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Abstract
Reference strains of *N. intermedia*

Perkins, D. D. and B.C. Turner. Reference strains of Neurospora intermedia.

Species-reference strains believed to be authentic derivatives of the type-material or of material conspecific with the type are available for N. sitophila and N. crassa (Perkins 1972 Neurospora Newsl. 19: 28 and 30). We believe that we have now established reliable standards also for the third described heterothallic species, N. intermedia.

The need for reference strains. Vegetative morphology is completely inadequate in our experience for distinguishing the established heterothallic species of Neurospora from one another. The published descriptions hove implicitly recognized this by placing greater emphasis on the sexual cycle than on vegetative traits. Taxonomic diagnosis stresses the size and shape of ascospores and perithecia. Yet even there structures may not be reliable because they can vary considerably within the same species, and although diagnostically useful quantitative differences may well exist between species, numerical values overlap even in published descriptions. Moreover, two hoploid isolates of opposite mating type must be available to complete the diploid phase of the life cycle before perithecio and ascospores can be obtained for measurement and diagnosis. Yet in collections from nature only a single mating type may be present.

Shear and Dodge recognized the boric importance of crossing behavior for taxonomy, and once having ret up representatives of sitophila and crassa, they assigned other strains to one or the other species, not on the basis of their morphology, but of their fertility with one or another of the established strains (Table 6. Shear and Dodge 1927 J. Agr. Res. 34: 1019). In our experience also, the production of fertile perithecia and abundant asci with viable black spores, when an isolate is crossed to authenticated species-reference strains, is the most practical and convenient method of determining that the isolate belongs to one or another of the established heterothallic species. It is probably also the most meaningful biologically.

Unfortunately, failure to cross is not in itself a sufficient basis for excluding that two isolates belong to the same species, because intraspecific factors sometimes impair fertility. However, if an unknown is infertile with one species-reference but maker viable black spores abundantly with another, there is no problem of assignment.

The search for an historically authentic strain of N. intermedia. When cultures labelled as N. intermedia from various culture collections were intercrossed, numerous combinations were infertile. Also, some strains labelled N. intermedio proved to be indistinguishable from tester strains known to be authentic N. crassa or N. sitophila, and were completely fertile with them. For example, "N. intermedio" strain 268.36, obtained by the Fungal Genetics Stock Center (FGSC#2055) in 1971 from Centraalbureau voor Schimmelcultures (CBS), Baarn, Netherlands, is indistinguishable from N. crassa and is completely fertile with standard crassa strains, producing nearly 100% black ascospores. Strain 10B (FGSC#580), obtained by FGSC from Dodge's laboratory after his death, and designated N. intermedia by FGSC on the basis of its label, is in fact N. ritophilo by the criterion of full fertility and black-spore production when crossed with the historically authenticated sitophila reference strain described in 1972 (Neurorporo Newsl. 19: 28). (Strain 10B was unfortunately selected by Dutta et al. to represent N. intermedia in their study of DNA differences between species, reported in 1971 (Neurospora Newsl. 18: 6) and 1972 (Molec. Gen. Genet. 114: 232).

From among the supposed intermedia stocks examined, we believe that two are authentic vegetative derivatives of the original strain described by Tai (1935 Mycologia 27: 328) and deposited in CBS by Dodge in April 1936 as strain 10B. The first is a strain designated N. intermedia Tai which was deposited in the Fungal Genetics Stock Center by J. Weijer, University of Alberta, in 1963, and assigned FGSC "962. The strain was from the collection of E.S. (Dowding) Keeping, University of Alberta, where it was labelled "N. intermedia, Washington, 1928, Wilcox. " E. S. Keeping has kindly written us: "I recall that in the twenties I received a culture from Dodge with a note that it was from 'war torn China. 'It may very well have been N. intermedia. " (M. S. Wilcox was associated with Dodge at the Bureau of Plant Industry, U.S. D.A., Washington, D.C. in 1928.)

A second probably authentic culture of N. intermedia was obtained from CBS in 1956 by A.M. Srb. This has since been maintained vegetatively at Cornell, and was deposited by Srb in 1969 in FGSC ("1754). (The CBS catalogue lists only one N. intermedia stock, 268.36, deposited by Dodge. Yet the stock obtained from CBS as intermedia by Srb in 1956 is markedly different from the stock obtained from CBS as intermedia by FGSC in 1971. We find that the latter is indistinguishable from N. crassa.)

The two putative intermedia stocks, from Alberta via FGSC, and from CBS via Srb, were obtained and examined by us in 1972. They ore strikingly similar to one another, and are uniquely different in appearance from any other Neurorporo strain we have handled. Both are a. Both are female-sterile. Surface growth is dense, with conidiation close to the surface and little aerial growth. Color is bright saffron (a golden yellow). The behavior of both is identical when they are crossed as fertilizing parents to numerous other strains used for testing. It is difficult to explain such remarkable similarity unless the two strains hod a common origin, representing subcultures that have been maintained separately since 1928.

The material on which Tai bored his brief 1935 description was isolated from a corn cob in Nanking, China in 1927. He stated that ascospores resembled ritophilo while perithecio resembled crassa, and that intermedia hybridized rather readily with the other species. Conidia of Tai's intermedia strain a were described as saffron-colored, and distinctly different in color from crassa or ritophilo. Mating type a is mentioned explicitly, but not A. Tai's description is not sufficiently detailed to tell whether the vegetative morphology resembled that of the saffron-colored a stocks we now hove from Weijer and Srb.

Tai published only one other paper on Neurospora (1936 Mycologia 28: 24), in which only the mating-type a strain of intermedia was used, although both A and a mating types were represented for other species involved in the some study. This suggests that only the a strain was still extant in 1936. All there facts ore consistent with our conclusion that the a strains from Weijer and from Srb are authentic representatives of the original N. intermedia material used by Tai.

Choice of A and a strains for use as testers. For several reasons it is not practical to use the original strain for carrying out routine mating tests to ascertain the species status of other strains. Only one mating type is represented, and it is female-sterile. Also, experience has shown that the original strain differs greatly in vegetative morphology from all of the many "normal"-appearing strains which we have collected from nature and with which it is highly fertile and apparently isosequential. (Similarity of chromosome sequence is inferred from the fact that ascospores are nearly 100% black, whereas the presence of gross rearrangements would result in at least 25% or 50% defective, white ascospores.) It is our belief that the particular strain on which the species description happened to be bored was morphologically highly atypical of the species as it is usually found in the wild. We have therefore chosen a Fair of highly fertile, morphologically "normal" A and a strains, which we propose to use on a trial basis as standard species-references for N. intermedia. Eventually it may be desirable to replace them with material from the original type-locality, but such strains are not now available.

Description and crossing behavior of the reference strains. The two strains proposed as intermedia standards ore:

P420 A FGSC#2316 Collected from burned sugar cane near Clewiston, Florida in March 1970.
P405 a FGSC#1940 Collected from burned grass near Lo Belle, Florida in March 1970.

These were selected from among other candidates because of high fertility, good spore production, and uniform morphology among progeny from crosses with each other. The new reference strains resemble the standard crassa and sitophila wild types in vegetative morphology and appearance. Conidia and mycelia ore usually orange, but con be distinctly yellow-tinged under some conditions, although never the intense yellow seen in the Weijer or Srb strains. Conidiation is better at 25°C and below than at 34° or above. Ascospores from P405 x P420 measure 28.5 x 16.5 µm (mean based on 125 spores, cross on synthetic cross medium, 25° C). Those from P420 X the strain from Srb measure 27.5 x 17.4 pm (50 spores). Ascospores from both crosses are thus larger than the size range given by Tai (19-26 x 12-15 pm), but the length/breadth ratio is similar.

Both strains are female-fertile. They are apparently isosequential with one another, and the A standard is fertile and isosequential with the strains from Weijer and Srb, which ore believed to be historically authentic. When progeny are obtained from crosses between the Weijer and Srb strains and the proposed references, a vast array of phenotypes is seen, varying markedly in pigment, morphology, conidiation, and vigor; and the saffron/orange carotenoid difference segregates.

Crosser between intermedio and standard crassa produce many perithecia. Ostioles are formed, and numerous ascospores are shot from the perithecia. But the ascospores ore nearly all white and inviable, with block spores ranging in frequency from <1% to perhaps 10%. This description applier both to crosses using the Weijer or Srb strains (as fertilizing parents), and the proposed new reference strains. Thus crassa X intermedia crosses ore often highly fertile if the criterion of fertility be numbers of productive perithecia and numbers of ascospores; but they are quite infertile with respect to the production of viable block ascospores.

Crosser have been made also between the intermedia references and strains which are believed to be authentic N. sitophila (P8085A and P8086a). There resemble the intermedia X crassa crosses in producing abundant perithecia, but they differ in being relatively barren and producing few ascospores.

General distribution of the species. A survey has been started crossing the reference standards to wild-collected strains from various sources. Isolates that are apparently N. intermedia hove been found among Neurospora collected both in the New World and in localities ranging from Japan to Australia, and from Hawaii to Indonesia and India. Samples of Neurospora collected by DDP from ontjom in and near Bogor, Indonesia, all appear to be N. intermedia, and intermedia appears to be the prevalent species in those ports of the Eastern Hemisphere where collections have been mode. Ho C. C. informs us that ontjom cultures recently collected by him in Java also behave in crosses as if they are conspecific with the proposed intermedio references.

The cultures listed below appear to be N. intermedia on the basis of their crossing behavior with the proposed references. The survey is incomplete, and other FGSC strains of uncertain species-status still remain to be tested. FGSC stocks classified as N. intermedia ore Numbers 573, 962, 1754, 1782-1785, 1789-1793, 1795-1813, 1820-1823, 1826-1827, 1830-1837, 1881-1883, 1939-1940, 2236-2237, 2316, 2360-2367.

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