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Segregation of genetic markers in the two-spored secondarily homothallic basidiomycete *Coprinus bilanatus*

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Summary. Secondarily homothallic basidiomycetes, of which the cultivated mushroom *Agaricus bisporus* is an example, produce both self-fertile and non self-fertile spores. The random migration of nuclei from the basidia to give binucleate spores provides the simplest explanation for the regulation of breeding behaviour in this group of fungi. To test the predictions of the random migration hypothesis, the segregation of mating-type, auxotrophy and antimetabolite resistance has been determined in the secondarily homothallic ink-cap fungus, *Coprinus bilanatus*. In 41 of a total of 56 spore progenies tested, the segregation ratios conformed to the predictions of the random migration hypothesis. Poor fits to the predicted ratios were, in many instances, associated with an adenine auxotrophy. On the basis of the data reported, random migration can be regarded as the primary control of secondary homothallism.

Key words: Basidiomycetes – *Coprinus bilanatus* – Secondary homothallism – Random nuclear migration

Introduction

Secondarily homothallic fungi have genetic incompatibility systems and characteristically produce both self-fertile spores (dikaryons, heteroallelic for mating-type) and non self-fertile spores (monokaryons, homoallelic for mating-type). Secondary homothallism was first described in the ascomycetes (Dodge 1927) and, soon afterwards, in the homobasidiomycete *Coprinus ephemerus* var. *bispora* (Lange) Konrad and Maubl., which is now known as *Coprinus bisporus* Lange (Sass 1928). Since that time many other basidiomycetes, both two- and four-spored, have been described as secondarily homothallic (Elliott 1986). Secondary homothallism in the basidiomycetes is of commercial importance because the cultivated mushroom *Agaricus bisporus* (Lange) Imbach (syn. *A. brunnescens* Peck; Malloch 1976) has a breeding system of this type (Miller 1971; Elliott 1972; Raper et al. 1972).

In two-spored species, such as *A. bisporus,* the reduction in spore number per basidium means that the four primary products of meiosis are available to migrate into just two spores. Despite the presence of an incompatibility system in these species, more spores are found to be self-fertile than are non self-fertile, so a mechanism which favours the association of nuclei heteroallelic for mating-type appears to operate.

The simplest explanation for the production of both self-fertile and non self-fertile spores is the random migration of nuclei from the basidium to give binucleate spores. This explanation has been dismissed in the past, because it was assumed that it would result in classic Mendelian ratios such as 3:1 (Miller et al. 1976; Elliott 1979) and 9:3:3:1 (Raper et al. 1972). A 1:1 ratio of spores heteroallelic to spores homoallelic for mating-type has also been postulated (Kuhner 1953; Burnett and Boulter 1963; Burnett 1976). However, most data reported do not fit these expected ratios, with between 60% and 90% of the cultures obtained from single spores being heteroallelic for mating-type (Skolko 1944; Kuhner 1953; Raper et al. 1972).

A re-interpretation of the consequences of random migration in secondarily homothallic basidiomycetes provides an explanation for the predominance of self-fertile spores found in these species (Langton and Elliott 1980). If random migration of the four meiotic products operates in a two-spored species, the association of unlike nuclei and, therefore, heteroallelism is favoured. In a

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unifactorial, two-spored species, two-thirds of a spore progeny can be expected to be heteroallelic for a given allele, i.e. a 2:1 ratio of self-fertile to non self-fertile progeny. In a bifactorial species, the proportions would be altered by the numer of tetratype meiotic tetrads resulting from second-division segregation. In the absence of second-division segregation, a 2:1 ratio would again be expected.

The proportions of dikaryons and monokaryons recovered in spore progenies have been reviewed in the context of the random migration hypothesis for a number of species (Elliott 1986). In five bifactorial species, the data give good fits to a 2:1 ratio. This suggests that if random migration does occur, there is little or no second-division segregation of the mating-type factors in these species.

Random pairing has predictable consequences for the segregation of other genetic markers (Langton and Elliott 1980). For a recessive biochemical marker such as auxotrophy, a 5:1 ratio of prototrophs to auxotrophs would be expected. With two independently segregating auxotrophic markers (aux^1 and aux^2), a 9:1:1:1 ratio can be expected (Prot: aux^1:aux^2:$aux^{1,2}$). Until recently, the only segregation data for auxotrophy in a secondarily homothallic species were those reported for *A. bisporus* (Raper et al. 1972). However, the poor and variable germination of spores found in this species make it an inappropriate organism in which to test the validity of the random migration hypothesis.

The two-spored secondarily homothallic ink-cap species, *Coprinus bilanatus* nom. prov. (Kemp 1974) has considerable advantages over *A. bisporus* as a laboratory test organism and is used as a genetic model in studies at IHR-L. An earlier evaluation of the random migration hypothesis using this species tested the segregation of auxotrophy and mating-type (Elliott and Challen 1983). The segregation of auxotrophs gave an excellent fit to the 5:1 test ratio. The data for the segregation of mating-type were less convincing.

Spore diad analyses in *C. bilanatus* have since been reported by Kemp (1985). These data were not consistent with the predictions of the hypothesis, and Kemp concluded that whatever mechanism was operating in *C. bilanatus*, it was not dependent on the random migration of the nuclei into the spores. To test further the random migration hypothesis, an extensive evaluation of the segregation of mating-type, auxotrophy and antimetabolite resistance has been made in a range of *C. bilanatus* dikaryons.

Materials and methods

Media, strains and antimetabolites

Malt extract medium (2% Boots malt extract w/v, buffered with 1 *M* KH_2PO_4 and 1 *M* Na_2HPO_4) or complete yeast extract medium, CYM (Raper et al. 1972), were used to grow mycelia and for spore germination (ca. 25 °C). Horse dung extract agar, HDE (Lange 1952), was routinely used for the production of sporophores at 25 °C in 12 h cycles of light and dark. The minimal medium, MM, of Raper et al. (1972) was used for the screening of auxotrophs.

Strains of *C. bilanatus* were kindly supplied by Dr. R. F. O. Kemp, University of Edinburgh. The dikaryon 488/1 was received in 1978 and used in our earlier analyses (Elliott and Challen 1983). Other dikaryons – 685/1 (ATCC 64529), 1230/1 (ATCC 64528), ade-1 × 778/4 (ATCC 64524) and ade-1 × 1230/4 (ATCC 64523) – were used by Kemp in his diad analyses (1985).

Three auxotrophic mutants were produced by treating oidial suspensions with UV radiation (Elliott and Challen 1983). One had a requirement for adenine *ade*, Cb.2(83) (ATCC 56225) and two required supplementation with arginine, *arg.*[1] Cb.2(18) (ATCC 56223) and *arg.*[2] Cb.2(62) (ATCC 56224). The two *arg.* mutants complement and can be distinguished by their response to supplementation with the arginine precursor citrulline (Challen 1988).

Sorbose is a paramorphogen for a number of filamentous fungi and its effects are well documented (Tatum et al. 1949; Moore and Stewart 1972; Trinci and Collinge 1973; Moore 1981). Sorbose resistance mutants of *C. bilanatus* were recovered following UV treatment of oidial suspensions (Challen 1988). Mutants were capable of growth on CYM or MM which contained 2% L-sorbose w/v in place of the usual D-glucose.

The fungicide carboxin is generally effective against basidiomycetes (Edgington et al. 1966) and resistance has been previously recovered in *A. bisporus* (Challen and Elliott 1987). Isolates of *C. bilanatus* with stable resistance to carboxin were selected without mutagenesis treatment (Challen 1988). Stock suspensions of carboxin were prepared and incorporated into CYM, as previously described, at a rate of 5 μg/ml (Challen and Elliott 1985).

Segregation tests and methods of analysis

Spore suspensions were obtained by shaking whole sporophores in sterile distilled water. Spore germination and isolation were carried out as previously described (Elliott and Challen 1983). Random spore progenies were collected from both Kemp's stock cultures and from novel dikaryons, constructed by mating homokaryons carrying auxotrophic and resistance markers (Challen 1988).

Segregation for mating-type was determined by scoring the relative numbers of monokaryons and dikaryons in a random spore progeny. Colony morphology was used as the principal criterion to distinguish dikaryons from monokaryons (Fig. 1). Typical dikaryotic morphology is often slow to develop (Elliott and Challen 1983) and, as a consequence, colonies were only scored after transfer to a second CYM plate (4–6 days after isolation. Occasional colonies were isolated that were difficult to categorise by eye. These were checked for the presence or absence of clamp connections using bright field microscopy (× 225 magnification).

Segregation of other markers was tested by subculturing all progeny onto MM and/or CYM supplemented with amino acid precursors and/or antimetabolites.

Temperatures during sporophore production could have differential effects on meiosis and the migration of nuclei and, in consequence, on the proportions of monokaryons and dikaryons. To investigate this, the dikaryon 488/1 was fruited at 15°, 20°, 25° and 30 °C, and the relative numbers of dikaryons and monokaryons were determined in random spore progenies.

All segregation data were tested for goodness of fit to expected ratios using Chi-squared analyses based on the maximum likelihood statistic.

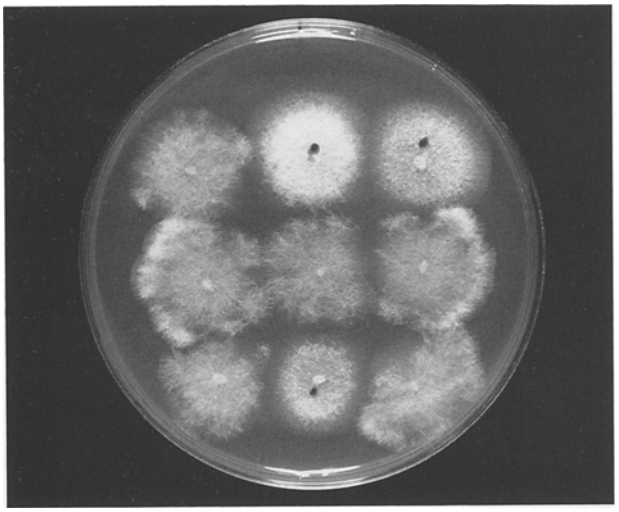

Fig. 1. Dikaryotic (D) and monokaryotic (M) morphologies in a random spore progeny of *Coprinus bilanatus*. Colony type is shown:

D M M
D D D
D M D

Results

A total of 56 experiments was carried out to test the segregation of mating-type, auxotrophy and resistance. Tables 1 to 5 summarise example data from single experiments or groups of replicated experiments. Data that do not fit the expectation are marked *, where $P \leq 0.05$.

Segregation of mating-type and the effect of temperature

The segregations of dikaryons to monokaryons in random spore progenies of 488/1 did not appear to be altered by growing sporophores at different temperatures (Table 1). In seven of the eight replicate progenies tested, the ratios of dikaryons to monokaryons were consistent with the 2:1 ratio predicted ($P \geq 0.05$). Only in one experiment at 20 °C was the number of dikaryons obtained higher than the expectation.

Table 2 summarises the results obtained with random spore progenies derived from the stock dikaryons provided by Kemp. Five of the ten progenies tested gave good fits to the predicted 2:1 ratio. Overall, the summed data for these dikaryons proved a good fit to the expectation. Three of the five individual cases where the data deviated from the expectation were due to higher numbers of monokaryons than predicted.

In six experiments, the monokaryotic progeny were further classified into specific mating-types by test mating with other monokaryons of known mating-type specificities (Table 3). These data were tested for goodness of fit to a 1:1:1:1 ratio. In all cases, mating-types occurred at equal frequencies, confirming that normal meiotic divisions had occurred.

Table 1. Effects of temperature on the segregation of mating-types in the *Coprinus dilanatus* dikaryon 488/1. Chi-squared goodness-of-fit tests for the predicted 2:1 ratio are shown for two replicate experiments at each of the four temperatures

Temp. (°C)	*N*	Dikaryons		Monokaryons		$\Sigma\chi^2$	*P*
		Obs.	Exp.	Obs.	Exp.		
15	173	127	115	46	58	3.73	0.053
	213	142	142	71	71	0.00	1.000
20	164	131	109	33	55	13.2	<0.001*
	223	137	149	86	74	2.92	0.087
25	167	110	111	57	56	0.03	0.862
	231	157	154	74	77	0.18	0.671
30	177	112	118	65	59	0.92	0.337
	87	55	58	32	29	0.47	0.493
Totals	1,874	1,263	1,250	611	624	0.41	0.522

N: number of progeny tested
Obs.: number in class observed
Exp.: number in class expected
* $P \leq 0.05$, i.e. not a good fit to predicted ratio

Table 2. Data of mating-type segregation in four different dikaryons of *Coprinus bilanatus* Chi-squared goodness-of-fit tests are for the predicted 2:1 ratio of dikaryons:monokaryons

Parental dikaryon	*N*	Dikaryons		Mono-karyons		$\Sigma\chi^2$	*P*
		Obs.	Exp.	Obs.	Exp.		
685/1	164	107	109	57	55	0.11	0.740
	190	106	127	84	63	10.5	0.001*
	165	94	110	71	55	6.98	0.008*
1230/1	210	142	140	68	70	0.09	0.764
	167	114	111	53	56	0.24	0.624
	208	142	139	66	69	0.20	0.655
ade-1 ×	250	214	167	36	83	39.8	<0.001*
778/4	202	136	135	66	67	0.03	0.862
ade-1 ×	170	97	113	73	57	6.76	0.009*
1230/04	148	111	99	37	49	4.39	0.036*
Totals	1,435	971	956	464	479	0.71	0.399

N: number of progeny tested
Obs.: number in class observed
Exp.: number in class expected
* $P \leq 0.05$, i.e. not a good fit to predicted ratio

Segregation of auxotrophic markers

Segregations for auxotrophy were initially tested in two dikaryons, ade-1 × 1230/04 and ade-1 × 778/4 (data not

Table 3. Segregation of mating-type factors in monokaryons obtained from random spore progeny of different parental dikaryons of *Coprinus bilanatus*. Chi-squared tests are for goodness of fit to the predicted 1:1:1:1 ratio

Dikaryon (Mating-type)		Monokaryotic progeny: no. in each mating-type				
Cb.P		A1B1	A2B2	A1B2	A2B1	
(A1B2/A2B1)	Obs.	17	15	13	8	$\Sigma\chi^2=3.37$
$N=53$	Exp.	13.25	13.25	13.25	13.25	$P=0.338$
Cb.Q		A1B1	A2B2	A1B2	A2B1	
(A1B2/A2B1)	Obs.	10	8	8	7	$\Sigma\chi^2=0.58$
$N=33$	Exp.	8.25	8.25	8.25	8.25	$P=0.901$
P1+2(18)		A1B1	A2B2	A1B2	A2B1	
(A1B1/A2B2)	Obs.	20	18	11	20	$\Sigma\chi^2=3.17$
$N=69$	Exp.	17.25	17.25	17.25	17.25	$P=0.366$
ade-1 × 1230/04		A2B2	A2B6	A3B2	A3B6	
(A2B6/A3B2)	Obs.	22	19	18	14	$\Sigma\chi^2=1.79$
$N=73$	Exp.	18.25	18.25	18.25	18.25	$P=0.617$
ade-1 × 1230/04		A2B2	A2B6	A3B2	A3B6	
(A2B6/A3B2)	Obs.	6	9	11	9	$\Sigma\chi^2=1.46$
$N=35$	Exp.	8.75	8.75	8.75	8.75	$P=0.692$
Cb.Z		A2B5	A2B6	A3B5	A3B6	
(A2B5/A3B6)	Obs.	17	13	16	20	$\Sigma\chi^2=1.52$
$N=66$	Exp.	16.5	16.5	16.5	16.5	$P=0.678$

N: number of progeny tested
Obs.: number in class observed
Exp.: number in class expected

shown). Both dikaryons are heteroallelic for auxotrophy and were constructed by Kemp (1985) using the *ade* mutant originally produced at IHR-L (Elliott and Challen 1983). Tests were conducted against a predicted 5:1 ratio of prototrophs to auxotrophs.

In one of four replicate experiments with ade-1 × 1230/04, there were more auxotrophic monokaryons than expected and the data were a poor fit to a 5:1 ratio. This, in turn, distorted the segregations for mating-type. Similarly, in two replicate experiments with ade-1 × 778/4, deviations were obtained from the 5:1 test ratio. Progenies from these two dikaryons accounted for the highest incidence of deviations from expected ratios.

In other dikaryons, auxotrophic markers did appear to segregate as expected. A good fit to a 5:1 ratio was obtained for the segregation of arginine auxotrophy (*arg.*^{1}) in a sorbose-resistant dikaryon P1+2(18) (Table 4).

Good fits to 9:3 segregation ratios were found for dikaryons containing two auxotrophic markers (Table 4). The carboxin-resistant cb.M carried both arginine requirements, *arg.*^{1} and *arg.*^{2}, but since these were not distinguished, the test ratio of 9:3 for prototrophs to auxotrophs was used instead of a 9:1:1:1 ratio. Another carboxin-resistant dikaryon Cb.O had arginine (*arg.*^{2}) and adenine (*ade*) requirements.

Other progeny from the dikaryon Cb.M were tested on MM supplemented with citrulline (Table 5). This test enabled *arg.*^{2} auxotrophs to be distinguished from those auxotrophs carrying *arg.*^{1} alone or those carrying both mutations together, *arg.*^{1,2}. The data were, therefore, tested against a 9:1:2 ratio (prot:*arg.*^{1}:*arg.*^{2} +*arg.*^{1,2}). In all these experiments using pairs of segregating auxotrophs, good fits were obtained to the predicted 9:3 and 9:1:2 ratios.

Segregation of resistance markers

The segregation for sorbose resistance was first tested in the dikaryon P1+2(18) (Table 4). Resistance segregated as a single-gene dominant mutation and a good fit was obtained to a 5:1 test ratio (resistant:sensitive). The segregation for sorbose resistance was also tested in two other dikaryons, Cb.P and Cb.Q. Progenies from these gave good fits to 5:1 ratios with Chi-squared values of 1.55 and 2.81, respectively ($P \geq 0.05$). Segregation for carboxin resistance was also tested against a 5:1 test ratio and was shown to behave as a single-gene dominant mutation (Tables 4 and 5).

Summary of segregation data

The majority of the data sets gave good fits to the predicted 2:1 test ratio for the segregation of mating-type. Poor fits to the predicted ratios were more frequent in the two dikaryons carrying adenine auxotrophy, ade-1 × 1230/04 and ade-1 × 778/4. In Table 2, three of the five

Table 4. Segregation of mating-type, auxotrophy and antimetabolite resistance in three dikaryons of *Coprinus bilanatus*, P1 + 2(18), Cb.M. and Cb.O. Chi-squared goodness-of-fit tests for predicted ratios of 2:1 for dikaryons:monokaryons, 5:1 and 9:3 for prototrophs:auxotrophs and 5:1 for antimetabolite resistant:sensitive progeny. Genotype of parental dikaryon is shown in brackets

P1 + 2(18) (*sorb.*^{R} × *arg.*^{1})
$N = 189$

	Dik.	Mono.		Prot.	Auxo.	Sorb.^{R}	Sorb.$^{-}$
Obs.	122	67	Obs.	155	34	150	39
Exp.	126	63	Exp.	158	32	158	32

Test ratio:

2:1	5:1	5:1
$\Sigma\chi^2 = 0.38$	$\Sigma\chi^2 = 0.19$	$\Sigma\chi^2 = 1.94$
$P = 0.538$	$P = 0.663$	$P = 0.164$

Cb.M (*carb.*^{R}, *arg.*^{2} × *arg.*^{1})
$N = 180$

	Dik.	Mono.		Prot.	Auxo.	Carb.^{R}	Carb.$^{-}$
Obs.	117	63	Obs.	145	35	141	39
Exp.	120	60	Exp.	135	45	150	30

Test ratio:

2:1	9:3	5:1
$\Sigma\chi^2 = 0.23$	$\Sigma\chi^2 = 2.96$	$\Sigma\chi^2 = 3.24$
$P = 0.632$	$P = 0.085$	$P = 0.072$

Cb.O (*carb.*^{R}, *arg.*^{2} × *ade*)
$N = 233$

	Dik.	Mono.		Prot.	Auxo.	Carb.^{R}	Carb.$^{-}$
Obs.	131	102	Obs.	88	28	186	47
Exp.	155	75	Exp.	87	29	194	39

Test ratio:

2:1	9:3	5:1
$\Sigma\chi^2 = 11.6$	$\Sigma\chi^2 = 0.04$	$\Sigma\chi^2 = 1.97$
$P = 0.001$ *	$P = 0.841$	$P = 0.160$

N: number of progeny tested
Obs.: number in class observed
Exp.: number in class expected
* $P \leqslant 0.05$, i.e. not a good fit to predicted ratio

progeny that failed to fit a 2:1 ratio of dikaryons to monokaryons were obtained from the dikaryons ade-1 × 778/4 or ade-1 × 1230/04. In other experiments, progeny from these two dikaryons consistently failed to segregate as expected for either mating-type and/or auxotrophy.

Table 6 provides a summary of all the segregation data obtained in the analyses of 56 random spore progenies from *C. bilanatus*. It includes a revised summary, excluding 20 experiments which involved the *ade* mutation.

Discussion

Mating-type, auxotrophy and resistance markers all appeared to segregate independently. There was no evidence of any linkage between the markers tested. The majority of the data sets fit the predicted ratios and are supportive of the random migration hypothesis (Table 6). However in a considerable number of experiments (15 out of 56), segregations failed to conform to the predicted ratios.

In the light of an earlier study (Elliott and Challen 1983), the results for segregation of auxotrophic markers were surprising. Only half of the data sets conformed to the predicted 5:1 test ratio. Each of the four experiments that failed to fit the 5:1 ratio involved the adenine (*ade*) marker. If the data involving the *ade* mutation is excluded, then the overall fit is dramatically improved. In all, 12 of the 15 progenies that did not fit the predicted ratios involved the segregation of *ade*. The *ade* mutation may have other pleiotropic effects associated with auxotrophy that affect, e.g. the migration of nuclei.

In principle, the occurrence of common-factor heterokaryons in *C. bilanatus* can provide an explanation for mating-type data sets that do not fit a 2:1 ratio (Elliott and Challen 1983). In a bifactorial, two-spored species, common-factor heterokaryons can only result from the formation of tetratype meiotic nuclei following the segregation of one of the mating-type factors at the second division of meiosis (Langton and Elliott 1980). However, common-factor heterokaryons which can be recognised on the basis of colony morphology (Challen 1988) were not observed in the course of this study or in other reported analyses of *C. bilanatus* (Kemp 1985; Stephens 1988). It seems likely that second-division segregation of the mating-type alleles occurs rarely, if at all. This lack of second-division segregation indicates that the mating-type loci are close to their respective centromeres, as suggested earlier (Elliott 1986). The occurrence of common-factor heterokaryons does not, therefore, provide a credible explanation for the mating-type data sets which do not conform to expectations in this study.

Second-division segregation for other markers has been observed in *C. bilanatus*. Recessive, extracellular enzyme mutants were found to segregate at the second division of meiosis by the recovery of dikaryons homoallelic for the mutation (Stephens 1988). Four types of enzyme mutant were tested and varying frequencies of second-division segregation were found ranging between 5.2% and 32.8% of the progenies tested. In our analyses, auxotrophic- and carboxin-sensitive dikaryons, which can only arise by second-division segregation, were very rare. These two mutations may also be close to their respective centromeres.

Diad analyses of *C. bilanatus* showed much higher proportions of dikaryons than the two-thirds expected if random migration was operating (Kemp 1985). In our experience, the same strains have not produced such high numbers of dikaryons. Of the 31 progenies tested, 11 did

Table 5. Segregation of mating-type, auxotrophy and carboxin resistance in the *Coprinus bilanatus* dikaryon Cb.M. Different arginine requirements are identified by their ability (*arg.*^{2}) or inability (*arg.*^{1} and *arg.*^{1,2}) to utilise citrulline. Chi-squared goodness-of-fit tests are for predicted 2:1 (dikaryons:monokaryons, 9:3 (prototrophs:auxotrophs), 9:1:2 (prototrophs:*arg.*^{2}:*arg.*^{1}, *arg.*^{1,2}) and 5:1 (resistant:sensitive) ratios

$N=188$	Dik.	Mono.	Prot.	Auxo.	Citrulline		Carb.^{R}	Carb.$^{-}$
					+ve	−ve		
Obs.	124	64	152	36	16	20	157	31
Exp.	125	63	141	47	16	31	157	31
Test ratio:								
	2:1		9:3		9:1:2		5:1	
	$\Sigma\chi^2=0.02$		$\Sigma\chi^2=3.43$		$\Sigma\chi^2=4.76$		$\Sigma\chi^2=0.000$	
	$P=0.888$		$P=0.064$		$P=0.093$		$P=1.000$	

N: number of progeny tested
+ve: positive growth response to citrulline, i.e. *arg.*^{2}
−ve: negative growth response to citrulline, i.e. *arg.*^{1}, *arg.*^{1,2}
Obs.: number in class observed
Exp.: number in class expected

Table 6. Summary of segregation test results and the effect of adenine auxotrophy (*ade* mutation) on segregation ratios in *Coprinus bilanatus*. **A.** Summary of all experiments conducted at IHR-L. **B.** Analyses from progeny not containing the *ade* mutation

Factors scored	Test ratio	**A.** (incl. *ade*)	**B.** (excl. *ade*)
		No. experiments/ No. failed *	No. experiments/ No. failed *
Dikaryons, monokaryons	2:1	31/11	20/3
Monokaryon mating-types	1:1:1:1	6/ 0	4/0
Prototrophy, auxotrophy	5:1	8/ 4	2/0
	9:3	3/ 0	2/0
	9:1:2	1/ 0	1/0
Carboxin resistance, sensitivity	5:1	3/ 0	3/0
Sorbose resistance, sensitivity	5:1	4/ 0	4/0
Totals		56/15	36/3

* Where result differed significantly from prediction ($P \leqslant 0.05$)

not conform to the predicted 2:1 ratio. These distortions were as likely to be caused by higher-than-expected numbers of monokaryons (5 cases) as by high numbers of dikaryons (6 cases).

The disparity in the ratios of dikaryons to monokaryons reported here and by Kemp are not easily reconcilable. Diad analyses involves micromanipulation of basidiospores from the surface of gill tissue. This is a complex and time-consuming procedure, which limits the size of progenies that can be screened. It is conceivable but unlikely for errors to occur during micromanipulation, e.g. non-sibling spores may be isolated as diads and/or immature spores may be isolated before migration is completed. Incomplete migration would, however, result in the production of higher numbers of monokaryons rather than dikaryons.

Neither random spore analyses nor diad analyses can distinguish between 'monokaryons', which form as a consequence of the random migration of pairs of nuclei (i.e. binucleate, homokaryotic spores) and those 'monokaryons' resulting from incomplete or uneven migration (uninucleate spores). Kemp (1985) has suggested that monokaryons originate as uninucleate spores. However, cytological studies of nuclear numbers in shed basidiospores of *C. bilanatus* have shown that uninucleate spores occur infrequently as compared with those containing two, three and four nuclei (Challen 1988).

Recently, in analyses of other random spore progenies of *C. bilanatus*, good fits were obtained to 2:1 ratios of dikaryons to monokaryons and 5:1 ratios of wild types to mutant in the segregation of recessive enzyme markers (Stephens 1988).

The data reported here and those of Stephens (1988) provide strong evidence for the random migration of nuclei in this secondarily homothallic species. Deviations from the predicted ratios do clearly occur, and some may result from incomplete or uneven migration of nuclei into the basidiospores. The bases of these distortions require further study but they would seem to be secondary to the primary control exercised by random migration.

References

Burnett JH (1976) Fundamentals of mycology. Edward Arnold, London

Burnett JH, Boulter ME (1963) The mating systems of fungi. II. Mating systems of the Gasteromycetes, *Mycocalia denudata* and *M. duriaeana*. New Phytol 62:217–236

Challen MP (1988) Biology and genetics of the secondarily homothallic basidiomycete *Coprinus bilanatus* nom. prov. MIBiol thesis, Institute of Biology, London

Challen MP, Elliott TJ (1985) The in vitro responses to a range of fungicides of two strains of the mushroom *Agaricus bisporus* and the pathogen *Verticillium fungicola*. Mycopathologia 90:161–164

Challen MP, Elliott TJ (1987) The production and evaluation of fungicide resistant mutants in the cultivated mushroom *Agaricus bisporus*. Trans Br Mycol Soc 88:433–439

Dodge BO (1927) Nuclear phenomena associated with heterothallism and homothallism in the Ascomycete *Neurospora*. J Agric Res 35:289–305

Edgington LV, Walton GS, Miller PM (1966) Fungicide selective for basidiomycetes. Science 153:307–308

Elliott TJ (1972) Sex and the single spore. Mushroom Sci 8:11–18

Elliott TJ (1979) A gene affecting gill-structure in *Agaricus bisporus*, the cultivated mushroom. Trans Br Mycol Soc 73:347–348

Elliott TJ (1986) Genetics and strain improvement in the genus *Agaricus*. PhD Thesis, University of Sussex

Elliott TJ, Challen MP (1983) Genetic ratios in secondarily homothallic basidiomycetes. Exper Mycol 7:170–174

Kemp RFO (1974) Bifactorial incompatibility in the two-spored basidiomycetes *Coprinus sassii* and *C. bilanatus*. Trans Br Mycol Soc 62:547–555

Kemp RFO (1985) Gene segregation in the 2-spored basidiomycete, *Coprinus bilanatus*. Heredity 54:391–395

Kuhner R (1953) L'amphithallie et ses causes dans la forme bisporique tetrapolaire de *Clitocybe lituus* (Fr.) Bull Soc Mycol Fr 69:307–325

Lange M (1952) Species concept in the genus *Coprinus*. A study on the significance of intersterility. Dan Bot Ark 14:1–164

Langton FA, Elliott TJ (1980) Genetics of secondarily homothallic basidiomycetes. Heredity 45:99–106

Malloch D (1976) *Agaricus brunnescens:* the cultivated mushroom. Mycologia 68:910–919

Miller RE (1971) Evidence of sexuality in the cultivated mushroom, *Agaricus bisporus*. Mycologia 63:630–634

Miller RE, Robbins WA, Kananen DL (1976) Inheritance of sporophore colour and 'wild' morphology in *Agaricus bisporus*. Mushroom Sci 9:39–45

Moore D (1981) Effects of hexose analogues on fungi: Mechanisms of inhibition and of resistance. New Phytol 87:487–515

Moore D, Stewart GR (1972) Effects of 2-deoxyglucose, D-glucosamine and L-sorbose on the growth of *Coprinus lagopus* hyphae. J Gen Microbiol 71:333–342

Raper CA, Raper JR, Miller RE (1972) Genetic analysis of the life-cycle of *Agaricus bisporus*. Mycologia 64:1088–1117

Sass JE (1928) Aberrant heterothallism in a homothallic *Coprinus*. Science 68:548

Skolko AJ (1944) A cultural and cytological investigation of a two-spored basidiomycete, *Aleurodiscus canadensis* n. sp. Can J Res Ser C 22:251–271

Stephens SK (1988) The production and characterisation of extracellular enzyme mutants in *Coprinus bilanatus*. PhD Thesis, University of Birmingham

Tatum EL, Barratt RW, Cutter Jrn VM (1949) Chemical induction of colonial paramorphs in *Neurospora* and *Syncephalostrum*. Science 109:509–511

Trinci APJ, Collinge A (1973) Influence of L-sorbose on the growth and morphology of *Neurospora crassa*. J Gen Microbiol 78:179–192