## Detailed Materials and Methods

## Sampling

From each population, pieces of about 8 mm diameter were collected from 100 thalli with sterile tools and transferred into sterile 2 ml tubes. The samples from mount Limbara (Sardinia, Italy) and Sierra de Gredos (Spain) were collected under the framework of Dal Grande et al. (2017) and Dal Grande et al. (2018), respectively. These studies performed extensive sampling of *U. pustulata* across the elevation gradients, collecting six populations from each gradient. The authors found that mid-elevation populations were admixed and comprised individuals from both, the warm-adapted and cold-adapted ecotypes. For the present study we thus selected only the populations from the highest and lowest site of each gradient to avoid potentially admixed populations (Fig. 1A).

## DNA extraction and genome sequencing

DNA was extracted from all the samples using a CTAB-based method (Cubero and Crespo, 2002). DNA concentration was measured with a Qubit fluorometer (dsDNA BR, Invitrogen). After extraction, equal amounts of DNA from each sample from a population were pooled. Library preparation (~400 bp insert size), sequencing on an Illumina HiSeq2000 with 100 bp paired-end chemistry at ~90x coverage per population, as well as tags and adaptor removal were performed by GenXPro GmbH (Frankfurt am Main, Germany).

**Homologous BGCs between warm and cold ecotype**

Homologous clusters were identified by performing reciprocal BLASTs between the BGCs inferred from the reference genomes of Mediterranean and cold-temperate *U. pustulata*. Homology between clusters was visualized via synteny plots using Easyfig v2.2.3 (Sullivan *et al.*, 2011). The GBK input files for Easyfig were generated with seqkit v0.10.1(Shen *et al.*, 2016) and the seqret tool from EMBOSS v6.6.0.0 (Rice *et al.*, 2000). Easyfig was run with tblastx v2.6.0+, a minimum identity value of 90 and a minimum length of 50 to draw the blast hits (Kjærbølling *et al.*, 2018). Clusters were manually matched for direction and organized to have the core gene with the same orientation.

Verification of presence/absence patterns

To map the Pool-seq reads against each reference genome we used Bowtie 2 v2.3.4.3 (Langmead and Salzberg, 2012) (details of the additional Pool-seq assemblies used only for the verification of presence absence pattern is given in Supporting Information Table S7). We performed “very sensitive local” alignments and reported only paired-end alignments (--*no-mixed*). The number of aligned reads was inferred using samtools flagstat v1.9 (Li *et al.*, 2009). For each Pool-seq dataset we report the number of reads that aligned against the gene of interest, i.e., core PKS gene of the BGC unique to a climate group (Table 2). For each Pool-seq dataset we report the number of mapped reads and its percentage on a total dataset basis as well as the mapping percentage ratio between Mediterranean and cold-temperate populations (Supporting Information Table S7).

## SNP analysis

We reconstructed the “meta-population” mycobiont genomes following the procedure described in Meiser et al. (2017). Briefly, we used SPAdes v3.12 with the flag “meta” for metagenomes and k-mer lengths of 21,33,55,77 (Bankevich *et al.*, 2012). We then ran DIAMOND v0.9.25 BLASTx in “more-sensitive” mode for longer sequences and a default e-value cut-off of 0.001 against the NCBI Genbank nr protein database (downloaded in August 2019). We parsed the results with MEGAN6 v.6.18.1 with max expected set to 1E-10 and using the weighted lowest common ancestor (LCA) algorithm (Huson *et al.*, 2016). Finally, we exported all contigs assigned to Ascomycota as representing the *U. pustulata* mycobiont. The candidate genes were searched in each reconstructed mycobiont genome using BLASTn v2.2.30. For each BLASTn search we reported the highest bit score and the coverage of the related scaffold.

We first filtered out reads shorter than 80 bp, reads with N's, and reads with average base quality scores less than 26 along with their pairs using FastQFS. Trimmed paired-end reads of each pool were mapped to the reference *U. pustulata* genome using BWA-MEM and default parameters (Li and Durbin, 2009). Unambiguously aligned reads with a minimum mapping quality of 20 were extracted using SAMtools v1.18 (H. Li *et al.*, 2009). Reads were sorted and duplicates were marked with Picard using the tools SortSam.jar and MarkDuplicates.jar. Single nucleotide polymorphisms were then called with SAMtools (mpileup, (R. Li *et al.*, 2009)). Indels were detected and masked with PoPoolation2 (Kofler, Orozco-terWengel, *et al.*, 2011) using the scripts identify-genomic-indel-regions.pl (--min-count 2 --indel-window 5) and filter-pileup-by-gtf.pl. During the conversion of the synchronized file into a gene-based synchronized file we extracted the SNPs present in the coding BGC regions (BGC-SNPs). For this we used the script create-genewise-sync.pl in PoPoolation2 (Kofler, Pandey, *et al.*, 2011) and a gff file with the genomic coordinates of all coding (CDS) BGC positions. The coverage for each population was reduced to a uniform coverage of 30 with PoPoolation2 using the resulting sync-file and the script subsample-synchronized.pl (--without-replacement, excluding the positions that exceeded the top 2% coverage.).

**Identifying functionally deleterious SNPs: PROVEAN**

We used PROVEAN to distinguish non-synonymous SNPs which were functionally deleterious from functionally/structurally neutral SNPs. PROVEAN is a species- and gene- independent software (<http://provean.jcvi.org/index.php>) which utilizes protein sequence conservation as well as the molecular effects, such as protein stability, solubility, function, and interactions, to quantify the deleterious effect of an amino acid substitution in form of PROVEAN score. The default threshold is -2.5, and a score ≤ -2.5 suggests deleterious variation whereas a score ≥ -2.5 is considered a neutral variation. In our analysis, we translated aforementioned CDS sequences for each population into amino acid sequences. We first used the protein sequence from the highland populations as reference and inferred the PROVEAN score for all the non-synonymous SNPs present in the low altitude populations. We then repeated the same procedure, but taking the low altitude protein sequence as reference and inferring the score of all the variant alleles in the highland populations. To increase the confidence in detecting the deleterious variants and reduce potentially false positives we used a stricter threshold (-4.0), and considered the score ≤ -4.0 as deleterious and ≥ -4.0 as neutral.

## HPLC analysis

Small thallus pieces were extracted for 1 hour at room temperature in 200 µl of methanol. Of this, 150 µl of the extract were centrifuged 1 min at 800 rpm through a Pall Acroprep Advance 0.2 μm polytetrafluoroethylene filter plate and diluted 10-fold with methanol. The samples were analyzed on an Agilent 1260 quaternary system with a quaternary pump, an incorporated degasser and using an Agilent Poroshell 120 EC-C18 column (2.7 µm, 3.0 x 50 mm). Substances were separated at 30°C using two solvent systems and a flow rate of 1.4 ml/min. Solvent A is aqua bidest, 30% methanol and 0.0658% trifluoroacetic acid, and solvent B is 100% methanol. The HPLC system was equilibrated to solvent A for 2 min and 2µl of extract was injected automatically after a needle wash. The runs continued isocratically for 0.18 min, solvent B was increased to 58% within 5 min, then increased to 100% within the next 5 min and isocratically maintained for 0.82 min. The runs ended with solvent A being increased back to 100% within 0.5 min. After the run the column was flushed for two minutes before the next run. Compounds were detected with a diode array detector (DAD) at 210, 254, 280 and 310 nm. The retention times and spectra (λ = 190-650 nm with 2 nm steps) were compared against a library of authentic products derived under identical conditions using the Agilent OpenLAB CDS ChemStation software. To find if the proportion of the compounds varied among Mediterranean and cold-temperate samples, we estimated relative amount of the compounds by accessing peak area of a compound at 254 nm as compared to the total peak area for all the compound in each sample (Seriña *et al.*, 1996).

UPLC- high resolution mass spectrometry analysis

ACN with 0.1% formic acid in H2O was used as solvent. The flow rate was set at 0.4 mL min-1 with a gradient from 5% ACN to 95% ACN over 15 min. The mass spectrometer was calibrated using 10 mM sodium formate before data acquisition. The MS method used for data acquisition also included an internal calibrant window before the data acquisition of each biological sample where 10 mM sodium formate were injected. The internal calibrant was used by Bruker DataAnalysis to correct the acquired mass data. The following MS settings were used for data acquisition: source settings: capillary voltage 4500 V, nebulizer nitrogen gas pressure 3 bar, ion source temperature 200 °C, dry gas flow 8 L min-1; scan settings: ion polarity positive, mass range 90 to 1500, spectra rate 3 Hz; tune parameters: transfer funnel 1 RF 300 Vpp, funnel 2 RF 300 Vpp, isCID off, hexapole RF 60 Vpp; stepping settings: (i) 0 ms, collision RF 500, transfer time 82.5 ms, collision energy 65%; (ii) 25 ms, collision RF 500, transfer time 82.5 ms, collision energy 85%; (iii) 45 ms, collision RF 500, transfer time 82.5 ms, collision energy 100%; (iv) 75 ms, collision RF 500, transfer time 82.5 ms, collision energy 130%; MS/MS settings: 8 precursor ions, threshold 1000 counts (absolute), activated active exclusion after 3 spectra and 0.5 min release time, active precursor reconsidering factor 4, smart exclusion 2 times.

GNPS Network Analysis

The raw MS-data acquired in the previous step was converted to .mzXML format using DataAnalysis 4.3 (Bruker). MS2-based network analysis was performed using the Molecular Networking workflow of the GNPS platform (Wang *et al.*, 2016). A molecular network was created using the online workflow at GNPS. The data was then clustered with MS-Cluster with a parent mass tolerance of 0.05 Da and a MS/MS fragment ion tolerance of 0.01 Da to create consensus spectra. Further, consensus spectra that contained less than 2 spectra were discarded. A network was then created where edges were filtered to have a cosine score above 0.7 and more than 6 matched peaks. Further edges between two nodes were kept in the network if and only if each of the nodes appeared in each other's respective top 7 most similar nodes. The spectra in the network were then searched against GNPS' spectral libraries. All matches kept between network spectra and library spectra were required to have a score above 0.7 and at least 6 matched peaks. The nodes were separated in three groups: specific to cold-adapted, specific to warm-adapted and not specific to either. Only subnetworks specific to one climate group containing at least 2 nodes were analyzed. False positives were excluded from the analysis after comparison of single spectra.

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