SHORT COMMUNICATIONS

Genetic Analysis of Pigmentation in *Arthroderma benhamiae*

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Although the study of dermatophyte genetics is in its infancy, it has already proved to be an important tool for assigning isolates to specific species (Padhye & Carmichael, 1969). Evidence for cross-fertility, which is sufficient to refer two varieties to the same species, can be shown by the production of fertile cleistothecia when an unknown strain is crossed with strains of known mating type. Such evidence does not require extensive genetic analysis of the organisms. However, the inheritance and control of expression of such traits as thallus morphology, pathogenicity, pigmentation and spore morphology are also significant, since these are characteristics which often are used as criteria for dermatophyte classification (Sekhon & Carmichael, 1972) especially in varieties for which no perfect stage has been discovered. Understanding the genetic and environmental control of such traits, as well as their degree of genetic stability, will be very useful in evaluating them as criteria for classification.

This report presents the results of a study concerning the genetics of pigmentation in the dermatophyte *Arthroderma benhamiae*, which is the perfect stage of *Trichophyton mentagrophytes* (Ajello & Shu-Lon, 1967).

METHODS

Cultures. The two strains of *A. benhamiae* used were obtained from Dr L. Ajello. Strain TM17 has the *a* compatibility allele. Strain TM20 carries the *A* compatibility allele. Both produce pigments, but pigmentation varies with the type of culture medium used for cultivation.

Production of single-spore isolates and maintenance of stock cultures. Single-spore isolates were obtained from cultures grown in 0.25 l prescription bottles on modified Sabouraud's dextrose agar supplemented with 1% (w/v) yeast extract. Spores were suspended in sterile saline solution by agitating with a Vortex mixer to release conidia. The spores were sedimented by centrifugation at 3000 g for 20 min, the supernatant decanted, and the spores resuspended in sterile saline containing 0.025% (v/v) Tween 80. Contaminating mycelia were removed by filtration through absorbant cotton (Emmons, 1932). The filtrate contained a mixture of microconidia and macroconidia. Macroconidia were removed by gravity sedimentation from the Tween 80 solution (Kwon-Chung, 1967).

Germination of single microconidia was observed after incubation for 18 h at 30 °C after transfer to sterile Mycosel agar plates.

After 10 days growth a microconidial suspension was made from each single-spore isolate thallus and stored in sterile glass-distilled water (Ajello & Shu-Lon, 1967). Stock suspensions of F1 monoascospore isolates were prepared by the same technique.

Pigmentation on chemically defined medium. The defined medium of Silva (1953) was used to determine the nutritional conditions most favorable for stable pigment production. Double-strength solutions of this medium were prepared, and equal volumes of single amino acid solution were added to give a final nitrogen concentration of 0.2 g/l.

The media, adjusted to pH 7.0, were dispensed in 10 ml quantities in 20 × 150 mm culture tubes and inoculated with 0.5 ml of spore suspension. Incubation was carried out for two weeks at 30 °C in a New Brunswick convection incubator shaker.

Production of the Ascigerous state. Portions of a thick suspension of spores of each mating type were inoculated onto plates of sifted soil-horsehair agar medium. The inoculated plates were wrapped in aluminum foil to exclude light and incubated at room temperature.

After 3 weeks' incubation cleistothecia were collected and any adhering conidia were removed by gently rolling the cleistothecia with a fine needle across the surface of 4 % water-agar plates. The cleaned cleistothecia were then transferred to another plate containing 4 % water-agar, prior to dissection.

Dissection of cleistothecia and asci. A single cleistothecium was transferred to a shell vial containing 0.5 ml distilled water, and the cleistothecium ruptured by crushing against the wall of the vial. The walls of the asci released from the ruptured cleistothecium were softened by treatment with 0.05 ml undiluted snail enzyme containing 179 500 units glucuronidase and 47 000 units sulphatase/ml (Glusulase, Endo Lab) for 15 to 30 min (Johnston & Mortimer, 1958).

Isolated asci were transferred to sterile Mycosel-yeast extract (1 %, w/v) agar blocks on Shoemaker slides using a Leitz micromanipulator. The asci were crushed and individual ascospores separated by dragging through a water droplet on the surface of the agar block. The spores on the slides were germinated in a moist chamber, transferred to modified Sabouraud's dextrose agar plates, and incubated until conidia were produced. Stock conidio-spore suspensions were made for each isolate. These spore suspensions were used as inocula for assaying the mating type and pigmentation characteristics of each ascospore produced in the isolated asci.

Determination of mating compatibility of F₁ ascospores. For our purposes the F₁ ascospores are defined as those ascospores resulting from the cross of the haploid parents TM17 and TM20. The mating type of each viable ascospore obtained from the F₁ generation was determined by back-crossing to parental strain TM17, which is the *a* compatibility type. Those F₁ progeny which produced fertile cleistothecia were considered to be *A* mating type, while those which did not were regarded as *a*.

RESULTS AND DISCUSSION

The production of pigments of *A. benhamiae* strains TM17 and TM20 on chemically defined media containing various amino acids as a nitrogen source was determined. Strain TM20 was either colourless or produced a yellow pigment on all the amino acids tested which supported growth (including DL-serine, L-lysine, glycine, L-isoleucine, L-asparagine, L-aspartic acid, L-glutamine, L-glutamic acid, L-arginine, L-arginine HCl, L-alanine and L-leucine). Strain TM17 produced a yellow pigment on L-glutamic acid, L-glutamine, L-asparagine, L-isoleucine and L-lysine. No pigment was produced on DL-serine, glycine or L-arginine. A brown pigment was produced on all other amino acids tested except for DL-threonine, L-methionine and L-histidine, which did not support growth of either strain.

The fact that the two strains produced different pigmentation under identical cultural conditions indicates that expression of pigmentation in these strains is a function of their

Table 1. *Tetrad analysis of mating compatibility and pigmentation alleles among monoascospore isolates obtained from a TM17 × TM20 cross*

Ascus	Spore number								Tetrad class
	1	2	3	4	5	6	7	8	
I	<i>AY</i>	<i>AB</i>	<i>aY</i>	<i>aB</i>	<i>aB</i>	<i>AY</i>	<i>AB</i>	—	TT
II	<i>AB</i>	<i>aB</i>	<i>AB</i>	<i>aY</i>	<i>AY</i>	<i>aB</i>	<i>AY</i>	—	TT
III	<i>AY</i>	<i>AY</i>	<i>AY</i>	<i>aB</i>	<i>aB</i>	<i>aB</i>	—	—	PD
IV	<i>aB</i>	<i>AY</i>	<i>aB</i>	<i>aB</i>	<i>AY</i>	<i>AY</i>	—	—	PD
V	<i>aB</i>	<i>AY</i>	<i>aB</i>	<i>AY</i>	<i>AY</i>	<i>aB</i>	<i>AY</i>	<i>aB</i>	PD
VI	<i>AB</i>	<i>aB</i>	<i>AY</i>	<i>aB</i>	<i>aY</i>	<i>AY</i>	<i>AB</i>	—	TT
VII	<i>AY</i>	<i>AY</i>	<i>aB</i>	<i>aB</i>	<i>AY</i>	<i>aB</i>	—	—	PD
VIII	<i>AY</i>	<i>aB</i>	<i>AY</i>	<i>AY</i>	<i>aB</i>	<i>AY</i>	<i>aB</i>	<i>aB</i>	PD
IX	<i>AY</i>	<i>aB</i>	<i>aB</i>	<i>AB</i>	<i>aY</i>	<i>aY</i>	—	—	TT
X	<i>AY</i>	<i>aB</i>	<i>AY</i>	<i>AY</i>	<i>aB</i>	<i>aB</i>	—	—	PD

A and *a*, mating compatibility types; *Y*, yellow pigmentation; *B*, brown pigmentation; —, no growth; TT, tetratype; PD, parental ditype.

genotype rather than phenotype, and should, therefore, be subject to genetic analysis. To test this hypothesis F₁ ascospores produced from a TM17 and TM20 cross were assayed for pigment production on the defined medium with L-leucine as the nitrogen source. On this medium TM17 produces a brown pigment (B) and TM20 is yellow (Y). The results of this experiment are given in Table 1.

Of the 80 ascospores isolated from ten asci, 67 germinated and could be assayed for pigmentation. Of those which germinated, 32 produced yellow pigment and 35 were brown. This closely approximates a 1:1 distribution of B and Y among the progeny. Additional evidence for 1:1 segregation of pigmentation is seen in asci 5 and 8. All eight ascospores produced from these two asci germinated and, for each ascus, 4 brown and 4 yellow progeny were obtained.

Inheritance of traits by the F₁ generation in a 1:1 ratio indicates that the traits are carried on homologous chromosomes. There is not yet sufficient information concerning pigmentation in *Arthroderma* to allow conclusions as to whether the loci determining brown or yellow phenotype are themselves alleles. This will require a more detailed understanding of the nature of the pigments and their mechanism of biosynthesis.

Ajello & Shu-Lon (1967) reported that inheritance of compatibility in *A. benhamiae* occurred through a one locus-two allele system, as indicated by the 1:1 ratio of *a* and *A* among F₁ progeny. Those F₁ progeny from the TM17 × TM20 cross which were scored for pigment production were also scored for mating compatibility. These results also appear in Table 1. Of the 67 ascospores which germinated, 34 were of the *A* compatibility type and 33 were *a*.

The results presented in Table 1 permit a tetrad analysis of the progeny *aA* YB ditypes produced from the TM17 × TM20 cross. *Arthroderma benhamiae*, along with several other *Arthroderma* and *Nannizzia* species, has a haploid chromosome number of four (Weitzman, Allerdice & Silva-Hufner, 1970). Tetrad analysis of the F₁ asci allows recognition of linkage between loci through observation of distribution of alleles among progeny ascospores. Strain TM17 has the genotype *aB*, and TM20 is of genotype *AY*. Three possible tetrad classes can be obtained from a cross between parental strains of these ditypes (Fincham & Day, 1971). These are the parental ditypes (PD) represented by the genotypes *aB*, *aB*, *AY* and *AY*, non-parental ditype (NPD) represented by the genotypes *aY*, *aY*, *AB* and *AB*, and tetratype (TT) with genotypes *AY*, *AB*, *aY* and *aB*.

The results from Table 1 show that four of the F₁ asci are tetratype (asci I, II, VI and IX) while the remainder are parental ditype. Since the number of NPD tetrads is much less than those of the PD type it appears that the mating compatibility and pigmentation alleles are linked. NPD tetrads can be formed only through recombination if loci are located on homologous chromosomes. Failure to obtain new tetrads also supports linkage of the pigmentation alleles, which was indicated by the 1:1 segregation patterns observed with the B and Y loci. However, data are not sufficient to allow an accurate estimation of linkage for these markers.

The degree of linkage can be estimated from the ratio of recombinant tetrads (0.5 TT + NPD) to the total number of tetrads (Fincham & Day, 1971). Applying this relationship to the data in Table 1, we obtain a recombination frequency of 20%. The pigmentation and mating compatibility alleles would then be located on the same chromosome, approximately 20 map units apart.

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