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Regular Articles

A detailed analysis of the recombination landscape of the button mushroom *Agaricus bisporus* var. *bisporus*

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ABSTRACT

The button mushroom (*Agaricus bisporus*) is one of the world's most cultivated mushroom species, but in spite of its economic importance generation of new cultivars by outbreeding is exceptional. Previous genetic analyses of the white *bisporus* variety, including all cultivars and most wild isolates revealed that crossing over frequencies are low, which might explain the lack of introducing novel traits into existing cultivars. By generating two high quality whole genome sequence assemblies (one *de novo* and the other by improving the existing reference genome) of the first commercial white hybrid Horst U1, a detailed study of the crossover (CO) landscape was initiated. Using a set of 626 SNPs in a haploid offspring of 139 single spore isolates and whole genome sequencing on a limited number of homo- and heterokaryotic single spore isolates, we precisely mapped all COs showing that they are almost exclusively restricted to regions of about 100 kb at the chromosome ends. Most basidia of *A. bisporus* var. *bisporus* produce two spores and pair preferentially via non-sister nuclei. Combined with the COs restricted to the chromosome ends, these spores retain most of the heterozygosity of the parent thus explaining how present-day white cultivars are genetically so close to the first hybrid marketed in 1980. To our knowledge this is the first example of an organism which displays such specific CO landscape.

1. Introduction

The button mushroom *Agaricus bisporus* has a long history of cultivation of more than 300 years, and the first commercial cultivation has been reported in France in the 18th century (Pardo et al., 2010; Tournefort, 1707). Button mushrooms are grown worldwide with a product volume of 3.9 million tonnes in 2009 (Sonnenberg et al., 2011) and a fast increasing production in China since the last few years (Zhang et al., 2014). The productivity and quality of the crop has been improved considerably during the last 30 years mainly by improving cultivation techniques, i.e., composting, casing, growing conditions, and mechanical spawning and harvesting. With a few exceptions, breeding has played a very minor role in the improvements. Genetic studies and breeding trials for advanced cultivars have been conducted meanwhile for cap colour (Callac et al., 1998), resistance to pathogens (Moquet et al., 1998; Foulongne-Oriol et al., 2012b), yield (Foulongne-Oriol et al., 2012a), earliness (Foulongne-Oriol et al., 2012a), and resistance to mechanical bruising (Gao et al., 2013, 2015). This has, however, not yet resulted in commercial cultivars that are substantially superior to the first hybrid strain Horst U1, which was marketed in 1980 (Fritsche, 1982). The cultivars released since 1980 were either genetically identical or very similar to the first hybrid (Sonnenberg et al., 2011). The main reason for the lack of introduction of new cultivars lies in the typical life cycle of the button mushroom. We have carried out a more detailed analysis of its meiosis as has been done so far to make clear what the obstacles are in breeding and also to explain the very narrow genetic base of almost all cultivars of the white button mushroom grown worldwide.

Sexual reproduction in fungi is mainly divided in homothallic (inbreeding/selfing) or heterothallic (outbreeding) sexuality (as reviewed recently by Ni et al., 2011). Compatibility between partners is determined by one (bifactorial) or two (tetrafactorial) mating loci where different mating types are needed for a successful

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mating. Homothallism has been defined historically in fungi as the ability of a single spore to produce a sexually reproducing colony when propagated in complete isolation (Blakeslee, 1904), since they have both mating type genes in a single genome, whereas in heterothallic organisms mating always takes place between individuals with different mating types. The species *A. bisporus* is mainly represented by two varieties, var. *bisporus* and the var. *burnettii* (Callac et al., 2003), both with a unifactorial mating system and displaying both types of sexuality and which can thus be designated as amphithallic (Kühner, 1977). Each variety, however, differs in ploidy level for the majority of its offspring, n + n in the *bisporus* and n in the *burnettii* variety (Kerrigan et al., 1994). As in all basidiomycetes, meiosis takes place in *A. bisporus* in basidia where, after fusion of the two different constituent haploid nuclei, meiosis I and II result in four haploid nuclei. In the majority of the basidia of *A. bisporus* var. *bisporus*, these nuclei are distributed over two spores with a preference of pairing non-sister nuclei (Callac et al., 1993; Summerbell et al., 1989; Kamzolkina et al., 2006). Germination of these spores results in heterokaryons able to produce fruiting bodies. Around 10–15% of the basidia produce three or four spores, the majority of this receiving one haploid nucleus which will germinate into homokaryons that need to be mated with a compatible partner to produce fruiting bodies. In *A. bisporus* var. *burnettii* approximately 90% of the basidia generate four spores (Kerrigan et al., 1994) where each spore receives one haploid nucleus and germinates into a homokaryon. There are indications that the heterokaryotic offspring of the var. *burnettii* also arises by pairing of non-sister nuclei (Kerrigan et al., 1994) but direct evidence for this has not been obtained yet by genotyping recovered constituent nuclei. The variety *bisporus* has thus mainly a homothallic sexuality whereas the variety *burnettii* is predominantly heterothallic. The meiotic behaviour of *A. bisporus* var. *bisporus* has been studied in the past using molecular markers (Royse and May, 1982; Allen et al., 1992; Summerbell et al., 1989). Genotyping of large numbers of random selections of single spore isolates (SSI) with a limited number of markers revealed that 95–100% retained complete heterozygosity. This indicates a low recombination frequency and pairing of non-sister nuclei. Kerrigan et al. (1993) studied segregation of a substantial number of markers in a set of homokaryotic offspring and showed that *A. bisporus* var. *bisporus* has a normal meiosis but here also a low recombination frequency was seen. Foulongne-Oriol et al. (2010, 2011) performed an extended linkage analysis using crosses between *bisporus* and *burnettii* varieties and showed a crossover landscape comparable to what is seen in other fungi and observed a strong impact of the genetic background on recombination ability, indicating a low recombination frequency in the *bisporus* and normal frequency in the *burnettii* variety. A thorough study of the meiosis of the variety *bisporus*, however, using large populations and many molecular markers has not been done. It is still unclear where and how frequent recombinations take place in the genome of the variety *bisporus*. Are crossovers (CO) indeed infrequent or do many CO result in lethal offspring? The utilisation, so far, of a limited number of offspring and markers also raises the question if all recombinations have been detected. Crossovers at extreme chromosome ends may not always be detected due to the presence of repetitive elements (Fulcher et al., 2014). It has been suggested that CO in *A. bisporus* var. *bisporus* are mainly localised at chromosome ends (Kerrigan et al., 1993; Foulongne-Oriol et al., 2009) which raises the question whether recombination is indeed suppressed in the variety *bisporus* or not always detected because they occur mainly at chromosome ends. The variety *bisporus* represents all commercial cultivars and most wild isolates. This variety is thus an important base for breeding and a more detailed understanding of its meiotic behaviour is important. By generating two high quality whole genome sequences (one *de novo* and the other by improving the existing reference sequence) of the first commercial white hybrid Horst U1, a thorough study was made of the CO landscape in offspring of this cultivar. A high number of SNPs in large haploid offspring and a detailed analysis with whole genome sequencing on a limited number of homo- and heterokaryotic single spore isolates was done to precisely map the CO landscape of the var. *bisporus* revealing a remarkable CO landscape which clearly demonstrates the origin of most of the present-day commercial white cultivars of the button mushroom.

2. Materials and methods

An overview of the genetic analyses and the strains involved is given in the supplementary data (Fig. S1).

2.1. Strains

All heterokaryons used in this study were obtained from the strain collection of Wageningen UR Plant Breeding (Table 1). Cultures of commercial cultivars have been obtained in the past via spawn ordered from companies and stored as vegetative cultures in the collection. SSI isolation and confirmation of ploidy level was done as described elsewhere (Gao et al., 2013). The recovery of constituent nuclei as homokaryons from heterokaryons was done by protoplasting as described previously (Sonnenberg et al., 1988).

2.2. DNA isolation

For sequencing, high molecular weight DNA of good quality and in high quantity is required. To obtain this, mycelium was harvested after growing for two weeks on agar plates covered with cellophane, subsequently lyophilized and grinded to a fine powder in an Eppendorf tube. One ml of DNA extraction buffer (200 mM Tris/HCl pH 8.0; 25 mM EDTA; 250 mM NaCl) was added and mixed thoroughly. Subsequently, 700 µl phenol and 300 µl SEVAG (Chloroform:isoamyl alcohol, 24:1 v/v) were added and mixed. After centrifugation for 1 h at 14,000 rpm (4 °C) the supernatant was transferred to a new tube, 12 µl RNase (10 mg/ml) was added and the mixture incubated at 37 °C for 30 min. One vol. of SEVAG was added, mixed gently and centrifuged for 30 min at 14,000 rpm (4 °C). The supernatant was transferred to a new tube and 0.55 vol. isopropanol (−20 °C) was added and gently mixed. After centrifugation for 10 min at max rpm (4 °C), the pellet was washed with 70% ethanol (−20 °C), dried and dissolved in 30 µl $T_{10}E_{0.1}$. For genotyping, genomic DNA was extracted from lyophilized mycelium using the Wizard® Magnetic 96 DNA Plant System (Promega) according to the manufacturer's protocol.

2.3. De novo sequencing and genome comparison

High molecular DNA of one of the constituent nuclei of Horst U1 (homokaryon H39) was *de novo* sequenced with the PacBio RSII technology using and p6-c4 chemistry (Pacific Biosciences, Menlo Park, California, USA). The sequence data (1,147,175 reads with a N50 length of 9461 nt, total 6,446,332,774 nt representing a coverage of 200×) were assembled into scaffolds using HGAP2 and polished with the Quiver protocol; both are part of SMRT® Analysis version 2.3.0.140936.p1.142411 algorithm (SMRT® portal, Pacific Biosciences, Menlo Park, California, USA). Resulting scaffolds were further assembled based on SMRT® View BridgeMapper (SMRT® portal, Pacific Biosciences, Menlo Park, California, USA), linkage map information (see Section 2 on linkage analysis) and the results of a BLAST search (CLC Genomics Workbench version 7.5.1, Qiagen, Aarhus, Denmark) of scaffold ends against all scaffolds.

Comparison between the genomes of homokaryons H39 and H97 was done using NUCmer (NUCleotide MUMmer) version 3.1 (Kurtz et al., 2004) with the following settings for the whole genome: mincluster = 1000, minmatch = 100; settings for scaffold-comparison: mincluster = 500 and nosimplify. A graphical presentation of repetitive elements for each chromosome in both H97 v3.1 and H39 v3.1 was done by Blasting individual chromosomes against the RepBase database (Temple et al., 2008), using the following settings: "sequence source" fungi and selecting "forced translated search". All other options were not used. The graphical output was sized to each chromosome and plotted along the respective chromosome in the MUMmer plots.

2.4. Annotation and graphical presentation of chromosome ends

Chromosome ends of each line (100 kb of each site) were Blasted against the whole genome using the Blast function in CLC Work Bench version 7.6.2 (Qiagen Aarhus A/S) to detect repetitive elements. The fragments of all repetitive elements detected, were used to reconstruct 3 types of *penelope* non-LTR elements. These were annotated on the genome and a graphical output was generated using CLC Main Work Bench 7.6.2 (Qiagen).

2.5. SNP detection

SNP between the constituent nuclei of the commercial cultivar Horst U1 were detected by resequencing homokaryon H39 (Illumina HiSeq 2000) and aligning the reads to the previous version of genome of the other constituent homokaryon H97 version 2.0 (http://genome.jgi-psf.org/) since this work was done before the latest version of H97 genome was available. SNP markers were designed and the genotyping were carried out at the Dr. van Haeringen Laboratorium B.V. Wageningen (The Netherlands) using the KASPar SNP Genotyping System (KBiosciences Competitive Allele Specific PCR SNP genotyping system).

2.6. Linkage analysis

The commercial cultivar Horst U1 was used to generate a linkage map. Hundred and thirty nigh homokaryotic offspring were genotyped using 629 SNP markers. Markers were chosen in such a way that they were evenly distributed over the genome varying from 83 (the longest chromosome 1) to 31 (the smallest chromosome 13) markers per chromosome. The linkage map was constructed using Joinmap 4.1 (Van Ooijen, 2011) with a haploid model (HAP) using Kosambi's mapping function and default parameters setting (independence LOD score; significance levels from 2.0 to 10.0 LOD). Groups were defined at the level of LOD $\geqslant$ 5, skewed segregation of markers was tested with chi-square ($P < 0.05$), and the crossover frequency was calculated as the crossover number per individual per chromosome. Due to a lack of recombination, the ranking order of markers within each linkage group deviates in Joinmap from their known position. For this reason we plotted the cumulative genetic distances (cM) of the SNP markers as calculated with Joinmap against their corresponding cumulative physical distance (kb).

2.7. Detection of crossovers with whole genome sequencing and graphical presentation

To exactly map crossovers, whole genomes were sequenced on a HiSeq 2000 instrument (Illumina) with a coverage of 50×. For this purpose, high molecular DNA was isolated (see Section 2) from two homokaryotic Horst U1 single spore progenies, four recovered constituent nuclei of two heterokaryotic Horst U1 progenies, and four recovered constituent nuclei of two commercial cultivars (Table 1). The reads were aligned to the H97 reference genome (repaired version 3.1) using the Burrows-Wheeler Alignment tool (BWA) backtrack algorithm of BWA version 0.7.7-r441 (Li and Durbin, 2009) and SAMtools version 0.1.19-44428 cd (Li et al., 2009) was used to position sort the aligned reads. Using Picard tools version 1.130 (http://picard.sourceforge.net/), the reads were reordered and duplicates removed. For the final indel realignment, GATK version 2.8-1-g932cd3a (McKenna et al., 2010) was used. All variants were called using Freebayes version v0.9.18-1-g4233a23 (Garrison and Marth, 2012). The resulting VCF file was filtered retaining only the positions of type "SNP" with a read depth >5. For the remaining positions, the data was converted to genotypes and only the positions that represent an SNP between H97 and H39 were retained. The dataset was filtered to retain only the positions that contained genotype data for all homokaryons. This final dataset was converted to be used as input for an adapted R-script (originally part of ReCombine (Anderson et al., 2011) and kindly provided to us by Dr. Carol M. Anderson, (University of California, San Francisco) to plot the genotypes of half tetrads.

3. Results

3.1. A comparison between the two genomes of the cultivar Horst U1

For a detailed analysis of meiotic recombination in the *bisporus* variety Horst U1, the availability of high-quality genomes of both

Table 1
Strains used in this study are all derived from the culture collection of Plant Breeding Wageningen UR. Except for Horst U1, all commercial lines were obtained by ordering spawn from the corresponding spawn company (year spawn obtained in parentheses). The constituent nuclei of the heterokaryotic SSI and the commercial lines were recovered as homokaryon by protoplasting. The method used for genotyping each haploid/nucleus is indicated.

Strain	Description	Constituent nuclei (recovered as homokaryons)	Genotyping
			Improved WGS from previous *de novo* sequence
H97	Constituent homokaryon of Horst U1	H97	
H39	Constituent homokaryon of Horst U1	H39	*De novo* sequence
BKR814	Homokaryotic SSI of Horst U1	BKR814	Resequenced (Illumina)
BKR099	Homokaryotic SSI of Horst U1	BKR099	Resequenced (Illumina)
BKR02	Heterokaryotic SSI of Horst U1	BKR02_p1 & BKR02_p2	Resequenced (Illumina)
BKR011	Heterokaryotic SSI of Horst U1	BKR011_p1 & BKR011_p2	Resequenced (Illumina)
Horst U1	First commercial white hybrid	H97 & H39	
Sylvan A15 (2002)	Commercial cultivar	A15_p1 & A15_p2	Resequenced & SNP
Amycel 2200 (1995)	Commercial cultivar	2200_p1 & 2200_p2	Resequenced & SNP
Sylvan 512 (1995)	Commercial cultivar	512_p1 & 512_p2	SNP
Amycel Delta (2004)	Commercial cultivar	Delta_p1 & Delta_p2	SNP
Intern. Spawn 643 (1989)	Commercial cultivar	IS_p1 & IS_p2	SNP
BKR population	139 haploid SSI of Horst U1	Haploid meiotic offspring Horst U1	SNP

constituent nuclei, i.e. homokaryons H97 and H39, is needed. One of the homokaryons (H97) has been sequenced by JGI (http://genome.jgi-psf.org/) and was described previously (Morin et al., 2012). This sequence shows some misassembly as mentioned earlier (Foulongne-Oriol et al., 2013). Using the linkage map of Horst U1 (see later), this misassembly was repaired: the left 197 kb of scaffold 12 was transferred to the end of chromosome 1 and the remainder of scaffold 12 was rearranged resulting in the repositioning of internal telomere sequences to both ends of this chromosome; scaffold 14 appeared to be a duplicate of scaffold 12 and has been removed. The new version has been deposited at JGI (H97 v3.1) and NCBI (see supplementary Table S1 for accession numbers).

The other constituent nucleus (homokaryon H39) was *de novo* sequenced using PacBio with a coverage of 200× (sequence submitted at NCBI; see supplementary Table S1 for accession numbers). A total sequence length of more than 31 Mb was generated with an average read length of >560 kb resulting in 60 scaffolds. Using the linkage map of Horst U1 (see later), the number of scaffolds was reduced to 16. Chromosomes 6, 11 and 13 each consist of 2 non overlapping scaffolds whereas all other scaffolds were assembled to represent full chromosomes. The right end of chromosome 9 is not yet identified but is expected to be one of the smaller not mapped scaffolds. This part contains the ribosomal DNA cluster and this might have caused problems in the assembling process. All chromosomes contain the typical *A. bisporus* telomere pattern (Foulongne-Oriol et al., 2013) on both sites except for the missing right end of chromosome 9. For the corrected version of H97 (v3.1) now also most chromosomes have telomeric sequences at the end except for the left side of chromosome 7 and the right side of chromosomes 9 and 13 (Table 2).

The alignment of both genomes allows the comparison of the genomic architecture between H97 and H39. A high level of collinearity is shown (Fig. 1A; Supplementary materials Fig. S2 for a one to one chromosome comparison). Some chromosomes show very small patches of inversions and only for chromosome 4, a large inversion on the chromosome of H39 is seen in comparison to chromosome 4 of H97 (Fig. 1B). This latter inversion may represent a real inversion but could also be due to a remaining misassembly in the H97 genome. In the alignments of each chromosome pair, gaps are seen. In a number of cases these gaps show also shifts in the alignment indicating unique sequences present in only one of the homokaryons (Supplementary materials Fig. S2). Most gaps coincide with clusters of repetitive elements. The reconstructed chromosomes 1 and 12 of H97 aligned well with the relevant *de novo* regions of H39 indicating the correct repair of H97.

Previously, the chromosome lengths of H97 and H39 were estimated using CHEF gel analysis (Sonnenberg et al., 1996). A comparison between the estimated lengths and the now available physical length of the H97 and H39 chromosomes shows that the length estimation by pulsed field electrophoresis has been quite accurate since the differences in lengths assessed by the two methods for each homolog varies between 1 and 3%. The small differences between the CHEF gel estimated length of the H39 chromosomes 6, 11 and 12 and the physical length of these chromosomes indicate that the gap between the two non-overlapping scaffolds on each chromosome is relatively small and coincide with a repeat cluster (Supplementary materials Fig. S2). The genome sequences alignment shows that the chromosome length polymorphism between *A. bisporus* strains H97 and H39 is almost exclusively due to differences in the amount of repetitive elements. The largest difference in length between homologs is seen for chromosome 9 and, next to the missing sequences at the end, is caused by the difference in the number of single rDNA units in the ribosomal cluster between H97 and H39 as reported previously (Sonnenberg et al., 1996).

Both genomes have a SNP density of 1 on every 110 bp and these are not evenly distributed over the genome. Some areas have a very low SNP density (shown later) but still allow the generation of sufficient SNP markers for linkage analysis. Except for chromosome 4 that has a putative inversion, all other chromosomes show a very good collinearity and interference of large differences in genome organisation on recombination is thus not expected.

3.2. A linkage map of the heterokaryon Horst U1

The commercial cultivar Horst U1 was used to generate a linkage map. The total map length is 321 cM and consists of 13 linkage groups according to the 13 chromosomes published previously (Kerrigan et al., 1993; Sonnenberg et al., 1996). The analysis of segregation ratios showed that 203 markers (32%) deviate from the expected 1:1 ratio (chi-square test, $P < 0.05$), mainly markers from chromosomes 3, 4, 5 and 7 (Table 3). In order to visualize the recombination along each chromosome, the cumulative genetic distance (cM) were plotted against their corresponding cumulative physical distance (Supplementary materials Fig. S3). These plots

Table 2
Details on length and completeness of chromosomes of the homokaryons H39 v3.1 and H97 v3.1, both the constituent nuclei of the first commercial variety Horst U1. Both genomes have been deposited at NCBI (submission numbers. . ..).

Homokaryon H39 v.3.1					Homokaryon H97 v3.1				
Chromosome	Estimated length (CHEF data)[a]	Size (bp)	Left telomere	Right telomere	Chromosome	Estimated length (CHEF data)[a]	Size (bp)	Left telomere	Right telomere
1	3.6	3,779,356	Yes	Yes	1	3.65	3,550,205	Yes	Yes
2	3.44	3,241,356	Yes	Yes	2	3.14	3,489,786	Yes	Yes
3	3.06	3,126,264	Yes	Yes	3	3.06	3,131,856	Yes	Yes
4	3.06	3,115,696	Yes	Yes	4	3.06	3,112,789	Yes	Yes
5	2.57	2,477,729	Yes	Yes	5	2.49	2,550,681	Yes	Yes
6[b]	2.32	2,476,259	Yes	Yes	6	2.49	2,329,815	Yes	Yes
7	2.32	2,233,015	Yes	Yes	7	2.23	2,323,169	No	Yes
8	1.9	2,155,647	Yes	Yes	8	2.11	1,953,186	Yes	Yes
9	2.13	1,624,235	Yes	No	9	1.89	1,688,379	Yes	No
10	1.81	1,787,784	Yes	Yes	10	1.75	1,762,792	Yes	Yes
11[b]	1.66	1,733,467	Yes	Yes	11	1.68	1,708,529	Yes	Yes
12[b]	1.51	1,576,767	Yes	Yes	12[c]	1.56	1,482,581	Yes	Yes
13	1.4	1,405,511	Yes	Yes	13	1.45	1,334,073	Yes	No
Total size		30,733,086			Total size		30,417,841		

[a] Chromosome length estimated by CHEF gel analysis (Sonnenberg et al., 1996).
[b] Chromosome consist of 2 non-overlapping scaffolds.
[c] Reconstructed/repaired compared to previous version (Morin et al., 2012).

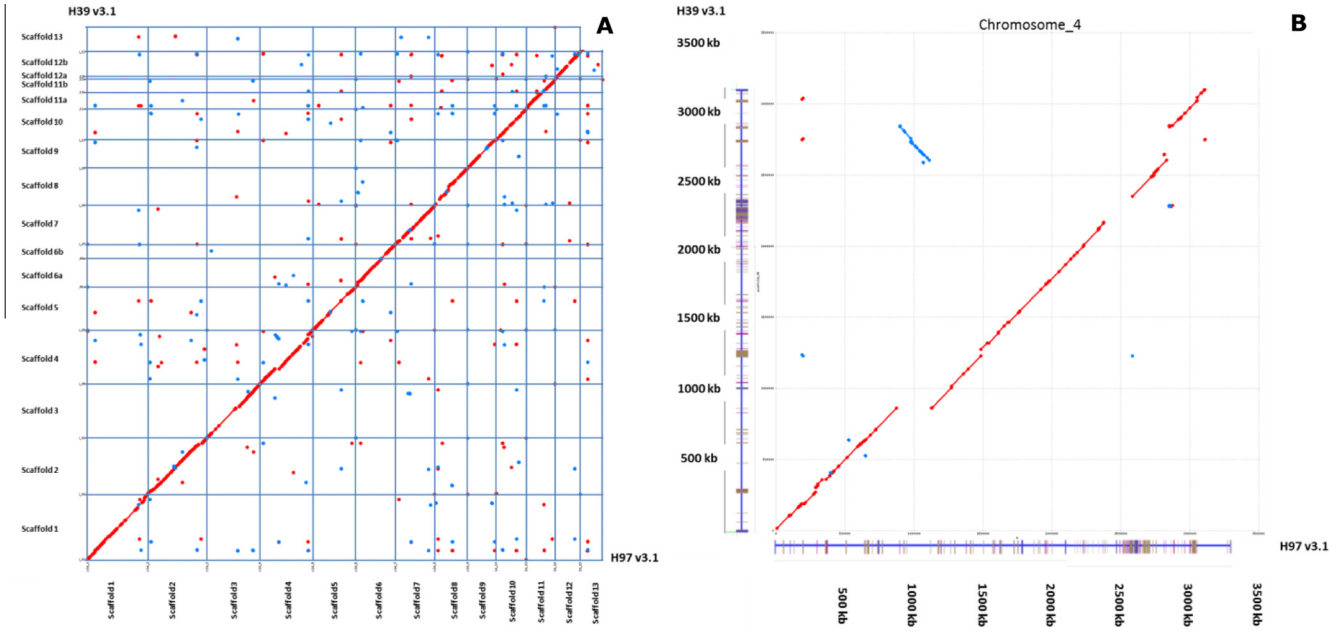

Fig. 1. Comparison of the genomes of H39 v3.1 and H97 v3.1 using MUMmer plots. (A) Comparison of all chromosomes of H39 and H97 genomes. The alignment of whole genomes shows a clear collinearity for all chromosomes. Chromosomes 6, 11 and 12 of H39 consist each of 2 scaffolds. Dots scattered throughout the figure represent repetitive sequences aligning on different genomic positions. Red: Sequences collinear; Blue: Sequences inverted in H39 compared to H97; The dots are red and blue due to different orientations of repetitive elements in the genome of both homokaryons. (B) MUMmer plot of the chromosomes 4 of both homokaryons. The plots shows an inversion (blue) in the H39 v3.1 relative to the relevant region in chromosome 4 of H97 v3.1. Details for all chromosome in supplemental materials Fig. S2.

Table 3
Map statistics of a segregating population of 139 homokaryotic SSI derived from Horst U1.

LG	Physical length of chromosomes (bp)			Marker range[a]	No. markers	No. distorted markers	No. of crossovers/ chromosome					CO frequency per chromosome	Ratio of end crossovers[d]	Parental types (%)
	H97vs3.1	H39vs3.1	Length (cM)				0 H39[b]	0 H97[c]	1	2	3			
I	3,550,205	3,779,356	22.6	5 kb to 3.4 Mb	83	0	50	62	20	0	0	0.30	1.00	0.85
II	3,489,786	3,241,356	3.6	60 kb to 3.4 Mb	73	1	75	61	3	0	0	0.04	1.00	0.98
III	3,131,856	3,126,264	21	23 kb to 3.1 Mb	53	53	71*	43	20	2	0	0.31	0.77	0.84
IV	3,112,789	3,115,696	6.8	123 kb to 30.5 Mb	62	62	58*	44	8	0	0	0.15	0.88	0.93
V	2,550,681	2,477,729	16.8	58 kb to 25.1 Mb	52	52	43*	73	19	2	0	0.29	0.62	0.85
VI	2,329,815	2,476,259	13.9	21 kb to 22.9 Mb	46	0	44	62	30	4	0	0.46	0.94	0.76
VII	2,323,169	2,233,015	37.5	49 kb to 22.9 Mb	60	27	44*	54	31	3	1	0.49	0.97	0.74
VIII	1,953,186	2,155,647	32.8	13 kb to 18.6 Mb	39	1	50	51	28	0	0	0.43	1.00	0.78
IX	1,688,379	1,624,235	5.7	59 kb to 16.0 Mb	33	0	55	68	7	0	0	0.11	0.71	0.95
X	1,762,792	1,787,784	58.7	17 kb to 17.4 Mb	36	2	44	35	41	8	0	0.70	0.94	0.62
XI	1,708,529	1,733,467	14.7	65 kb to 168.9 Mb	35	2	59	58	18	0	0	0.27	0.78	0.87
XII	1,482,581	1,576,767	42.3	34 kb to 147.2 Mb	26	2	49	50	39	2	0	0.57	1.00	0.71
XIII	1,334,073	1,405,511	45.3	14 kb to 132.3 Mb	31	1	60	59	45	3	0	0.56	0.98	0.71
Totals			321.7		629	203	702	720	309	24	1	Averages	0.89	0.81

[a] Marker range relative to the H97 v3.1 genome.
[b] Number of individuals with intact linkage group of H39.
[c] Number of individuals with intact linkage group of H97.
[d] End crossover is defined as a crossover within 100 kb from chromosome ends.
* Significant deviation from the expected 1:1 ratio (chi-square test, $P < 0.05$).

show clearly a restriction of CO at chromosome ends. The ratio of number of crossovers (COs) occurring in the first/last 100 kb of each chromosome over all COs varies from 0.62 to 1 with an average of 0.89 for all COs (Table 3). Covering the chromosome ends with suitable markers was not always possible due to the presence of telomeres and associated repeats. These consist almost exclusively of non-LTR *Penelope* sequences that are found in subtelomeric regions of many eukaryotic genomes (Gladyshev and Arkhipova, 2007; for an annotation of chromosome ends see Supplemental materials Fig. S4). Three classes of *Penelope* have been found, exclusively in association with telomere sequences and not present in other parts of the genome. They all have a reverse transcriptase motive and are likely involved in the maintenance of telomeres as in other eukaryotes (Fulcher et al., 2014). The regions containing *Penelope* copies vary from a few kb up to 40 kb. Thus, not all COs at chromosome ends might have been reg-

istered due to these repeats and as a consequence, more post-meiotic chromosomes are completely parental types (81%) than expected. Since the majority of the COs is confined to chromosome ends, most chromosomes are very similar to either one of the parental types (visualized in the genotype file of the 139 homokaryotic offspring in Supplemental materials Fig. S5).

3.3. Assessing crossovers using whole genome sequencing (WGS)

For a detailed analysis of COs, whole genomes of two homokaryotic (BKR814 and BKR099) and two heterokaryotic (BKR02 and BKR011) single spore isolates derived from Horst U1 were resequenced using Illumina HiSeq 2000. The same was done for two heterokaryotic commercial lines, Sylvan A15 and Amycel 2200 (Table 1 and Fig. S1). The constituent nuclei of all heterokaryons were recovered as homokaryons before sequencing using the protoplasting technique (Sonnenberg et al., 1988). Since in general both constituent nuclei from heterokaryons of *A. bisporus* var. *bisporus* result from pairing of non-sister nuclei, half tetrades can be analysed in this way, i.e. two post-meiotic nuclei derived from the same meiotic event. If chromatids involved in the same CO are paired into one spore, it will allow a detailed analysis of the CO region. That will reveal if DNA repair mechanisms of meiosis in the button mushroom will lead to gene conversion, a recombination with a nonreciprocal exchange of DNA resulting in a 3:1 segregation of the region involved (Mercier et al., 2015). All 10 homokaryons mentioned above were resequenced (Illumina HiSeq 2000) and reads were aligned to the reference genome H97 v3.1. A first analysis showed that the constituent nuclei of the cultivars Sylvan A15 and Amycel 2200 were completely identical. For further processing of data we, therefore, excluded Amycel 2200. The genotype and position of each SNP was plotted for each half tetrade using an adapted R script (plotTetradeSeg described in Anderson et al., 2011) resulting in a graphical presentation of the genotype of each homokaryon in each half tetrade (Fig. 2 and Supplemental Fig. S6). For all three half tetrades (BKR02, BKR11 and Sylvan A15) the major part of all chromosomes in both constituent nuclei were parental types and complementary, i.e. a H39 genotype in one nucleus is always opposed by a H97 genotype in the other nucleus and *vice versa* except for regions were a non-reciprocal CO has occurred. Non-sister nuclei where thus paired in these heterokaryons. The graphical annotations also show clearly the regions where SNP density is low resulting in gaps in the graphs. For each of the eight homokaryons (two homokaryotic SSI and the homokaryons derived from two heterokaryotic SSI and the commercial cultivar Sylvan A15) the position of COs was defined as equal to the position of the SNP preceding the genotype change. In this way, 71 COs were scored of which 20 are reciprocal. Of all COs, 92% are located within a distance of 85 kb from a chromosome end (Fig. 3). This demonstrates that in offspring of Horst U1, COs are mainly confined to the extreme ends of chromosomes as seen in the Horst U1 map discussed above. The single spore homokaryons and homokaryons recovered from two heterokaryotic SSIs of Horst U1 show for each chromosome end just one or no CO with one exception (chromosome 1 of BKR02-p1 shows a double recombination). For the constituent homokaryons of the cultivar Sylvan A15 a number of chromosomes show two CO at the end of chromosomes close to each other (14–39 kb distance between each pair of COs).

Of the 20 reciprocal COs, six showed a gene conversion (GC) (Fig. 4). The number of homozygous SNPs in the GC tracts varies from 1 to 3 SNPs with an average distance between the SNPs flanking the CO of 276 bp (ranging from 146 to 469 bp). This is the maximum region of GC. The minimum region is determined by the outermost SNPs within the GC tract and this is for the 3 GC where 2 or 3 SNP are homozygous on average 26 bp (varying 20–37 bp). The distance between flanking SNPs in a CO where no GC is seen is on average 984 bp. The region involved in gene conversion is thus small in the button mushroom and might have not been detected in reciprocal CO where the flanking SNP are too far apart. There are a number of regions in the three heterokaryons that show homozygosity for just 1 SNP. These were not scored as GCs and although the high sequence coverage indicates that all detected SNP are reliable we cannot exclude that single SNP may have been caused by sequence errors. Only one putative GC was seen not associated with a CO where two adjacent SNP are homozygous and one region with a more complex CO pattern (Fig. 4).

3.4. Pairing of non-sister nuclei and the origin of present-day commercial lines of white button mushrooms

The WGS of offspring of the cultivar Horst U1 described in the previous paragraph showed a pairing of non-sister nuclei and

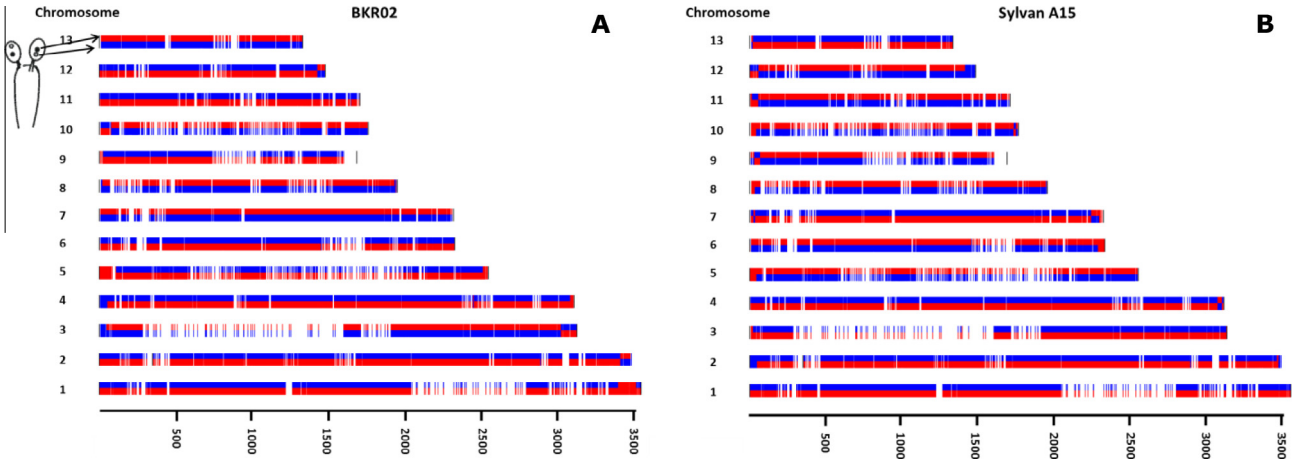

Fig. 2. Graphical representation of the genotype of all chromosomes of the constituent nuclei of 2 heterokaryons (red = H39 v3.1; blue = H97 v3.1). The X-axis indicates the physical position on each chromosome (nt × 1000); the Y-axis indicates the chromosome number. A: Genotypes of the constituent nuclei of a heterokaryon directly derived from Horst U1. Single crossovers are seen at most chromosome ends and one double crossover at the right end of chromosome 1. B: Genotypes of the constituent nuclei of the commercial variety Sylvan A15. Double crossovers are seen at the end of a number of chromosomes indicating that this line is a second generation isolate from Horst U1. Blank areas represent regions with no or very low SNP density. Genotypes of the other 4 nuclei can be found in Fig. S6. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

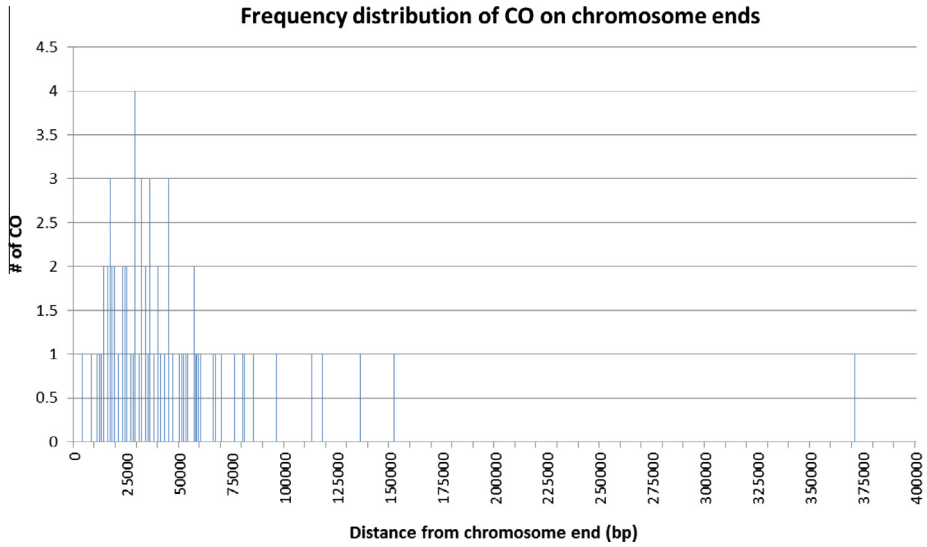

Fig. 3. Distribution of 71 CO in 6 resequenced homokaryotic offspring of the bisporic variety Horst U1 and the commercial variety Sylvan A15. The position of COs is expressed in bp from a chromosome end using the genome of H97 as the reference.

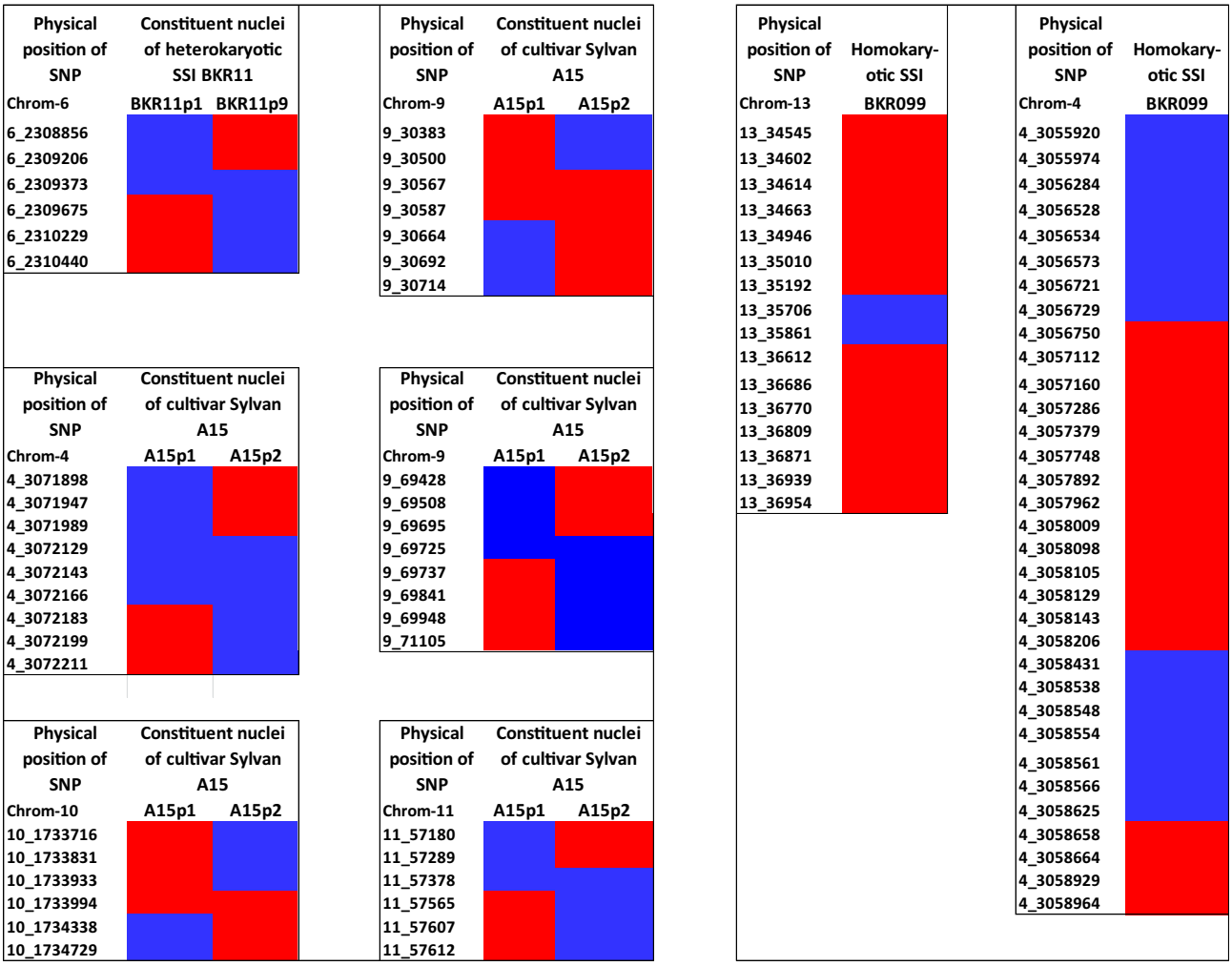

Fig. 4. Eight regions of gene conversions at reciprocal COs in the constituent nuclei of Sylvan A15 and BKR099. The position of each SNP is given per chromosome at the left side of each CO region (the first digit) followed by the position on that particular chromosome. The CO region can be defined as the region between the flanking SNPs (maximum) or the region between the outermost SNPs within the CO region. The left panel represent COs with a gene conversion; the right panel a gene conversion not associated with a CO and a complex CO.

restriction of COs to chromosome ends resulting in heterokaryotic offspring that has a genotype very similar to the parent since heterozygosity is preserved for most parts of all chromosomes. To support that this is the general rule in *A. bisporus* var. *bisporus* an additional 16 heterokaryotic single spore cultures of Horst U1 were genotyped. They were protoplasted to recover the constituent nuclei as homokaryons and genotyped using 4–5 markers per chromosome. Since a major part of each chromosome in the offspring has a parental genotype, a low number of markers is sufficient to see which parental type is inherited and if the constituent nuclei in each heterokaryon have complementary genotypes. Some markers (especially those at chromosome ends) failed in some samples. Since also the heterokaryons were genotyped with the same markers, in most cases the genotype of each line (the heterokaryon and both constituent homokaryons) could be deduced where only one marker was missing in one of the homokaryons. Markers of chromosome 8, however, dropped out too many times and this chromosome was omitted in the analyses. All constituent nuclei of each heterokaryon showed a complementary genotype except for regions were a non-reciprocal recombination had occurred (Fig. 5A). Only 10 recombinations were seen, of which three were reciprocal. These recombinations all occur for markers near chromosome ends similar to the BKR heterokaryotic offspring discussed in the previous paragraph. To demonstrate that, next to Sylvan A15 and Amycel 2200, most commercial lines of the white button mushrooms used now or in the last 20 years are derived

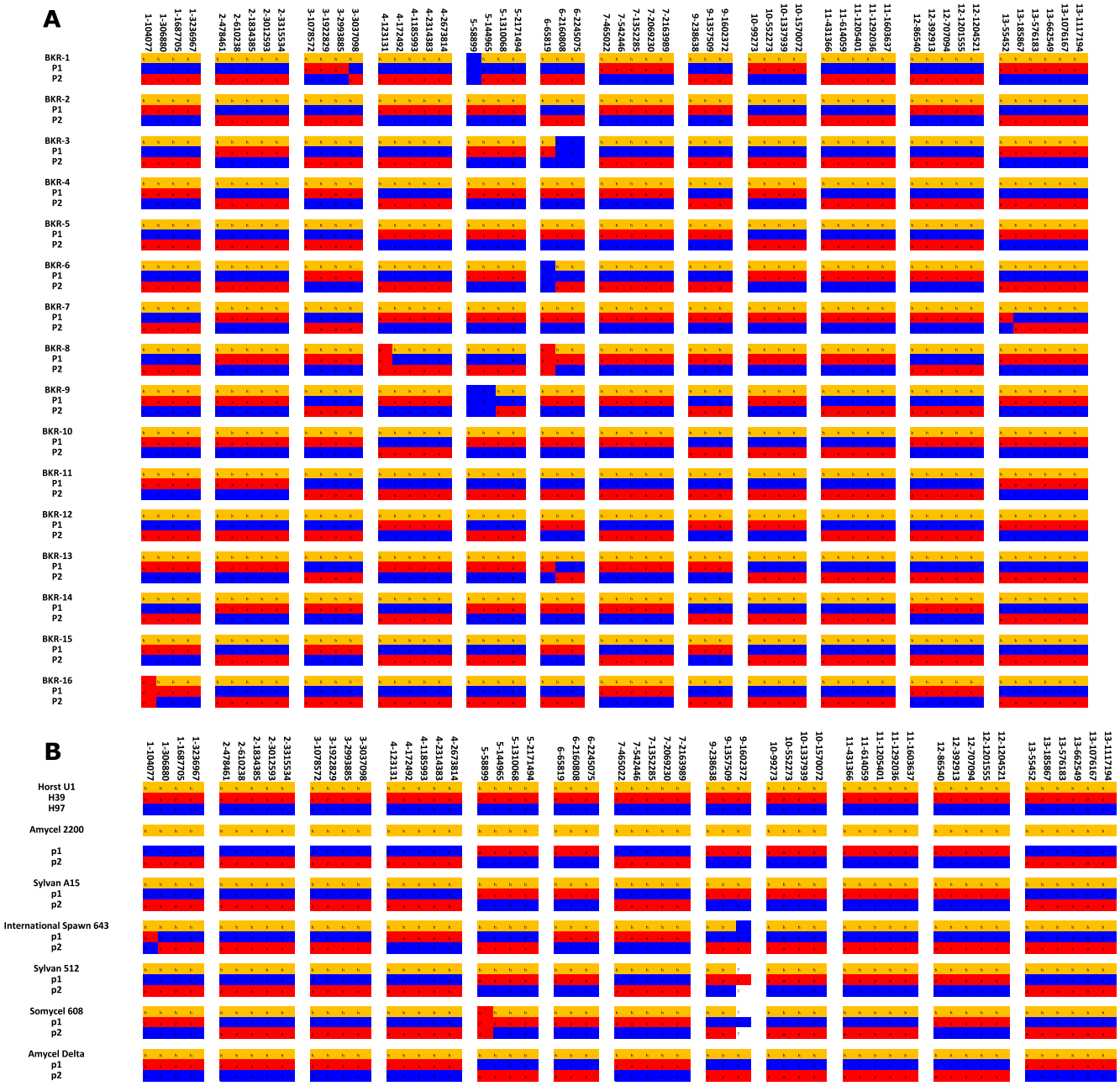

Fig. 5. Genotype for each chromosome of heterokaryons and their constituent nuclei with a restricted number of SNP for each chromosome (chromosome 8 is missing due to dropout of markers). The first row in each line is the genotype of the heterokaryon (mostly yellow = heterozygous), the constituent nuclei have mostly the genotype of H39 (red) or the genotype of H97 (blue). Each column represent a chromosome with on top of each column the position of markers used. (A) 16 heterokaryons derived from Horst U1. (B) 6 commercial varieties of the white button mushroom and their constituent nuclei. Varieties tested are Horst U1, Amycel 2200, Sylvan A15, International Spawn 643, Sylvan 512, Somycel 608 and Amycel Delta).

from the first hybrid Horst U1, both constituent nuclei of an additional four cultivars, Int. Spawn 643, Sylvan 512, Somycel 608 and Amycel Delta, were genotyped using the same markers as used for genotyping the heterokaryotic offspring. In each of the six commercial lines (including Amycel 2200 and Sylvan A15) the constituent nuclei had complementary Horst U1 genotypes except for regions were recombination had occurred (Fig. 5B). Only Sylvan A15 and Amycel 2200 were identical whereas the other cultivars were different due to a different distribution of parental types over the two nuclei and due to COs.

4. Discussion

Two high quality genomes representing the first commercial hybrid of the white button mushroom are now publically available . . .(H97 v3.1 & H39 v3.1) and certainly can contribute to research and breeding for this commercially important edible mushroom. The high collinearity of these genomes indicate that it is unlikely that structural differences have influenced position and frequencies of the CO or could be the cause of skewed segregation. The alignment study revealed also that the physical length differences between the homologs in H97 and H39 are almost exclusively due to differences in the amount of repetitive elements, mostly present in small or larger clusters (Supplemental Fig. S2). A BLAST of the large clusters showed that most elements in these clusters are LTR-retrotransposons of the *copia* and *gypsy* type (varying 60–80% among chromosomes).

Gao et al. (2015) used H97 and H39 in QTL mapping of bruising sensitivity in *A. bisporus* var. *bisporus* for generating two segregating populations with H97 and H39 as parental lines. The second parent of each population was a homokaryon each originating from *A. bisporus* var. *bisporus* heterokaryons collected from the wild and genetically not related to any commercial variety. The linkage maps of these populations were also very short (166 and 86 cM using 95 and 76 SNP markers, respectively) and recombinations were mainly seen with markers at chromosome ends. The similarity of the recombination landscape in these three populations involving four genetically non-related *A. bisporus* var. *bisporus* homokaryons as parents indicates that restriction of recombination mainly to chromosome ends might be typical for this variety.

The WGS of eight homokaryons allowed a more precise mapping of COs showing that 92% of the 71 COs mapped occurs in the first/last 85 kb of each chromosome. The majority of the chromosomes in these first generation SSI from Horst U1 (two homokaryotic SSI and four recovered homokaryons of two heterokaryotic SSI) are parental types or show one CO (45% of each class), whereas 9% have two COs and only 1% three COs. Assuming that each bivalent has at least one (obligate) CO involving two of the four chromatids (and a single crossover is also the most frequent type seen), the number of complete parental type expected would be approximately 50%, close to what is observed in the sequenced offspring. It is likely that some COs have not been detected because of missing sequences of chromosome ends. No COs were seen for the right end of chromosomes 9 and 13 where no telomere and the telomere associated *Penelope* sequences were seen in the H97 genome sequence. The number of chromosomes with one CO at both chromosome ends might thus be somewhat higher, suggesting that WGS detects all COs if high quality reference genomes are available. This in contrast to genotyping offspring with a limited number of SNP markers as was done for the homokaryotic offspring of Horst U1 (629 SNP on 139 homokaryotic SSI). Here, 81% of the chromosomes were parental types and thus a low fraction of chromosomes with a CO was seen indicating that with SNP markers many COs at chromosome ends are not detected. Single SNPs fail thus easily whereas WGS generate sufficient data to reliably genotype chromosome ends. Covering the chromosome ends with suitable markers is not always possible due to the presence of telomeres and associated repeats.

Previous segregation analyses of *A. bisporus* suggest that recombination frequency in the *bisporus* variety is overall lower than in the *burnettii* variety (Callac et al., 1997; Foulongne-Oriol et al., 2010). Foulongne-Oriol et al. (2010) have generated a near-saturated linkage map using offspring of a cross between a *bisporus* and a *burnettii* homokaryon. The results denote that the *burnettii* partner in the cross is responsible for a map extension to 1156 cM. Recombination sites were found more or less over the whole genome with clustering in some regions. Extensions in the map near the telomeres show a higher CO frequency in these regions than in other chromosomal regions suggesting that the *burnettii* variety might have a more or less equal distribution of recombination sites over all chromosomes and that the CO landscape in the offspring of the inter-varietal cross is intermediate to that of both parents. However, a proper comparison between CO landscapes of the inter-varietal and the *bisporus* variety offspring can only be made if all COs are detected. A comparison of the frequencies of parental and non-parental post-meiotic chromosomes between the inter-varietal offspring (Foulongne-Oriol et al., 2010) and the Horst U1 offspring, the latter using SNP markers and WGS, demonstrates this well (Table 4). A much higher parental type of chromosomes was found in the *bisporus* offspring than in the inter-varietal offspring when a limited number of markers are used. Data obtained for Horst U1 with WGS, however, are very similar to the data of the inter-varietal offspring, i.e. a similar percentage of parental chromosomes and a considerably higher number of chromosomes with COs. For the inter-varietal map some CO might have been missed at chromosome ends because here no WGS were used but since COs in the offspring of the inter-varietal cross are more evenly distributed over the genome, the number of COs at chromosome ends is considerably less than in the *bisporus* offspring and consequently not many COs might have been missed. The difference in COs between the *bisporus* and the *burnettii* variety is thus mainly the position and not frequencies of COs. An almost exclusive recombination at the very tips of chromosome ends is, as far as we know, never demonstrated before in other organisms.

A remarkable observation was the occurrence of adjacent recombinant sites at chromosome ends in the commercial cultivar Sylvan A15. Each of these double CO is located in proximity of its counterpart (14–39 kb). Assuming that the COs are type I showing interference (Mercier et al., 2015), the most plausible explanation is that each CO in these doublets were generated in consecutive meiotic events. Sylvan A15 is thus likely generated by selecting a heterokaryotic SSI from a previously selected heterokaryotic SSI derived directly from Horst U1. Sylvan A15 can thus be considered

Table 4
A comparison of the frequencies of the type of post-meiotic chromosomes found in offspring of the intervarietal cross (Foulongne-Oriol et al., 2010) and Horst U1 (this study). The differences between the percentages of parental type of chromosome found in the variety bisporus is clearly influenced by the genotyping method. Only the whole genome sequencing (WGS) detects all COs indicating that the frequency of CO in the offspring of the intervarietal cross is similar to that of the bisporic variety but they differ mainly in position of COs.

	bisp × burnetii	H97 × H39	
	AFLP, microsatt. & CAPS markers (%)	SNP genotyping (%)	WGS genotyping (%)
Parental types	48	81	45
1 CO	31	18	45
2 CO	13	1	9
3 CO	4	0	1
4 CO	2	0	0
>5 CO	1	0	0

as a second generation SSI. Four nuclei of the two first generation homokaryons derived from Horst U1 (BKR heterokaryons, see Table 1) have on average nine COs per homokaryon whereas the Sylvan A15 constituent homokaryons show 18 COs per homokaryon supporting the assumption that Sylvan A15 is a second generation SSI of Horst U1 and the two subsequent meiosis generated twice the number of COs.

The genotyping of the constituent nuclei in 16 heterokaryotic SSI shows the preference for pairing of non-sister nuclei and also a balanced chromosome distribution. Aneuploidy is rarely seen in the heterokaryotic SSI (unpublished data, Sonnenberg) indicating that each bivalent shows at least one "obligate" CO. Why this CO is preferentially located at chromosome ends in the variety *bisporus* is not clear. Only a few cytological observations have been done in the past on meiosis in the button mushroom. Evans (1959) was the first to make a detailed meiotic study of this mushroom. He detected 12 chromosomes and reported that after nuclear fusion, the 24 chromosomes are always associated as 12 bivalents at meiosis. The limited optical resolution of his microscopic images, however, did not allow describing precise morphology of the chromosome pairing and chiasma formation. Saksena et al. (1976), who used an improved fluorescence microscopic technique showed nine chromosome pairs at meiotic prophase, but further details were lacking due to limits in resolution. Mazheika et al. (2006) used electron microscopy of silver stained spread protoplasted meiotic prophase I hymenium of the *bisporus* and the *burnettii* variety and demonstrated that in the *burnettii* variety, homologs formed regular synaptonemal complexes (SCs), whereas the *bisporus* variety showed only very short patches of axial elements and synaptonemal complexes. The authors concluded that the formation of axial structures in prophase I is disrupted in the *bisporus* variety leading to a reduced recombination frequency. Our research, however, indicate that the position and not frequency of recombination differs between the *bisporus* and *burnettii* variety. How to interpret the cytological observation of Mazheika and co-authors with this respect is unclear. The authors mention "Some nuclei contained small completely mature SCs among short asynaptic AEs. In some nuclei, very short SCs were observed together with long AEs with SC fragments near the telomeres". One option would be that, after the formation of DSB and single-stranded DNA overhangs, possibly at a number of positions along the chromosome, the search for homology starts at chromosome ends where chromosome alignment starts first followed by synapsis, also started from chromosome ends as has been suggested by Harper et al. (2004). The formation of bouquet, a clustering of chromosome ends at the nuclear enveloped could play a role in this. Although the bouquet formation itself does not seem to be a prerequisite for homologous pairing and SC formation it might direct the start of the SC formation to chromosome ends. Xiang et al. (2015) showed recently that in flatworms, after the clustering of chromosome ends in a bouquet, the SC formation is initiated at or near the paired telomeric region and extended outward to bring arms into full synapsis. If the formation of SC in the *bisporus* variety is somehow disrupted in an early state, recombination sites can only be solved in a CO in regions where SC is formed, in this case at chromosome ends. A higher CO frequency at chromosome ends is more often seen in other organisms (Mézard et al., 2015) but an almost exclusive position of COs at the extreme ends of chromosome has to our knowledge not been reported before. A more detailed cytological study and genetic analysis will be needed to unravel the mechanism that restricts the formation of COs to the extreme chromosome ends in the variety *bisporus*.

The question remains why the *bisporus* variety retains most of its genome heterozygous by restricting recombination to a small chromosome area while combining non-sister nuclei. One explanation is that the heterozygous state can shelter deleterious alleles (haplo-lethal) so that heterozygosity for these alleles contributes somehow to fitness. Extensive suppression of recombination can indeed lead to the generation of lethal alleles as was shown for the non-recombining mating type region in the smut-fungus *Microbotryum violaceum* (Fontanillas et al., 2014). The failure to isolate one of the mating types in the haplotypes underlines this (Hood and Antonovics, 2000). Such alleles should be eliminated by strong selection in the haploid phase but *M. violaceum* often undergoes mating among the immediate products of meiosis and thus avoids the haploid phase. This intratetrad mating also occurs often in the *bisporus* variety. However, haplotypes/homokaryons can be easily isolated in this strain and, although a few chromosomes are biased towards one parental type, all parental types of chromosomes were observed in non-recombined haploid offspring, thus suggesting that sheltering of lethal alleles might not be a complete explanation. It is still possible that certain combinations of parental types are not viable in a homokaryon (epistatic effects) but that requires screening of a large set of homokaryons to reveal such unique combinations. Alternatively, one might think that the intratetrad mating is beneficial because it eliminates the need to find a mating partner. Heterokaryons also have a higher growth rate than homokaryons and might have an advantage when spread in nature.

The typical CO landscape of the *bisporus* variety has severe complications for breeding. The introduction of new traits requires the introgression of certain parts of donor genome, usually of wild isolates. These have often a low quality and suboptimal yields. The typical CO landscape of the variety *bisporus* will cause considerable linkage drag, i.e. unwanted introduction of wild genome and often result in lower quality mushrooms than the present-day hybrids (unpublished data from our own breeding programs). Previous research has shown that using offspring of bisporic varieties QTL can only be assigned to chromosome and not precisely mapped on chromosomes (Gao et al., 2015). This study can contribute to the understanding of the mechanisms for the typical CO landscape in the variety *bisporus* and comparison to the "normal" CO landscape in the variety *burnettii* might lead to detect the genetic determinants involved in the different mechanisms and introduce the proper components into breeding stock of this commercially important mushroom.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fgb.2016.06.001.

References

Allen, J.J. et al., 1992. Persistent meiotic arrest in basidia of *Agaricus bisporus*. Mycol. Res. 96, 125–127.

Anderson, C.M. et al., 2011. ReCombine: a suite of programs for detection and analysis of meiotic recombination in whole-genome datasets. PLoS ONE 6 (10), e25509.

Blakeslee, A.F., 1904. Sexual reproduction in the Mucorineae. Proceedings of the American Academy of Arts and Sciences, vol. 40, pp. 205–319.
Callac, P. et al., 1993. Morphological, genetic, and interfertility analyses reveal a novel, tetrasporic variety of *Agaricus bisporus* from the Sonoran desert of California. Mycologia 85, 835–851.
Callac, P. et al., 1997. Conservation of genetic linkage with map expansion in distantly related crosses of *Agaricus bisporus*. FEMS Microbiol. Lett. 146, 235–240.
Callac, P. et al., 2003. A novel homothallic variety of *Agaricus bisporus* compromises rare tetrasporic isolates from Europe. Mycologia 96, 222–231.
Callac, P. et al., 1998. Evidence for PPC1, a determinant of the pilei-pellis color of *Agaricus bisporus* fruitbodies. Fungal Genet. Biol. 23, 181–188.
Evans, H.J., 1959. Nuclear behaviour in the cultivated mushroom. Chromosoma 10, 115–135.
Fontanillas, E. et al., 2014. Degeneration of the nonrecombining regions in the mating-type chromosomes of the anther-smut fungi. Mol. Biol. Evol. 32 (4), 929–943.
Foulongne-Oriol, M. et al., 2009. Novel microsatellite markers suitable for genetic studies in the white button mushroom *Agaricus bisporus*. Appl. Microbiol. Biotechnol. 84, 1125–1135.
Foulongne-Oriol, M. et al., 2010. An expanded genetic linkage map of an intervarietal *Agaricus bisporus* var. *bisporus* × *A. bisporus* var. *burnettii* hybrid based on AFLP, SSR and CAPS markers sheds light on the recombination behaviour of the species. Fungal Genet. Biol. 47, 226–236.
Foulongne-Oriol et al., 2011. Comparative linkage mapping in the white button mushroom *Agaricus bisporus* provides foundation for breeding management. Curr. Genet. 57, 39–50.
Foulongne-Oriol, M. et al., 2012a. QTL mapping of yield-related components and oligogenic control of the cap colour in the button mushroom *Agaricus bisporus*. Appl. Environ. Microbiol., 2422–2434
Foulongne-Oriol, M. et al., 2012b. Relationship between yield components and partial resistance to *Lecanicillium fungicola* in *Agaricus bisporus* assessed by QTL mapping. Appl. Environ. Microbiol. 78, 2435–2442.
Foulongne-Oriol et al., 2013. Genome-wide survey of repetitive DNA elements in the button mushroom *Agaricus bisporus*. Fungal Genet. Biol. 55, 6–21.
Fritsche, G., 1982. Genetics and breeding of the cultivated mushroom. In: Buenemann, G. (Ed.), Proceedings of the 21st International Horticultural Congress, Hamburg, Germany, pp. 665–676.
Fulcher, N. et al., 2014. If the cap fits, wear it: an overview of telomeric structures over evolution. Cell. Mol. Life Sci. 71, 847–865.
Gao, W. et al., 2013. Genetic variation and combining ability analysis of bruising sensitivity in *Agaricus bisporus*. Plos One 8 (10), e76826.
Gao, W. et al., 2015. Quantitative trait locus mapping for bruising sensitivity and cap color of *Agaricus bisporus* (button mushroom). Fung. Genet. Biol. 77, 69–81.
Garrison, E., Marth, G., 2012. Haplotype-based Variant Detection from Short-read Sequencing. arXiv.:1207.3907 [q-bio.GN].
Gladyshev, E.A., Arkhipova, I.R., 2007. Telomere-associated endonuclease-deficient *Penelope*-like retroelements in divers eukaryotes. Proc. Nat. Acad. Sci. 104, 9352–9357.
Harper, L. et al., 2004. A bouquet of chromosomes. J. Cell Sci. 117, 4025–4032. http://dx.doi.org/10.1242/jcs.01363.
Hood, M.E., Antonovics, 2000. Intratetrad mating, heterozygosity, and the maintenance of deleterious alleles in *Microbotryum violaceum* (*=Ustilaga violocea*). Heredity 85, 231–241.
Kamzolkina, O.V. et al., 2006. Karyological evidence for meiosis in the three different types of life cycles existing in *Agaricus bisporus*. Mycologia 98 (5), 763–770.
Kerrigan, R.W. et al., 1993. Meiotic behaviour and linkage relationships in the secondarily homothallic fungus Agaricus *bisporus*. Genetics 133, 225–236.
Kerrigan, R.W. et al., 1994. The heterothallic life cycle of *Agaricus bisporus* var. burnettii, and the inheritance of its tetrasporic trait. Exp. Mycol. 18, 193–210.
Kühner, R., 1977. Variation of nuclear behaviour in the homobasidiomycetes. Trans. Brit. Mycol. Soc. 68, 1–16.
Kurtz, S. et al., 2004. Versatile and open software for comparing large genomes. Genome Biol. 5, R12.
Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754–1760.
Li, H. et al., 2009. The Sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079.
Mazheika, I.S. et al., 2006. Abnormal meiosis in *bisporus* strains of white button mushroom *Agaricus bisporus* (Lange) Imbach. Russ. J. Genet. 42, 279–285.
McKenna, A. et al., 2010. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20, 1297–1303.
Mercier, R. et al., 2015. The molecular biology of meiosis in plants. Ann. Rev. Plant Biol. 66, 297–327.
Mézard, C. et al., 2015. Where to cross? New insight into the localisation of meiotic crossovers. Trends Genet. 31, 393–401.
Moquet, F. et al., 1998. Selfproduction induces variability in agronomic traits for a wild *Agaricus bisporus*. Mycologia 90, 806–812.
Morin, E. et al., 2012. Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. Proc. Natl. Acad. Sci. USA 109, 17501–17506.
Ni, M. et al., 2011. Sex in fungi. Annu. Rev. Genet. 45, 405–430.
Pardo, A. et al., 2010. Modelling the effect of the physical and chemical characteristics of the materials used as casing layers on the production parameters of *Agaricus bisporus*. Arch. Microbiol. 192, 1023–1030.
Royse, D.J., May, B., 1982. Use of isozyme variation to identify genotypic classes of *Agaricus brunnescens*. Mycologia 74, 93–102.
Saksena, K.N. et al., 1976. Study on development of *Agaricus bisporus* by fluorescent microscopy and scanning electron microscopy. J. Bacteriol. 126, 417–428.
Sonnenberg, A.S.M. et al., 1996. Isolation of expressed sequence tags of *Agaricus bisporus* and their assignment to chromosomes. Appl. Environ. Microbiol. 62, 4542–4547.
Sonnenberg, A.S. et al., 1988. An efficient protoplasting regeneration system for *Agaricus bisporus* and *Agaricus bitorquis*. Curr. Microbiol. 17, 285–291.
Sonnenberg, A.S.M. et al., 2011. Breeding and strain protection in the button mushroom *Agaricus bisporus*. In: Savoie, Foulongne-Oriol, M., Largeteau, M., Barroso, G. (Eds.), Proceedings of the7th International Conference on Mushroom Biology and Mushroom Products, Arcachon, France, pp. 7–15 <https://colloque4.inra.fr/icmbmp7/Downloadproceedings>.
Summerbell, R.C. et al., 1989. Inheritance of restriction fragment length polymorphisms in *Agaricus brunnescens*. Genetics 123, 293–300.
Temple, S. et al., 2008. VisualRepbase: an interface for the study of occurrence of transposable element families. BMC Bioinformatics 9, 345. http://dx.doi.org/10.1186/1471-2105-9-345.
Tournefort, M., 1707. Description dún champignon extraordinaire. Mémoires de l'Academie royale des sciences vol 1, 101–103 (Source gallicalabs.bnf.fr/Archives de l'Académie des Sciences).
Van Ooijen, J.W., 2011. Multipoint maximum likelihood mapping in a full-sib family of an outbreeding species. Genet. Res. 93 (5), 343–349.
Xiang, Y. et al., 2015. Synaptonemal complex extension from clustered telomeres mediated full-length chromosome pairing in *Schmidtea mediterramea*. Proc. Nat. Acad. Sci. 17, ES5159–ES5168.
Zhang, Y. et al., 2014. Edible mushroom cultivation for food security and rural development in China: bio-innovation, technological dissemination and marketing. Sustainability 6, 2961–2973.