**Supplemental Materials**

**Supplemental Methods**

*P. destructans Isolates*

Isolates originated from four Canadian Provinces: Ontario (11 isolates), New Brunswick (18 isolates), Nova Scotia (1 isolate), and Prince Edward Island (PE, 10 isolates) and six US States: New York (3 isolates), North Carolina (3 isolates), West Virginia (5 isolates), Ohio (1 isolate), Vermont (2 isolates), and Pennsylvania (1 isolate). Isolates from New Brunswick were collected from live bats, arthropod species, and cave surface soils within seven hibernacula sites. The remaining isolates from Nova Scotia, Ontario, PE, and the US were from diseased bats due to WNS infections.

*Spot densitometry*

By measuring the light emission for each colony, the amount of colouration is estimated via Integrated-light Density Values (IDV), which provide data on the reflectiveness of the total colony. Here, darker cultures reflect less light than lighter colonies, where a value of 0 is assigned to white growth. To measure the extent of pigment secretion and diffusion into the agar medium we followed protocols described by Vogan *et al.* (1). We first subtracted any interfering background noise from the solid agar media. The background IDV of the agar medium was used as a threshold for determining the edge of pigment diffusion on each plate. The area of pigment diffusion was then measured for each colony.

*Sequencing*

Raw reads were filtered using trimmomatic; adaptor sequences and regions with low quality scores were trimmed. Reads were corrected with a k-mer-based algorithm using SGA (2), contigs were assembled with the IDBA-UD algorithm (3). At this point, mis-assembled regions were corrected and the final scaffolds were constructed. We then aligned the raw reads from all isolates to our *de novo* build using the Burrows-Wheeler Alignment MEM algorithm (V0.7.15; 4), with shorter duplicate hits flagged as secondary alignments. We used PicardTools (V1.131; 5) to sort BAM files, remove PCR duplicates, and add read group identifiers. Lastly, we masked repetitive regions in the *de novo* assembly using RepeatMasker (V3.0; 6).

Variant calling was conducted on concatenated BAM files using Freebayes V1.1.0 (7). We first excluded calls that lacked coverage and quality scores. Further quality-based filtering of variant calls was conducted with bcftools V1.3 (4), with lenient parameters (QUAL & DP > 20). With this final set, we performed sequence homology search of fungal protein database, mapping of GO terms, and functional annotation using the tools available on the Blast2GO platform (8). We then conducted an enrichment analysis using Fisher's Exact test to identify statistically significant enrichment within genes that contains variants. We investigated 30 loci that contained putative mutations based on genome sequence comparisons through locus-specific PCR and sequencing. The sequencing results identified a false positive rate of 30%, resulting in our final set of 23 confirmed mutations among the three isolates. In addition, the same sites were also investigated in the isolate US8, as its genome was not sequenced in the original Illumina MiSeq run.

Enrichment analysis determines the probability that the GO Terms within a given subset of genes, compared to a background set of genes, were assigned by chance. Using the built-in

functions in Blast2GO, we compared the set of genes containing variants (Table 1) to all other genes that were assigned GO Terms. The results from Fisher's Exact Test were corrected for FDR, with a significance cut-off of < 0.05.

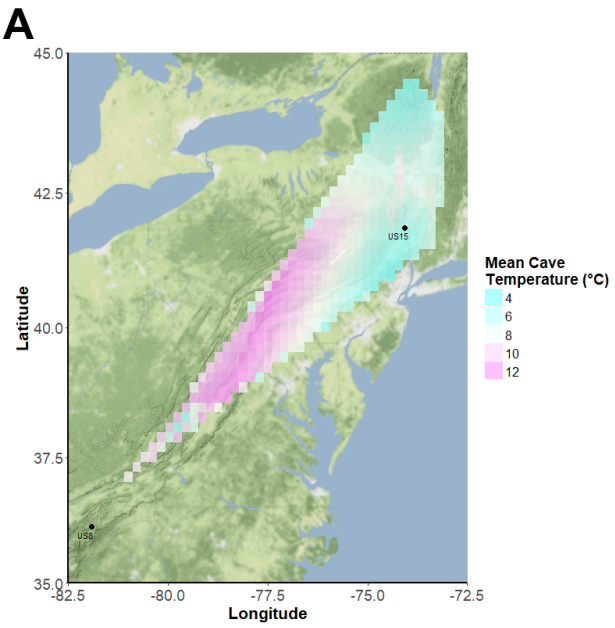
*Statistical Analysis*

We used a mixed-effects linear model to conduct a multivariate analysis of trait variance. Phenotype measures were scaled, with each trait having a mean of zero, and transformed prior to running of the multivariate mixed model. The resulting model followed the following design: *lmer*(*value* ~ *trait* - *1* + *trait-1*|*isolate*). This represents a model measuring the variance/covariances of traits among the random effect, the identity of isolates. By default, lmer also includes a term for residual variance (9). We then computed a matrix of correlation p-values using cor_pmat (10), with a significance threshold of 0.05.

Table S1: Results from a genome comparison survey of *P. destructans* isolates based on data from our isolates and those retrieved from the Sequence Read Archive in NCBI. The retrieved reads were aligned to our de novo assembly and screened for the variants at our previously identified SNP sites. Base calls matching the reference genome are designated as "REF", while "ALT" indicates base calls that match mutations described in this paper. "N/A" indicates sequence information missing at the locus.

| Accession (SRR) | ID | Collection Date | Collection Location | Scaffold/Position | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 4871 | 720 | 6034 | 355 | 1928 | 3235 | 1536 | 1498 | 2133 | 4883 |
| | | | | Reference/Alternative | | | | | | | | | |
| | | | | T/G | C/T | G/T | G/A | T/C | C/T | T/A | T/C | G/A | G/A |
| SRR1952982 | 20631-21 | 2008 | USA: New York, Williams Hotel | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR6011474 | 20674-9 | 2008-03-18 | USA: Vermont, Bennington County | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR6011482 | 22884-4W | 2010-01-27 | USA: Vermont, Rutland County | REF | REF | REF | REF | N/A | ALT | REF | N/A | REF | REF |
| SRR6011480 | 22949-4 | 2010-03-05 | USA: Maryland, Allegany County | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR6011488 | W41203 | 2012-04-12 | Canada: New Brunswick, Dorchester Mine | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR6011494 | 23877-1 | 2008-02-02 | Near Rosendale NY | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR6011470 | 22442-2 | 2011-09-22 | USA: Grafton, MA | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR6011477 | 20682-10 | 2008-03-21 | USA: Massachusetts, Berkshire County | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR5757331 | Pd 52201 | 2012 | N/A | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR6011471 | 22429-8 | 2009-01-30 | USA: West Virginia, Pendleton County | REF | REF | REF | REF | N/A | REF | REF | N/A | REF | REF |
| SRR5755626 | Pd 681103 | 2012-04-22 | N/A | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR6011467 | GU999986 | 2009-04-09 | Germany: Thueringen | REF | REF | REF | REF | N/A | REF | REF | N/A | REF | REF |
| SRR5755631 | Pd 692102 | 2013 | N/A | REF | REF | REF | REF | REF | ALT | REF | REF | REF | REF |
| SRR5755630 | Pd H07218 | 2013 | N/A | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR5755633 | Pd M53205 | 2013 | N/A | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR5755618 | Pd UWMM.03 | 2013 | N/A | REF | REF | REF | REF | N/A | REF | REF | REF | REF | REF |
| SRR5755620 | Pd X4702.13.1 | 2013 | N/A | REF | REF | REF | REF | N/A | REF | REF | REF | REF | REF |
| SRR6011473 | 22948-1 | 2014-04-12 | N/A | REF | REF | REF | REF | N/A | REF | REF | N/A | REF | REF |
| SRR5755623 | Pd UWMM.14 | 2014-04-12 | N/A | REF | REF | REF | REF | N/A | REF | REF | REF | REF | REF |
| SRR6011466 | 20693-1 | 2008-03-21 | USA: Massachusetts, Hampden County | REF | REF | REF | REF | N/A | REF | REF | REF | REF | REF |
| SRR5755627 | Pd 102203 | 2015 | N/A | REF | REF | REF | REF | N/A | REF | REF | REF | REF | REF |
| SRR5755624 | Pd WO2109 | 2015 | N/A | REF | REF | REF | REF | N/A | REF | REF | REF | REF | REF |
| SRR6011478 | 22004-1 | 2008-04-01 | USA: Connecticut, Litchfield County | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR6011475 | 22426-2 | 2009-01-27 | USA: Connecticut, Litchfield County | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR6011476 | 22469-1 | 2009-03-03 | USA: Virginia, Giles, County | REF | REF | REF | REF | N/A | REF | REF | REF | REF | REF |
| SRR6011469 | 22504-1 | 2009-03-25 | USA: Pennsylvania, Centre County | REF | REF | REF | REF | N/A | REF | REF | REF | REF | REF |
| SRR6011479 | 22930-2 | 2010-02-08 | USA: Tennessee, Sullivan County | REF | REF | REF | REF | N/A | REF | REF | N/A | REF | REF |
| SRR6011472 | 22971-3 | 2010-03-10 | Canada: Ontario, Kirkland Lake | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR6011483 | 22972-2W | 2010-03-15 | Canada: Ontario | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR6011484 | 22997-1 | 2010-04-08 | USA: Tennessee, Fentress County | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR6011493 | 23414-1W | 2011-01-13 | USA: Indiana, Washington County | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR6011491 | 23444-1 | 2011-02-09 | USA: Tennessee, Montgomery County | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR6011490 | 23455-1 | 2011-02-16 | USA: Virginia, Wise County | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR6011495 | 23874-1 | 2011-12-12 | USA: Maine, Hancock County | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR6011489 | 23897-2 | 2012-03-19 | USA: Missouri, Lincoln County | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR6011468 | GU350433 | 2009-04-01 | Switzerland: Aargau | REF | REF | REF | REF | N/A | REF | REF | N/A | REF | REF |
| SRR6011465 | GU350434 | 2009-03-29 | Hungary: Kislod | REF | REF | REF | REF | N/A | REF | REF | N/A | REF | REF |
| SRR5755625 | Pd 671202 | N/A | N/A | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR5755629 | Pd European | N/A | N/A | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR5755634 | Pd N. American | N/A | Illinois | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |
| SRR5755619 | Pd UWMM.13 | N/A | N/A | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR5755622 | Pd X4148.13 | N/A | West Virginia | REF | REF | REF | REF | REF | REF | REF | N/A | REF | REF |
| SRR5755621 | Pd X4702.13.2 | N/A | N/A | REF | REF | REF | REF | REF | REF | REF | REF | REF | REF |

## Supplemental Figures



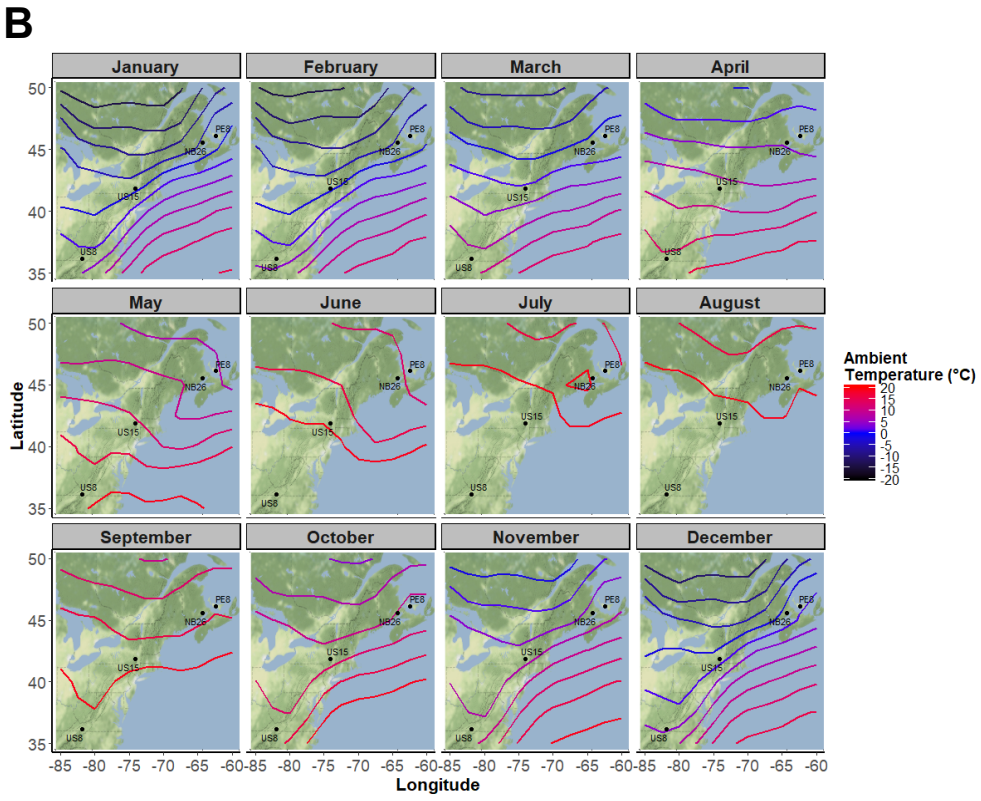



Fig. S1: A) Measurements of cave temperatures were collected from Figure 18 A & B in Swezey & Garrity (11). We used the data collected from WNS-infected sites to estimate the average cave conditions across the space spanning sampling areas, displayed here using a heat map. B) Records for monthly average air temperature was retrieved using R package *RNCEP* (12). In this figure, the distance between contour lines represents 2°C. We displayed the temperature trends over geographical space using the R package *ggmap* (13) with Stamen maps.

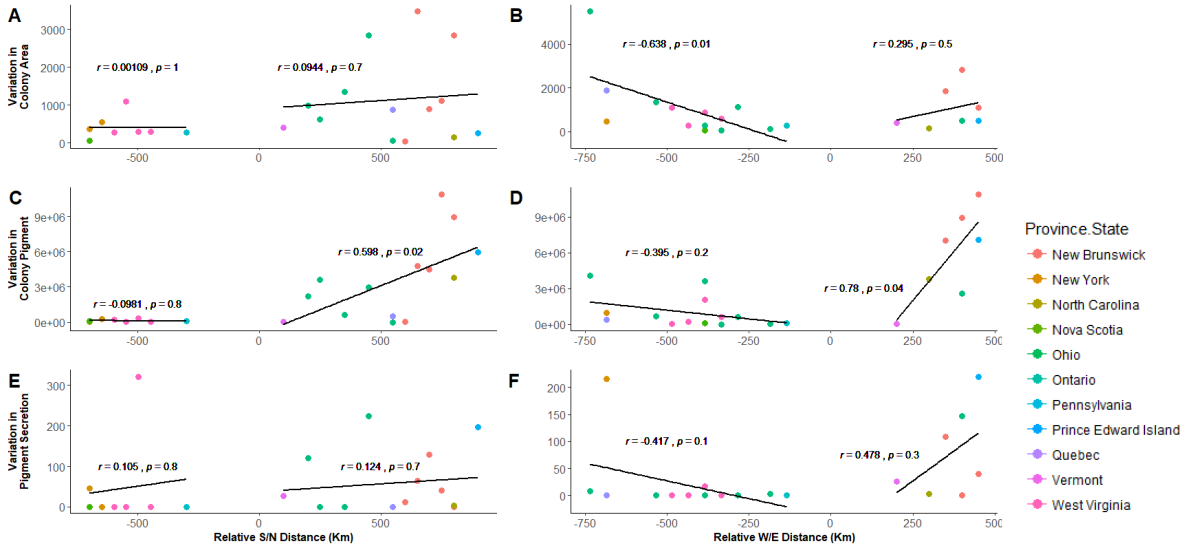



Fig. S2: Measurements of *P. destructans* colony phenotypic traits at 14°C after 28 days of growth. A: Variation of fungal colony area on agar plates along south/north (S/N) relative distance. B: Variation of fungal colony area on agar plates along west/east (W/E) relative distance. C: Variation of colony surface pigmentation along S/N relative distance. D: Variation of colony surface pigmentation along W/E relative distance. E: Variation in colony pigment diffusion along S/N relative distance. F: Variation in colony pigment diffusion along E/W relative distance.

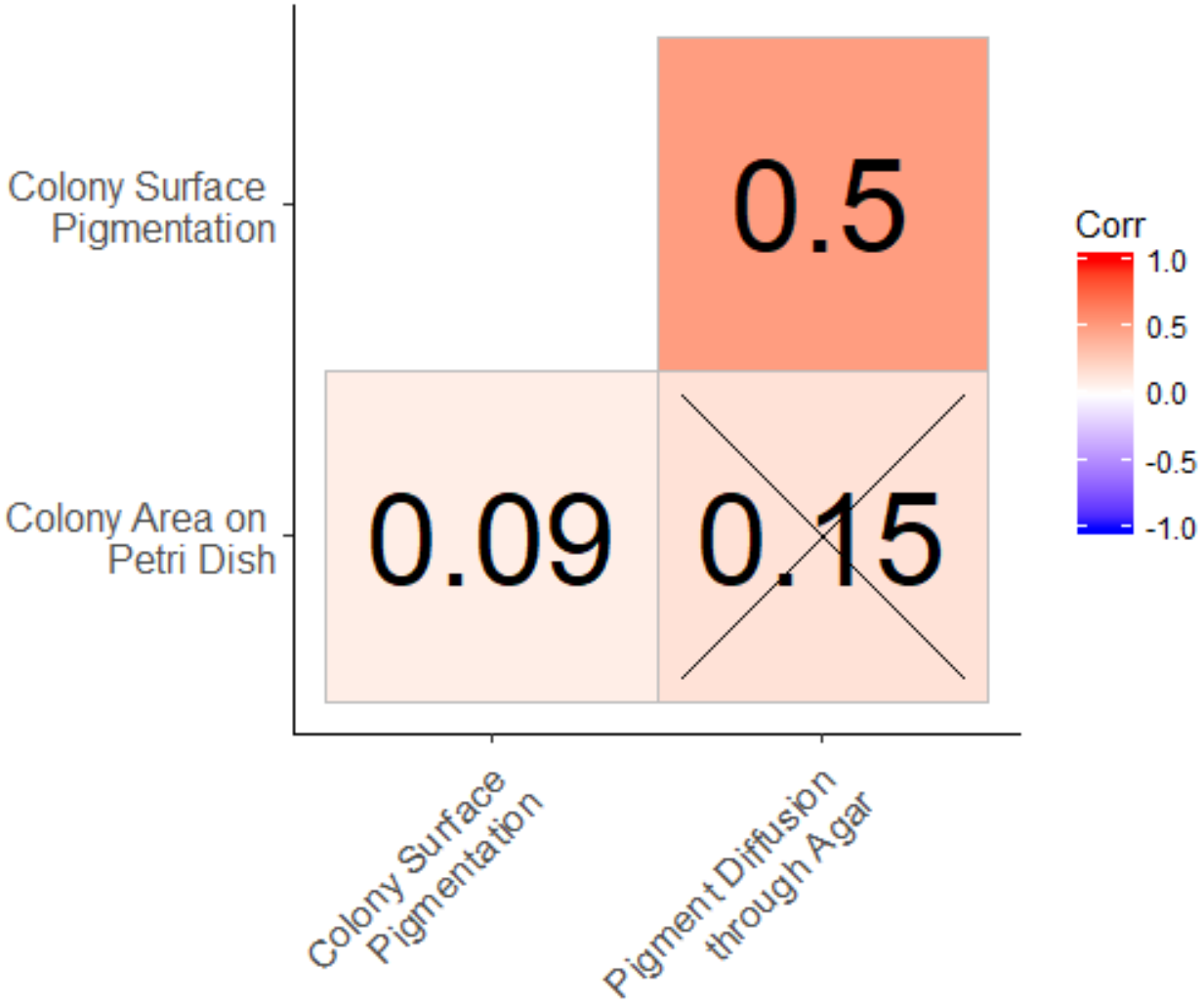



Fig. S3: Results from multivariate linear mixed model demonstrate patterns of the correlation between pairs of traits. Labels and colours of squares represent correlation coefficients, while an "X" represents p-values greater than a significance level of 0.05. This figure was generated using the R package *ggcorrplot* (14).

## Supplemental References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