Running title: eGWA of Botrytis-Arabidopsis co-transcriptome

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

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**ABSTRACT**

A disease symptom arises from the interaction of the host and pathogen genomes. However, little is known about how genetic variation in the interaction leads to shifts in the transcriptomes, especially in polygenic interactions like those between generalist pathogens and their plant hosts. To elucidate how the *Botrytis cinerea* on *Arabidopsis thaliana* interaction is shaped by directional influences of genetic variation on pathogen virulence, we measured the co-transcriptome across a genotyped and genetically diverse collection of 96 *B. cinerea* isolates infected on the Arabidopsis wildtype, Col-0. Using the *B. cinerea* genomic variation, we performed genome-wide association (GWA) for each of 23,947 variable transcript expression profiles in the host, and 9,267 transcripts in the pathogen. This analysis identified mostly *trans*-eQTL in the pathogen and found eQTL hotspots dispersed across the pathogen genome affecting both the pathogen’s and the host’s transcriptomes. There was a relative absence of *cis*-eQTL that is likely explained by allelic heterogeneity and structural variants. The gene membership in the *trans-*eQTL hotspots suggests links to several known and many novel mechanisms of virulence in the plant-pathogen interaction. Genes annotated to these hotspots provide potential targets for blocking manipulation of the host response by this ubiquitous generalist pathogen. This shows that regulation of the co-transcriptome shows a polygenic nature that is similar to the virulence outcome in the *Botrytis cinerea* on *Arabidopsis thaliana* interaction.

**INTRODUCTION**

Infectious disease is an interaction between host and pathogen, determined by the genetics of both organisms and their interactions. Plant-pathogen interactions are often divided into qualitative, in which few genetic variants interact to determine binary disease outcomes, or quantitative, in which a spectrum of outcomes may occur due to polygenic variation in the host and pathogen. The past decade has begun unraveling the molecular basis of large-effect loci on both the host side and the pathogen side that control qualitative interactions (Giraldo and Valent 2013, Marone, Russo et al. 2013, Meng and Zhang 2013, Cui, Tsuda et al. 2015, Lo Presti, Lanver et al. 2015). In this model, these genes, via recognition events surrounding their proteins, create sweeping changes in the transcriptome and phenotype of both the host and pathogen. However, a large number of important plant-microbe interactions are quantitative (Poland, Balint-Kurti et al. 2009, Kou and Wang 2010, Lannou 2012). In contrast to qualitative systems, quantitative plant-pathogen interactions exhibit a lack of large effect virulence/ resistance genes (Poland, Balint-Kurti et al. 2009, Kou and Wang 2010, St. Clair 2010, Roux, Voisin et al. 2014). 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). Recent evidence is suggesting that the virulence of the pathogen is equally polygenic (Bartoli and Roux 2017, Wu, Sakthikumar et al. 2017, Atwell, Corwin et al. 2018, Soltis, Atwell et al. 2019). When working on variation in both the host and pathogen in these settings, evidence suggests that the pathogen dominates the system (Bartha, McLaren et al. 2017, Wang, Roux et al. 2018), though there is some evidence for a balanced contribution of plant and pathogen genetics (Soltis, Atwell et al. 2019). In both the host and pathogen, these polygenic architectures impact numerous loci that in turn influence a wide array of potential mechanisms that extend far beyond perception events. It is, however, unclear how polygenic architectures in both the host and pathogen interact to alter higher-order phenotypes such as virulence or even more direct phenotypes like the transcriptome of both species. Thus, there is a need to develop genomic approaches to understand how polygenic information is transmitted between the pathogen and the host to shift the genomic response of both organisms.

Hypothetically, polygenic variation in the pathogen will lead to genetic variation in numerous genes that will alter the transcriptome of the pathogen and differentially alter the expression of various virulence mechanisms. This variation in virulence mechanism will then differentially impact the host and lead to shifts in the resistance transcriptome. Thus, by measuring the transcriptome in both the pathogen and the host, it should be possible to map how genetic variation in the pathogen is conveyed through the pathogen’s transcriptome and concurrently how the host’s transcriptome responds. Recent work has shown that it is possible to measure the pathogen’s transcriptome in planta in *A. thaliana* -*Pseudomonas syringae* (Nobori, Velásquez et al. 2018). To date in plants, co-transcriptome work where both the host’s and pathogen’s transcripts have been measured has been shown in the *A. thaliana* - *B. cinerea* system through single sample RNA-Seq (Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018). Using this co-transcriptome approach has shown that both the host and pathogen transcriptomes are highly dependent on variation in the *B. cinerea* genome (Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018). It was possible to use the co-transcriptome to coalesce the pathogen and host transcriptomes into defined modules and to identify co-expression interactions between these host and pathogen modules (Zhang, Corwin et al. 2018). However, these studies did not assess if it was possible to identify the candidate genes within the pathogen whose genetic variation may be causing these co-transcriptome interactions.

One way to untangle these connections is to utilize GWA to identify expression quantitative trait loci (eQTL), SNPs correlated with variation in transcript expression profiles. These SNPs are candidates for polymorphisms that are causing the differential transcript accumulation and can be parsed into either *cis* or *trans* effects. Locally acting (*cis*) eQTL may indicate regulatory variation within or near the expressed gene itself. *trans*-eQTL indicate SNPs that are acting at a distance and are often considered to be polymorphisms that affect regulatory processes influencing the expression of the transcript. If a *trans*-eQTL affects many transcripts, it is classified as a hotspot and the SNP may influence a regulatory process that in turn influences a large number of transcripts.

eQTL analysis has been utilized to study host-pathogen interactions, albeit with a focus on host or pathogen. Frequently, these studies focus on the host’s response, such as mapping how host loci control host gene expression over time using either traditional QTL mapping or GWA analysis (Chen, Hackett et al. 2010, Hsu and Smith 2012, Zou, Chai et al. 2012, Allen, Carrasquillo et al. 2016, Christie, Myburg et al. 2017). Additional studies have begun to invert this scheme by looking at how genetic variation in the pathogen influences the host transcriptome 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 studies show that it is possible to identify pathogen loci that influence host gene expression, but they have thus far addressed pathogen populations with limited genetic variation (Wu, Cai et al. 2015, Guo, Fudali et al. 2017). Expanding these approaches would require conducting a co-transcriptome analysis wherein both the host and pathogen transcriptomes are measured using a natural population of the pathogen.

To begin mapping how genetic variation in the pathogen can impact both the host and pathogen transcriptomes, we conducted GWA of 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 dominated by complex small-effect loci that display a high degree of interaction between the host and pathogen (Denby, Kumar et al. 2004, Rowe and Kliebenstein 2008, Zhang, Corwin et al. 2017, Atwell, Corwin et al. 2018). The genomes of both the host and the pathogen harbor extensive genetic diversity that has been successfully used for GWA to identify loci controlling virulence (Atwell, Corwin et al. 2018, Soltis, Atwell et al. 2019). Further, the virulence outcome of the interaction is easily measured via high-throughput digital imaging allowing for a large body of molecular information to underpin any hypothesis generation from GWA (Denby, Kumar et al. 2004, Rowe and Kliebenstein 2008, Zhang, Corwin et al. 2017, Atwell, Corwin et al. 2018, Soltis, Atwell et al. 2019). Finally, it is possible to measure both the host and pathogen transcriptomes in the same sample (Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018).

Thus, we conducted a GWA analysis of the pathogen and host transcriptomes to identify loci in *B. cinerea* that may be modulating this interaction. We utilized a previous co-transcriptome dataset of variation in individual transcript expression profiles of diverse *B. cinerea* isolates infecting the wildtype host Col-0 *A. thaliana* (Zhang, Corwin et al. 2017, Zhang, Corwin et al. 2018). These SNPs have an explicit directionality to them, as causality must arise within the pathogen and then extend to the host. This analysis found mostly small-effect polymorphisms dispersed throughout the *B. cinerea* genome, with several hotspots of *trans*-eQTL activity. These hotspot loci could be linked to affecting specific host or pathogen transcript modules and to variation in lesion size. There was no identifiable overlap in the hotspots that linked to the host or the pathogen, suggesting a somewhat 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. 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 expression GWA 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 Col-0 *A. thaliana* genes,each as individual traits across 96 diverse *B. cinerea* isolates. 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 (Figure N1). For these genes with significant SNPs, there was a median of 10 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 SR1a, SR1b). Further, the distribution of p-values for significant SNPs found little evidence for large effect polymorphisms suggesting a highly polygenic basis of loci modulating transcriptome variation (Figure SR1c, SR1d).

Given the scale of this dataset, it was not viable to estimate empirical significance thresholds for every transcript using 1,000 or more permutations. However, we permuted the whole dataset across each of the tens of thousands of traits five times and repeated 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 SR2). 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, Rivas, Beaudoin et al. 2011, Visscher, Wray et al. 2017). 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 by our analysis (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 lack of dominant *cis*-effect SNP patterns much like 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 SNP *cis-*effects, and 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 overlaps in the top SNP per transcript (Figure N6). By permuting the SNP positions, we identified maximum permuted hotspot sizes as a 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.

**eQTL hotspot modules**

To better understand the transcriptome modules that are being influenced by these hotspots, we examined the genes influenced by each hotspot. We first utilized the gene ontology (GO) annotations within each species to better assess if there was any common functionality. The *B. cinerea* GO annotations showed a preponderance of enzyme and transcription factor annotations but no specific molecular insights arose largely because the majority of genes had no annotation (Table N1 and N2). In contrast, GO analysis of the *A. thaliana* transcripts showed that three of the hotspots have an overrepresentation of photosynthesis-related functions within their targeted genes (Table N1 and N3). Two of the hotspots predominantly affect *A. thaliana* genes associated with abiotic stress responses. Only two of the hotspots are linked to expected plant defense loci, including chitin response and microbe defenses. This suggests that the *B. cinerea* genes underlying these hotspots have specific effects on defined networks within the host and are not causing nonspecific responses.

In previous work, we had defined key transcript modules within both the host and pathogen transcriptomes that could be linked to virulence. Thus we proceeded to test if any of these trans-eQTL hotspots were associated with the previously defined transcript modules (Zhang, Corwin et al. 2017, Atwell, Corwin et al. 2018, Zhang, Corwin et al. 2018) (Table X1). Nine of the 11 *B. cinerea* eQTL hotspots were enriched for transcripts present 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. Similarly, nine of the *A. thaliana* eQTL hotspots were enriched for transcripts from two of the major *A. thaliana* co-expression networks when infected with *B. cinerea* (Figure N8). These two modules contain genes that function in jasmonate and salicylic acid signaling processes and camalexin biosynthesis (Network I), or photosynthesis (Network IV). Interestingly, these links are not limited to a single hotspot, but are strong connections across a number of different hotspots suggesting that these modules have a polygenic architecture underlying them(Figure N8). These frequent links suggest that the identified 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.

**eQTL hotspot candidate genes**

To better understand what the causal basis of these hotspots might be, we investigated the candidate genes associated with the SNPs. The 12 *B. cinerea* hotspots linked to *A. thaliana* transcripts, annotated to 11 genes, included 4 enzymes and 2 genes associated with isolate compatibility (Table N1). The 13 *B. cinerea* hotspots linked to *B. cinerea* expression profiles were associated to 11 genes, including 4 enzymes (Table N1). However, only one of these 22 genes had any previous published information linking them to virulence functions in *B. cinerea* or other fungi; a glycoside hydrolase whose homolog shows increased expression in virulent strains of *Ustilago maydis* on *A. thaliana* (Bccwh41) (Martínez-Soto, Robledo-Briones et al. 2013). To test if any of these 22 eQTL hotspot genes may have a link with virulence in *B. cinerea*, we compared their expression in the co-transcriptome data to existing virulence measurements. The virulence was measured on different leaves, and not on the same leaves as the co-transcriptome. Three of the *B. cinerea* hotspot genes and two of the *A. thaliana* hotspot genes are strongly positively correlated to lesion size variation, and none are negatively correlated with lesion size (Table N1) (Zhang, Corwin et al. 2018). Further, we utilized a previous GWA of virulence of these same isolates on *A. thaliana* to test if there was any overlap. 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). Together, this suggests that these genes are likely candidates for controlling transcriptome responses in both the host and pathogen.

**DISCUSSION**

**Dispersed interactions across host and pathogen genomes**

Using co-transcriptome GWA, we identified 25 *trans-*eQTL hotspots dispersed across the genome, with half the chromosomes in the *B. cinerea* genome harboring one or more loci that modulate either the host or pathogen transcriptomes. This contrasts with previous studies in cross-species eQTL, which identified one or only a few cross-species eQTL hotspots (Guo, Fudali et al. 2017). 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*-co-expression networks (vesicle/virulence, translation/growth, exocytosis regulation, peptidase). This distributed spread of candidate polymorphisms shows no evidence for clustering of the genetic variation in *B. cinerea*. This contrasts with the expectation that most filamentous fungi have multiple-speed genomes, 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. It will require conducting a similar analysis in the multi-speed genome filamentous fungi to test whether eQTL in a pathogen with a multi-speed genome cluster within the highly polymorphic regions.

**Complications in detection of *cis*-acting loci**

In most eQTL studies, there is a dominant signature of *cis*-acting loci. However, in this pathogen, the dominant pattern was one of *trans*-eQTL with few *cis*-eQTL. A deeper investigation suggested that this may be due to genetic factors that complicate our ability to identify the *cis*-acting SNPs. *B. cinerea* has high haplotype diversity, and in the three gene clusters investigated, there were potential rare *cis*-acting variants that fall below the minor allele cutoff for GWA. Further, the identified *cis*-acting variants were often deletions that create allelic heterogeneity and are not incorporated into the GWA algorithm, thus complicating the GWA analysis of these regions. To fully understand the pattern of potential *cis*-acting loci in *B. cinerea* would require a deeper investigation of structural variation by incorporating long-read sequencing. Additionally, the GWA algorithms would need to be recoded to allow for simultaneous use of both SNP and presence/ absence polymorphism data (Wang, Roux et al. 2018).

**Polygenic modules and pleiotropy in cross-species eQTL**

Individual genes in the host displayed a polygenic basis of expression modulation from many significant transcript-SNP associations. This contrasts with previous studies in which each host expression profile was explained by only a single major-effect pathogen locus (Guo, Fudali et al. 2017). This polygenic pattern suggests that the modulation of host genes by pathogen genetics are robust to mutation or silencing of individual pathogen genes.

Further, we saw a pleiotropic effect of hotspots on host networks. Many pathogen eQTL hotspots targeted several genes with each host network. Previous studies also identified multiple targets within a host network per each pathogen eQTL (Wu, Cai et al. 2015). This suggests that the modulating effects of pathogen genetics on the host networks are robust to mutations and deletions of individual host 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. In effect, we see polygenicity of host expression regulation by the pathogen at the gene level, and at the network level, suggesting robustness in these connections in the face of changes to the pathogen or host genetics.

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

The detected eQTL hotspots often showed strong links to co-expression networks and showed functional enrichment in their sets of genetic targets. This points toward specificity in the targets of each eQTL, and specificity in the responses to this expression modulation.

The 13 *B. cinerea* hotspots were annotated to 7 gene functions, 4 of which were 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*.

The 12 *A. thaliana* hotspots were annotated to 11 gene functions (Table N1). Among these, four are enzymatic. 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. Further, three of the hotspots showed an overrepresentation of photosynthesis functions among their *A. thaliana* target genes. Future studies into proteomics of *B. cinerea* may elucidate direct vs. indirect effects of pathogen genes on host metabolism and physiology.

Additional *B. cinerea* hotspots may alter the transcription machinery or affect the number of nuclei per *B. cinerea* mycelial cell, potentially altering the virulence of the pathogen (Bcin12g00330, Topoisomerase II-associated protein PAT1; Bcin09g06590, Helicase) (Table N1).

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 (Table N1, Table N2). 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**

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). This study begins to establish causal inference from genome to transcriptome to phenotype, 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.

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.

**Conclusion**

This study, to our knowledge, is one of the first to conduct a genome-wide mapping of loci controlling variation in the transcriptomes of both the host and the pathogen. This showed a preponderance of *trans*-acting polymorphisms with predominantly moderate to small effects suggesting that a polygenic architecture underlies the transcriptome variation, similar to the virulence interaction. Using previously defined transcriptome modules showed that there may be a modular structure to these effects, with specific pathogen SNPs linking to specific modules in either the host or the pathogen. These SNPs identify key candidate genes that may be modulating the host-pathogen interaction and future studies will need to assess if this is the case and how these cross-species regulatory interactions are being mediated.

**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.

**FIGURE LEGENDS**

**Figure 1. 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, from AT1G01010.

**Figure 2. *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 3. *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. Panel c is the gene models of the biosynthetic gene network, with the cluster 3 deletion indicated as a triangle.

**Figure 4. Manhattan-type plot of GEMMA results of transcriptome-wide *B. cinerea* and *A. thaliana* 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 5. 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 6. 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.

**TABLES**

**Table N1. Annotation of the hotspots identified from *B. cinerea* and *A. thaliana* eQTL.** Each row identifies one of the significant eQTL hotspot SNPs from association to transcripts in *B. cinerea* or in *A. thaliana*. Gene functions are from BotPortal, Arabidopsis GO overrepresentation is from PANTHER. Additional annotation includes the overlap of hotspot target genes with gene lists from previous studies of *B. cinerea* virulence- associated genes and *B. cinerea* x *A. thaliana* co-expression analysis.

[ TABLE GOES HERE ]

**SUPPLEMENTAL FIGURE AND TABLE LEGENDS**

**Table SN2a. Annotation of the *B. cinerea* genetic targets of *B. cinerea* hotspots.** Columns include the hotspot SNP and the nearest gene, and all other columns pertain to the target gene modulated by the eQTL. Additional information about the target gene includes gene name, gene function, annotation as an enzyme, and target transcript. Gene functions are IPR numbers from the InterPro database.

**Table SN2b. Functional summary of the *B. cinerea* genetic targets of *B. cinerea* hotspots.** These count occurrences of major functional categories among the hotspot target genes.

**Table SN3a. Annotation of the *A. thaliana* genetic targets of *B. cinerea* hotspots.** Columns include the *B. cinerea* hotspot SNP and the nearest *B. cinerea* gene, and the *A. thaliana* target gene.

**Table SN3b. Gene ontology analysis of the *A. thaliana* genetic targets of *B. cinerea* hotspots.** These include all PANTHER overrepresentation test outputs for target gene sets within each eQTL hotspot. Hotspots are labeled by SNP and nearest B. cinerea gene. All calculations are from Bonferroni-corrected Fisher’s exact tests, and only significant GO categories are presented.

**Figure S1. Distribution of number of associations and p-values for SNP-transcript associations**. *B. cinerea* transcripts are a and c, *A. thaliana* transcripts are b and d. In a and b, histograms depict the distribution of number of significant SNP associations per each transcript. In c and d,boxplots encompass the top 1 SNP associated with each transcript. Box edges delimit the first and third quartile, the thick center line delimits the median. Whiskers extend to 1.5 times the interquartile range and additional points indicate outliers.

**Figure S2. Distance between transcript center and top SNP location for all *B. cinerea* expression profiles on Col-0 *A. thaliana*.** Distances are in Mb, including only top SNPs on the same chromosome as the focal gene. Panel a data include the top 1 SNP identified by GEMMA association with each transcript expression profile (lowest p-value for association). Panel b describes the length of individual chromosomes. Panel c data include the shortest distance between transcript genomic location and top 1 SNP identified by GEMMA association with each transcript expression profile (lowest p-value for association) out of 5 permutations.

**Figure S3. Manhattan-type plot of GEMMA results of transcriptome-wide *B. cinerea* expression phenotypes.** Each point represents a single transcript-SNP p-value of association. 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 S4. *cis*-effect analysis of the botrydial biosynthetic gene network.** Panel a is hierarchical clustering of *B. cinerea* isolates from SNPs within the botrydial 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 botrydial network-level expression within *B. cinerea* clusters. Isolates are clustered based membership in groups defined by hierarchical clustering of the SNPs within the botrydial biosynthesis network.

**Figure S5. *cis*-effect analysis of the cyclic peptide biosynthetic gene network.** Panel a is hierarchical clustering of *B. cinerea* isolates from SNPs within the cyclic peptide 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 cyclic peptide network-level expression within *B. cinerea* clusters. Isolates are clustered based membership in groups defined by hierarchical clustering of the SNPs within the botrydial biosynthesis network.

**Figure S6. Interspecific hotspot comparison on the *B. cinerea* genome with the top 10 genes per SNP.** 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.

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