**Dominance of *trans*-eQTL over *cis*-eQTL patterns**

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 many of the cis-acting loci are better detected through presence/ absence polymorphisms; future eQTL studies within *B. cinerea* would benefit from the use of both SNP and presence/ absence polymorphism data. Some studies have found this pattern of dominant *trans*-effects as well … XXX

Previous co-expression studies in *B. cinerea* also identified five major co-expression networks with genes disbursed across the genome of *B. cinerea* (Zhang, Corwin et al. 2018). This provides additional evidence for trans-regulation of gene expression in *B. cinerea* virulence interactions. In particular, our eQTL hotspots contained many genes from the trans-GCNs (vesicle/virulence, translation/growth, exocytosis regulation, peptidase) but none of the cis-GCNs containing tandem gene clusters.

**Cross-species *trans*-eQTL patterns**

~~Work by other groups has termed these loci trans-species eQTL (ts-eQTL) and hotspots of these loci as Host Expression Modulators (HEM) (Wu, Cai et al. 2015, Guo, Fudali et al. 2017).~~

~~Previous studies have identified a small subset of parasite chromosomes that interact with the host genome (i-chromosomes), even though the affected plant genes are dispersed across the host genome (Guo, Fudali et al. 2017). However, in the case of~~ *~~B. cinerea~~*~~, half the chromosomes in the genome appear to harbor one or more loci with expression modulation of~~ *~~A. thaliana~~* ~~genes.~~ ~~Expanding analysis to~~ ~~additional hosts may reveal specific chromosomes with more common expression modulation effects.~~

We do find some specificity in the expression modulation interactions between pathogen and host loci.

Within our hotSNP analysis, each of the targeted *A. thaliana* gene expression profiles was uniquely linked to a single SNP in *B. cinerea*. Similarly, the system of (nematode spp. ) x Medicago truncatula, each host expression profile was explained by only a single major-effect pathogen locus (Guo, Fudali et al. 2017). This may suggest one-to-one interactions between the host and pathogen genomes, not at the gene-to-gene level as seen for specialist pathogen systems, but at the gene-to-network level. Interestingly, while the expression modulation of individual host transcripts is polygenic, this variation may be dominated by only a single hotspot eQTL.

Genes within the same host network often share eQTL in the pathogen, as observed in previous studies of host-pathogen interspecific eQTL (Wu, Cai et al. 2015).

Overarching pattern of polygenicity; both the host and pathogen appear to draw from extensive genetic variation to determine disease outcomes.

Can be interpreted as direct or indirect effects.

One mechanism: pathogen eQTL may produce phytotoxins with strong host responses.

Indirect mechanism: a transcription factor in B. cinerea may control a pathway for a secreted toxin, causing expression responses in A. thaliana. The interpretation is dependent on which genes are linked to the variation in the TF genes.

Future directions: eQTL direction and magnitude of effect on targets?

Directionality- is pathogen targeting these host networks, or is host countering pathogen attacks?

Host immunity can alter B. cinerea expression response

B. cinerea can modify virulence strategy in response to host

Changes in mRNA level could be attributed to the expression levels of individual genes across net cell population, or a change in the distribution of cell types within the population.

These pathways may identify novel receptors and adaptors in the pattern recognition and signal transduction pathways across interacting organisms.

**Bot/ boa/ net5**

BOT biosynthetic GCN is comprised of tandemly clustered genes, suggesting cis- regulation. Isolate 94.4 lacks the BOT pathway, 19 isolates lack the BOA pathway, 24 isolates lack the cyclic peptide pathway (Zhang, Corwin et al. 2018).

**3. Potential mechanisms of eQTL hotSNPs**

The 12 *A. thaliana* hotSNPs were annotated to 11 gene functions (Table N1). Among these, 4 were 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. In fact, one A. thaliana hotSNP is annotated to a secreted glycoside hydrolase, which may directly interact with the 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* hotSNPs, 4 were annotated to *B. cinerea* enzymes (Table N1). Further, the targets of these hotSNPs are often enzymes (Table N2). These hotSNP enzymes may alter major branches of the *B. cinerea* metabolic pathways active during the infection of *A. thaliana*.

Two of the B. cinerea hotSNPs 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.

Two of the *A. thaliana* hotSNPs are annotated to genes that have been previously shown to predict isolate crossing compatibility and mating type (Atwell, Corwin et al. 2015). These loci may structure some of the pathogen diversity, and as such may be major vectors of pathogen variation affecting host response.

From the current analysis and limited annotation information in *B. cinerea*, we cannot distinguish between direct and indirect effects of *B. cinerea* hotSNPs on the *A. thaliana* transcriptome.

**3. Annotation of genes targeted by eQTL hotSNPs**

The frequency of enzymes in the *B. cinerea* eQTL hotspot target genes suggests a role of *B. cinerea* metabolic shifts as the fungal infection progresses *in planta*.

The presence of many transcription factors among the hotspot targets indicates that the eQTL hotspots may control major networks of *B. cinerea* gene expression variation. The hotSNPs may affect variation in a hierarchical manner, with the targets of the eQTL also regulatory genes modulating the expression of genes downstream. The eQTL hotspots may be near the top of a hierarchical gene regulatory structure.

Two of the *B. cinerea* genes downstream of eQTL hotspots peak in their expression during the outset of colonization (BcPIO5, BcPIO10) (Gioti, Simon et al. 2006). An additional gene is a homeobox transcription factor induced early in infection (BcPIE1, BcHOX8). We detected expression of these genes at a later intermediate infection stage in our experiment, so these genes may play an ongoing role in the progression of infection or remain active at the newly colonized cellular edge of a growing necrotic lesion.

Two of the *B. cinerea* genes are mitogen-activated protein kinases, active in signaling cascades within the fungus to regulate major cellular pathways. These may point to pathways of signal transduction and decision-making as infection of the host progresses.

Looking at the genes targeted by a single hotSNP can reveal potential mechanistic patterns as well. One hotSNP from the *A. thaliana* transcriptome (Bcin16g01950, Bccwh41, glycoside hydrolase, family 63) targets many photosynthesis-related genes (Table N3), as well as members of multiple pathogen defense pathways. These patterns are further reinforced as this hotspot contains several genes from the major photosynthetic co-expression network and the JA/ SA/ Camalexin defense co-expression network (Figure N8).

The two hotSNPs identified on chromosome 12 from the *B. cinerea* transcriptome each target many enzymes, and one targets three genes expressed in planta during infection (Table N2). Both hotSNPs are annotated to the same gene (Bcin12g00330, Bcpat1, Topoisomerase II-associated protein PAT1), whose expression is highly correlated with *B. cinerea* lesion size; this locus may be a control point for major *B. cinerea* metabolic and expression shifts, potentially feeding into virulence strategies.

The two *A. thaliana* co-expression networks connected to our hotspot target lists are highly central within the host-pathogen dual transcriptome interaction network. Our results point to novel B. cinerea factors likely linking the host and pathogen co-expression. One of these networks contains nuclear photosynthesis genes, and photosynthetic chloroplast reaction centers, suggesting shifts to photosynthetic function over the course of infection (Zhang, Corwin et al. 2017). Numerous studies have identified downregulation of photosynthesis in host plants infected by *B. cinerea* (Berger, Papadopoulos et al. 2004, Govrin, Rachmilevitch et al. 2006, Mulema and Denby 2012, Windram, Madhou et al. 2012).

In previous studies, the host gene lists targeted by pathogen eQTL included functional enrichment for transcription factors, defense-related enzymes, enzymes in essential amino acid biosynthesis, ATP response, metabolic functions, and antimicrobial and inflammatory immune response (Wu, Cai et al. 2015, Guo, Fudali et al. 2017)

In previous studies, genes from the same host network often shared the same eQTL (Wu, Cai et al. 2015).

**4. Triangle of interaction: genotype to expression to phenotype**

DNA polymorphisms alter protein structure, causing phenotypic effects. Among these is the trait of gene expression, which can cause phenotypic effects as well.

From previous studies, gene expression plasticity is more sensitive to isolate x host interactions than is lesion size (Zhang, Corwin et al. 2017). As such, eQTL analysis has the potential to identify loci determining the outcome of plant-pathogen interaction that would be masked in GWA analysis of lesion size. The major plant defense pathways select between alternate molecular pathways for canalized isolate defense (Zhang, Corwin et al. 2017). Here, we gain some insight in to the B. cinerea modulation of these genetic pathways. Both pathways do provide some defense against B. cinerea (Zhang, Corwin et al. 2017), so it will be interesting to see what additional gene-expression-phenotype connections are elucidated by eQTL analysis in the mutant hosts.

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). Zhang found connections between early transcript abundance and later lesion development. 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.

The first of the two major host networks tagged by the hotSNPs contains genes whose expression response early in infection predicts resistance at 72 hpi, and the direction of this interaction depends upon host genetics (Zhang, Corwin et al. 2017). In this study, we identify strong links to draw the connection from genetic change in the pathogen, to expression pathway responses in the host, to phenotypes of virulence.

**4. Future directions**

How robust are these patterns to host immunity? Which are conserved or specialized. Which pathways are sensitive to host. Future studies can expand this work to incorporate the effect of host immune pathway knockouts. We can explore the interaction term in the genetic control of host and pathogen gene expression, to ask how the B. cinerea eQTL change in response to host immune pathways. Some B. cinerea genetic effects may be constitutive in infection, while others may be specialized at the gene level, conditional on host immune pathway or host species.

On the flip side, host defense pathways that are linked to B. cinerea hotSNPs in a genotype-dependent manner may be potential points in which the host defense pathways are targeted by the B. cinerea genome.

We can further expand this work to look for signs of conserved plant genes interacting with this virulence variation in B. cinerea. For the genes in A. thaliana that are linked to B. cinerea eQTL hotSNPs, are there homologs in other plant species that are also differentially expressed over the course of pathogen infection? Co-expression studies over other host- B. cinerea systems can look for conserved plant immunity genes, as well as pathway structure. For example, eQTL genes identified from experiments in Medicago and P. spp. were enriched among differentially expressed host genes in tomato - P. spp. coexpression analyses (Guo, Fudali et al. 2017).

Host side of interaction – points of molecular communication between host and pathogen

Evidence of pathogen-specific host defense genetics (Zhang, Corwin et al. 2018).

Dual GWA and/or incorporation of deletion polymorphisms (Wang, Roux et al. 2018)

We can perform validation studies of these hotSNPs, tracing the effect of B. cinerea knockouts on expression variation in the host and pathogen, and connecting this to phenotypic changes.

**~~Conclusion~~**

~~hotSNPs provide potential targets for breeding disease resistance. May be factors controlling modules of virulence strategies- decision points?~~

Within a generalist pathogen species, there are several theories for how one species can adapt to attack a broad host range. Perhaps the pathogen has evolved a singular virulence gene of broad effect that disables core defense pathways across diverse plant host species; this could be considered a “silver bullet” virulence gene. This mechanism would predict low diversity in the pathogen as the silver bullet locus is effective across the host range and is fixed in the pathogen population. A second prediction for an extreme generalist species is specialization at the level of the individual. This would predict high population structure and moderate diversity. However, we observe low population structure in *B. cinerea*, high diversity, and a lack of evidence for individual specialization to hosts. Our final prediction is specialization at the gene or allele level, ~~which would select 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 2007; Rowe 2010; Atwell 2015; Corwin 2016; Zhang 2016}.~~

~~32% of our hotSNP 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~~

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