SHORT COMMUNICATION

Maleness in *Allomyces arbuscula*

By MUKTI OJHA

Laboratoire de Microbiologie générale, Département de Biologie végétale, Université de Genève,
CH-1211 Genève 4, Switzerland

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Coloured gametangia in the male and female hybrid *Allomyces arbuscula* × *A. macrogynus* and in various mutants of *A. arbuscula* were essentially of two sizes, large and small, measuring approximately $1847 \pm 347 \mu\text{m}^2$ and $1032 \pm 179 \mu\text{m}^2$ respectively. The large coloured gametangia were the same size as those of the female wild-type. The carotene content of the culture increased with masculinization and with increases in the proportion of large coloured gametangia. Relative gametangial size alone is not a reliable phenotypic marker of sexuality in *Allomyces*.

INTRODUCTION

Allomyces is a monoecious, hermaphroditic fungus. The gametophyte is characterized by superimposed male and female gametangia whose positioning is species specific (Emerson & Wilson, 1954). Morphological characteristics of these gametangia include their size and colour, the females being twice as large as males, colourless and synthesizing a sesquiterpene (C_{12}) hormone, sirenin. The males are bright orange due to massive synthesis and accumulation of a tetraterpene (C_{40} , mainly γ -carotene). It appears that the differential size of the gametangia also determines the size of cytoplasmic inclusions, particularly lipid crowns (aggregates of large lipid granules around nuclei in differentiated gametangia), nuclei and nuclear caps which are larger in the female than in the male gametangia. In wild-type strains the ratio of males to females is 1:1. However, the ratio is altered in certain segregants of interspecific hybrids (Emerson & Wilson, 1954) and nuclear (Stumm, 1958; Olson *et al.*, 1982) or cytoplasmic male mutants (Turian *et al.*, 1969; Ojha, 1983). If gametangial size and colour, the two predominant phenotypic markers of sexuality in *Allomyces*, are associated with the genes controlling male- or femaleness, then unisexual mutants should possess either large or small gametangia. We have measured the size of large and small gametangia, their frequency, and the total carotenoid content of a wild-type and some male strains.

METHODS

Strains. *Allomyces arbuscula* Bali wild-type, hybrid male and female strains (*A. arbuscula* × *A. macrogynus*, obtained by courtesy of the late Professor R. Emerson), an X-ray induced male mutant of *A. arbuscula* ['mas' (masculine), from Professor C. Stumm] and two mutants induced by treatment with ethidium bromide in this laboratory were maintained on YPSS slopes (yeast extract, 0.2%; K_2HPO_4 , 0.05%; soluble starch, 0.75%; Emerson, 1941) at room temperature (approx. 22 °C).

Mutagenesis with ethidium bromide. Meiospores were liberated from dried resistant sporangia, filtered, pelleted and washed with dilute salts solution (DS, pH 5.5; Machlis, 1953) by centrifugation at 500 *g*. The spores were counted and inoculated onto ethidium bromide-containing ($100 \mu\text{g ml}^{-1}$) YPSS plates solidified with agar as described earlier (Turian *et al.*, 1969). After two weeks, a check was made of the sex ratio of the gametangia from the colonies visually selected to be males which were more pigmented than the wild-type. Colonies with a male:female ratio of over 90% were selected and subcultured. The estimation of gametangial size (length × width) and the sex ratio (percentage of coloured gametangia in the total population) were measured from samples of 7- to 10-d-old culture grown at room temperature.

Table 1. Measurements of gametangial size, ratio of small to large coloured gametangia, yield of carotenes and percentage of males in strains of *A. arbuscula*

Measurements were made in the wild-type *A. arbuscula* Bali 1, hybrid male and female strains (*A. arbuscula* × *A. macrogynus*; Emerson & Wilson, 1954) and some male mutants of *A. arbuscula* Bali 1. The measurements of gametangial sizes and sex ratios represent a mean of at least 100 gametangia. Gametangial sizes are given as the product of length × width ± SD.

Strain	Mean size of gametangia (μm ²)			Ratio of small:large coloured	Yield of γ-carotene [ng (mg dry wt) ⁻¹]	Percentage of males
	Large, colourless	Large, coloured*	Small, coloured†			
Wild-type	1524 ± 300	—	1040 ± 213	1:1	—	—
'mas'	—	2183 ± 518	1162 ± 200	1:1	91	—
2-2	—	1962 ± 517	1056 ± 190	1:3	—	96
1-4	—	2033 ± 254	978 ± 198	1:3	112	95
Male hybrid	1530 ± 280	1597 ± 275	979 ± 166	1:3.6	64	90
Female hybrid	1445 ± 195	1459 ± 171	988 ± 142	1:3	12	10

* The mean size of gametangia from the mutant strains was 2059 ± 421; the overall mean size of gametangia from the hybrids and masculinized strains was 1847 ± 347.

† The mean size of gametangia from the mutant strains was 1065 ± 196; the overall mean size of gametangia from the hybrids and masculinized strains was 1032 ± 179.

Extraction of carotene. Discs 4 mm in diameter from actively growing culture on solid YPSS medium in Petri plates were subcultured on fresh medium overlaid with dialysis membrane. The mycelia were scraped off and lyophilized and the carotene was extracted and estimated according to Davis (1976).

RESULTS AND DISCUSSION

In the male hybrid and mutant strains, gametangia are heterogeneous in size, whether they are produced singly or in pairs. When present in pairs they are morphologically similar to the wild-type, except for the presence of carotenoids. Table 1 shows the measurement of gametangial size, the ratio of small to large coloured gametangia, the yield of carotene and the proportion of males in various strains. In the wild-type the mean size ± SD of large colourless female and small coloured male gametangia was 1524 ± 300 μm² and 1040 ± 213 μm² respectively. In the hybrid female strain the size of female and large male gametangia was 1445 ± 195 μm² and 1459 ± 171 μm² respectively whereas the size of small male gametangia was 988 ± 142 μm². The large colourless females and coloured males in the hybrid male strain were of approximately equal size, 1530 ± 280 μm² and 1597 ± 275 μm² respectively, and the small coloured male gametangia were 979 ± 166 μm². The masculinized mutants also had large coloured male gametangia produced singly or in chains. The mean size of the large and small gametangia from the mutant strains was 2059 ± 429 and 1065 ± 196 μm² respectively. The overall mean size of the coloured gametangia from the hybrids and masculinized mutants was 1847 ± 347 μm² and 1032 ± 179 μm² for the large and small respectively. Their ratio in the wild-type and 'mas' mutant was 1:1, but 3:1 in the ethidium bromide induced mutants (strains 1-4 and 2-2). The yield of carotene depended upon the sex ratio and the ratio of large to small coloured gametangia. Thus the mutant 1-4 had more carotenes than the 'mas' mutant or the hybrid male strain.

These results show that the increase in the number of coloured gametangia in the male strains was probably due to the acquisition of the synthetic capacity for γ-carotene in otherwise female cells. Such cells normally synthesize sirenin, an oxygenated sesquiterpene, while the males synthesize γ-carotene, a tetraterpene. The carotene is unlikely to be a precursor of the oxygenated sesquiterpene sirenin, since carotenization does not precede differentiation of the female organ in *Allomyces*. This is in contrast to the Mucorales where β-carotene is the precursor of the sex hormone trisporic acid (C₁₆ terpenoid). Thus masculinization in *Allomyces* may involve removal of the block in γ-carotene biosynthesis in the large gametangial region which normally synthesizes sirenin. In conclusion, it appears that the relative gametangial size is not a reliable phenotypic marker of sexuality in *Allomyces*.

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