**DISCUSSION**

1. **Dispersed interactions across host and pathogen genomes**

Theories of filamentous pathogen genomics suggest a two-speed genome model, in which diverse fungal virulence effectors are enriched in regions of the genome containing repetitive sequences and transposable elements {Dong 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. Previous studies in cross-species eQTL conformed with this expectation, as in the system of *Medicago truncatula* x *Meloidogyne hapla*, in which few cross-species eQTL hotspots (termed Host Expression Modulators) clustered on only a few of the parasite chromosomes (termed i-chromosomes) {Guo 2017}. The targeted plant genes, on the other hand, were dispersed across the host genome {Guo 2017}. Our findings contrast these expectations; half the chromosomes in the *B. cinerea* genome appear to harbor one or more loci with expression modulation of *A. thaliana* genes which are dispersed across the host genome. 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, as we identified mostly *trans*-eQTL hotSNPs. Previous co-expression studies in *B. cinerea* also identified five major co-expression networks with genes dispersed 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*-networks (vesicle/virulence, translation/growth, exocytosis regulation, peptidase) but none of the *cis*-networks comprised of tandem gene clusters.

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

Our results are suggestive of the highest-diversity model of a generalist pathogen, in which specialization occurs 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}.

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 in some cases 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. However, this lack of detectable ­*cis*-effect variation also suggests high haplotype diversity.

Individual genes in both host and pathogen displayed a highly 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). However, within the hotSNP analysis each targeted *A. thaliana* gene was uniquely linked to a single hotSNP in *B. cinerea*. This suggests that the hotSNPs are host expression modulators with independent genetic targets within target networks. A single hotSNP can target multiple genes within a single host network, as observed in previous studies of host-pathogen interspecific eQTL (Wu, Cai et al. 2015). Further, multiple hotSNPs may target unique components within a single network. Additional eQTL may act in a more restricted manner, to regulate expression of relatively few genes. 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. This gives us an overarching pattern of polygenic genetic regulation, as both the host and pathogen appear to draw from extensive genetic variation to determine disease outcomes.

1. **Detection of pathogenicity genes and novel loci**

The 12 *A. thaliana* hotSNPs 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. In fact, one A. thaliana hotSNP 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* hotSNPs, 4 were annotated to *B. cinerea* enzymes (Table N1). Further, the targets of these hotSNPs are often enzymes, suggesting a role of *B. cinerea* metabolic shifts as the fungal infection progresses *in planta* (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.

Approximately 1/3 of our hotSNP loci and 1/5 of the hotSNP target genes currently lack gene ontology information (Table N1, Table N2). This study is additionally identifying a large number of novel virulence-associated loci within *B. cinerea*.

1. **Connecting from genome to transcriptome to phenotype (future directions)**

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 hotSNPs 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**

The 25 hotSNPs 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.