Melanotus eccentricus: cultural characters and mating system

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Utilizing five collections of *Melanotus eccentricus* from Puerto Rico, cultural and mating characteristics are described. Dikaryon cultures produce laccase and tyrosinase. The species is sexually unifactorial (= bipolar), with complex mating-type genes.

Keywords: Mating systems, cultural characters, *Melanotus*, Agaricales, Genetics.

Mating data and cultural characters were provided for *Melanotus defraudatus* Horak & al. (1990) and *M. hartii* Ammirati et al. (1979) with their original descriptions. This paper intends to furnish similar data for *M. eccentricus* (Murr.) Singer, a common subtropical agaric fruiting throughout the Caribbean area. Identification was made using the generic monograph by Horak (1977). Dr. Horak subsequently confirmed the identification of the 1989 collections.

Materials and methods

Basidiomata . – Specimens were photographed, notes were made on morphological characters, and basidiomata were dried over warm air.

Cultures. – Single-spore isolates were obtained by suspending a portion of pileus, including several lamellae, over malt extract (Difco, 1.5%) agar (Difco Bacto-agar, 2.0%; MEA). When a spore deposit was barely visible on the agar surface, the pileus tissue was discarded. Spores were allowed to germinate (5-20 hours), and single-spore germlings were harvested and transferred separately to MEA Petri dishes for further growth. When resultant colonies were 6-8 mm diameter, they were transferred to slanted tubes of MEA for storage at 4 C. Polyspore cultures were harvested after spore germlings had become overgrown, and were treated in the same way as single-spore isolates.

Self-crosses. – Twelve single-spore isolates from each collection were chosen (from the 20 usually originally established and

stored) for self-cross experiments. Small squares of actively growing mycelium were cut and paired in all combinations, being placed at a distance of 12-15 mm. Care was taken to allow time not only for mycelial confrontation, but for possible contact zone morphology differentiation (usually totalling not more than three weeks).

Compatible crosses were inferred by presence of clamp connections on hyphae in the contact zone. Notes were made on clamp connections on the obverse sides of inoculum blocks as inferential evidence of nuclear migration. When self-cross experiments were analyzed, two isolates of each mating type were chosen as tester strains, based on their vigor and their ability to dikaryotize their mates. Tester strains for collections 2443 and 2499 have been deposited at ATCC.

Intercollection matings were performed using tester strains from each collection. These were mated in all combinations, as well as with those of the same collection (as a confirmation of previous self-cross data), using the same methods as those for self-crosses.

Because of data generated in matings between collections 3487 and 3488, a full complement of single-spore isolates of these collections were mated, using the same methods as for self-crosses.

Lethal reactions. – Especially in, but not limited to, contact zone morphology below, extensive hyphal lysis was noted. In self-crosses of collection 2443 these reactions were subjectively judged on a scale of 0-2 (0 = no lethal reaction; 1 = weak to moderate; 2 = strong).

Macrochemical reactions. – Dikaryon (polyspore) cultures of collections 2443 and 2493 were assayed for presence of laccase and tyrosinase. Colonies on MEA, potato dextrose agar (Difco; PDA) and Emerson's YPsS agar (Emerson, 1958) were grown in the dark for six weeks (following the regimen by Nobles, 1948). Colonies were sliced from inoculum block to periphery, with each slice divided into three lengths representing vigorous marginal mycelium, middle age, and old mycelium, each section approximately 3 × 15 mm. These sections were placed in empty separate Petri dishes, flooded with syringaldazine (for presence of laccase) and d-cresol (for presence of tyrosinase), and observed for 30 minutes for color changes (bright purple-pink for laccase, tan to reddish tan for tyrosinase, generally following the protocol by Marr, 1979, 1984; Marr & al., 1986).

Colors within quotation marks are from Ridgway (1912); alphanumerical notations are references to plates by Kornerup & Wanscher (1967). DAPI: 4'-6-diamidino-2-phenylindole; TENN: University of Tennessee herbarium; RHP: the author.

Material examined. – PUERTO RICO: Cayey Co., Bosque Carite, 2.11.89, coll. D.J. Lodge and RHP, field no. 2443 (TENN no. 48727); Junquillo Mts., El Verde

Biological Station, trail to tower, 6.11.89, coll. RHP, on rotting vine, field no. 2493 (TENN no. 48858); same location, 7.11.89, coll. RHP, on rotting palm, field no. 2499 (TENN no. 48744); same location, 3.12.90, coll. RHP, field no. 3446, on leaves of Musaceae (TENN no. 48859); Cayey Co., Reserve Forestal Carite, 8.12.90, coll. RHP & S. Gordon, field no. 3487, on rotting palm (TENN no. 48860); same location, 8.12.90, coll. RHP & D.J. Lodge, field no. 3488, on rotten palm (TENN no. 48861).

Results

Basidiomata. – Because descriptions of the species furnished by Murrill (1917), Singer (1947) and Horak (1977) were less than precise, the following somewhat expanded description of basidiomata is furnished, based on notes from fresh specimens.

Pileus up to 15 mm broad, opaque, abruptly inrolled when young; surface minutely scurfy to matted-tomentose, often concentrically areolate, approximately 'cinnamon buff' (6B4) to 'light ochraceous buff' (5A4) when young, mellowing to more or less 'vinaceous buff' (9B2) or 'tilleul buff' (5A2) or occasionally 'pale olive buff' (3B2) by maturity. – Lamellae decurrent, narrow, fragile, concolorous with pileus, 'pale pinkish buff' to 'pinkish buff' (6A3) or 'tilleul buff' (5A2) when young, darkening with spores to more or less 'avellaneous,' (7B3) 'drab' (6D3) or 'wood brown' (7C4) by maturity. – Stipe poorly developed to virtually absent, concolorous with lamellae apically, off-white at base, minutely downy, eccentric to nearly lateral. – Spore print (on pileus) 'bone brown' (7F8) to 'walnut brown' (7D6). – Odor and taste negligible.

Cultural characters. – All isolates grew rapidly (90 mm in 3-5 days). Single-spore isolates uniformly produced copious white aerial mycelium, usually semi-appressed/cottony, and very tardily developing a pallid tan color at the Petri dish rim. Mycelium of monokaryon isolates and dikaryon cultures was sticky - obviously involved in a coating of viscous material which remained liquid even after six weeks in sealed Petri dishes. In addition, random lengths of hyphae were covered with crystalline deposit as a crust. Similar crystals were precipitated in the MEA agar around these encrusted hyphae. No evidence of nematode-trapping devices was seen. Clamp connections were abundant and obvious on dikaryon aerial hyphae, hyphae at the agar surface, and submerged hyphae, although on the latter, clamp connections were fewer and less easily observed. Colony odor was negligible, except on YPsS agar, where odor was somewhat musty.

Asexual propagules (Fig. 1) were produced by most single-spore isolates. Sporogenesis commenced with the production of a narrow, circinate side-branch of a major hypha (Fig. 1A). When the circinate hyphae had curled from about 0.7-1.2 gyres, cross-walls

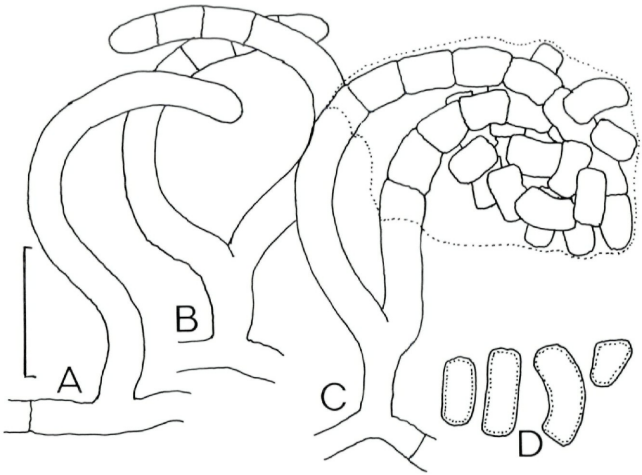

Fig. 1. – *Melanotus eccentricus*: Asexual propagule formation (collection 3487). – A. Initial sporophore without transverse septa. – B. Double sporophore with early transverse septa. – C. Small aggregation of propagules in mucoid droplet. – D. Various shapes of propagules. – Bar = 10 μm.

were formed, dividing the hypha into lengths approximately 4-7 μm long (Fig. 1B), which subsequently disarticulated into separate propagules. Often 2-5 such circinate hyphae were formed from the same point, all acting in similar fashion. Growth of the circinate sporophore continued, apparently from the base, for additional spores were produced after initial disarticulation (Fig. 1C). These propagules were involved in a mucoid or viscous droplet which, in time, spread over surrounding hyphae or on the agar surface. Each disarticulated propagule was cylindrical to truncate-reniform (Fig. 1D). Germination was observed when the propagules were transferred to fresh agar. In only one single-spore isolate (3488:10) was germination observed *in situ*. Observation of sporophores and propagules under epifluorescence microscopy revealed that the sporophore was uninucleate, as were those spores whose nuclei could be seen. Most spores, however, were impermeable to the buffer and/or DAPI stain, and no nuclei could be observed.

Enzyme analysis. – Laccase: Reactions on MEA were weak to absent for all mycelial ages, and over time through 30 mins. On PDA, reactions were stronger. Collection 2443 showed strong positive reaction for all mycelial ages in less than 1 min, remaining so through

the test period, while collection 2493 showed slower reactions, stronger in older and middle-age areas, weakest at colony margin. Colonies on YPsS reacted moderately to strongly for all mycelial ages, but not as strongly as colonies on PDA. Tyrosinase: Colonies on MEA showed no reaction for any mycelial age over the test period. Collection 2443 showed no reactions on PDA, but collection 2493 exhibited a slowly-developing (30-60 min) mottled, weak to moderate reaction from all mycelial ages. For YPsS colonies, collection 2493 showed slow positive reaction in youngest mycelium, while collection 2443 showed a slow brown to red-brown reaction only on the oldest mycelium.

Self-crosses. – Crosses consistently revealed a unifactorial mating system (Fig. 2). Fig. 4 shows preliminary assignment of mating types to tester strains of various collections.

	9	2	3	11	5	7	8	4	10	1	12	13
9		–	–	–	+	+	+	+	+	+	+	+
2	–		–	–	+	+	+	+	+	+	+	+
3	–	–		–	+	+	+	+	+	+	+	+
11	–	–	–		+	+	+	+	+	+	+	+
5	+	+	+	+		–	–	–	–	–	–	–
7	+	+	+	+	–		–	–	–	–	–	–
8	+	+	+	+	–	–		–	–	–	–	–
4	+	+	+	+	–	–	–		–	–	–	–
10	+	+	+	+	–	–	–	–		–	–	–
1	+	+	+	+	–	–	–	–	–		–	–
12	+	+	+	+	–	–	–	–	–	–		–
13	+	+	+	+	–	–	–	–	–	–	–	

Fig. 2. – *Melanotus eccentricus* (collection 3487): self-crosses of 12 single-spore isolates. – + = compatible cross; - = incompatible cross. Note unifactorial mating system.

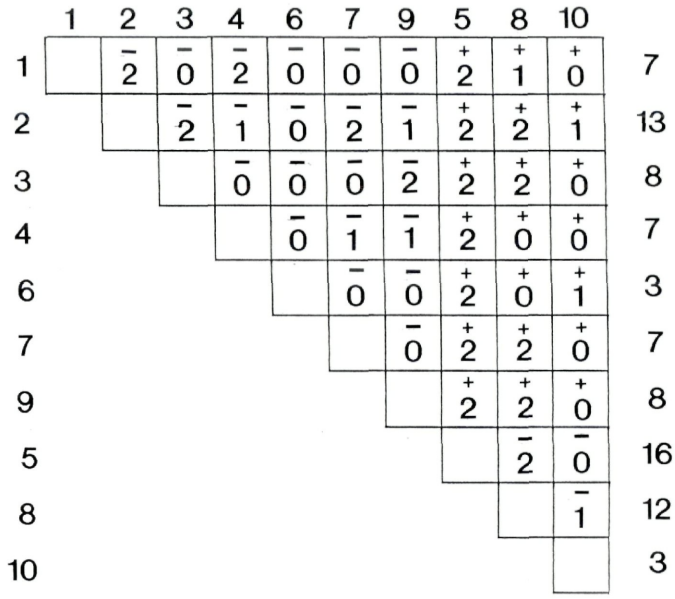

Fig. 3. – *Melanotus eccentricus* (collection 2443): self-crosses of 10 single-spore isolates plus lethal reactions. – + = compatible cross; - = incompatible cross; 0 = no lethal reaction; 1 = weak to moderate lethal reaction; 2 = strong lethal reaction. Scores for each isolate at right.

Contact zone morphology was noted, and could be described in the following categories: 1) overgrown, in which no differentiated contact zone was observed. Usually hyphae were sparse directly between inoculum blocks but denser outward in the contact zone. Such contact zones occurred in compatible and incompatible crosses, and so were not diagnostic. 2) The contact zone was bounded by two narrow bands of submerged, congestedly branched mycelium, reminiscent of 'barrage' reactions. This morphology could not be patterned, however. 3) The narrow contact zone exhibited very few hyphae, with the zone margins somewhat abrupt, but not dissimilar from the donor colonies. At first, this morphology was noted as 'flat,' but again, it could not be patterned, occurring on incompatible and compatible crosses.

Mating types for each collection were assigned arbitrarily (see Fig. 4, top).

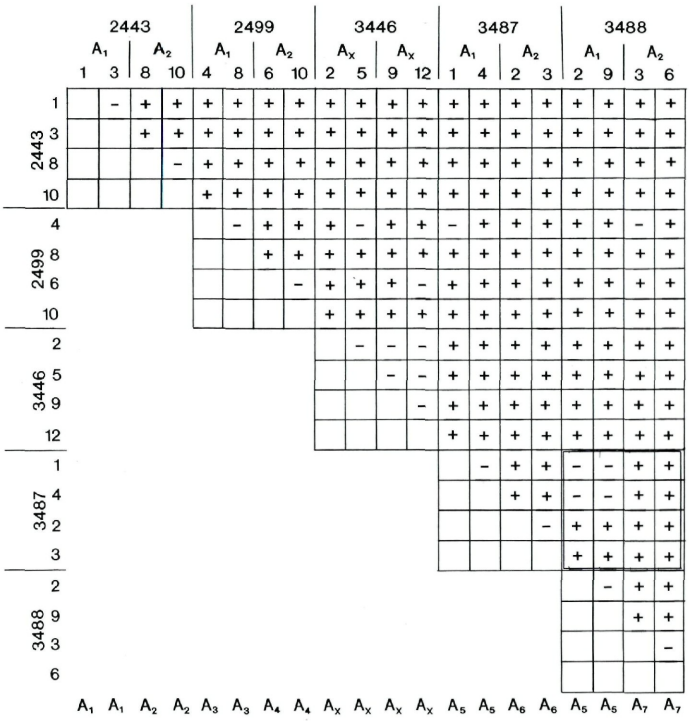

Fig. 4. – *Melanotus eccentricus*: intercollection matings. – + = compatible mating; - = incompatible mating. Preliminary mating type assignments at top; mating type reassignment after intercollection matings at bottom. Note shared mating type gene between 3487 and 3488.

Intercollection matings. – When tester strains of all collections were mated, virtually universal intercompatibility was observed (Fig. 4). Redundant self-crossed tester strains verified the unifactorial mating system inferred in self-crosses.

Within the intercollection mating grid, tester strains of 3487 × 3488 (Fig. 4, lower right) showed a pattern of shared mating type genes. Isolates 3487:1/4 × 3488:2/9 evidently were of the same mating type. To test this hypothesis, a full complement of single-spore

isolates (3487:1-15 × 3488:1-12,15) were mated. The resultant grid (Fig. 5) confirmed the data derived from tester strain matings. Based on these intercollection mating data, mating types were reassigned, as shown for tester strains in Fig. 4, bottom.

Lethal reactions. – Fig. 3 shows a mating grid for collection 2443, in which lethal reactions are superimposed on compatibility. When individual mating scores were summed for each isolate (Fig. 3, right) a wide range of variation was noted, in which specific strains caused consistent, strong lysis (2443:2, 5, 8) while others were involved very little (2443:6, 10).

3487 \ 3488	1	3	4	6	7	10	2	5	9	11	12	15	8
			A_7						A_5				
1	+	+	+	+	+	+	–	–	–	–	–	–	+
4	+	+	+	+	+	+	–	–	–	–	–	–	+
5	+	+	⊞	+	+	+	–	–	–	–	–	–	+
6	+	+	+	+	+	⊞	–	–	–	–	–	–	+
7	+	⊞	+	+	+	+	–	–	–	–	–	–	+
A_5 8	+	+	+	+	+	+	–	–	–	–	–	–	+
10	⊞	⊞	+	+	+	+	–	–	–	–	–	–	+
12	+	+	⊞	+	+	+	–	–	–	–	–	–	+
13	+	⊞	+	+	+	+	–	–	–	–	–	–	+
14	+	+	+	+	+	+	–	–	–	–	–	–	+
15	+	+	+	+	+	+	–	–	–	–	–	–	+
2	–	–	–	–	–	–	+	⊞	+	+	+	+	+
A_6 3	–	–	–	–	–	–	+	⊞	+	+	+	+	+
9	–	–	–	–	–	–	+	+	+	+	+	+	+
11	+	–	–	–	–	–	+	+	+	+	+	+	+

Fig. 5. – *Melanotus eccentricus*: expanded intercollection matings between collections 3487 and 3488. – + = compatible mating; - = incompatible mating; squared + = few, scattered clamp connections. 3488:8 is dikaryon. Mating type assignments at top and left.

Discussion

Culture characters

Production of asexual propagules was virtually identical to that described for *M. hartii* (Ammirati & al., 1979, where propagules were termed 'oidia'). Because these spores are involved in mucoid droplets, air dispersal is prohibited. Nonetheless, through either direct water dispersal, or especially through insect vectors, production of asexual propagules greatly enhances exploitation of ecological niches and long-distance distribution, as well as survival and importance of the monokaryon generation to the life-cycle of the species.

Equally interesting is the rapidly developing impermeability of walls of asexual propagules to water, exemplified by the inability to visualize nuclei using watery mountants under epifluorescence microscopy. One conclusion might be that such spores are insured of not germinating *in situ*, and therefore of not competing directly with the mycelium which produced them. Supporting this idea, asexual spores of only a single monokaryon isolate were seen to germinate *in situ*.

Isozyme analysis

Marr & al. (1986) offered a scheme in which higher basidiomycetous fungi were categorized by production of laccase and/or tyrosinase. The smallest group produced both enzymes, based on the same macrochemical reactions as utilized here, but applied to basidiomata. Vegetative dikaryon mycelium of *M. eccentricus* seems to produce both classes of compounds, but apparently both quantitative amounts and production location (old, middle-age, young mycelium) vary. This enzyme analysis coincides with the report by Horak & al. (1990) for *M. defraudatus*. In both cases, PDA was superior over YPsS and MEA for production of laccase and tyrosinase. Ammirati & al. (1979) reported positive syringaldazine reactions for *M. hartii*, but negative results with p-cresol.

Self-crosses

Of three species of *Melanotus* for which mating systems have been ascertained, one, *M. defraudatus* Horak & al. (1990) is bifactorial (= tetrapolar), while *M. hartii* (Ammirati & al., 1979) and *M. eccentricus* are unifactorial. Such variation is not uncommon (see Boidin, 1971, 1986), but with such a small sampling, no evolutionary extrapolation can be safely offered here.

Lack of differentiated contact zone morphology seems typical of unifactorial mating systems. Although some suggestion of 'barrage'

and 'flat' morphologies was observed, these did not sort into patterns, and therefore were not relevant in assigning mating types to isolates.

Lethal reactions

The commonly encountered lethal reactions were similar to those seen in other fungi (e.g. *Xeromphalina campanella* and other species of that genus; cf. Petersen, 1990). In bifactorial fungi, such lethality seems most expressed in 'flat,' or common-A matings, contributing significantly to the 'crevasse' between mated colonies.

Using the data on relative strength of lethal reactions presented in Fig. 3, three groups of isolates could be envisioned (e.g. group I, with score of 3; II, with scores of 7-8; and III, with scores of 12-16). Such a distribution could be explained by two loci (four alleles) governing some physiological phenomenon in which homozygous 'weak' produced group I, heterozygosity produced group II, and homozygous 'strong' produced group III. Because interpretation of lethal reactions is subjective, confidence in the assignment of scores is not high, and alternative explanations are surely possible. With lethal reactions occurring in both compatible and incompatible crosses, however, the independence of these genes from mating type factors cannot be doubted. Moreover, unlike 'fertility' genes in *Heterobasidion* (Chase & Ullrich, 1990a, b), lethal factors in *Melanotus eccentricus* do not prevent anastomosis or dikaryotization.

Intercollection matings

In spite of uniformly incompatible results of self-crosses in collection 3446, and arbitrary selection of tester strains, all tester strains were intercompatible with those from the other collections (Fig. 4). These results support the concept of complex mating-type genes, in which intercollection variation overrides within-collection incompatibility.

Collections 3487 and 3488 were gathered within 25 meters of one another, so it is not remarkable that they shared a mating type gene. Full-complement matings (Fig. 5) confirmed the limited tester strain data. These matings could be sorted by contact zone morphology into precisely the categories outlined above. Moreover, lethal reactions also occurred, similar to those reported above for self-crosses of collection 2443.

Using data from the intercollection mating grid, mating type genes could be reassigned. Fig. 4, bottom, shows this reassignment, including arbitrary numerical assignment of subunits in 3487 and 3488. Collection 3446, with no internal indication of mating types, could not be assigned or reassigned.

Acknowledgments

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