of photosynthesis-related functions among their targeted genes. Two of the hotspots show many target genes with abiotic stress response functions. Only two of the hotspots are linked to expected plant defense loci, including chitin response and microbe defenses.

**DISCUSSION**

**Dispersed interactions across host and pathogen genomes**

We identified 25 eQTL hotspots dispersed across the genome, with half the chromosomes in the *B. cinerea* genome harboring one or more loci with expression modulation of *A. thaliana* genes dispersed across the host genome. This contrasts with previous studies in cross-species eQTL, in which few cross-species eQTL hotspots (termed Host Expression Modulators) clustered on only a few of the parasite chromosomes (termed i-chromosomes) (Guo, Fudali et al. 2017). Our findings are surprising considering the two-speed genome model of filamentous pathogen genomics, in which diverse fungal virulence effectors are enriched in regions of the genome containing repetitive sequences and transposable elements (Dong, Raffaele et al. 2015). This predicts patterns of virulence loci in small regions of the genome with high mutation rates, and slower evolution in the rest of the genome, with little virulence effect. Expanding analysis to additional hosts could reveal specific chromosomes with more common, or more concentrated, expression modulation effects, but thus far we have not found evidence of the two-speed genome for *B. cinerea* expression modulation on *A. thaliana*.

Further, most of the controlling variation detected in our study is distant from the affected transcripts, located in *trans*-eQTL hotspots. Previous co-expression studies in *B. cinerea* also identified five major *trans-* co-expression networks with genes dispersed across the genome of *B. cinerea* (Zhang, Corwin et al. 2018). These findings together provide evidence for *trans*-regulation of gene expression in *B. cinerea* virulence interactions. In particular, our eQTL hotspots contained many genes from the *trans*-networks (vesicle/virulence, translation/growth, exocytosis regulation, peptidase) but none of the *cis*-networks comprised of tandem gene clusters.

**Haplotype diversity and polygenic genetic modulation of expression**

Our results of many significant SNPs linked to expression variation in transcripts of host and pathogen, suggest the highest-diversity model of a generalist pathogen, in which specialization occurs at the gene or allele level and selects for very high diversity and low population structure as the different genetic strategies are intermixed within individuals. This is consistent with the high SNP diversity and low population structure observed in previous studies of *B. cinerea*, due to a combination of random mating and frequent recombination (Williamson, Tudzynski et al. 2007, Rowe, Walley et al. 2010, Atwell, Corwin et al. 2015, Corwin, Subedy et al. 2016, Zhang, Corwin et al. 2017).

Both the genome-wide patterns of eQTL and the network-level focus on haplotype structure and polymorphisms find a signal of SNPs tagging many *trans*-eQTL and few *cis*-eQTL in this study. Network-level focus suggests that some *cis*-acting loss-of-expression polymorphisms would be detectable through analysis of structural variation; future eQTL studies within *B. cinerea* would benefit from the use of both SNP and presence/ absence polymorphism data (Wang, Roux et al. 2018). Future studies could incorporate long-read sequencing to better address these questions. However, this lack of detectable ­*cis*-effect variation also suggests high haplotype diversity, in which the *cis*-acting variation is made up of rare variants that fall below the minor allele cutoff for GWA.

Individual genes in both host and pathogen displayed a polygenic basis of expression modulation from many significant transcript-SNP associations. This contrasts previous studies in which each host expression profile was explained by only a single major-effect pathogen locus (Guo, Fudali et al. 2017). Within the hotspot analysis, multiple eQTL hotspots targeted each co-expression network, but no two eQTL hotspots targeted the same *A. thaliana* gene. Previous studies have also identified multiple targets within a host network per each pathogen eQTL {Wu 2015}.

This may suggest that the hotspots are host expression modulators with parallel network interactions, but independent genetic targets within each host network. In effect, we see polygenicity of host expression regulation by the pathogen at the gene level, and at the network level. Pathogen eQTL hotspots have pleiotropic effects on multiple host genes, and multiple host networks. However, each host gene within these networks may be sensitive to a single pathogen eQTL hotspot. Within this narrowed context, we find some evidence for gene-to-gene interactions between the host and pathogen genomes, as is commonly reported for large-effect loci in specialist pathogen systems. However, due to the redundancy between pathogen-host interactions, it remains to be seen whether these interactions cause noticeable phenotypic effects.

Non-hotspot eQTL may act in a more restricted pleiotropic manner, to regulate expression of relatively few genes. This gives us an overarching pattern of polygenic and pleiotropic genetic regulation, as both the host and pathogen appear to draw from extensive genetic variation to determine disease outcomes.

**Detection of known pathogenicity genes and novel loci**

The 12 *A. thaliana* hotspots were annotated to 11 gene functions (Table N1). Among these, 4 are enzymatic, including a glucose/ ribitol dehydrogenase and a glycoside hydrolase. These *B. cinerea* enzymes may alter pathogen metabolism to elicit host responses, detected here as transcriptional regulation. Alternately, a more direct effect is possible if any of these enzymes are secreted, and function in the digestion of host polysaccharides or other metabolites. Future studies into proteomics of *B. cinerea* may elucidate direct vs. indirect effects of pathogen genes on host metabolism. In fact, one *A. thaliana* hotspot is annotated to a secreted glycoside hydrolase, which may directly interact with host metabolism. Either mechanism would likely stimulate major host responses and thus an expression response (Bcin16g01950, glycoside hydrolase, family 63). Among the 13 *B. cinerea* hotspots, 4 were annotated to *B. cinerea* enzymes (Table N1). Further, the targets of these hotspots are often enzymes, suggesting a major role of *B. cinerea* metabolic shifts as the fungal infection progresses *in planta* (Table N2). These hotspot enzymes may alter major branches of the *B. cinerea* metabolic pathways active during the infection of *A. thaliana*.

Two of the *B. cinerea* hotspots may have direct effects on the transcription machinery (Bcin12g00330, Topoisomerase II-associated protein PAT1; Bcin09g06590, Helicase)(Table N1). Alternately, these genes may affect the number of nuclei per *B. cinerea* mycelial cell, potentially altering the virulence of the pathogen. Some loci show clearer links to virulence, including eQTL hotspots whose expression is positively correlated with lesion size, or whose targets include members of major virulence co-expression networks, and genes with known virulence mechanisms. Further, approximately 1/3 of our hotspot loci and 1/5 of the hotspot target genes currently lack gene ontology information (Table N1, Table N2). As such, this study identifies a large number of novel virulence-associated loci within *B. cinerea*.

**Drawing connections from genome to phenotype**

This work provides some directionality in interspecific genetic interactions, as we detect pathogen loci modulating host and pathogen gene expression. However, future validation work will be required to further understand the directionality and mechanism of this crosstalk. For pathogen eQTL affecting host networks, mutants in the eQTL and the host target genes could elucidate whether the pathogen is specifically targeting host networks, or whether the host is sensing and countering the pathogen attack in response to particular signals.

Previous work in the *B. cinerea* – *A. thaliana* pathosystem established connections between host polymorphisms and lesion growth, between gene expression and lesion size, and between transcriptomes of the host and pathogen (Corwin, Subedy et al. 2016, Zhang, Corwin et al. 2017, Fordyce, Soltis et al. 2018, Zhang, Corwin et al. 2018). To begin establishing causal inference from genome to transcriptome to phenotype, the results of this work fill the gap of connecting genetic variation in the pathogen to expression changes in the interacting transcriptomes. This work builds our functional knowledge of cross-kingdom communication between host and pathogen.

One of the major host networks targeted by the hotspots contains genes with an early expression response that predicts plant resistance at 72 hpi. This study points to pathogen loci that are potentially modulating these host pathway responses to define virulence outcomes.

**Conclusion**

This study is, to our knowledge, the first to look for genome-wide signatures of expression modulation in a plant-pathogen system. Further, it is one of the first studies of how pathogen genomics regulate pathogen and host expression variation over the course of disease.

The 25 eQTL hotspots identified in this study provide potential targets for breeding low-virulence *B. cinerea*. These loci may control modular virulence strategies, serving as decision points in the course of *B. cinerea* infection on *A. thaliana*. The target genes in plants, and their associated networks, may provide targets for disease resistance in plants.

**METHODS**

**Experimental design**

We used a previously described collection of *B. cinerea* genotypes that were isolated as single spores from natural infections of fruit and vegetable tissues collected in California and internationally (Atwell, Corwin et al. 2015, Zhang, Corwin et al. 2017, Fordyce, Soltis et al. 2018, Zhang, Corwin et al. 2018). We focused analysis on the *A. thaliana* accession Columbia-0 (Col-0), and all plants were grown as described in a previous study, with 4-fold replication of the full randomized complete block experimental design across two independent experiments (Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018). The original study included wildtype Col-0 A. thaliana hosts, as well as knockouts to the salicylic acid pathway (*npr1-1*) and to jasmonic acid sensitivity (*coi1-1*). Leaves were harvested 5 weeks after sowing, and inoculated in a detached leaf assay with spores of each of 96 *B. cinerea* isolates (Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018).

**Expression analysis**

RNASeq libraries were prepared as previously described (Kumar, Ichihashi et al. 2012, Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018). Briefly, we prepared mRNA from leaves frozen at 16 hours post inoculation, and pooled amplified, size-selected libraries into four replicate groups of 96 barcoded libraries. Sequencing was completed on a single Illumina HiSeq 2500 (San Diego, CA) lane as single 50bp reads at the U.C. Davis Genome Center- DNA Technologies Core (Davis, CA). Individual libraries were then separated by adapter index from fastq files, evaluated for read quality and overrepresentation (FastQC Version 0.11.3, www.bioinformatics.babraham.ac.uk/projects/), and trimmed (fastx, http://hannonlab.cshl.edu/fastx\_toolkit/commandline.html). Reads were aligned to the *A. thaliana* TAIR10.25 cDNA reference genome, followed by the *B. cinerea* B05.10 cDNA reference genome, and we pulled gene counts (Langmead, Trapnell et al. 2009, Li, Handsaker et al. 2009, Van Kan, Stassen et al. 2017, Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018). We summed counts across gene models, and normalized gene counts as previously described (Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018).

We used as input the model-adjusted means per transcript from negative binomial linked generalized linear models in previously published studies in the *A. thaliana* transcriptome and *B. cinerea* transcriptome (Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018). *A. thaliana* and *B. cinerea* transcript phenotypes were from least square means of normalized gene counts in a negative binomial generalized linear model (nbGLM) (Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018). We calculated linear models from the transcript data including the effects of isolate and host genotype. We z-scaled all transcript profiles prior to GWA.

**Genome wide association**

For GEMMA mapping, we used 95 isolates with a total of 237,878 SNPs against the *B. cinerea* B05.10 genome (Atwell, Corwin et al. 2018). We used haploid binary SNP calls with MAF > 0.20 and <20% missingness. We ran GEMMA once per phenotype, across 9,267 *B cinerea* gene expression profiles and 23,947 *A. thaliana* gene expression profiles.

**Genome wide association of permuted phenotypes**

To validate SNPs as significantly associated with transcript variation, we performed a comparative analysis of randomized phenotypes. Taking each transcriptional profile, we randomized the assignment of phenotypes across the 96-isolate collection. This analysis includes 9,267 randomized *B. cinerea* phenotypes and 23,947 randomized *A. thaliana* phenotypes, one from each measured expression profile. We repeated this randomization in a 5x permutation. We ran GEMMA on each of these permutations, and plotted SNP p-value vs. position (Figure N5). To threshold our individual expression profile GEMMA outputs, we considered p-values below the average 5% permutation threshold as significant; p < 1.96e-05 for *B. cinerea* and p < 2.90e-05 for *A. thaliana*. Permutation approaches are often more effective than p-value thresholding for determining significance across GWA studies with many phenotypes (Evans and Cardon 2006).

**Defining significant hotspots**

We plotted the number of transcripts linked to each SNP, summed across all 5 permutations, to calculate permuted hotspot size. For any SNPs that linked to permuted hotspots of over 5 transcripts in *B. cinerea* or 10 transcripts in *A. thaliana*, we removed these SNPs from downstream analysis as likely false positives. The maximum hotspot size across any of the 5 permutations was 11 genes in *B. cinerea* and 80 genes in *A. thaliana*. We then conservatively defined significant hotspots as SNP peaks exceeding 20 transcripts in *B. cinerea* and 150 transcripts in *A. thaliana*. We further annotated hotspot SNPs to the nearest gene within a 2kb window. The average LD decay in the B. cinerea genome is < 1kb, so we can be relatively confident of SNPs tagging particular genes at the hotspot peaks (Atwell, Corwin et al. 2018). Three genes are annotated to pairs of neighboring hotspots, the rest are unique genes. Two genes on chromosome 12 denoting hotspots from *A. thaliana* gene expression appear closely linked; in fact, they are separated by ~80kb on the *B. cinerea* genome.

**Annotation of gene ontology and network membership**

*A. thaliana* co-expression analysis identified 131 genes across four major networks (Zhang, Corwin et al. 2017). Network architecture varied by plant host, but a constitutive core was conserved across *A. thaliana* genotypes. We compared our eQTL hotspots (both the gene at eQTL hotspot SNP and all associated transcript profiles) to the largest *A. thaliana* network lists (*npr1-1* background) to estimate all possible regulatory ties. We identified gene overlap with two of the major networks; Network I, camalexin biosynthesis; Network IV, chloroplast function.

*B. cinerea* co-expression analysis identified ten major co-expression networks containing 5 to 242 genes (Zhang, Corwin et al. 2018). We identified gene overlap with four of these networks, including one likely involved in fungal vesicle virulence processes including growth and toxin secretion (vesicle/ virulence), one involved in translation and protein synthesis (translation/ growth). These networks maintained a consistent core across the 3 *A. thaliana* host genotypes, but linkages varied; as such we compared our gene lists with the networks across all 12 hosts and included both host-dependent and host-independent annotations of our hotspots.

We looked for functional overrepresentation among the genes targeted by each *A. thaliana* eQTL hotspot using the PANTHER overrepresentation test implemented by plant GO term enrichment from TAIR (Lamesch, Berardini et al. 2011, Mi, Muruganujan et al. 2013).

**Pathway focus**

We focused further *cis*-effects analysis on three networks which were highly conserved across *B. cinerea* isolates (Zhang, Corwin et al. 2018). We clustered isolates by SNP data within focal networks. Hierarchical clustering was computed using the R package pvclust based on mean linkage (UPGMA), with correlation distance and 1000 bootstrap replications (Suzuki and Shimodaira 2015). AU p-values are reported in red, BP values in green. Edges with high AU values are considered strongly supported by the data, and clustering is drawn according to these edges with AU > 95%.

For botcinic acid biosynthesis, the major deletion extends 53.5 kb and includes SNP 4kb from the 5’ end of the chromosome, indicating a teleomeric loss on chromosome 1. We selected a focal region encompassing the deletion endpoints (1.4029, 1.82614) and an additional 2 genes beyond the deletion boundaries (Bcin01g00170, Bcin01g00190) (Figure N4c). We removed 10 SNPs that were likely miscalled (SNP state ~ inverse compared to surrounding region) and called all SNPs within the deletion region as missing.

**TABLE LEGENDS**

**Table N1. Annotation of the hotspots identified from *B. cinerea* and *A. thaliana* eQTL.**

**Table N2. Annotation of the *B. cinerea* genetic targets of *B. cinerea* hotspots.**

**Table N3. Annotation of the *A. thaliana* genetic targets of *B. cinerea* hotspots.**

**FIGURE LEGENDS**

**Figure N1. Manhattan plot examples for 1 transcript per species.** Panel a is an example plot of p-values for all *B. cinerea* SNP associations to a single *B. cinerea* transcript, from Bcin01g00170. Panel b is an example plot of p-values for all *B. cinerea* SNP associations to a single *A. thaliana* transcript.

**Figure N2. *cis*-diagonal plot comparing *B. cinerea* gene center to position of top associated SNP, for all 9,284 transcripts.** We retained only the SNPs with highest probability (lowest p-value) of significant effect on expression for each transcript. Panel a depicts the single top SNP per transcript. Panel b depicts the top 10 SNPs per transcript. Chromosomes are delimited by red bars along the x-axis. Vertical striping of SNP positions indicates genomic locations of putative *trans*-eQTL hotspots.

**Figure N3. Distance between transcript center and top SNP location for all *B. cinerea* expression profiles on Col-0 *A. thaliana*.** Data include the top 1 SNP identified by GEMMA association with each transcript expression profile (lowest p-value for association). Distances are in Mb, including only top SNPs on the same chromosome as the focal gene.

**Figure N4. *cis*-effect analysis of the botcinic acid biosynthetic gene network.** Panel a is hierarchical clustering of *B. cinerea* isolates from SNPs within the botcinic acid biosynthetic gene network. Clustering was based on mean linkage (UPGMA), with correlation distance and 1000 bootstrap replications. AU p-values are reported in red, BP values in green. Edges with high AU values are considered strongly supported by the data, and clustering is drawn according to these edges with AU > 95%. Panel b is Violin plots of botcinic acid network-level expression within *B. cinerea* clusters. Isolates are clustered based membership in groups defined by hierarchical clustering of the SNPs within the botcinic acid biosynthesis network (Figure X5). Panel c is the gene models of the biosynthetic gene network, with the cluster 3 deletion indicated as a triangle.

**Figure N5. Manhattan-type plot of GEMMA results of transcriptome-wide *B. cinerea* expression phenotypes.** Panel a is a Manhattan-type plot of the top 1 SNP hit per *B. cinerea* transcript on Col-0 *A. thaliana*. Panel b is a Manhattan-type plot of the top 1 SNP hit per *A. thaliana* transcript when infected by *B. cinerea*.

**Figure N6. All eQTL hotspots across the *B. cinerea* and *A. thaliana* transcriptomes.** We counted the number of genes (transcripts) associated with each SNP. Panel a is for all *B. cinerea* transcripts, panel b is all *A. thaliana* transcripts.

**Figure N7. Interspecific hotspot comparison on the *B. cinerea* genome.** For each SNP that is a top hit for one or more transcripts, the number of associated transcripts is counted, across both the *B. cinerea* transcriptome and the *A. thaliana* transcriptome.

**Figure N8. Genes linked to eQTL hotspots are in virulence and defense co-expression networks.** Circles along the *B. cinerea* genome map are eQTL hotspots, centered at the gene containing the eQTL and with radius proportional to the number of transcripts linked to this hotspot. The gene center is marked with a white dot. Hotspots for *B. cinerea* transcripts are drawn in blue, hotspots for *A. thaliana* transcripts are drawn in green. The *A. thaliana* networks depicted are the most inclusive of the host-dependent networks, from *npr1-1*. Links between hotspots and co-expression networks are drawn according to the number of genes shared between them. Variable line weight represents the percent of hotspot target genes shared with the co-expression network; 1-25% is dashed, 25-50% is dotted, 50-75% is solid, 75-100% is heavy solid.

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