RESULTS

We focused our analysis on *B. cinerea* genes with variable expression in *B. cinerea* on *A. thaliana*, and *A. thaliana* genes with variable expression in this system. Of these 9,284 *B. cinerea* genes, 74% (5,244) varied significantly in their expression due to *B. cinerea* genetic variation, with an average broad-sense heritability of 0.152 {Zhang, 2018}.

We associated these expression profiles of these 9,267 genes to genome-wide SNP variation in the *B. cinerea* genome. To associate this expression variation with *B. cinerea* genomic variation, we performed Genome-wide Efficient Mixed Model Association (GEMMA). We first controlled for the effects of population structure within our *B. cinerea* isolates by calculating and including a relatedness matrix in the downstream analysis. GEMMA estimates the significance of effects of each SNP on the focal phenotype as a p-value.

To search for hotspots of quantitative trait loci (QTL) potentially controlling expression variation, for the SNP with the strongest evidence (lowest p-value) of association by GEMMA per transcript, we plotted the p-value and location of each top SNP. Hotspots are visualized as peaks composed of many high p-value SNPs.

To validate SNP peaks in our hotspot analysis, 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 9267 randomized 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 significance vs. position (Figure XX). We then defined hotspots in our data as any peaks formed by SNPs with p-values below a 100% threshold defined by a 5x permutation of randomized transcript profiles.

These randomized analyses do not produce any eQTL hotspots, thus we are confident the hotspots detected in our original analysis cannot be explained by random associations between transcriptional variation and genomic variation.

We identified regulatory hotspots on most chromosomes, except chromosome 11, 17, and 18 (Figure X7a). These hotspots of controlling variation may be due to *cis*- or *trans*-acting loci. Genome-wide, we identified XX hotspots, from X to X per chromosome. Hotspots were defined by X to X SNPs, with an average length of X kb.

To look for *B. cinerea* loci that are *trans*-acting to control expression variation in the affected host, we also examined patterns of association between *A. thaliana* expression variation and *B. cinerea* genomic variation. We identified hotspots putatively controlling *A. thaliana* expression variation for all *B. cinerea* chromosomes except 17 and 18 (Figure X9a). These *B. cinerea* loci may contribute to regulation of gene expression in the host. We looked for evidence of eQTL hotspots that are common across both the *B. cinerea* and *A. thaliana* analysis by… XXXX.

To search for a pattern of transcriptome-wide *cis* effects, 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 X4a). These distances are similar to those from the association of random transcript profiles to top SNPs (Figure X4b). As such, we do not see evidence for *cis*-effect loci overrepresented in the top candidates for control of expression variation. Rather, most control of gene expression variation in *B. cinerea* on *A. thaliana* appears to be *trans*-acting.

In these transcript-to-SNP associations, we find that *cis*- control of loci is largely drowned out by patterns of *trans*-acting variation. 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 to *cis*-acting 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, we find that few genes have a top SNP hit within the same chromosome, and even fewer within 1Mb (Figure X5). We again conclude that most of this genetic variation is *trans*-acting.

As we did not detect a transcriptome-wide pattern of *cis*-effect variation controlling expression patterns of *B. cinerea* on *A. thaliana*, we narrowed our focus to a subset of localized gene networks to identify the role of *cis*-eQTL in controlling this expression variation. These focal networks were among those most highly conserved across *B. cinerea* isolates {Zhang, 2018}. These include the botcynic acid biosynthetic pathway (X genes, X Mb), botrydial biosynthesis (X genes, X Mb), and Network 5 {Zhang, 2018}, which contains XX (X genes, X Mb).

[Primary analysis on all 3 of these networks]

We focused further analysis on the botcynic acid biosynthetic pathway. Upon visualizing SNP-level variation surrounding genes in this pathway, we found evidence of a deletion common to 12 of the 96 isolates. We selected a focal region encompassing the deletion endpoints (X,X) and an additional 2 genes beyond the deletion boundaries (Figure X1). We removed X SNPs that were likely miscalled (SNP state ~ inverse compared to surrounding region) and called all SNPs within the deletion region as missing. Hierarchical clustering based on these loci assigned the 96 isolates into three major clusters, and one small two-isolate cluster (B05.10, Fd1) (Figure X2). The largest cluster (cluster 4) includes a sub-cluster containing all of the isolates in the deletion group (cluster 3). We removed isolates with the whole-region deletion, then performed ANOVA across the 3 major clusters (1, 4, 5) to see whether SNP variation within the botcynic acid biosynthesis region predicts expression level of genes within the region. We found no significant effect of cluster membership on expression profile (F(1,74)=0.36, p=0.55). This suggests that the major *cis*-effect loci controlling expression variation in this cluster are not captured by SNP-level variation.

We examined mean botcynic acid pathway expression across all isolates, grouped by cluster membership. Expression levels across the network are reduced in the deletion group (cluster 3) but we also see independent low-expression isolates outside of this deletion group (Noble Rot, 01.04.03, Apple 517, 02.04.09) (Figure X3). These isolates also contain deletions within the botcynic acid region (Figure X1), likely of independent origin. This deletion extends X kb and includes SNP X at the X’ end of the chromosome, indicating a teleomeric loss on chromosome X. As such, the major *cis­*-effect variation controlling expression in the botcynic acid region appears to be explained by deletion events, which are not captured by our SNP analysis. If insertion and deletion events account for the majority of localized control of expression variation, our GWA analysis will not detect these *cis*-effect loci.