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The Buller phenomenon in *Armillaria heimii* Pegler, a bipolar diploid basidiomycete

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Abstract – The Buller phenomenon consists in the dikaryotisation of a monokaryon by a heterocaryon, a process widespread among Basidiomycetes. The species of genus *Armillaria,* generally hetothallic tetrapolar, are characterized by a secondary mycelium made of diploid and uninucleate cells. However, a process analogous to the Buller phenomenon has been observed in the tetrapolar *Armillaria* species. In the present study, the Buller phenomenon was investigated in *Armillaria heimi* Pegler, an African species consisting of bipolar and homothallic populations. It was shown that the single spore, haploid mycelia from the bipolar populations can be diploidized by diploids from both bipolar and homothallic populations. This result confirms that the bipolar and homothallic populations belong to the same species. Fertile basidiomes were obtained from the diploidized haploids. Molecular analysis with RAPD markers showed that at least in one case, the diploid originating from the Buller phenomenon resulted from a recombination between the genotypes of the donor diploid and the haploid. The diploid was never modified by the haploid.

***Armillaria* / Basidiomycetes / Buller phenomenon / RAPD / Africa**

Résumé – Chez les Basidiomycètes, le « phénomène de Buller » consiste en la dicaryotisation d'un mycélium primaire par un mycélium secondaire (généralement dicaryotique). Les espèces du genre *Armillaria* présentent la caractéristique d'avoir un mycélium secondaire diploïde et non dicaryotique, le phénomène de Buller n'en existe pas moins dans ce genre. Chez les Armillaires, ce phénomène pose des problèmes nucléaires particuliers du fait du caractère diploïde et unicléé des articles du mycélium secondaire. L'étude du phénomène de Buller a été entreprise chez l'espèce africaine *Armillaria heimii* Pegler qui est constituée de populations qui sont les unes homothalles, les autres hétérothalles bipolaires. Il a été montré que le phénomène de Buller existe aussi chez cette espèce et que les haplontes issus des populations bipolaires peuvent être diploïdisés y compris par des diplontes appartenant aux populations homothalles, résultat qui confirme au passage l'unicité de l'espèce *A. heimii*. Des carpophores fertiles ont été obtenus à partir des subcultures issues des haplontes diploïdisés. L'utilisation des marqueurs RAPD a montré dans un cas que le diplonte issu du phénomène de Buller résultait d'une recombinaison entre le diplonte donneur et l'haplonte. En revanche, au contact de l'haplonte, le diplonte ne subit aucune modification.

***Armillaria* / Basidiomycètes / phénomène de Buller / RAPD / Afrique**

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INTRODUCTION

The Buller phenomenon consists in a mating between a monokaryon and a heterokaryon, resulting in the transformation of the monokaryon into a heterokaryon (Raper 1966). The Buller phenomenon exists only in Basidiomycetes, it was discovered by Buller (1931) and named "Buller phenomenon" by Quintanilha (1937).

In a typical Basidiomycete, the secondary mycelium is heterokaryotic, with two types of complementary nuclei, associated by pairs (dikaryons) in each cell or distributed by chance among multinucleate cells. Several processes can explain the Buller phenomenon: (i) one of the two types of nuclei of the heterokaryotic mycelium migrates into the mycelium of the homokaryon and associates with the native nuclei, (ii) a dikaryon from the donor migrates into the homokaryon and replaces the native nuclei ("Rawitscher's scheme"), (iii) recombination takes place between the two nuclei of the donor to give rise to recombined nuclei which associate with the nuclei of the receptor. Quintanilha (1937) and Papazian (1950) showed that the first situation is by far the most common, but that the other two can also be observed.

The species of genus *Armillaria* are unique among Basidiomycetes because the secondary mycelium is diploid, with uninucleate cells. First postulated by Hintikka (1973), the diploid state of the nuclei of *Armillaria* spp. has been demonstrated by several lines of evidence: (i) direct measurement of DNA content (Franklin et al. 1983, Peabody and Peabody 1985), (ii) mating of auxotrophic haploid mutants giving rise to a prototrophic secondary mycelium subcultured from a single nucleus (Anderson and Ullrich 1982), (iii) reconstruction of the complete sexual cycle in the laboratory with obtention of four mating types from a basidiome coming from a single nucleus originating from an artificial mating between two haploids (Guillaumin 1986).

Korhonen (1978) and Anderson and Ullrich (1982) showed that a process analogous to the Buller phenomenon exists in the tetrapolar *Armillaria* species, though the donor mycelium is diploid; when a homokaryon is paired with a diploid of the same species, the growing peripheral parts of the homokaryon change their morphology after about fifteen days and, when subcultured, they appear made of diploid nuclei. The process is so regular that it was at the base of a routine method for identification of the tetrapolar *Armillaria* species (Guillaumin and Berthelay 1981): an unknown diploid culture successively paired with haploid testers of different *Armillaria* species will diploidize the testers belonging to only one species and will show incompatible reactions with the others.

The nuclear significance of the Buller phenomenon in *Armillaria* species was investigated using different types of markers: mating type alleles (Guillaumin 1986), isozymes (Rizzo and Harrington 1992, Rizzo and May 1994), RFLP markers (Carvalho *et al.* 1995). All these studies led to the same conclusions: in a large majority of cases, the nuclei of the "diploidized haploid" which were analysed were found identical to those of the donor diploid (the analogous of the "Rawitscher's scheme"). However, in a few cases, an association of the markers from the diploid and the haploid was found in the diploidized haploid, involving recombination. In addition, Carvalho *et al.* (1995) showed that the mitochondria of the diploidized haploid were the same as those of the haploid, proving that only the nuclei of the diploid had migrated.

Although tetrapolar heterothallism is the rule among *Armillaria* species from the temperate zones, two temperate taxa were found homothallic: *Armillaria*

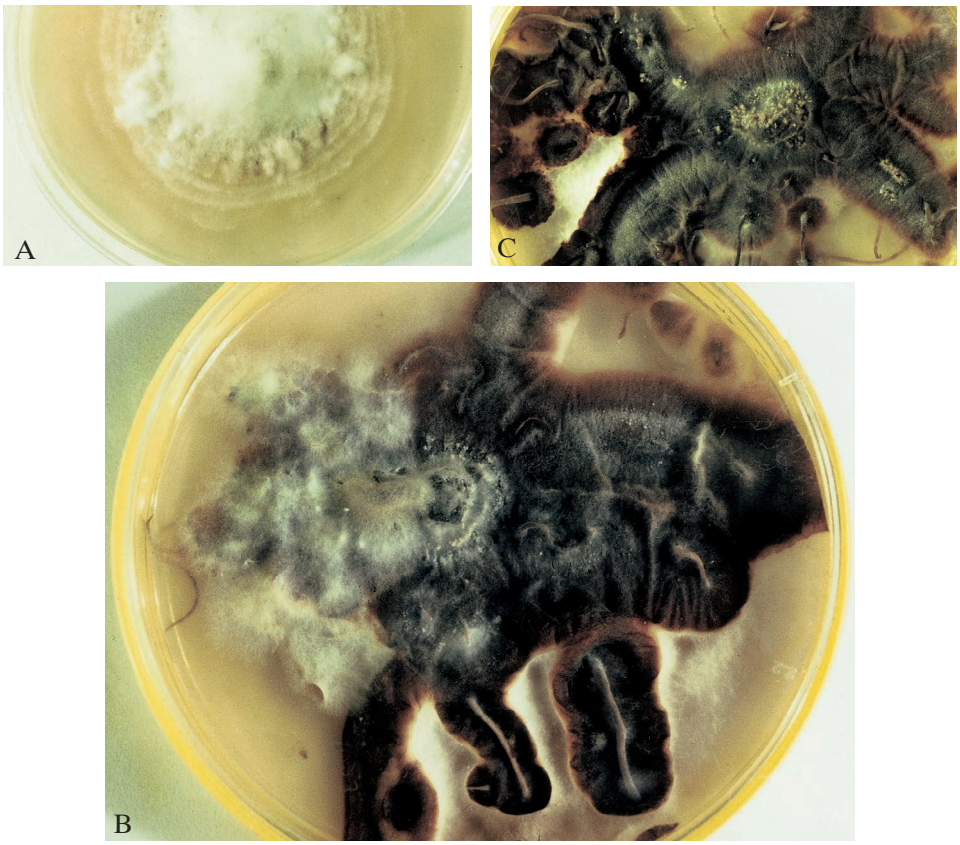

Fig. 1. Buller phenomenon between a haploid from Cameroon and a diploid from Gabon. **A.** Control culture of the haploid CA1-5. **B.** Transformation of CA1-5 (left side of the dish) by pairing with the diploid G9 (right side of the dish, not modified). **C.** Control culture of the diploid G9.
Phénomène de Buller entre un haplonte du Cameroun et un diplonte du Gabon. ***A.*** *Culture témoin de l'haplonte CA1-5.* ***B.*** *Transformation de l'haplonte CA1-5 (à gauche) par appariement avec le diplonte G9 (à droite, non modifié).****C.*** *Culture témoin du diplonte G9.*

ectypa (Fr.) Lamoure (Zolciak *et al.* 1996) and *Armillaria mellea* ssp. *nipponica* Cha and Igarashi (Cha and Igarashi 1995). Among the tropical taxa, *Armillaria puiggarii* Speggazini and *A. mellea* ssp. *africana* were found homothallic (Abomo-Ndongo *et al.* 1997), it was ulteriorly demonstrated that the latter is just a clone of *A. mellea* ssp. *nipponica* (Ota *et al.* 2000).

The situation is more complex as concerns *Armillaria heimii* Pegler, a widespread and highly variable African species (Mohammed and Guillaumin 1993): some populations of this species were found homothallic while others were heterothallic and bipolar (Abomo-Ndongo *et al.* 1997): up to now, *A. heimii* is the only taxon within genus *Armillaria* for which a bipolar (unifactorial) pattern was demonstrated. Bipolar populations were found in Cameroon, Ivory Coast, Liberia, Gabon and Kenya while homothallic populations were reported from the Congo, Zimbabwe, Malawi, Zambia, South Africa, Tanzania, Kenya, Madagascar and La

Réunion (Mohammed and Guillaumin 1993). These two sets of isolates were compatible with one another in somatic pairings while they were all somatically incompatible with the isolates of two other African *Armillaria* species (*A. mellea* ssp. *nipponica* and "SIG III", an unnamed taxon). This observation had led to consider them as belonging to the same species *Armillaria heimii* despite their different sexual patterns (Abomo-Ndongo and Guillaumin 1997).

The discovery of bipolar populations in an *Armillaria* species asked the question of the occurrence of the Buller phenomenon in these populations. In addition, as homothallic and bipolar populations coexist within the same species, it was also interesting to investigate the possibility of diploidization of a homokaryon (from a bipolar isolate) by a homothallic diploid. Such a possibility was demonstrated by Ainsworth *et al.* (1990) between outcrossing and non outcrossing populations of *Stereum hirsutum*. However, the secondary mycelium of *S. hirsutum* is multinucleate while it is diploid in *Armillaria heimii*.

We investigated the possibility to diploidize haploid mycelia from bipolar isolates of *A. heimii* by pairing with diploids belonging to bipolar or homothallic populations of the same species. Then we tried to obtain basidiomes from some of the diploids originating from these confrontations. In a third step, we tried to elucidate by molecular analysis (RAPD) the origin of the nuclei of three isolates originating from the diploidization of a haploid through the Buller phenomenon.

MATERIAL AND METHODS

Material

All the *Armillaria* strains used in this study had been described previously by Abomo *et al.* (1997). They included the following:

* Natural diploid isolates belonging to *Armillaria heimii*. Two were homothallic: U1 (Uganda) and C1 (Congo), others were heterothallic bipolar: CA1 (Cameroon), G2 (Gabon), G9 (Gabon) and K69 (Kenya).

* Single spore isolates originating from basidiomes from the diploids CA1, G2 and K69 fruited in the laboratory. These single-spore isolates were fluffy and putatively haploid, for each of these two series, they were distributed between two mating types (bipolar pattern).

* Synthetic diploid strains obtained from matings between the single spores from CA1, G2 or K69. Some matings were carried out between two compatible single spores from the same basidiome (sib-matings, ex: G2-3 × G2-5), others between single spores originating from different basidiomes (ex: G2-9 × CA1-2).

* Diploids belonging to tropical *Armillaria* species other than *A. heimii*: K5 from Kenya, belonging to *A. mellea* ssp. *nipponica*, K10 from Kenya, belonging to "SIG III" and GUA, from La Guadeloupe, West Indies, belonging to *A. puiggarii* Speggazini.

For RAPD analysis, only three associations were used: the single spore, haploid isolate CA1-5 was paired with the three diploids G9 (Gabon), K69 (Kenya) and C1 (Congo) the first two being bipolar and C1 homothallic.

Methods

1) Matings between haploid and diploid isolates

A piece of inoculum 40-50 mm^3 of a fluffy haploid mycelium was paired with a similar piece of inoculum from a crustose diploid isolate in a Petri dish on malt-agar medium (2% w/v malt extract and 1.5% w/v agar). These inoculums were taken from the margin of 3 week old cultures, they were placed side-by-side in the centre of the dish. The pairings (each repeated twice) were incubated at 24 ± 1°C in darkness. Separate cultures of the haploid and the diploid isolates were also grown as controls in other Petri dishes, on the same medium and in the same conditions. The cultures were observed for morphological changes after 16 days of growth, then after one month to confirm the results.

At the moment of the second observation, four subcultures were made from each of the two partners of each pairing, the cuttings being taken at the margin of the two colonies, as far as possible from the partner.

2) Fruiting *in vitro*

The ability for fruiting was tested for eight diploidized haploids according to the method described by Abomo-Ndongo et *al*. (1997): the isolates were grown in 250 ml erlenmeyers flasks on orange fragments with sterile water. During a preliminary incubation at 24° C in the dark, the medium was completely colonized by the mycelium and the rhizomorphs. Then during summer, the flasks were put on the benches of the laboratory, without controlling light or temperature, they fruited from September to December.

3) Single spore isolation

Single spore isolations were carried out as described by Guillaumin and Berthelay (1981).

4) RAPD analysis (Random amplification of Polymorphic DNAs)

a) culture of the rhizomorphs "*subcorticalis*"

For each selected isolate, flat white rhizomorphs of the "*subcorticalis*" type were obtained according to the method described by Guillaumin, 1986: a sterilized fragment of orange surmounting a column of sterile water in a large glass tube 300 × 75 mm was inoculated with a fresh culture of *A. heimii*. The fungus colonized the orange fragment, then initiated flat rhizomorphs growing downwards in the water column. These rhizomorphs were collected after one month and lyophilised.

b) DNA extraction

Total DNA was extracted according to the method described by Mohammed (1994), slightly modified: each lyophilised sample (10 to 20 mg) was put in an Eppendorf tube of 1,5 ml with 500 µl of TES buffer (100 mM Tris Hcl pH 7.5, 10 mM EDTA, 2% SDS). The sample was ground with sterile sand. Then, 5 µl proteinase K were added (20 µg/ml) and the sample was incubated for 1 h at 55-60°C, then 100 µl of CTAB 10%-NaCl were added (CTAB 10 g, NaCl 4.1 g, water qsq 100 ml). After an incubation of 10 mn at 65°C, DNA was extracted with 700 µl of a mixture chloroform/isoamylic alcohol (24:1)-SEVAG.

After 30 mn at – 20°C, 10 mn centrifugation at 13 500 rpm led to a separation into two phases, the upper, aqueous phase was transfered to a new Eppendorf tube, DNA was precipitated with 1 ml of ethanol 70 % at – 20°C, dried and dissolved in 50 µl TE buffer. The optical density of each sample was measured with a spectrophometer at wavelength 260 nm. The samples ready for RAPD analysis were prepared by dilution so as to obtain about 60 ng of DNA in each test tube.

c) RAPD test

The amplification reaction was conducted according to Williams *et al.* (1990), with slight modification, in a volume of 25 µl with 10 mM Tris-HCl pH 9, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% triton X_{100}, 0.2 mM of the primer; 0.5 unit of Taq-polymerase (Appligène) and about 60 ng of DNA.

Amplification was carried out in a 9600 Perkin Elmer Cetus thermal cycler as follows: initial denaturation for 5 mn at 93°C, then 40 cycles with three steps: denaturation 1 mn at 91°C, annealing 1 mn at 36°C, polymerisation 2 mn at 70°C. The 40 cycles were followed by final extension for 5 mn at 70°C.

Twenty-four different primers were tried: three of them R 25, R 28, UBC 31 had been selected by J. Anderson's team at the University of Toronto. These three primers had revealed a high variability among the isolates of *Armillaria* spp. (Smith et *al.* 1992, Guillaumin et *al.* 1996, Zolciak *et al.* 1997). The other 21 primers were from the kits of Operon Technology Co., they had revealed some variability in different groups of fungi.

d) DNA electrophoresis

The amplified DNA was deposited on an agarose gel (1.4 % agarose) prepared in buffer TAE 1X (0.04 Triacetate, 0.001 EDTA). DNA migrated during 4 hours under 70 volts. DNA fragments were coloured with Ethidium bromide (0.5 g/l), observed under UV and photographied. The size of the fragments was evaluated by reference to a size scale (DNA ladder, Gibco BRL)

Results

1) Evidence of Buller phenomenon

a) reactions observed

Three different reactions were observed (Table 1):

(i) the haploid isolate, initially fluffy and white, became brown and crustose on the major part of its surface. However it remained clearly distinct from the diploid although the latter showed also a flat, brown and crustose morphology which had not been modified by the pairing with the haploid.

These putatively diploidized haploids were subcultured (four explants per colony) and the subcultures were observed after 16 days. The pairing was considered as positive (+ in Table 1) when all four subcultures were crustose.

(ii) the haploid isolate was not modified and remained fluffy, the diploid also conserved its crustose morphology. The subcutures from the unmodified haploid were all fluffy and white. These reactions were regarded as negative (- in Table 1).

(iii) questionable reactions were observed, corresponding to two different situations:

Table 1. Buller phenomenon: pairings between haploids G2 (Gabon), CA1 (Cameroon) and several diploids of *Armillaria.*
Tableau 1. Phénomène de Buller : confrontations entre les haplontes G2 (Gabon) et CA1 (Cameroun) et divers diplontes d'*Armillaria.*

		Armillaria heimii									***Armillaria spp***		
		Heterothallic bipolar pattern							Homothallic pattern				
		G2(1)	CA1(1)	K69(1)	G9(1)	G2-9xCA1-2(2)	G9-8xCA1-6(2)	G2-3xG2-5(2)	G9-1xK69-1(2)	U1(1)	C1(1)	*A. puiggarii.*	*A. mellea spp. nip.*	*A. sp.* (SIG III)
Haploids from G2														
Mating-type 1	G2-1	+ +	- ?	? +	/ /	- -	/ /	+ ?	+ +	+ +	+ +	- -	- -	- -
	G2-4	+ +	+ +	- -	/ /	? ?	/ /	+ +	+ +	- -	+ +	- -	- -	- -
Mating-type 2	G2-2	+ +	- -	+ +	/ /	+ +	/ /	+ +	+ +	+ +	+ +	- -	- -	- -
	G2-10	+ +	+ +	- -	/ /	+ -	/ /	- -	+ +	+ +	+ +	- -	- -	- -
Haploids from CA1														
Mating-type 1	CA1-2	? +	+ ?	- -	+ +	/ /	- -	/ /	/ /	+ ?	+ +	- -	- -	- -
	CA1-9	+ +	+ +	+ +	+ +	/ /	+ +	/ /	/ /	+ +	+ +	- -	- -	- -
Mating-type 2	CA1-5	+ +	+ +	+ +	+ +	/ /	+ +	/ /	/ /	+ +	+ +	- -	- -	- -
	CA1-6	- -	+ +	? ?	+ +	/ /	+ +	/ /	/ /	+ +	+ +	- -	- -	- -

(1) natural diploid (2) synthetic diploid.
Pairings (2 repeats for each pairing) : + = positive ; - = negative; ? = doubtful; / = not made.
(1) = diplonte naturel (2) = diplonte artificiel.
Confrontations (2 répétitions pour chaque confrontation) : + = positive - = négative; ? = douteuse; / = non effectuée.

– one of the two partners was rapidly invaded by the growth of the other, only one colony was present after one month.

– the limit between the colonies was unclear, often because rhizomorphs from one partner had merged with the other.

These reactions were considered as uncertain and noted (?) in Table 1. The pairings corresponding to positive reactions, but which had led to at least one fluffy subculture (out of four) were also noted (?).

Each association (haploid - diploid) was represented by two repeats.

b) results of mating with different types of diploids

The haploids G2-n belonging to the two mating-types of the G2 series, when mated with their natural parent (diploid G2), were diploidized in all cases. The result was the same when the haploids CA1-n were mated with their natural diploid parent CA1 (with the exception of one repeat of the mating CA1-2 × CA1).

Positive reactions were also generally obtained when the haploids were mated with other natural diploids with a bipolar heterothallic pattern (G2-n with CA1 and K69, CA1-n with G2, G9 and K69). However, some questionable and even negative results were also observed (Table 1).

The four haploids of the G2 series were also mated with three synthetic diploids obtained from the mating of two haploids and the four haploids of the series CA1 were mated with one such synthetic diploid. A majority of positive results were observed (Table 1).

When the haploids were mated with the two homothallic isolates C1 and U1, a large majority of positive results were obtained (Table 1).

The control matings with diploids belonging to different tropical *Armillaria* species *(A. puiggarii, A. mellea* ssp. *nipponicana, Armillaria* sp. or SIG III) gave negative results (Table 1). Moreover, black lines (typical of antagonistic reaction between incompatible *Armillaria* isolates) were observed in the confronting zone.

2) Fruiting *in vitro* of diploids originating from the Buller phenomenon

Subcultures of the diploidized haploid of six positive haploid-diploids pairings were submitted to fruiting in the laboratory. These matings were as follows: CA1-5 × K69, CA1-5 × CA1, the two repeats of CA1-5 × G9, CA1-2 × U1 and CA1-5 × U1. Mature basidiomes were obtained with the diploids originating from CA1-2 × U1, CA1-5 × U1 and the two repeats of CA1-5 × G9.

Single spore isolates from CA1-5 × U1 were all identical and displayed the same crustose morphology as the single spore isolates from the basidiomes of U1, showing that the new diploid obtained from the haploid x diploid mating was homothallic.

In contrast, the single-spore isolates from the CA1-5 × G9 basidiomes displayed the same cotonous and fluffy morphology as the single-spore isolates originating from the diploid G9. When mated with each other, these isolates gave rise to crustose isolates in about half of the matings. These cultures fell into two mating types, showing a bipolar pattern, as for G9.

3) Molecular analysis of the diploidized haploid

In the three matings CA1-5 × G9, CA1-5 × K69 and CA1-5 × C1, the modified haploid was analysed through the RAPD method.

Of the 24 primers tested, some failed to provoke any amplification or gave patterns difficult to interpretate, while others gave the same patterns for the haploid and the diploid. Only 10, 10 and 15 primers respectively revealed a diffe-

rent pattern for the haploid and the diploid donor in the three 3 pairings CA1-5 × G9, CA1-5 × K69 and CA1-5 × C1 (Table 2). These 35 cases were distributed between two situations:

(i) in a majority of cases, the diploidized haploid showed the same pattern as the donor diploid (fig. 2A).

(ii) in some cases, the diploidized haploid showed a recombined pattern between the haploid and the donor (fig. 2B and 2C).

As shown in table 2, for the mating CA1-5 × C1, all the RAPD patterns of the diploidized haploid were identical to the patterns of the donor diploid, except in one case when CA1-5 × C1 differed from C1 by one band only. The same situation was observed for CA1-5 × G9. In contrast, for the diploid originating from the diploidization of CA1-5 by K69, 7 patterns were recombined (generally for more than 1 band) while for the other 8 primers, the patterns were identical for the diploidized haploid and the donor diploid.

Table 2. Results of the different RAPD profiles obtained from the diploidization of the haploid CA1-5 *(A. heimii)* by 3 diploids G9, C1, K69
Tableau 2. Résultats des différents profils RAPD obtenus de la diploïdisation de l'haplonte CA1-5 d'*A. heimii* par 3 diplontes G9, C1, K69

Primer	*Sequence 5' → 3'*	*Diploids*		
		G9	*C1*	*K69*
R 25	ACTTGAGGCG	**1**	**1**	**2**
R 28	ATGGATCGGC	**0**	**0**	**1**
UBC 31	CCGGCCTTCC	**1**	**1**	**2**
OPD 20	ACCCGGTCAC	**1**	**1**	**2**
OPE 06	AAGACCCCTC	**(2)**	**?**	**1**
OPE 07	AGATGCAGCC	**1**	**1**	**?**
OPE 08	TCACCACGGT	**0**	**0**	**1**
OPE 10	CACCAGGTGA	**1**	**1**	**2**
OPE 12	CAGCTCACGA	**0**	**0**	**1**
OPE 13	CCCGATTCGG	**1**	**1**	**1**
OPF 01	ACGGATCCTG	**1**	**1**	**2**
OPF 05	CCGAATTCCC	**1**	**1**	**(2)**
OPG 12	CAGCTCACGA	**0**	**0**	**1**
OPG 14	GGATGAGACC	**0**	**(2)**	**1**
OPG 16	AGCGTCCTCC	**1**	**1**	**1**

1 = identical RAPD profile between the diploidized haploid and the diploid donor.
2 = recombined RAPD pattern of the diploidized haploid between the haploid and the diploid donor.
(2) = similar to case 2, the difference based on 1 polymorphic band.
0 = identical RAPD pattern between the haploid and the diploid donor.
? = unclear pattern.

1 = profil de l'haplonte diploïdisé identique à celui du diplonte diploïdisant.
2 = profil de l'haplonte diploïdisé recombiné entre les profils de l'haplonte et du diplonte diploïdisant.
(2) = cas 2 reposant sur une difference d'une seule bande.
0 = identité de profil RAPD entre l'haplonte et le diplonte diploïdisant.
? = profil RAPD non interprétable.

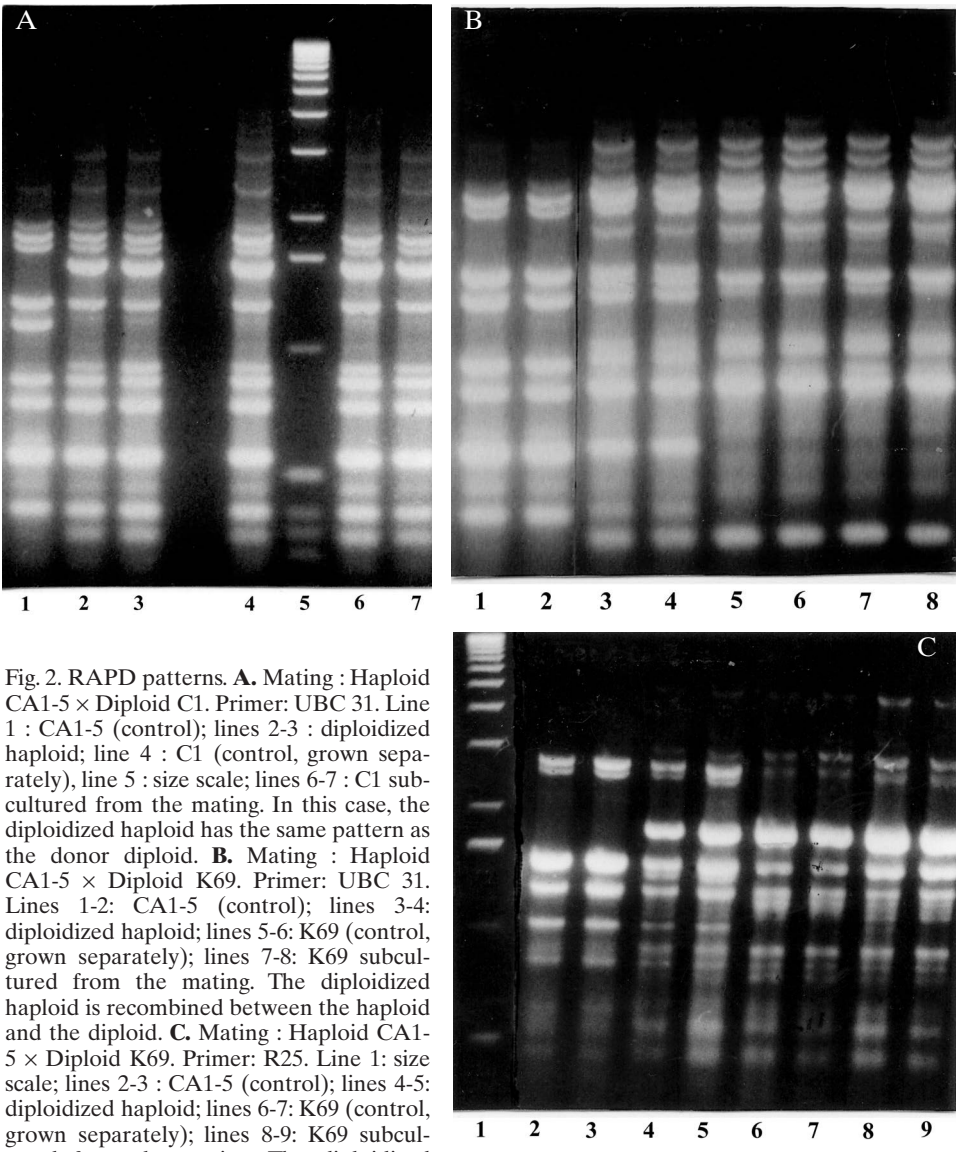

Fig. 2. RAPD patterns. **A.** Mating : Haploid CA1-5 × Diploid C1. Primer: UBC 31. Line 1 : CA1-5 (control); lines 2-3 : diploidized haploid; line 4 : C1 (control, grown separately), line 5 : size scale; lines 6-7 : C1 subcultured from the mating. In this case, the diploidized haploid has the same pattern as the donor diploid. **B.** Mating : Haploid CA1-5 × Diploid K69. Primer: UBC 31. Lines 1-2: CA1-5 (control); lines 3-4: diploidized haploid; lines 5-6: K69 (control, grown separately); lines 7-8: K69 subcultured from the mating. The diploidized haploid is recombined between the haploid and the diploid. **C.** Mating : Haploid CA1-5 × Diploid K69. Primer: R25. Line 1: size scale; lines 2-3 : CA1-5 (control); lines 4-5: diploidized haploid; lines 6-7: K69 (control, grown separately); lines 8-9: K69 subcultured from the mating. The diploidized haploid is recombined between the haploid and the diploid.

*Profils RAPD. **A.** Croisement haplonte CA1-5 × Diplonte C1. Amorce : UBC 31. Piste 1 : témoin CA1-5; pistes 2-3 : Haplonte diploidisé; piste 4 : témoin C1 (cultivé seul); piste 5 : échelle de taille; pistes 6-7 : C1 (repiqué à partir du croisement). L'haplonte diploïdisé a le même profil que le diplonte donneur. **B.** Croisement haplonte CA1-5 × Diplonte K69. Amorce : UBC 31. Pistes 1-2 : témoin CA1-5; pistes 3-4 : Haplonte diploidisé; pistes 5-6 : témoin K69 (cultivé seul); pistes 7-8 : K69 (repiqué à partir du croisement). L'haplonte diploïdisé est recombiné. **C.** Croisement haplonte CA1-5 × Diplonte K69. Amorce : R25. Piste 1 : échelle de taille; pistes 2-3 : témoin CA1-5; pistes 4-5 : Haplonte diploidisé; pistes 6-7 : témoin K69 (cultivé seul); pistes 8-9 : K69 (repiqué à partir du croisement). L'haplonte diploïdisé est recombiné.*

With all the primers tested, the diploid donor (subcultured from the mating) and a control consisting of the same diploid isolate grown separately showed completely identical patterns.

CONCLUSIONS AND DISCUSSION

The Buller phenomenon is widespread in the tetrapolar *Armillaria* species of temperate areas (Korhonen 1978, Anderson & Ullrich 1982). The results obtained in the present study show that this 'diploid-haploid mating' also exists in the bipolar populations of the African species *Armillaria heimii* Pegler, the only *Armillaria* species presently known to include bipolar forms.

The haploids which were chosen for this study have a stable, cottonous morphology in culture without appearence of crusts even when they are grown side-by-side with an isolate belonging to a different *Armillaria* species. The modification of the morphology of these haploids, which become crustose after sixteen days when mated with diploids of the same species, is a strong presumption for their diploidization. In addition, the subculture of the peripheral parts of this transformed colony (the parts the most remote from the diploid colony with which the haploid is paired) generally generates a crustose colony. A hypothesis alternative to the Buller phenomenon would be that the hyphae of the diploid isolate paired with the haploid isolate have grown among the hyphae of the haploid colony and that their tips have emerged at its periphery. This hypothesis would involves a strong increase of the growth of the diploid hyphae in contact with the haploid hyphae, a process never observed in *Armillaria*s.

The haploid mycelia of *A. heimii* (which can be obtained only from heterothallic populations of this species) can be diploidized not only by the diploid isolates of these heterothallic populations, but also by diploid isolates from the homothallic populations. This result is a the further argument (in addition to the absence of somatic incompatibility between the diploids, Abomo-Ndongo & Guillaumin 1997) in favour of a single species *Armillaria heimii* despite its extensive variability in many fields (Mohammed et Guillaumin 1993, Abomo-Ndongo 1997). Mwenje and Ride (1996, 1997) described in Zimbabwe three different *Armillaria* groups, among which at least the groups I and III probably belong to this '*A. heimii sensu lato'* defined by the two criteria: 1) absence of somatic incompatibility within the species and 2) sexual compatibility with the haploids of the heterothallic populations of the species (through the Buller phenomenon).

The results show that the ability of the haploids to be diploidized by the diploids is variable (even among the siblings haploids from the same fruiting body). Symmetrically, the diploids are inequal in their capacity to diploidize a given haploid. Surprisingly, in our experiments, the isolate which was the most successful in diploidization of haploids was a homothallic isolate, C1, from Congo.

In a number of pairings, the different subcultures gave different results, some pairings showing the fluffy morphology of a non-transformed haploid, some others showing the diploid, crustose morphology. As stated by Carvalho et al. (1995), the migration of the diploid nuclei into the haploid colony is probably slow and gradual, and it is likely that a mixture of haploid and diploid nuclei subsists for some time in the cytoplasm of the haploid. According to the place where the cutting is taken, the result of the subculture may be different. However, only one

generation of subculture is generally sufficient to obtain a homogenous morphology (haploid or diploid), showing that the process of selection of one type of nuclei was completed in the subcultured colony.

To try to understand the origin of the diploid nuclei which are generated in the Buller phenomenon in *Armillaria heimii*, we analysed with RAPD markers three isolates originating from the diploidization of the same haploid by three diploids.

For two of these associations, a large majority of primers showed the same bands in the donor, diploid, isolate and in the diploidized haploid. Their patterns were different only with one primer and this difference dealt with only one band. In both cases, the asssertion of recombination between the haploid and the diploid would be questionable, since it would rest on one pattern only, and on one band in the pattern. For the third association, recombined patterns were observed for 7 primers out of 15, showing that the diploid originating from the Buller phenomenon was recombined, as it was already established for the tetrapolar species *A. ostoyae* (Rizzo & Harrington 1992) and *A. gallica* (Carvalho *et al.* 1995).

The diploid involved in the diploid-haploid matings was not modified by the Buller phenomenon. This observation suggests that the migration of the nuclei is unidirectional and does not involve the haploid nuclei. The same situation had been observed in the other diploid-haploid matings already analysed in *Armillarias*.

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