

CULTURE STUDIES IN APHYLLOPHORALES

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SUMMARY: The use of diagnostic culture studies for taxonomical purposes is discussed as well as pairing tests for investigation of species delimitations and microevolution.

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Morphology of basidiocarps has traditionally been fundamental for the taxonomy of Aphyllophorales fungi. It is, however, apparent that this information frequently is insufficient for the delimitation of both species and genera. Classification to any higher rank is mostly very hazardous. Studies of fungal mycelia in pure cultures offer a new set of characters which are independent of those obtained from the basidiocarps. Crossing tests between haploid, single-spore isolates are excellent instruments in the delimitation of closely related species. Recent improvements of DNA-techniques have made extensive work in this field possible which seem to be very promising. However, even if more and mutually independent characters are being gathered to descriptions of species and genera, phylogenetic relationships will not be revealed by itself. Increased knowledge on biology of individual species, speciation processes, biogeography, etc. will help us understand and interpret characters obtained from various studies. Taxonomy mainly based on fruitbody morphology has given us a fundamental basis for further studies and as shown here, culture studies have supplied further characters and a better understanding of taxonomy of Aphyllophorales. Two different applications of culture studies for taxonomical purposes are discussed: diagnostic culture studies and pairing tests.

DIAGNOSTIC CULTURE STUDIES

By addition of the new characters obtained from cultures, a more natural and less artificial classification would be possible. However, culture characters are to some extent dependant on environmental conditions in the culture and for the purpose of sys-

tematics, standardized protocols are necessary. Such protocols are given by NOBLES (1965, emendated by NAKASONE, 1990), STALPERS (1978) where necessary specifications for a standardized test are given as well as code symbols for recorded characters.

However, even if great efforts are made to keep uniform environmental conditions in the cultures, there is always a certain variation in the culture characters within a certain species. This variation may be discouraging but, on the other hand, the variation itself can be analyzed and be of importance for ecological and physiological studies. In general, this intraspecific variation can be observed in three different ways:

- Variation in growth rate, also between different subcultures.
- The mycelium may adopt different growth forms. A uniform and slowly growing mycelium may suddenly change its growth mode in a certain sector of the culture. Production of conidia may be present or absent.
- Production of extracellular oxidases may vary, even between different strains of the same species.

The degree of variation in culture characters varies for different species and genera.

After these, somewhat bothering comments it is proper to say that some culture characters indeed are useful in systematics. From our systematic studies based on fruit-body morphology we know that some genera appear to be well-founded, others are more badly defined. By studying culture characters and their variation in well-founded genera or in groups of closely related species it is possible to evaluate the variability of individual characters. The following examples illustrate the value of diagnostic culture studies:

Hyphodontia - *Schizopora*. The main difference between the two genera is the presence of skeletal hyphae and a tougher consistency in the latter. The two genera are closely related which is further underlined by culture characters. Representatives of both genera are tetrapolar, have a normal nuclear behaviour, their hyphae may be provided with the small peculiar structures, malocysts and drepanocysts.

Vararia - *Dichostereum*. The two genera were earlier united but separated because of different spore characters. Culture characters underline the differences. Cultured mycelia in all species of *Dichostereum* have oedocephaloid conidiophores.

Athelia decipiens (Hohn. & Litsch.) J. Erikss. - *A. epiphylla* (Pers). These two species are closely related differing mainly by the absence of clamps in basidiocarps of *A. decipiens*. However, its mycelia in culture have the same kind of scattered clamps on wide hyphae as in *A. epiphylla* which emphasizes a still closer relationship than believed earlier.

Steccherinum ochraceum (Fr.) S.F. Gray - *Junghuhnina nitida* (Fr.) Ryv. Basidiocarps of the two species are very similar, differing mainly in the shape of the hymenophore which is odontoid in *S. ochraceum*, poroid in *J. nitida*. Still, the two species are kept in different genera and by some authors even in different families. Culture characters are very similar between the two species which further underlines the close relationship.

Even if this kind of results may appear convincing, we must also consider the practical aspects of generic divisions. In fact, it is true that even a badly delimited genus may be a better taxonomical solution than creating a good segregate genus of closely related species while leaving a bad rest product.

EXPERIMENTAL STUDIES BY THE USE OF PAIRING TESTS

While diagnostic culture studies are very useful in classification, they are not always efficient on species level or below. In experimental studies we use the ability of a living fungal culture to recognize other cultures by different kinds of responses upon confrontations. Two haploid mycelia of the same species which are crossed, will mate and produce a secondary mycelium if:

- the two mycelia are not identical (i.e. not having the same mating type factors),
- the species is heterothallic,
- no special factors are present which could prevent mating from taking place.

Pairing tests which include a number of isolated specimens of one species/species complex, representing a variety of habitats in nature and a wide geographic distribution, are very useful for comparative purposes. From the extensive collections of Aphyllophorales –at least over the N. Hemisphere– we know that the majority of species have a circumpolar distribution. The individual species also seem to occupy the same kind of ecological niches wherever they are collected in this region. This situation has to a great extent been verified by the numerous pairing tests performed during the last decades. However, in a number of cases also a cryptic speciation has been detected, so called sibling species. They are characterized by more or less identical fruitbody morphology but separated by sterility barriers. In all experiments with mating tests it is essential to make comparisons between fruitbodies from which the cultures have been isolated. From such comparisons we can learn about distribution, morphological variation, character of the pertaining substrate, etc. Such comparisons are especially valuable when comparing sibling species. The most extensive results hitherto comes from experiments with a corticioid species growing on branches of broad-leaved trees, *Peniophora cinerea* (Fr.) Cooke. In this species an intersterile sibling has been detected in Central Europe which is ecologically specialized, confined to decorticated *Fagus* branches. The main form of the species grows on other kinds of deciduous substrates. The two siblings are mutually intersterile with exception for a few specimens from North Europe, which show a high degree of intercompatibility with all tested specimens of the *P. cinerea*. Moreover, both European siblings are intercompatible with N. American isolates. In Ontario, Canada, a similar situation has been found. One of the specimens isolated from that area is only compatible with other specimens from that region (Ontario), while intersterile with all other representatives. The explanation which we can trace is that speciation has occurred due to occupation of a new niche. It is quite obvious that the biological species concept does not work well. The sterility barriers may be parts of a process, parts of a species character itself. A similar situation was found in the pathoge-

nic polypore, *Heterobasidion annosum* Bref., by CHASE & ULLRICH (1990). While the sterility factors seem to be mixed in N. American populations, apparently rather randomly, they are in Europe fixed to facilitate the development of substrate-specific siblings: one is restricted to spruce, another prefers pine as substrate but is also found on broad-leaved trees. Such sterility barriers seem to be of simple genetic origin, which Chase and Ullrich also showed in detail for *H. annosum*. Creation of sterility barrier could be looked upon as part of a species strategy for survival and for expansion to occupy new niches. This raises another question. How can a genetical divergent form survive when surrounded by a great number of normal individuals of the same species? An answer was given by EHRLICH & RAVEN (1969), who found that the actual distribution of diaspores was far more restricted than commonly thought. If a genetical divergent form would develop on a new kind of substrate in a delimited area where other members of the same species cannot survive, there are good possibilities for this new form to stabilize genetically, and to develop sterility barriers towards the main population. This is possible if easily established sterility genes are available –and among Aphyllophorales fungi, they obviously are.

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