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The Two Life Cycles of *Agaricus bisporus*

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ABSTRACT: The basidiomycete *Agaricus bisporus*, the button mushroom, has an amphithallic life cycle, i.e. secondarily homothallic or heterothallic, according to the 'ploidy' level of the spores which can be respectively, heterokaryotic ($n+n$) or homokaryotic (n). In this species, the ploidy level of the spores is correlated to the basidial spore number. For the var. *bisporus*, most of the basidia are bisporic and the secondarily homothallic life cycle predominates. In contrast, for the var. *burnettii*, recently described for specimens discovered in the Sonoran Desert of California, most of the basidia are tetrasporic and the heterothallic life cycle predominates. For the intervarietal hybrids, the dominance of the tetrasporic trait is generally well expressed and the heterothallic life cycle predominates.

All these observations, previously based on the study of some wild strains and intervarietal hybrids, are now confirmed by the study of several hundred wild strains and hybrids at INRA-CTC and at Sylvan. Based on the analysis of several basidial spore components, a synthesis of both new and old data permits a more robust summary of the characteristic reproductive behavior of the two varieties.

1 INTRODUCTION

The basidiomycete *Agaricus bisporus* (Lange) Imbach, the button mushroom, has an amphithallic life cycle, i.e. secondarily homothallic or heterothallic, according to the 'ploidy' level of the spores which can be respectively heterokaryotic ($n+n$) or homokaryotic (n) (see Lange 1952, Kühner 1977). For the var. *bisporus*, most of the basidia are bisporic and produce heterokaryotic spores which confer upon this variety a predominant secondarily homothallic life cycle (Raper *et al.* 1972).

Based on morphological, biological, and genetic studies, the var. *burnettii* Kerrigan & Callac was recently described for specimens found in the Sonoran Desert of California (Callac *et al.* 1993). Most of the basidia of this variety are tetrasporic and, correlatively, its life cycle is predominantly heterothallic (Kerrigan *et al.* 1994). Moreover, the two varieties appeared to be completely interfertile and it was shown that the elevated basidial spore number condition (in which tetrasporic, or occasionally trisporic basidia predominate) was transmitted as a dominant trait, with variable degrees of expression, to the intervarietal hybrids (Kerrigan *et al.* 1994, Imbernon *et al.* 1995). In a more recent work, we show that the basidial spore number trait is primarily determined by the *BSN* locus (Imbernon *et al.* submitted).

Since 1990, many wild specimens have been collected in France (Callac 1994) and about 1% of them appear to be tetrasporic. Preliminary studies have shown that the genetic determinant of their tetrasporic trait could also be the *BSN* locus (Callac 1995). In all cases, the average spore number of basidia (ASN: Kerrigan and Ross 1987) has proven to be a useful variable for phenotypical discrimination and for study of the segregation of the basidial spore number trait in offspring.

Through the previous works cited above and others in progress at INRA-CTC as well as at Sylvan, 516 strains have been examined for their basidial spore number. Here, by grouping all these data, we propose to give a general view of the balance between the two life cycles of the species, for each of the two varieties and for the intervarietal hybrids. This will be based on the study of wild isolates from the Californian desert presumed to belong to the var. *burnettii*, wild isolates from France presumed to belong to the var. *bisporus*, and intervarietal hybrids obtained through various experiments.

2 MATERIALS AND METHODS

2.1 *Strains*

Among the 516 strains studied, 217 are field isolates from France, 61 are field isolates from California, and 238 are intervarietal hybrids. French strains, belonging to the INRA-CTC collection, were isolated by tissue culture from wild specimens collected in numerous sites since 1990 (Callac 1994, 1995). Isolates from the Sonoran Desert of California were prepared from spore cultures from lamellae of dried specimens, most of which had been contributed to the Agaricus Resource Program (Kerrigan 1991), or were prepared from tissue cultures of fresh specimens, from several sites in an area of about 100 km^2 in eastern Riverside Co., California.

Intervarietal hybrids were obtained by confrontations between homokaryons from tetrasporic isolates of the var. *burnettii* and bisporic isolates of the var. *bisporus*. They have three different origins: (1) 91 issued from crosses between two tetrasporic strains (JB 2, JB 3) and seven bisporic strains (data previously reported by Kerrigan *et al.* 1994), (2) 123 come from confrontations between homokaryotic single spore isolates of 45 different tetrasporic strains and a single homokaryon (U1-7) obtained by the protoplast method from the bisporic cultivar U1 (data partly previously reported by Imbernon *et al.* 1995), (3) 24 drawn from crosses between homokaryons from 10 additional unrelated tetrasporic strains and two unrelated bisporic strains

2.2 *Basidial spore number*

Fruiting tests and counts of basidia in *n*-spored classes were conducted as described by Callac *et al.* (1993). To simplify the analyses, the two variables 'bispored class' (= BSC) and 'tetraspored class' (= TSC), previously defined by Kerrigan *et al.* (1994), were used. For a given strain, BSC is the percentage of (infrequent) monosporic plus bisporic basidia, and TSC is the percentage of tetrasporic basidia plus (infrequent) basidia bearing more than four spores. Therefore, it is possible to represent each strain by one point in a (BSC,TSC) system of axes. The percentage of the remaining trisporic basidia or tri-spored class (3SC) is: 3SC = 100 - BSC - TSC. In our analysis, the average spore number (ASN) can vary from 2 to 4 and can be deduced from BSC and TSC: ASN = (300 + TSC - BSC) / 100. Therefore, it is also possible to represent each strain by one point in a (3SC, ASN) system of axes.

In the graphic representation used here, the two systems of axes are combined (see Figs. 1, 2). For any strain, the values taken by the four variables (BSC, TSC, 3SC, ASN) can be represented by a single point.

3 RESULTS

3.1 *French isolates*

The distribution of spore number data is represented in the triangular part of Fig. 1. The two isolates Bs 261 and Bs 423 appeared clearly tetrasporic and plotted very far from the other French isolates. The remaining 215 isolates were more bisporic than tetrasporic (BSC > TSC and ASN < 3) and were therefore considered to belong to the var. *bisporus* even though ten of them (4.6 %) were predominantly trisporic (3SC > BSC). The mean values of the four basidial spore number variables for these 215 isolates of the

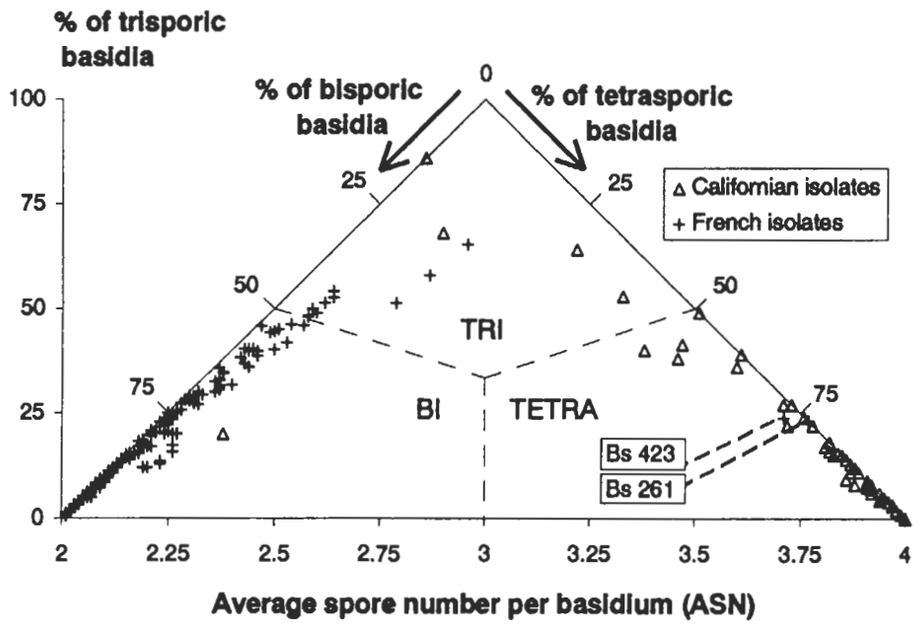

Fig. 1. Graphic representation of the distribution of ASN, and the percentages of bi-, tri-, and tetra-sporic basidia, for 217 wild French isolates and 61 isolates from the Californian desert. The strains preponderantly bi-, tri-, or tetra-sporic are located, respectively, in the corresponding areas of the triangular part of the graphic.

var. *bisporus* are given in Table 1. The percentage of tetrasporic basidia that produce homokaryotic spores, which are very useful for breeding work, was very low (1.3 % on average).

3.2 *Californian isolates*

Figure 1 shows that a single strain from the desert appeared to be clearly bisporic but two others were also more bisporic than tetrasporic. Among the 58 remaining ones, considered as unequivocally belonging to the var. *burnettii* (TSC > BSC and ASN > 3), two (3.4 %) were predominantly trisporic (3SC > TSC). Table 1 shows that the proportions of bisporic and tetrasporic basidia are reversed in this variety. The percentage of tetrasporic basidia is, on average, 84.9 %.

Table 1. Mean values of four basidial spore number variables for the var. *bisporus*, the var. *burnettii*, and the intervarietal hybrids

Sample	Number of isolates	Average spore number per basidium (ASN)	Percentage of bisporic basidia[a]	Percentage of trisporic basidia	Percentage of tetrasporic basidia[b]
Var. *bisporus*[c]	215	2.21	80.40	18.33	1.27
Var. *burnettii*[d]	58	3.84	0.98	14.14	84.88
Hybrids	238	3.68	2.63	27.12	70.25

[a]Including rare monosporic basidia
[b]Including rare basidia bearing more than four spores
[c]Typical French field isolates having their ASN values lower than three
[d]Typical field isolates solates from the Sonoran Desert of California having their ASN values greater than three.

3.3 *Intervarietal hybrids*

For all the tetrasporic parental strains, most of the single spore isolates were homokaryotic. This confirms the correlation between the 'tetrasporic' trait and heterothallism.

The distribution of spore number data is represented in Fig. 2. As expected, all 238 hybrids were predominantly tetrasporic (or trisporic: 20, or 8.4 %). This confirms the dominance of the elevated spore number trait. The mean values of the basidial spore number variables, given in Table 1, were relatively near the values estimated for the var. *burnettii*. However, the percentage of tetrasporic basidia slightly decreased, while trisporic basidia increased.

4 DISCUSSION

It was previously shown that strains of the Californian desert were tetrasporic, predominantly heterothallic, and belonged to the var. *burnettii*. However, while the present analysis of the basidial spore number trait in different populations generally confirms our previous data, it also illuminates two less-prominent features of these populations: the existence, at low frequencies, of atypical isolates, and the relatively high frequencies of trisporic basidia.

4.1 *Atypical isolates*

Rare 'tetrasporic' isolates among the 'bisporic' French population, and rare 'bisporic' isolates among the tetrasporic population of the desert, represent about 2% (5/278) of the studied isolates. Assignment to the var. *bisporus*

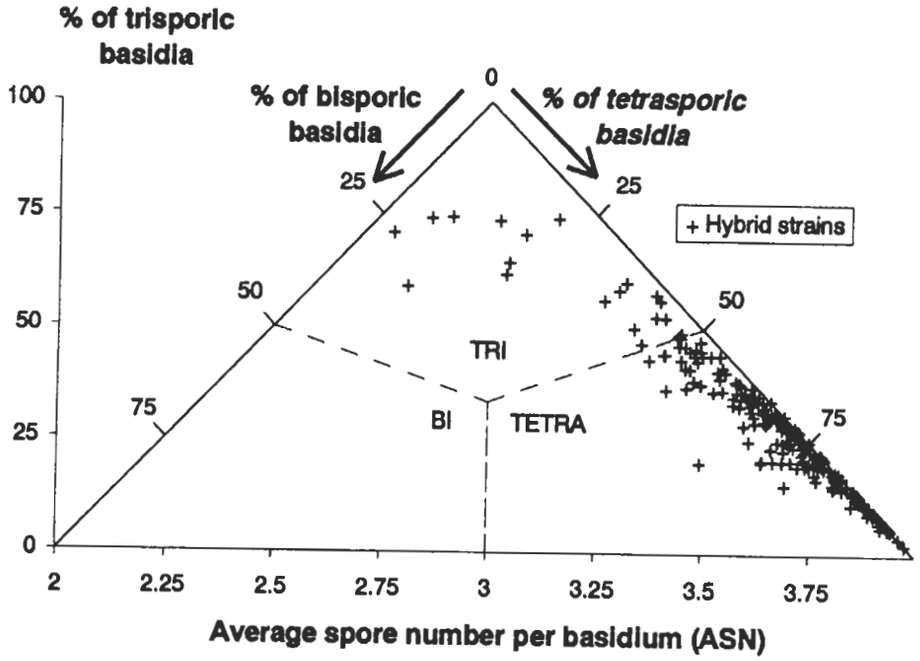

Fig. 2. Graphic representation of the distribution of ASN, and the percentages of bi-, tri-, and tetra-sporic basidia, for 238 intervarietal hybrids. The strains preponderantly bi-, tri-, or tetra-sporic are located, respectively, in the corresponding areas of the triangular part of the graphic.

or the var. *burnettii* is not exclusively based on the criterion of basidial spore number taxonomic criterion. If this criterion does not agree with the geographical origin of the strains, complementary genetic studies become necessary to assess their origin before assigning them to a taxonomic category. The question is whether if the 'bisporic' and 'tetrasporic' traits really coexist, at extremely different frequencies, in a single population, or whether the atypical isolates could have a particular and more or less recent origin as a migration from another population. The ancestral relationships of the five isolates noted above remain to be determined.

4.2 *Trisporic basidia*

The importance of these basidia has been generally underestimated in previous studies about the life cycle of the species (except Pelham 1967, Elliott 1972). In the present analysis on large samples, it is clear that the production of trisporic basidia is important in the 'bisporic' population as well as in the 'tetrasporic' one (on average, respectively 18 and 14 % of the basidia were trisporic). Moreover about 4% of the isolates are predominantly trisporic in the two populations. This reflects the variations of the ASN variable and is particularly visible in distribution of the intervarietal hybrids (Fig. 2). In a recent work (Imbernon *et al.*, submitted) we analyzed first and second generation hybrids and found a major genetic determinant of the basidial spore number trait. However, it was clear that other genetic and environmental effects were also responsible for variations in the value of ASN variable among the studied progenies.

Since the trisporic basidia are numerous, it becomes useful to know the ploidy level of the spores borne by these basidia. In the simple cytogenetic model used by Kerrigan and Ross (1987) then by Kerrigan *et al.* (1994), the bi-, tri-, and tetra-spored basidia produced, respectively, 2, 1, and 0 heterokaryotic spores and 0, 2, and 4 homokaryotic ones. This model allowed calculation of the theoretical fraction of homokaryotic spores (FSH = 2 - 4/ASN). Table 2 summarizes data from previous works (Kerrigan *et al.* 1994, Imbernon *et al.* submitted) where the predicted fractions were relatively near the observed fractions of homokaryons in the single spore offspring of predominantly bisporic or tetrasporic strains. Predominantly trisporic strains remain in need of study.

Table 2. Theoretical and observed fractions of homokaryons among single spore offspring.

Parental strains	ASN[a]	Theoretical FSH[b]	Observed FSH[c]
JB 3 (var. *burnettii*)	3.86	0.96	0.90 (273)
JB 3-83 x U 1-7 (intervarietal hybrid)	3.67	0.91	0.77 (200)
H 136 x U 1- (second generation hybrid)	3.66	0.91	0.81 (99)
H 292 x U 1-2 (second generation hybrid)	2.24	0.21	0.25 (100)

[a]Average spore number of the parental strain
[b]Theoretical fraction of homokaryotic spores (FSH = 2 - 4 / ASN)
[c]Fraction of homokaryotic single spore isolates estimated by several methods (growth rate mycelium tests, mating tests, and/or genotypic tests). Parenthetical values are the numbers of single spore isolates tested.

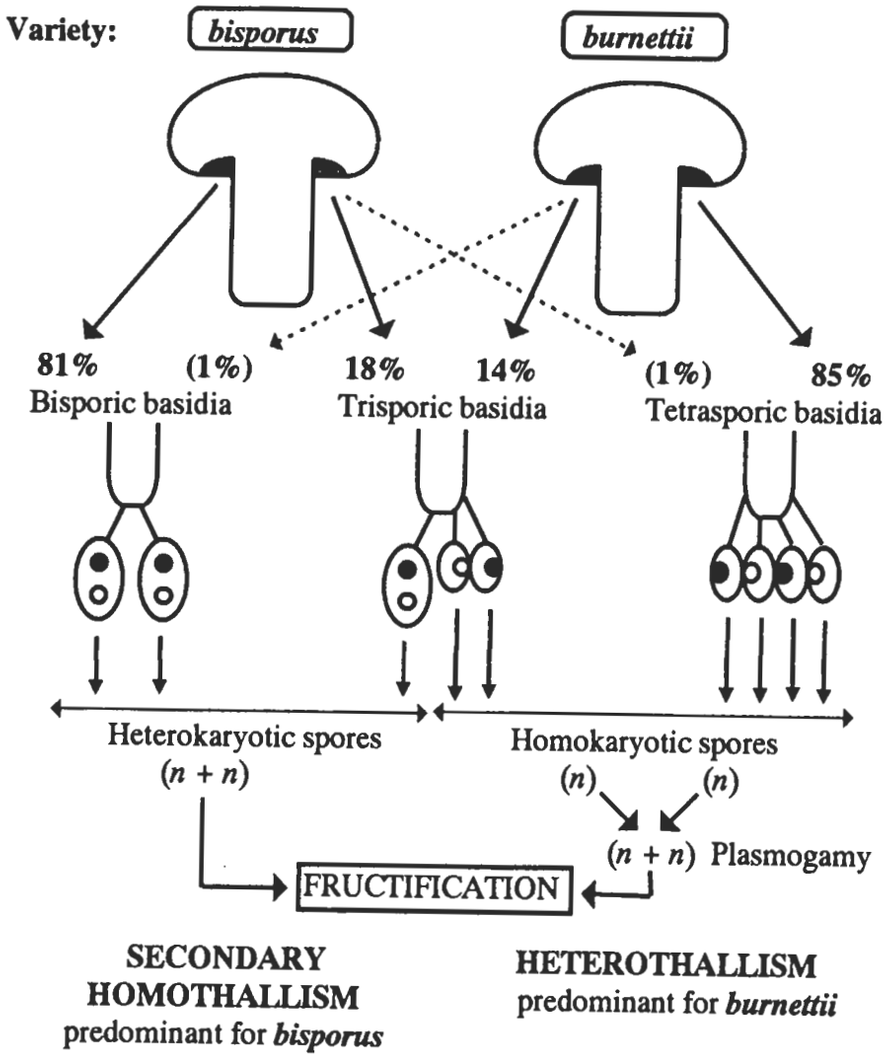

Figure 3. Schematic representation of the two life cycles of the two varieties, *bisporus* and *burnettii*, of *Agaricus bisporus*.

4.3 *New vision of the two life cycles of A. bisporus*

Figure 3 gives a schematic representation of the two life cycles of the vars. *bisporus* and *burnettii*. For this, we used the percentage of each type of basidia calculated from our two samples: the French sample for the var. *bisporus*, and the sample of the desert for the var. *burnettii*. Moreover, the five atypical isolates were excluded and the cytogenetic

model presented above was used. The theoretical fraction of homokaryotic spores calculated by using this model is 0.19 for the var. *bisporus* and 0.96 for the var. *burnettii*. However, the expected fraction of homokaryotic single spores is probably overestimated for several reasons (see Kerrigan *et al.* 1994) as for example the greater mortality of the homokaryotic spores.

All the heterokaryons are represented as fertile in the Fig. 3. *A. bisporus* has a unifactorial incompatibility system and for the var. *bisporus* the spores on the bisporic basidium typically receive two post-meiotic nuclei, which complement each other at the mating type locus (Sass 1929, Evans 1959, Kerrigan *et al.* 1993) and therefore produce fertile heterokaryons. For the var. *burnettii*, such a non random migration of nuclei has to be demonstrated and, presently, the fertility of almost all the heterokaryotic single spore isolates remains hypothetical.

4.4 *Conclusion*

Secondary homothallism and heterothallism, respectively, predominate in the var. *bisporus* and the var. *burnettii*. Consequently, outbreeding must play a more important role in the tetraporic population of the desert. This is a dramatic example of diversity within a species.

The adaptative value of the different reproductive modes presumably depends upon differences in ecological factors affecting the environments of the two populations. Simple genetic determination of the basidial spore number trait, the presence of alleles associated with atypical phenotypes, and existence of at least four common mating type alleles in both populations (see Imbernon *et al.* 1995), suggest that,although extant populations are strongly divergent for spore number and life cycle traits, selection could potentially lead to rapid changes in the reproductive behavior of a population.

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