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Genetic isolation between two recently diverged populations of a symbiotic fungus

Sara Branco¹, Pierre Gladieux², Christopher E. Ellison³, Alan Kuo⁴, Kurt LaButti⁴, Anna Lipzen⁴, Igor V. Grigoriev⁴, Hui-Ling Liao⁵, Rytas Vilgalys⁵, Kabir G. Peay⁶, John W. Taylor¹, Thomas D. Bruns¹

¹ Department of Plant and Microbial Biology, University of California, Berkeley, California, United States of America

² Laboratoire d'Ecologie, Systematique et Evolution, Universite Paris Sud, Orsay, France

³ Department of Integrative Biology, University of California, Berkeley, California, United States of America

⁴ Department of Energy, Joint Genome Institute, Walnut Creek, California, United States of America

⁵ Department of Biology, Duke University, Durham, North Carolina, United States of America

⁶ Department of Biology, Stanford University, Stanford, California, United States of America

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Corresponding author – Sara Branco; sara.mayer.branco@gmail.com

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Abstract

Fungi are an omnipresent and highly diverse group of organisms, making up a significant part of eukaryotic diversity. Little is currently known about the drivers of fungal population differentiation and subsequent divergence of species, particularly in symbiotic fungi. Here we investigate the population structure and environmental adaptation in *Suillus brevipes* (Peck) Kuntze, a wind-dispersed soil fungus that is symbiotic with pine trees. We assembled and annotated the reference genome for *S. brevipes* and re-sequenced the whole genomes of 28 individuals from coastal and montane sites in California. We detected two highly supported populations with very low divergence and no evidence for migration. However, we detected highly differentiated genomic regions, most notably a region that contains a gene encoding for a membrane Na^+/H^+ exchanger known for enhancing salt tolerance in plants and yeast. Our results are consistent with a very recent split between the montane and coastal *S. brevipes* populations, with few small genomic regions under positive selection and a pattern of dispersal and/or establishment limitation. This is the first population genomics study comprising a member of the phylum Basidiomycota or of a mycorrhizal fungus. It documents the least differentiated fungal populations currently known and identifies a

putatively adaptive gene that motivates further functional tests to link genotypes and phenotypes and shed light on the genetic basis of adaptive traits.

Introduction

The way organisms are distributed across the landscape is the result of their ability to reach, establish and persist in suitable habitats. Factors such as dispersal and establishment limitation influence these abilities, shaping the genetic diversity of populations in nature. Fungi are no exception. They have simple morphologies allied to well-defined ecological niches, comprise considerable diversity and tend to have small genomes. These features make them good models for studying population differentiation and adaptation in nature (Gladieux *et al.*, 2014). Unlike most animals and plants, the vast majority of fungi produce copious amounts of microscopic airborne propagules and are often assumed to have exceptional long-range dispersal capabilities (Brown *et al.*, 2013) leading to wide distributions and large population sizes (Green & Bohannan, 2006). This idea has however been recently challenged by ecological evidence that dispersal limitation at the landscape scale affects community structure and richness (Peay *et al.*, 2012) and that there is widespread fungal regional endemism (Taylor *et al.*, 2006, Amend *et al.*, 2010, Talbot *et al.*, 2014). Even though fungi are good models for understanding the genetic basis of evolutionary change, it is not yet clear how fungal adaptive divergence occurs and whether it is linked to dispersal, natural selection, or both.

Environmental factors are known to drive fungal adaptation, with temperature as a recurrent feature associated with wild fungal population differentiation even in host-

associated fungi (Vacher *et al.*, 2008, Frenkel *et al.*, 2010, Ellison *et al.*, 2011, Mboup *et al.*, 2012). However, fungal population genomics and adaptation have been studied in a very small set of fungi (Liti *et al.*, 2009, Neafsey *et al.*, 2010, Ellison *et al.*, 2011, Rosenblum *et al.*, 2013). This limited sample leaves out an immense diversity of fungal groups and life histories, including all symbiotic basidiomycete mushroom-forming fungi. The phylum Basidiomycota is one of the largest fungal phyla, comprising about 30,000 species (James *et al.*, 2006) and including the majority of mycorrhizal species, which are soil fungi living in obligate symbiotic associations with vascular plants (Smith & Read, 2010). In particular, ectomycorrhizal (EM) fungi are an important group associated with a wide range of plant families including tree species dominant in many biomes. EM fungi play a crucial role in forest ecosystems by providing water and nutrients to their host trees, for which they receive carbohydrates in return (Smith & Read, 2010). Although there have been attempts to understand EM fungal population genetics (Douhan *et al.*, 2011), little is currently known about the patterns of population structure in these fungi or on the relative role of dispersal limitation and local adaptation in shaping fungal diversity.

Here we report on the first population genomics study of an EM basidiomycete fungus. We compile a new fungal reference genome, document fungal population structure and investigate candidate genomic regions for local adaptation. We focused on *Suillus brevipes*, a widespread North American EM fungus associated with pine trees (Smith & Thiers, 1964). *S. brevipes* is a soil fungus and reproduces by forming fruiting bodies that produce and disperse in the order of 10^8 airborne spores per mushroom (Dahlberg & Stenlid, 1994). It plays an important ecological role as a pioneer species,

colonizing pine seedlings and young trees (Peay *et al.*, 2012) and contrary to most EM species, is amenable for *in vitro* cultures, making it possible to document population differentiation and to unveil its causal mechanisms. We assembled and annotated the *S. brevipes* reference genome and re-sequenced the genomes of isolates collected from two contrasting environments in California, coastal mixed *Pinus muricata* and *P. contorta* and mountain *P. contorta* forests separated by 300 km of inhospitable habitat, lacking either plant host. Given the inherent differences between coastal and montane environments, we expected to find clearly differentiated and locally adapted populations, with strong signatures of positive selection in genomic regions related to factors known to be relevant for fungal ecology and evolution, such as host tree, climate, and/or soil type. We also hypothesized ongoing gene flow between the coast and mountains, facilitated by the massive amounts of airborne spores produced by *S. brevipes*. Surprisingly, we detected distinct yet very little diverged coastal and montane populations, with only 155 reciprocally fixed SNPs. Demographic modeling pointed to isolation as the most likely model, with the absence of gene flow between the two populations. We also identified a small set of highly differentiated genomic regions, including a gene involved in salt tolerance, indicating positive selection plays a role in shaping these populations and suggesting the emergence of local adaptation in *S. brevipes*.

Materials and Methods

Isolate collection, culturing, and DNA extraction and sequencing

Suillus brevipes (Peck) Kuntze individual sporocarps were collected from pine forests from the coast and mountains of Northern California (Table S1). We harvested 11 coastal individuals in Mendocino County, Salt Point and Point Reyes National Seashore from forests dominated by *Pinus muricata* with residual *P. contorta* in the Mendocino site (under the Scientific Research and Collecting Permit # PORE-2011-SCI-0014). These sites are characterized by a Mediterranean climate with cool summers and mild winters with temperatures rarely reaching below freezing. We also collected 17 *S. brevipes* individuals in the Sierra Nevada mountains (Yosemite National Park; under the Scientific Research and Collecting Permit # YOSE-2013-SCI-0071) from *P. contorta* forests, around 300 km East from the coastal sites. These mountains also offer a Mediterranean climate but winters include below freezing temperatures and most precipitation arrives as snowfall. Sporocarps were collected at least 10 meters apart to ensure selection of different genets (Bergemann & Miller, 2002, Dahlberg & Stenlid, 1994), with some mountain individuals separated by up to 30 miles and coastal individuals separated by over 50 miles. The coastal and mountain collection sites are separated by dry, non-forested and inhospitable Californian Central Valley habitat.

We obtained pure cultures from each individual by placing a piece of each sporocarp in full strength Modified Melin-Norkrans (MMN) agar plates. Pure mycelium was harvested after no more than two weeks of growth, flash frozen in liquid N, and stored at -80C. Genomic DNA was obtained for each isolate using a CTAB-based extraction protocol. Genomic libraries were generated with Apollo 324 NGS Library Prep System (Wafergen Biosystems) and samples were sequenced in 3 lanes of Illumina HiSeq2000, 100bp paired-end with a 350bp insert size at the QB3 Vincent J. Coates Genomics

Sequencing Laboratory at the University of California, Berkeley. The raw reads were deposited in the Short Read Archive and the accession numbers are listed in Table S1. One mountain isolate (Sb2) was selected as the reference genome and deposited in the CBS Collection of Fungi, with accession number CBS 139023. DNA was extracted as above, however library preparation, sequencing, genome assembly and annotation were carried out by the US DOE Joint Genome Institute under the 1000 Fungal Genomes project (Grigoriev *et al.*, 2013). The genome was sequenced using Illumina HiSeq platform. Paired-end reads from 270bp insert size standard Illumina library were filtered for artifact/process contamination and subsequently assembled as a diploid using AllPathsLG release version R43839 (Gnerre *et al.*, 2011). The final assembly of 1550 scaffolds has an N50 of 159 kbp and an L50 of 83. The assembly was repeat-masked and annotated using the JGI Annotation Pipeline (Kuo *et al.*, 2014). Briefly, the Pipeline computes multiple gene models at each locus using a variety of RNASeq-based, protein-based, and *ab initio* gene predictors, characterizes those models by predicting functional domains and signal peptides, and selects the highest quality model at each locus based on expression, similarity to known proteins, and domain content. In addition, the Pipeline compares the draft gene set to those of other genomes and builds a draft set of gene families. Finally, the Pipeline exports the assembly and annotation to an interactive Portal in MycoCosm (Grigoriev *et al.*, 2013). The *Suillus brevipes* Sb2 Portal can be found at http://genome.jgi.doe.gov/Suillus_brevipes/

Read filtering, mapping and variant calling

Isolate re-sequencing produced over 60 million sequences. Reads were filtered based on quality and all duplicates and reads with more than 10% Ns, and with at least one base with phred score smaller than 20 were discarded. Read pairs were re-grouped and orphan reads were also discarded. All good quality paired reads were then aligned to the reference genome using Bowtie2. 2.1.0 (Langmead & Salzberg, 2012). Alignments were sorted with SAMtools (Li *et al.*, 2009) and duplicate and poor quality alignments removed with Picard (<http://picard.sourceforge.net>) and BamTools (Barnett *et al.*, 2011). Realignment, indexing and SNP calling was performed with the Genome Analyses Toolkit (GATK; McKenna *et al.*, 2010), using unified genotyper. We discarded singletons, poor quality SNPs (<30) and SNPs including Ns using VCFtools (Danecek *et al.*, 2011) and in-house scripts. Per individual genome coverage was calculated using GATK.

Population structure

Population structure was inferred using both clustering and phylogenetic inference. We used 567192 SNPs (excluding singletons) to compute a neighbor-joining tree with PHYLIP (Felsenstein, 2002) including all the *S. brevipes* isolates. We also used FRAPPE to compute global ancestry from maximum likelihood estimates of model parameters (Tang *et al.*, 2005) and assess population structure. Two coastal isolates, Sb7 and Sb10, were collected just 10m apart in Mendocino. These individuals shared 97% SNPs, so we considered them to be clones and removed Sb7 from further analyses.

Polymorphism and genome scans for divergence

Gene and genome wide polymorphism were assessed using egglib (Mita & Siol, 2012) and in-house scripts. We calculated the average 5kb window and gene number of segregating sites and the whole genome and genic nucleotide diversity (π), including silent and non-silent π in each population. We also calculated Tajima's D to test for neutrality and Zeng's E to compare for high- and low-frequency variants (Zeng *et al.*, 2006). In addition, we calculated the average number of nucleotide substitutions per site between populations (Dxy) for 5Kb windows and measured population differentiation due to genetic structure by computing the average genome-wide Weir Cockerham's Fst and average Fst per 5Kb and 1Kb windows with VCFtools (Danecek *et al.*, 2011). We calculated fixed and exclusive differences as well as expected and observed heterozygosity across the two populations with in-house scripts.

Demography

Population demography was analyzed using a diffusion-based approach implemented in the program $\partial a \partial i$ (Gutenkunst *et al.*, 2009). Demographic parameters were estimated from the joint mountain and coastal *S. brevipes* allele frequency spectra polarized with the *S. luteus* genome sequence. Given the risk for potential misidentification of ancestral states, we also fitted the model using folded allele frequency spectra. Conventional bootstrapping (100 replicates) was calculated to estimate parameter uncertainties.

Natural selection

To detect signals of positive selection we identified outlier Dxy and Fst values within each population, performed McDonald Kreitman tests and computed a selective sweep analysis. We selected the top 1% Dxy values for 5Kb windows and top 0.1% Fst values for 5Kb and 1Kb windows to search for differentiated regions across the two populations and respective genes. The functions of these highly differentiated genes were inferred by retrieving gene ontology categories from MycoCosm (Grigoriev *et al.*, 2013).

We also identified genes showing an excess of amino acid substitutions using the MacDonal-Kreitman test (McDonald & Kreitman, 1991), with *S. luteus* as the outgroup. We filtered the results to exclude genes that showed limited variability and corrected for multiple hypothesis testing using the Benjamini-Hochberg method (Benjamini & Hochberg, 1995).

We searched for selective sweeps by measuring haplotype homozygosity using an in-house script (Wilson & Messer, unpublished). This program calculates an H statistics to detect hard and soft sweeps by measuring the length of pairwise homozygosity stretches in a population sample generated by elevated linkage disequilibrium around adaptive sites.

Results

Suillus brevipes genomes

The *S. brevipes* reference genome (obtained from Sb2, a mountain individual included in this study) was assembled into 1550 scaffolds totaling 51.7 Mbp and annotated. The 22,453 predicted genes, represent 2313 distinct protein domains, and

cluster into 2844 multigene families. Details on the genome assembly and annotation can be found in Table S2.

The average sequence coverage per individual varied from 7X to 26X (Table S1). We recovered a total of 1254275 single nucleotide polymorphisms (SNPs) across the 28 *S. brevipes* genomes, with 2.4% of the genome as variable sites.

Population subdivision

Using a neighbor-joining approach we found two distinct and highly supported *Suillus brevipes* montane and coastal populations (Fig. 1a). The same two populations were found with a maximum likelihood population structure analysis using FRAPPE, which emphasized the shared ancestry of the two populations (Fig. 1b). All individuals collected from montane Yosemite National Park clustered together, and individuals from coastal Mendocino, Point Reyes, and Salt Point formed another cluster with no evidence of geographic substructure.

Genomic diversity, differentiation and divergence

Genomic summary statistics were estimated using 5kb sliding windows (6387 windows for the mountain population and 6301 for the coast population) and the genic regions (22453 genes) (Table 1). The two populations presented comparable levels of genetic variation, and each population showed close to 2% polymorphism across the genome and 0.2% in the genic regions. The average observed heterozygosity was similar in both populations ($H_{oCoast} = 0.32 \pm 0.19$, $H_{oMountain} = 0.28 \pm 0.18$), and only slightly higher than the heterozygosity expected under Hardy Weinberg equilibrium ($H_{eCoast} =$

0.20 $\pm$ 0.21; $H_{e\text{Mountain}} = 0.21 \pm 0.21$). Nucleotide diversity (π) was also similar between coast and mountain (Table 1). Tajima's D was slightly negative, indicating a genome-wide excess of low frequency polymorphism compared to neutral expectations, however this was not observed when including only genic regions (Table 1). Zeng's E statistics, which measures positive selection, was negative in both coastal and montane populations, indicating an excess of high frequency derived polymorphisms in both populations. In fact, testing the fit between the observed site frequency spectra and spectra generated with a constant size model using the PYTHON package *∂a∂i* (Gutenkunst *et al.*, 2009) detected departures from the standard neutral model in both populations (mountain χ^2 , d.f. = 32, $P = 0$; coast χ^2 , d.f. = 20, $P = 0$).

Most of the SNPs were shared between the two populations (44%) and only 155 (0.01%) of the sites were reciprocally fixed for different alleles in the two populations. The remaining SNPs were variable in one population and fixed in the other (Table 2). This distribution of polymorphism and divergence suggests a recent split between the two populations, the prevalence of gene flow, or both. The mean pairwise nucleotide difference between populations (D_{xy}) for 5kb windows was 0.008, consistent with the results of the clustering analyses.

Demography

Demographic inference of coastal and montane populations indicated isolation (absence of gene flow) between the mountain and coastal populations as the most likely demographic model (Table 3). Inference using folded site frequency spectra also pointed to the isolation model as the best fit to the data (isolation likelihood = -32595.1;

migration likelihood = -239130.9), suggesting that model choice was not biased by multiple mutations along the outgroup branch.

The maximum-likelihood parameter estimates for the unfolded isolation model are reported in Table 4. The coastal population is estimated to have a smaller effective population size compared to the montane population and the estimated divergence time is 25000 generations. Even though this is the most likely of all tested demographic models, the unfolded modeled site frequency spectrum did not fit the observed data well (chisquare test mountain: d.f. = 33, $P = 0$; chisquare test coast: d.f. = 21, $P = 0$). The poor fit was due to a deficit of high frequency derived alleles in simulated site frequency spectra compared to the observed dataset (Fig S1).

Genome scans for natural selection

In order to test for natural selection, we investigated genomic regions with a high proportion of reciprocally fixed SNPs, regions identified as outliers for Dxy and Fst (Noor & Bennett, 2009, Cruickshank & Hahn, 2014). We also used McDonald-Kreitman tests and selective sweep analysis to identify signals of positive selection across the genome.

Out of the detected 155 reciprocally fixed differences, 30 were in genic regions. Scaffold 16 hosted 30% of these, with 5 non-synonymous fixed differences, and scaffold 12 included 20%, of which 4 were non-synonymous. Three of these genes had known functions. Scaffold 16 includes gene 669775, which codes for a protein kinase. Scaffold 12 includes three adjacent genes with six fixed differences. 826957 has no known function, 826959 codes for a cysteine-type endopeptidase and 826958 is involved in pH

regulation. The latter is invariable in the coastal individuals (Fig. 2) and codes for a Na^+/H^+ exchanger, which is a membrane protein involved in salt tolerance in *Sacchromyces cerevisiae* and *Arabidopsis* (Nass *et al.*, 1997, Apse *et al.*, 1999). The *S. cerevisiae* homolog *Nha1* encodes for a membrane protein involved in Na^+ and K^+ efflux needed for yeast to tolerate alkali cation tolerance at acidic pH (Bañuelos *et al.*, 1998). Gene 826958 will hereafter be referred to as *Nha1-like*.

We used Dxy outliers as an absolute divergence metric to scan the genome for regions under divergent selection (Noor & Bennett, 2009, Cruickshank & Hahn, 2014). Table S3 reports the genes located in the top 1% 5kb window Dxy values. These encode proteins with a wide variety of functions, with 20% involved in transporter activity. *Nha1-like* and flanking genes in scaffold 12 (coding for an endopeptidase and another unknown protein) were included in the Dxy outlier list.

We also investigated Fst across the genome. On average Fst values per SNP were low (average Fst = 0.053, s.d. = 0.115), as expected given the low level of differentiation between populations. Fst was also calculated for 5 Kb and 1Kb genomic sliding windows and compared to the list of Dxy outliers. The majority of windows showed low Fst values yet some regions had high relative divergence. Fig. 3 depicts the 1Kb window Fst values, showing 807 windows displaying Fst values above 0.5. We found 24 genes associated with the top 0.1% of 1kb windows with highest Fst values (Table 5). These are involved in a variety of cellular functions, namely transporter, catalytic, oxidoreductase activities and DNA binding. Four Dxy outliers were also on this list, including one gene in scaffold 66 and the three Dxy outlier genes in scaffold 12 (these were not listed as Fst outliers

when scanning the genomes with 5Kb windows). Gene *Nha1-like* fell in the window with the maximum F_{st} value ($F_{st} = 1$).

Using McDonald Kreitman tests, we found four genes displaying significant differences between ratios of non-synonymous to synonymous substitutions within and between species, which are consistent with positive selection (Table S4). These are involved in heterokaryon incompatibility (also listed as a Dxy outlier), carbohydrate metabolic processes, and post-translational processes in organelles.

We did not find signals of recent selective sweeps across the genome. We quantified haplotype homozygosity using an H statistics that measures the length of pairwise homozygosity stretches in a population sample generated by elevated linkage disequilibrium around adaptive sites (Wilson and Messer, unpublished). The highest H values are listed in Table S5 and show no evidence for selective sweeps.

Discussion

Our study revealed emerging differentiation of *Suillus brevipes* populations in two contrasting environments at a relatively small spatial scale. We found a very recent split between montane and coastal populations in California with no evidence for post-divergence migration, as indicated by low population divergence, the presence of very few reciprocally fixed differences and genetic isolation as the most likely demographic model. Despite the low overall genomic divergence, we detected a small set of highly differentiated and positively selected genes across the two populations. These results are consistent with the early stages of environmental adaptation.

Our results point to the existence of dispersal and/or colonization limitation in *Suillus brevipes*, which can result from constraints in physically moving spores or challenges in spore germination and mycelium persistence in new habitats. Although *Suillus* is known to be a prolific disperser, producing annual spore loads on the order of trillions of spores per km², spore loads decrease exponentially with distance from their source, rendering colonization of new host trees and establishment in new habitats less effective than expected (Peay *et al.*, 2012). This finding makes spore dispersal limitation a good candidate explanation for the lack of gene flow between coastal and montane populations in California. These habitats are separated by the Central Valley, which comprises an approximate 300 km non-forested gap that based on our results appears to represent an effective geographic barrier to migration between the coastal and montane populations of *S. brevipes*.

Despite demographic modeling pointing to two allopatric *S. brevipes* populations as the most likely demographic model, this model had a poor fit to the data due to an excess of high frequency derived alleles in both populations (Fig. S1). This pattern could be an artifact derived from misidentification of ancestral alleles or a true biological pattern, explained by more complex demographic scenarios (including bottlenecks) or genome-wide positive selection, such as selective sweeps (Ellison *et al.*, 2011, Caicedo *et al.*, 2007). We had access to a single outgroup (*S. luteus*, the sister species to *S. brevipes*), making it challenging to control for errors in calling ancestral alleles. However, results from demographic modeling without an outgroup (using a folded site frequency spectra) also pointed to absence of migration as the most probable scenario. Furthermore, we

failed to detect selective sweeps indicating genome-wide positive selection is probably not the cause for the non-monotonic observed site frequency spectra.

The few available studies investigating wild EM fungal population structure report mixed trends, ranging from panmixis to strong population differentiation (Douhan *et al.*, 2011). Microsatellite approaches conducted in several EM fungal species point to examples of lack of population structure, extensive gene flow and little genetic differentiation both at local and regional spatial scales (Zhou *et al.*, 2001, Kretzer *et al.*, 2005, Bergemann *et al.*, 2006, Muller *et al.*, 2007, Roy *et al.*, 2008, Rivera *et al.*, 2014). However, most of these studies were based on a very small number of markers that might have been insufficient to detect emerging population differentiation. In fact, several other examples point to high levels of population differentiation in EM fungi (Bergemann & Miller, 2002, Grubisha *et al.*, 2007, Carriconde *et al.*, 2008, Amend *et al.*, 2010, Dunham *et al.*, 2013). In this regard, population genomics of the saprobe *Neurospora*, detected structure hidden in microsatellite analysis (Ellison *et al.*, 2011). Unfortunately, the causes underlying population differentiation are not always clear.

The few highly differentiated genomic regions are consistent with the very early steps in local adaptation in *Suillus brevipes*. In particular, the reduced nucleotide diversity among coastal individuals at the *NhaI-like* gene is consistent with recent positive selection in the coastal population, making it tempting to hypothesize that the coastal allele of this gene spread by natural selection for adaptation to the stress of saline soil. Consistent with this hypothesis, soil analyses from at least the Point Reyes area confirm high Na content, with an average of 170 ppm, and acidic pH (average pH = 5.1; Peay *et al.*, 2010). Basidiomycetes are in general relatively salt-intolerant and saline soils are

known to host poorly diverse EM fungal communities (Schechter & Branco, 2014 and references therein). Further research will be needed to fully understand the role of gene *Nha1-like* for salt tolerance in *Suillus brevipes*.

Our study is one of the few available fungal population genomic studies so far and provides the earliest example of divergence and emergence of environmentally adapted fungal populations. Other studies also reported evidence of adaptive evolution, revealing more complex patterns of genomic differentiation, demographics and positive selection. A pioneer population genomics study on wild and domesticated yeasts (*Saccharomyces cerevisiae* and *S. paradoxus*) found worldwide population differentiation correlated to phenotypic variation (Liti *et al.*, 2009). Ellison *et al.*, 2011 found evidence of cryptic population differentiation and local adaptation in *Neurospora crassa*. Their genome scans revealed distinct populations at different latitudes, with up to 9% genomic divergence, asymmetrical migration and areas of genomic differentiation including genes related to temperature and circadian rhythm, showing environmental factors shape fungal evolution. *Batrachochytrium dendrobatidis*, the amphibian-killing chytrid fungus, was found to include high genetic variation across populations and a complex evolutionary history, including differences in chromosome copy numbers and heterozygosity across isolates as well as signatures of selection in genes involved in amphibian skin infection (Rosenblum *et al.*, 2013). Whole genome data has also revealed population structure, strong genomic divergence (~50%) and signals of introgression in the human pathogens *Coccidioides immitis* and *C. posadasii* (Neafsey *et al.*, 2010). Comparing to these studies, *Suillus brevipes* is an example of a much more recent population split, as demonstrated

by the very low amount of reciprocally fixed alleles (0.01%), low Dxy and low overall Fst.

This study documents the first steps of fungal population differentiation, reporting the most recent split between fungal populations to date. Our results corroborate the existence of dispersal limitation in fungi and show positive selection associated with abiotic factors as a driver for population differentiation in fungi, including a gene involved in salt tolerance not previously hypothesized to be relevant in EM fungi. Further functional tests can clarify the importance of this gene and shed light on the genetic basis of adaptive traits in fungi. Furthermore, a broader geographic sampling, including samples from other environments will aid in understanding the evolution of *S. brevipes* in particular and of EM fungi in general.

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Figures and figure legends:

Figure 1 – Evidence for population structure between the coastal and montane *Suillus brevipes* populations. a) Neighbor-joining tree based on 567192 SNPs after singleton exclusion; green branches depict individuals from the mountains (Yosemite National Park, CA) and blue branches refer to coastal individuals (Sb20 was collected from Pt Reyes, Sb21 from Salt Point, and all others from Mendocino Co, CA); Sb7 and Sb10 are clones. * indicates bootstrap support values of 100. b) FRAPPE diagrams showing genetic differentiation between coastal and montane individuals.

Figure 2 – Genomic regions of divergence genotype matrices. Columns depict polymorphic sites and rows the genotypes for the different individuals. The shaded areas represent flanking regions surrounding the divergence outliers and non-colored bases are heterozygous sites. The location of introns is not shown. Individuals are grouped by population of origin (coast and mountain). The matrix shows three adjacent genes including 20% of genic reciprocally fixed SNPs in scaffold 12. 826959 codes for a cysteine-type endopeptidase, *Nha1-like* (826958) for a Na^+/H^+ exchanger, and 826957 has an unknown function. These three genes are both Dxy and Fst outliers (see text for details).

Figure 3 – Average Fst values for 1kb windows across the *S. brevipes* genome.

Tables

Table 1 - Genomic summary statistics for the montane and coastal *Suillus brevipes* populations with number of segregating sites, nucleotide diversity (π), π for silent sites, Tajima's D, and Zeng's E for 5Kb genomic windows and genes.

	Mountain (5kb windows)	Mountain (genes)	Coast (5kb windows)	Coast (genes)
Segregating sites	52.2	10.7	48.8	12.7
π	2.7×10^{-3}	2.1×10^{-3}	3.2×10^{-3}	2.0×10^{-3}
π Silent	-	5.3×10^{-3}	-	5.0×10^{-3}
π non-Silent	-	1.2×10^{-3}	-	1.1×10^{-3}
Tajima's D	-0.15	0.31	-0.4	0.36
Zeng's E	-	-2.01	-	-1.94

Table 2 – Number and percentage of polymorphic and fixed SNP between the coastal and mountain populations. (Total number of SNPs = 1254275).

	Number of SNP	Percentage
Fixed in both populations	155	0.01%
Polymorphic in both populations	701640	44.0%
Fixed in mountain only	410696	32.7%
Fixed in coast only	141784	11.3%

Table 3 - Optimized log-likelihoods for demographic models as provided by $\partial a \partial i$ (using the unfolded site frequency spectra). Tested models included isolation, migration, migration and exponential growth, migration with two epochs, and migration with two epochs and exponential growth. The isolation model is the most likely model and is highlighted in bold.

Model	Optimized log-likelihood	Theta
Isolation	-32595.1	135137.6

Migration	-76454.7	112079.5
Migration/Exponential	-62192.6	17137.5
Migration 2 epochs	-141888.1	20751.7
Migration 2 epochs/ Exponential	-117173.2	21888.5

Table 4 - Maximum-likelihood demographic parameter estimates for the isolation model to the joint allele frequency spectrum for the mountain and coastal *S. brevipes* populations, with respective means and standard deviation (SD). $\mu=10^{-9}$; divergence time in generations.

Parameter	No Migration model		
	Estimate	Mean	SD
Ancestral N_e	6.53×10^5	4.1×10^5	608.1
Mountain N_e after split	3.87×10^5	2.3×10^5	3824.1
Coast N_e after split	1.16×10^5	7.3×10^4	1353.9
Divergence time	2.5×10^4	1.6×10^4	291.5

Table 5 – 1kb window Fst gene 0.1% outliers, with localization in genome and protein function (with respective description) if available.

Scaffold	Protein ID	Function	Description	Obs
2	763737	Translation elongation factor		
2	948321	Proton-transporting ATPase activity, rotational mechanism		
4	713130	Transporter activity	MFS general substrate transporter	
8	814720	Purine ribonucleoside monophosphate biosynthetic process	Adenosine/AMP deaminase	
8	853122	GTPase activity	Translation elongation factor EFG/EF2, C-terminal	
8	814728	Catalytic activity	Alcohol dehydrogenase, zinc-binding	
9	860915	Sequence-specific DNA binding transcription factor activity	Zinc finger, GATA-type	

12	826957	-		Dxy outlier; gene with fixed differences
12	826958	Regulation of pH, solute:hydrogen antiporter	Sodium/hydrogen exchanger	Dxy outlier; gene with fixed differences
12	826959	Cysteine-type endopeptidase	Peptidase C1B, bleomycin hydrolase	Dxy outlier; gene with fixed differences
14	718701	Glucose-6-phosphate dehydrogenase		
16	795305	Nucleotide binding, nucleotide-excision repair		
22	862126	Oxidoreductase activity	Pyridine nucleotide- disulphide oxidoreductase, class I	
32	830321	tRNA processing		
32	830324	Oxidoreductase activity	Aldo/keto reductase	
41	831287	Catalytic activity	Phospholipase D/Transphosphatidylase	
41	855962	Protein modification process	Ubiquitin	
42	831382	Catalytic activity	Serralysin-like metalloprotease, C- terminal	
63	733034	Transporter activity	MFS general substrate transporter	
66	804723	L-serine ammonia- lyase activity	Iron-sulphur-dependent L-serine dehydratase single chain form	Dxy outlier
66	779351	DNA ligase (ATP) activity, DNA replication, DNA repair		
66	733526	Nucleic acid binding, zinc ion binding		
85	834749	Two-component response regulator activity		

123 783492 Transferase activity
nitrogenous groups

Data Accessibility

DNA sequences : Short Read Archive accessions in table S1

Suillus brevipes reference genome and annotation:

http://genome.jgi.doe.gov/Suillus_brevipes/

S. brevipes raw single nucleotide polymorphism list : Dryad doi XXXXX

S. brevipes neighbor-joining tree : Dryad doi XXXXX

Author Contributions

SB, TDB and JWT designed the study and collected samples. SB, PG and CEE performed analyses. AK, KL, AL and IVG assembled and annotated the *Suillus brevipes* reference genome. SB, PG, CEE, H-L L, RV, KGP, JWT and TDB wrote the paper.

Supporting Information

Table S1 - *Suillus brevipes* isolate location, habitat, pine host, coordinates and elevation, number of raw and high quality reads, % alignment rate, genome coverage, and Short Read Archive accession numbers. * Sb2 was the isolate used to generate the reference genome for the species (<http://genome.jgi-psf.org/Suibr1/Suibr1.home.html>). See text for details.

Table S2 - 5kb window Dxy gene 1% outliers, with localization in genome and protein function and description. Genes in scaffold 12 include 20% of the reciprocally fixed differences falling in genic regions and gene 828720 was found to under positive selection by McDonald Kreitman test.

Table S3 - Genes under positive selection as inferred by McDonald-Kreitman tests on genes using *S. luteus* as the outgroup. *P* values were adjusted using the Benjamini-Hochberg correction.

Table S4 – Selective sweep analysis; top five H value peaks for coastal and mountain *Suillus brevipes* populations.

Figure S1 – Observed and estimated site frequency spectra under the isolation model from coastal and montane *Suillus brevipes* populations.