Genic introgression from an invasive exotic fungal forest pathogen increases the establishment potential of a sibling native pathogen

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Abstract

Significant hybridization between the invasive North American fungal plant pathogen Heterobasidion irregulare and its Eurasian sister species H. annosum is ongoing in Italy. Whole genomes of nine natural hybrids were sequenced, assembled and compared with those of three genotypes each of the two parental species. Genetic relationships among hybrids and their level of admixture were determined. A multi-approach pipeline was used to assign introgressed genomic blocks to each of the two species. Alleles that introgressed from H. irregulare to H. annosum were associated with pathways putatively related to saprobic processes, while alleles that introgressed from the native into the exotic species were mainly linked to gene regulation. There was no overlap of allele categories introgressed in the two directions. Phenotypic experiments documented a fitness increase in H. annosum genotypes characterized by introgression of alleles from the invasive species, supporting the hypothesis that hybridization results in putatively adaptive introgression. Conversely, introgression from the native into the exotic species appeared to be driven by selection on genes favoring genome stability. Since the introgression of specific alleles from the exotic H. irregulare into the native H. annosum increased the invasiveness of the latter species, we propose that two invasions may be co-occurring: the first one by genotypes of the exotic species, and the second one by alleles belonging to the exotic species. Given that H. irregulare represents a threat to European forests, monitoring programs need to track not only exotic genotypes in native forest stands, but also exotic alleles introgressed in native genotypes.

* These authors equally contributed to this work.
Keywords
Biological invasions, fungi, *Heterobasidion*, hybridization

Introduction

Introgression, i.e. the exchange of genetic material between interfertile species through hybridization, is recognized as a significant catalyst of evolution of eukaryotes (Arnold et al. 2008; Edelman et al. 2019). Thanks to increasing world trade, to advances in genetic characterization and to the democratization of genomic research technologies, the frequency and estimates of hybridization events across all phyla have risen exponentially in recent years (Rieseberg et al. 2000; Barton 2001; Seehausen 2004; Mallet 2005; Le Gac and Giraud 2008; Dasmahapatra et al. 2012; Clarkson et al. 2014).

Although the interspecific transfer of genetic material can be a stochastic process not necessarily involving genes responsible for phenotypic adaptive variation (Suarez-Gonzalez et al. 2018), there are many cases in which introgressive events have been documented to provide novel genes and alleles or to generate favorable allelic combinations that confer adaptive advantages to the recipient organism (Suarez-Gonzalez et al. 2018). Introgression may also increase the fitness of hybrids, allowing them to be competitive against parental species, particularly in novel ecological niches (Mallet 2007), thus facilitating successful invasions (Schierenbeck and Ellstrand 2009; Lee 2002). In plants, it has been well documented that hybridization may result in evolutionary changes increasing invasiveness (Schierenbeck and Ellstrand 2009; Abbott et al. 2003). It is also recognized that hybridization can lead to the emergence of new pathogenic fungal species (Brasier 2000, 2001; Fisher et al. 2012; Giraud et al. 2008; Gladieux et al. 2014; Stuckenbrock 2016).

Stuckenbrock (2016) suggested that studies integrating genomic and experimental data are pivotal to understand the evolution of fungal hybrids in nature. Whereas fungal hybrid genotypes can be generated and studied in vitro (Olson and Stenlid 2001; Giordano et al. 2018), hybrids resulting from natural selection are difficult to find in nature, given that only a few cases of ongoing hybridization are known (Garbelotto et al. 1996; Gonthier and Garbelotto 2011; Hughes et al. 2013; Pryszcz et al. 2014; Menardo et al. 2016; Sillo et al. 2019).

The fungus *Heterobasidion irregulare* Garbel. & Otrosina is a pathogen of pines in North America that was accidentally introduced into central Italy in 1944, during World War II (Gonthier et al. 2004). The pathogen has become invasive, (Gonthier et al. 2014), and is now included in the A2 list of organisms recommended for regulation by the European and Mediterranean Plant Protection Organization (EPPO 2015). In Central Italy, where *H. irregulare* has been reported so far, its Eurasian sister species *H. annosum* (Fr.) Bref. is also present; however the invasive exotic species is significantly more abundant than the native one (Gonthier et al. 2007). The invasiveness of the exotic species has been experimentally shown to depend not on pathogenicity, which is similar between the two species (Garbelotto et al. 2010), but on its greater ability...
to saprobically colonize wood and to produce more fruiting bodies, thus increasing its sporulation rate compared to the native sister species (Giordano et al. 2014). Primary infection by *Heterobasidion* is indeed effected by basidiospores colonizing exposed woody surfaces of stumps and wind-thrown trees (Garbelotto and Gonthier 2013), hence an enhanced ability to saprobically colonize wood would confer significant advantages to individuals carrying them. Genomic analyses have corroborated the above hypothesis by showing that genes involved in saprobic growth are significantly more divergent between the two species than expected by genetic drift, while, on the contrary, genes involved in pathogenicity appear to be rather conserved between the two, having undergone purifying selection (Sillo et al. 2015). A recent phenotypic and transcriptomic characterization of artificial *Heterobasidion* hybrids has also determined that *H. irregulare* nuclear genes related to nucleus-mitochondrion communication may confer an advantage to hybrid genotypes compared to *H. annosum* genes involved in the same function (Giordano et al. 2018).

The two species have evolved allopatrically for 34–41 millions of years, but have remained interfertile (Garbelotto and Gonthier 2013). Their recent sympatry has resulted in one of the few known current examples of fungal hybrid swarms (Gonthier and Garbelotto 2011). A study of over 260 genotypes from the hybrid zone in Italy using AFLP and multi gene phylogenies has indicated that, depending on site, up to 42% of the genotypes had an admixed genome, with levels of genomic admixing varying between 5% and 45% (Gonthier and Garbelotto 2011). Sequencing of 11 loci has indicated that in 85% of cases, introgression involved alleles of one species being absorbed by the other, while intragenic recombination was detected only in 15% of cases (Gonthier and Garbelotto 2011). Thus, it is expected that the introgression of whole alleles, and not recombination, may be the dominant process reshaping genomes through hybridization between *Heterobasidion* species. This study may confirm or disprove such expectations.

Two studies using different genetic markers (Gonthier and Garbelotto 2011; Garbelotto et al. 2013) have shown that there is no significant genetic structuring within the invasive and the native species (Gonthier et al. 2014, 2015). Thus, despite the relatively large area of the hybrid zone, it can be assumed that a single population of *H. irregulare* is hybridizing with a single population of *H. annosum*. The lack of population structure further simplifies assignment of alleles to either species, given that a genome-wide comparative study has determined that interspecific DNA divergence between the two taxa is high and ranges between 20.0 and 40.0 SNPs/Kbp, while intraspecific divergence within either species ranges only between 1.0 and 4.0 SNPs/Kbp (Sillo et al. 2015).

The model system represented by these interbreeding species with a comparable biology and epidemiology provides a unique opportunity to study the genomics of hybridization and introgression in a natural habitat. The main overarching goal of this study was to investigate a fungal invasion at the gene, rather than at the species level (Petit 2004). Theory predicts that the number of introgression events should be significantly larger from the native into the exotic species, if the latter is in demographic
expansion (Currat et al. 2008; Excoffier et al. 2009). Despite its relatively recent introduction, the exotic fungus is overwhelmingly dominant and its geographic range is increasing, hence, it can be safely assumed that it is in demographic expansion. Our first prediction, thus, is that the number of chromosomal blocks introgressed from the native into the exotic species should be larger than the number of chromosomal blocks introgressed from the exotic into the native species.

Theory predicts that the introgression of alleles between species may be driven by the adaptive advantages such alleles may provide (Clarke et al. 2002; Fitzpatrick et al. 2010). In our case, invasiveness of the exotic species has been associated with genes involved in saprobic wood decay and in nucleus-mitochondrion communication, hence our second prediction is that alleles of genes involved in these two functions would be introgressed from the exotic into the native species, and that, vice versa alleles involved in these two functions would not be lost by H. irregulare when hybridizing with H. annosum.

Although we have yet to identify H. annosum alleles that may provide an advantage to H. irregulare genotypes acquiring them, we can hypothesize that alleles of regulatory genes evolutionarily adapted to the Italian landscape, or that genes putatively related to hybrid genome stability may be good candidates as alleles introgressing into H. irregulare. The latter group of alleles may enhance the survival of these hybrids by counteracting the effects of genomic instability caused by the large number of introgression events, as has been shown in other cases (e.g. in animals; Walsh et al. 2018). Finally, our last prediction is that H. annosum genotypes that have absorbed H. irregulare alleles involved in saprobic decay and nucleus-mitochondrial communication should display an increased saprobic ability when grown on woody substrates. Conversely, given that virulence does not differentiate the two species, there should be no clear association between genomes of hybrids and this trait. Nonetheless, because all genotypes tested in this study were established in nature and thus naturally viable, we predict pathogenicity should be within the range displayed by pure parental genotypes. In addition, H. irregulare genotypes that have absorbed a large number of chromosomal blocks from H. annosum may display decreased saprobic growth, pathogenicity or both, due to the effects of large-scale structural genomic rearrangements caused by the large extent of genetic introgression from H. annosum.

As stated above, the main overarching goal of this study was to investigate a fungal invasion at the gene level, rather than at the species level (Petit 2004). Are exotic alleles introgressed in the native species, and do these alleles confer an adaptive advantage to genotypes of the native species that acquire them? Given that the exotic fungal species is a forest pathogen that has been identified as a serious threat to the European region and that a strict monitoring program has been recommended for the timely detection of the enlargement of its range (Gonthier et al. 2014; EPPO 2015), this gene centric approach may also indicate whether the monitoring should be expanded to include the detection of specific exotic H. irregulare alleles as they introgress in native H. annosum populations both within and outside the current zone of infestation by the exotic fungus.
Materials and methods

Biological materials and DNA extraction

An extensive sampling of *Heterobasidion* genotypes was performed in 2005–2006 in the invasion area of *H. irregulare* in Italy where *H. annosum* was also present. Single *Heterobasidion* basidiospores were collected on woody spore traps following the sampling method of Gonthier et al. (2007). Subsequently, fungal genotypes were identified through taxon-specific PCR (Gonthier et al. 2007) as belonging to *H. irregulare* or *H. annosum* and genotyped through Amplified Fragment Length Polymorphism (AFLP) (Gonthier and Garbelotto 2011). Among all sampled genotypes, nine haploid homokaryon hybrid genotypes showing different levels of genetic admixture based on the previous AFLP genotyping (Gonthier and Garbelotto 2011) were selected for the current study (Suppl. material 4: Table S1). Mycelia of fungal genotypes were grown in 2% malt extract liquid medium at 25 °C for ten days before being harvested. For the harvesting, approximately 200 mg of fungal mycelium were collected using a vacuum pump, freeze dried overnight and ground using glass beads (diameter 0.2 mm and 0.4 mm) in a FastPrep™ Cell Disrupter (FP220-Qbiogene). Total DNA extraction was performed using DNeasy Plant Mini Kit (Qiagen Inc., Valencia, California, USA), following manufacturer instructions. DNA quantification was carried out by using the ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). DNA quality was assessed by using a chip-based microcapillary electrophoresis system (Experion, Bio-Rad Laboratories, Hemel Hempstead, UK).

Sequencing, mapping, and genotype calling

Paired-End (PE) 100bp DNA libraries were prepared for each genotype and sequenced using an Illumina HiSeq2000 platform at the Vincent J. Coates Genomics Sequencing Laboratory (Berkeley, CA, USA). Low quality reads (Q score < 20) as assessed by using FASTQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/) were removed by using Trim-Galore! v. 0.5.0 with default parameters (Krueger F. Trim-Galore!, available at http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Reads of each genotype were aligned to the reference *H. irregulare* TC 32-1 genome (Olson et al. 2012) available in the JGI database using the BWA-MEM algorithm of bwa aligner v. 0.7.15 (r1140) optimized for 100bp PE reads (Li and Durbin 2009), with default parameters. The output was used to generate mapping files in SAM format, which were converted in BAM format, sorted, and indexed by SAMtools v.1.3 (Li et al. 2009). The SNP calling was performed by using the mpileup command of SAMtools v.1.3 and the call command of bcftools v. 1.10.2 (default parameters). The VCF output files of each genotype were merged into one VCF file which was filtered by using VCFtools 0.1.12b (Danecek et al. 2011) with “--minQ” set to 25 and “--min-meanDP” set to 5. The SNP panels obtained on the six pure genotypes used in the work by Sillo et al. (2015) were also included in the VCF file, for a total of 15 genotypes. In order to prune SNPs affected by linkage
disequilibrium (LD), i.e., to obtain a set of independent SNPs, the software *plink* v1.07 was used. The VCF file containing the SNP panel was first filtered by removing InDels (by using the vcftools command `--remove-indels`) and then converted on *plink* format by VCFTools. Independent SNPs (unlinked SNPs) were subsequently selected by setting an LD ($r^2$) threshold of 0.8 for SNP pairs through the *plink* command “`--indep-pairwise`”, setting a windows size of 500 bp and a step size of 50. BAM files were submitted to the EMBL database ENA as a project under the PRJEB36378 accession number.

**Genetic relationship among genotypes**

In order to infer the genetic relationship among genotypes and to determine the level of admixture, a phylogenomic analysis and a PCoA were performed on the distance matrix obtained by the unlinked SNP panel of the above mentioned 15 genotypes, i.e. six pure and nine hybrids. Analysis was carried out by using REALPHY v. 112 (Bertels et al. 2014). Reads of each genotype were aligned to the *H. irregulare* reference genome via *bowtie2* (embedded in REALPHY software), and then were used by REALPHY to infer a bootstrap consensus phylogenetic tree (1000 bootstrap replicates, initial tree BioNJ, model of nucleotides substitution GTR) via PhyML (Guindon et al. 2010). The tree was midpoint rooted. The distance matrix was then used as input for a Principal Coordinate Analysis (PCoA) performed by using R.

Results from phylogenomic and PCoA analyses, combined with the outcomes of reads assignment by the software *sppIDer* (see below), were used to decide the taxonomic status of hybrids, i.e. hybrids showing clustering with *H. irregulare* pure genotypes and showing >50% of their genomes as belonging to *H. irregulare* were considered as “*H. irregulare* hybrids”, while hybrids showing clustering with *H. annosum* pure genotypes and showing >50% of their genomes as belonging to *H. annosum* were considered as “*H. annosum* hybrids”.

**Detection of introgressed regions and identification of putative introgressed alleles**

In order to detect regions putatively introgressed from one to the other parental species in hybrid genotypes, i.e. from *H. irregulare* into *H. annosum* and vice versa, we used a conservative approach informed by the outcomes of three distinct analyses, in order to reduce any bias due to the use of any single test for introgression. Tests were done separately for the two groups of hybrid genotypes, that is the group including the five *H. irregulare* genotypes receiving alleles introgressed from *H. annosum*, and the four *H. annosum* genotypes receiving alleles introgressed from *H. irregulare* (see results).

First, the genome-wide four taxon Patterson’s D statistics (ABBA/BABA test) was used to detect hybridization events along genomes. The ABBA/BABA test compares patterns of ancestral and derived alleles within four taxa. Equal numbers of sites for each pattern are expected if gene flow is absent. A positive/negative value of D is generally related to an excess of ABBA/BABA sites and might indicate introgression. In
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**Figure 1.** Phylogenomic analysis of the nine hybrid genotypes and PCoA. A, Phylogenomic relationship within reference genome, pure and hybrid genotypes as inferred by REALPHY. Bootstrap values higher than 50% are showed. B, PCoA on the distance matrix obtained by analysis of unlinked SNPs. Red and blue dots represent pure *H. irregulare* and *H. annosum* genotypes, respectively. Pink and cyan dots represent hybrid genotypes assigned as *H. irregulare* and *H. annosum*, respectively. Clusters were circled as follows: red for *H. irregulare* cluster, blue for *H. annosum*, and gray (dashed line) for F1 hybrids.

In detail, the test was performed on each of the 14 scaffolds for each hybrid genotype by *angsd* v.0.700 (http://www.popgen.dk/angsd/) using the following parameters: -doAb-bababa 1 -doCounts 1. The parameter -blocksize was adjusted according to the dimension in bp of each scaffold. Input files were the BAM files obtained from the alignment of reads against the reference genome. The four-taxon D-statistic test was computed using the following combination: *H. annosum* (P1), hybrid genotypes (P2), *H. irregulare* (P3), and *H. occidentale* (O) as outgroup (*H. occidentale* genotype UC1935443 – Holotype at the Berkeley herbarium – which was sequenced and aligned to the reference *H. irregulare* using the same bioinformatic pipeline performed for the other 15 genotypes). The significance of statistics was assessed using the method of weighted block jackknifing.

Second, reads obtained from the hybrid genotypes were aligned on two reference genomes of the parental species, reconstructed by starting from *de novo* assembly of six pure genotypes published by Sillo et al. (2015). The software *sppIDer* (Langdon et al. 2018) was used to accomplish this task. This software maps the reads of hybrids to a concatenated genome built from the reference genomes of the two parental species, to assess the genomic contribution of *H. irregulare* and *H. annosum* in hybrid genotypes. In order to build the combination reference genome, *de novo* assembly of three pure *H. irregulare* and three *H. annosum* genotypes were used to create two distinct consensus genomes of the two parental species. *De novo* assembly of reads from the three pure genotypes of the two parental species was performed using Velvet v.1.2.08 (Zerbino and Birney 2008) with optimized k-mer = 39 bp, and refined by using IMAGE2 (https://...
sourceforge.net/projects/image2/), which was set up at twenty iterations, and used as inputs both unassembled reads and contigs in FASTA format generated by the assembly process (Tsai et al. 2010). The software Contiguator v. 2.7.4 (Galardini et al. 2011) was used to perform the final assembly in order to reconstruct the 14 scaffolds for each genotype. A consensus reference genome for *H. irregulare* and for *H. annosum* was built in order to take into account the intraspecific diversity within species. Indexed VCF files with SNPs of pure genotypes from Sillo et al. (2015) were used as inputs, along with *de novo* refined assemblies, for *bcftools* v. 1.10.2 *consensus* command and a single *consensus* fasta file for each species was obtained. The two consensus genomes were concatenated by using the script *combineRefGenomes.py* embedded in *sppIDer*.

Third, to assess the presence and dimension of introgressed blocks, we used the R package *introgress* v. 1.2.3. (Gompert and Buerkle 2010) on the panel of unlinked SNPs (VCF file) previously obtained. The package was used to generate a graphical representation of SNP ancestry allowing for inspecting the different patterns of introgression. *De novo* assemblies were used to assign the introgressed blocks to one species or the other, in order to quantify both in size and in number the introgression events. Two-tailed T-tests were used to determine the significance of the difference between the average size of introgressed blocks of the two species, respectively, as the Shapiro-Wilk normality test for size of blocks confirmed the normal distribution of this dataset. A Wilcoxon rank sum test (Mann–Whitney U test) was used to assess the significance of difference in the number of introgressed blocks of the two species, as the Shapiro-Wilk normality test for number of blocks confirmed the not normal distribution of this dataset. All tests were performed with R language.

In *H. annosum* hybrid genotypes with different levels of admixture caused by different levels of introgression from *H. irregulare*, regions in scaffolds showing excess of ABBA sites and aligning with *H. irregulare* genome were considered to be regions putatively introgressed from the invasive species to the native. In *H. irregulare* hybrid genotypes, regions in scaffolds showing excess of BABA excess and aligning with *H. annosum* genome were considered to be regions putatively introgressed from the native species to the invasive. Bed files resulting from the alignment of hybrid genotype reads to the concatenated reference genome were used to assign reads to one species or the other, and to extract fasta formatted aligned sequences (through the bedtools v. 2.19 *getfasta* command; Quinlan and Hall 2010). These sequences were searched through *blastn* algorithm (v. 2.2.31) in the gene catalogue of the established manual annotated genome of *H. irregulare* TC32-1 (Olson et al. 2012) with e-value < 0.05 and nucleotide identity > 98%, in order to identify putative genes in the regions assigned to the two different parental species. Related transcripts were identified by ID, and the sequences of their corresponding encoded proteins were analyzed with Blast2GO v.4.1.9 (Conesa et al. 2005) to search for homologs and to determine their Gene Ontology (GO). At the same time, all gene models of the *H. irregulare* reference genome were annotated through Blast2GO v.4.1.9. Fisher enrichment test (False Discovery Rate < 0.05) was performed with Blast2GO v.4.1.9 to search significant differences in frequencies of GO terms compared to all *H. irregulare* gene models (over-representation).
Phenotypic experiment testing virulence and saprobic ability

In order to assess the fitness of hybrid genotypes relative to the fitness of pure genotypes of each one the two species, virulence on host plant and saprobic growth on wood substrate were assessed by means of two distinct phenotypic experiments. All genotypes collected in the invasion area in Central Italy (nine hybrids and four pure genotypes) were used in the assays. For both assays, genotypes were first cultured in Petri dishes filled with Malt Extract Agar (MEA; malt extract agar 33.6 g/L).

The virulence assay was performed by inoculating *P. pinea* germlings, previously grown in sterile conditions, in a microcosm by using a novel and optimized method. Healthy and uniform seeds of *P. pinea* removed from the cones were washed for 10 minutes in running water, the surface was disinfected for 60 minutes in 30% hydrogen peroxide solution, rinsed twice (10 minutes each) with sterile distilled water and deprived of their lignified tegument (free seeds). Subsequently, they were aseptically transferred into glass jars containing 100 mL of water-agar medium (15 g agar, 1 L distilled water). One seed was inserted in each glass jar. After fifteen days at room temperature (25 ± 2 °C), germlings were inoculated by placing on the medium close to their root collar two mycelial plugs (6 mm diameter) obtained from the edges of actively growing fungal cultures. Eight replicates for each fungal genotype were prepared and incubated at room temperature (25 ± 2 °C). As negative controls, eight additional non-inoculated germlings were grown in the same conditions; they remained alive and uncontaminated until the end of the experiment. The germlings were inspected daily for the presence of disease symptoms, including root browning (visible through the agar), needle discoloration, decline in vitality and collapse. The virulence of fungal genotypes was determined on the base of rapidity to death, expressed as the number of days elapsed since pathogen inoculation.

The saprobic assay was performed by measuring the *in vitro* growth rate of each genotype on agar medium mixed with *P. pinea* sawdust to simulate the natural wood substrate as described by Giordano et al. (2018). Sawdust was prepared by cutting a fresh log using a circular saw and collecting the resulting mixture of heart- and sapwood sawdust in a plastic bag. Petri dishes (90 mm diameter) filled with *P. pinea* sawdust and water agar (15 g sawdust, 12 g agar, 1 L distilled water) were inoculated in the center with a single mycelial plug (6 mm diameter) obtained from the edges of actively growing fungal cultures. Ten replicates for each fungal genotype were incubated at 25 ± 2 °C in the dark. The radial growth of fungal cultures was measured every 48 hours for 10 days along two perpendicular lines. Growth curves from the saprobic assay were compared by performing the permutation test based on t-statistics embedded in the *statmod* R package. The number of permutations was set at 100.

To compare the performance of fungal genotypes in both assays, a pairwise distance matrix of measurements between all possible pairs of genotypes was calculated. For the virulence assay, the distance between two genotypes was calculated as the difference between the average number of days elapsed since pathogen inoculation and death of the germling. For the saprobic assay, the distance between two genotypes
was calculated as the difference between the average radial growth at the end of the experiment. Matrices were used as input for Principal Coordinates Analysis (PCoA) performed by using R v.4.0.3 (function `pcoa`).

**Results**

**Sequencing, mapping, and genotype calling**

The sequenced reads for each genotype were around 8 million (estimate coverage ranging from 17X to 21X). The proportion of reads that aligned to the reference *H. irregulare* genome ranged from 76.20% to 83.14% in four genotypes (118NB, 144SE, 45EH, 49OE), while in five genotypes (136SE, 41NB, 150EA, 115OG and 51ED) ranged from 89.38% to 91.38% (Suppl. material 4: Table S1).

**Phylogenomic and PCoA analyses**

The phylogenomic analysis generated a consensus midpoint rooted tree separating two distinct clades, one including pure genotypes of *H. irregulare* and five hybrids (136SE, 41NB, 115OG, 51ED, and 150EA), and one including pure genotypes of *H. annosum* and four hybrids (144SE, 45EH, 118NB, and 49OE).

In the PCoA, *H. annosum* genotypes were distinguishable from *H. irregulare* types by their position along the PC1. However, an additional third cluster was visible within the *H. annosum* cluster, distinguishable from the other two clusters by its position along the PC2. This third cluster included two F1 hybrid genotypes containing comparable levels of the genomes of both species, but still harboring a majority of *H. annosum* SNPs. Genotype 150EA, a putative F1 hybrid harboring a majority of *H. irregulare* SNPs, was in an intermediate position, belonging to both the *H. irregulare* cluster and the cluster including the other two F1 hybrid genotypes.

**Detection of introgressed regions and identification of putative introgressed alleles**

In order to detect which alleles were consistently transferred between species and to compare introgression levels in the two directions, we focused on the identification of those alleles that were present in all hybrid genotypes belonging to each one of the two groups of hybrids, by intersecting the outcomes of three distinct approaches.

The ABBA/BABA test performed on each scaffold identified an excess of ABBA and Baba sites that can be interpreted as a footprint of introgressive events. On average, an excess of ABBA sites was detected. ABBA/BABA test results across genomes are shown in Figure 2. The excess of ABBA sites was detected in almost all scaffolds, with the exception of scaffolds 2, 7, 12 and 14, suggesting a genome-wide mosaic architecture of hybrid genomes. Interestingly, two blocks important for pathogenic
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Figure 2. Results of Patterson D statistics (ABBA/BABA test) along scaffolds for all hybrid genotypes. For the four taxon test, population were as follows: *H. annosum* (P1), hybrid genotypes (P2), *H. irregulare* (P3), and *H. occidentale* (O) as an outgroup. ABBA excess D>0 indicate the level of introgression between *H. irregulare* and hybrid genotypes; BABA excess D<0 indicate the level of introgression between *H. annosum* and hybrid genotypes. Gray line represent average D along the 14 scaffolds.

interactions with Norway spruce and Scots pine have been identified in scaffold 12 (Olson et al. 2012).

The sppIDer analysis correctly assigned genomic regions of hybrids to one of the two parental species and is visualized in Suppl. material 3: Figure S1. Detailed alignments are reported in Suppl. material 3: Figures S2–10 (a representative example is reported in Figure 3). Thus, this analysis not only identifies the species of origin of all genomic regions in admixed genomes but also confirmed the species assignment of hybrids, based on the percentage of the inherited parental genome (Suppl. material 3: Figure S1). The package introgress was used on a panel of 50 559 unlinked SNPs and identified the number and size of introgressed blocks in both directions, i.e. from the native into the exotic pathogen and from the exotic to the native pathogen. (Figure 4). The number of introgressed blocks per genotype and scaffold is reported in Table 1. Introgressed *H. annosum* blocks in *H. irregulare* hybrid genotypes had a maximum size of 7 007 bp and a minimum size of 21 bp (average size in all 5 *H. irregulare* hybrids = 85.04 bp). The total number of introgressed blocks was 14 088. Conversely, *H. irregulare* blocks into *H. annosum* hybrid genotypes had a maximum
size of 16,536 bp and a minimum size of 64 bp (average size 890.51 bp), and the total number of introgressed blocks was 5,791. In both cases, some scaffolds showed a low number of introgressed blocks, i.e., 6, 10, 12, and 14 (Table 1). When comparing the size of introgressed *H. annosum* blocks vs. the size of introgressed *H. irregulare* blocks, the two were significantly different (two-tailed Student T test p-value $2.2 \times 10^{-16}$, $t = -63.021$, df = 19,878). The number of introgressed blocks from one species into the other was also different (Wilcoxon rank-sum test p-value = 0.031746, df = 125, Test statistic $W = 19$). In summary, *H. annosum* hybrids harbored fewer but larger introgressed blocks than *H. irregulare* hybrids.

In *H. irregulare* and *H. annosum* hybrids, the portions of the genome unaffected by introgression were 2,187,985 bp and 7,255,121 bp in size, respectively. These results suggest that intralocus recombination may be occurring only in a minority of the genome, e.g. within a maximum of 2 Mbp and 7 Mbp in *H. irregulare* and *H. annosum*, respectively. Considering all introgression events in all four *H. annosum* hybrid genotypes, a total of 2,384 introgressed *H. irregulare* alleles were found (Suppl. material 1: Dataset S1). A Fisher enrichment test identified GO terms that were overrepresented in this set, including catabolic pathways, i.e., peroxidases, heterocyclic compound binding, mitochondrial transport and vesicle trafficking (Table 2). Conversely, a total of 1,418 introgressed *H. annosum* alleles were found in *H. irregulare* hybrids (Suppl. material 2: Dataset S2). Fisher enrichment tests on this set determined that overrepresented GO terms were mainly related to nuclear functions including RNA processing, gene expression, nucleotide binding (Table 3). No alleles showing the same GO were ever found to be introgressed in both directions between the two species.

Figure 3. Representative example (hybrid genotype 118NB) of whole genome comparative analysis through sppIDer. Average depth coverage of reads after the alignment with the two parental species along the 14 scaffolds of the two genomes.
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Table 1. Summary of number of introgressed blocks in *Heterobasidion* hybrids.

| Scaffold ID | 118NB | 49OE | 45EH | 144SE | Sum | 136SE | 41NB | 51ED | 115OG | 150EA | Sum |
|-------------|-------|------|------|-------|-----|-------|------|------|-------|-------|-----|
| scaffold_01 | 118   | 50   | 36   | 491   | 695 | 139   | 121  | 90   | 130   | 186   | 666 |
| scaffold_02 | 254   | 51   | 48   | 202   | 555 | 165   | 249  | 315  | 233   | 274   | 1236 |
| scaffold_03 | 243   | 39   | 44   | 47    | 373 | 154   | 281  | 1171 | 237   | 225   | 2068 |
| scaffold_04 | 476   | 33   | 42   | 165   | 716 | 302   | 492  | 161  | 438   | 164   | 1557 |
| scaffold_05 | 159   | 35   | 26   | 43    | 263 | 190   | 378  | 73   | 327   | 220   | 1188 |
| scaffold_06 | 67    | 25   | 30   | 119   | 241 | 44    | 77   | 320  | 87    | 49    | 577  |
| scaffold_07 | 204   | 29   | 41   | 72    | 346 | 124   | 226  | 448  | 220   | 53    | 1071 |
| scaffold_08 | 209   | 28   | 31   | 189   | 457 | 108   | 152  | 145  | 179   | 272   | 856  |
| scaffold_09 | 238   | 24   | 129  | 417   |     | 118   | 228  | 69   | 243   | 100   | 758  |
| scaffold_10 | 461   | 31   | 29   | 65    | 586 | 111   | 192  | 188  | 176   | 101   | 768  |
| scaffold_11 | 178   | 26   | 24   | 136   | 364 | 113   | 206  | 220  | 242   | 109   | 890  |
| scaffold_12 | 214   | 27   | 26   | 16    | 283 | 82    | 143  | 330  | 157   | 50    | 762  |
| scaffold_13 | 119   | 23   | 18   | 92    | 252 | 103   | 163  | 499  | 115   | 177   | 1057 |
| scaffold_14 | 179   | 22   | 16   | 26    | 243 | 104   | 111  | 91   | 167   | 161   | 634  |
Virulence and saprobic ability of fungal genotypes

In the virulence assay, *in vitro* mortality of pine germlings was recorded for a period of 40 days. Forty days post inoculation (DPI), each fungal genotype had killed all germlings it had been inoculated onto, but there was significant variability among genotypes in the average DPI needed to kill all germlings. Seven virulent genotypes (one pure *H. annosum* and six hybrids) were able to induce damping-off of germlings within 20 DPI (Suppl. material 4: Table S2). By contrast, three genotypes (one pure *H. irregulare* and two hybrids) killed germlings after 30 DPI. PCoA on
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Table 3. Gene Ontology terms over-represented in the dataset of 1,418 introgressed *H. annosum* alleles in hybrid *H. irregulare* genotypes. FDR: False Discovery Rate (FDR) corrected p-value.

| GO-ID            | Term                                      | Category                  | FDR          | Sequences (N°) in Test Group | Sequences (N°) in Reference Group |
|------------------|-------------------------------------------|---------------------------|--------------|-----------------------------|-----------------------------------|
| GO:0008152       | metabolic process                         | BIOLOGICAL_PROCESS       | 4.201E-10    | 824                         | 3403                              |
| GO:0000166       | nucleotide binding                        | MOLECULAR_FUNCTION       | 5.605E-07    | 258                         | 886                               |
| GO:0032553       | ribonucleotide binding                    | MOLECULAR_FUNCTION       | 1.131E-04    | 193                         | 670                               |
| GO:009987        | cellular process                          | BIOLOGICAL_PROCESS       | 1.166E-04    | 576                         | 2457                              |
| GO:005488        | Binding                                   | MOLECULAR_FUNCTION       | 1.342E-04    | 641                         | 2781                              |
| GO:0032555       | purine ribonucleotide binding             | MOLECULAR_FUNCTION       | 2.269E-04    | 184                         | 646                               |
| GO:0035639       | purine ribonucleoside triphosphate binding| MOLECULAR_FUNCTION       | 2.269E-04    | 183                         | 641                               |
| GO:0017076       | purine nucleotide binding                 | MOLECULAR_FUNCTION       | 2.961E-04    | 185                         | 655                               |
| GO:0043167       | ion binding                               | MOLECULAR_FUNCTION       | 4.752E-04    | 461                         | 1942                              |
| GO:0005622       | intracellular                             | CELLULAR_COMPONENT       | 7.746E-04    | 266                         | 1033                              |
| GO:0005524       | ATP binding                               | MOLECULAR_FUNCTION       | 8.246E-04    | 158                         | 552                               |
| GO:0032559       | adenyl ribonucleotide binding             | MOLECULAR_FUNCTION       | 8.330E-04    | 159                         | 557                               |
| GO:0010467       | gene expression                           | BIOLOGICAL_PROCESS       | 9.074E-04    | 151                         | 524                               |
| GO:0030554       | adenyl nucleotide binding                 | MOLECULAR_FUNCTION       | 1.122E-03    | 160                         | 566                               |
| GO:0043039       | tRNA aminoacylation                       | BIOLOGICAL_PROCESS       | 1.529E-03    | 17                          | 19                                |
| GO:0043038       | amino acid activation                     | BIOLOGICAL_PROCESS       | 1.529E-03    | 17                          | 19                                |
| GO:0016070       | RNA metabolic process                     | BIOLOGICAL_PROCESS       | 2.027E-03    | 122                         | 411                               |
| GO:0004812       | aminoacyl-tRNA ligase activity            | MOLECULAR_FUNCTION       | 2.537E-03    | 16                          | 18                                |
| GO:006418        | tRNA aminoacylation for protein translation| BIOLOGICAL_PROCESS       | 2.537E-03    | 16                          | 18                                |
| GO:0019787       | ubiquitin-like protein transferase activity| MOLECULAR_FUNCTION       | 6.277E-03    | 10                          | 7                                 |
| GO:0017111       | nucleoside-triphosphatase activity        | MOLECULAR_FUNCTION       | 9.330E-03    | 75                          | 235                               |
| GO:0034660       | ncRNA metabolic process                   | BIOLOGICAL_PROCESS       | 2.207E-02    | 31                          | 73                                 |
| GO:0005634       | nucleus                                   | CELLULAR_COMPONENT       | 2.251E-02    | 122                         | 447                               |
| GO:0006399       | tRNA metabolic process                    | BIOLOGICAL_PROCESS       | 4.406E-02    | 24                          | 53                                 |

Virulence data differentiated two large groups, one including more virulent genotypes and one including less virulent genotypes (Suppl. material 3: Figure S11), however there was no clear correlation between group assignment and genomic background of the genotypes.

In the saprobic assay, the extent of *in vitro* mycelial growth on a wood-rich substrate was assessed for a period of ten days. Only one pure *H. irregulare* genotype (9OA) was able to fully colonize the Petri dishes within the time frame of the experiment, while other genotypes varied in the extent of their growth (Suppl. material 4: Table S3). PCoA on saprobic assay data differentiated genotypes based on their growth rate. Three *H. annosum* hybrids had a growth rate comparable to that of all pure *H. irregulare* genotypes. All the remaining hybrids and the *H. annosum* genotype were placed in a cluster of genotypes characterized by lower growth rates (Figure 5). Permutation tests determined that growth rates of *H. annosum* hybrids were significantly
higher than those of *H. irregulare* hybrids (observed value of the t-statistic = 6.506, p-value adjusted for multiple testing = 0.00043). The saprobic growth of *H. annosum* hybrids was no different from that of *H. irregulare* pure genotypes (p-value adjusted for multiple testing > 0.05). On the other hand, the growth rate of *H. irregulare* hybrids was significantly lower than that of pure *H. irregulare* genotypes (observed value of the t-statistic = -2.439, p-value adjusted for multiple testing = 0.000051).

**Discussion**

The introgression of both exotic genotypes and exotic alleles in native populations, where exotic is defined here as pertaining to an exotic species, is knowingly associated with biological invasions. During interspecific hybridization, the interspecific introgression of alleles bears significant evolutionary implications, shifting the focus from the more traditional “species-centric” to a “gene-centric” concept of invasion (Crispo et al. 2011). In this study, we have shed light on the dynamics of hybridization-mediated allelic introgression between an invasive and a native fungal species. We performed
the study by sampling pure and admixed genotypes in an area in Italy where the North American forest pathogen *H. irregulare* has been introduced and is hybridizing with the native Eurasian forest pathogen *H. annosum* (Gonthier et al. 2007; Gonthier and Garbelotto 2011). This system is particularly interesting as the precise date and location of introduction are known (Gonthier et al. 2004), the biology and population structure of pathogens are also known, the two species are fully interfertile, and 15 years of research have identified some of the likely mechanisms behind the clear demographic dominance of the exotic species compared to the native one.

For this study, we selected a set of 15 natural genotypes with levels of admixture ranging between zero (pure genotypes) and 45% (fully admixed) based on a large number of anonymous AFLP markers. The fact that all studied genotypes had successfully established themselves on wood in a natural setting suggested they were all sufficiently fit to survive in nature. Our expectation that these genotypes would display variably admixed genomic regions (Elgvin et al. 2017) and variable phenotypic fitness proved correct, allowing us to associate genomic variability and phenotypic fitness with specific introgression histories.

Phylogenomic analyses confirmed that the selected natural hybrids varied in the level of genomic admixture. Three hybrid genotypes (118NB, 150EA and 49OE) had a balanced level of admixture, suggesting either a recent hybridization event between two pure parental genotypes or balanced backcrosses with both parental species. The remaining six genotypes had signs of introgression in a relatively small portion of their genome, indicative of multiple backcrosses with a single parental species, to which they could be assigned. Some scaffolds (e.g. scaffolds 6, 12 and 14) showed significantly lower levels of introgression, suggesting these scaffolds contain genes regulating essential functions or species-specific genes (Olson et al. 2012).

The genomes of *H. irregulare* hybrids were profoundly affected by introgression from *H. annosum*, and only approximately 2 Mbp appeared to have been left untouched, while the remaining 30 Mbp were subject to allelic replacement. A significantly larger amount (about 7 Mbp) of the genome of *H. annosum* hybrids, instead, had been left untouched by the introgression of *H. irregulare* alleles, suggesting a smaller scale process in terms of genomic rearrangement. Regions not affected by alleles introgression might be subjected to intra locus recombination, however, in this study, these regions were estimated to be only approximately 24% and 7% of the genome in *H. annosum* and *H. irregulare*, respectively. The effects of intralocus recombination, despite their significance, are not treated in this work and these regions were excluded from the quantification of allelic introgression.

The size of blocks introgressed from the exotic *H. irregulare* into the native *H. annosum* was on average one order of magnitude larger than the size of those introgressed from *H. annosum* into *H. irregulare*. Small block dimensions (about 85 bp on average) and high number of genomic blocks (more than 14 000) introgressed into the exotic species may result in the excessive fragmentation of chromosomal blocks. As a result, exons may be partial, non-functional and may be more likely to be lost during recurrent recombination events (Sachdeva and Barton 2018). On the other hand, a low
number (about 6000) of larger blocks (on average 890 bp in size) introgressed from *H. irregulare* may suggest that putatively adaptive and functional alleles may be truly integrated and preserved into the recipient *H. annosum* genomes. It is known that large genomic blocks have a better chance to be maintained through multiple generations when compared to small blocks (Baird et al. 2003; Sachdeva and Barton 2018).

Alleles contained in the larger blocks introgressed from *H. irregulare* into *H. annosum* showed a GO term enrichment related to catabolic pathways, i.e. peroxidases, heme binding proteins, oxidoreductases, metal ion binding proteins and to vesicle trafficking. Oxidoreductases and heme peroxidase genes have been identified among those that best differentiate the highly saprobic *H. irregulare* from the less saprobic *H. annosum* (Sillo et al. 2015), and some of them, e.g. manganese peroxidases, are known to play a role in lignin degradation during saprobic colonization of wood by *Heterobasidion* species (Yakovlev et al. 2013). All experimental evidence has indicated that higher saprobic potential explains much of the invasiveness of *H. irregulare* (Giordano et al. 2014; Giordano et al. 2019). Another category of over-represented GO terms among *H. irregulare* alleles introgressed into *H. annosum* was related to mitochondrial transport. Interestingly, this is another category of genes that well differentiates the two species at the genomic level (Sillo et al. 2015), with *H. irregulare* nuclear genes better at regulating mitochondrial functions in admixed genomes than *H. annosum* nuclear genes (Sillo et al. 2015). Based on the preexisting literature, the introgression of alleles belonging to these gene groups above into *H. annosum* had been predicted. Alleles involved in clathrin-mediated vesicular trafficking were also significantly enriched among alleles introgressed in *H. annosum* hybrids: this catabolic process plays a role in the breakdown of host defenses and in the development of polarized fungal hyphal growth (Steinberg 2007; Shultzhaus et al. 2017). To explain the advantages provided by the presence of these alleles, it should be noted that mutants of the fungus *Candida albicans* (C.P. Robin) Berkhout with modifications in genes affecting vesicular trafficking are plagued by defects in the cytoskeleton (Epp et al. 2010). Finally, alleles related to “iron-sulphur cluster binding” and “membrane” were found to be introgressed in *H. annosum*. Iron-sulphur cluster enzymes are known to play different roles in cells, including a role in regulating genomic integrity (Stehling et al. 2012) and in detoxification (Haas et al. 2008). Maintaining genomic integrity is obviously an important function in admixed hybrid genomes, while detoxification may be relevant, for example, during delignification processes (Sista Kameshwar and Qin 2020). Moreover, genes related to membrane may affect the activity of extracellular enzymatic activities, required by wood decay fungi to delignify the substrate in which they live (Wu et al. 2020).

Conversely, directional allelic introgression from *H. annosum* to *H. irregulare*, although larger in scale, was limited to DNA-methylation and transcription factor genes. Genes related to DNA-repair have been reported in hybridization events in animals, e.g. sparrows, and it has been hypothesized that they may be selected by nature to modulate epistatic interactions following genomic alterations caused by hybridization (Walsh et al. 2018). The re-patterning of DNA methylation has been also recently
Hybridization and directional genic introgression in Heterobasidion spp. documented in plants (Li et al. 2019). This phenomenon is a process affecting gene expression and silencing of mobile elements in the genome (Martienssen and Colot 2001). The introgression of these alleles can be regarded as the result of a selection of H. annosum alleles involved in epistatic regulation within the highly mosaic-like H. irregulare hybrid genotypes. In other words, given that the consequence of mosaic architecture of hybrid genomes can be the alteration of transcriptomic profiling and/or proliferation of mobile elements, methylation and regulation of genes may contribute to the stability of the hybrid genome. Regulatory genes may also be important in optimizing gene expression and adaptation in a microbial species facing a new environment (Elena and Lenski 2003), and it has been hypothesized that evolution may promote adaptations through already available and even acquired genetic tools rather than generating novel catabolic components (Lavoie et al. 2010). In addition, H. annosum GO terms involved in tRNA aminoacylation processes were identified as being overrepresented in H. irregulare hybrids. It is worth noting that tRNA aminoacylation is not only involved in protein translational fidelity that is indispensable for cell survival, but also allows for the tolerance of low levels of mistranslation, a predicament that may occur during stressful exposure to new environments (Wiltrout et al. 2012; Pan 2013). Thus, it could be inferred that the introgression of these alleles may be beneficial to H. irregulare hybrids constantly facing exposure to novel environments. A further transcriptomic analysis (i.e. RNA-sequencing) will be pivotal to clarify the functional role of introgressed alleles during different life phases of the hybrids (e.g. wood colonization, infection).

The sabrobic in vitro assay revealed that H. annosum hybrids containing H. irregulare alleles involved in saprobic decay had an increased saprobic ability, comparable to that of pure H. irregulare genotypes. Based on the results of the saprobic assay, it is reasonable to hypothesize these H. annosum hybrid genotypes may be competitive against pure H. irregulare genotypes. By contrast, H. irregulare hybrid genotypes showed a decrease in saprobic growth when compared to pure H. irregulare genotypes. We believe the impact of large-scale introgression is costly to hybrid H. irregulare genotypes, resulting in a reduction in fitness. We suggest that viability may be maintained only in those H. irregulare hybrids that: first, absorb transcription and methylation related genes from the native species stabilizing their genomic instability caused by large genomic alterations, and, second, maintain their alleles involved in production of peroxidase and heme-binding proteins, mitochondrial transport and vesicle trafficking. This last point is corroborated by the fact that the alleles involved in catabolic pathways were not affected by introgressive events, i.e. these alleles were never replaced by H. annosum alleles in any of the H. irregulare hybrids. Negative epistatic interactions and the breakdown of advantageous gene complexes (Lynch 1991; Guerrero et al. 2017) in H. irregulare recipient genomes, highly modified by large scale introgression events, are two plausible explanations for this detrimental effect on fitness.

Virulence assays in the past have not been able to differentiate the two species (Garbelotto et al. 2010; Pepori et al. 2019). The results of the virulence assay performed in
this study further confirmed that virulence may be a genotype-specific trait, rather than a species-level trait as previously suggested (Garbelotto et al. 2010). We note, though, that all hybrids fell within the range of virulence identified for the pure parental genotypes used in the study, and so all can be regarded as ecologically viable. This result confirms that by using genotypes that have established themselves on wood in a natural setting, we obtained a representative sample of functionally and ecologically viable genotypes. In addition, the result also confirms that hybrid swarms represent a viable genetic bridge for the transfer of genetic material between the two species (Brasier 2001).

Although we recognize that the number of genotypes characterized as pure H. annosum and H. irregulare used in our phenotypic tests is limited, the genotypes employed here represented a select sample representative of each species, and results were in agreement with our expectations. In a previous comparative genomic study, genes involved in virulence have been reported to be conserved between the two species (Sillo et al. 2015), resulting in indistinguishable virulent phenotypes (Garbelotto et al. 2010; Pepori et al. 2019). Conversely, genes involved in catabolic processes, including wood decay, and genes regulating nucleus-mitochondrion communication have been shown to diverge (Sillo et al. 2015) and to be associated with different phenotypes (Giordano et al. 2014; 2018). Results of this study are in agreement with a role of selection driving introgression: alleles from divergent loci such as those involved in catabolic processes were significantly enriched as a result of introgression and caused a measurable phenotypic change in the species receiving them by increasing its saprobic ability. On the other hand, we could not document introgression-related enrichment of genes directly involved in virulence of live plants and no measurable change in the associated phenotype could be measured.

It is known that competition with parental species living in the same environment may negatively affect the success of hybrids (Stukenbrock 2016). In the pathosystem studied here, the exotic Heterobasidion species may outcompete or even replace the native species, possibly due to its higher saprobic and sporulation potential (Gonthier et al. 2014). Our results suggest that H. annosum genotypes hybridizing with H. irregulare may become as competitive as those belonging to the exotic species, by receiving alleles that provide them with adaptive phenotypic advantages, including an increased saprobic wood decay potential. If this suggested scenario was correct, these alleles introgressed from H. irregulare into H. annosum, including those related to the saprobic ability among others, may move into H. annosum populations both inside and outside the hybrid zone. The rapid absorption of advantageous or adaptive exotic alleles through introgressive hybridization has been previously documented for many organisms (Fitzpatrick et al. 2010; Crispo et al. 2011) including the fungi (Hessenauer et al. 2020). Thus, H. annosum hybrids, with some specific H. irregulare alleles introgressed in their genome, may themselves represent a threat to the stability of European forests. On the other hand, introgression from the native into the invasive species affected a larger portion of the genome and involved alleles exclusively associated with gene regulation and transcription processes, resulting in lower saprobic growth, but ensuring the viability of hybrid genotypes.
Conclusion

Overall, our results show that mating events leading to viable and fertile *Heterobasidion* hybrids occur frequently in the Latium region of Italy where the exotic forest pathogen *H. irregulare* is now sympatric with the native forest pathogen *H. annosum*. The resulting allelic introgression has identified specific classes of adaptive *H. irregulare* alleles that confer an advantage to recipient native *H. annosum* genotypes. Given the phenotypic outcomes of these introgression events including a measurable increase in saprobic potential of *H. annosum* individuals, we propose an operational shift in the monitoring of the invasion process to include not only the detection of exotic *H. irregulare* genotypes, but also the presence of adaptive exotic *H. irregulare* alleles or genes spreading into native *H. annosum* populations. A gene centric approach to the study of invasions has been previously advocated (Petit, 2004) and clear adaptive implications of genic introgression between tree pathogens have been previously documented for the two fungi causing Dutch elm disease (Hessenauer et al. 2020).

In conclusion, despite the limited number of sequenced hybrid genotypes, this study suggests that horizontal allelic movement occurred as a result of interspecific hybridization, but it was qualitatively and quantitatively different when comparing the two directions of introgression. A large population genomics survey in and near the invasion area in Central Italy targeting introgressed alleles identified in this study will be pivotal to further validate these results.

Acknowledgments

This work was supported by the Italian Ministry of Education, University and Research, within the FIRB program (grant number RBFR1280NN). This work used the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley, supported by NIH S10 Instrumentation Grants S10RR029668 and S10RR027303.

Sequence data were submitted to the EMBL database ENA - European Nucleotide Archive as a project under the PRJEB36378 accession number. All other data were available within the article or its supplementary materials.

References

Abbott RJ, James JK, Milne RI, Gillies AC (2003) Plant introductions, hybridization and gene flow. Philosophical Transactions of the Royal Society B: Biological Sciences 358: 1123–1132. https://doi.org/10.1098/rstb.2003.1289

Arnold ML, Sapir Y, Martin NH (2008) Genetic exchange and the origin of adaptations: prokaryotes to primates. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 2813–2820. https://doi.org/10.1098/rstb.2008.0021
Baird SJE, Barton NH, Etheridge AM (2003) The distribution of surviving blocks of an ancestral genome. Theoretical Population Biology 64: 451–471. doi:10.1016/S0040-5809(03)00098-4

Barton NH (2001) The role of hybridization in evolution. Molecular Ecology 10: 551–568. doi:10.1046/j.1365-294x.2001.01216.x

Bertels F, Silander OK, Pachkov M, Rainey PB, van Nimwegen E (2014) Automated reconstruction of whole-genome phylogenies from short-sequence reads. Molecular Biology and Evolution 31: 1077–1088. doi:10.1093/molbev/msu088

Brasier CM (1995) Episodic selection as a force in fungal microevolution, with special reference to clonal speciation and hybrid introgression. Canadian Journal of Botany 73:1213–1221. doi:10.1139/b95-381

Brasier CM (2000) The rise of the hybrid fungi. Nature 405: 134–135. doi:10.1038/35012193

Brasier CM (2001) Rapid evolution of introduced plant pathogens via interspecific hybridization: Hybridization is leading to rapid evolution of Dutch elm disease and other fungal plant pathogens Bioscience 51: 123–133. doi:10.1641/0006-3568(2001)051[0123:REOIPP]2.0.CO;2

Clarke KE, Rinderer TE, Franck P, Quezada-Euán JG, Oldroyd BP (2002) The Africanization of honeybees (Apis mellifera L) of the Yucatan: a study of a massive hybridization event across time. Evolution 56: 1462–1474. doi:10.1111/j.0014-3820.2002.tb01458.x

Clarkson CS, Weetman D, Essandoh J, Yawson AE., Maslen G, Manks M, Field SG, Webster M, Antão T, MacInnis B, Dominic Kwiatkowski D, Donnelly MJ (2014) Adaptive introgression between Anopheles sibling species eliminates a major genomic island but not reproductive isolation. Nature Communications 5: e4248. doi:10.1038/ncomms5248

Conesa A, Götz S, García-Gómez JM, Terol J, Talón, M, Robles M (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21: 3674–3676. doi:10.1093/bioinformatics/bti610

Crispo E, Moore JS, Lee-Yaw JA, Gray SM, Haller BC (2011) Broken barriers: human-induced changes to gene flow and introgression in animals: an examination of the ways in which humans increase genetic exchange among populations and species and the consequences for biodiversity. BioEssays 33: 508–518. doi:10.1002/bies.201000154

Currat M, Ruedi M, Petit RJ, Excoffier L (2008) The hidden side of invasions: massive introgression by local genes. Evolution 62: 1908–1920. doi:10.1111/j.1558-5646.2008.00413.x

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R (2011) The variant call format and VCFtools. Bioinformatics 27: 2156–2158. doi:10.1093/bioinformatics/btr330

Dasmahapatra KK, et al. (2012) Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature 487: e94. doi:10.1038/nature11041

Deprotter JR, Seidl MF, Wood TA, Thomma BP (2016) Interspecific hybridization impacts host range and pathogenicity of filamentous microbes. Current Opinion in Microbiology 32: 7–13. doi:10.1016/j.mib.2016.04.005

Edelman NB, Frandsen PB, Miyagi M, Clavijo B, Davey J, Dikow RB, García-Accinelli G, Van Belleghem SM, Patterson N, Neafsey DE, Challis R, Kumar S, Moreira GRP, Salazar
Hybridization and directional genic introgression in *Heterobasidion* spp.

C, Chouteau M, Counterman BA, Papa R, Blaxter M, Reed RD, Dasmahapatra KK, Kronforst M, Joron M, Jiggins CD, McMillan WO, Di Palma F, Blumberg AJ, Wakeley J, Jaffe D, Mallet J (2019) Genomic architecture and introgression shape a butterfly radiation. Science 366: 594–599. https://doi.org/10.1126/science.aaw2090

Elena SF, Lenski RE (2003) Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. Nature Reviews Genetics 4: 457–469. https://doi.org/10.1038/nrg1088

Elgvin TO, Trier CN, Tørresen OK, Hagen IJ, Lien S, Nederbragt AJ, Sætre GP (2017) The genomic mosaicism of hybrid speciation. Science Advances 3:e1602996. https://doi.org/10.1126/sciadv.1602996

Epp E, Vanier G, Harcus D, Lee AY, Jansen G, Hallett M, Sheppard DC, Thomas DY, Munro CA, Mullick A, Whiteway M (2010) Reverse genetics in Candida albicans predicts ARF cycling is essential for drug resistance and virulence. PLoS Pathogens 6: e1000753. https://doi.org/10.1371/journal.ppat.1000753

EPPO (2015) Pest risk analysis for *Heterobasidion irregulare*. EPPO, Paris. http://www.eppo.int/QUARANTINE/Pest_Risk_Analysis/PRA_intro.htm [last accessed January 12th, 2021]

Excoffier L, Foll M, Petit RJ (2009) Genetic consequences of range expansions. Annual Review of Ecology, Evolution, and Systematics 40: 481–501. https://doi.org/10.1146/annurev.ecolsys.39.110707.173414

Feurtey A, Stukenbrock EH (2018) Interspecific gene exchange as a driver of adaptive evolution in fungi. Annual Review of Microbiology 72: 377–398. https://doi.org/10.1146/annurev-micro-090817-062753

Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ (2012) Emerging fungal threats to animal, plant and ecosystem health. Nature 484: 186–194. https://doi.org/10.1038/nature10947

Fitzpatrick BM, Johnson JR, Kump DK, Smith JJ, Voss SR, Shaffer HB (2010) Rapid spread of invasive genes into a threatened native species. Proceedings of the National Academy of Sciences 107: 3606–3610. https://doi.org/10.1073/pnas.0911802107

Galardini M, Biondi EG, Bazzicalupo M, Mengoni A (2011) CONTIGuator: a bacterial genomes finishing tool for structural insights on draft genomes. Source code for biology and medicine 6: 1–11. https://doi.org/10.1186/1751-0473-6-11

Garbelotto M, Ratcliff A, Bruns TD, Cobb FW, Otrosina WJ (1996) Use of taxon-specific competitive-priming PCR to study host specificity, hybridization, and intergroup gene flow in intersterility groups of *Heterobasidion annosum*. Phytopathology 86: e543. https://doi.org/10.1094/Phyto-86-543

Garbelotto M, Gonthier P (2013) Biology, epidemiology, and control of *Heterobasidion* species worldwide. Annual Review of Phytopathology 51: 39–59. https://doi.org/10.1146/annurev-phyto-082712-102225

Garbelotto M, Linzer R, Nicolotti G, Gonthier P (2010) Comparing the influences of ecological and evolutionary factors on the successful invasion of a fungal forest pathogen. Biological Invasions 12: 943–957. https://doi.org/10.1007/s10530-009-9514-4

Giordano L, Sillo F, Garbelotto M, Gonthier P (2018) Mitonuclear interactions may contribute to fitness of fungal hybrids. Scientific Reports 8: 1–7. https://doi.org/10.1038/s41598-018-19922-w
Giordano L, Gonthier P, Lione G, Capretti P, Garbelotto M (2014) The saprobic and fruiting abilities of the exotic forest pathogen Heterobasidion irregulare may explain its invasiveness. Biological Invasions 16: 803–814. https://doi.org/10.1007/s10530-013-0538-4

Giraud T, Refrégier G, Le Gac M, de Vienne DM, Hood ME (2008) Speciation in fungi. Fungal Genetics and Biology 45: 791–802. https://doi.org/10.1016/j.fgb.2008.02.001

Gladieux P, Ropars J, Badouin H, Branca A, Aguileta G, De Vienne DM, Giraud T (2014) Fungal evolutionary genomics provides insight into the mechanisms of adaptive divergence in eukaryotes. Molecular Ecology 23: 753–773. https://doi.org/10.1111/mec.12631

Gompert Z, Alex Buerkle C (2010) INTROGRESS: a software package for mapping components of isolation in hybrids. Molecular Ecology Resources 10: 378–384. https://doi.org/10.1111/j.1755-0998.2009.02733.x

Gonthier P, Warner R, Nicolotti G, Mazzaglia A, Garbelotto MM (2004) Pathogen introduction as a collateral effect of military activity. Mycological Research 108: 468–470. https://doi.org/10.1017/S0953756204240369

Gonthier P, Nicolotti G, Linzer R, Guglielmo F, Garbelotto M (2007) Invasion of European pine stands by a North American forest pathogen and its hybridization with a native interfertile taxon. Molecular Ecology 16: 1389–1400. https://doi.org/10.1111/j.1365-294X.2007.03250.x

Gonthier P, Garbelotto M (2011) Amplified fragment length polymorphism and sequence analyses reveal massive gene introgression from the European fungal pathogen Heterobasidion annosum into its introduced congener H. irregulare. Molecular Ecology 20: 2756–2770. https://doi.org/10.1111/j.1365-294X.2011.05121.x

Gonthier P, Anselmi N, Capretti P, Bussotti F, Feducci M, Giordano L, Honorati T, Lione G, Luchi N, Michelozzi M, Paparatti B, Sillo F, Vettraino AM, Garbelotto M (2014) An integrated approach to control the introduced forest pathogen Heterobasidion irregulare in Europe. Forestry 87: 471–481. https://doi.org/10.1093/forestry/cpu015

Gonthier P, Sillo F, Lagostina E, Rocotelli A, Santa Cacciola O, Stenlid J, Garbelotto M (2015) Selection processes in simple sequence repeats suggest a correlation with their genomic location: insights from a fungal model system. BMC Genomics 16: e1107. https://doi.org/10.1186/s12864-015-2274-x

Guerrero RF, Muir CD, Josway S, Moyle LC (2017) Pervasive antagonistic interactions among hybrid incompatibility loci. PLoS Genetics 13: e1006817. https://doi.org/10.1371/journal.pgen.1006817

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59: 307–321. https://doi.org/10.1093/sysbio/syq010

Haas H, Eisendle M, Turgeon BG (2008) Siderophores in fungal physiology and virulence. Annual Review of Phytopathology 46: 149–187. https://doi.org/10.1146/annurev.phyto.45.062806.094338

Hessenauer P, Fijarczyk A, Martin H, Prunier J, Charron G, Chapuis J, Bernier L, Tanguay P, Hamelin RC, Landry CR (2020) Hybridization and introgression drive genome evolution of Dutch elm disease pathogens. Nature Ecology and Evolution 4: 626–638. https://doi.org/10.1038/s41559-020-1133-6
Hybridization and directional genic introgression in *Heterobasidion* spp.

Hughes KW, Petersen RH, Lodge DJ, Bergemann SE, Baumgartner K, Tulloss RE, Lickey E, Cifuentes J (2013) Evolutionary consequences of putative intra-and interspecific hybridization in agaric fungi. Mycologia 105: 1577–1594. https://doi.org/10.3852/13-041

Langdon QK, Peris D, Kyle B, Hittinger CT (2018) sspIDer: a species identification tool to investigate hybrid genomes with high-throughput sequencing. Molecular Biology and Evolution 35: 2835–2849. https://doi.org/10.1093/molbev/msy166

Lavoie H, Hoggles H, Mallick J, Sellam A, Nantel A, Whiteway M (2010) Evolutionary tinkering with conserved components of a transcriptional regulatory network. PLoS Biology 8: e1000329. https://doi.org/10.1371/journal.pbio.1000329

Lee CE (2002) Evolutionary genetics of invasive species. Trends in Ecology and Evolution 17: 386–391. https://doi.org/10.1016/S0169-5347(02)02554-5

Le Gac M, Giraud T (2008) Existence of a pattern of reproductive character displacement in Homobasidiomycota but not in Ascomycota. Journal of Evolutionary Biology 21: 761–772. https://doi.org/10.1111/j.1420-9101.2008.01511.x

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Goncalo Abecasis G, Durbin R (2009) The sequence alignment/map format and SAMtools. Bioinformatics 25: 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics. 25: 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li N, Xu C, Zhang A, Lv R, Meng X, Lin X, Gong L, Wendel JF, Liu B (2019) DNA methylation repatterning accompanying hybridization, whole genome doubling and homoeolog exchange in nascent segmental rice allotetraploids. New Phytologist 223: 979–992. https://doi.org/10.1111/nph.15820

Lynch M (1991) The genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45: 622–629. https://doi.org/10.1111/j.1558-5646.1991.tb04333.x

Mallet J (2007) Hybrid speciation. Nature 446: 279–283. https://doi.org/10.1038/nature05706

Mallet J (2005) Hybridization as an invasion of the genome. Trends in Ecology and Evolution 20: 229–237. https://doi.org/10.1016/j.tree.2005.02.010

Martienssen RA, Colot V (2001) DNA methylation and epigenetic inheritance in plants and filamentous fungi. Science 293: 1070–1074. https://doi.org/10.1126/science.293.5532.1070

Menardo F, Praz CR, Wyder S, Ben-David R, Bourras S, Matsumae H, McNally KE, Parlange F, Riba A, Roffler S, Schaefer LK, Shimizu KK, Valenti L, Zbinden H, Wicker T, Keller B (2016) Hybridization of powdery mildew strains gives rise to pathogens on novel agricultural crop species. Nature Genetics 48: 201–205. https://doi.org/10.1038/ng.3485

Olson Å, Stenlid J (2001) Mitochondrial control of fungal hybrid virulence. Nature 411: 438–438. https://doi.org/10.1038/35078147

Olson Å, Aerts A, Asiegbu F, Belbahri L, Bouzid O, Broberg A, et al. (2012) Insight into trade-off between wood decay and parasitism from the genome of a fungal forest pathogen. New Phytologist 194: 1001–1013. https://doi.org/10.1111/j.1469-8137.2012.04128.x

Pan T (2013) Adaptive translation as a mechanism of stress response and adaptation. Annual Review of Genetics 47: 121–137. https://doi.org/10.1146/annurev-genet-111212-133522

Pepori AL, Michelozzi M, Santini A, Cencetti G, Bonello P, Gonthier P, Sebastiani F, Luchi N (2019) Comparative transcriptional and metabolic responses of *Pinus pinea* to a native and a non-native Heterobasidion species. Tree Physiology 39: 31–44. https://doi.org/10.1093/treephys/tpy086
Petit RJ (2004) Biological invasions at the gene level. Diversity and Distributions 10: 159–165. https://doi.org/10.1111/j.1366-9516.2004.00084.x

Pryszcz LP, Németh T, Gácser A, Gabaldón T (2014) Genome comparison of Candida orthopsilosis clinical strains reveals the existence of hybrids between two distinct subspecies. Genome Biology and Evolution 6: 1069–1078. https://doi.org/10.1093/gbe/evu082

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ, Sham PC (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. American Journal of Human Genetics 81: 559–575. https://doi.org/10.1086/519795

Quinlan AR, Hall IM (2010) BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics. 26: 841–842. https://doi.org/10.1093/bioinformatics/btq033

Rieseberg LH, Baird SJ, Gardner KA (2000) Hybridization, introgression, and linkage evolution. Plant Molecular Biology 42: 205–224. https://doi.org/10.1023/A:1006340407546

Sachdeva H, Barton NH (2018) Introgression of a block of genome under infinitesimal selection. Genetics 209: 1279–1303. https://doi.org/10.1534/genetics.118.301018

Salazar C, Baxter SW, Pardo-Diaz C, Wu G, Surridge A, Linares M, Bermingham E, Jiggins CD (2010) Genetic evidence for hybrid trait speciation in Heliconius butterflies. PLoS Genetics 6: e1000930. https://doi.org/10.1371/journal.pgen.1000930

Scharf CL, Craven KD (2003) Interspecific hybridization in plant-associated fungi and oomycetes: a review. Molecular Ecology 12: 2861–2873. https://doi.org/10.1046/j.1365-294X.2003.01965.x

Schierenbeck KA, Ellstrand NC (2009) Hybridization and the evolution of invasiveness in plants and other organisms. Biological Invasions 11: e1093. https://doi.org/10.1007/s10530-008-9388-x

Schultzhaus Z, Johnson TB, Shaw BD (2017) Clathrin localization and dynamics in Aspergillus nidulans. Molecular Microbiology 103: 299–318. https://doi.org/10.1111/mmi.13557

Seehausen O (2004) Hybridization and adaptive radiation. Trends in Ecology and Evolution 19: 198–207. https://doi.org/10.1016/j.tree.2004.01.003

Sillo F, Garbelotto M, Friedman M, Gonthier P (2015) Comparative genomics of sibling fungal pathogenic taxa identifies adaptive evolution without divergence in pathogenicity genes or genomic structure. Genome Biology and Evolution 7: 3190–3206. https://doi.org/10.1093/gbe/evv209

Sillo F, Gonthier P, Lockman B, Kasuga T, Garbelotto M (2019) Molecular analyses identify hybridization-mediated nuclear evolution in newly discovered fungal hybrids. Ecology and Evolution 9: 6588–6605. https://doi.org/10.1002/ece3.5238

Sista Kameshwar AK, Qin W (2020) Systematic metadata analysis of brown rot fungi gene expression data reveals the genes involved in Fenton’s reaction and wood decay process. Mycology 11: 22–37. https://doi.org/10.1080/21501203.2019.1703052

Steenkamp ET, Wingfield MJ, McTaggart AR, Wingfield BD (2018) Fungal species and their boundaries matter – Definitions, mechanisms and practical implications. Fungal Biology Review 32: 104–116. https://doi.org/10.1126/science.1219723

Stehling O, Vashisht AA, Mascarenhas J, Jonsson ZO, Sharma T, Netz DJ, Pierik AJ, Wohlschlegel JA, Lill R (2012) MMS19 assembles iron-sulfur proteins required for DNA metabolism and genomic integrity. Science 337: 195–199. https://doi.org/10.1016/j.fbr.2017.11.002
Hybridization and directional genic introgression in *Heterobasidion* spp.

Steinberg G (2007) Hyphal growth: a tale of motors, lipids, and the Spitzenkörper. Eukaryotic Cell 6: 351–360. https://doi.org/10.1128/EC.00381-06

Stukenbrock EH, McDonald BA (2008) The origins of plant pathogens in agro-ecosystems. Annual Review Phytopathology 46: 75–100. https://doi.org/10.1146/annurev.phyto.010708.154114

Stukenbrock EH (2016) The role of hybridization in the evolution and emergence of new fungal plant pathogens. Phytopathology 106: 104–112. https://doi.org/10.1094/PHYTO-08-15-0184-RVW

Suarez-Gonzalez A, Lexer C, Cronk QC (2018) Adaptive introgression: a plant perspective. Biology Letters 14:pi: 20170688. https://doi.org/10.1098/rsbl.2017.0688

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123: 585–595. https://doi.org/10.1093/genetics/123.3.585

Tsai IJ, Otto TD, Berriman M (2010) Improving draft assemblies by iterative mapping and assembly of short reads to eliminate gaps. Genome Biology 11: R41. https://doi.org/10.1186/gb-2010-11-4-r41

Walsh J, Kovach AI, Olsen BJ, Shriver WG, Lovette IJ (2018) Bidirectional adaptive introgression between two ecologically divergent sparrow species. Evolution 72: 2076–2089. https://doi.org/10.1111/evo.13581

Wiltrout E, Goodenbour JM, Frechin M, Pan T (2012) Misacylation of tRNA with methionine in Saccharomyces cerevisiae. Nucleic Acids Research 40: 10494–10506. https://doi.org/10.1093/nar/gks805

Wu H, Nakazawa T, Takenaka A, Kodera R, Morimoto R, Sakamoto M, Honda Y (2020) Transcriptional shifts in delignification-defective mutants of the white-rot fungus Pleurotus ostreatus. FEBS Letters. https://doi.org/10.1002/1873-3468.13890

Yakovlev IA, Hietala AM, Courty PE, Lundell T, Solheim H, Fossdal CG (2013) Genes associated with lignin degradation in the polyphagous white-rot pathogen *Heterobasidion irregulare* show substrate-specific regulation. Fungal Genetics and Biology 56: 17–24. https://doi.org/10.1016/j.fgb.2013.04.011

Zerbino DR, Birney E (2004) Velvet: Algorithms for de novo short read assembly using de Bruijn. Genome Research 18: 821–829. https://doi.org/10.1101/gr.074492.107

**Supplementary material I**

**Dataset S1. List of introgressed *H. irregulare* alleles**
Authors: Fabiano Sillo, Matteo Garbelotto, Luana Giordano, Paolo Gonthier
Data type: dataset
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Link: https://doi.org/10.3897/neobiota.65.64031.suppl1
Supplementary material 2

Dataset S2. List of introgressed *H. annosum* alleles
Authors: Fabiano Sillo, Matteo Garbelotto, Luana Giordano, Paolo Gonthier
Data type: dataset
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Supplementary material 3

Figures S1–S11
Authors: Fabiano Sillo, Matteo Garbelotto, Luana Giordano, Paolo Gonthier
Data type: images
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Supplementary material 4

Tables S1–S4
Authors: Fabiano Sillo, Matteo Garbelotto, Luana Giordano, Paolo Gonthier
Data type: tables
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