

An annotated pedigree of *Neurospora crassa* laboratory wild types, showing the probable origin of the nucleolus satellite and showing that certain stocks are not authentic.

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Abstract

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and E.G. Barry

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apparently authentic stocks of Lindegren 1A have been located, and we have concluded that FGSC 354 is indeed mislabeled. There are reasons to believe that all the stocks judged nonauthentic were incorrectly labeled prior to receipt by FGSC.

The nucleolus satellite is present in some laboratory strains (sat⁺) but not in others (sat⁻). Examinations of many wild types by Barry indicated that absence of the satellite is the norm. The N. crassa laboratory wild types that were tested are shown in Figure 1. Six wild-collected strains were also tested [N. crassa strains Panama A (FGSC 1131), Costa Rica A (FG SC 851), Puerto Rico 18a (FGSC 429) and Mauriceville-1cA (FGSC 2225); N. intermedia Honduras x6a (FGSC 1300); and N. discreta Kirbeyville-6A (FGSC 3228)]. All six wild-collected strains lack the satellite. With two exceptions, all sat⁺ strains could be traced back to the Abbott wild types. The exceptions were Lindegren (-) a (FGSC 541) and Lindegren 1A (FGSC 354); both were sat⁺. If either of these stocks were authentic, it would imply two independent origins of the satellite. A dual origin seems unlikely because all sat⁺ strains that have been tested are alike in containing two HindIII sites in the rDNA spacer, while most sat⁻ strains have only one (Metzenberg, in Table 4 of Reference 18).

Lindegren (-) a (FGSC 541) had previously been judged nonauthentic by FGSC because it was albino and morphologically abnormal, and by D.G. Catcheside because its rec genotype was not compatible with those of its supposed progeny (6). Apart from the sat problem, we had no doubt about Lindegren 1A (FGSC 354) until the study of Raju et al. (19). They found that, with one exception, inbreeding was completely correlated with a high frequency of bubble asci (defective asci containing eight tiny transparent spores). The exception was Lindegren 1A (FGSC 354) x Lindegren 25a (FGSC 353). These stocks were supposedly derived from inbred Lindegren stocks, yet crosses between them have few bubble asci.

We therefore sought other stocks of Lindegren 1A. Two were found, from J.F. Wilson and from the collection of E.L. Tatum. They are both sat⁻ and both give the expected high frequency of bubble asci when crossed with Lindegren 25a (FGSC 353). Furthermore, the Lindegren 1A from Wilson was not heterokaryon-compatible when tested with Lindegren 1A (FGSC 354) (Wilson, personal communication). Thus the suspect 1A (FGSC 354) is clearly different from 1A (Wilson) and from 1A (Tatum); the latter two strains are alike to the extent that they have been tested.

Certain other laboratory wild stocks had previously been considered nonauthentic because their rec or scot genotypes were inconsistent with those of stocks that were reported to be their ancestors or their progeny (6, 16). The laboratory wild types are heteromorphic for other naturally occurring variants (Table 1). Figure 1 shows these traits for all stocks in which they have been determined.

Our pedigree follows previous authors (see Figure 1 legend) except that we have made two corrections as follows:

Two studies have suggested that Lindegren 1A (FGSC 354) is not authentic, perhaps as a result of mislabeling, mixup, or contamination. One study concerns the origin of the nucleolus satellite, described here. The other concerns the correlation of inbreeding with a high frequency of bubble asci (19). A few other wild type stocks deposited with the Fungal Genetics Stock Center (FGSC) have previously been judged nonauthentic. We have therefore prepared an annotated pedigree of the laboratory wild types

in an attempt to evaluate authenticity on the basis of genotype, using naturally occurring variants such as het, rec, sat and scot.

Two

First, it is unlikely that the FGSC stocks of Lindegren (+) and (-), now called A and a, were the immediate ancestors of Lindegren 1A and 25a. The FGSC stocks of Lindegren (+) and (-) were obtained from CBS (Centraalbureau voor Schimmelcultures). The only Lindegren (+) and (-) cultures listed by CBS were deposited in 1937. At that time Lindegren et al. had completed a maximum of four generations of an inbreeding program undertaken to eliminate several sterility factors (11). By 1940, about seven more generations of inbreeding had been completed, which resulted in greatly improved fertility (12). Beadle obtained Lindegren's stocks early in 1941. (2). (Letters to D.D. Perkins from C.C. Lindegren and W.S. Malloch make it clear that Beadle's N. crassa stocks were obtained directly from Lindegren, and not from Lindegren via Malloch as stated in reference 4.) These stocks were crossed to produce Lindegren 1A and 25a (4). It seems unlikely that Lindegren would have sent Beadle his relatively infertile 1937 stocks instead of his fertile 1940 stocks.

The second correction involves Ema (FGSC 692) and EmA (FGSC 691), which were deposited by D.G. Catcheside. FGSC files include an updated note from Noreen Murray quoting from a letter from Catcheside, as follows: "Ema is descended from 5297a by numerous transfers of conidia. EmA is descended from 5256A, but a female-fertile extract was substituted in Australia because the original went female sterile. I think it was crossed to Ema and then a extracts crossed to EmA and finally a decent A picked." Catcheside (1986 personal communication) recalls that this problem occurred in 1952, i.e. before his stocks were deposited in FGSC.

The stocks judged nonauthentic or doubtful are stippled in the figure, and are discussed below. The occurrence of a few nonauthentic stocks should not be surprising. Most or all of their original authentic counterparts were isolated when stocks were still kept by serial transfers, long before FGSC was established. Serial transfers gave ample opportunity for mislabelling, contamination and the accumulation of spontaneous mutations. Distribution of original stocks to secondary users may have introduced errors in stock identification. Some of the stocks may have been outcrossed to remove mutations, the fl's being substituted for the originals.

Lindegren (-) a, FGSC 541: FGSC stock 541 had been judged nonauthentic for several reasons. Its rec genotype was incompatible with those of 1A (FGSC 354 and 25a (FGSC 353). Since we believe 1A (FGSC 354) is itself nonauthentic, this reason no longer applies. However, FGSC 541 is also suspect because it is albino and some cultures of it have been morphologically abnormal. A request by FGSC for a replacement from CBS produced an albino strain; a subsequent replacement was morphologically very abnormal. Since the FGSC and CBS stocks were both albino, it appears that the CBS culture was contaminated or much mutated before it was deposited in FGSC. The CBS culture was not lyophilized until 1958 (2.) There was thus ample opportunity for contamination and mutation. However, we do not know whether CBS ever had a sat⁺ a stock with which their Lindegren (-) a could have been contaminated, in order to account for the sat⁺ in FGSC 541.

Lindegren 1A: FGSC 354 clearly differs from the other 1A stocks as shown above. We have concluded that the Wilson 1A strain is probably authentic, and that FGSC 354 is not, for two reasons. (a) The Wilson strain is like Lindegren 25a and the RL wild types in all genes that have been tested, consistent with the fact that these stocks were highly inbred, whereas FGSC 354 differs from 25a and the RL wild types in at least four genes. (b) Crosses of Wilson 1A x 25a (FGSC 353) give few mature asci and many bubble asci, as expected of an inbred cross. In fact, Beadle obtained Neurospora from Abbott because Beadle's old stocks, i.e. the Lindegren inbreds, gave few asci with eight ripe spores (3). This description could apply to crosses of Wilson 1A x 25a; it could not apply to crosses of 1A (FGSC 354) x 25a. (Note: Bubble asci are a much less serious problem for geneticists than the kind of sterility that caused Lindegren to inbreed, but they might be a nuisance to people who were isolating ordered tetrads, as Beadle and his colleagues were doing.)

Lindegren (+) A —X— Lindegren (-) a

(853) *sal⁺scot⁺rec-1⁺2⁺3⁻*

(541) *sal⁺scot⁺rec-1⁺2⁺3⁺*

Abbott 12a —X— Lindegren 1A —X— Lindegren 25a —X— Abbott 4A

(353) *sal⁺scot⁺rec-1⁺2⁺3⁻*
 (1748) *sal⁺scot⁺rec-1⁺2⁺3⁻*
 (1752) *sal⁺scot⁺rec-1⁺2⁺3⁻*
 (1758) *sal⁺scot⁺rec-1⁺2⁺3⁺*
 (1759) *sal⁺scot⁺rec-1⁺2⁺3⁺* (J) Really *sc*
 (687) *sal⁺scot⁺rec-1⁺2⁺3⁻* Really *sc*

(354) *sal⁺scot⁺rec-1⁺2⁺3⁻rec-1⁺(J)*
 not heli-compatible with CDE
 (ELT) *sal⁺scot⁺rec-1⁺2⁺3⁻*
 (JFW) *sal⁺scot⁺rec-1⁺2⁺3⁻rec-1⁺* CDE

(353) *sal⁺scot⁺rec-1⁺2⁺3⁻rec-1⁺(J)*
 (JFW) *m^r* CDE

(1228) *sal⁺scot⁺rec-1⁺2⁺3⁻rec-1⁺(J)*
 (1757) *rec-1⁺2⁺3⁺*
 (1758) *rec-1⁺2⁺3⁺*
 (1757) *sal⁺scot⁺rec-1⁺2⁺3⁺*
 (757) *sal⁺scot⁺rec-1⁺2⁺3⁺*

RL3-8A

(2218) *rec-1⁺2⁺3⁻*
 (JFW) CDE
 (JFW derivative) *m^r*
 (SB) *sal⁺scot⁺*

RL21a

(2219) *rec-1⁺2⁺3⁻*
 (JFW) CDE
 (JFW derivative) *m^r*
 (SB) *sal⁺scot⁺*

Emerson 5297a

(SE) *rec-1⁺*
 (527) *sal⁺scot⁺* (J)
 (352) *sal⁺rec-1⁺2⁺3⁻m^r*
 (592) *scot⁺rec-1⁺2⁺3⁻m^r5⁻c*
 (NM) *c*

Emerson 5256A

(SE) *rec-1⁺*
 (626) *sal⁺scot⁺*
 (AMJ) (J)
 (ATCC) (J)
 (424) *sal⁺scot⁺rec-1⁺2⁺3⁻m^r5⁻c*

ST74A

(DCC) *rec-1⁺2⁺3⁺*
 (DDP) *Cde*

STA4

(StL) *Cde*
 (262) *m^r*

OR74A

74-OR8-1a

(StL) *Cde*
 (988) *m^r*
 (FJD) *sal⁺*

74-OR23-1A

(StL) *Cde*
 (986) *m^r*
 (FJD) *sal⁺*
 (987) (J)

74-OR23-1VA

(OM) *sal⁺scot⁺m^r*

ORSa

(OM) *sal⁺scot⁺m^r*

74-ORS-6a

EmA

(691) *sal⁺scot⁺rec-1⁺2⁺3⁺m^r* Cr

Figure 1. Solid arrows indicate single crosses. Dotted arrows indicate conidial reisolations (5; 14; Newmeyer unpublished). Dashed arrows indicate two or more crosses, as follows: Lindegren (+) A and Lindegren (-) a were probably not the immediate ancestors of Lindegren 1A and Lindegren 25a (see text). EmA (FGSC 691) was obtained by a cross of Emerson 5297a x Emerson 5256A, followed by one or more backcrosses to Emerson 5256A (see text). [Catcheside's Ema (FGSC 692) is really a stock of Emerson 5297a, not obtained by crossing to Emerson 5256A, contrary to the pedigree in reference 1 (see text).] ST74A and ST73a were derived from Emerson 5297a and Emerson 5256A by P. St. Lawrence via two or three generations of inbreeding (see Reference 1). 74-OR8-1a was obtained by a cross of SL73a x ST74A (carrying a spontaneous pan), followed by two backcrosses to ST74A or OR74A (5). ORSa was obtained by seven backcrosses to 74-OR23-1A (14). 74-ORS-6a was obtained by six backcrosses to 74-OR23-1VA (9).

The symbol het is omitted before the genes het-c, het-d, het-e and the symbol rec is omitted before rec-2 and rec-3. [I] and [III] indicate mitochondrial DNA types. Stippling indicates stocks that are probably not authentic.

The strains actually used for scoring are given in parentheses on the same line with the specific variants scored. Numbers in parentheses are FGSC numbers. "1757" and "1758 X" (see reference 6) are apparently both really FGSC 1758. "1757X" and "1758" are apparently really FGSC 1757. Evidently the numbers for the A and a stocks were interchanged and later corrected, with an "X" added to indicate the correction. Stocks not obtained from FGSC are indicated as follows: (AML) = Rockefeller University to A.M. Lambowitz. (ATCC) = ATCC 10815; this stock from ATCC became FGSC 626. (DDP) = P. St. Lawrence to D. Newmeyer to D.D. Perkins to J.F. Wilson. (DGC) = D.G. Catcheside. (ELT) = from E.L. Tatum collection (held by FGSC); his Lindegren 1A was later deposited as FGSC 5410. (FJD) = F.J. de Serres to D.D. Perkins to E.G. Barry. (JFW) = L. Garnjobst to J.F. Wilson; Wilson's Lindegren 1A was later deposited as FGSC 5222. (NM) = D.G. Catcheside to N.E. Murray to D.D. Perkins; should be the same as FGSC 692. (OM) = O.M. Mylyk to D.D. Perkins and later deposited as FGSC 2489 and 2490. (SB) = S. Brody to D. Newmeyer to D.D. Perkins to E.G. Barry. (SE) = S. Emerson's original isolates. (St.L) = P. St. Lawrence to J.F. Wilson.

The stocks listed here as Lindegren 1A and 25a were called Beadle and Tatum 1A and 25a by (6) and (16).

References for scoring of variants:

sat: E.G. Barry in (18), Barry unpublished, P. St. Lawrence unpublished. scot: (16), Perkins unpublished. rec: (6). The rec genotype of FGSC 424 in Fig. 1 of reference (6) is a misprint (D.G. Catcheside, personal communication); the genotype shown in our pedigree is correct. mei-1: (8,22), Perkins unpublished. ttc: (14,23). het: (24,15), J.F. Wilson personal communication; mitochondrial DNA: (13) and references therein, A.M. Lambowitz personal communication.

*(757) was deposited as Abbott wild A and is only presumed to be Abbott 4A

Abbott 12a: Two strains labeled Abbott 12a (FGSC 687 and 739) cannot be authentic because they are the wrong mating type. Two other strains labeled Abbott 12a (FGSC 351 and 1758) are doubtful for several reasons. (a) They are scot⁻, whereas a genuine Abbott 12a should be scot⁺ to account for the occurrence of scot⁺ in Emerson 5297a. This is true regardless of which strain of Lindegren 1A was crossed to Abbott 12a to generate Emerson 5297a. (b) The original literature describing mei-1 (then called the Abbott abortion gene) speaks as if mei-1 was present in both Abbott 12a and Abbott 4A, although actual evidence is given only for Abbott 4A (8, 21). The Abbott wild types were not inbred, but they were isolated from perithecia from the same soil sample (7,3,4). Thus both could have carried mei-1. (c) If the Lindegren 1A that was crossed to Abbott 12a to generate Emerson 5297a was like 1A (Wilson), then authentic Abbott 12a should be het-e, to account for the presence of this allele in Emerson 5297a. (d) On the other hand, if the Lindegren 1A that was crossed to Abbott 12a was like 1A (FGSC 354) then authentic Abbott 12a should be sat⁻, to account for the presence of sat⁻ in Emerson 5297a. [We assume that the Emerson 5297a stocks are valid because they explain the presence of ttc^s in 74-OR8-1a and of het-e in the OR and ST74 wild types and in EmA (FGSC 691)].

Emerson 5256A: Catcheside (6) judged FGSC 424 to be nonauthentic because it is rec-2⁻, whereas its parents were both rec-2⁺. It should also have been rec-1⁻ to account for the rec-1⁻ in EmA (FGSC 691). Differences in mitochondrial DNA between stocks labeled Emerson 5256A confirm that at least one 5256A stock cannot be authentic.

Table I
Variants segregating in laboratory wild types

Chromosomal	Mitochondrial
IL - rec-3, ttc#	DNA types I and II
IIL - het-c	
IIR - het-d	
IVR - mei-1	
VL - sat	
VR - scot, rec-1, rec-2	
VIII - het-e	

mei-1⁺ was purposely selected when Emerson 5297a and Emerson 5256A were isolated, het-compatibility with ST74A was selected when 74-OR8-1a was isolated, and ttc^r was selected when ORS_a was isolated. In the remaining cases there was no intentional selection for any of the variants listed above, many of which were unknown when the stocks were isolated, but there was often selection for other purposes, e.g. to eliminate a female-sterile character in the isolation of EmA (691), ST73a and ST74A; to eliminate a peach-like morphological mutation in the isolation of STA4; and to improve fertility in the inbreeding of the Lindegren strains.

ttc signifies triphenyltetrazolium chloride resistance, listed in Reference 17 as a probable allele of tet.

Conclusions:

Pedigrees of the old stocks should be used with caution. Probably none of the stocks indicated by stippling in Figure 1 is authentic. Because there is no authentic Abbott 12a stock, we cannot determine whether the genuine or the spurious Lindegren 1A was crossed to Abbott 12a to generate Emerson 5297a.

There is no evidence that the nucleolus satellite arose more than once, inasmuch as Lindegren 1A (FGSC 354) sat⁺ seems not to be authentic and Lindegren (-) a (FGSC 541) sat⁺ is clearly contaminated and/or mutated. The satellite is probably derived from an ancestor of the Abbott stocks.

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