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## Continental-level population differentiation and environmental adaptation in the mushroom

### *Suillus brevipes*

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**Continental-level population differentiation and environmental adaptation in the mushroom *Suillus brevipes***

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**Abstract**

Recent advancements in sequencing technology allowed researchers to better address the patterns and mechanisms involved in microbial environmental adaptation at large spatial scales. Here we investigated the genomic basis of adaptation to climate at the continental scale in *Suillus brevipes*, an ectomycorrhizal fungus symbiotically associated with the roots of pine trees. We used genomic data from 55 individuals in seven locations across North America to perform genome scans to detect signatures of positive selection and assess whether temperature and precipitation were associated with genetic differentiation. We found that *S. brevipes* exhibited overall strong population differentiation, with potential admixture in Canadian populations. This species also displayed genomic signatures of positive selection as well as genomic sites significantly associated with distinct climatic regimes and

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abiotic environmental parameters. These genomic regions included genes involved in transmembrane transport of substances and helicase activity potentially involved in cold stress response. Our study sheds light on large-scale environmental adaptation in fungi by identifying putative adaptive genes and providing a framework to further investigate the genetic basis of fungal adaptation.

## Introduction

The environment in which organisms live plays an important role in shaping the distribution of genetic variability within and between populations across time and space. Environmental adaptation in natural populations leaves genomic signatures of positive selection that shed light on the origins and maintenance of genetic variation within and between populations (Dobzhansky 1948, Novembre & Di Rienzo 2009). Natural selection affects the levels of genetic variability and can lead to selective sweeps, where sites linked to a selected mutation show reduced within-population variability (Nielsen 2005). One way to studying environmental adaptation is to detect genomic regions under positive selection. This approach can be done by using large-scale single nucleotide polymorphism (SNP) datasets, detecting statistical outliers and characterizing genes under selection. Another method to studying environmental adaptation is ecological association, in which one detects allele frequencies that exhibit significant statistical association with environmental variables that are used as proxies for natural selection (Frichot *et al.* 2013, Frichot & François 2015). Genome scans for environmental adaptation produce lists of genetic polymorphisms potentially involved in local adaptation that can be compared to genomic regions under positive selection and provide a better assessment of environmental adaptation.

Studies on the genomics of environmental adaptation are very often conducted in biological systems with pre-defined ecotypes that are recognized based on obvious morphological traits, ecological preferences, or both (e.g. Jones *et al.* 2012, Laurent *et al.* 2015, Nachman *et al.* 2003, Schweizer *et al.* 2016). Such studies aim to document the genomic basis of ecotypic differences and are based on *a priori* ideas on relevant population units and environmental parameters that might cause genetic

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divergence. For most taxa, including the vast majority of microbiological diversity, *a priori* information on ecotypic variation is scarce if available at all, making studies of environmental adaptation more challenging to implement. Fungi in particular offer little obvious phenotypic variation potentially linked to fitness differences at the population level and there are only a few documented cases of fungal local adaptation relating to environmental differences, such as heavy metal contaminated soils (Colpaert *et al.* 2011), genetically different plant pathogenic hosts (e.g. Salvaudon *et al.* 2008, Enjalbert *et al.* 2005) and thermal clines (Mboup *et al.* 2012). Recent ecological genomic approaches have revealed additional evidence of fungal adaptation related to subtle environmental variation and allowed candidate genomic regions involved in environmental adaptation to be identified. Such regions provide indirect evidence for fungal adaptation to environmental conditions (an approach coined ‘reverse ecology’; Li *et al.* 2008) and can be the basis for developing tests aimed at revealing the mechanisms responsible for the process of fungal adaptation. Relevant examples include ecotypic variation in the saprobe *Neurospora crassa* linked to temperature gradients (Ellison *et al.* 2011), adaptation to cheese environment in the genus *Penicillium* (Ropars *et al.* 2015), host specificity in the wheat pathogen *Zymoseptoria graminicola* (Stukenbrock *et al.* 2011) and adaptation to soil chemistry in the ectomycorrhizal fungus *Suillus brevipes* (Branco *et al.* 2015). The latter study focused on the same species that we examine here and documented two recently diverged and isolated populations in an obligate fungal symbiont of pine from coastal and montane California. The two populations showed very limited genetic differentiation, but a gene homologous to *Nha-1*, a membrane Na<sup>+</sup>/H<sup>+</sup> exchanger involved in salt tolerance in plants and *Saccharomyces cerevisiae* (Nass *et al.* 1997, Apse *et al.* 1999) differed between the two populations and showed evidence of positive selection. This report was the first to find salt as a potential driver for ectomycorrhizal fungal population differentiation, even though soil chemistry is long known to affect fungal physiology and growth (Smith & Read 2010).

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Here we investigated the genomic basis of adaptation to climate at the continental scale in *S. brevipes* by expanding the genomic dataset studied of Branco *et al.* (2015). Adding to the previously documented coastal and montane California populations, we sequenced the genomes of individuals from five additional sites in Canada (two locations were sampled), Colorado, Minnesota and Wyoming (United States of America), totaling 55 *S. brevipes* individuals. This expanded sample encompassed a wide range of environments and enabled us to test whether *S. brevipes* genomes display signatures of adaptation to distinct climatic regimes. Specifically, we asked whether there is evidence for local adaptation in this species across North America by detecting selective sweeps across the *S. brevipes* genome and assessing whether large-scale climatic parameters such as temperature and precipitation are associated with genetic differentiation. We hypothesized that this wind-dispersed species would show both strong population structure across the continent and a pattern of climatic adaptation, with genomic signatures of positive selection in regions correlated to temperature and/or precipitation and involved in environmental response. We found strong population differentiation across North America as well as significant genotype-climate associations in genomic regions under strong positive selection. These regions included genes involved in transmembrane transport and helicase activity that are known from other systems to be involved with cold stress response.

## Materials and methods

### *Isolate collection, culturing and DNA extraction and sequencing*

We collected fruitbodies of *Suillus brevipes* and cultured dikaryotic individuals from them from seven pine forests selected from the western and northern range of the species. Specifically we sampled two sites in California (USA), two sites in Alberta (Canada), and one site, each, in Wyoming, Colorado and Minnesota (USA, Fig. 1). A total of 55 dikaryotic *S. brevipes* individuals were included in our study. This sample contained 28 dikaryotic *S. brevipes* cultures from California that we had been previously sequenced and reported on Branco *et al.* (2015), and 27 new individuals from the other

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parts of range. We sequenced the whole genomes of the new cultures with pair-end 100bp Illumina technology following the protocol described in Branco *et al.* (2015). We combined these new data with the sequences generated for the *S. brevipes* populations from coastal and montane California studied in Branco *et al.* (2015) to produce a total sample of 55 *S. brevipes* individuals. We used four individuals as outgroups, one *S. luteus* individual and three individuals of *Suillus* sp. (S106, S108, S110), a species closely related to *S. pseudobrevipes* (Nguyen *et al.*, in press). *Suillus luteus* reads were obtained from the reference genome (<http://genome.jgi.doe.gov/Suillu1/Suillu1.home.html>) and we sequenced the *Suillus* sp. (S106, S108, S110) whole genomes from fruitbodies collected in Florida and processed in the same manner as *S. brevipes* individuals. All details on collection sites for all individuals are summarized in Table S1. Raw reads were deposited in Short Read Archive and accession numbers can also be found in Table S1.

#### *Read filtering, mapping and variant calling*

We used a Galaxy workflow to perform read filtering and mapping (Goecks *et al.* 2010, Blankenberg *et al.* 2010, Giardine *et al.* 2005; Supplement 1). In short, we filtered the reads using Trim Galore! 0.3.7 ([http://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore/](http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)) and additionally filtered by quality with a cut-off value of 20. Filtered paired reads were then mapped to the *S. brevipes* reference genome ([http://genome.jgi.doe.gov/Suillus\\_brevipes/](http://genome.jgi.doe.gov/Suillus_brevipes/), assembled in 1555 scaffolds with a N50 of 159 kbp) using Bowtie 2.2.3 (Langmead & Salzberg 2012). Alignments were sorted with SAMtools 0.1.19 (Li *et al.* 2009) and paired with Paired Read Mate Fixer in Picard 1.129 (<http://picard.sourceforge.net>). Duplicates and poor alignments were removed also using Picard, as well as filtered for uniquely mapped reads. The genome alignment rate was assessed with Picard and genome coverage with SAMtools. Variant calling was performed using ANGSD 0.801 (Korneliussen *et al.* 2014), a program based on genotype likelihoods, the marginal probability of the sequencing data given a genotype in a particular individual for a particular site. With our moderate genome coverage data, variant calling comes associated with uncertainty that can lead to biased estimates of allele

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frequencies and population genetic parameters. ANGSD takes base calling, mapping and alignment errors into account, making it ideal to analyze the genomes included in this study. This software, which was developed for low and medium coverage data and diploid organisms, is equally useful for studying dikaryons.

#### *Population structure and individuals genealogy*

We used RAxML (Stamatakis 2014) to infer the *S. brevipes* individuals maximum-likelihood genealogy based on the full set of SNPs (using the model GTRGAMMA and 100 bootstrap replicates). Population structure was studied using principal components analysis (PCA) with ngsCovar implemented in ngsTools (Fumagalli *et al.* 2013) and NgsAdmix (Skotte *et al.* 2013), a software for assigning individuals to differentiated genetic clusters and inferring admixture of related individuals using genotype likelihood data. Analyses were performed with the number of clusters, K, ranging from two to seven and with five replicates per K value. Average likelihoods were compiled using the Evanno method (Evanno *et al.* 2005) to identify genetically homogeneous groups of individuals. The divergence metrics  $F_{ST}$  and  $D_{xy}$  were computed for the homogeneous populations only (the two California populations, Colorado and Minnesota) using ANGSD and ngsStat, respectively.

#### *Summary statistics and linkage disequilibrium*

Summary statistics and linkage disequilibrium decay were estimated for the four homogenous populations (the two California populations, Colorado and Minnesota). We computed nucleotide diversity ( $\pi$ ), Watterson theta ( $\theta$ ) and Tajima's D with ANGSD (Korneliussen *et al.* 2014) and heterozygosity for all populations using ngsStat in ngsTools (Fumagalli *et al.* 2013). To assess linkage disequilibrium (LD) decay, we calculated the correlation coefficient ( $r^2$ ) between any two loci across

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the genome using genotypes.  $r^2$  was calculated using VCFTools 0.1.13 (Danecek *et al.* 2011) with a maximum of 5000bp between SNPs.

#### *Association between genotypes and environmental variables*

Genotype-environment associations were assessed using Latent Factor Mixed Models 1.4 (LFMM, Frichot *et al.* (2013)), a univariate mixed-model approach that estimates the effects of hidden factors representing background residual levels of population structure. We acquired environmental parameters for each *S. brevipes* homogeneous population using geo-referenced environmental layer datasets (Hijmans *et al.* 2005). We used a set of 19 climate variables available in BIOCLIM (Hijmans *et al.* 2005): annual mean temperature, mean diurnal range, isothermality, temperature seasonality, maximum temperature of warmest month, minimum temperature of coldest month, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation seasonality, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter.

We normalized the environmental variables (by subtracting each value by the parameter mean and dividing this amount by the corresponding standard deviation) and performed a PCA to reduce the number of variables to be tested (hereafter envPCA). envPC1 and envPC2 explained 95% of the climate variation (envPC1: 66%; envPC2: 29%, Fig S1) and were selected as the environmental variables for LFFM analyses. These principal components depict a combination of temperature and precipitation (Table S2), with envPC1 being heavily influenced by how cold and dry the winters are (with minimum temperature of coldest month, mean temperature of coldest quarter and precipitation of coldest quarter with the highest loadings) and envPC2 roughly informing on weather heterogeneity throughout the year (with mean diurnal range, mean temperature of wettest quarter and annual precipitation with the highest loadings).

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In LFMM the optimal latent factor number (K) influences the false discovery rate and should be selected based on the mean genomic inflation factor ( $\lambda$ ), which is the median of the squared z scores divided by 0.456. Based on 5 LFMM runs, K=6 had the  $\lambda$  estimates closer to 1.0 for both envPC1 and envPC2. We obtained the median z scores of all five runs and re-adjusted the p-values using the Benjamini-Hochberg correction procedure and set the false discovery rate to 10% as recommended in the program manual. We selected the top 5% SNPs significantly correlated with envPC1 and envPC2 to investigate gene ontology enrichment (see below).

### *Genome scans for selective sweeps*

We used SweeD 3.2.11 (Pavlidis *et al.* 2013) to search for selective sweeps. These genomic regions are defined by reduced genetic variation (with an excess of rare variants and of high frequency derived variants) around mutation sites due to strong and recent positive selection that has led to the fixation of a single allele (Nielsen 2005). SweeD implements a composite likelihood ratio (CLR) test based on the SweepFinder algorithm (Nielsen *et al.* 2005). The CLR uses the variation of the whole or derived site frequency spectrum of a genomic sequence to compute the ratio of the likelihood of a selective sweep at a given position to the likelihood of a null model without selective sweep. The null hypothesis relies on the site frequency spectrum (SFS) of the whole genomic sequence rather than on a standard neutral model, which makes it more robust to demographic events such as population expansions (Pavlidis *et al.* 2013, Nielsen *et al.* 2005). SweeD was run both separately for each of the populations identified using analyses of population structure to detect population-specific selective sweeps, and on all samples to detect species-wide selective sweeps. We used the empirical derived site frequency spectra of the whole genome as a background for all scaffolds, using the four outgroup individuals to orient SNPs. We considered only scaffolds where the first and last SNPs were more than 3 kb distant, and computed CLR every 1 kb.

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CLR values not belonging to the 95th percentile were considered as outliers. As the CLR is not designed to test if close outlier values belong to the same selective sweep, we simply defined genomic intervals affected by selective sweeps by merging consecutive outlier positions into single intervals and adding 500 bp at the flanks of each interval.

#### *Outlier Gene Ontology (GO) enrichment analysis*

We conducted gene ontology (GO) enrichment analyses on selective sweeps and LFMM outliers using ClueGO 2.2.4, a Cytoscape plugin Java tool that extracts knowledge on putative function for large clusters of genes. We modified the default settings to include minimal number and percentage of genes for GO term/pathway selection and the minimal and maximal level for GO Tree interval as one and twelve respectively. Significance of GO terms was calculated with a two-sided hypergeometric test and p-values corrected using the Benjamini-Hochberg approach. We also identified enrichment of functional domains associated to the two environmental principal components. We used a hypergeometric test to assess differences in functional domains associated with envPC1 and envPC2. The geometric test was further applied to each domain with genetic variation regarding envPC1 and envPC2, with a total of 862 domains examined. Individual functional domains with FDR higher or equal than 0.05 were likely observed by chance after correcting for multiple comparisons.

## **Results**

### *Suillus brevipes genomes*

We obtained over one billion raw Illumina reads and over 900 million high-quality reads from 55 *S. brevipes* individual genomes, that yielded 1,495,484 high quality single nucleotide polymorphisms across the 52.7Mb genome. Polymorphism level in this species was close to 3% segregating sites. Table S1 summarizes all information on per-individual number of generated raw reads, number of high-quality reads, alignment rate and genome coverage (which varied between 21x and 110x).

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We found distinct and clearly differentiated *S. brevipes* populations across North America, including the two Californian populations previously described in Branco *et al.* (2015). The genealogy of individuals based on the full set of SNPs (Fig. 2) showed the Canadian samples as paraphyletic and sister to all other populations, with Minnesota nested within Castle Rock and Whitecourt basal to Wyoming, Colorado and coastal and montane California.

The ngsAdmix analyses were consistent across distinct K values, revealing coastal California, montane California, Colorado and Minnesota as genetically homogeneous (Fig. 3, Figure S2).

Individuals from the two populations in Canada and Wyoming showed substantial membership in clusters identified in other populations suggesting potential admixture (Fig. 3).

The SNP-based PCA also showed *S. brevipes* population genetic structure (Fig. 4), with the two Californian populations previously analyzed appearing as clearly differentiated but accounting for a small portion of the genomic variability encompassed in the sampled populations. Apart from Minnesota and Colorado, the remaining populations were not as clearly differentiated. Individuals from the two Canadian sites and Wyoming showed some degree of overlap in the ordination space and Canadian individuals spread over both PC1 and PC2 indicating high genetic variability.

California, Colorado and Minnesota populations showed similar per-base nucleotide diversity ( $\pi$ ), Watterson theta ( $\theta$ ) and heterozygosity (Table 1). Linkage disequilibrium (LD) decay curves were very similar across populations (Fig. 5), showing a strong sharp decay in the first hundreds of base pairs. Such pattern indicates very small linkage blocks and therefore pervasive outcrossing in this species.

Genomic pairwise  $F_{ST}$  revealed high levels of differentiation between populations of *S. brevipes* (Table 2). The California montane and coastal populations were the least differentiated (with  $F_{ST\ CA-CM} = 0.09$ ), while Minnesota was the most differentiated, with  $F_{ST}$  values of 0.3 and over when compared to California and Colorado. These results are in accordance with both the phylogeny and admixture analyses.

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## *Selective sweep detection and association between genomic sites and environmental variables*

Selective sweep analyses revealed over 1500 genomic regions with signatures of positive selection in each population and all populations combined. Only 95 sites were recovered as swept in all analyses.

Sweeps were prevalent across the majority of scaffolds, with larger scaffolds containing more swept sites (Fig. S3). Gene ontology (GO) enrichment analyses failed to detect enriched GO categories across the sweeps, indicating that sites under positive selection span a wide range of functions.

LFMM analyses found 18744 sites significantly associated with envPC1 (which is heavily influenced by winter temperature and precipitation) and 6776 sites with envPC2 (which is correlated with weather heterogeneity throughout the year). GO enrichment analyses on these sites revealed that the categories significantly enriched for envPC1 were ATPase-driven transmembrane transporters and ATPase-dependent helicases (Fig. 6a), while envPC2 was significantly enriched with protein transmembrane transporter activity (Fig 6b).

We found a total 862 functional domains in the LFMM sites correlated with the two environmental principal components (727 in envPC1, 459 in envPC2, with an overlap of 324 domains). A hypergeometric test revealed significant differences between the domains in envPC1 and envPC2 ( $p = 1.8e-93$ ). Of 862 functional domains with genetic variation either in envPC1 or envPC2, three were identified as not randomly enriched in envPC1 or/and envPC2 (Table 3;  $FDR < 0.05$ ;  $p < 0.05$ ). The domains' functions were protein kinase, WD40 repeats and nucleic acid binding and DNA/RNA helicase.

We found 43 individual genomic sites that were both significantly associated with environmental parameters and showed signatures of positive selection as assessed by LFMM and selective sweep analysis (Table 4). These sites were located across the scaffolds and most were outliers restricted to a single population. These results suggest the existence of local adaptation, with different alleles being selected for in different populations. Eleven genomic sites currently have no predicted function and four sites were included in start/stop codons. The latter were adjacent to transporter genes and hydrolases, indicating potential loss-of-function mutations. Notably, the  $Na^+/H^+$  exchanger associated

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with salt tolerance found to be fixed in the coastal California population in Branco *et al.* (2015) was also found to be a relevant genomic region for that population only, and it was under strong positive selection and strongly associated with both envPC1 and envPC2.

## Discussion

### *Suillus brevipes* biogeography

*Suillus brevipes* is an obligate mycorrhizal associate of *Pinus* subgenus *Pinus* and is widespread in North America (Smith, A. H. & Thiers, H. D. 1964, Nguyen *et al.*, in press), tightly associated with the range of its hosts. This species is one of the most ruderal of the genus and is commonly found with young pine trees at forest margins and also with introduced and invasive pines (Ashkannejhad & Horton 2006, Peay *et al.* 2007, Hynson *et al.* 2013). This high level of host specificity limits the species distribution and creates a mosaic continental structure of habitat for *S. brevipes*.

In spite of the prolific production of aerially dispersed meiotic spores in *Suillus* spp. (Peay *et al.* 2012, Dahlberg & Stenlid 1994), there is mounting evidence that effective dispersal is limited to a fairly fine spatial scale, which is consistent with the idea that ‘almost nothing is everywhere’ (Gladieux *et al.* 2015a, Taylor *et al.* 2006, Peay *et al.* 2010). In fact, recent work showed that the spore loads of ectomycorrhizal fungi, including *Suillus* species, decrease exponentially as spores travel away from their source, support the hypothesis that these fungi are not very effective at dispersing over long distances (Peay *et al.* 2012). For example, even though *S. pungens* was estimated to produce 8 trillion spores/km<sup>2</sup> within its host forest, its spore load decreased exponentially with distance from the forest edge, with spore levels insufficient to consistently colonize pine seedlings at distances greater than 1km (Peay *et al.* 2012). Such observed dispersal limitation at the local scale is also consistent with both the population genomics results of Branco *et al.* (2015) revealing two isolated and locally adapted *S. brevipes* populations in California and the results reported here. Long distance spore dispersal in *S. brevipes* is thus likely to be rare, and if it occurs, immigrant genotypes may be less fit

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than local ones. This explanation is consistent with our finding that North American *S. brevipes* is subdivided into multiple, clearly circumscribed genetic clusters.

The least distinct and most genetically diverse populations occur in the two Canadian sites and to a less extent in Wyoming. The finding of individuals assigned to multiple clusters in Canada might suggest admixture between individuals from differentiated populations. This hybridization idea is consistent with the known postglacial history of *P. banksiana* and *P. contorta*, the only hosts in this area. In fact, these pine species form their own hybrid zone in western Alberta within the Whitecourt site that we sampled (Yeatman 1967). Population genetic studies (Godbout *et al.* 2010) and pollen records (Williams *et al.* 2004) suggest that these pine species met in Alberta as the glaciers retreated, by reinvasion from Eastern North America (*P. banksiana*) and from one or more western mountain corridors (*P. contorta*) (Critchfield 1985).

Alternatively, Canadian admixture can be interpreted as Alberta hosting the oldest *S. brevipes* populations, and therefore the most diverse, from which all other populations would be derived. This interpretation is consistent with the individuals genealogy and with previous findings of artifactual inference of admixture in ancestral populations in other organisms (Kauer *et al.* 2003, Schöfl & Schlotterer 2006, Gladieux *et al.* 2008), but would require the presence of a pine refugia in this area during glaciation..

#### *Adaptation in Suillus brevipes*

We found evidence consistent with abiotic environmental adaptation in *S. brevipes* populations across North America. Genes found both under positive selection and significantly associated with climatic environmental variables were mainly involved in transmembrane transport of substances and helicase activity. Even though these gene categories are involved in multiple cell functions, we argue that they can be directly related to environmental adaptation, namely in the physiological responses to conditions determined by variations in temperature and precipitation.

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Transmembrane transporter genes are known to play important roles, including responding to environmental stress in wide variety of organisms (Chan *et al.* 2011, Krulwich *et al.* 2011, Mansour 2014, Chen *et al.* 2003, Apse *et al.* 1999, Rizzello *et al.* 2013, Mahajan & Tuteja 2005, Heide & Poolman 2000). In fungi, transporters are involved in environmental sensing and are crucial for detecting and responding to a wide variety of cues, including chemistry, temperature and water availability (Aguilera *et al.* 2007, Bahn & Mühlischlegel 2006, Bahn *et al.* 2007, Maldonado-Mendoza *et al.* 2001, Javelle *et al.* 2003, Harrison *et al.* 2002, Dietz *et al.* 2011, Pettersson *et al.* 2005, Peter *et al.* 2016). Temperature and precipitation differences across the sampled sites can therefore be directly linked to the enriched transporter gene categories detected in our study. Cold stress in particular affects the activity of membrane transporters due to changes in membrane fluidity that can have multiple nefarious consequences, including decreased nutrient uptake (Aguilera *et al.* 2007). Water stress on the other hand can be mitigated by membrane proteins that mediate water transfer (aquaporins, Carbrey & Agre 2009). These transporters are known to occur in fungi (Pettersson *et al.*, 2005), being involved in the transport of both water and ammonia in ectomycorrhizal fungi and presumed to be directly linked to mechanisms of environmental adaptation (Dietz *et al.* 2011, Peter *et al.* 2016).

Helicases are motor proteins that separate nucleic acids strands. These enzymes are involved in many fundamental processes such as replication, transcription, translation and nucleic acid repair and are known to be involved in environmental stress response. RNA helicases play a role in responding to abiotic stressors by changing the export of nuclear mRNA, translation initiation, mRNA decay, rRNA processing, transcription and the progression of cell cycle (Owtrim 2006). RNA helicases are known to be involved in adaptation to cold in bacteria and fungi (Barria *et al.* 2013, Pandiani *et al.* 2010, Aguilera *et al.* 2007). In the aforementioned “reverse ecology” study of *Neurospora crassa* populations (Ellison *et al.* 2011), an RNA helicase was one of several genes hypothesized to be important to cold adaptation and one of only two genes whose role in cold adaptation could be confirmed by gene deletion. Cold stress can lead to the stabilization of RNA secondary structure, impairing transcription, translation and degradation, and RNA helicases act to melt such structures

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allowing for RNA degradation (Barria *et al.* 2013). In *Saccharomyces cerevisiae*, RNA helicases are induced during the early stages of cold stress response (Aguilera *et al.* 2007).

DNA helicases are involved in DNA repair, as they allow repair machinery to access the single stranded DNA (Goldman *et al.* 2002), and are also induced by cold stress (Vashisht *et al.* 2005), allowing for repair mechanisms to act on this molecule. Further functional tests addressing the role of candidate genes are needed to investigate potential differences in environmental adaptation (including cold and water stress response) in *S. brevipes* individuals across North America.

The membrane  $\text{Na}^+/\text{H}^+$  exchanger described in Branco *et al.* (2015) to be under strong positive selection, was again found here among the highly differentiated genes and strongly associated with envPC1 and envPC2 in the coastal California population. This gene is homologous to *Nha-1*, known for enhancing salt tolerance in plants and *S. cerevisiae* (Apse *et al.* 1999, Nass *et al.* 1997) and a good candidate for local adaptation in *S. brevipes*. It is therefore not surprising that this gene is located in a putative selective sweep region and located in a  $D_{xy}$  outlier genomic window. However, our LFMM analyses were restricted to climatic variables and did not include soil parameters, so possible explanations for the strong correlation with envPCs include pleiotropic functional properties or linkage to another gene truly correlated with weather variability.

### **Concluding remarks**

Our study provides a unique large-scale view of within species evolution and sheds light on the factors driving environmental adaptation in fungi. We used a large-scale dataset to detect genomic regions correlated to climatic parameters and harboring signatures of positive selection without any prior information on phenotypic differences, population units or environmental factors that could underlie potential adaptation. Such reverse ecology approach is very suitable for microbial systems, where there is very little *a priori* information. Fungi in particular have small genome sizes, simple morphologies, short generation times and a multitude of life styles, being exceptional models for studying adaptation in eukaryotes (Gladieux *et al.*, 2014). Reverse ecology has in fact revealed hidden

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fungus environmental adaptation in several species so far (e.g. Branco *et al.* 2015, Ellison *et al.* 2011, Gladieux *et al.* 2015b and Badouin *et al.* submitted to this issue). Genome scans are therefore a useful framework for identifying putative adaptive genes responsible for local adaptation, and the results can motivate functional tests aimed at linking genotypes to phenotypes and are crucially needed for a full understanding of the genetic basis of adaptation.

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#### **Author contributions**

S.B., J.W.T. and T.D.B. designed the study, S.B., N.N., R.V., K.G.P., J.W.T. and T.D.B. collected samples, S.B. processed the samples, S.B., K.B., H-L.L., P.G., C.E.E and H.B. performed analyses, S.B. wrote the manuscript with comments from all co-authors.

#### **Data accessibility**

DNA sequences: Short Read Archive accessions in Table S1 (Supporting information).

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## Supporting information

Table S1 - *Suillus brevipes* isolate location, pine host, coordinates and elevation, number of raw reads, average genome coverage, and Short Read Archive accession numbers.

Table S2 - Loadings for each principal component (envPC) in the environmental PCA for the studied *Suillus brevipes* populations.

Figure S1 - PCA plot on normalized environmental variables for the studied *Suillus brevipes* populations.

Figure S2 - Likelihoods per K in each admixture run.

Figure S3 - Number of swept sites per genome scaffold. A - All populations, B - coastal California, C - montane California, D - Colorado, E - Minnesota.

Supplement 1 - Galaxy workflow for cleaning and mapping *Suillus* reads to reference genome.

## Figures

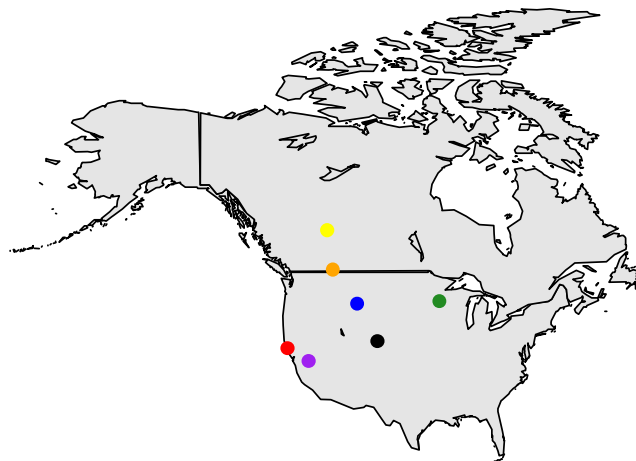


Figure 1 – Sampling localities of the studied *Suillus brevipes* populations in North America. Red - coastal California (16 ind); purple - montane California (9 individuals); yellow – Canada (Alberta), Whitecourt (5 individuals); orange – Canada (Alberta), Castle Rock (7 individuals); blue - Wyoming (7 individuals); black - Colorado (5 individuals); green - Minnesota (6 individuals).

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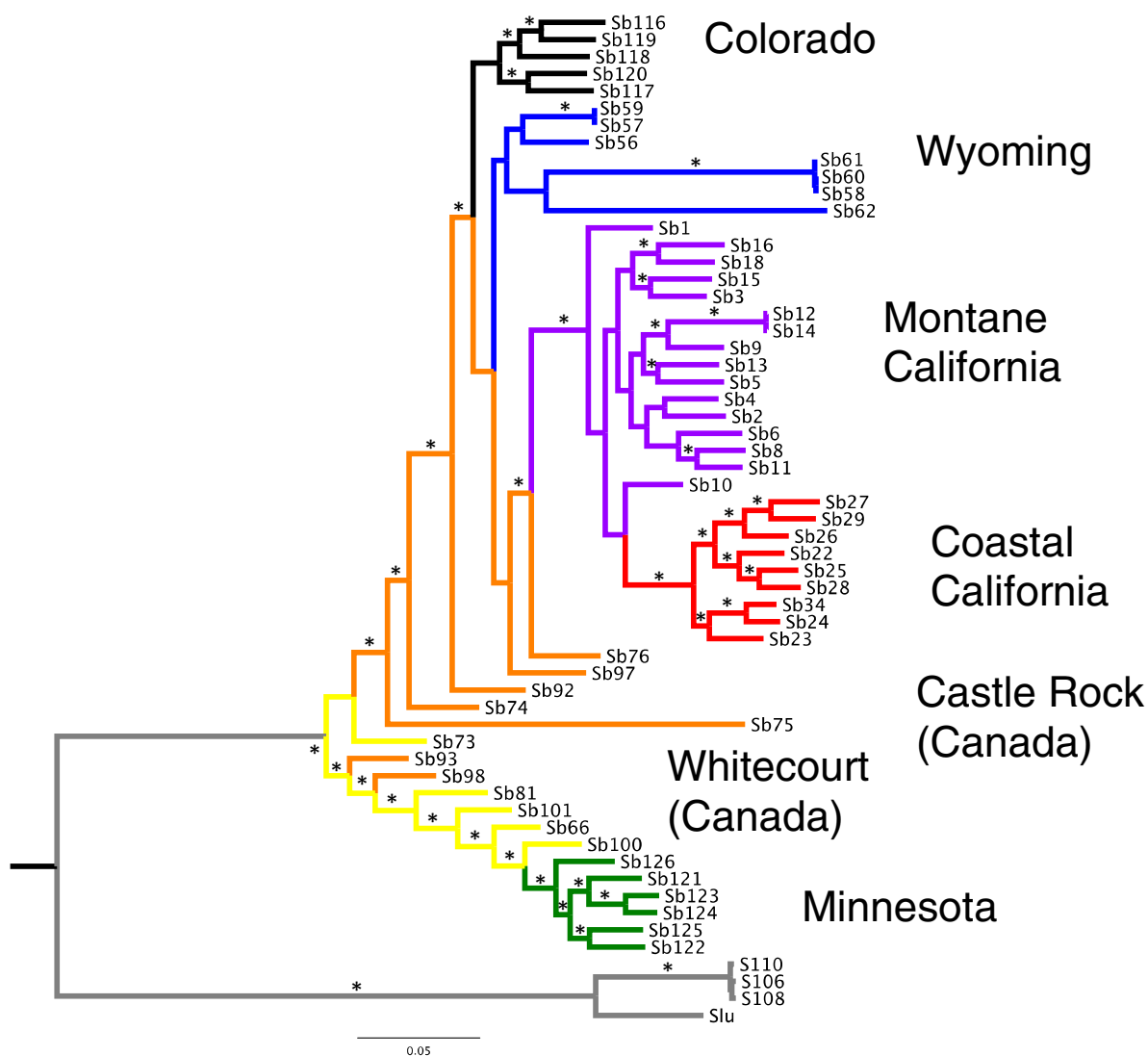


Figure 2 – Rooted maximum-likelihood genealogy inferred from the full SNP dataset. We used one individual of *S. luteus* (Slu) and three individuals of *Suillus* sp. as outgroups (in gray). The remaining colors correspond to Fig. 1: Red - coastal California; purple - montane California; yellow – Canada (Alberta), Whitecourt; orange – Canada (Alberta), Castle Rock; blue - Wyoming; black - Colorado; green - Minnesota. \* indicates bootstrap support value >90.

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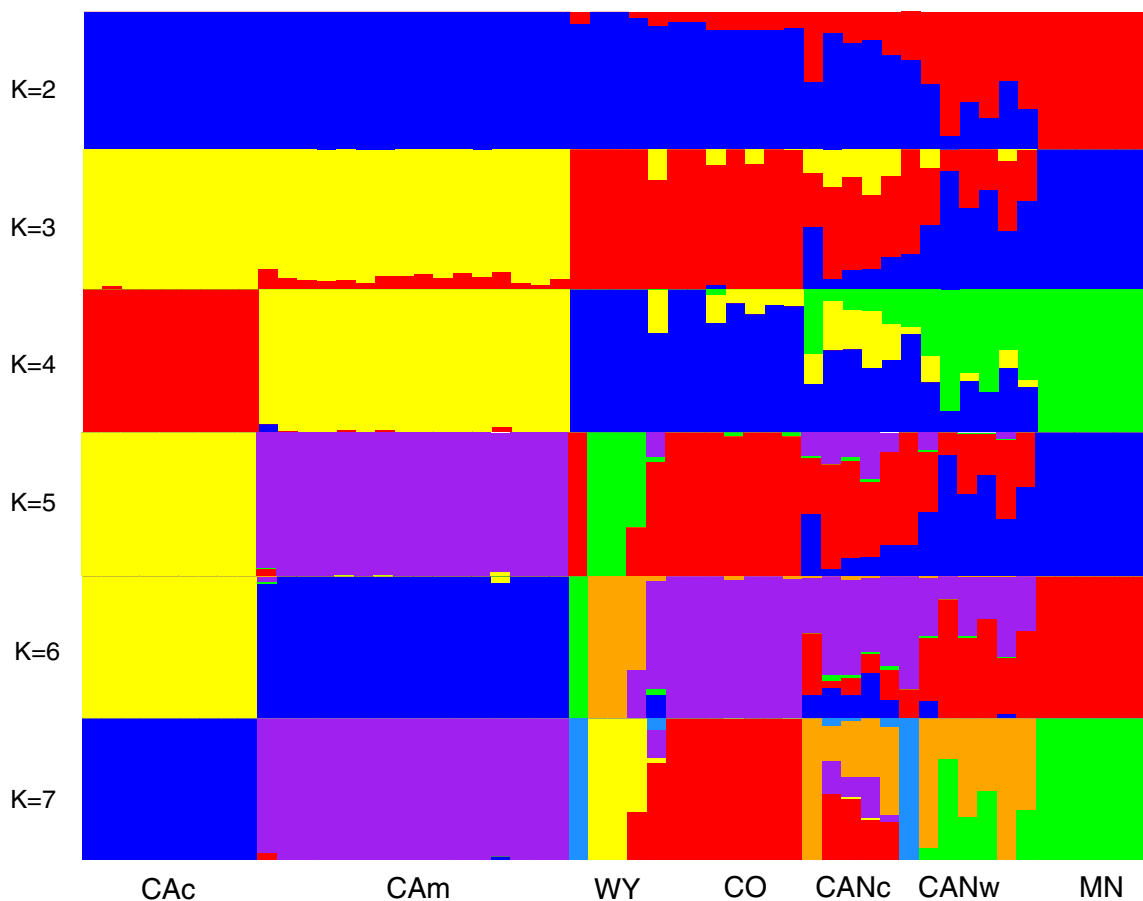


Figure 3 – Estimated *S. brevipes* population structure as inferred by NGSAdmix. Each individual is represented by a bar partitioned into K color segments (K=2 to K=7). CAC - coastal California; CAM - montane California; CANc – Canada (Alberta), Castle Rock; CANw – Canada (Alberta), Whitecourt; CO – Colorado; MN – Minnesota; WY -Wyoming.

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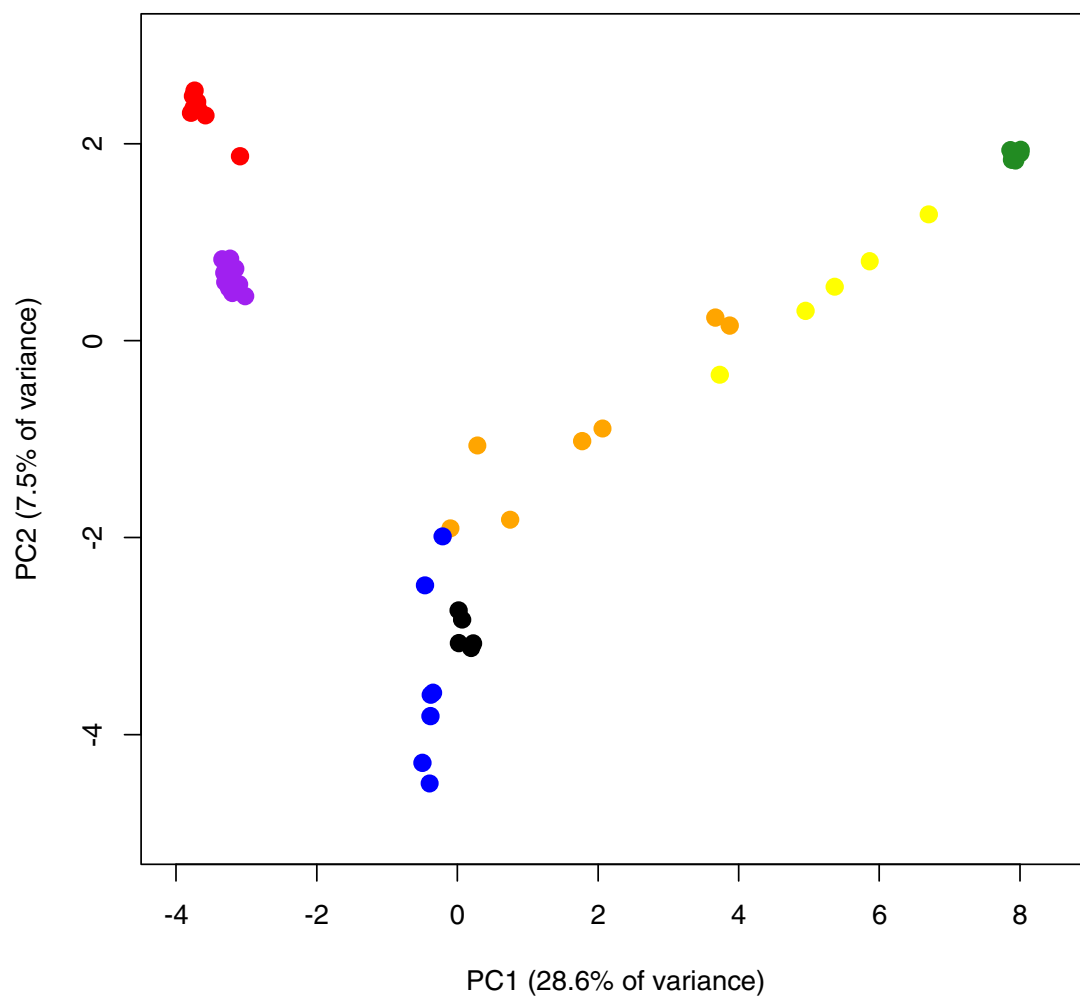


Figure 4 – PCA of sampled *S. brevipes* populations with percentage of variance explained by each principal component. Colors correspond to Fig. 1: Red - coastal California; purple - montane California; yellow – Canada (Alberta), Whitecourt; orange – Canada (Alberta), Castle Rock; blue - Wyoming; black - Colorado; green - Minnesota.

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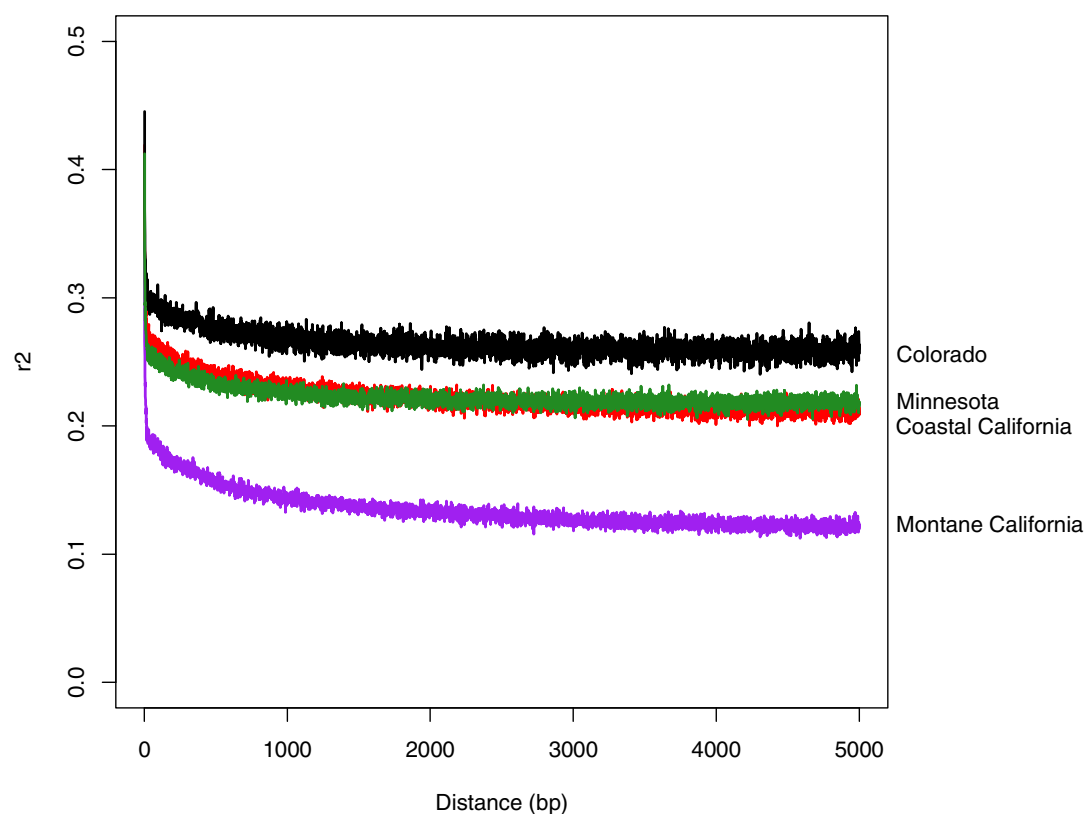
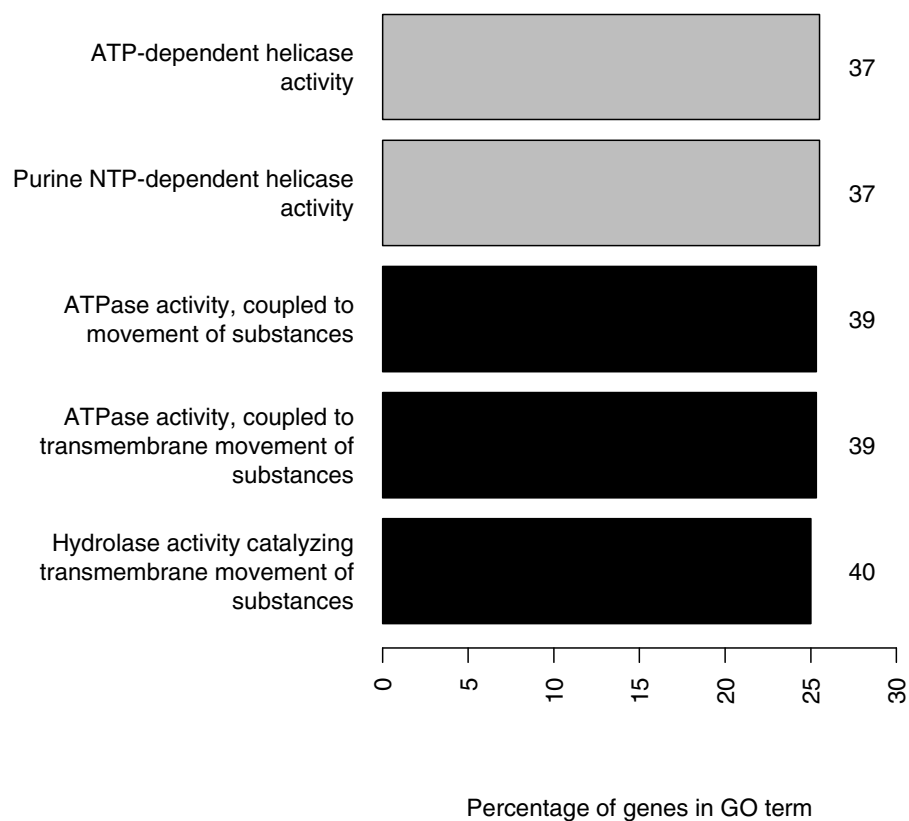


Figure 5 – Linkage disequilibrium decay in each population across the whole genome. Populations are color-coded as in Fig. 1: Red - coastal California; purple - montane California; black - Colorado; green - Minnesota.

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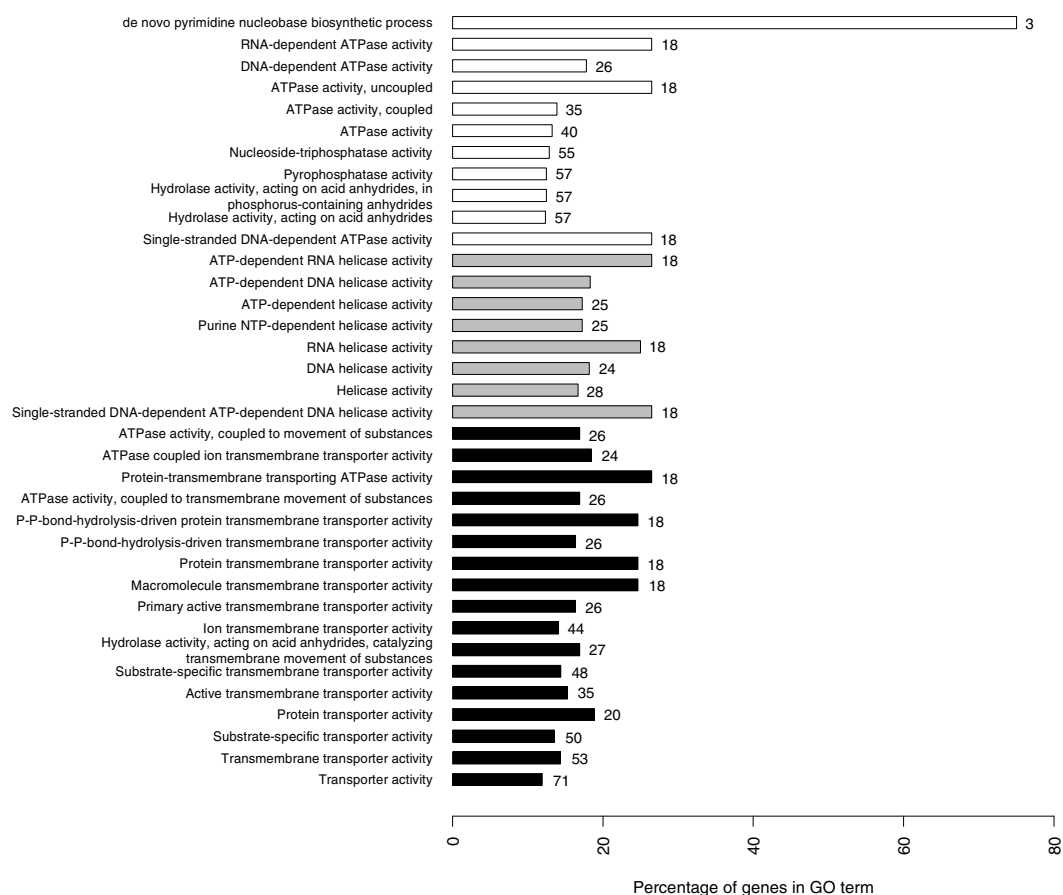


Figure 6 – Percentage and number of genes assigned to different gene ontology categories significantly correlated with envPC1 (a) and envPC2 (b) as assessed by ClueGO ( $p < 0.005$ ). Only the genes that were significantly correlated with one envPC compared to the other were listed. ‘Percentage of genes in GO term’ refers to the percentage of genes from the uploaded cluster that were associated with the term, compared to all *S. brevipes* genes associated with the same term. The numbers on bars correspond to the numbers of genes from the analyzed cluster associated with each category. Gray - helicase activity; black - transmembrane transporters; white - other categories.

## Tables

Table 1 – Average per-base nucleotide diversity ( $\pi$ ), Watterson theta ( $\theta$ ) and heterozygosity across *Suillus brevipes* populations. CAc - California coast, CAm - California mountain, CO - Colorado, MN - Minnesota.

	Nucleotide diversity	Watterson theta	Heterozygosity
<b>CAc</b>	0.098	0.093	0.093
<b>CAm</b>	0.106	0.113	0.104
<b>CO</b>	0.108	0.110	0.1
<b>MN</b>	0.123	0.142	0.115

Table 2 - Pairwise  $F_{ST}$  across *Suillus brevipes* populations. CAc - California coast, CAm - California mountain, CO - Colorado, MN - Minnesota.

	CAc	CAm	CO	MN
<b>CAc</b>	-			
<b>CAm</b>	0.09	-		
<b>CO</b>	0.19	0.12	-	
<b>MN</b>	0.38	0.35	0.3	-

Table 3 - Significantly enriched pfam domains in envPC1 and envPC2, with respective function overlap and counts.

ID	p-value	Function	Overlap (envPC1 vs envPC2)	Total count	envPC1 count	envPC2 count
PD000001	1.62E-05	Protein kinase	12	338	54	22
PD000018	1.94E-09	WD40 repeats	15	565	56	28
PF00270	1.55E-05	Nucleic acid binding, DNA/RNA helicase	9	65	16	11

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Table 4 - Outlier genomic positions in both LFMM and selective sweeps, with location, protein identification number, population, function, association to environmental PC and whether they are located in Dxy outlier windows. Grey highlights the sodium/proton exchanger described in (Branco *et al.* 2015).

Population	Scaffold	Position	Protein ID	Function	PC	Dxy outlier
CAC	8	148810	1781890	Binding	1	
CAC	50	112014	420413	DNA binding, transcription	1	
CAC	32	38903	798540	GTPase activity, transcription factor binding	1	
CAC	15	190772	186662	Hydrolase activity	1	yes
CAC	12	293833	718100	Integral to membrane; Na <sup>+</sup> /H <sup>+</sup> exchanger	1, 2	yes
CAC	42	167903	952639	No annotation	1	
CAC	50	143767	1853970	No annotation	1	
CAC	60	35465	833182	No annotation	1, 2	
CAC	12	323480	186662	Oxidoreductase activity	1	yes
CAC	27	176597	871448	Prenyltransferase activity, integral to membrane	1	
CAC	42	47281	1864144	Protein kinase activity	1	
CAC	32	190685	-	Start codon	1	
CAC	60	38348	804009	Tetrapeptide transporter, OPT1/isp4	1	
CAC	32	189047, 189371, 190230	798695	Vacuolar proton-transporting V-type ATPase, V1 domain	1	
CAC, CAm, CO	42	203805	856219	Fructose-bisphosphate aldolase	1, 2	yes
CAC, CO	63	173649	732802	Hydrolase activity	1	
CAC, CO, MN	23	88396	722280	Small oligopeptide transporter	1	yes
CAC, MN	19	109622	950841	Oxidoreductase activity	1	
CAC, MN	12	250675	-	Stop codon	1	
CAm	94	92412	857933	Beta-glucan synthesis-associated, SKN1	2	
CAm	32	34301	871507	Calcium ion binding	1	
CAm	17	242097	770019	No annotation	2	
CAm	43	47379	1839120	No annotation	1	
CAm, CO	36	193163	831272	No annotation	1	
CAm, CO	106	47087	739551	No annotation	2	yes
CAm, CO	22	52119	-	Stop codon	1	
CAm, MN	8	71288	853314	Alpha/beta hydrolase fold-1	1, 2	
CAm, MN	78	96366	738222	No annotation	1	
CO	11	182993	716688	No annotation	1, 2	
CO	20	126831	770517	Nucleotide catabolic process	1	

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CO	50	110208	729737	Oxidoreductase activity	1, 2
CO	17	137848	769788	Regulation of transcription, translational initiation	1
CO	1	353572	1926079	Ubiquitin-protein ligase activity	2
MN	199	58134	747856	Cyclic nucleotide biosynthetic process	2
MN	8	41947	715982	Ion channel activity	1, 2
MN	1	838431	824286	Mitochondrial intermembrane space protein transporter complex	1
MN	110	82327	1053000	NADH quinone oxidoreductase	1
MN	181	54297	878990	No annotation	1, 2
MN	11	367299	793247	Phosphoglycerate mutase	1
MN	86	10054	834508	Protein binding	1
MN	40	165975	800418	Protein kinase activity	1
MN	63	70718	-	Stop codon	1
MN	86	5096	857630	Transporter activity	1

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