Evolutionary genetics and systematics of fungi in the *Collybia dryophila* group.

by

Rytas J. Vilgalys

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APPROVED:

O. K. Miller, Jr., chairman

J. O. Falkinham

K. W. Hilu

R. J. Stipes

B. J. Turner

B. Wallace

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(ABSTRACT)

I have undertaken an evolutionary and systematic study of fungi in the Collybia dryophila group (Basidiomycetes: Tricholomataceae). Species in the C. dryophila group occur worldwide as primary litter decomposers, yet little is known about their systematics.

In a previous study, the C. dryophila group in North America was shown to consist of four biological species, identifiable on the basis of mating compatibility and certain taxonomic features (Vilgalys, 1983). In this study, crosses made using isolates from over 80 individuals of the C. dryophila group from Europe indicate that there are four species from that continent as well. All four species are rigorously intersterile, while intra-specific matings occur freely. One exceptional case of reduced intra-group mating compatibility is reported, which may be the result of divergence due to geographic isolation.

Morphological studies indicate the four European biological species are all characterized by a high level of

polymorphism, frequently involving features previously used to distinguish taxa. Because of this, diagnostic taxonomic features of the European species were reevaluated, and a revised taxonomy presented.

Representative tester strains from North America, Europe, Asia, and Australia were crossed to determine world-wide mating relationships in the C. dryophila group. Six major compatibility groups are identified on the basis of frequent mating compatibility. In several instances, populations of different geographic origin are mating-compatible, suggesting that these allopatric populations might be conspecific. Intercompatible allopatric populations are not always similar in their fruit body morphology, however, suggesting that extensive geographic differentiation has occured in the C. dryophila group.

Genetic similarity of different populations was measured by the method of DNA hybridization. In general, there was good correspondence between mating compatibility and base sequence homology in the C. dryophila group. Allopatric, intercompatible populations, however, showed reduced homology when compared to that observed within sympatric populations. This suggests that geographic isolation may play a primary role in the evolution of different species in the C. dryophila group. Finally, a phylogeny based on DNA hybridization data is presented.

In summary, several lines of evidence suggest an allopatric mode for speciation in the C. dryophila group.

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INTRODUCTION

The nature and origin of species continue to puzzle evolutionary and systematic biologists. For systematists, an understanding of the nature of species is critical for constructing logical, natural classifications. Evolutionary biologists, on the other hand, are primarily concerned with the forces which shape species and other groups of related organisms. Although evolutionary and systematic biology represent different disciplines, both utilize similar types of data to study similar phenomena. Contemporary evolutionary studies regularly employ biochemical and molecular data to determine genetic relatedness among groups of organisms. Biochemical data, based upon electrophoretic analysis of isozymes, has provided a clear picture of the nature of genetic differentiation in animals (Ayala, 1976) and plants (Gottlieb, 1977). Molecular evidence, particularly DNA reasociation data, has provided an even more powerful method for elucidating relationships in both animal (Brownell, 1983; Sibley & Ahlquist, 1983, 1984) and plant (Belford & Thompson, 1981a, 1981b) groups as well.

One group conspicuously absent from modern evolutionary studies are the higher fungi. As pointed out by Burnett (1983), mycology has contributed little to the synthesis of genetic and evolutionary theory known as Neodarwinism

(Dobzhansky, 1971; Mayr, 1963; Stebbins, 1950). Species concepts in higher fungi are still largely based upon traditional morphological studies, which are hampered by a limited number of useful morphological characters. The utility of morphological features for evolutionary and phylogenetic studies in fungi is further diminished by a lack of knowledge concerning character homology in many groups (Vilgalys, in press).

Despite these drawbacks, higher fungi present an appealing group for evolutionary studies, particularly at the species level. Knowledge about the operation of mating incompatibility mechanisms in higher fungi (Raper, 1966; Burnett, 1975) makes interfertility (or intercompatibility) studies possible for many fungi that can be grown in culture. The large number of basidiomycete species studied to date all have similar homogenic mating systems which govern intra-specific mating compatibility (Burnett, 1975). Inter-specific mating relationships have been studied in a large number of basidiomycete genera as well (Boidin, 1980). The vast majority of these studies indicate that basidiomycetes form discrete intersterile groups, or biological species. To date, not a single case of bona fide interspecific hybridization is known for natural populations of basidiomycetes (Ullrich & Raper, 1974). In many cases, morphological differentiation between intersterile groups of fungi is slight or absent, making taxonomic distinction

difficult. Examples of such 'species complexes' in fungi are common (Boidin, 1980; Burnett, 1983).

Species complexes in fungi present a challenge for taxonomists, and also to evolutionary biologists. In my study, I have investigated the evolution and systematics of several fungi in the genus Collybia (Fr.) (Basidiomycetes: Tricholomataceae) closely related to C. dryophila (Bull. : Fr.)Kumm. Members of the C. dryophila group occur commonly as litter-decomposers worldwide, and are easily cultured in the laboratory.

Results of mating studies using North American material demonstrated the presence of four intersterile mating groups in the C. dryophila group (Vilgalys, 1982; Vilgalys & Miller, 1983). Morphological studies also indicated consistent morphological differences among the four species. In a subsequent study, morphological and ecological features of the four North American species were compared with other Collybia taxa using phenetic and cladistic methods (Vilgalys, in press). In that study, the C. dryophila group was clearly recognized as a monophyletic group by both phenetic and cladistic analyses. Relationships among the four North American species, however, could not be unequivocally determined, because of a high level of homoplasy in the cladograms (Vilgalys, in press). Relationships of the four North American species with taxa from other regions of the

world were not immediately apparent from the descriptive data (Vilgalys & Miller, 1983).

The objectives of my present study are to:

1. Determine mating relationships of additional populations in the C. dryophila group from Europe, Asia, and Australia.
2. Compare morphological features of these populations.
3. Assess the level of genetic divergence among these populations.
4. Evaluate the validity of current morphological and biological species concepts as they apply to this group and to other fungi.
5. Discriminate among possible modes of speciation which operate in these fungi.

CHAPTER 1. SYSTEMATICS OF THE COLLYBIA DRYOPHILA GROUP IN EUROPE. 1. MATING RELATIONSHIPS.

A large body of systematic studies of fungi have employed mating data for delimiting species and establishing stable taxonomic concepts. Mating studies can also provide useful data for evolutionary studies of fungi, particularly concerning speciation (Burnett, 1975; 1983). Evidence from many studies suggest that most homobasidiomycetes belong to discrete mating groups which are mutually intersterile. Mating within a single group occurs frequently, while inter-group matings are nearly always incompatible.

Species in the Collybia dryophila (Bull. : Fr.)Kumm. group occur as primary litter decomposers in forests throughout Europe. Although numerous taxa are recognized in the C. dryophila group from Europe, their circumscription remains difficult. This is primarily because of a high level of phenotypic plasticity in the group, making morphological comparisons difficult.

In North America, the C. dryophila group consists of four intersterile species (Vilgalys and Miller, 1983). The four North American species can also be distinguished on the basis of their basidiocarp features (Vilgalys & Miller, 1983). Relationships between the four North American species and taxa from Europe are not immediately apparent from fruit body

morphology. For this reason, we have undertaken a study of evolutionary systematics in the C. dryophila group. In this study, we report on the mating relationships among a sample of European members of the C. dryophila group.

MATERIALS AND METHODS

ORGANISMS AND CULTURE CONDITIONS

Organisms used in this study are listed in Table 1, along with data concerning their origin and other collection data. Material for mating studies consists of spore print stocks obtained by placing fresh pilei of Collybia species over paper or plastic slides. Each spore print stock was assigned a number by its collector, shipped to Blacksburg, Virginia, USA, and stored at 2° C until used. Voucher specimens for each spore print were assigned the same number and are deposited at VPI. Culture conditions for germination of spores and mating studies are detailed in Vilgalys & Miller (1983). All cultures were grown (at 20° C) and maintained (at 2° C) on Noble's medium (1.5% malt extract; 1.5% agar).

MATING STUDIES

Mating types were determined by pairing up to 11 monosporous isolates from a single stock in every combination (Vilgalys

& Miller, 1983). The presence of clamp connections in newly formed dikaryons provided the criterion for determining compatibility. In some cases, additional isolates (up to 31) were also tested to insure recovery of all mating types. Interstock crosses were made by pairing one or two monosporous isolates from varying numbers of stocks in every combination. Testers representing different incompatibility groups from several crosses were then mated to determine the total number of groups present (Vilgalys & Miller, 1983). Intersterility groups were identified this way from a preliminary sample of 37 different stocks. 46 additional stocks were screened by crossing one or two randomly selected monosporous isolates with at least two testers representing each intersterility group. In case of dubious or anomalous results, matings were repeated until the true mating reaction became evident.

RESULTS

INTRA-STOCK MATINGS

The type of mating system controlling compatibility was determined for 23 stocks, indicated in Table 1. From these stocks, usually three or four mating types were identified, indicating that mating compatibility is under bifactorial control. Incomplete recovery of all four mating types in

some cases was attributable to the small sample sizes used for intra-stock matings. With 11 monosporous isolates, the probability of recovering all four mating types is only 83% (assuming that four mating types assort randomly and intra-factor recombination is low). In one case (stock M-83/1) where the sample size was increased to 31 isolates, the four mating types were found to assort randomly (X^2=1.90, .25 < p < .4). Formation of pseudoclamps or unilateral nuclear migration corresponding to common-factor matings were not observed in any of our intra-stock crosses. For this reason, we have been unable to detect hemi-compatible matings when they might occur.

The limited sample sizes in our intra-stock crosses were necessitated because of poor recovery of monokaryons from spore platings. We have observed a tendency for basidiospores from spore prints to aggregate when suspended in solution, resulting in adequate germination percentages but low numbers of monosporous isolates being obtained during isolation. Addition of a surfactant to the spore suspension along with agitation helps, but does not always alleviate this problem.

INTER-STOCK MATINGS

The results of several inter-stock crosses are shown in Table 2. Monosporous isolates from 37 stocks belong to one of four

major intersterility groups of the C. dryophila group from Europe. These are designated as groups E-I through E-IV in Table 1. Inter-group and intra-group mating frequencies from the crosses in Table 2 are summarized in Table 3. All four European groups are virtually intersterile: the small proportion of matings observed between groups (less than 1% of the total number of interstock pairings) are not repeatable, and probably do not represent true mating reactions. Within-group matings generally occured frequently (84-100%), because of polymorphism at the two mating loci.

Testers representing intersterility groups E-I through E-IV were used to screen 44 additional stocks for their mating specificity. With the exception of one stock (for which only a single monokaryotic strain was isolated), each of these stocks crossed with at least one tester from a single intersterility group.

In all, isolates from 80 stocks could be assigned to one of the four intersterility groups. The numbers of distinct mating factors controlling mating compatibility in each group were not determined, because of the limited number of mating types used from each stock and our inability to determine hemi-compatible matings. Intra-group incompatibility attributable to common mating factors was relatively infrequent, and suggests that a large number of mating allelomorphs exist as in other species (Raper, 1966).

Several isolates from group E-III showed a reduced frequency of intercompatibility with other isolates from that group. These isolates are indicated by an asterisk in Table 1. Most of these isolates were obtained from stocks collected from Sardinia, as well as from Bavaria and Czechoslovakia. Matings among isolates from these stocks (designated E-IIIb in Table 3) occured at high frequency and are apparently under control of the same type of homogenic mating system as the rest of group E-III. Compatible crosses between these isolates and the rest of group E-III occur infrequently (at a frequency of 33%), and usually result in the production of pseudo-clamped hyphae only (figure 1). Repeated subculturing from the junction line of such crosses always resulted in sectoring (figure 2), and stable, clamped dikaryons did not form. Crosses using isolates known to have different mating factors from groups E-IIIa and E-IIIb indicate that incompatibility in this case is not attributable to common mating factors. We have never observed pseudoclamps in any of our intra-stock crosses, so it is unlikely in this case that pseudoclamped hyphae are attributable to common mating factors, as in other fungi (examples in Raper, 1966). Although we suspect a genetic basis for this observed heterogenic mating behavior, our inability to fruit species of Collybia has prevented more complete study of this phenomenon.

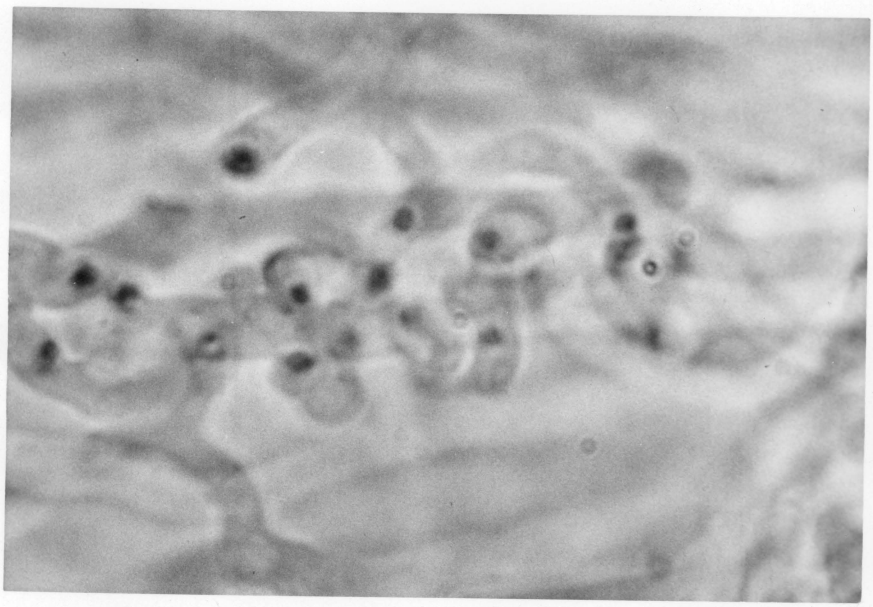

Figure 1. Pseudoclamped hyphae formed after crossing incompatible stocks (nos. 46 X 65) from group E-III.

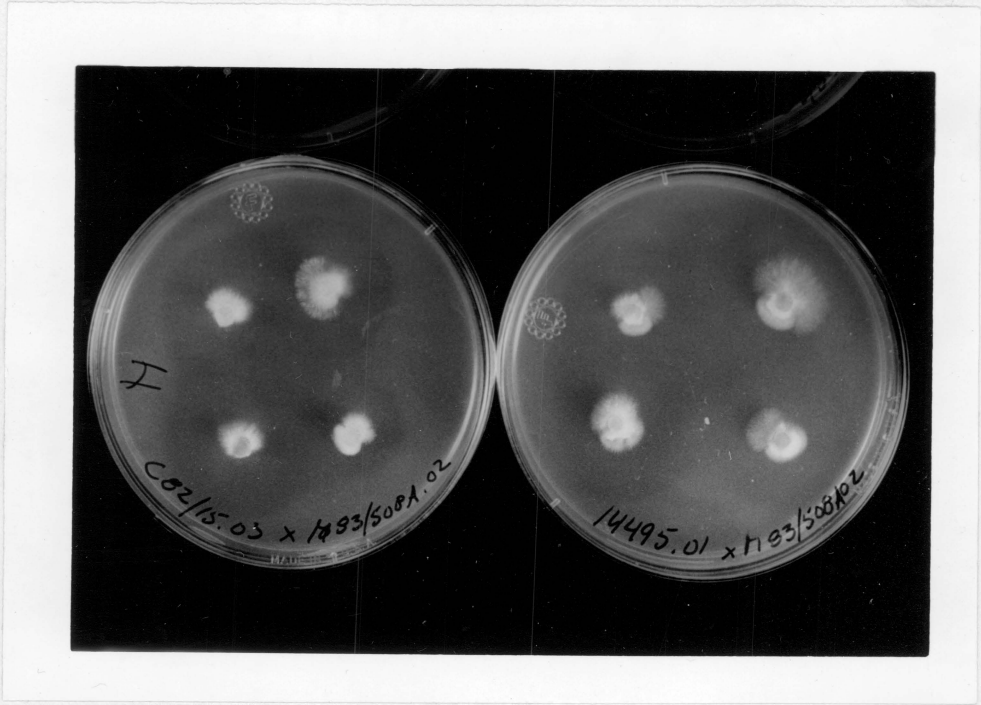

Figure 2. Sectoring observed after crossing incompatible stocks from group E-III.

DISCUSSION

MATING COMPATIBILITY STUDIES

Mating patterns from intra-stock crosses in this study conformed to a bifactorial mating system in every case (Table 1). The pattern of dikaryotization in European isolates was similar to that of North American material (Vilgalys & Miller, 1983). In numerous intra-stock crosses with the C. dryophila group, we have never observed production of pseudoclamps which might indicate common-B factor matings (Raper, 1966). Nor have we been able to verify nuclear migration between component monokaryons in compatible crosses. When two compatible monokaryons are confronted, dikaryon formation occurs only at the junction line, and the dikaryotic colony proceeds to grow from it. Establishment of clamped, dikaryotic hyphae within either monokaryotic colony occurs at a very slow rate, suggesting growth of the dikaryon rather than nuclear migration. A similar pattern of dikaryotization also occurs in other species of Collybia (Arnold, 1935), as well as a number of other genera (Aschan, 1954; Ullrich, 1973; Burnett, 1975).

NOTES ON THE INTERSTERILITY GROUPS

As with the North American species in the C. dryophila group (Vilgalys & Miller, 1983), the four European groups (Table 3) are also reproductively isolated. A detailed morphological comparison of each group is presented elsewhere (Vilgalys & Miller, Chapter 1). Several notable features of each group, however, are presented in Table 1 and discussed below.

Members of groups E-I, E-III, and E-IV were generally found in mesic regions of continental Europe and Great Britain, including both deciduous and coniferous forests. Group E-II was found in sub-alpine or sub-arctic habitats and appears to be restricted to cooler regions of Europe usually characterized by dwarf vegetation. Most collections of E-II came from Scotland and Scandinavia, although a single collection was made from the Tirollean Alps. Judging from the data in Table 1, no clear distinction between groups is apparent on the basis of substrate. Fungi in all four groups were found fruiting on soil as well as on litter or decayed wood, although certain groups (E-I and E-IV) did appear to favor a soil substrate more than others.

Fungi belonging to groups E-I and E-IV usually fruited earlier than other groups in any given locality (May to June in most areas). The longest fruiting season is that of group E-III, which was found from June through November, depending

upon the locality. In general, fruiting by members of E-III continued later into the season than in other groups. Group E-II generally fruited for the duration of the short growing season where it was found.

BIOLOGICAL SPECIES IN C. DRYOPHILA

Intersterility among the four groups identified from this study is almost absolute. Intra-group mating compatibility occurs at high frequency, suggesting that a high level of outcrossing is achieved through a multiple allelic mating system. The combination of these two factors supports the application of a biological species concept in the C. dryophila group. The biological species is formally defined by Mayr (1963) as "groups of interbreeding natural populations that are reproductively isolated from other such groups". This definition is more explicitly defined by Dobzhansky (1970) as "the most inclusive Mendelian population". A number of similar definitions of biological species have been formulated in the mycological literature as well by Anderson & Ullrich (1978), Petersen (1977), Burnett (1983), and others. By any of these definitions, many intersterile mating groups of basidiomycetes meet the criteria necessary to be considered biological species. As pointed out by Anderson, et al. (1981), populations of fungi from a single geographic area (i.e., sympatric populations)

nearly always belong to discrete biological species. Thus, at a local level, a biological species concept is reliable and useful for determining systematic relationships among fungi.

Whether or not gene flow occurs freely in these species, or in other fungi, is currently unknown. The concept of gene flow is central to any definition of biological species (Grant, 1980). 'Good' biological species do not exchange genetic material with other species; gene flow within a biological species, is not limited, resulting in a high level of genetic uniformity. Gene flow within and intersterility group is usually assumed in fungi with mating systems which favor outcrossing, and because mating factors are often widely distributed (Raper, Krongelb, & Baxter, 1958). The assumption of gene flow in biological species of fungi may not always be warranted. Factors restricting gene flow, such as heterogenic incompatibility (Esser & Blaich, 1973) or geographic isolation (Simchen, 19676; Parker-Rhodes, 1949; 1950) can result in divergence between conspecific populations. A possible example of restricted gene flow within a common mating group is represented by the reduction in mating frequency among certain members of group E-III (Table 3). Numerous cases of endemism resulting from isolation between island populations are known for Mediterranean species of higher plants (Cardona & Contandriopoulos, 1979). Populations from Sardinia, at

least, may be so isolated from the continental populations of group E-III enough that a reduced level of mating compatibility now occurs because of heterogenic incompatibility. Sporocarps of all three Sardinian collections are macroscopically distinct from other continental populations of group E-III (personal observation), which supports this hypothesis.

It is interesting to note that intersterility barriers in Collybia and other basidiomycetes are nearly always absolute and leads to the conclusion that interspecific hybridization does not occur in natural populations of homobasidiomycetes. Likewise, there is currently no evidence for hybrid zones where different species overlap, as there is for higher plants (Grant, 1981). The factors causing rigorous pre-zygotic reproductive isolation among species of basidiomycetes are currently unknown. Further studies on the extent and nature of genetic variation in populations of different intersterility groups from North America and Europe may help understand how species in the C. dryophila group arose.

Table 1. Sources of stock material used for mating studies.

Group	Stock no.	Collection[1]	Season	Geographic origin	Substrate, habitat	mating types[2]
E-I	1	B 82/63	June	Austria	soil, *Picea*	
	2	B 83/1	June	Austria	soil, garden	2
	3	B 83/3	June	Austria	soil, *Pinus*	0
	4	B 83/4	June	Austria	in grass, *Corylus*	4
	5	B 82/6/26	June	Austria	soil, coniferous woods	
	6	B 'exc'	June	Austria	moss, conif. wds.	4
	7	M 83/1	June	Austria	in moss, *Pinus*	4
	8	B 81/2	May	Italy	soil, *Crataegus* & *Robinia*	4
	9	B 83/6	June	Italy	in moss, *Picea*	4
	10	RV 84/63	June	France	deep moss, swamp	
	11	RV 84/64	June	Italy	deep moss, swamp	
	12	RV 84/98	June	France	soil, deciduous wds.	
	13	RV 84/99	June	France	soil, decid. wds.	
	14	RV 84/100	June	France	soil, decid. wds.	
	15	RV 84/101	June	France	litter & soil, decd. wds.	
	16	RV 84/102	June	France	soil, decid. wds.	
	17	HK FRBG	June	Denmark	soil, *Tilia*	
	18	HK ALDM	June	Denmark	on wood, *Fagus*	
	19	RV 84/123	June	Denmark	soil & litter, *Thuja*	
	20	RV 84/124	June	Denmark	soil, *Thuja*	
	21	RV 84/125	June	Denmark	soil, conif. wds.	
	22	RV 84/126	June	Denmark	litter, conif. wds.	
	23	RV 84/128	June	Denmark	litter, conif. wds.	
	24	RV 84/129	June	Denmark	soil, conif. wds.	
	25	RV 84/130	June	Denmark	soil, conif. wds.	
	26	RV 84/132	June	Denmark	soil, conif. wds.	
	27	RV 84/134	June	Denmark	soil, conif. wds.	
	28	RV 84/135	June	Denmark	litter, conif. wds.	
	29	RV 84/136	June	Denmark	soil, conif. wds.	
	30	RV 84/179	June	Denmark	litter, *Fagus*	
	31	RV 84/180	June	Denmark	soil, *Fagus*	
	32	RV 84/185	June	Sweden	on wood, mixed wds.	
	33	RV 84/186	June	Sweden	on wood, mixed wds.	
	34	RV 84/189	June	Sweden	soil, mixed wds.	
	35	RV 84/208	June	Sweden	moss, *Picea*	
	36	RV 84/211	June	Sweden	humus, *Picea* & decid. wds.	
	37	RV 84/217	June	Sweden	litter, *Picea*	
	38	RV 84/222	June	Sweden	litter, *Pinus*	
	39	HM 84/02	Jul.	Germany	soil, *Picea* & *Pinus*	

Table 1 (cont.)

Group	Stock no.	Collection	Season	Geographic origin	Substrate, habitat	mating types
E-II	40	M 1b/289	Aug.	Sweden	litter, *Salix* & *Betula*	
	41	RV 84/182	June	Sweden	moss, mixed wds.	
	42	OKM 19024	Aug.	Austria	soil, *Picea*	4
	43	W 14513	Sep.	Scotland	litter, *Betula*	
	44	W 14514	Sep.	Scotland	moss, *Betula*	3
	45	M 84/136	Aug.	Norway	litter, *Betula*	
E-III	46	C 82/15	Jul.	Switzerland	soil, *Fagus*	3
	47	I-St. Luc	Sep.	Switzerland	litter, *Vaccinium*	4
	48	I-Gemmi	Aug.	Switzerland	soil, open grassland	3
	49	I-Bern	Aug.	Switzerland	litter, *Fagus* & *Picea*	3
	50	W 14495	Sep.	Scotland	moss, *Betula*	
	51	W 14497	Sep.	Scotland	litter, *Betula*	
	52	W 14498	Sep.	Scotland	litter, *Picea*	4
	53	W 14499	Sep.	Scotland		
	54	W 14500	Sep.	Scotland		
	55	B 83/2	June	Austria	soil, *Pinus*	3
	56	M 83/2	June	Austria	soil, *Pinus*	
	57	B 83/5A	June	Italy	moss, *Picea*	3
	58	HM 84/01	Jul.	Germany	soil, *Pinus*	
	59	HM 84/03	Jul.	Germany	litter, in grass	
	60	HR 84/90	Sep.	France	sphagnum, conif. wds.	
	61	[3]OKM 20693	Sep.	Czekoslovakia	litter, *Fagus*	
	62	[3]OKM 20711	Sep.	Czekoslovakia	soil, *Fagus*	4
	63	[3]M 83/508	Oct.	Sardinia	soil, *Quercus*	2
	64	[3]M 83/525	Nov.	Sardinia	soil, *Quercus*	
	65	[3]M83/11/03	Nov.	Sardinia	soil, *Cistus*	
	66	[3]HM 84/04	Jul.	Germany	litter, *Fagus*	
E-IV	67	B 81/3	June	Austria	soil, *Pinus*	
	68	M 82/15	May	Italy	soil, mixed wds.	
	69	B 81/1	May	Italy	humus, *Pinus* & *Picea*	3
	70	B 82/6/10	June	Italy	soil, conifers	
	71	RV 84/35	June	France	soil, decid. wds.	
	72	RV 84/36	June	France	soil, decid. wds.	
	73	L 81/310	June	France	litter, *Pseudotsuga*	
	74	RV e251	June	France	humus, decid. wds.	3
	75	RV 84/61	June	France	litter, *Pinus*	
	76	RV 84/62	June	France	moss, bog	
	77	RV 84/65	June	France	litter, *Pinus*	
	78	W 14496	Sep.	Scotland	litter	4
	79	W 14501	Sep.	Scotland	moss, *Betula*	4
	80	RV 84/131	June	Denmark	litter, conif. wds.	

Table 1 (cont.)

[1]Collection designations preceded by collector's initials (see acknowledgements). [2]number of mating types identified from intra-stock crosses as described in text. [3]stocks showing reduced intra-group compatibility with other stocks from group E-III.

Table 2. Results of inter-stock crosses among Collybia species. 0=all crosses incompatible, 1=some crosses compatible, 2=all crosses compatible.

Group		E-IV								E-III					E-II				E-I	
	Stock	79	78	74	73	70	69	68	67	53	52	51	50	46	44	43	42	40	8	6
E-I	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	
	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
E-II	40	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2			
	42	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2				
	43	0	0	0	0	0	0	0	0	0	0	0	0	0	2					
	44	0	0	0	0	0	0	0	0	0	0	0	0	0						
E-III	46	0	0	0	0	0	0	0	0	1	2	1	2							
	50	0	0	0	0	0	0	0	0	2	2	2								
	51	0	0	0	0	0	0	0	0	1	0									
	52	0	0	0	0	0	0	0	0	2										
	53	0	0	0	0	0	0	0	0											
E-IV	67	2	2	2	2	2	2	2												
	68	2	2	2	2	2	2													
	69	2	2	2	2	2														
	70	2	2	2	2															
	73	2	2	2																
	74	2	2																	
	78	2																		

Table 2 (cont.). 0=all crosses incompatible, 1=some crosses compatible, 2=all crosses compatible.

Group		E-I						E-III						
	Stock	2	3	4	9	7	1	49	48	47	55	57	63	64
E-III	65	0	0	0	0	0	0	0	1	0	0	1	2	2
	64	0	0	0	0	0	0	0	0	0	1	1	2	
	63	0	0	0	0	0	0	0	1	0	1	1		
	57	0	0	0	0	0	1	2	2	2	2			
	55	0	0	0	0	0	0	1	1	2				
	47	0	0	0	0	0	0	2	2					
	48	0	0	0	0	0	0	1						
	49	0	0	0	0	0	0							
E-I	1	2	2	1	2	1								
	7	2	1	2	2									
	9	2	2	1										
	4	2	2											
	3	1												

Table 2 (cont.). + = compatible, - = incompatible, r = rare clamps observed, p = prolific pseudoclamps formed.

Group		E-I			E-III							
	Stock	1	2	9	55	47	57	63	65	64	61	62
E-I	5	+	-	+	-	-	-	-	-	-	-	-
	8	+	+	+	-	-	-	-	-	-	-	-
E-II	40	-	-	-	-	-	-	-	-	-	-	-
	42	-	-	-	-	-	-	-	-	-	-	-
	43	-	-	-	-	-	-	-	-	-	-	-
E-III	46	r	-	-	+	+	+	+	+	+	+	+
	50	-	-	-	+	+	+	p	p	+	p	p
	61	-	-	-	p	p	r	r	+	-	-	+
	62	-	-	-	p	p	p	+	+	+	+	+
E-IV	69	-	-	-	-	-	-	-	r	-	-	-
	73	-	-	-	-	-	-	-	-	-	-	-

Table 3. Inter- & Intra-group mating compatibility frequency among European mating groups. (Based upon data from 979 crosses among monokaryotic isolates from 37 stocks.)

	E-IV	E-IIIb	E-IIIa	E-II	E-I
E-I	0[1]	0.01	0.01	0.03	0.91
E-II	0	0	0	1.00	
E-IIIa	0	0.33	0.84		
E-IIIb	0.17	0.84			
E-IV	1.00				

[1]Proportion of compatible matings.

CHAPTER 2. SYSTEMATICS OF THE COLLYBIA DRYOPHILA GROUP IN EUROPE. 2. MORPHOLOGICAL STUDIES.

Collybia dryophila (Bull. : Fr.)Kumm. is generally regarded as a polymorphic species in Europe. The confusion surrounding this species dates back to Bulliard's (1789) original plate describing *Agaricus dryophilus* (Figure 3). This plate shows at least several taxa, reflecting Bulliard's broad concept of this species. Although numerous varieties and related species have been described from the *C. dryophila* group since, their identities remain unclear.

I have demonstrated (in chapter 1) that European fungi from the *C. dryophila* group belong to one of four biological species. Biological species concepts have proven useful for guiding morphological studies and establishing clear taxonomic relationships in a large number of higher fungi (Boidin, 1980). The purpose of this study was to describe morphological variation within and among different biological species of the *C. dryophila* group from Europe. The value of traditional morphological criteria used to distinguish taxa in the *C. dryophila* group is assessed with respect to the biological species concept. A revised taxonomic arrangement of the *C. dryophila* group in Europe is also presented.

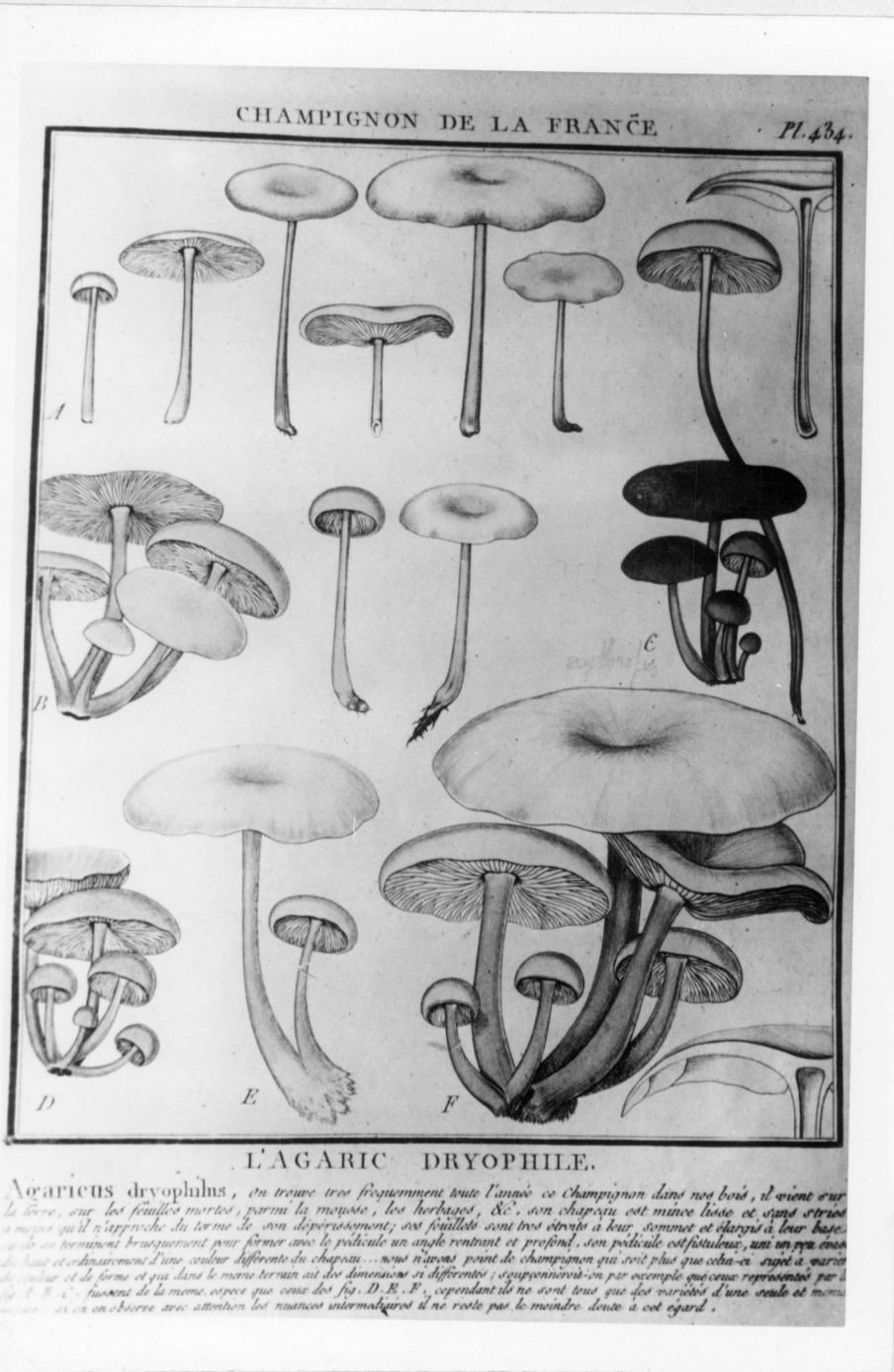

Figure 3. Original illustration of Agaricus dryophilus taken from Bulliard (1789) showing his broad concept of this species.

MATERIALS AND METHODS

The material for this study consists of voucher collections from my previous mating study (chapter 1), as well as additional collections made by myself and those sent to me by other mycologists from a number of localities in Europe. In most cases, the mating specificity of a collection was known. In the case of a large number of fruitings from a single location, the mating specificity of known collections with additional collections was assumed based upon morphological similarity and proximity. Colors given in descriptions represent plate, column and row (e.g., 4A3) from Kornerup & Wanscher (1978).

Spore measurements were determined by measuring from 10 30 basidiospores from the pileus of a single sporocarp from each collection. Coefficients of variation (CVs) for spore measurements from a single collection ranged up to 20%, but were usually much lower (less than 10%). When the mean values for different collections of a single species were averaged, CVs were reduced to less than 5%.

Cluster analysis was performed using the NT-SYS package of computer programs developed by Rohlf, Kishpaugh & Kirk (1979). Pairwise similarity matrices between OTUs (Operational Taxonomic Units- collections in this case) were generated using an algorithm based on the simple matching coefficient (defined as the proportion of character states

shared by two OTUs). Hierarchial clustering using unweighted arithmetic averages (Sneath & Sokal, 1973) was used to construct the resulting phenogram.

RESULTS

SPORE MEASUREMENTS

Spore measurements were determined for 120 collections which could be referred to one of four biological species in the *C. dryophila* group. The mean spore length and width for each collection are plotted by mating group (E-I to E-IV) in figure 4. Circles were drawn around the plotted points from each mating group to aid comparison.

Summary statistics for each mating group are presented in Table 4. Mean basidiospore length and width were found to be linearly related ($r=0.44$, $p<0.001$). Slight, but apparent differences do exist in spore sizes between the different mating groups. Spores of group E-II were consistently larger than those from other groups, although a certain amount of overlap with group E-I is evident. Spores of group E-III have a lower size range than any other group, despite a large amount of overlap with groups E-I and E-IV. A statistical analysis on spore measurements was performed. Because of the linear relationship of length and width significant heterogeneity existed between variances from different

collections. A non-parametric test did reveal significant differences in median lengths and widths from each group (Kruskal-Wallis test, $p < 0.001$ for both mean length and mean width). Quotient values (Q) did not differ significantly among groups ($p < 0.22$). Although these differences are statistically significant, they have little predictive value for taxonomy. Larger spores (average length > 7.0 µm) do appear to be useful for discriminating fungi belonging to group E-II.

MORPHOLOGICAL FEATURES

Sporocarps of each mating group showed considerable variation, even within a single population. Polymorphism for size and colour was evident in each group to varying degrees, and frequently included features traditionally used to distinguish taxa in the C. dryophila group. Three examples are discussed here: 1) A striate pileus margin is often used in keys to delimit C. aquosa (Bull. : Fr.)Kumm.. The limited taxonomic value of this feature has been discussed by Halling (1983), who pointed out that almost all species of Collybia have a translucent-striate pileus margin when fresh and moist. 2) Yellow lamellae are diagnostic for several taxa in the C. dryophila, including C. dryophila var. funicularis Fr. and C. luteifolia Gillet. Sporocarps from a single population of group E-I were found to have either white and

yellow lamellae in varying proportions. 3) Huge sporocarps with an enlarged basal bulb are diagnostic for C. dryophila var. oedipus Quel.. Collections of group E-IV from a single geographic region show a range of sporocarp types which may or may not possess a large basal bulb. These three features, although frequently present in certain biological species, are nevertheless shown to be polymorphic, and have little predictive value by themselves for identifying species.

Despite the high level of polymorphism present in these fungi, several morphological differences appear to be generally present among the different mating groups. Based upon preliminary study of voucher collections, and subsequent field observations, eight characters were chosen for their potential diagnostic value in species delimitation. These characters, scored as present or absent, are 1) dark pileus, 2) enlarged basal bulb, 3) pinkish rhizomorphs, 4) yellow lamellae, 5) large spores (mean length >7 µm), 6) soil as a substrate, 7) inflated cheilocystidia, and 8) early spring fruiting. These eight characters were used because they appeared to show minimal variation due to environmental influences, or because they could be easily observed in well preserved (and annotated) herbarium materials. As a test of the utility of these characters, 39 collections were arbitrarily chosen to represent the four mating groups from a variety of geographic locations. Each collection was then scored for each of the eight characters, and the data used

to construct the phenogram pictured in figure 5. In almost every case, collections referable to the same mating group were found to cluster together. Several collections were found to deviate from the overall pattern, however, these represent a minority. The use of additional collections in the data matrix did not change the overall branching pattern significantly. Thus, the eight characters chosen are demonstrably reliable for distinguishing species in the C. dryophila group.

The phenetic manner in which species of the C. dryophila group are recognized by numerical taxonomy (figure 5) points out two important features of their systematics. Firstly, no character can be used alone to consistently distinguish one species from another: a suite of characters, however, almost always discriminated among different species. Secondly, the ability to reliably discriminate among taxa is dependent upon a representative survey of morphological variation within and among different populations.

Deviate clustering of several collections in figure 5 was not unexpected. In most cases, it could be attributed to extreme phenotypic variation within a mating group. For example, two Scottish collections (W 14496 & W 14501) which had dark capped sporocarps and fruited late in summer failed to cluster with other collections from group E-IV, to which these belong. All other collections identified as belonging to group E-IV usually have lighter pilei, and generally fruit

in the springtime on bare soil. An alternative explanation could be that these collections represent a population which has diverged from the rest of group E-IV due to geographic isolation.

When identification is critical, questionable material should be identified by appropriate crosses against testers strains of each species, as mating studies still provide the most reliable way to distinguish species in the C. dryophila group.

THE TAXONOMIC IDENTITY OF COLLYBIA DRYOPHILA

The C. dryophila group is beset by nomenclatoral confusion, since many taxa in the group are based on features now known to be polymorphic. Bulliard's original concept of A. dryophilus (figure 3) is very general, and applies equally well to any of the taxa distinguished by the present study, and possibly other species of Collybia as well. Fries was apparently familiar with variation in the group, and recognized at least one related taxon, A. xanthopus Fr., at an early date in his career (Fries, 1815). In his Systema Mycologicum (1821), Fries recognized a number of taxa in the group: A. xanthopus Fr., A. aquosus (Bull. : Fr.) and the

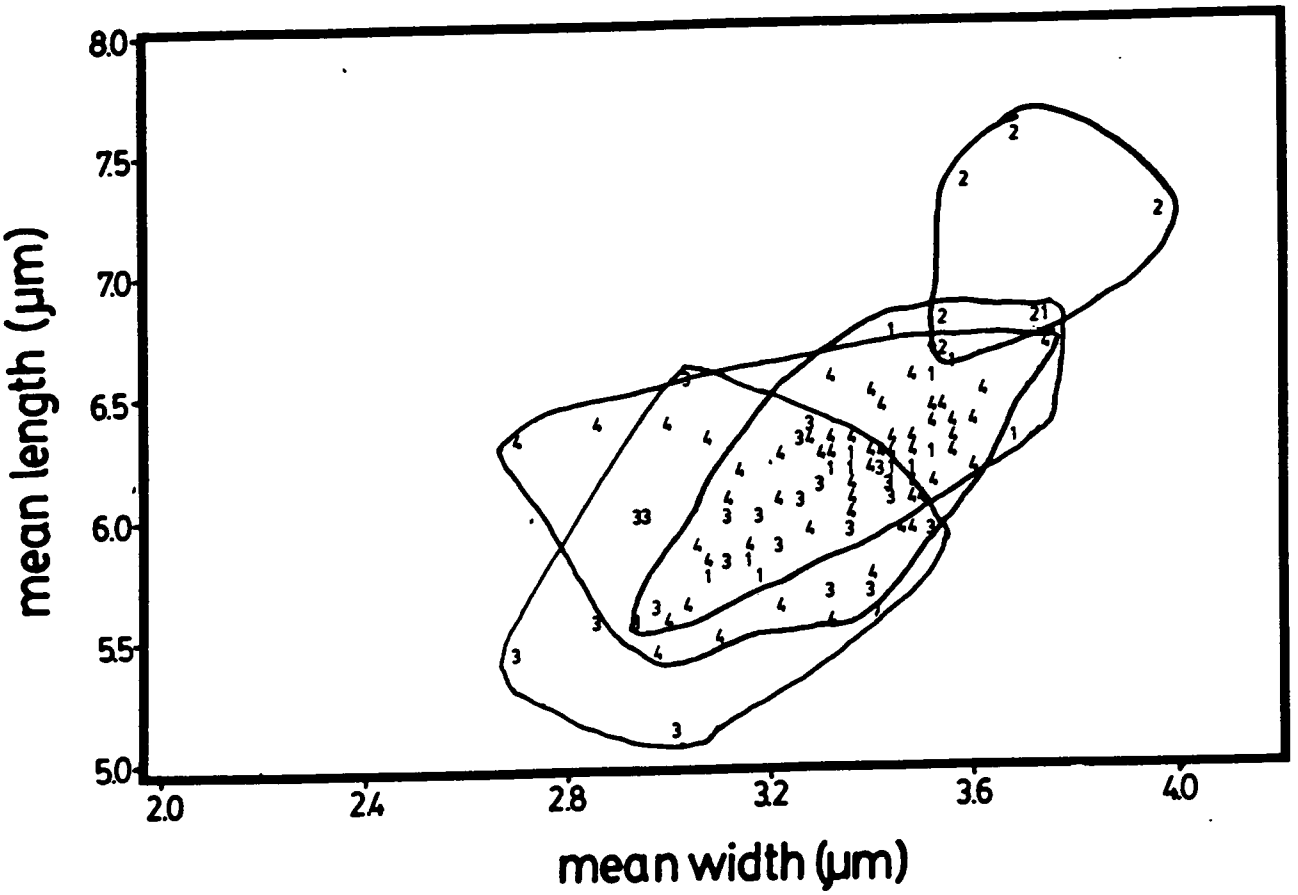

Figure 4. Plot of mean basidiospore length vs. width for collections studied. Numbers (1-4) correspond to different biological species (E-I to E-IV, respectively).

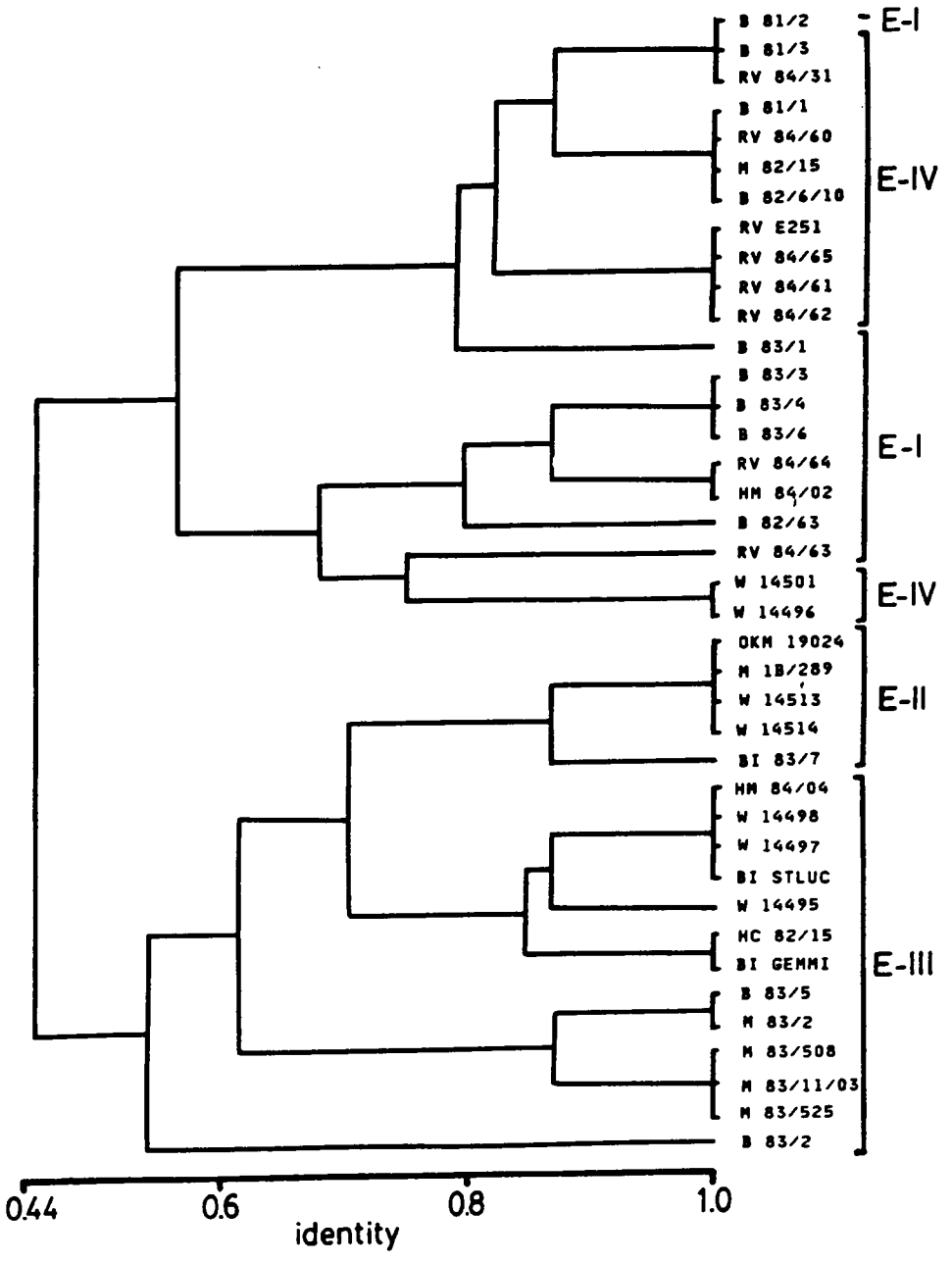

Figure 5. Phenogram of different collections in the C. dryophila group. Similarity is based upon the simple matching coefficient of Sneath & Sokal (1973).

more common A. dryophilus, as well as a variety with yellow lamellae fruiting on wood, A. dryophilus β funicularis Fr.. Later, Fries (1938) recognized additional taxa closely related to A. dryophilus, including A. exculptus Fr., A. succineus Fr.. Several illustrations of these taxa were also published (Fries, 1882), but these were based upon drawings sent to Fries from other collectors and do not necessarily represent authenticated material. Neither a type nor an authentic collection (verified by either Fries or Bulliard) exists for C. dryophila. Based upon my studies, all of Fries' taxa are based at least partly on features which are known to be polymorphic within a single mating group: striate pileus margin, pileus color, wood substrate, etc. For this reason, it is necessary to either select neotypes for the taxa in this study, or to redescribe the taxa as new.

Several Swedish collections from the C. dryophila group have been distributed as exsiccata by Lundell & Nannfeldt (1934-1942). These exsiccata were found to represent at least two of the species recognized by this study. The first collection (L&N 704a, collected 27 June 1938 in Bondkyrka parish), which fruited on soil and possesses a dark pileus along with inflated cheilocystidia, almost certainly represents group E-I from my study, which I also collected in the vicinity of Uppsala on several occasions during June of 1984. A second collection (L&N 1722, 11 Sept. 1946 from Wekinge) fruited on litter, has a lighter pileus and

filamentous, diverticulate cheilocystidia typical of group E-III from the present study. I have found and collected representatives of group E-III from Uppsala and vicinity as well. The third exsiccata (L&N 704b, 19 July 1938, Lena parish) posseses a dark pileus with inflated cheilocystidia typical of group E-I, but as it fruited during the mid-summer, I am less certain about its identity.

The light coloured collection (L & N 1722) corresponds well with the current concept of C. dryophila, as it is presently used in Europe (Moser, 1983; Kuhner & Romagnesi, 1978), and North America (Halling, 1983; Vilgalys & Miller, 1983). Since this material is not inconsistent with either Fries' or Bulliards concepts of A dryophilus, I propose that it be designated as the neotype for C. dryophila.

TAXONOMIC TREATMENT

The *Collybia dryophila* groups is restricted to medium sized species in section *Levipedes* (Fr.)Quel. of *Collybia* possessing light colored lamellae, a glabrous stipe and '*dryophila*'-type structure (Singer, 1975) in the pileus cuticle.

KEY TO EUROPEAN TAXA IN THE *COLLYBIA DRYOPHILA* GROUP

1. Pileus dark when fresh (chestnut, fuscous)..... 2
1. Pileus lighter when fresh (ochraceous, tawny)..... 3
   2. Cheilocystidia inflated-clavate, average basidiospore length < 6.5 µm long, pileus dark but fading with drying (often with pinkish tints), usually fruiting early in the season (May-June)...(group E-I) *C. ocior*
   2. Cheilocystidia diverticulate-filamentous, average basidiospore length > 6.5 µm, pileus dark and not fading .....(group E-II) *C. alpina*
3. Cheilocystidia inflated-clavate, sporocarps light colored with a distinct basal bulb and pink rhizomorphs, usually fruiting early in the season (May-June).....(group E-IV) *C. aquosa*
3. Cheilocystidia diverticulate-filamentous, sporocarps with

with well developed ochraceous colours, usually lacking a basal bulb, and fruiting later (June-late autumn).... .....(group E-III) C. dryophila

COLLYBIA ALPINA VILGALYS & MILLER, NOM. PROV.

Pileus relatively small, 1-3.5 cm broad, convex with inrolled margin becoming upturned with undulating margin; surface lubricous when moist, hygrophanous, becoming dull upon drying; margin translucent striate when very moist; color deep red brown (brick red, reddish date, or chestnut) when fresh (7D8-7F8,7D7-7F7,6D5-6D8,6E5-6E8,6F5-6F8), slightly darker over disk, paling upon drying, but becoming dark again if remoistened; context thin (2-3 mm over disk), pallid or white (6B2-5B3), becoming darker and eventually concolorous below cuticle.

Lamellae very close to crowded (approximately 15 through lamellae), with numerous tiers of lamellulae, adnexed-seceding, up to 3 mm broad, white (to 4A3); margin entire.

Stipe 2.5-4 cm long, 2-4 mm broad, cylindric, pliant-fibrous, hollow at maturity, expanded at base but without a distinct basal bulb; surface glabrous above with scant canescence near base, pallid above and below (4A3-4A4)

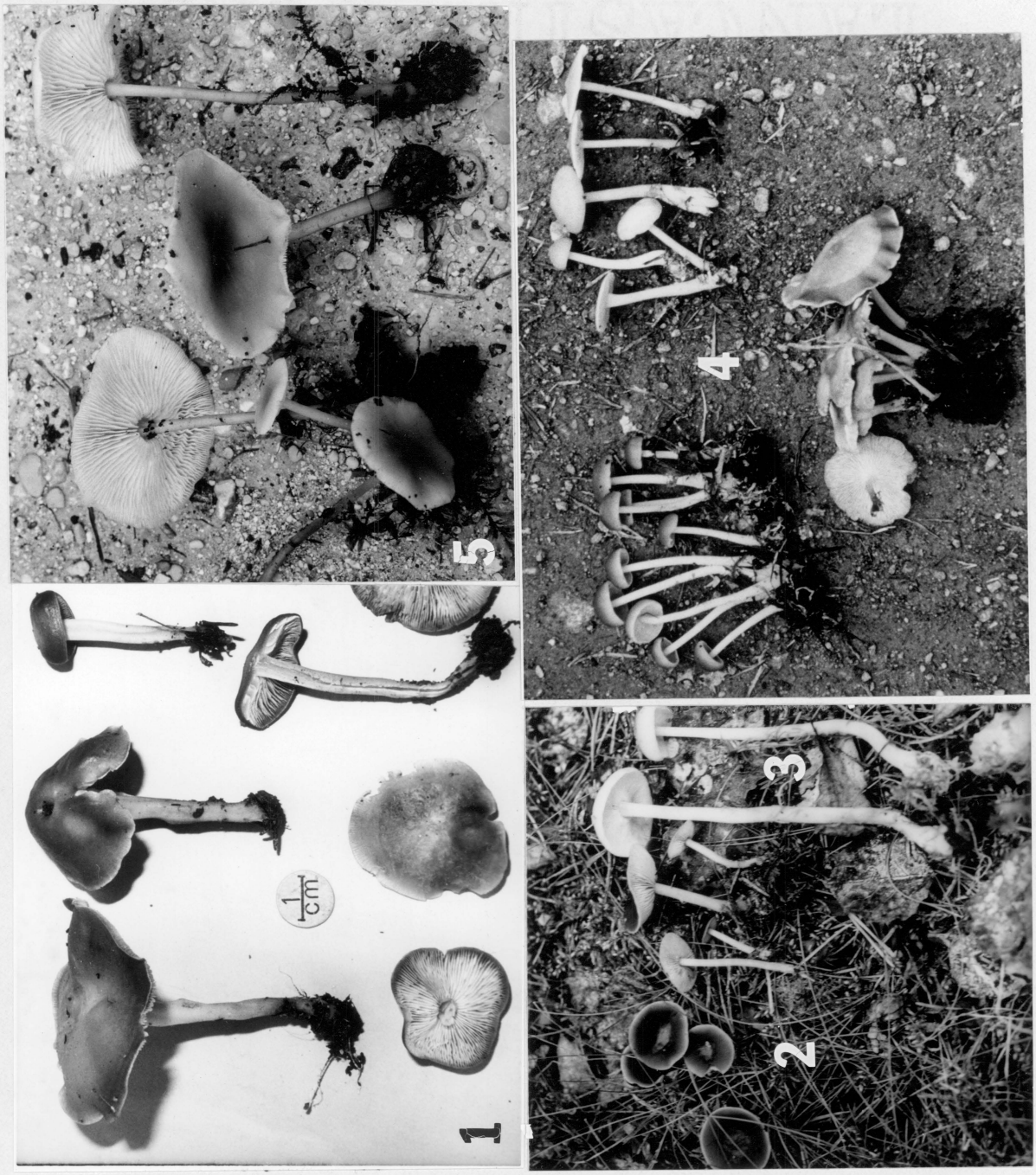

Figure 6. Collybia species. Sporocarps. 1) C. ocior (B EXC, x 3/4), 2) C. alpina (RV 84/182, x 1/3), 3) C. dryophila (RV 84/181, x 1/3), 4) C. dryophila (RV 84/213, 84/214, 84/215, x 1/3), 5) C. aquosa (RV 84/78, x 3/4).

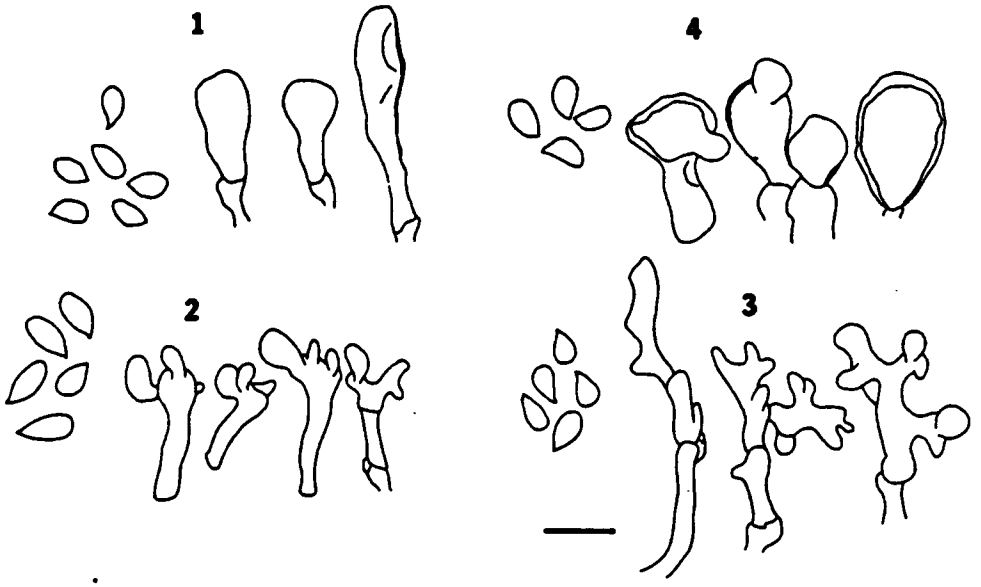

Figure 7. Collybia species. Microscopic features. 1) C. ocior (Lundell & Nannfeldt 704a), 2) C. alpina (holotype), 3) C. dryophila (Lundell & Nannfeldt 1722, neotype), 4) C. aquosa (M 82/15). Solid bar = 10 µm. Basidiospores shown on left, cheilocystidia on right.

when young, turning darker toward base (to 5B5, 6C5-6), but never distinctly ochraceous. Extreme base attached to scant fine white rhizomorphs.

Chemical reactions: $FeSO_4$= -, phenol= pileus cuticle pink, gum guiac= very mild, localized near pileus cuticle.

Basidiospores 6.0-8.4 x 2.8-4.7 µm, ellipsoid in profile and face view, thin walled, smooth, hyaline, unreactive in KOH and Melzer's reagents, acyanophilous. Basidia 20-34 x 3-10 µm, clavate, thin walled, tetrasterigmate. Pleurocystidia absent or else indistinguishable from basidioles. Cheilocystidia inconspicuous, frequently collapsed along lamellar edge, up to 35 µm long, 3-8 µm broad, scattered to aggregated, consisting of diverticulately branched terminal cells similar to pileipellis hyphae, only narrower. Pileipellis a dense myxocutis of interwoven hyphae with a 'dryophila'-type structure; hyphae 4-8 µm broad, thin walled and hyaline, or else slightly thick walled, frequently encrusted with a granular or plate-like pigment which is reddish brown in water mounts, discoloring to a light brownish-olivaceous hue in aqueous alkali; densely pigmented hyphae frequently forming coralloid clusters; insoluble amorphous pigment globules up to 50 µm diam often scattered among pileipellis hyphae. Pileus trama of less densely interwoven hyphae, 4-8 µm broad, thin walled, occasionally with light encrusting pigments present. Lamellar trama composed of parallel, thin walled hyphae, 4-8 µm broad,

mostly hyaline or occasionally with very light encrusting pigments present, pigments dissolving in aqueous alkali. Stipitipellis composed of parallel, vertically oriented thin walled hyphae; surface composed of scattered, narrow diverticulately branching hyphae, 2-4µm broad, hyaline or else slightly pigmented similarly to pileipellis hyphae. Clamp connexions present in all tissues.

Fruiting singly or in small groups under Pinus and/or dwarf shrubs (Betula, Salix, Vaccinium) in sub-arctic woodlands or (occasionally?) sub-alpine regions from June through September.

Material examined: Austria: OKM 19024; Greenland: TB 78.18 (C), P. Milan Petersen 72.86 (C), P. Milan Petersen 72.370 (C); Netherlands: C. Bas 25-V-1955 (L); Norway: BI 81/7, M 84/136, R. J. D. McBeath 680 (E); Sweden: MM 1b/289, RV 84/182, RV 84/221, RV 84/222; United Kingdom (Scotland): RW 14513, RW 14514 (All VPI, unless noted).

Collybia alpina is one of the more distinctive members of the C. dryophila group encountered in my studies. It is best distinguished by its large spores and dark pileus, which does not fade with drying. The Scottish collections (W 14513 & W 14514) had slightly smaller spores than the other material, but were similar in other respects.

Field collections of C. ocior also have a dark pileus and may be confused with C. alpina in the field. I have found both species fruiting in the same vicinity near Uppsala during June of 1984, and could not distinguish collections based on macroscopic features. Yellow lamellae characteristic of some collections of C. ocior have not been observed in C. alpina, and could be used to distinguish field collections. In most cases, the difference in cystidial morphology and spore size (outlined in the key) serve to distinguish these two species.

A number of ecological and floristic studies from subarctic regions of Europe have noted a form of C. dryophila with a dark pileus fruiting under dwarf shrubs (Kallio & Kankainen, 1964, 1966; Lange, 1955; Lamoure, Lange & Petersen, 1982). Kallio & Kankainen (1964) reported larger spores for the dark capped form (5.8 x 3.0 µm) than in the typical lighter form (4.0 x 2.5 µm). Gulden & Lange (1971) also noted a dark capped form in their studies on Norwegian fungi, but did not find any difference in spore size with the lighter form. From my observations, a dark pileus is characteristic of two separate species, one with larger spores (group E-II, C. alpina), and another with smaller spores (group E-I, C. ocior). Mating studies demonstrate that both species are present in Scandinavia, and it is probable that both reports correspond to different species recognized in this report. Although the values reported by

Kallio & Kankainen (1964) are less than mine, a larger spore size along with a dark pileus are consistent with the features described here for C. alpina.

COLLYBIA AQUOSA (BULL. : FR.)KUMM.

=Agaricus aquosus Bull. f. 12, 1780.

≡C. dryophila var. aquosa (Bull. : Fr.)Quel., 1886.

≡C. dryophila ssp. aquosa (Bull. : Fr.)Konr. & Maub.

≡Marasmius dryophilus var. aquosus (Bull. : Fr.)Rea, 1922.

=C. dryophila var. oedipus Quel., Encher. 1888.

≡C. oedipus (Quel.)Metrod, 1952

=?Agaricus ventricosus Bulliard, Hist. Champ. Fr., t. 911, f. 1 (1780)

≡Collybia ventricosa (Bull.)Gillet, Champ. Fr., p. 324, 1874.

=?Agaricus tuberosus Schum. non auctore, Soell. p. 276.

Pileus medium to large, 2-7 cm broad, convex with an inrolled margin, becoming plane or upturned with a wavy margin; surface smooth, glabrous, strongly hygrophanous, lubricous when fresh and moist, dull with drying, pallid yellow to yellow-ochraceous (5E5-7), occasionally with greyish or pinkish (salmon) cast (particularly in larger specimens), fading with age and drying (4A2-3,4B2-3).

Context thin, whitish. Odor and taste mild to slightly fungoid.

Lamellae adnexed-seceding, crowded, relatively shallow (3-4 mm), thin; white to pale yellowish (never distinctly yellow); margin even to slightly eroded.

Stipe 3-10 cm long, 3-5 mm broad, cylindric or curved-tortuous, pliant-fibrous, slightly flared at apex, enlarged into a distinct basal bulb (to 2 cm broad); pallid above (5A4,4A4) to concolorous with pileus below, becoming ochraceous (5C8,6C8) in age toward base; coarse ochraceous-pinkish (6B4-5) rhizomorphs frequently attached to base.

Chemical reactions: phenol, 3% KOH, gum guiac all negative.

Spore print white to cream-coloured. Basidiospores 5-7.5 x 3-4 µm broad, ellipsoid in profile and face view, thin walled, hyaline, inamyloid. Pleurocystidia absent. Cheilocystidia 15-45(-65) x 4-14 µm, scattered to abundant, occasionally crowded, inflated-clavate to sphaeropedunculate, thin walled at first but soon becoming irregularly thickened, appearing somewhat echinulate, occasionally with proliferating lobed diverticulae. Lamellar trama of parallel, cylindric hyphae, 4-18 µm broad, thin walled., Pileipellis a myxocutis of interwoven hyphae with a 'dryophila'-type structure; hyphae 3-30 µm broad, thin walled, occasionally encrusted with a light yellowish

pigment; scattered yellowish pigment globules (to 100 µm broad) frequently present. Pileus trama composed of less densely interwoven, thin walled hyphae. Stipitipellis composed of vertically oriented, parallel, cylindric hyphae 3-13 µm broad; surface covered with scattered narrow, diverticulate elements (to 5 µm broad, occasionally higher). Rhizomorphs composed of interwoven thin walled hyphae, 3-5 µm broad, with scattered yellowish incrustation; inflated elements (to 10 µm broad) scattered within. Clamp connexions present in all tissues.

Fruiting singly or gregariously usually on bared soil. Most frequent in spring (May-June), but may also fruit later in the season. Widely distributed through most of Europe and Great Britain, except in northern Scandinavia (rare or absent?).

Material examined: Austria: B 81/3; Italy: M 82/15, B 81/1; France: L 81/301, RV E251, RV 84/16-RV 84/36, RV 84/52-RV 84/58, RV 84/60-RV 84/62, RV 84/65, RV 84/67-RV 84/97; Denmark: RV 84/123-RV 84/127, RV 84/131, RV 84/138-RV 84/160, RV 84/167-RV 84/173, RV 84/177, RV 84/178, T. Laessoe 3-VI-1984 (C); Netherlands: C. Bas 6323 (L), United Kingdom (Scotland): W 14496, W 14501. (All VPI, unless noted).

Although C. aquosa is often distinctive in its typical form, it could easily be mistaken for lighter forms of C.

dryophila, particularly on the basis of macroscopic features. Several features which I found to be diagnostic include the combination of light colors, inflated cheilocystidia and basal bulb, which is usually attached to pinkish rhizomorphs on mineral soil.

Traditionally, C. aquosa was distinguished by having a translucent striate pileus margin. A distinctive striate pileus margin was observed in a number of well developed collections of C. aquosa collected by myself and H. Romagnesi near Paris, France. A striate pileus margin was not characteristic of all the material in my study, however. Halling (1983) has discussed the limited utility of this feature, as most species of Collybia possess a translucent-striate pileus margin when moist.

Well developed specimens of C. aquosa with an enlarged basal bulb (to 20 mm) are apparently common in some regions of Europe during wet seasons. In the past, these collections have been referred to C. dryophila var. oedipus Quel.. Both large and medium size forms were found to be mating-compatible (chapter 1), and I have no doubt as to their conspecificity.

COLLYBIA OCIOR (PERSOON)VILGALYS & MILLER, STAT. NOV.

≡Agaricus ocior Persoon, Myc. Eur., p. 151, 1828.

=A. dryophilus β funicularis Fr., Syst. Myc., 1821.

≡A. dryophilus var. funicularis (Fr.)Fr., Epicr., p. 93, 1938.

≡C. dryophila ssp. funicularis (Fr.)Konrad, 1927.

≡C. funicularis (Fr.)Konrad & Maublanc, Encyc. Mycol. XIV, p. 281, 1948.

≡Marasmius funicularis (Fr.)Karsten, 1879.

≡M. dryophilus var. funicularis (Fr.)Rea, 1922.

=?Agaricus exculptus Fries, Epicr., p. 93, 1838.

≡C. exculpta (Fr.)Gillet, Champ. Fr., 1874.

≡Marasmius exculptus (Fr.)Rea, 1922.

=Agaricus extuberans Battara, Fung. Arim. Hist., t. 28, f. 1, 1755.

≡A. extuberans (Batt. : Fr.), Epicr., p. 93, 1838.

≡Collybia extuberans (Batt. : Fr.)Quel., Mem. Soc. Montbeliard I, p. 62, 1872.

=?Agaricus succineus Fries, Epicr., p. 91, 1838.

≡Collybia succinea (Fr.)Quelet, Mem. Soc. Montbeliard I, 1872.

≡C. extuberans var. succinea (Fr.)Quel., Fl. Mycol., 1888.

=?Collybia papillata Gillet, Champ. Fr., 1874.

=Collybia luteifolia Gillet, Champ. Fr., p. 328, 1874.

Pileus 1-5(-6) cm broad, convex with inrolled margin when young becoming plane to upturned; margin translucent-striate when moist, even or undulating; surface glabrous,

hygrophanous, slightly lubricous when moist, dull when dry; color variable, reddish brown to chestnut when fresh (6D7-8,6E7-8,6F7-8,7D7-8) with or without a light margin, fading upon drying to buff (4A2-3,4B4-6,5A3-4,5B4,), frequently with a distinct lilaceous tint (pink or violaceous), becoming dark again upon moistening; context thin, white, odor and taste mild.

Lamellae adnexed but soon seceding from stipe, crowded or very close (30-35 thru-lamellae), narrow to moderately broad, relatively shallow (3-4 mm), numerous tiers of lamellulae present; variously colored, ranging from pure white to various shades of yellow (cream to lemon yellow, 2A2-2A3,3A1-4,4A3); margin even or minutely fimbriose to serrulate.

Stipe to 7 cm long, 4-8 mm broad, usually slender, cylindric, straight or slightly fluted, pliant-fibrous, slightly flared at apex, expanding near base into a small bulb or remaining broadly fusiform, sometimes joined into fasciculate clusters; pallid at apex (3A2,4A2-3,4A5-6), darker below (ochraceous to reddish brown, 4B5-6,5B4-5). Base often attached to white or noticeably pigmented (pinkish ochraceous, 5A4) rhizomorphs.

Spore print white. Basidiospores 5.0-6.5 x 3-4 µm, ellipsoid in profile and face view, aguttulate, thin walled, hyaline, inamyloid. Basidia 18-25 x 3-7 µm, tetrasterigmate, thin walled, asiderophilous. Pleurocystidia absent or

indistinguishable from basidia. Cheilocystidia 18-47 x 5-16 µm, abundant to crowded, inflated-clavate to subglobose or sphaeropedunculate, frequently proliferating into additional inflated diverticulae; mostly thin walled, but also with irregularly thickened walls, hyaline, inamyloid. Lamellar trama of parallel hyphae, 4-16 µm broad, thin walled, hyaline, inamyloid. Pileipellis a 'dryophila' type cutis; hyphae 3-15 µm broad, thin walled, with scattered areas of lightly incrusted hyphae, incrustation olivaceous brown in aqueous alkali, occasionally with scattered amorphous brownish pigment globules (to 50 µm diam). Stipitipellis composed of vertically oriented, parallel hyphae; hyphae 4-7 µm broad, thin walled, cylindric, not encrusted; surface with scattered thin walled narrow diverticulate end cells, 2-3 µm broad. Clamp connexions present in all tissues.

Fruiting singly to gregariously on soil, litter, and decaying wood, usually early in the season (May-June). Widely distributed in Europe.

Material examined: Austria: B 82/63, B 82/6/26, B 83/1, B 83/3, B 83/4, B 'exc', M 83/1; Italy: B 81/2, B 83/6; France: RV 84/37-RV 84/42, RV 84/49-RV 84/51, RV 84/63, RV 84/64, RV 84/98-RV 84/103; Denmark: S. Klug-Anderson & H. Knudsen 3-VI-1984 (C), B. Ronne 2-VI-1984 (C), E. Rald 27-V-1983 (C), RV 84/123-RV/126, RV 84/128-RV 84/130, RV 84/132-RV 84/136,

RV 84/174-RV 84/176, RV 84/179, RV 84/180, Netherlands: C. Bas 2248 (L), J. Schutte 2-VI-1965 (L), Kits v. Waveren 7-VI-1958 (L), Kits v. Waveren 10-VI-1960 (L), A. J. P. Oort 31-V-1962 (L), Rv. Crevel 8-VI-1973 (L); Sweden: RV 84/185, RV 84/186, RV 84/188, Rv 84/189, RV 84/194, RV 84/196, RV 84/211, RV 84/214, RV 84/217, RV 84/218, RV 84/221, RV 84/222, Lundell & Nannfeldt 704a (UPS), Lundell & Nannfeldt 704b (UPS), G. Stahlberg 5-VI-1944 (UPS) (All VPI, except where noted).

Collybia ocior is a very common, yet poorly known, species in Europe. Almost all the previous nomenclatoral confusion surrounding C. ocior can be attributed to the unreliability of morphological features previously used to distinguish this taxon, particularly gill colour. Lamellae of C. ocior may be either white or yellow, and both colour forms are usually present in large fruitings. The basis for gill color polymorphism in C. ocior is not known at present. No apparent correlation with substrate type was apparent in field collections, nor have I been able to notice any difference in cultures from yellow or white gilled forms.

An apparently consistent feature of C. ocior is its brown pileus, which fades upon slow drying, revealing distinct pinkish-lilaceous hues. I have noticed that the dark color returns to partially dessicated specimens if the sporocarps are remoistened with water. This feature is quite striking,

and could serve to distinguish C. aquosa and C. dryophila from C. ocior in the field, since those species are generally light coloured when moist. Collybia alpina, which also possesses a dark pileus, is harder to distinguish from C. ocior, although the features given in the key ought to be sufficient in most cases.

A good photo illustration of the yellow-gilled form of C. ocior with a dark pileus has been published (as C. dryophila) by Cetto (1977, pl. 545).

COLLYBIA DRYOPHILA (BULL. : FR.)KUMMER

≡Agaricus dryophilus Bulliard, Hist. Champ., t. 434, 1780.

≡A. dryophilus Bull. : Fries, Syst. Mycol. I, p. 124, 1821.

≡Marasmius dryophilus (Bull. : Fr.)Karsten, Krit. Ofvers. Finland Basidsvamp, p. 103. 1889.

≡Collybidium dryophilum (Bull. : Fr.)Murrill, Mycologia 3: 101. 1911.

≡Gymnopus dryophilus (Bull. : Fr.)Murrill, N. Amer. Fl. 9: 362. 1916.

=?Collybia aurata Quelet, Encher., p. 31, 1886.

=Agaricus xanthopus Fries, Obs. Myc., 1815.

≡A. xanthopus Fr. : Fries, Syst. Mycol. I, p. 124, 1821.

≡A. xanthopoda (misspelled) in Saccardo, Sylloge Fungorum v. 5 (1882-1928).

Pileus to 4 cm broad, convex to broadly convex with or without a slightly raised disk; margin inrolled when young, becoming wavy or upturned at maturity, translucent-striate when moist; surface smooth, lubricous when moist, strongly hygrophanous; color yellow to ochraceous when fresh (4A3-4A6, 4B4-4B6, 5B5-5B6, 5A7, 6C8), either fading or else becoming ochraceous in age. Context thin (< 5 mm at disk), spongy, pallid, odor and taste mild or slightly fungoid.

Lamellae slightly adnexed, seceding as the pileus expands, crowded, thin, relatively shallow (2-4 mm), with numerous tiers of lamellulae; color white when fresh, usually remaining pallid or sometimes becoming yellowish to ochraceous only with age (2A2, 3A1-3A3, 4A2-4, 5A5); margin entire and even or else slightly fimbriate.

Stipe to 13 cm long, to 6 mm broad, pliant-fibrous, usually slender, cylindric or slightly compressed above, slightly flared at apex, expanding at base, with or without a small basal bulb present (to 10 mm broad); surface glabrous above, glabrous or finely pubescent toward base where in contact with substrate; color pallid or yellow when fresh (2A3, 3A3, 4A3-4), soon developing strong ochraceous hues toward base and eventually over entire length (5A2-5A6, 5B6,

6B5-6B7). Base sometimes attached to white or pale ochraceous (5A3) rhizomorphs.

Spore print white or cream coloured. Basidiospores 5.1-6.6 x 2.7-3.5 µm, mostly short ellipsoid in face and profile view, thin walled, hyaline, inamyloid. Basidia 18-27 x 5-7 µm, thin walled, tetrasterigmate (rare one and two spored basidia noted), asiderophilous. Pleurocystidia absent. Cheilocystidia present, usually embedded along lamellar edge, abundant or scattered and then inconspicuous, consisting of characteristic long (to 40 µm), narrow diameter hyphae (usually less than 7 µm broad) with numerous diverticulate branchlets; hyphae thin walled, hyaline, inamyloid. Lamellar trama composed of parallel cylindric hyphae, 4-11 µm diam, smooth, thin walled, inamyloid. Pileipellis composed of densely interwoven hyphae with a 'dryophila'-type structure; hyphae mostly thin walled, to 10(-15) µm broad at their terminus, not usually encrusted, yellowish in water and KOH. Pileus trama of less densely interwoven, thin walled hyphae. Stipitipellis composed of parallel, cylindric hyphae, 4-12 µm broad, with smooth, slightly thickened walls, yellowish or hyaline in KOH, inamyloid; surface covered with scattered to abundant caulocystidia, consisting of long, narrow hyphae (to 7 µm broad), frequently diverticulate as in cheilocystidia. Clamp connexions present in all tissues.

Macrochemical reactions- gum guiac, phenol, $FeSO_4$ all negative.

Material examined: Austria: B 83/2, M 83/2; Czekoslovakia: OKM 20693, OKM 20711; Denmark: B. Hansen 12-X-1980 (with Christiansenia tumefaciens Ginns & Sunhede) (C), K. Haverslev 26-VIII-1981 (with C. tumefaciens) (C); France: HR 84/90, HM 84/07; Germany: HM 84/01, HM 84/03, HM 84/04; Italy: B 83/5, M 83/508, M 83/525, M 83/11/03; Greenland: P. Milan Petersen 73-205 (C); Netherlands: RV 84/109, RV 84/114-RV 84/120, C. Bas 1342 (L) C. Bas 7790 (L), P. B. Jahnsen 24-VI-1955 (L), K. Booy 27-VII-1976 (L), E. Arnolds 323 (L); Sweden: RV 84/181, RV 84/190-RV 84/193, RV 84/195, RV 84/197-RV 84/207, RV 84/209, RV 84/210, RV 84/212, RV 84/213, RV 84/215, RV 84/216, Lundell & Nannfeldt 1722 (NEOTYPE, UPS). Switzerland: BI Gemmi, BI St. Luc, BI Bern, HC 82/15; United Kingdom (Scotland): W 14495, W 14497 (All VPI, unless noted).

Despite a common mating specificity, group E-III collections referable to C. dryophila show considerable morphological variation, both within and between populations. The most consistent feature among the collections I observed are the narrow, diverticulate cheilocystidia, which are also similar to those described from North American collections (Halling, 1983; Vilgalys & Miller, 1983). Most European collections of C. dryophila which I observed were largely

similar to North American material in having well developed ochraceous colors in the pileus. Dark pileus colors characteristic of many North American collections (Vilgalys & Miller, 1983) were only rarely observed in European material, however.

Sporocarps of C. dryophila may occasionally be parasitized by Christiansenia mycetophila Ginns & Sunhede, resulting in the production of large teratological galls (Ginns & Sunhede, 1978). Based on my observations, the Christiansenia parasite appears to be restricted to sporocarps of group E-III (C. dryophila). Additional study of parasitized material may indicate if other members of the C. dryophila are also infected by Christiansenia species.

I have already noted the diminutive stature of several collections made from Sardinia (chapter 1). Superficially, these collections are similar to C. earleae (Murr.)Murr., a North American taxon. Microscopically, however, the Sardinian material was found to possess the filamentous cheilocystidia characteristic of C. dryophila. Additional studies of material collected from the Mediterranean region might indicate whether a diminutive stature is characteristic of C. dryophila from this region.

Table 4. Summary of spore measurement data by species

species	n[1]	variable	min(mean)max	CV[2]
E-I	18	length	5.5(6.2)6.8 µm	5.5%
		width	2.9(3.4)3.7	4.6
		q	1.7(1.8)2.0	3.2
II	7	length	6.7(7.1)7.6	4.6
		width	3.5(3.8)4.2	6.6
		q	1.7(1.9)2.1	6.5
III	23	length	5.1(5.9)6.6	5.4
		width	5.1(5.9)6.6	6.6
		q	1.7(1.9)2.2	6.8
IV	69	length	5.5(6.2)6.7	4.6
		width	2.7(3.4)3.7	5.9
		q	1.7(1.9)2.3	6.4

[1]n=number of collections from which mean values were used to compute means for each species.

[2]CV=coefficient of variation

CHAPTER 3. INTERCOMPATIBILITY, CULTURAL FEATURES AND MORPHOLOGY OF ALLOPATRIC SPECIES IN THE COLLYBIA DRYOPHILA GROUP.

Taxonomic studies of closely related fungi are frequently confounded by a high degree of phenotypic variation, making species circumscription difficult. Mating studies, however, have proven to be useful for determining taxonomic relationships in a number of groups (Boidin, 1980).

Fungi identified as C. dryophila (Bull. : Fr.) Kumm. have been described from around the world, where they occur as primary litter decomposers. I have previously demonstrated the presence of discrete intersterility groups, or biological species, in the C. dryophila group from North America (Vilgalys, 1982; Vilgalys and Miller, 1983), and from Europe (this volume, chapter 1). Both Europe and North America each possess at least four biological species, identifiable on the basis of mating compatibility. Different biological species from either continent also show consistent morphological differences, which allow them to be identified from their sporocarps (Vilgalys & Miller, 1983; this volume, chapter 2).

Cultural and nutritional features have been shown to have potential value for taxonomy of a number of agarics (Miller, 1971), but have not been applied to the C. dryophila group. Slight cultural differences among the North American species

were apparent in my previous study (Vilgalys and Miller, 1983), but were not used as taxonomic features since I did not evaluate their variability.

Lindeberg (1946) demonstrated a requirement for biotin and thiamine for three isolates identified as Collybia dryophila from Sweden. While almost all fungi require thiamine for growth, biotin heterotrophy is apparently rare in agarics, and is not known for other species of Collybia (Lindeberg, 1946). At least three species of the C. dryophila group are now known to occur in Sweden (this volume, chapter 2). Whether biotin and thiamine heterotrophy are characteristic of only one or all species in the C. dryophila group has not been investigated.

Relationships among European and North American biological species are not readily apparent from simple comparison of their fruit bodies (chapter 2, this volume). In this study, I report on mating compatibility between North American and European biological species in the C. dryophila group, along with several additional isolates of this group from Asia and Australia. Nutritional requirements and cultural morphology of representative isolates from each group are also compared and evaluated for their use as taxonomic features.

Table 5. Origin of tester strains used for mating studies.

Stock number	Collection number	Geographic origin
	Group N-IV. *C. brunneola* Vilg. & Mill.	
1	RV 139	Virginia, USA
	Group N-III. *C. earleae* (Murr.)Murr.	
2	RV 153	Virginia, USA
3	GB 266	Virginia, USA
4	GB 263	Virginia, USA
5	GB 272	Virginia, USA
	Group N-II. *C. subsulphurea* Peck	
6	RV 149	Virginia, USA
7	RV 157	Virginia, USA
8	RV 213	Virginia, USA
	Group N-I. *C. dryophila* (Bull. : Fr.) Kumm.	
9	RV 161	West Virginia, USA
10	Halling 3307	Massachusetts, USA
11	RV 148	Virginia, USA
12	RV 83/180	Virginia, USA
	Group K.	
13	OKM 20263	Gyeongbugr Province, Korea
	Group J.	
14	OKM 19797	Kyoto, Japan
	Group A.	
15	OKM 19449	Perth, Australia
	Group E-I. *C. ocior* (Persoon)Vilg. & Mill., stat. prov.	
16	B 81/2	Tirol, Austria
17	B 82/63	Tirol, Austria
18	B exc	Tirol, Austria
	Group E-II. *C. alpina* Vilg. & Mill., nom. prov.	
19	OKM 19024	Tirol, Austria
20	W 14513	Aberdeenshire, Scotland
21	M 1b/289	Lapponia, Sweden (UK)
	Group E-III. *C. dryophila*	
22	W 14495	Perthshire, Scotland (UK)
23	C 82/15	Switzerland
24	OKM 20711	Bratislava, Czekoslovakia
25	B 83/2	Italy
26	BI StLuc	St Luc, Switzerland
27	M 83/525	Sardinia
28	M 83/508	Sardinia
29	M 83/11/03	Sardinia
	Group E-IV. *C. aquosa* (Bull. : Fr.)	
30	W 14501	Perthshire, Scotland (UK)
31	B 82/6/10	Tirol, Austria
32	L 81/301	Lyon, France

Table 6. Intercompatibility relationships between stocks originating from different biological species of the C. dryophila group from Europe, North America, Asia, and Australia.

group		N-IV	N-III				N-II			N-I				K	J	A
	stock	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
E-I	16	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	17	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E-II	19	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
	20	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
	21	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
E-III	22	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-
	23	-	-	-	-	-	-	-	-	+	-	*	+	+	+	-
	24	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-
	25	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-
	26	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-
	27	-	-	-	-	-	-	-	-	+	+	+	*	+	*	-
	28	-	-	-	-	-	-	-	-	+	+	+	+	+	*	-
	29	-	-	-	-	-	-	-	-	+	-	+	+	+	*	-
E-IV	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K	15	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-
J	14	-	-	-	-	-	-	-	-	+	+	+	+	+	+	*
A	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Legend: (+) clamp connections normally produced (strains intercompatible), (*) pseudoclamps formed in some crosses (strains intercompatible), (-) all strains incompatible.

MATERIALS AND METHODS

Mating studies.-The origin of monokaryotic tester strains used for mating studies is given in Table 5. These testers represent each of four biological species from both North America (designated as groups N-I through N-IV), Europe (E-I through E-IV), and also additional material obtained from Korea (K), Japan (J), and Australia (A). Representative tester strains from each group are maintained at 2° C on Nobles (N) medium (1.5% malt extract, 1.5% agar) in the Virginia Tech Culture Collection, and will eventually be distributed to other major culture collections. Methods used for single-spore culture isolation and mating studies were given previously (Vilgalys & Miller, 1981; Vilgalys & Miller, 1983). Clamp connections provided the criterion for compatibility in all crosses.

Nutritional studies.-Representative dikaryotic strains of each group (except group A, for which no dikaryon was available) were obtained from multispore isolations on Nobles Medium. Two isolates (RV 148 - group N-I, and RV 149 - group N-II) were initially tested for biotin and thiamine heterotrophy as described by Lindeberg (1946). The following version of Lindeberg's nutrient solution B (Lindeberg, 1944) was used: glucose 1 g, NH_4-tartrate 5 g, KH_2PO_4 1 g, $MgSO_4$-$7H_2O$ 0.5 g, $CaCl_2$ 50 mg, NaCl 2.5 mg, 2 ml

micronutrient solution ($FeNO_3$-$9H_2O$ 725 mg/l, $ZnSO_4$-$7H_2O$ 440 mg/l, $MnSO_4$-H_2O 160 mg/l), and distilled H_2O to 1 liter. Inoculum was prepared by growing isolates on nutrient solution B to which biotin (1 µg / liter) and thiamine-HCl (50 µg / liter) were added before sterilization, along with 1.5% agar. Triplicate flasks containing 25 ml of nutrient solution with and without biotin and/or thiamine (1 µm/l and 50µm/l, respectively) were inoculated with single 5 mm plugs taken from the periphery of inoculum plates and placed in the dark at room temperature. After sufficient growth had occurred in the controls, mycelial mats were harvested on filter paper, washed with water, and weighed (both wet and dry weight were measured). To determine the best time for harvesting the mycelium, additional flasks containing solution B (with both vitamins) were inoculated, and triplicate flasks harvested at periodic intervals to determine growth curves for both strains.

The remaining isolates were tested for their nutritional requirements by inoculating 4 mm plugs (taken from inocula prepared as before) onto solid media (1.5% agar in nutrient solution B with and without the two vitamins). Growth of each strain was scored relative to the growth of the control plate containing both thiamine and biotin.

Cultural studies.-The effect of media on colony morphology was studied by growing isolates of each group on the

following media: L+, solid nutrient solution B with biotin and thiamine, (same as that used for nutritional studies above); SCM, Schizophyllum complete medium (Stevens, 1981); SFM, Schizophyllum fruiting medium (Stevens, 1981); PDA, Potato dextrose agar (BBL); PYA, PDA above supplemented with 0.2% yeast extract; MYA, N above supplemented with 0.2% yeast extract; CZD, Czapek Dox agar (Difco); YpSs, Emerson's YpSs agar (Stevens, 1981); MMN, Modified Melin Norkrans (Molina and Palmer, 1982); PRUNE, Prune agar (Difco). All cultures were grown in the dark at 20° C for 4-5 weeks, and observed for differences in cultural morphology.

RESULTS

Mating studies.-The North American and European groups used in this study were previously determined to have a bifactorial mating incompatibility system with multiple alleles (Vilgalys & Miller, 1983; this volume, chapter 1). Intra-stock crosses using monosporous isolates from groups K, J, and A also showed a tetrapolar arrangement of mating types in every case, indicating that a bifactorial mating system operates in these groups as well.

The results of inter-stock crosses between stocks listed in Table 5 are shown in Table 6. Several pairs of European and North American biological species are intercompatible (N-I with E-III, N-II with E-II, and N-IV with E-I). In

addition, groups K and J are intercompatible with both N-I and E-III. Groups N-III, E-IV, and A were each intersterile with all other groups. The results shown in Table 6, which represent some 600 unique intercrosses, were repeatable in numerous smaller sets using additional isolates during the course of these studies. Illegitimate matings between intersterile groups, expressed as rare clamp connections formed at the junction line between intersterile monokaryons, were occasionally observed, but these matings did not form stable dikaryotic mycelia when transferred to media and were not repeatable.

In most cases where allopatric populations were compatible, matings occurred at a high frequency, conforming to a multiple allelic mating incompatibility system. Several crosses between intercompatible groups N-I, E-III, K and J formed abundant, proliferating pseudoclamps at their junction line. These crosses are indicated by an asterisk in Table 6. Normal clamp connections were rare or absent in these crosses, and subcultures from these matings usually produced sectored colonies lacking clamp connections. Normal dikaryotic colonies were recovered in at least one instance, but only after repeated subculturing from the junction line (cross between RV 148, group N-I and Sardinian stock M 83/525).

Nutritional studies.-Figure 8 shows the growth curves for isolates RV 148 and RV 149 grown in synthetic media. Both strains grew rather slowly, with maximal growth occuring after about 50-60 da. The growth curves for wet and dry weight were both similar (only the dry weight curve is shown). Increased growth also coincided with a decrease in pH of the nutrient solution (figure 8). The results of ANOVA on growth with and without the various vitamin treatments are given in Table 7. A limited amount of growth occurred in both isolates without either biotin or thiamine. Significantly more growth resulted with the addition of both biotin and thiamine to the growth solution. While thiamin by itself had no effect, the addition of biotin had an intermediate effect on growth.

Enhanced growth on solid media containing both biotin and thiamine was observed in all isolates tested (Table 8 and figure 9). The degree to which growth was enhanced in different isolates varied, since a certain amount of growth on the control medium was evident in almost every case. This is presumably due to carry-over of nutrients from the inoculum source, although limited heterotrophy in certain isolates is also possible. In either case, thiamine and biotin heterotrophy is evidently characteristic of all the groups studied.

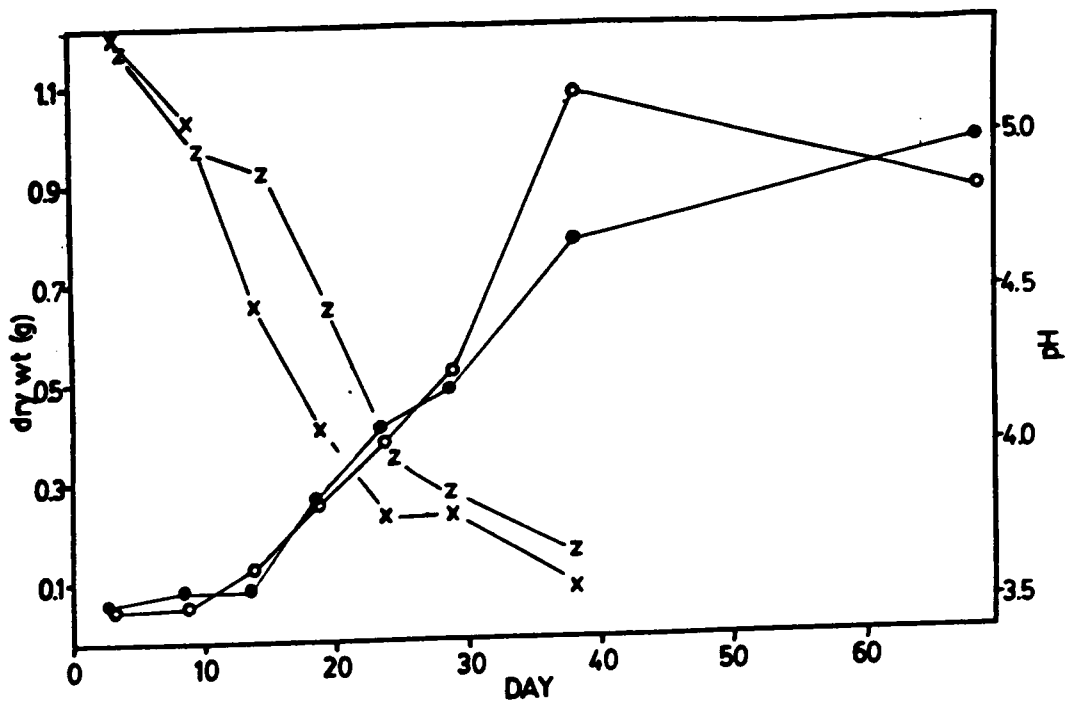

Figure 8. Growth curves for two Collybia isolates grown in liquid media(Dry wt: ● = RV 148, o = RV 149; pH: X = RV 148, Z = RV 149).

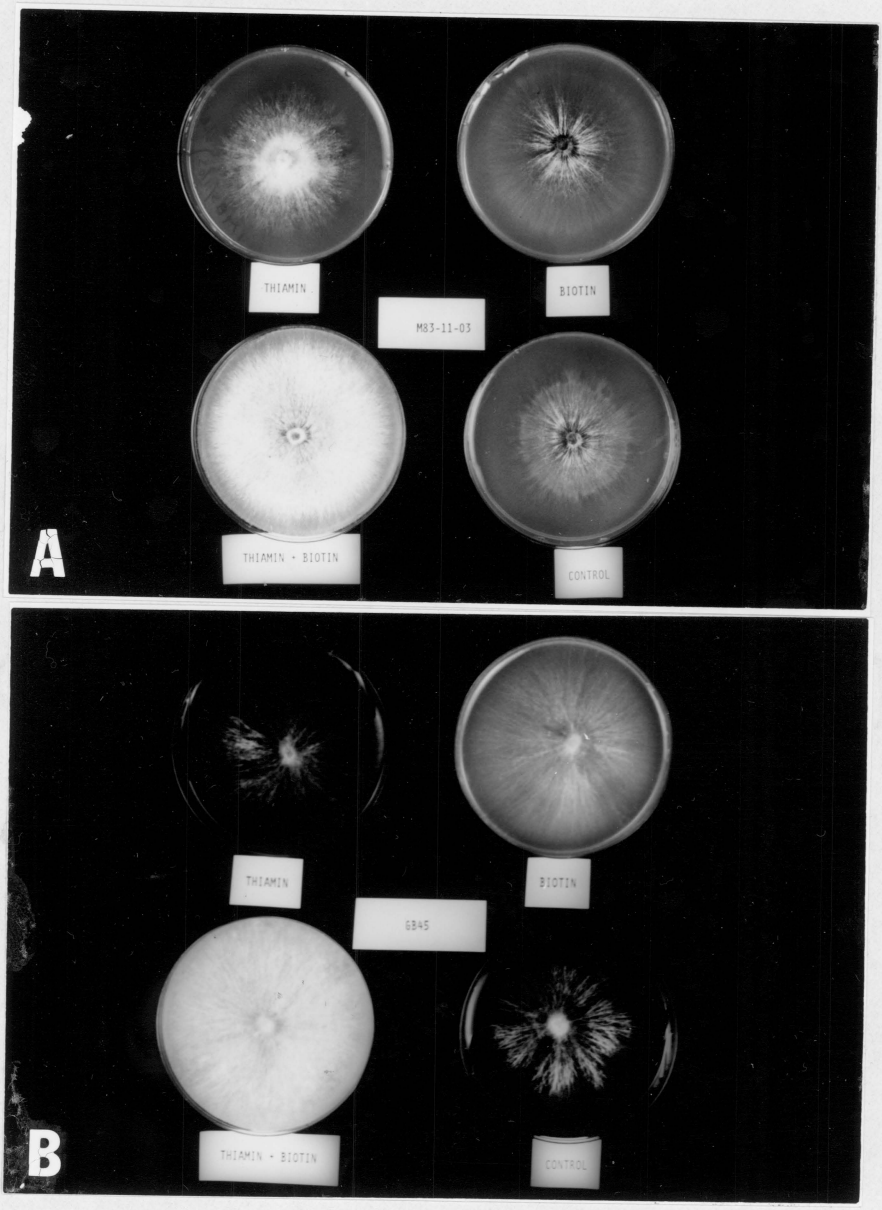

Figure 9. Effect of thiamine and biotin on growth in Collybia isolates on agar media. A) M 83-11-03 (Group E-III), B) GB 45 (Group N-IV).

Cultural studies.-All isolates of Collybia were able to grow on the media tested with the exception of CZD. On YPSS, a clear zone surrounding the cultures appeared, indicative of of extracellular amylase activity in all isolates.

Considerable heterogeneity in cultural morphology was evident when isolates were grown on different media. In some cases, phenotypic variation in a single isolate was striking (figure 10). The greatest difference in cultural morphology occurred when isolates were grown on media containing yeast extract (SFM, PYA, MYA). Addition of yeast extract resulted in a dense penicillioid growth pattern, frequently associated with the production of dark pigments in the medium along with pigment drops on the mat surface (figure 11). The degree of pigment production could not be correlated with any specific biological species of Collybia, although it does appear to occur primarily in isolates originating from collections possesing dark pilei.

Isolates representing intercompatible biological species from different continents often differed in their macroscopic cultural morphology (figure 11, intercompatible isolates from groups E-II and N-II). However, I did not study enough isolates to ascertain whether these differences were constant within a given species. In light of the apparent diversity observed when a single isolate was grown on various media, further study of additional cultural features was not undertaken.

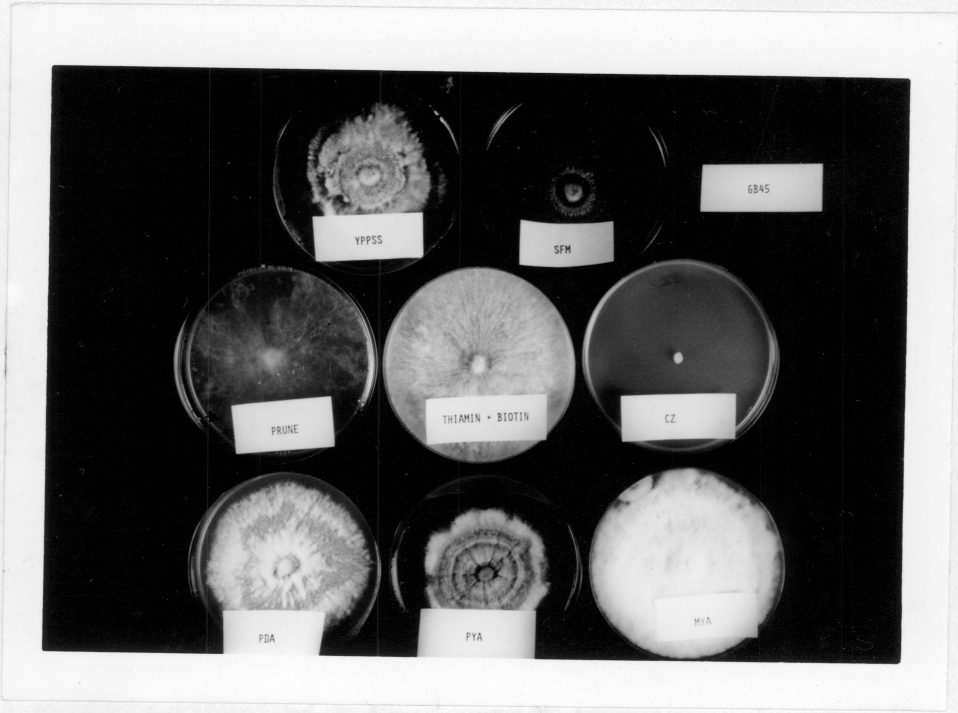

Figure 10. Variation within a single isolate (GB 45) of Collybia grown on different media.

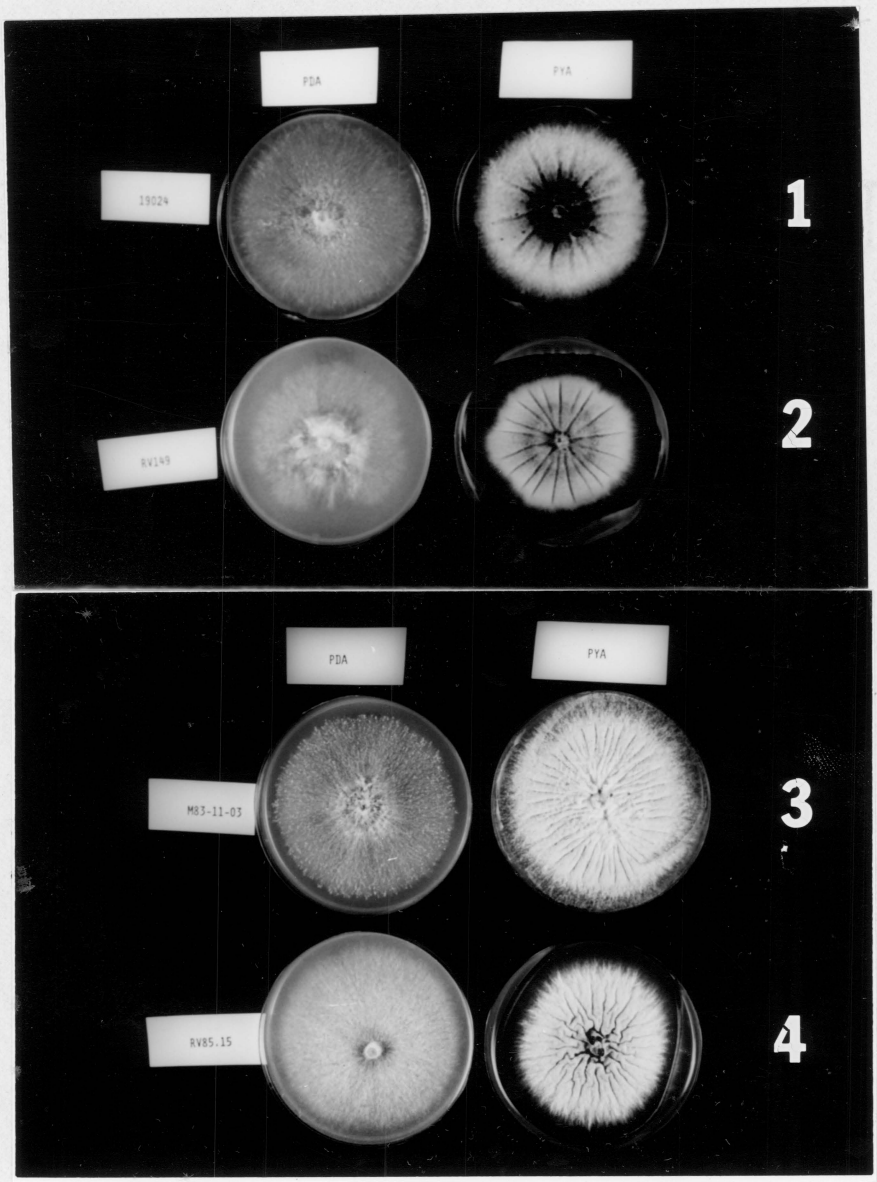

Figure 11. Effect of yeast extract on colony morphology and pigment production of Collybia isolates. Left side - without yeast extract added, right side - with yeast extract. (1) OKM19024, (2) RV 149, (3) M 83-11-03, (4) OKM 18761.

DISCUSSION

Results of this and previous studies suggest that all of the members of the C. dryophila group share a bifactorial mating system with multiple alleles. The results of intercompatibility tests among a worldwide sample of isolates (Table 6) also demonstrate that several allopatric populations of the C. dryophila group are closely related, if not conspecific. Three pairs of intercompatible biological species exist between North America and Europe. In addition, two groups from Europe and North America (E-III and N-I) are also compatible with isolates from Japan and Korea. Three biological species studied are intersterile with all others tested (N-III, E-IV, and A).

In most cases where allopatric populations were intercompatible, matings occurred at a high frequency conforming to a multiple allelic mating system, and were otherwise similar phenotypically to intra-population crosses among sympatric isolates. In some crosses between groups N-I, E-III, J and K, an apparent reduction in intercompatibility was evident, manifested by the production of anomalous pseudo-clamped hyphae. I have also observed similar types of reactions in crosses with certain isolates of group E-III (chapter 1, this volume). Pseudoclamp production in other fungi is usually associated with common B-factor matings (Raper, 1966), and with certain strains

carrying modified B-factors in Schizophyllum (Raper & Raper, 1964). Pseudoclamps indicative of common-factor matings have not been observed in any of my intra-stock-crosses with the C. dryophila group. Pseudoclamp formation in this instance appeared to be associated with isolates originating from particular stocks, and occurred even when mating types were known to be different, suggesting another reason for their production. The presence of modifier mutations affecting expression of the B-factor (Raper & Raper, 1964) could explain why certain monokaryotic strains might produce pseudoclamps. This explanation is unlikely, however, since such B-modifier mutations have only been obtained by mutagenesis using laboratory strains, and have never been demonstrated in natural populations. The significance of this reaction is unclear, although it may represent a secondary incompatibility reaction (such as heterogenic incompatibility) superimposed on the regular homogenic mating system (this volume, chapter 2).

The results of nutritional studies indicate that the C. dryophila group is generally heterotrophic for both thiamine and biotin. This extends Lindeberg's (1946) previous observation on this group from Sweden. Although these features cannot serve to distinguish members of the C. dryophila group, a requirement for biotin may be useful for distinguishing the group from other species of Collybia, which do not require this vitamin (Lindeberg, 1946).

The level of phenotypic plasticity exhibited by isolates of the C. dryophila group on various media (figure 10) was unexpected. On Noble's medium, cultures of the C. dryophila group are quite similar, differing mainly by the amount of aerial mycelium produced (Vilgalys and Miller, 1983). Based on my observations, utility of cultural characters for delimiting taxa in this group is not likely without further studies on the range of intra-group variation.

An important question arising from this study concerns the taxonomic identity of the various mating groups in the C. dryophila group. Speciation is a complex evolutionary event that is not well understood in fungi. A number of species concepts are used by mycologists (Clemencon, 1977), which are based upon morphology, mating compatibility, or a combination of both. A species concept based solely on a single feature of speciation is probably overly simplistic, and may not reflect what are really diverse systematic relationships.

Based upon my previous studies (Vilgalys, 1982; Vilgalys & Miller, 1983; this volume, chapter 2), biological species within either Europe or North America were referable to distinct taxonomic species on the basis of one to several morphological features. Despite intra-group variation, small yet consistent morphological differences do exist among the sympatric biological species from a single continent. This particularly holds true for collections made within a restricted geographic locality.

Within a geographic region, then, morphological observations can support a species concept based upon mating compatibility. Stated in other words, sympatric populations form good taxonomic and biological species. Do geographically isolated (allopatric) populations also make good species?

Intercompatibility relationships in the C. dryophila group are summarized in figure 12. A total of six major groups can be distiniguished on the basis of sterility with one another. At first glance, it might seem that intercompatible mating groups from different continents are a single species. Morphological studies do not always support this view, however.

The possibility that intercompatible allopatric populations in the C. dryophila group are divergent became evident after several European mycologists tried to use my keys developed for North American biological species (Vilgalys and Miller, 1983). Diagnostic features used to identify North American taxa are either absent (e.g., tawny strigose hairs at the stipe base typical of C. earleae (Murr.)Murr.) or else widely distributed among the European species (e.g., pinkish rhizomorphs characteristic of C. subsulphurea Peck). Another example of incongruity between morphology and mating compatibility in allopatric populations involves lamellar color. Yellow lamellae are diagnostic for North American group N-II (C. subsulphurea), and also for

European group E-I (C. ocior), yet these groups are intersterile.

Intercompatible taxa from North America and Europe show varying degrees of morphological dissimilarity. In the case of groups N-II and E-II, morphological divergence is particularly striking. Collybia subsulphurea (N-II) is common early in the collecting season throughout North America, and is characterized by its strong yellow colors throughout the sporocarp and by its pink rhizomorphs (Vilgalys and Miller, 1983). It is intercompatible with C. alpina (E-II), which is distinguished by an extremely dark pileus, restricted distribution limited to higher European latitudes and altitudes, and larger spore size than any other species (this volume, chapter 2).

Other pairs of intercompatible taxa show lesser degrees of morphological divergence. Whether morphological differences are indicative of genetic differences in this case is not certain. A genetic basis for divergence between two allopatric strains of Pleurotus ostreatus (from Europe and Japan) has been demonstrated by Prillinger and Mollitoris (1979), who correlated morphological differences with different laccase isozymes. Although their study was only based upon two strains, it demonstrates that fixation for different alleles is a possibility for populations of fungi existing in allopatry. I have also observed differences between groups N-II and E-II at two different biochemical

loci (unpublished data), suggesting that allopatric groups are genetically divergent. Certainly additional quantitive studies of genetic distance are necessary before more is known about the extent of divergence between these populations of fungi.

In light of these uncertainties, it would be unwise to make any final judgements about the taxonomic status of these groups at this time. The current scheme (figure 12), whereby sympatric biological species are given taxonomic recognition within either Europe or North America, is not taxonomically inconsistent, and is therefore retained for the present time. Any decision about the taxonomic status of the Asian and Australian isolates must, however, await additional study of population material from these areas.

Whether or not differences in sporocarp morphology have a genetic basis, the striking differences reported here raise a question about the validity of morphology as a feature reflecting systematic relations. Likewise, a species concept based strictly on mating compatibility may also be overly simplistic, since it ignores the possibility of independent evolution of genetically isolated populations. Instead, the reality of species themselves might be a more appropriate issue in such cases.

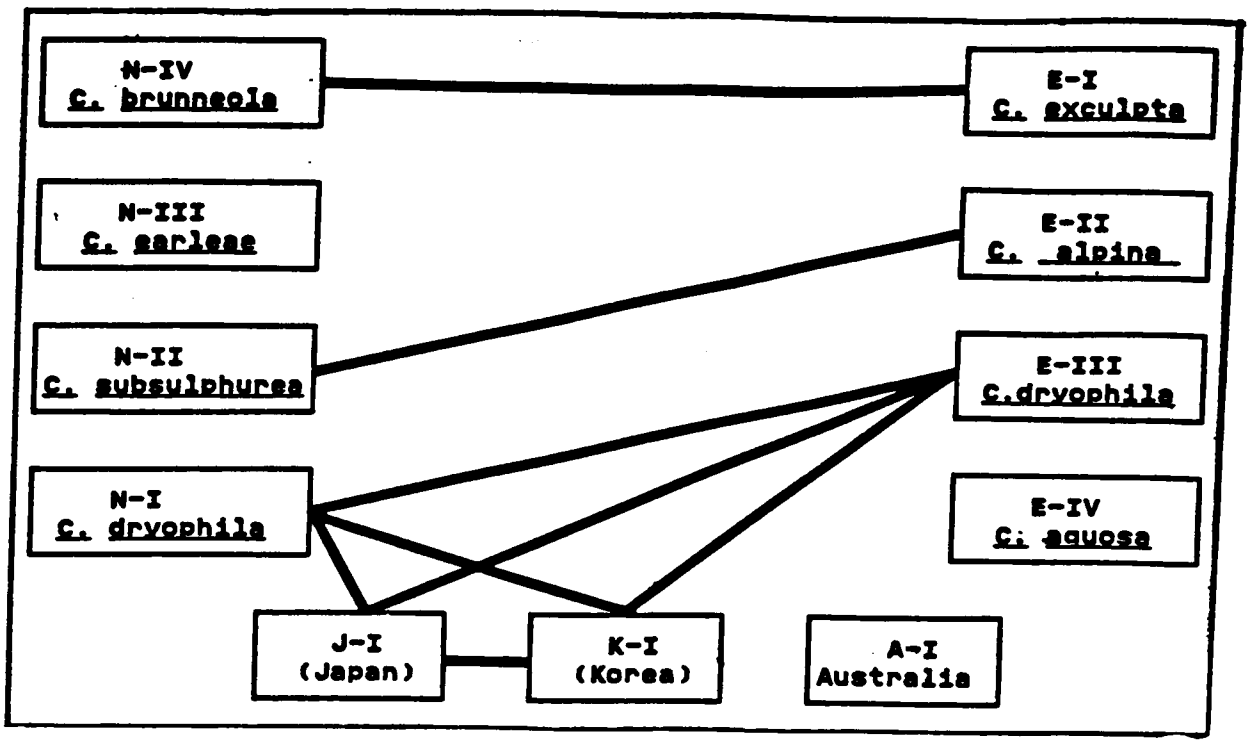

Figure 12. Summary of intercompatibility relationships in the Collybia dryophila group.Solid lines signify mating compatibility.

Table 7. Effect of thiamine & biotin on growth of Collybia isolates grown in liquid culture.

species (strain)	treatment[1]	yield[2] wet wt	yield[2] dry wt
C. dryophila (RV 148)			
	thiamine and biotin	2.97 g	96.4 mg
	biotin only	0.28	24.7
	thiamine only	0.13	8.5
	control	0.15	9.5
C. subsulphurea (RV 149)			
	thiamine + biotin	3.24 g	88.7 mg
	biotin only	0.48	65.0
	thiamine only	0.42	41.4
	control	0.22	41.5

[1]50 µg thiamine hydrochloride and 1 µg biotin per liter.

[2]Yields are mean values; means connected by the same solid line are not significantly different (Duncan's multiple range test, α=.01)

Table 8. Effect of thiamine and biotin on growth of Collybia species.

			Growth			
Group	Strain	Origin	min[1]	min+t[2]	min+b[3]	min+t+b[4]
N-I	RV 148	USA	+[5]	+	+	++
N-II	RV 149	USA	+	+	+	++
N-III	OKM 18671	USA	+	+	+	++
N-IV	GB 45	USA	+p	+p	+	++
E-I	B 81/2	Europe	+	+p	++	++
E-II	OKM 19024	Europe	+	+	++	++
E-III	W 14495	Europe	+p	+p	++	++
E-III	M 83/11/03	Europe	+	+	++	++
E-IV	DL 81/301	Europe	+	+	+	++
K	OKM 20263	Korea	+	+	++	++
J	J 8	Japan	-	+	++	++

[1]minimal media without vitamins, [2]minimal media plus thiamine, [3]minimal media plus biotin, [4]minimal media plus thiamine and biotin.

[5]- = no growth, + = limited growth, ++ = maximum growth, p = pigment production.

CHAPTER 4. EVOLUTIONARY RELATIONSHIPS OF MUSHROOMS IN THE COLLYBIA DRYOPHILA GROUP BASED ON DNA HYBRIDIZATION.

Almost nothing is known about rates and modes of evolution in fungi. As pointed out in a recent review (Burnett, 1983), mycology has contributed little to the ideas which culminated in the neodarwinian synthesis of the 1940s and 1950s. Despite this shortcoming, many fungi do lend themselves well for evolutionary studies, particularly at the species level. A great deal is already known about the genetics of mating systems in fungi (Raper, 1966). Knowledge about fungal mating systems has permitted the study of interbreeding and intersterile populations. Mating studies have been shown to be exceptionally useful for determining relationships in many problematic groups of Basidiomycetes, which are noted for their phenotypic plasiticity (Boidin, 1980). Substantiated reports of interspecific hybridization in basidiomycetes are rare, and are almost entirely based upon matings among allopatric populations. Sympatric populations of higher fungi have always been found to belong to discrete biological species (Anderson, et al., 1980; Chapter 3, this volume). The genetic basis for such rigorous pre-zygotic isolation is not known. In addition, the role of intersterility barriers in the evolution of fungal species is not presently understood.

I have undertaken a study of several closely related fungi belonging to the Collybia dryophila (Bull. : Fr.)Kumm. group (Basidiomycetes: Tricholomataceae). Collybia dryophila is traditionally viewed as a single polymorphic species, but I have shown (Vilgalys and Miller, 1983; Chapters 1 & 3) that the group contains a number of intersterile groups defineable on the basis of mating compatibility tests. Worldwide intercompatibility relationships in the C. dryophila group are summarized in figure 12. Several populations from different parts of the world are intercompatible, suggesting conspecificity. Morphological studies (Chapters 2 and 3), however, indicate that striking morphological differences can exist between intercompatible populations which are allopatric. As a result, I suspect that geographic isolation might play a significant role in the group's evolution.

To investigate phylogenetic relationships of the C. dryophila group, I have compared isolates representing allopatric populations from different biological species by DNA hybridization. DNA hybridization studies have been applied to all major groups of fungi, including ascomycetous yeasts (Bicknell and Douglas, 1970; Kurtzman, et al., 1983; Price, et al., 1978), Ascomycetes (Dutta, 1976; Dutta, et al., 1976), several orders of zoosporic fungi (Ojha, et al., 1975), and Basidiomycetes (Horgen, et al., 1984).

The relationship between interfertility and genic identity in fungi has not been extensively studied. In ascomycetous

yeasts, interfertile strains belonging to the same species usually show 80-100% base sequence complementarity (as revealed by DNA hybridization), while intersterile strains mostly show less than 25% hybridization (Price, et al., 1978). Kurtzman, et al. (1980), however, reported partial interfertility (6% viable F_1 ascospores) among different strains of the yeast genus Issatchenkia Kudriavzev which had only 25% base sequence complementarity. Such cases of intermediate DNA homology values are rare for yeasts, however, and their significance is not known. Three heterothallic species of Neurospora studied by Dutta (1976) show greater than 80% DNA hybridization, yet two of these species are intersterile (both species do cross with the third). Horgen, et al. (1983) studied DNA homology among several species of mushrooms. They reported 56% hybridization between two intersterile species of Agaricus, while DNAs from two presumably interfertile strains showed greater than 96% hybridization. Besides these reports, which involve only a few species, few studies have attempted to correlate breeding data and genic identity in fungi.

MATERIALS AND METHODS

OVERVIEW

The conditions controlling DNA hybridization have been extensively reviewed (Britten, et al., 1974; Wetmur and Davidson, 1968). Of the various measures used to determine genetic relatedness, both normalized percent hybridization (NPH, the ratio of heterologous:homologous hybridization corrected for self-renaturation of the labelled tracer DNA) and decrease in melting temperature of heterologous hybrid DNA (delta-T) are most commonly used. A number of various delta-T statistics are used (based on mean, median or modal delta values) which differ in their resolution of genetic similarity as evolutionary distance increases (Sibley and Ahlquist, 1983). NPH values have a larger range and are harder to reproduce than delta T values, but directly reflect genetic relatedness under constant conditions of hybridization (Sibley and Ahlquist, 1983), and were therefore chosen for this study.

ORGANISMS STUDIED

Table 9 lists the isolates used in this study and the mating groups they represent. Dikaryotic isolates were obtained from multispore platings on 1.5% malt agar as

described in Chapter 3. Starter cultures of each isolate were inoculated into 50 ml 1.5% malt extract broth and allowed to grow for 3 to 5 weeks. Starter cultures were then used to inoculate 2 liter Erlenmeyer flasks containing 700-800 ml malt extract broth and grown on an orbital shaker in the dark at room temperature for 2 weeks. For each isolate, 7-10 2 liter flasks were necessary to produce enough mycelium (30-100 g wet wt) for DNA isolation. Mycelium was harvested by suction through Miracloth (Calbiochem), washed with saline-TE (0.15 M NaCl, 0.05 M Tris, 0.05 M EDTA, pH 8.0). If necessary, mycelium was frozen at -20° C and lyophilized until use.

DNA ISOLATION AND HYBRIDIZATION

DNA was isolated by one of two methods. For the majority of the study, a modification of the hydroxylapatite (HA) method described by Johnson (1981) was used. Fresh mycelium (30 g) was suspended in saline-EDTA and 200 µl diethylpyrocarbonate added to inhibit nuclease activity. The suspension was blended for 1 minute at high speed using a Waring blender, and then passed through a French Press at 16,000 psi. After phenol extraction, the lysate was precipitated with two volumes ice-cold ethanol, resuspended in 30 ml 0.1 SSC (1X SSC = 0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0), and incubated with RNAse A (50 µg/ml) for

2 hr at 37 C. The lysate was then adjusted to 0.1 M phosphate and collected on HA as described (Johnson, 1981). In the second method, high molecular DNA was isolated according to Specht, et al. (1982), with the following modifications: (1) 50 µg/ml Pronase was added to the ground mycelial suspension, and the DNA was extracted for only 24 hours, (2) after RNAse treatment and chloroform extraction, DNA was spooled onto glass rods, resuspended in TE (10 mM Tris, 1 mM EDTA, pH 8.0) and further purified by one or two cycles of isopycnic centrifugation using CsCl. Although both these methods yield DNA suitable for reasociation studies, more consistent purity was obtained by the HA method (purity higher than 70% by the method of Johnson, 1980). Yield varied with the isolate used, with an upper range of 30-40 µg DNA/g fresh wt mycelium. With some isolates of Collybia, we frequently encounter difficulty with contaminating polysaccharide and pigments which tenaciously bind the DNA. DNAs from these isolates were used only if they demonstrated the ability to reassociate. All DNAs were stored in 0.1X SSC at -20° C until used.

All DNAs were sheared before hybridization by dropwise passage through a French Press at 16,000 psi to a modal fragment size of 500 nucleotides, as determined by alkaline agarose electrophoresis.

The thermal melting point (Tm) of each DNA in 0.5X SSC was measured spectrophotometrically and used to determine

guanine-plus-cytosine (G+C) content as described by Johnson (1981), with Escherichia coli b DNA (50% G + C) as standard. The species of Collybia used in this study range in G+C content from 44-47% (Table 9).

Reasociation kinetics of Collybia DNA were determined spectrophotometrically. Approximately 50 µg/ml of fungal or E. coli (standard) DNA in 6X SSC with 20% dimethylsulfoxide (DMSO) was placed in stoppered quartz microcuvettes (final A_{260}=1.5), and heated at 1 degree C/min to 10° C above Tm while recording the absorbance at A_{260}. After rapid cooling to Tm - 25° C (usually about 2 min), the decrease in absorbance due to renaturing DNA was recorded until fungal DNAs had reassociated halfway (usually within 5 to 9 hrs). The reassociation data for each DNA was corrected for collapse chromicity and standardized for C_0t (C&sub0t is reported as the product of initial concentration of moles nucleotides per liter and time in seconds), and is presented as a modified Wetmur-Davidson plot (Wetmur and Davidson, 1968) in figure 13.

DNA was chemically labeled in vitro with ^{125}I by the methods of Selin, et al. (1983) to an average specific activity of 3 x 10^6 cpm/µg. DNA hybridization was performed using the S1-nuclease procedure described by Johnson (1981),. Hybridizations were carried out in 1.17 M NaCl-.010 M HEPES, pH 7.0 at 65° C for 24 hours. The higher salt concentrations used in our experiments were found to accelerate the rate of

reasociation by a factor of about 9-10 relative to 0.12 M phosphate buffer commonly used by others (Britten, et al., 1974), permitting the use of shorter incubation periods with less DNA. For comparison with other data, C_0t values attained in our experiments were multiplied by 10 to give equivalent (EC_0t) values. The ratios of unlabelled (driver) DNA to labelled (tracer) DNA in the experiments were were in excess of 1000, and the incubations were carried out to a minimum EC_0t of 1000 (except where noted), which provided for essentially complete reanealling of homologous DNAs used in the experiments. S1 Nuclease-resistant duplexes were precipitated and harvested according to Johnson (1981), except that 0.5 ml of 1N HCl containing 1% dibasic sodium phosphate and 1% sodium pyrophosphate were used to precipitate the DNA. The DNA was then harvested on Whatman GF/F glass fiber filters, and washed with a 1:4 dilution of the acid solution before drying. Radioactivity was counted using a Beckman model 5500 gamma counter. Normalized percent hybridization (NPH) was determined as the ratio of heterologous:homologous binding (corrected for self-renaturation of the homologous tracer DNA).

Table 9. List of isolates used for DNA hybridization studies.

Species	Strain[1]	Collection[2]	Mating group[3]	Base composition[4]
C. dryophila	VT 1158	RV 148	N-I	45(0.6)
C. dryophila	VT 1379	RV 83-180	N-I	
C. dryophila		W 14495	E-III	44(0.7)
C. dryophila	VT 1237	HC 82-15	E-III	
C. dryophila		MM 83-11-03	E-III	
C. dryophila		OKM 20263	K	
C. dryophila	VT 1219	OKM 19790	J	
C. subsulphurea	VT 1152	RV 149	N-II	45(0.4)
C. earleae	VT 1147	OKM 18761	N-III	46(0.1)
C. brunneola	VT 1159	GB 45	N-IV	45(0.3)
C. funicularis		EXC	E-I	45(0.4)
C. funicularis	VT 1163	B 81-2	E-I	
C. alpina	VT 1154	MM 1b-289	E-II	47(0.2)
C. alpina	VT 1155	OKM 19024	E-II	47(0.9)
C. alpina		RW 14513	E-II	
C. aquosa	VT 1162	DL 81-301	E-IV	46(0.3)
C. aquosa	VT 1241	RW 14501	E-IV	

[1]isolate numbers from Virginia Tech culture collection. [2]collection numbers for voucher specimens deposited at VPI. [3]designated mating groups. [4]base composition reported as percent guanine plus cytosine (average of three determinations rounded to nearest integer; standard error given in parentheses).

Table 10. Estimates of repetitive DNA content and genome size (of single copy portion) for Collybia species based on reassociation kinetics.

group	species (isolate)	% repetitive DNA	genome size (base pairs)
N-II	C. subsulphurea (RV 149)	16-22 %	$4.3\text{-}5.2(\bar{x}=4.8) \times 10^7$
N-I	C. dryophila (RV 83-180)	18	7.5×10^7
N-I	C. dryophila (RV 148)	7	4.0×10^7

Range of experimental values and mean are shown for RV 149 (from three determinations). Other values are based on a single determination.

Table 11. Effect of C_0t value on NPH values.

Tracer	Driver	EC_0t	NPH[1]	Δ NPH[2]
14501	14501	700	100(4.3)	9.9
		300	90.1(1.5)	
14501	L81/301	700	88.8(0.4)	8.8
		300	81.0(0.8)	
GB 45	19024	700	65.9(1.1)	7.6
		300	58.3(1.2)	
GB 45	C82/15	700	50.9(0.2)	7.3
		300	43.6(0.8)	
RV 149	19024	700	80.5(3.1)	8.1
		300	72.4(1.7)	

[1]normalized percent hybridization with respect to homologous DNA standard incubated at high EC_0t (standard error given in parentheses). [2]decrease in low EC_0t NPH relative to high EC_0t NPH value.

Table 12. DNA hybridization among species in the C. dryophila group

Driver DNAs[1]:		Reference (Tracer) DNAs:							
Group	Strain	RV 149 N-II	19024 E-II	GB 45 N-IV	HC82/15 E-III	RW14501 E-IV	RV148 N-I	18761 N-III	BEXC E-I
N-II	RV 149	100	80	61[2]	49	68	53	50	69
E-II	19024	81	100	66	50	78	57	44	63
N-IV	GB 45	61[2]	63	100	53	62	64	50	107
E-III	HC82/15	47	46	51	100	46	ND[3]	ND	ND
E-IV	W14501	73	76	73	54	100	41	37	55
N-I	RV 148	56	50	ND	83	64	100	38	48[1]
N-III	OKM18761	ND	42	42[2]	41	39	74	100	52
E-I	BEXC	ND	ND	ND	ND	ND	36	39	100
E-I	B81/2	53[2]	57	88	46	ND	35[2]	34	75
E-III	W14495	62	58	67	97	56	72	38	63
E-IV	L81/301	73	77	76	55	89	43	40	71

[1]reference DNA's incubated with at least 1000-fold excess driver DNA's in 1.17 M NaCl-1 mM HEPES (pH 7.0) to a minimum ECot of 1000.

[2]values based upon a single determination. All other values are based upon avarages of two determinations.

[3]ND = not determined

Table 13. Matrix of average NPH values among different *Collybia* species.

	N-I	N-II	N-III	N-IV	E-I	E-II	E-III
E-IV	0.490	0.715	0.388	0.703	0.633	0.765	0.526
E-III	0.743	0.527	0.372	0.567	0.538	0.516	
E-II	0.513	0.800	0.410	0.647	0.630		
E-I	0.416	0.613	0.416	0.976			
N-IV	0.638	0.611	0.461				
N-III	0.495	0.503					
N-II	0.545						

Table 14. DNA hybridization between intercompatible populations from different continents.

Driver DNAs[1]:			Reference DNAs:	
Group	Strain	Origin	HC82/15 (Europe)	RV148 (USA)
N-I	RV148	Virginia,USA	83	100
N-I	RV83-180	Virginia,USA	ND[2]	93
E-III	W14495	Scotland,UK	97	72
E-III	M83-11-03	Sardinia	ND	68
K	OKM20263	Korea	ND	67
J	J-8	Japan	ND	78

[1]reference DNA's incubated with at least 1000-fold excess driver DNA's in 1.17 M NaCl-1 mM HEPES (pH 7.0) to a minimum ECot of 1000.

[2]ND = not determined

RESULTS AND DISCUSSION

CHARACTERIZATION OF FUNGAL DNA

The reassociation plot for whole cell *Collybia* DNA is shown in figure 13. The straight line shown for *E. coli* is typical of unique DNA with second-order kinetics (Wetmur-Davidson, 1968). The inverse of the intersect for the linear portion of each fungal DNA reasociation curve (corresponding to single copy DNA) indicates approximately 80% unique sequences (20% repetitive DNA).

The genomes of all eukaryotes contain varying amounts of repetitive sequences which reassociate faster than single copy sequences (Britten and Kohne, 1968). Because repeated sequences may often obscure the measurement of genetic relatedness based upon DNA hybridization, most DNA hybridization studies with eukaryotes use labelled DNA preparations in which the repetitive portion has been partially removed by incubation to low C_0t values (details given by Britten, et al., 1974). Compared with other eukaryotes, the genome of *Collybia* and all other fungi that have been studied contain relatively small amounts of repetitive DNA, usually less than 20% (Dutta, 1974). This amount is not large when one considers that even fractionated 'unique' mammalian DNA (incubated to high C_0t values and passed through hydryoxyapatite) may often still contain

considerable amounts (>20%) of moderately repetitive DNA (Ivanov and Markov, 1978; Deininger and Schmid, 1979).

The kinetic complexities of the single-copy portions of representative Collybia DNAs were determined from the slope of their reasociation plots relative to that of E. coli, summarized in Table 10. The single copy genome size of species in the C. dryophila group ranges from 4-7 X 10^7 nucleotides, which is similar to values reported for other Basidiomycetes (Ullrich & Raper, 1977).

Because DNA preparations from the species used in this study contain relatively little repetitive DNA, we did not separate DNAs into unique and repetitive fractions before labeling them with ^{125}I. Since the presence of repeated DNA in our samples could possibly obscure the measure of genetic relatedness, a series of low C_0t incubations were included in the study to determine what effect repetitive DNA had on heterologous hybridization. The results of reduced C_0t values on NPH in homologous and heterologous hybrids are shown in Table 11. A similar depression in NPH (of about 10 %) was observed regardless of whether hybrids were homologous or heterologous. The NPH values for low C_0t incubations in Table 11 still incorporated a large amount of hybridization by single-copy DNA, so an additional series of experiments were performed using two heterologous DNA tracers. Two heterologous tracers were hybridized to the same DNAs to a high EC_0t of 1300 (complete renaturation) and a low EC_0t of

10 (only repetitive DNA renatured). NPH values for both low and high C_0t incubations are plotted by tracer in figure 14. Plots for both tracers had identical regression lines (r=0.77) and nearly identical slopes (m=0.51+0.53). In spite of heterogeneous error variances, these lines are not significantly different. Although repetitive DNA apparently evolves about twice as fast as single copy DNA (based on the slope of the data in figure 13), correlation of NPH with genetic distance is not affected at high C_0t NPH values above 25%. The effect of repetitive DNA on the measurement of genetic relatedness might be greater when DNAs from more distantly related organisms (e.g., other species of Collybia) are compared. We have data (unpublished) based on DNA hybridization with additional outgroup species which indicates that at lower NPH values (below 20%), the linearity shown in figure 13 apparently disappears as the repetitive DNA assumes a higher proportion of the total hybridization detected. For the NPH values observed in this study, however, the presence of repetitive DNA in our labelled DNAs does not appear to affect the relative degree of genetic similarity observed among species. Similar congruity between hybridization values for repetitive and whole DNA fractions have also been reported for Drosophila species by Entingh (1970).

INTERSPECIFIC HYBRIDIZATION AND PHYLOGENETIC RELATIONSHIPS

Table 12 shows the matrix of NPH measurements obtained with DNAs from each biological species from Europe and North America. The average standard error for replicate measurements was about 6%. In general, we obtained very good agreement between reciprocal NPH values among the reference DNAs. Several non-reciprocal NPH values were observed, however (e.g., between OKM 18761 and RV 148). It is possible that these non-reciprocal values are due to experimental error, but they may also represent differences in in genomic DNA content of these species (Brownell, 1983; Belford and Thompson, 1981a).

NPH values in Table 12 show a complete range of values from 34% to 107%. Within a single biological species, NPH values ranged down to 75 percent, but were usually above 90 percent.

Although hybridization values usually have rather large experimental errors associated with them, they still directly reflect genetic relatedness and can therefore be used to reconstruct phylogenetic relationships. Sibley and Ahlquist (1983) stated that phylogenetic trees reconstructed from NPH values have identical branching patterns to those using delta-T values. Average interspecific NPH values are shown in Table 13. A phylogram based on the average NPH values in Table 13 was constructed using the NT-SYS package of Rohlf,

et al. (1979) by the unweighted pair group method using arithmetic averages (UPGMA), and is presented in figure 15. The correlation between the patristic distances shown in the tree and the input data (Table 13) is 0.95, and suggests there is little distortion of the actual phylogenetic relationships due to experimental errors or other factors (e.g., non-reciprocity, heterogeneous rates of divergence within different lineages).

The relationship of mating compatibility to genetic similarity is indicated by a horizontal bar above intercompatible species in figure 15. Mating compatibility generally corresponds with high levels of DNA hybridization, supporting the view that mating compatibility is indicative of a genetic similarity. However, there does not appear to be a single NPH value below which these fungi are found to be intersterile. For example, NPH values between the intercompatible groups E-III and N-I range down to 72%. Similar NPH values exist between group E-IV and two intercompatible groups, E-II and N-II, with which it is intersterile. In fact, no clear pattern of phylogenetic relationship is evident for groups E-IV, E-II, and N-II, since their relative branch lengths fall within the range of experimental error noted for our data. Phylogenetically, these three groups are more realistically viewed as a trichotomy.

Incongruity between genic identity and mating compatibility has also been shown in other fungi. In the yeast genus Issatchenkia, Kurtzman, et al. (1980) have observed partial fertility of ascospores from crosses between strains having as little as 25% nucleotide complementarity. Taken together with my results above, these observations suggest that the development of intersterility barriers during speciation could possibly occur independently of genetic divergence.

The data from this study demonstrate that intercompatible populations existing allopatrically are more divergent than sympatric populations of a single biological species. In two of the three cases where European and American biological species are intercompatible (N-I with E-III, and N-II with E-II), lower NPH values were obtained in allopatric comparisons. Table 14 gives additional NPH data for several isolates which are compatible with groups N-I and E-III, showing lower NPH values for allopatric DNA hybrids than for sympatric ones (>90%). These observations suggest that species in the C. dryophila group may have arisen primarily via an allopatric mode of evolution.

The case of groups N-IV and E-I represents the single exception to the trend for higher divergence among allopatric populations. I failed to demonstrate reciprocity for NPH values between DNA hybrids formed between these two groups: the North American isolate of group N-IV showed 107% NPH with tracer DNA from group E-I, while the reciprocal comparison

was only 88%. Since only a limited number of comparisons were made for these groups, the high value could be attributable to experimental error. An alternative explanation might be that species N-IV and E-I differ in genome size through polyploidy or aneuploidy. Future studies are necessary before any conclusion can be reached in this special case.

A final remark concerns the levels of divergence observed by this study, which are considerably higher than that observed in other groups of eukaryotes. Since DNA hybridization is highly dependent upon reaction conditions used for reassociation (Britten, et al., 1974), data from different laboratories should only be compared with caution. The NPH values reported from this study ranged down to 37%. Similar NPH values in other eukaryotes correspond to different subfamilies of rodents (Brownell, 1983), orders of birds (Sibley and Ahlquist, 1983), and superfamilies of primates (Benveniste and Todaro, 1974). and different sections of a single genus in higher plants (Belford and Thompson, 1981a). This level of divergence is startling for a group conventionally viewed as a single species! Using other outgroup species of Collybia, I have obtained undetectable levels of homology within this single genus (unpublished results). Similar low hybridization values have also been reported for other of Basidiomycetes (Horgen, et al., 1983; Kuninaga and Yokosawa, 1982), and for fungi in

general (Kurtzman, 1984). Two explanations could account for such a high level of genomic divergence: (1) Rates of molecular evolution may be significantly higher in fungi than in other eukaryotes. This would contrast with an apparently slow rate of organismal evolution reflected by the high phenotypic similarity exhibited by different mushroom species. (2) These groups represent ancient lineages. The scanty fossil data for Basidiomycetes places them back to the Pennsylvanian, 300 million years before present (Dennis, 1969). If Sibley and Ahlquist's (1984, 1983) calibrations are correct and applicable to other organisms, then the age of the C. dryophila group is on the order of about one hundred million years! It is tempting to speculate that in the fungi, which have such a small genome (<0.05 pg), only a minute proportion is responsible for the genetic information necessary to produce such phenotypically similar organisms.

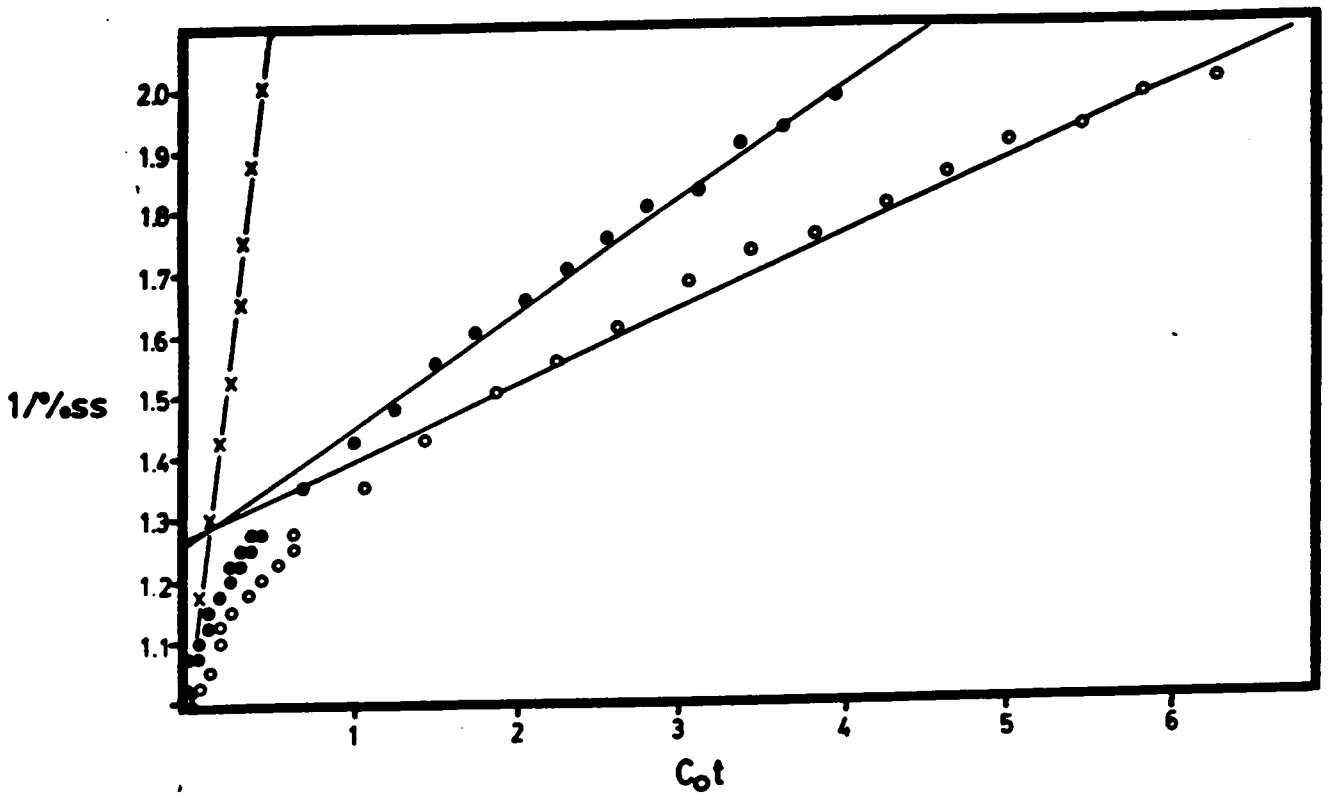

Figure 13. Reassociation plots for selected Collybia DNAs. (X)=DNA of E. Coli, (•)=whole cell DNA of RV 149 (group N-II), (o)=whole cell DNA of VT 1216 (Collybia subnuda, not included in the present study).

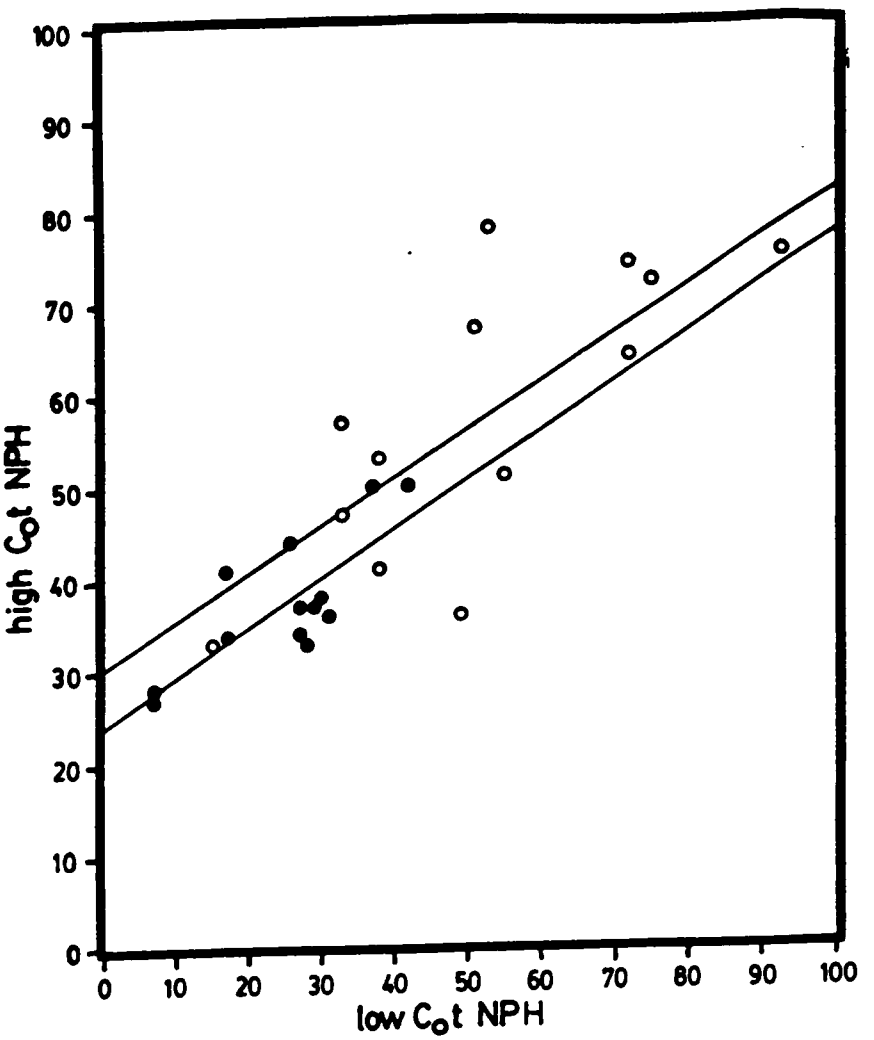

Figure 14. Effect of C_0t on hybridization values. Labelled DNAs: (o)=RV 148 (group N-I), (•)=OKM 18761 (group N-III). Regression lines for each labelled DNA do not differ.

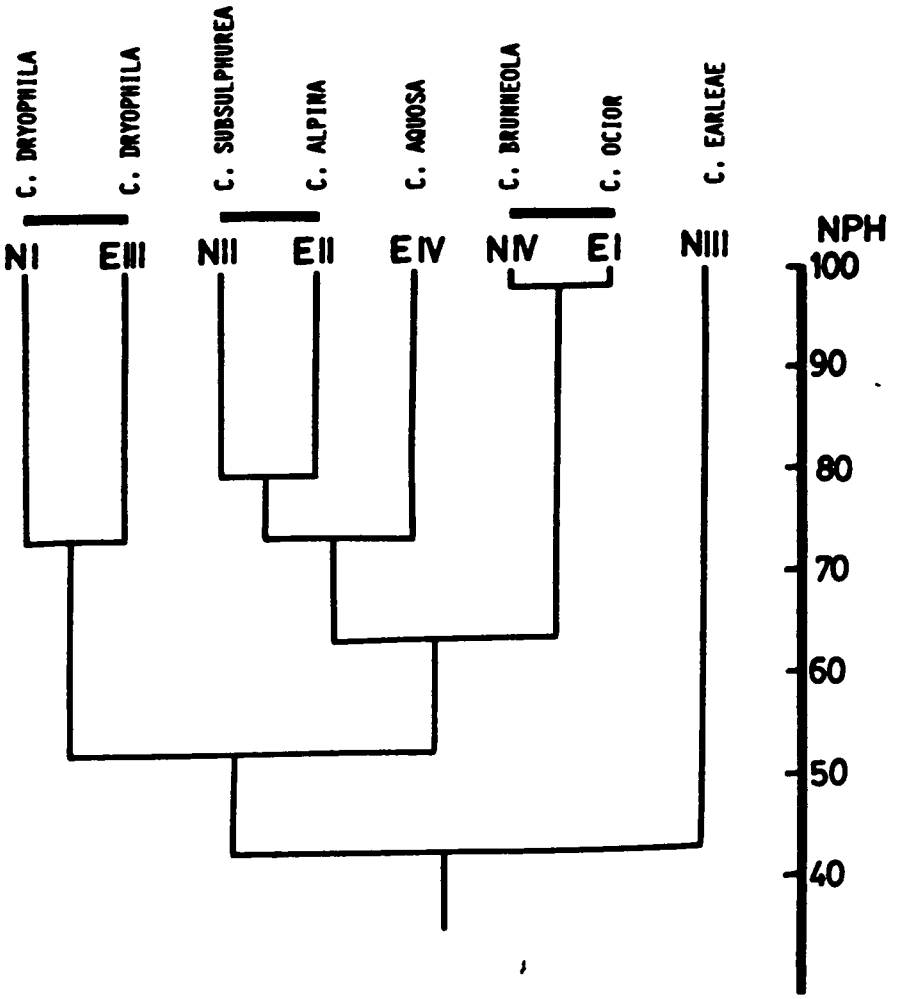

Figure 15. Phylogram of the C. dryophila based upon normalized percent hybridization.

CONCLUSIONS

Judging from both morphological and genetic evidence, the C. dryophila group is obviously in a dynamic state of evolution. A high level of phenotypic diversity in this group originally warranted a study of mating compatibility to determine how many intersterile groups were present. Concurrent studies of morphological and genetic variation have also provided insight into the nature of species in this group, and, more indirectly, in other fungi as well.

A primary factor determining the genetic structure of a species is the type of mating system that it possesses. All species in the C. dryophila group have a bifactorial mating system with multiple alleles. The bifactorial mating system of the dryophila group is similar, but not entirely the same as that of other species, such as Schizophyllum (Raper, 1966). In numerous intra-stock crosses, I have never observed the formation of pseudo-clamped hyphae corresponding to common B-factor matings (Pseudo-clamps have been observed in other interstock matings, but their occurrence does not correspond with common mating factors and probably represents another type of reaction between mating partners). I have also been unable to verify nuclear migration between component monokaryons in compatible crosses. When two compatible monokaryons grow together, dikaryon formation

occurs only at their junction line. The newly-formed dikaryon subsequently outgrows its parental monokaryotic colonies. Clamped dikaryotic hyphae eventually do form within the monokaryotic colonies, but their slow rate of establishment suggests that this is due to the dikaryon growing into its component monokaryons rather than by nuclear migration. A similar pattern of dikaryon formation is apparently present in many fungi (see chapter 1), for which nuclear migration may not be a significant factor in the establishment of dikaryotic mycelia. A reduction or absence of nuclear migration between mycelia growing in nature has added significance, since it would affect the structure of populations.

Since multiple allelic mating systems greatly favor outcrossing (Burnett, 1975), each species in the C. dryophila group must represent at least several interbreeding populations. Although gene flow certainly occurs within these species, the amount of interbreeding between different populations of the C. dryophila group is not clear. Compatible matings among isolates from a single geographic region (i.e., sympatric populations) nearly always occur at high frequency, supporting the view that these fungi do form good biological species at the local level, at least. Despite a considerable degree of phenotypic variation, fruit bodies belonging to the same species from a given location often show enough similarity to be identifiable from their

morphology. This general observation does not hold, however, when geographic isolation is a factor. For example, I have observed abnormal mating behavior in certain crosses among isolates originating from certain geographic regions in Europe, particularly the island of Sardinia. A similar reduction in intercompatibility between what are perceived to be allopatric populations has also been reported in Armillaria (Anderson, et al., 1980), Auricularia (Duncan and McDonald, 1967), and Pleurotus (Hilber, 1984).

Based on evidence from DNA hybridization (chapter 3), populations from different land masses (i.e., allopatric populations) have greater genetic dissimilarity, and are almost certainly genetically isolated, despite that they are also intercompatible. This conclusion is also supported by evidence for considerable morphological divergence between certain allopatric species which are intercompatible (e.g., groups E-II and N-II). My conclusions about allopatric evolution in the C. dryophila group are further supported by isozyme data (unpublished), which indicate fixation for different isozyme allelomorphs in allopatric populations. Mating compatibility, therefore, appears to be a primitive feature which may still be common among certain genetically isolated populations.

It is interesting to note that intersterility barriers in Collybia and other basidiomycetes are nearly always absolute: interspecific hybridization is unknown for natural

populations of homobasidiomycetes (Ullrich and Raper, 1977). Likewise, there is currently no evidence for hybrid zones where different species overlap, as for higher plants (Grant, 1981). The origin of rigorous pre-zygotic reproductive isolation among species of fungi is still unknown, although several theories have been put forward. Kemp (1975, 1977) proposed that intersterile groups of fungi may arise sympatrically, by mutation of genetic factors responsible for self recognition. Other modes of speciation based upon geographic speciation (Burnett, 1983) and heterogenic incompatibility (Hoffman & Esser, 1976) have also been suggested for fungi. Although different modes of speciation within a group of fungi are not mutually exclusive, the data from this study indicate that speciation in the C. dryophila group is primarily allopatric. The pattern of genetic diversity in the C. dryophila complex is consistent with neodarwinian theories concerning geographic speciation (Mayr, 1963; Dobzhansky, 1970; Grant, 1981). Under an allopatric mode of speciation, intersterile groups in the C. dryophila group could have arisen by the gradual divergence of populations existing in allopatry. Mating barriers, however, would not be selected for until divergent groups again became sympatric. The genetic factors which determine intersterility are apparently very strong in Collybia and other basidiomycetes, since naturally occuring hybrids or hybrid zones are not known at present.

In conclusion, this study demonstrates several dynamic aspects of speciation as it might occur in Collybia and other fungi. The evolutionary consequences of biogeographic variation should be particularly apparent to taxonomists working with fungi: a species concept based solely on morphology or mating compatibility may not be realistic in some situations. Instead, a reappraisal of what a species is may be more rewarding and appropriate.

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