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Deoxyribonucleic Acid of Fungi

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INTRODUCTION

The base composition of deoxyribonucleic acid (DNA) from bacteria (24, 34, 43) and from blue-green algae (21) has been the object of extensive analyses in the last 10 years. These analyses have revealed that the mean guanine plus cytosine content in moles per cent (%GC) of procaryotic DNA has a range which extends from 25 to 75%, and they have confirmed the results obtained in 1956 (27) showing that the DNA base compositions from related organisms are similar and can be used for taxonomic and phylogenetic purposes. In comparison, the information about DNA from eucaryotic microorganisms is scarce, judging from recent compilations (29, 43).

With regard to fungi, the subject of the present paper, the first attempt to assess the value of DNA base composition for systematics is to be credited to Belozersky and his collaborators (7, 48, 50). Their survey was limited to 14 species distributed as follows: two *Myxomycetes*, two *Phycomycetes*, four *Ascomycetes*, two *Deuteromycetes*, and four *Basidiomycetes*. The GC contents ranged from 34 to 57%. DNA with less than 50% GC was found almost exclusively in the first two classes. For the *Ascomycetes* and *Deuteromycetes*, the GC contents were close to 50%, whereas for *Basidiomycetes* they were significantly higher than 50%. These few results suggested to the Russian workers that determination of DNA base composition showed some promise of value for studies of systematic and phylogenetic relationships. Twenty-three additional species belonging to four classes of the *Eumycotina* were included in a survey a few years later by Storck (45). Base composition was inferred from buoyant density and melting temperature measurements, and the values obtained by these two methods were found not to differ on the average by more than 1% GC. Also, the base composition values obtained by chemical determination were found in most cases to agree well with those calculated from physicochemical measurements. The results of this survey, together with those of the Russian workers and the few isolated values scattered in the literature, revealed that the %GC in fungi ranged from 34 to 63% and showed that as for procaryotes, related organisms tended as a rule to have similar GC contents. For example, in the *Mucorales*, the overall range of 8% (38 to 46% GC) decreased to 2% for the four *Mucor* species analyzed. In contradistinction, a difference of 13% GC was found to exist between two species assumed to belong to the genus *Sporobolomyces*. Values of 63 and 62% GC were found for *Sporobolomyces salmonicolor* and *Rhodotorula mucilaginosa*, respectively. This finding lent tentative support to the hypothesis (28),

based on criteria other than GC content of DNA, that some *Rhodotorula* species might originate from *Sporobolomyces* species through loss of the ability to produce ballistospores. Clearly, these examples suggested that further exploration of fungal DNA base composition might be taxonomically rewarding. Although limited to some 40-odd species, these studies showed yet another promising facet. When the averages of GC content of the classes were compared, it appeared that the *Zygomycetes* had the lowest average, whereas the *Basidiomycetes* had the highest one. Among the *Ascomycetes*, GC contents lower than 50% were found only for the *Hemiascomycetidae* for which the range in GC content was similar to that for the *Zygomycetes*. Thus, not only was there a suggestion for a dichotomous GC-contents distribution among the *Ascomycetes* but perhaps for a "link" between them and the *Zygomycetes*. These speculations found further support in the observation that *Dipodascus uninucleatus*, which is regarded to be a key genus in the phylogenetic scheme seeking the origin of the *Ascomycetes* in the *Zygomycetes* (1), has a GC content of 43%.

The signs of promise shown by these preliminary investigations prompted several workers, including ourselves, to analyze systematically large numbers of species in selected taxonomic groups. While the work described in the present paper was in progress, there have been several publications on the yeasts and the yeastlike fungi. Meyer and Phaff (36) found that the GC contents of 15 species of ascosporogenous yeasts and their anascosporogenous counterparts ranged from 34.9 to 48.3%. They fit well, therefore, with the group of lower values (38 to 48%) found earlier (45). The same can be said for 15 of the 18 *Candida* species investigated by Stenderup and Bak (44) and for a larger number of ascosporogenous and some of the anascosporogenous yeasts analyzed by Nakase and Komagata (38). Recently (39), these last authors determined the base ratios of the DNA of 26 species or varieties of *Hansenula* and found a GC content range of 28.5 to 46.3%. It was previously reported (45) that among yeasts there was another group of GC values, distinct from the one mentioned above in that its range was from 49 to 63%. This group included as we have already seen, two species of *Sporobolomyces* and one of *Rhodotorula* and in addition one species of *Cryptococcus* and one of *Torulopsis*. Values in the higher range have been reported for *Candida* (38, 44), some species of *Torulopsis* and *Trichosporon*, and for all species studied of *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces* (38). More recently (46), an analysis of additional species and varieties of *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces* has shown that with one exception, a *Cryptococcus* species, the %GC was in all cases higher than 50 and averaged 58.1. All these investigations reinforce the contention formulated earlier (45) that yeast and yeastlike fungi which are related to the *Heterobasidiomycetes* have a GC content which is significantly higher than that of those organisms classified as *Hemiascomycetidae* or their anascosporogenous counterparts. Worth mentioning, finally, is the fact that these studies on yeasts have demonstrated that there could be a significant intrageneric variation in DNA GC content expressed by the existence of a dichotomous distribution, suggesting that the taxonomic status of these genera should be reappraised.

Apart from that on yeast and yeastlike fungi, no other systematic study has been made except one on *Homobasidiomycetidae* (Storck et al., *in preparation*). It involved 30 species totaling 50 isolates distributed between *Hymenochaetaceae* and *Polyporaceae*. In the first family, the GC contents ranged from 50.0 to 51.5%, and in the second from 51.0 to 59.5%. Added to others, these results reinforce the fact that *Basidiomycetes* have average GC contents higher than 50%.

In this paper, we give the results of a survey started in September 1965 and covering 322 species totaling 492 isolates belonging to *Oömycetes*, *Zygomycetes*, *Ascomycetes*, and *Deuteromycetes*. This survey, together with the others reported above, provides us for the first time with enough values to warrant a serious attempt to assess in a quantitative and critical way the taxonomic and phylogenetic value of DNA base composition in fungi.

MATERIALS AND METHODS

Culture Conditions

The sources of the organisms used are listed in the tables of %GC values presented under Results and Discussion. The isolates were maintained in culture tubes on a solid medium containing 0.3% yeast extract (Difco), 1.0% peptone (Difco), 2% D-glucose, and 1.5% agar. From 100 to 200 ml of the same medium, agar-free, was inoculated with the spores or the mycelium contained in one or two tubes, or both. These liquid starter cultures were shaken at room temperature (25 to 30 C) on a rotary shaker for 36 to 48 hr. A microscopic check of these cultures was made prior to their transfer into 1,000 ml of the same medium contained in baffled, long-necked, 2-liter Erlenmeyer flasks which were shaken under conditions similar to those for the starter flasks for 48 to 72 hr, depending on the rate of growth. The cultures were checked microscopically prior to harvest. Mycelium was harvested by filtration,

washed with 200 ml of a saline solution containing 0.15 M NaCl and 0.1 M ethylenediaminetetraacetate (EDTA; *p*H 8.0) (saline-EDTA) and stored in the freezer.

Extraction and Purification of DNA

Mycelium was ground in liquid nitrogen. This results in a quick disruption of cell walls, does not require elimination of an abrasive, and, more important, yields DNA preparations with a high molecular weight suitable for analysis by buoyant density centrifugation and melting temperature determination. The procedure used for DNA extraction and purification is a combination of the method of Cheng and Sueoka (14) and of that of Marmur (33). Although it has been described in detail elsewhere (51), one might mention that it involves the use of Pronase and amylase in order, respectively, to decrease the number of protein extractions and to eliminate or reduce contamination of DNA preparation by polysaccharides which, as we will show later, may introduce artifacts in centrifugation profiles. DNA concentration was estimated by optical density measurements at 260 nm (OD_{260}) using the equivalent of 50 μg of DNA/ml per unit OD_{260}. The purity of the DNA preparation was routinely estimated by establishing ratios of absorption at 230, 260, and 280 nm (33).

Determination of GC Contents

The method of Meselson et al. (35) was used for the determination of buoyant densities. The conversion into %GC was calculated according to the method of Schildkraut et al. (42). SP8 bacteriophage DNA, kindly supplied by M. Mandel (M. D. Anderson Hospital, Houston, Tex.), was used as a reference. All DNA buoyant densities were related to that of *Escherichia coli*, which was taken to be 1.710 g/cc (42). To assess the value of the experimental error, repeated measurements were made on DNA extracted from *Penicillium atramentosum* and from *Syncephalastrum racemosum*. For each of these DNA preparations, the %GC values obtained were grouped into frequency distributions and their statistical indices were calculated (Table 1). Thus, the precision of our measurements is the same as that reported by other investigators (31, 42).

All the %GC values reported here represent an average of at least two determinations of the buoyant density of the same DNA sample. In some instances, the %GC values listed are an average of several determinations made on DNA preparations extracted from two or more cultures of the same isolate. The value of the buoyant density was not affected by variation of the amount of DNA per centrifuge cell or by the presence of two or even three different DNA populations in the same cell. Also, the buoyant density was the same when DNA was extracted from spores, or yeastlike cells (in the case of *Mucor* isolates) rather than from mycelium. Similarly, within a period of a week, age had no effect on the buoyant density and the same was true for temperature and medium. All these examples serve to reemphasize the fact that DNA is metabolically stable, in comparison to other macromolecules, and therefore that its base composition can be regarded as a good identification index.

Two types of satellite DNA forms were occasionally encountered in the centrifugation profiles. The first type, which was deoxyribonuclease-sensitive, had a buoyant density varying between 1.685 and 1.700 g/cc. Since it was shown in a survey of 14 fungal species (51) that DNA extracted from mitochondria had a buoyant density varying from 1.688 to 1.703 g/cc, it was inferred that this type of satellite band had a mitochondrial origin. This satellite represented but a small proportion of the total extractible DNA and it was sometimes present, sometimes absent in extracts from the same isolate. However, the buoyant density of the minor band was constant from one preparation of the same isolate to another. The second type of satellite banded at densities less than 1.680 g/cc and was not sensitive to deoxyribonuclease. Unlike the first type, it showed considerable variation in buoyant

TABLE 1. *Statistical indices of the frequency distribution of GC content values obtained by repeated measurements on the same DNA sample*[a]

Organism	N	$\bar{X}$	R	s^2	s	SE	PE
Penicillium atramentosum	26	49.8	49–51	0.29	0.54	0.11	0.36
Syncephalastrum racemosum	26	48.3	47–51.5	0.83	0.91	0.18	0.61

[a] Abbreviations: N, number of measurements; $\bar{X}$, arithmetic average; R, range; s^2, variance; s, standard deviation; SE, standard error; PE, probable error.

density from one preparation of the same isolate to another. In addition, it banded, unlike the first type of satellite and the main DNA band, as early as 14 hr after the beginning of the centrifugation. It was inferred on the basis of studies made on *Mucor subtillissimus* DNA preparations (R. C. Moyer, Ph.D. thesis, University of Texas, 1965) that this second type of satellite was a polysaccharide contaminated with DNA. It might be added that since its discovery in a fungus, the contamination of a DNA preparation by polysaccharides has been reported for bacteria (54), blue-green algae (21), algae, protozoa, plants, and animals (31).

Comparability of Nucleotide Composition Analyses by Different Methods

We indicated in the Introduction that in a previous survey (45) DNA samples were analyzed by buoyant density and melting temperature measurements and that the results did not differ on the average by more than 1%. Only in one case was a difference as high as 5% found. The comparison of these values with those available in the literature and obtained from either indirect physicochemical or direct chemical measurements further failed to indicate significant discrepancies. It was concluded, therefore, that fungal DNA, like bacterial DNA, does not contain large proportions of odd bases or sugars, or both. However, these conclusions should be reexamined because differences which appear to be significant have now been found in data accumulated since these earlier surveys (Table 2). For each value, the method used is specified. Discrepancies between GC content values obtained from different laboratories have been observed not only in fungi but also in bacteria (30). In both cases, the major sources for these discrepancies appear to be the analytical methods employed and also the use of different reference DNA forms. This last source for errors is especially relevant in the case of indirect physicochemical methods. It should be mentioned in passing that it is this type of method which is in greater use nowadays as can be seen by perusal of Table 2. Comparison between data originating from different laboratories is rendered even more difficult, if not impossible, because the exact origin of the isolates or cultures analyzed is not given; also, because of changes in existing classifications, different organisms may be listed with the same denomination or the same organism may be named differently by different investigators. In view of these difficulties, the data found in the literature have been treated separately from those which are original to the present study. As this paper will show, the taxonomic resolving power of the GC content of DNA is minimal at the species level.

Statistical Calculations

To facilitate the comparison of the results and their interpretation, the values listed in Table 2 were grouped into frequency distributions. Statistical indices were calculated and are listed in Table 3. It is important to mention that the number of species (N_s) was used in the calculations. Thus, when several isolates of the same species had been studied (listed as individual values in Table 2) an average was calculated. This average was entered as one value in the calculation of the statistical indices. This explains why N_s is either equal to or less than the number of isolates (N_i). It should also be noted that not all the genera analyzed were entered in Table 3, and this explains, for example, why N_s for *Saccharomycetaceae* is 52, whereas the sum of the number of *Hansenula* and *Saccharomyces* species is only 33. As an inspection of Table 2 reveals, the deletion of a taxon from Table 3 is due to the fact that the number of species in that taxon was judged to be too small to justify the calculations of statistical indices. The following formula was used in these calculations: variance

$$s^2 = \frac{\Sigma(X_i - \bar{X})^2}{N_s}$$

where X_i is an individual %GC value for a species, $\bar{X}$ the arithmetic average, standard deviation or $s = \sqrt{s^2}$, standard error or $SE = s/\sqrt{N}$, and probable error or $PE = 0.6745s$. Unless otherwise indicated, the same procedure for selection of data and for calculation of indices was used for the preparation of Tables 4, 5, 7, 9, 11, and 13.

RESULTS AND DISCUSSION

Taxonomic Significance of the Diversity in GC Content of Fungal DNA

Before attempting to analyze in detail for each taxonomic group the significance of DNA base ratios, it seems desirable to try first to formulate some general principles which could apply to given groups regardless of their existing systematic position. To do so, we must concentrate on the diversity of GC content at various levels of taxonomic grouping.

By diversity or heterogeneity we refer specifically to the variation in %GC values that is observed within taxonomic groups. This diversity can be expressed quantitatively by using indices of dispersion such as range, variance, standard deviation, etc. These indices can be calculated

TABLE 2. *GC content of DNA from fungi as reported in the literature*

Organism	Buoyant density	T_m[a]	C[b]	Reference
Oömycetes				
Phytophthora infestans		47.5		15
Sapromyces sp.	27			43
Zygomycetes				
Mucorales				
Cunninghamellaceae				
Cunninghamella echinulata	34			51
Mucoraceae				
Absidia glauca	44	48		45
Absidia sp.			39	50
Mucor fragilis	39			51
M. racemosus	38			45
M. rouxianus	39			45
M. rouxianus	38	41		45
M. rouxii	39			45
M. rouxii	37			51
M. subtilissimus	39	39		45
M. subtilissimus	39	40		45
Phycomyces blakesleeanus (−)	43	44		45
P. blakesleeanus (−)			39	48
P. blakesleeanus (+)			39	48
Rhizopus nigricans	49		45	20
Zygorhynchus moelleri	35	40		45
Syncephalastraceae				
Syncephalastrum racemosum	48	47		45
Ascomycetes				
Endomycetales				
Ascoideaceae				
Dipodascus albidus		33		38
D. uninucleatus	43			45
Endomycetaceae				
Endomyces reesii	39	41		45
Endomycopsis capsularis		40		38
E. fibuligera		37.5		38
E. muscicola		34.5		38
Saccharomycetaceae				
Citeromyces matritensis		42.5		38
Debaryomyces globosus		45		36
D. hansenii		34.5		38
D. hansenii		36.5		36
D. kloeckeri (*hansenii*)	40	40		45
Hanseniaspora valbyensis		30		38
Hansenula angusta (*polymorpha*)		45		39
H. angusta (*polymorpha*)		45.5		39
H. anomala		32.5		38
H. anomala		32.5		39
H. anomala		33.5		39
H. anomala		34		39
H. beckii (*Endomycopsis bispora*)		34		39
H. beckii (*E. bispora*)		33.5		39
H. beijerinckii		40		39
H. bimundalis		38.5		39
H. bimundalis var. *americana*		40		39
H. californica		41.5		39
H. californica		41.5		39
H. canadensis		35.5		39
H. canadensis		36		39
H. capsulata		42.5		38
H. capsulata		44		39
H. capsulata		42.5		39
H. ciferrii		30.5		39
H. fabianii		42.5		39
H. fabianii		41.5		39
H. fabianii		43		39
H. fabianii		43		39
H. fabianii		43		39
H. holstii		34		39
H. jadinii		41		39
H. minuta		43.5		39
H. minuta		44.5		39
H. mrakii		40		39
H. petersonii		41		39
H. petersonii		39		39
H. platypodis (*E. platypodis*)		32		39
H. platypodis (*E. platypodis*)		33.5		39
H. platypodis (*E. platypodis*)		29		39
H. saturnus		39.5		39
H. saturnus		41.5		39
H. schneggii (*H. anomala* var. *schneggii*)		33.5		39
H. schneggii (*H. anomala* var. *schneggii*)		32.5		39
H. silvicola		31.5		39
H. silvicola		32		39
H. silvicola		32.5		39
H. silvicola		32.5		39
H. subpelliculosa		31		39
H. wickerhamii		41.5		39
H. wingei		37		39
Kluyveromyces polysporus		31		38
Lipomyces starkeyi		45.5		38
Lodderomyces elongasporus		40		36
L. elongasporus		39.5		36
Metschnikowia bicuspidata		44.5		36
M. pulcherrima		48.5		36
M. pulcherrima		42.5		36
M. pulcherrima		44.5		36

[a] Temperature corresponding to midpoint transition from double helix to random coil.
[b] Chemical determination.

TABLE 2.—*Continued*

Organism	Buoyant density	T_m[a]	C[b]	Reference
M. reukaufii		42		36
Naganishia globosus		47.5		38
Pachysolen tannophilus		40		38
Pichia kluyverii		26		38
P. kluyverii		27		38
P. membranaefaciens		40		38
P. membranaefaciens	46	44		45
P. vini		34.5		38
Saccharomyces bisporus		44.5		36
S. carlsbergensis	40			51
S. carlsbergensis	40			51
S. cerevisiae	38			51
S. cerevisiae	41			51
S. cerevisiae	42			51
S. cerevisiae	42			51
S. cerevisiae	42			51
S. cerevisiae	40			51
S. cerevisiae	40			51
S. cerevisiae		37		36
S. cerevisiae		36		38
S. cerevisiae		36.5		38
S. cerevisiae		36.5		38
S. cerevisiae		36		38
S. cerevisiae		37		36
S. cerevisiae		36		36
S. cerevisiae		38		45
S. cerevisiae	40	41		45
S. cerevisiae			36	45
S. cerevisiae	39			45
S. delbrueckii		32		38
S. exiguus		30.5		38
S. florentinus		38.5		38
S. fragilis		41		36
S. fragilis	42	42		45
S. inconspicuus		46.5		36
S. rosei		44		36
S. rosei		40		38
S. vini	41			43
Schizosaccharomyces octosporus	40	42		45
S. pombe		42		45
Schwanniomyces occidentalis		31.5		38
Wickerhamia fluorescens		35		38
Euascomycetidae				
Plectomycetes				
Microascales				
Ophiostomataceae				
Ceratocystis ulmi	56			51
Pyrenomycetes				
Chaetomiales				
Chaetomiaceae				
Chaetomium globosum	58			51
Clavicipitales				
Clavicipitaceae				
Claviceps purpurea			53	50
Sphaeriales				
Sordariaceae				

TABLE 2.—*Continued*

Organism	Buoyant density	T_m[a]	C[b]	Reference
Gelasinospora autosteira	54			51
G. calospora			52	20
G. calospora	55			51
G. calospora	55	53		45
G. cerealis	55			51
G. tetrasperma			50	20
Neurospora crassa	53		53	20
N. crassa	52	55		45
N. crassa	54			45
N. crassa			54	45
N. crassa	54			51
N. intermedia	53		52	20
N. sitophila	55			51
N. tetrasperma			50	20
Sordaria macrospora	54			51
Discomycetes				
Helotiales				
Sclerotiniaceae				
Sclerotinia libertiana			46	48
Pezizales				
Helvellaceae				
Helvella esculenta			50	50
Loculoascomycetidae				
Pleosporales				
Sporormiaceae				
Sporormia sp.	51	51		45
Deuteromycetes				
Melanconiales				
Melanconiaceae				
Colletotrichum lagenarium	53		50	20
Moniliales				
Cryptococcaceae				
Brettanomyces bruxellensis		35		38
Candida albicans		32.5		38
C. albicans		35		44
C. atmosphaerica		41		36
C. atmosphaerica		40		44
C. brumptii		54		44
C. catenulata		54.5		44
C. claussenii		35		44
C. diddensii		40		36
C. humicola		60		38
C. gelida		52		38
C. koshuensis		30		38
C. krusei		38		38
C. krusei		39.5		44
C. lipolytica		49.5		3
C. melinii		41		44
C. melinii		36.5		38
C. oregonensis		48		36
C. parapsilosis		40		36
C. parapsilosis		41		44
C. parapsilosis var. *hokkai*		52		38
C. pelliculosa		37		44
C. pelliculosa		34		39

TABLE 2.—*Continued*

Organism	Buoyant density	T_m[a]	C[b]	Reference
C. pseudotropicalis		41.5		44
C. pulcherrima		48		44
C. pulcherrima	46	48		45
C. pulcherrima		44.5		38
C. punicea		51		38
C. rugosa		47		38
C. stellatoidea		35.5		44
C. tenuis		44		44
C. tropicalis		35		44
C. tropicalis		35		36
C. truncata		37		44
C. utilis		46		44
C. utilis		40		39
C. zeylanoides		57.5		44
C. zeylanoides		51.5		38
C. zeylanoides		51.5		38
Cryptococcus albidus	55	55		45
C. flavus syn. *Rhodotorula flava*	55			45
C. gastricus	51			46
C. gastricus	65.5			46
C. laurentii		56		38
C. laurentii var. *magnus* syn. *Torula heveanensis*	49			46
C. laurentii var. *flavescens* syn. *Rhodotorula peneaus*	58			46
C. melibiosum syn. *Torulopsis melibiosum*	61			46
C. neoformans	51.5			46
C. neoformans		46		38
C. skinneri	53			46
C. terreus	59.5			46
C. uniguttulatus syn. *C. neoformans* var. *uniguttulatus*	51.5			46
C. uniguttulatus	58			46
Geotrichum candidum		40.5		38
Kloeckera apiculata		30		38
Rhodotorula glutinis		64		38
R. graminis	70			46
R. minuta var. *minuta*	53			46
R. minuta var. *texensis* syn. *R. texensis*	54			46
R. minuta var. *texensis* syn. *R. tokyoensis*	52.5			46
R. mucilaginosa	61	63		45
P. pallida	54.5			46
R. pallida	63.5			46
R. rubra		63.5		38
R. slooffii		47.5		38
R. texensis		48		38
Torulopsis aeria		52.5		38
T. colliculosa		40.5		38
T. glabrata		35.5		38
T. pinus		34.5		38
T. stellata		40.5		38

TABLE 2.—*Continued*

Organism	Buoyant density	T_m[a]	C[b]	Reference
T. stellata	50	48		45
T. torresii		48.5		38
Trichosporon behrendii		32.5		38
T. cutaneum		59		38
T. cutaneum		59		38
T. pullulans		54		38
Trigonopsis variabilis		44		38
Moniliaceae				
Aspergillus nidulans			47	20
A. niger	52	52		45
A. niger			50	48
A. oryzae			46	43
Botrytis cinerea			50	50
Fusarium oxysporum f. lycopersici			48	20
Penicillium chrysogenum	52	52		45
P. notatum	53	51		45
Trichothecium roseum			50	50
Heterobasidiomycetidae				
Aureobasidium pullulans		51.5		38
Sporobolomyces alborubescens	63			46
S. holsaticus	62			46
S. holsaticus	64			46
S. holsaticus	65			46
S. holsaticus syn. *S. coralliformis*	64.5			46
S. odorus	65			46
S. pararoseus	51.5			46
S. pararoseus syn. *S. marcillae*	55			46
S. pararoseus	60.5			46
S. roseus	50	50		45
S. roseus	56			46
S. roseus syn. *S. salmoneus*	55.5			46
S. roseus syn. *S. tenuis*	53.5			46
S. roseus	55			46
S. salmonicolor		57		38
S. salmonicolor	63	63		45
S. salmonicolor	63.5			46
S. salmonicolor	64.5			46
Tremella fuciformis		54.5		38
Homobasidiomycetidae				
Agaricaceae				
Agaricus bisporus			44	48
Agaricus (Psalliota) campestris			44	7
Amanita muscaria			57	50
A. strobiliformis			58	45
Coprinus lagopus	53		52	20
Pleurotus ostreatus	51.5			—[c]
Hymenochaetaceae				
Inonotus dryophilus	50			—[d]
I. dryophilus	51			—[d]

[c] Storck, *unpublished data.*

[d] R. Storck, M. K. Nobles, and C. J. Alexopoulos, *in preparation.*

TABLE 2.—*Concluded*

Organism	Buoyant density	T_m[a]	C[b]	Reference
Phellinus ferruginosus	50			—[d]
P. gilvus	50.5			—[d]
Lycoperdaceae				
Bovista sp.			51	50
Polyporaceae				
Bjerkandera adusta	57.5			—[d]
B. adusta	56			—[d]
B. fumosa	52			—[d]
Ceriporiopsis placenta	53			—[d]
C. placenta	54			—[d]
C. placenta	56.5			—[d]
Daedalea confragosa	57			51
D. quercina	55.5			—[d]
Daedaleopsis confragosa	59			—[d]
Elfvingia applanata	58			—[d]
E. applanata	59.5			—[d]
Fomes fraxineus	51			—[d]
F. fraxineus	56			—[d]
F. fraxinophilus	54.5			—[d]
Fomitopsis pinicola	56.5			—[d]
F. pinicola	57			—[d]
F. pinicola	57			—[d]
Ganoderma tsugae	54			—[d]
Gloeophyllum saepiarium	54			—[d]
G. saepiarium	54			—[d]
Irpex lacteus	54			—[d]
I. lacteus	54.5			—[d]
Laetiporus sulphureus	54			—[d]
L. sulphureus	56			—[d]
Laricifomes officinalis	54.5			—[d]
Lenzites betulina	59			—[d]
L. betulina	59			—[d]
Meruliopsis taxicola	55.5			—[d]
Phaeocoriolellus trabeus	59			—[d]
Phaeolus schweinitzii	55			—[d]
Piptoporus betulinus	57			—[d]
P. betulinus	56.5			—[d]
Polyporus balsameus	56.5			—[d]
P. brumalis	59			—[d]
P. brumalis	58.5			—[d]
P. palustris	53.5			—[d]
P. palustris	57.5			—[d]
P. versicolor			57	45
Poria carbonica	54.5			—[d]
P. cinerascens	57			—[d]
P. cinerascens	57.5			—[d]
P. rivulosa	55.5			—[d]
P. sequoiae	59.5			—[d]
Pycnoporus cinnabarinus	59			—[d]
P. sanguineus	58.5			—[d]
P. sanguineus	59			—[d]
Spongipellis galactinus	53			—[d]
S. galactinus	54			—[d]
Schizophyllaceae				
Schizophyllum commune	61			51
S. commune	58	58		45
S. commune			57	50

provided enough representatives of a given taxon have been analyzed. Since there is an enormous difference between the total number of existing fungal species and the number that can possibly be analyzed, it is necessary to limit the survey of each class to a few orders or families to be able to study quantitatively the diversity in GC content.

In the present work, only one species of *Entomophthorales* was studied and all the remaining *Zygomycetes* were *Mucorales*. Of the 220 isolates of *Deuteromycetes*, there were 56 of *Aspergillus* and 90 of *Penicillium*. Also, the survey of the *Ascomycetes* involved mostly members of the families *Eurotiaceae* and *Gymnoascaceae* and 13 species of *Chaetomium*. These limitations should thus be kept in mind when one is to generalize on the basis of the present study and the data from the literature.

One prerequisite for the use of GC content measurement as a tool for taxonomy is that the compositional diversity be as great as possible for the group of organisms to be studied. This prerequisite is satisfied for fungi since, as will be shown here, their range in %GC extends from 26 to 70. (*Acrasiales* and *Myxomycetes* are excluded from this survey because of their uncertain taxonomic status.) Recent compilations for protozoa and algae (29) indicate ranges of 22 to 68 and 37 to 68% GC, respectively. The %GC for bacterial DNA extends from 25 to 75% (24) and for blue-green algae DNA from 35 to 71% (21). Thus, the compositional diversity in eucaryotic microorganisms is the same as that in procaryotic organisms.

Another prerequisite for taxonomic usefulness is the need for organisms which are described under the same specific epithet to show a minimum heterogeneity in their DNA base composition. Among bacteria, the %GC values for organisms in one genus are as a rule so close together that differences between species are usually not distinguishable, and when organisms, and there are exceptions, have a %GC value falling out of the range of the genus they are usually found to have been incorrectly classified (18). As shown in Table 5, more than six isolates were analyzed for several species and statistical indices were calculated. These indices should be compared with those listed in Table 1. It is seen that in the case of *Syncephalastrum racemosum* the indices are of the same order of magnitude. Appraisal of intraspecific variation in GC content was also made for all other species studied in the present survey, regardless of their taxonomic position, by establishing a frequency distribution of range size for those species for which at least two isolates had been studied. As shown in Fig. 1, more than 80%

TABLE 3. *Statistical indices of frequency distribution of the GC content values reported in the literature*[a]

Taxonomic group	N_s	N_i	$\bar{X}$	R	s^2	s	SE	PE
Mucorales	12	17	40.4	34–47.5	16.17	4.02	1.16	2.71
Endomycetales	58	107	38.5	26.5–47.5	24.91	4.99	0.65	3.36
Ascoideaceae	2	2	38	33–43				
Endomycetaceae	4	4	38	34.5–40				
Saccharomycetaceae	52	101	38.6	26.5–47.5	26.39	5.14	0.71	3.46
Hansenula	23	45	37.8	30.5–45	19.63	4.43	0.92	2.98
Saccharomyces	10	30	39.5	30.5–46.5	22.60	4.75	1.50	3.20
Euascomycetidae	14	20	52.9	46–58	8.61	2.93	0.78	1.97
Moniliales	69	88	47.9	30–70	81.09	9.00	1.08	6.07
Cryptococcaceae	60	78	47.7	30–70	97.42	9.87	1.27	6.65
Candida	27	38	43.7	30–60	57.82	7.60	1.46	5.12
Cryptococcus	11	14	55.3	49–61	13.65	3.69	1.11	2.48
Rhodotorula	9	11	57.8	47.5–70	54.12	7.36	2.45	4.96
Torulopsis	6	7	42.8	34.5–52.5	43.15	6.57	2.68	4.43
Moniliaceae	9	10	49.7	46–52	4.40	2.10	0.70	1.41
Heterobasidiomycetidae	8	20	58.7	51.5–65	24.87	4.99	1.76	3.36
Sporobolomyces	6	18	60.6	54–65	18.04	4.25	1.73	2.86
Homobasidiomycetidae	42	62	55	44–59.5	12.88	3.59	0.55	2.42
Agaricaceae	7	7	51.1	44–58	26.48	5.15	1.94	3.47
Hymenochaetaceae	3	4	50.5	50–50.5				
Polyporaceae	31	48	56.2	52–59.5	4.29	2.07	0.37	1.39

[a] Abbreviations: N_s, number of species analyzed; N_i, number of isolates analyzed.

TABLE 4. *Statistical indices of the frequency distribution of GC content values for fungal classes*[a]

Class	N_s	N_i	$\bar{X}$	R	s^2	s	SE	PE
Oömycetes	24	27	53	40.5–62	28.15	5.31	1.08	3.58
Zygomycetes	66	155	42.6	27.5–59	58.02	7.62	0.94	5.14
Ascomycetes	69	90	53.4	48.5–60	6.10	2.47	0.30	1.67
Deuteromycetes	163	220	52.1	35.5–64.5	10.67	3.27	0.26	2.21
All classes	322	492	50.5	27.5–64.5	36.55	6.05	0.34	4.08

[a] Abbreviations are indicated in Tables 1 and 3.

TABLE 5. *Statistical indices of the frequency distribution of GC content values for different isolates of the same species*[a]

Species	N	$\bar{X}$	R	s^2	s	SE	PE
Actinomucor elegans	12	41.3	40.5–43	0.52	0.72	0.20	0.48
Mycotypha microspora	7	45	42–47.5	2.86	1.69	0.63	1.14
Radiomyces embreei	8	46.1	44–50	2.78	1.67	0.59	1.12
Rhizopus oligosporus	12	40	38.5–41	0.58	0.76	0.21	0.51
Syncephalastrum racemosum	13	50.3	48.5–52.5	1.60	1.26	0.34	0.85
Thamnidium anomalum	12	45.2	44.5–46	0.26	0.51	0.14	0.34
T. elegans	8	51	37–60.5	56.50	7.52	2.65	5.07

[a] Abbreviations are indicated in Tables 1 and 3.

of all the species surveyed have a range of GC content values which is less than 5%. Only two species have a range greater than 10% GC. One of these two species is *Thamnidium elegans* (Table 5). The individual values for the eight isolates of this species are listed in Table 8. One can see that five out of these eight values are narrowly clustered between 53.0 and 55.5%, with the three remaining values equaling 37.0, 40.5, and 60.5% GC, respectively. Essentially, the distribution appears to be composed of at least two clusters of nonoverlapping values. The other case

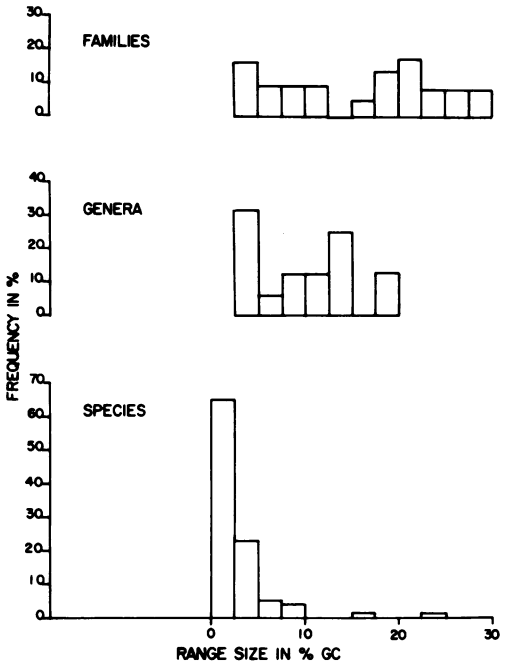

FIG. 1. *Frequency distribution (expressed in per cent) of range size in %GC within the species, genera, and families studied in the present work.*

in which an intraspecific variation greater than 10% GC was encountered is that of *Helminthosporium speciferum* (Table 12) for which the respective values of %GC of the four isolates analyzed were 52.5, 53.0, 53.0 and 69.5. Such high intraspecific diversity in DNA base composition has been encountered only rarely except in some yeasts (46).

In a recent study (Storck et al., *in preparation*), of 3 species of *Hymenochaetaceae* and 29 of *Polyporaceae* there were 16 species for which 2 or more isolates were analyzed. Out of these, only four species had a range of %GC greater than 1.5. From all evidence available at the present time (*see* Fig. 2), one is compelled to conclude that as a rule intraspecific variation in GC content in fungi is almost always less than 10% when expressed in range. The present survey provides us with a first example of a study of intraspecific heterogeneity and indicates that it might be possible and useful to attempt to define species and, for that matter, other taxa by the average value of indices such as variance or standard deviation. If this is done with the values of Table 5, excepting *T. elegans*, one would reach the conclusion that a species is characterized by a heterogeneity in the composition of its population of DNA which has a standard deviation of less than 2% GC. The exceptional cases in which this condition is not fulfilled will need additional information. In the meanwhile, it is conceivable that extreme values are due to original misidentification or subsequent chance contamination.

As it was already suggested in a previous but limited survey (45), the compositional diversity of fungal DNA decreased from classes to subclasses to genera and species. This is definitely the case from the results of the present survey (Fig. 1). Eight genera and 12 families for which more than one representative species had been studied were grouped according to frequency of the range size of %GC regardless of taxonomic position. Comparison with species clearly shows that the diversity in composition increases from species to family. The same conclusion is reached for the values pooled from the literature (Fig. 2).

The highest intrageneric variation we recorded was 19.5% for the genus *Mucor* represented by 17 species (Table 9). Only the lowest value of the distribution, namely 29.5%, was isolated, whereas all other values from 34.5 up to 49.0% were closely grouped by intervals of 0.5 to 1% only (Table 8). It would appear that the true range extends from 35 to 49% with an average of 41.0%. Three other genera included in this survey had ranges higher than 10% GC, namely *Chaetomium* (Table 11), *Aspergillus*, and *Penicillium* (Table 13), with values of 11.5, 13, and 13%, respectively. In two cases (Fig. 3), the frequency distribution is unimodal. This situation also prevails in the

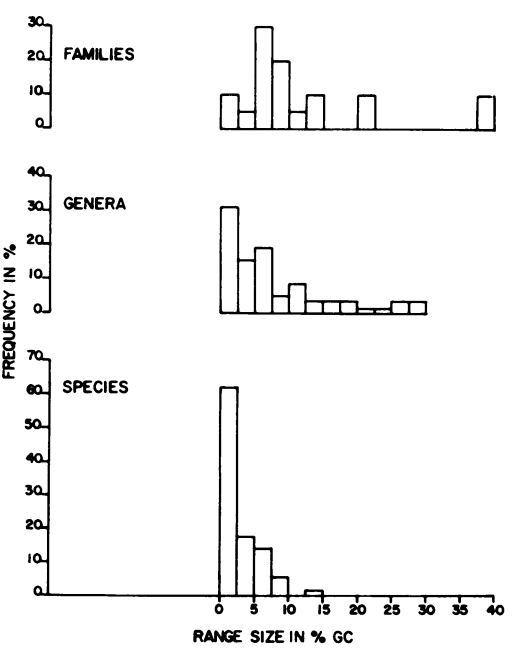

FIG. 2. *Frequency distribution (expressed in per cent) of range size in %GC within species, genera, and families listed in Table 2.*

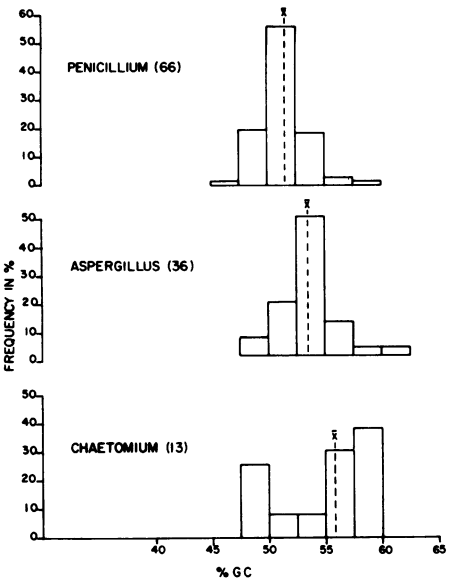

FIG. 3. *Frequency distribution (expressed in per cent) of the GC content values in the genera Penicillium, Aspergillus, and Chaetomium. Numbers in parentheses indicate the number of species analyzed in each genus.* $\bar{X}$ *= average.*

studies on other genera available in the literature. Some genera of yeasts, however, owe their high diversity in GC content to the existence of a bimodal frequency distribution (39, 44, 46; *see* Fig. 4 and 5).

It was indicated earlier, in the analysis of intraspecific diversity, that on the basis of the study of those species for which six or more isolates were determined (*see* Table 5), it was possible in all cases pertainine to the present study, with but one exception, to define a fungal species as a DNA population with a standard deviation of less than 2% GC. For those genera for which the standard deviation could be calculated with some reliance, we found the value of this dispersion index to vary from 0.88 to 6.34% GC. If, for taxonomic purposes, we were to attempt to give a definition of a genus on the basis of its population of DNA molecules, we would say that it is characterized by a standard deviation value of less than 10% and on the average close to 3% GC.

If the values for the standard deviation for families are grouped, one finds that they range from 1.00 to 7.17%, with an average value of 4.2%. It will, of course, be necessary to accumulate more values before a final attempt is made to define quantitatively a taxon by the dispersion indexes of the distribution of its GC content. It remains, nevertheless, that the diversity in DNA base composition increases, as would have been expected, from species to class.

With the considerations outlined above, it is now possible to proceed with the analysis of each taxonomic group separately.

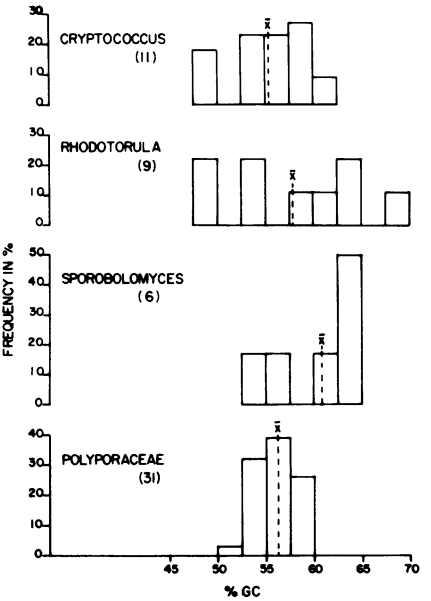

FIG. 4. *Frequency distribution (expressed in per cent) of the GC content values in the genera Cryptococcus, Rhodotorula, and Sporobolomyces, and in the family Polyporaceae. Numbers in parentheses indicate the number of species analyzed in each taxonomic group.* $\bar{X}$ *= average.*

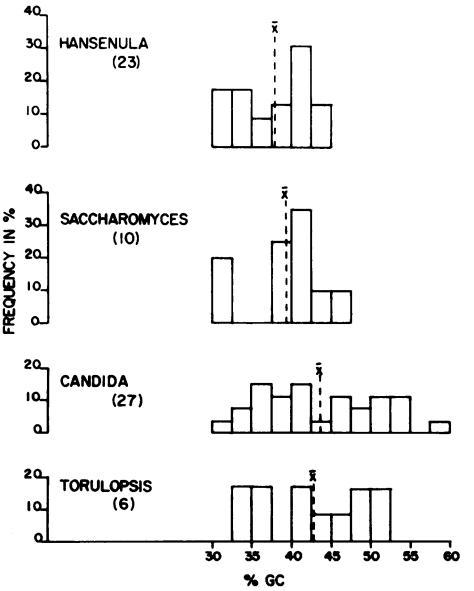

FIG. 5. *Frequency distribution (expressed in per cent) of the GC content values in the genera Hansenula, Saccharomyces, Candida, and Torulopsis. Numbers in parentheses indicate the number of species analyzed in each genus.* $\bar{X}$ *= average.*

TABLE 6. *GC content of DNA from Oömycetes*

Organism	Source[a]	GC content
Saprolegniales		
Saprolegniaceae		
Achlya ambisexualis	ESB	54.5
A. benekei	ESB	62
A. flagellata	ESB	55
A. klebsiana	ESB	44.5
A. oviparvula	ESB	46.5
Dictyuchus pseudoachlyoides	ESB	40.5
Isoachlya subterranea (received as *Isoachlya itoana*)	ESB	61.5
Protoachlya paradoxa	ESB	60.5
Saprolegnia ferax	ESB	49.5
S. hypogyna	ESB	55.5
S. parasitica	ESB	60.5
Thraustotheca primoachlya	ESB	45.5
Peronosporales		
Pythiaceae		
Phytophthora boehmeriae	Gal-N34	52.5
P. cactovorum	Gal-N261	53.5
P. calocasiae	Gal-N315	58
P. cinnamomi	Gal-N33	57
P. cinnamomi	Gal-N38	52
P. cinnamomi	Gal-N39	49
P. cinnamomi	Gal-N53	57
P. cryptogea	Gal-N57	52
P. fragariae	Gal-N72	54
P. heveae	Gal-N331	55
P. infestans	Gal-63B	54
P. palmivora	Gal-N137	53
P. parasitica	Gal-N211	50.5
P. parasitica-nicotianae	Gal-N15	49
Pythium pulchrum	ESB	51.5

[a] Abbreviations: ESB, E. S. Beneke, Biology Research Center, Michigan State University, East Lansing 48823; Gal, M. E. Gallegly, Department Plant Pathology, West Virginia University, Morgantown 26506.

Oömycetes

In the case of the *Oömycetes*, two families were analyzed, the *Saprolegniaceae* and the *Pythiaceae*. Individual values are listed in Table 6. To the best of our knowledge, published studies on *Oömycetes* are limited to a *Sapromyces* species (43) with 27% GC and to *Phytophthora infestans* (15) with 47.5% GC.

The values listed in Table 6 have been grouped, and statistical indices have been calculated and are listed in Table 7. Inspection of this table shows that the two families analyzed have the same average GC content but that they differ greatly with respect to the values of their dispersion indices. Clearly, a comparison between the two families and, by the same token, the respective order to which they belong is limited by the fact that only two genera of the *Pythiaceae* were surveyed as opposed to six of the *Saprolegniaceae*.

Our results indicate that the *Saprolegniaceae* analyzed fall into three groups, with GC ranges of 40.5 to 49.5, 54.5 to 55.5, and 60.5 to 62. Although this distribution is not an expression of traditional classification there are, nevertheless, some interesting coincidences which could prove to be of significance if a wider survey of the family were undertaken.

In terms of GC content, *Achlya klebsiana* and *A. oviparvula* appear to belong to one group, *A. ambisexualis* together with *A. flagellata* to to another, and *A. benekei* to a third. If the five species of *Achlya* analyzed are grouped in accordance with the currently accepted classification (26), we would have genus *Achlya*: subgenus *Achlya*, *A. klebsiana* (GC 44.5), *A. oviparvula* (GC 46.5), *A. ambisexualis* (GC 54.5), *A. flagellata* (GC 55.0); subgenus *Centroachlya*, *A. benekei* (GC 62). However, *A. klebsiana* is closer to *A. flagellata* morphologically than it is to *A. oviparvula* with which it shares a comparable GC content.

Again, of interest is the similarity of the GC content of *A. benekei* (GC 62), *Protoachlya paradoxa* (GC 60.5), and *Isoachlya subterranea* (GC 61.5).

Coker (16) states: "The relationships of the genus (*Protoachlya*) are not obvious. Either *Dictyuchus* or the *Racemosa* group of *Achlya* seems nearest." The *Racemosa* group is what

TABLE 7. *Statistical indices of the frequency distribution of GC content values for Oömycetes*[a]

Taxonomic group	N_s	N_i	$\bar{X}$	R	s^2	s	SE	PE
Oömycetes	24	27	53	40.5–62	28.15	5.31	1.08	3.58
Saprolegniales	12	12						
Saprolegniaceae	12	12	53	40.5–62	51.46	7.17	2.07	4.83
Achlya	5	5	52.5	44.5–62	40.1	6.34	2.83	4.27
Saprolegnia	3	3	55.2	49.5–60.5				
Peronosporales	12	15						
Pythiaceae	12	15	53.1	49–58	4.83	2.20	0.63	1.48
Phytophtora	11	14	53.2	49–58	5.02	2.24	0.67	1.51

[a] Abbreviations are indicated in Tables 1 and 3.

TABLE 8. *GC content of DNA from Zygomycetes*

Organism	Source[a]	GC content
Mucorales		
Choanephoraceae		
Blakesleea trispora	NRRL-2456	39
Choanephora cucurbitarum	NRRL-2744	40
Gilbertella persicaria	NRRL-1546	40
Cunninghamellaceae		
Cunninghamella baineri	ATCC-6796B	32
C. bertholletiae	HLL	27.5
C. blakesleeana	NRRL-1368	31
C. blakesleeana	ATCC-8688b	34
C. echinulata	ATCC-8688A	33.5
C. echinulata	ATCC-11585A	32.5
C. echinulata	ATCC-11585B	32.5
C. echinulata (−)	QM-6783, NRRL-1387	30.5
C. elegans	ATCC-6795B	31
C. elegans	ATCC-10025A	31
C. homothallica	NRRL-2365	29.5
C. verticillata	ATCC-8983	31
C. vesiculosa	NRRL-3009	28
Mycotypha africana	RSA-1193	43
M. microspora	UT-266	47.5
M. microspora	RSA-716	44.5
M. microspora	RSA-774	45.5
M. microspora	RSA-775	42
M. microspora	RSA-1183	46
M. microspora	RSA-1522	46
M. microspora	RSA-1559	43.5
Kickxellaceae		
Coemansia braziliensis	RSA-77	50.5
C. mojavensis	RSA-71	54.5
C. spiralis	UT-268	49
C. spiralis	RSA-1278	50.5
Dipsacomyces acuminosporus	RSA-1012	52.5
Linderina macrospora	RSA-1724	56
L. pennispora	NRRL-A12619	42
L. pennispora	RSA-3	30
Mortierellaceae		
Haplosporangium bisporale	NRRL-2493	52
Mortierella claussenii	NRRL-2760	50
M. isabellina	QM-6826, NRRL-1757	50
M. minutissima	NRRL-2591	49
M. parvispora (−)	NRRL-2942	50.5
Mucoraceae		
Absidia blakesleeana	QM-6774, NRRL-1304	52
A. cylindrospora (a)	NRRL-A12905	41.5
A. cylindrospora (b)	NRRL-A12872	40.5
A. regneri	QM-45b	59
A. spinosa	NRRL-2797	41
Actinomucor elegans	UT-199	40.5
A. elegans	RKB-12	41.5
A. elegans	RKB-162	40.5
A. elegans	RKB-349	40.5
A. elegans	RKB-605	41.5
A. elegans	RKB-860	42
A. elegans	RKB-1062	41
A. elegans	RKB-1702	43
A. elegans	RKB-1703	41
A. elegans	RKB-1704	41
A. elegans	RKB-1705	41
A. elegans	RKB-1706	42
Circinella linderi	QM-762, NRRL-2342	53.5
C. minor	QM-6939 NRRL-1453	53.5
C. muscae	QM-629	36
C. muscae	QM-629M	39.5
C. muscae	QM-7788	35.5
C. umbellata	USDA-A-12910	54.5
Mucor ambiguus	NRRL-1644	43.5
M. angulisporus	NRRL-2657	46
M. azygospora	NRRL-3068	36
M. bacilliformis	NRRL-2346	34.5
M. circinelloides	NRRL-223	43
M. fragilis	USDA-A-12253	39.5
M. genevensis	UT-121	40
M. griseo-cyanus	NRRL-1413	44
M. hiemalis	NRRL-1417	42.5
M. hiemalis	NRRL-1419	43.5
M. indicus	NRRL-555	40
M. jansseni	NRRL-2629	44
M. mucedo	NRRL-1425	29.5
M. pusillus	QM-436	48
M. racemosus	QM-79j	41
M. ramannianus	QM-6832, NRRL-1839	49
M. recurvatus	UT-122	35.5
M. subtilissimus	NRRL-1909	40
M. subtilissimus	QM-1060	41.5
Phycomyces blakesleeanus (+)	UT-151a	37.5
P. blakesleeanus (−)	UT-151b	39.5
P. blakesleeanus	NRRL-1464	41
Rhizopus arrhizus	UT-62-1	38
R. arrhizus	NRRL-1437	42
R. arrhizus	NRRL-2542	38
R. oligosporus	NRRL-514	40.5

[a] Abbreviations: NRRL, Northern Utilization Research and Development Division of the USDA, Peoria, Ill. 61604; ATCC, American Type Culture Collection, Rockville, Md. 20852; HLL, H. L. Lewis, National Cotton Council of America, Memphis, Tenn. 38101; QM, Pioneering Research Center, U.S. Army Natick Laboratories, Natick, Mass. 01760; RSA, Rancho Santa Ana Botanic Garden, Claremont, Calif. 91711; UT, University of Texas Isolate, Austin 78712; RKB, R. K. Benjamin (address same as RSA); USDA, (address same as NRRL).

TABLE 8.—*Continued*

Organism	Source[a]	GC content
R. oligosporus	NRRL-2549	38.5
R. oligosporus	NRRL-2710	40
R. oligosporus	NRRL-10.455	40.5
R. oligosporus	NRRL-A9848	40.5
R. oligosporus	NRRL-A9865	39.5
R. oligosporus	NRRL-A9867	38.5
R. oligosporus	NRRL-A9868	40
R. oligosporus	NRRL-A10.456	40.5
R. oligosporus	NRRL-A10.457	41
R. oligosporus	NRRL-A10.458	40.5
R. oligosporus	NRRL-A11.126	40
R. oryzae	UT-62-18	37.5
R. oryzae	NRRL-1526	40
Zygorhynchus moelleri	UT-193	39
Pilobolaceae		
Pilaira anomala	NRRL-2289	45.5
Syncephalastraceae		
Syncephalastrum racemosum	UT-184	49
S. racemosum (−)	RSA-24	50.5
S. racemosum (−)	RSA-229	51
S. racemosum (−)	RSA-232	50
S. racemosum (+)	RSA-235	51
S. racemosum (+)	RSA-236	51.5
S. racemosum (+)	RSA-237	50
S. racemosum (−)	RSA-673	51.5
S. racemosum (+)	RSA-674	52.5
S. racemosum (−)	RSA-699	48.5
S. racemosum (+)	RSA-702	51
S. racemosum	QM-709	48.5
S. racemosum	QM-8011	48.5
Thamnidiaceae		
Chaetocladium brefeldii	NRRL-2508	41
Cokeromyces poitrassi	RKB-903	37
C. poitrassi	RKB-1095	36
C. poitrassi	RKB-1245	36.5
C. poitrassi	RKB-1264	38
C. poitrassi	RKB-1267	36.5
C. recurvatus	RSA-1	31.5
C. recurvatus	RSA-918	32
Helicostylium piriforme (+)	RSA-537	50
H. piriforme (+)	RSA-866	54.5
Radiomyces embreei	RKB-914	46
R. embreei	RKB-984	50
R. embreei	RKB-985	46
R. embreei	RKB-1186	44.5
R. embreei	RKB-1372	44
R. embreei	RKB-1373	46
R. embreei	RKB-1374	46
R. embreei	RKB-1458	46
R. spectabilis	UT-242	47.5
R. spectabilis	RSA-1620	44
Thamnidium anomalum	RKB-80	46
T. anomalum	RKB-88	45.5

TABLE 8.—*Concluded*

Organism	Source[a]	GC content
T. anomalum	RKB-109	45
T. anomalum	RKB-110	44.5
T. anomalum	RKB-169	44.5
T. anomalum	RKB-356	45
T. anomalum	RKB-357	45
T. anomalum	RKB-358	45.5
T. anomalum	RKB-359	45
T. anomalum	RKB-360	45.5
T. anomalum	RKB-361	44.5
T. anomalum	RKB-362	46
T. elegans (−)	RKB-40	40.5
T. elegans (−)	RKB-74	55.5
T. elegans (−)	RKB-140	53.5
T. elegans (+)	RKB-166	55
T. elegans (−)	RKB-257	60.5
T. elegans (+)	RKB-258	37
T. elegans (+)	RKB-653	54
T. elegans	NRRL-2467	53
Entomophthorales		
Basidiobolus ranarum	UT-32	38

Johnson (26) separates as the subgenus *Centroachlya* in which *A. benekei* certainly belongs. (Although in the original description the oöspores of *A. benekei* are described as centric, in a recent article (19) they are stated to be centric or subcentric.) Our results would seem to support a *Protoachlya-Centroachlya* relationship rather than a *Protoachlya-Dictyuchus* affinity. *I. subterranea* has centric or subcentric oöspores as do most species of *Isoachlya*. Its GC content places it close to the subgenus *Centroachlya* of *Achlya* and to *Protoachlya*. This seeming correlation between GC content and centric oöspores species breaks down, however, when the three species of *Saprolegnia* analyzed are considered. *S. ferrax* (GC 49.5) and *S. hypogyna* (GC 55.5) both have centric oöspores. Obviously, a much larger number of species in the *Saprolegniaceae* must be analyzed for GC content before any conclusions as to their affinities can be reached.

The GC range in the *Pythiaceae*, based almost entirely on the genus *Phytophthora*, is 49 to 58%. One could postulate several groups were it not for the fact that the range within a single species (*P. cinnamomi*) covers almost the entire range for the genus. Therefore, no taxonomic or phylogenetic conclusions can be drawn without a much larger survey of this family.

Zygomycetes

As already indicated, all isolates analyzed were *Mucorales* with the exception of *Basidiobolus ranarum*. The values for all isolates listed in Table 8 were grouped and used for calculation of

TABLE 9. *Statistical indices of the frequency distribution of GC content values for Zygomycetes*[a]

Taxonomic group	N_s	N_i	$\bar{X}$	R	s^2	s	SE	PE
Zygomycetes	66	155	42.6	27.5–59	58.02	7.62	0.93	5.14
Mucorales	65	154	42.7	27.5–59	58.58	7.65	0.94	5.16
Choanephoraceae	3	3	39.5	39–40				
Cunninghamellaceae	10	21	33.2	27.5–47.5	32.16	5.67	1.79	3.82
Cunninghamella	8	13	30.4	27.5–32.5	3.15	1.77	0.62	1.19
Mycotypha	2	8	44	43–47.5				
Kickxellaceae	6	8	49.9	36–56	43.12	6.57	2.68	4.43
Coemansia	3	4	51.1	49–54.5				
Linderina	2	3	42.7	30–56				
Mortierellaceae	5	5	50.3	49–52				
Mortierella	4	4	49.9	49–50.5				
Mucoraceae	31	63	42.8	29.5–59	40.99	6.40	1.14	4.31
Absidia	4	5	46.8	40.5–59				
Circinella	4	6	45.4	35.5–54.5				
Mucor	17	19	41	29.5–49	23.78	4.88	1.18	3.29
Rhizopus	3	17	39.1	37.5–42				
Thamnidiaceae	8	40	43.8	32–52	41.37	6.43	2.27	4.33
Cokeromyces	2	7	35.4	31.5–38				
Radiomyces	2	10	46	44–50				
Thamnidium	2	20	48.1	37–60.5				

[a] Abbreviations are indicated in Tables 1 and 2.

statistical indices as shown in Table 9. In total, 154 isolates distributed among 65 species of *Mucorales* were analyzed. All the families of this order are represented with the exception of *Dimargaritaceae*, *Endogonaceae*, and *Piptocephalidaceae*. The number of genera and species analyzed varies from one family to another. In total, 65 species belonging to 24 different genera were studied. The survey of this large group of easily available organisms was given particular attention, and some interesting observations can be made on the basis of the data obtained. First, all the species for which more than six isolates were analyzed (*see* Table 5) yielded with one exception not much more compositional diversity than was found when repeated GC determinations were made on the same DNA sample (*see* Table 1). The exception mentioned above, namely, *T. elegans* for which eight isolates were studied, yielded a variance greater than 3.0. This does not appear to be a generic trait since the variance for the distribution of 12 isolates of *T. anomalum* is equal to 0.26. There were 23 species of *Mucorales*, including those listed in Table 5, for which at least 2 isolates were analyzed. (Table 8). In nine out of these 23 species, the range is equal to or less than 2.0, for six others it lies between 2.5 and 3.5, for four others it ranges from 4 to 4.5, and there were four other species with range equal to 5.5, 6, 12, and 23.5%. If one compares these various range values to those listed in Table 1, it appears that range values varying between 0 and 4.5 are not statistically significant. On this basis, one would conclude that the intraspecific compositional diversity among *Mucorales* is as a rule small. It is tempting to suggest that the narrow range indicates evolutionary stability and the wide range evolutionary activity within a species.

The average GC content in the various families of the *Mucorales* progresses from a low of 33.2 for the *Cunninghamellaceae* to a high of 50.3 for the *Mortierellaceae* and *Syncephalastraceae*, with the other families in between. This does not correlate with Benjamin's (8) concept of the relationships of these families (Fig. 6) in which the *Mucoraceae* and *Syncephalastraceae* are placed at approximately the same evolutionary level, with the *Mortierellaceae* at the next level and the *Kickxellaceae* at the top. Again, many more isolates in each family should be examined before any conclusions on the basis of GC content can be drawn.

Perhaps the most striking result of our survey of the *Mucorales* is the GC value for *Cunninghamella* (27.5 to 32.5) as compared to that for *Mycotypha* (43 to 47.5) which is now included in the same family. On this basis, it would appear that the position of *Mycotypha* in the *Cunninghamellaceae* should be re-examined. (It was recently suggested that the genus *Mycotypha* should be classified in the *Thamnidiaceae* on the basis of a study of spore structure (53).)

Most of the values for GC content of *Mucorales* species which are available in the literature and which originate from the laboratory of the senior

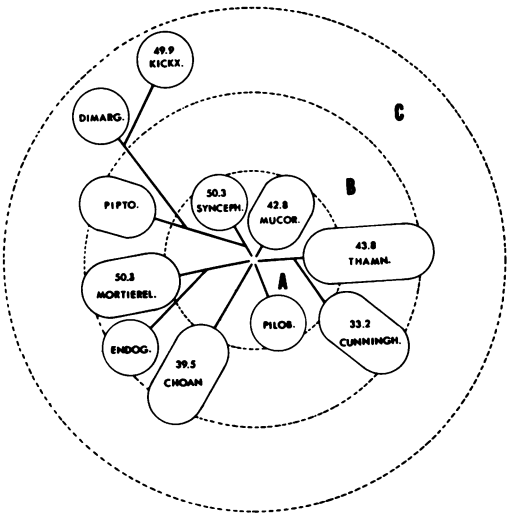

FIG. 6. *Phylogenetic relationships among the families of the Mucorales, according to Benjamin (8). In the figure above, some of the abbreviated family names are the arithmetic average of GC content found in Table 9. The abbreviations are as follows: Kickx., Kickxellaceae; Dimarg., Dimargaritaceae; Pipto., Piptocephalidaceae; Synceph., Syncephalastraceae; Mucor., Mucoraceae; Thamn., Thamnidiaceae; Mortierel., Mortierellaceae; Endog., Endogonaceae; Choan. Choanephoraceae; Pilob., Pilobolaceae; Cunningh., Cunninghamellaceae. (Reproduced with the author's permission.)*

author (45, 51) are in good agreement with those listed in Table 8. The same can be said for the few others also listed in Table 2. By comparing Tables 3 and 9 it appears that average %GC values for the *Mucorales* are very close. Thus, we can state that *Mucorales* and perhaps all *Zygomycetes* (*see B. ranarum*, Table 8) are, as a group, characterized by DNA with average GC content significantly lower than 50%.

Ascomycetes

A total of 90 ascomycete isolates distributed among 69 species was studied. With the exception of *Eremascus fertilis* and of two species of *Loculoascomycetidae*, however, the survey was limited to *Euascomycetidae*. The individual GC content values for the isolates are listed in Table 10. As for the other two classes previously analyzed, statistical indices were calculated (Table 11). The overall range is from 48.5 to 60.0. There was only one isolate out of 90 which had a %GC of 60.0, and only six had %GC values above 57.5 (Table 10). Thus, the range for the group of *Ascomycetes* analyzed here is quite narrow as compared to that for other classes (*see* Table 4 and Fig. 7). Most of the values of GC content for

TABLE 10. *GC content of DNA from Ascomycetes*

Organism	Source[a]	GC content
Hemiascomycetidae		
Endomycetales		
Endomycetaceae		
Eremascus fertilis	QM-6887	54
Euascomycetidae		
Plectomycetes		
Eurotiales		
Eurotiaceae		
Anixiopsis stercoraria	QM-8503	50.5
A. stercoraria	QM-8504	55
Aspergillus alliaceus[b]	QM-1885	52
A. aureolus[b]	QM-1906	54
A. niveo-glaucus[b]	UT-6	54
A. quadrilineatus[b]	QM-7465	52.5
A. violaceus[b]	QM-1905	52
Backusia terricola	QM-8602	52
B. terricola	QM-8603	52
Emericella nidulans	UT-23	51
E. nidulans	UT-24	54
E. nidulans (received as *Aspergillus nidulans*)	QM-1985	53
E. rugulosa	UT-65-6	54
E. variecolor (received as *A. variecolor*)	QM-1910	53
Emericellopsis salmosynnemata (syn. *Cephalosporium salmosynnematum*)	QM-6889	53.5
Eupenicillium baarnense (received as *Penicillium baarnense*)	QM-1871	54.5
E. brefeldianum (received as *P. brefeldianum*)	QM-1872	51
E. egyptiacum (received as *P. egyptiacum*)	QM-7553	48.5
E. javanicum (received as *P. javanicum*)	UT-135	51.5
E. javanicum (received as *P. javanicum*)	UT-136	53
Eurotium amstelodami (received as *Aspergillus amstelodami*)	QM-8405	56
E. amstelodami (received as *A. amstelodami*)	QM-8486	54
E. amstelodami (received as *A. amstelodami*)	QM-8486a	52

[a] Abbreviations: QM, Pioneering Research Center, U.S. Army Natick Laboratories, Natick, Mass. 01760; UT, University of Texas isolate, Austin 78712; ATCC, American Type Culture Collection, Rockville, Md. 20852.

[b] These are species of *Aspergillus* which form ascocarps but which have not been transferred to ascomycete genera.

TABLE 10.—*Continued*

Organism	Source[a]	GC content
E. herbariorum (received as *A. mangini*)	QM-7419	55
E. repens	UT-273	54.5
E. rubrum (received as *A. ruber*)	QM-360	53
E. rubrum (received as *A. ruber*)	QM-1973	54
Pseudeurotium multisporum	QM-7781	54
P. zonatum	QM-8030	57
Sartorya fumigata (received as *A. fischeri*)	UT-13	54.5
S. fumigata var. *glaber* (received as *A. fischeri* var. *glaber*)	QM-1903	51.5
Talaromyces avellaneus	UT-126	54.5
T. avellaneus (received as *P. avellaneum*)	QM-1849	52.5
T. avellaneus (received as *P. avellaneum*)	QM-7490	52
T. stipitatus	ATCC-10500	50
T. stipitatus	UT-144	49
T. vermiculatus	UT-230	49
T. vermiculatus (received as *P. vermiculatum*)	QM-1858	57
T. wortmanii	QM-7322	50
Thielavia sepedonium	QM-46a	55.5
Gymnoascaceae		
Amauroascus verrucosus	QM-1802M	53
A. verrucosus	QM-8502	53
Arachniotus flavoluteus	QM-8505	53
A. flavoluteus	QM-8506	52.5
A. reticulatus	QM-8507	52
Auxarthon brunneum	QM-8508	52
A. californiense	QM-8509	50
A. reticulatum	QM-8512	52.5
A. zuffianum	QM-8514	52
Byssochlamys fulva	QM-6766	51.5
Ctenomyces serratus	QM-8516	52.5
Eidamella deflexa	QM-8468	55
Gymnoascus reessii	QM-8517	54.5
G. reessii	QM-8521	55
G. uncinatus	QM-8590	51.5
Myxotrichum stipitatum	QM-8525	55.5
Petalosporus anodosus	QM-8526	53
Pseudoarachniotus citrinus	QM-8528	53
P. reticulatus	QM-7891	52.5
Pseudogymnoascus roseus	QM-6969	50.5
Toxotrichum cancellatum	QM-8534	50
Phaeotrichaceae		
Pycnidiophora dispersa	QM-7827	54
Microascales		
Ophiostomataceae		
Ceratocystis ulmi	QM-8426	53
Pyrenomycetes		

TABLE 10.—*Concluded*

Organism	Source[a]	GC content
Chaetomiales		
Chaetomiaceae		
Chaetomium bostrychodes	QM-6711	59
C. brasiliense	QM-623	51.5
C. caprinum	QM-6695	57.5
C. causiaeformis	QM-949	60
C. elatum	QM-606	56.5
C. fusisporum	QM-7960	49.5
C. globosum	QM-6694	57.5
C. globosum	QM-8199	57.5
C. globosum	QM-8402	58
C. globosum	QM-8495A	58
C. indicum	QM-46b	58.5
C. indicum	QM-8014	50
C. mollipilium	QM-1007	57
C. murorum	QM-6709	48.5
C. sub-spirilliferum	QM-8180	58.5
C. succineum	QM-1044	56.5
C. tenuissimum	QM-8178	57.5
Hypocreales		
Hypocreaceae		
Hypocrea chlorospora (received as *Trichoderma* sp.)	QM-1221	52
Sphaeriales		
Sordariaceae		
Gelasinospora autosteira (a)	UT-CR-4a	53
G. autosteira (A)	UT-CR-7A	53
G. autosteira (A)	UT-CR-10A	53.5
G. autosteira (a)	QM-7817A	53
Sordaria humana	QM-819	50.5
S. macrospora	QM-794	54
Loculoascomycetidae		
Pleosporales		
Pleosporaceae		
Leptosphaeria millefolii	QM-1285	56.5
Sporormiaceae		
Sporormia minima	QM-8592	53.5

Euascomycetidae listed in Table 2 originate from the laboratory of the senior author (45, 51); these and a few others scattered in the literature are in good agreement individually with those obtained in the present survey. These, as a rule, suggest that the subclass *Euascomycetidae* is characterized by a GC content higher than 50%.

Not enough families of *Acomycetes* were included in this survey to permit us to draw meaningful conclusions about the class as a whole. This short discussion will therefore center around the two orders best represented by the cultures analyzed.

Order Eurotiales. Of the four families (*Ascosphaeraceae*, *Gymnoascaceae*, *Eurotiaceae*, and *Phaeotrichaceae*) comprising the order *Eurotiales*,

TABLE 11. *Statistical indices of the frequency distribution of GC content values for Ascomycetes*[a]

Taxonomic group	N_s	N_i	$\bar{X}$	R	s^2	s	SE	PE
Ascomycetes	69	90	53.4	48.5–60	6.10	2.47	0.29	1.66
Eurotiales	48	62	52.7	48.5–57	3.08	1.75	0.25	1.18
Eurotiaceae	29	40	52.9	48.5–57	3.55	1.88	0.34	1.26
Aspergillus[b]	5	5	52.9	52–54				
Emericella	3	5	53	51–54				
Eupenicillium	4	5	51.5	48.5–54.5				
Eurotium	4	7	54.1	52–56				
Pseudeurotium	2	2	55.5	54–57				
Talaromyces	4	8	51.8	49–57				
Gymnoascaceae	18	21	52.5	50.5–55.5	2.32	1.52	0.35	1.02
Arachniotus	2	3	52.5	52–53				
Auxarthon	4	4	51.6	50–52.5				
Gymnoascus	2	3	53.6	51.5–55				
Pseudoarachniotus	2	2	53	52.5–53				
Chaetomiales	13	17	55.7	48.5–60	12.52	3.54	0.98	2.38
Chaetomium	13	17	55.7	48.5–60	12.52	3.54	0.98	2.38
Sphaeriales	3	6	52.5	50.5–54				
Sordaria	2	2	52	50.5–53				
Pleosporales	2	2	55	53.5–56.5				

[a] Abbreviations are indicated in Tables 1 and 3.

[b] These are species of *Aspergillus* which form ascocarps but which have not been transferred to ascomycete genera.

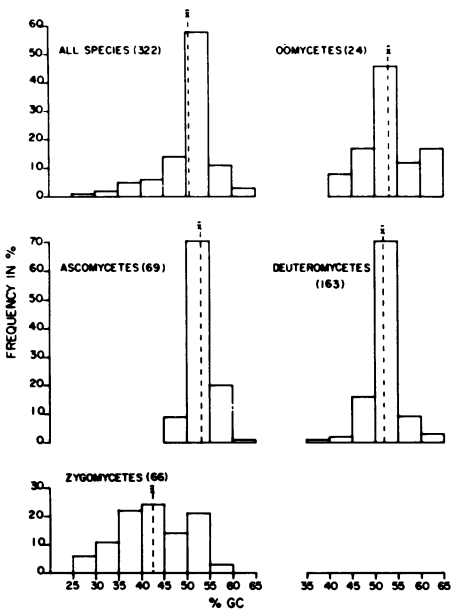

FIG. 7. *Frequency distribution (expressed in per cent) of the GC content values in the totality of the species and in the classes studied in the present work. Numbers in parentheses indicate the number of species analyzed in each taxonomic group.* $\bar{X}$ = *average.*

three are represented in this study but only two by enough species to make any discussion meaningful. [While this manuscript was approaching completion, a new family in the *Eurotiales*, the *Amorphothecaceae*, was described (D. G. Parbery, Aust. J. Bot. **17**:345, 1969). It consists of *Amorphotheca resinae*, represented in this survey by its conidial states *Cladosporium resinae* and *C. resinae f. avellaneum*, listed under *Deuteromycetes* in Table 12.]

With 12 of its genera included in this survey, the family *Gymnoascaceae* is well sampled even if only a few species—sometimes only one from each genus—were analyzed. The narrow range of GC content (50.5 to 55.5%) in the family indicates a very homogeneous taxon and makes it futile to speculate on relationships on a generic or specific level on this basis.

The same general comments are applicable to the *Eurotiaceae* with at least nine genera represented. (The perfect stages of the species of *Aspergillus* listed in Table 10 under *Eurotiaceae* have not yet been classified in any ascomycete genus. Two or more ascomycetous genera are probably represented.) The spread here, however, is somewhat greater than in the *Gymnoascaceae* and there is little overlapping between the two extremes (*Eupenicillium*, with a range of 48.5 to 54.5% GC, and *Pseudeurotium* with 54 to 57% GC). It is possible that analysis of many more cultures will give a better picture of evolutionary development in this family.

Order Chaetomiales. This is a small order with the single family *Chaetomiaceae* including a total of three genera, according to Ames (2), of which *Chaetomium* is by far the largest and the only one studied by us. The GC range in the 17 cultures

TABLE 12. *GC content of DNA from Deuteromycetes*

Organism	Source[a]	GC content
Melanconiales		
Melanconiaceae		
Pestalotiopsis sp.	QM-178	56
Moniliales		
Dematiaceae		
Bispora punctata	QM-7369	52.5
Cercospora salina	QM-8322	64.5
Chloridium viride	QM-1103	49.5
Cladosporium cladosporioides	QM-71d	49
C. cladosporioides	QM-489	50
C. herbarum	QM-3167	55
C. resinae	QM-8598	54
C. resinae f. avellaneum	QM-8042	51.5
Curvularia eragrostidis	QM-7931	53.5
C. lunata	QM-3728	53
C. maculans	QM-666	53.5
C. maculans	QM-4761	53.5
C. maculans	QM-6208	53
C. siddiquii	QM-8356	56
Curvularia sp.	QM-8133	52
C. verruciformis	QM-8326	52
Helicoma isiola	QM-760	54.5
Helminthosporium specifierum	QM-8535	53
H. speciferum	QM-8536	69.5
H. speciferum	QM-8562	52.5
H. speciferum	QM-8563	53
Humicola fuscoatra	QM-997	52.5
H. grisea (thermophilic)	QM-228	45.5
Nigrospora oryzae	QM-7977	42
N. sphaerica	QM-1253	62
Phialophora fastigiata	QM-265	49.5
P. lagerbergii	QM-267	40.5
P. verrucosa	QM-264	51
Spondylocladium atrovirens	QM-1793	52
Stachybotrys atra	QM-94d	49
S. atra	QM-1297	55.5
S. atra	QM-8497	55
Stemphylium callistephi	QM-1326	53.5
Stemphylium sp.	QM-1484	57.5
Torula ramosa	QM-1030	48.5
Moniliaceae		
Acrothecium arenarium	QM-8024	45
Arthrobotrys superba	QM-1688	50.5
Aspergillus ambiguus	QM-8155	54
A. avenaceus	QM-6741	51
A. awamori	QM-6949	52.5
A. awamori	QM-7397	52.5
A. candidus	QM-1997	53.5
A. carbonarius	QM-331	54
A. carbonarius (received as *Aspergillus fonsecaeus*)	UT-16	56
A. clavatus	UT-10	55
A. clavatus	QM-6884	52.5
A. conicus	QM-7405	61
A. fasciculatus	QM-6950	51
A. flavipes	UT-14	58
A. flavus	UT-15	51
A. flavus	QM-10e	51.5
A. flavus	QM-7637	50.5
A. flavus	QM-8190A	50
A. fumigatus	QM-1981	53
A. giganteus	UT-19	52
A. giganteus	QM-1970	52.5
A. giganteus	QM-7974	54.5
A. japonicus	QM-332	54.5
A. kanagawaensis	QM-7396	53
A. katsuobushi	QM-8157	54.5
A. luchuensis	QM-5565	54.5
A. niger	QM-1999	52.5
A. niger	QM-7922	54
A. niger	QM-8404	54
A. niger	QM-8487	53.5
A. niger	QM-8195A	52.5
A. niger var. *cinnamomeus*	QM-326	52.5
A. niveus	QM-6855	55
A. niveus var. *bifidus*	QM-7213	54
A. nutans	QM-8159	48
A. ochraceus	UT-22	54
A. ochraceus	QM-58c	52.5
A. ochraceus	QM-6731	53.5
A. oryzae	QM-1273	52.5
A. parvulus	QM-7955	48.5
A. phoenicis	QM-329	52.5
A. proliferans	QM-7462	55
A. quadricinctus	QM-6874	55
A. restrictus	QM-7305	52
A. sydowi	QM-4d	52.5
A. tamarii	QM-506	49
A. tamarii	QM-1223	49.5
A. tamarii	QM-6733	50.5
A. terreus	UT-29	56.5
A. terreus	QM-1991	55
A. terreus	QM-1992	55
A. terreus var. *boedijni*	QM-7473	57
A. terreus var. *floccosus*	QM-7474	55
A. unguis	QM-8f	54
A. unguis	QM-25b	53.5
A. ustus	QM-7477	55
A. versicolor	QM-4g	53
A. versicolor	QM-432	51.5
Beauveria tenella	QM-7954	53
Cylindrocephalum aureum	QM-523	54
Dactylium dendroides	QM-513	51.5
Gliocladium nigrum	QM-1240	60
Monosporium apiospermum	QM-7218	53.5
Paecilomyces varioti	QM-6764	50.5
P. varioti	QM-8377	50.5
P. varioti	QM-8492	51.5
Penicillium abeanum	QM-8154	51
P. adametzi	QM-1916	49.5
P. aeneum	QM-7290	53
P. atramentosum	QM-7483	50
P. braziliense	QM-7493	55

TABLE 12.—*Continued*

TABLE 12.—*Continued*

Organism	Source[a]	GC content
P. brevi-compactum	QM-7497	53
P. brevi-compactum	QM-8406	51
P. brevi-compactum	QM-8488A	52
P. capsulatum	QM-26c	51.5
P. capsulatum	QM-4869	52
P. casei	QM-7309	52.5
P. chrysogenum	UT-129	52
P. chrysogenum	QM-941	51
P. chrysogenum	QM-942	52
P. chrysogenum	QM-6861	51.5
P. chrysogenum	QM-7500	54.5
P. citreo-viride	QM-5720	53
P. claviforme	UT-128	52
P. claviforme (received as *P. silvaticum*)	QM-8040	50
P. clavigerum	QM-1918	52.5
P. corylophilum	QM-7510	52
P. cyaneo-fulvum	QM-7514	48.5
P. cyclopium	QM-8403	51
P. cyclopium	QM-8491	51
P. cyclopium	QM-8491A	52
P. daleae	QM-7551	52.5
P. decumbens	QM-1920	52
P. digitatum	UT-131	52
P. digitatum	UT-132	52
P. diversum	QM-1921	50
P. fellutanum	QM-5716	53.5
P. fellutanum	QM-7554	52.5
P. frequentans	UT-133	51
P. funiculosum	QM-28b	49.5
P. funiculosum	QM-8496A	50
P. funiculosum	QM-8496B	49.5
P. granulatum	QM-6868	50.5
P. griseo-azureum	QM-8150	51
P. griseolum	QM-7523	53
P. herquei	QM-7568	48.5
P. humuli	QM-7570	51.5
P. implicatum	QM-7573	54
P. italicum	UT-134	51.5
P. janthinellum	QM-6865	53.5
P. janthinellum	QM-8464	54
P. japonicum	QM-7298	50.5
P. jenseni	QM-7587	50.5
P. kojigenum	QM-7957	53
P. lilacino-echinulatum	QM-7289	49
P. lilacinum	QM-4e	61
P. lilacinum	QM-7592	59
P. lividum	QM-1930	51
P. luteo-caeruleum	QM-8151	50
P. martensii	QM-50a	50
P. megasporum	QM-6879	51
P. melinii	QM-1931	52
P. namyslowskii	QM-1932	55.5
P. notatum	UT-140	52.5
P. notatum	QM-946	51.5
P. notatum mut. *fulvescens*	QM-7296	51.5
P. ochro-chloron	QM-7604	47
P. olivino-viride	QM-7605	51

TABLE 12.—*Concluded*

Organism	Source[a]	GC content
P. oxalicum	QM-7606	53
P. phoeniceum	QM-7608	48.5
P. piscarium	QM-471	52.5
P. puberulum	QM-1556	52.5
P. puberulum	QM-7615	49
P. pulvillorum	QM-1935	51
P. purpurogenum	UT-141	54.5
P. purpurogenum var. *rubri-sclerotium*	QM-8042	48.5
P. raciborskii	QM-7620	52
P. radulatum	QM-7526	52
P. raistrickii	QM-1936	50
P. resedanum	QM-6966	50.5
P. roqueforti	UT-143	51
P. rugulosum	QM-7302	50.5
P. rugulosum	QM-7660	50
P. rugulosum	QM-7661	55
P. sclerotiorum	UT-227	50
P. simplicissimum	QM-6881	52
P. terlikowskii	QM-7687	50
P. variable	QM-2809	50
P. varians	QM-7691	49
P. velutinum	QM-7686	52
P. verruculosum	QM-3203	50.5
P. verruculosum	QM-3698	49.5
P. verruculosum	QM-7713	49
P. verruculosum	QM-7999	49
P. viridicatum	QM-7595	54
P. wentii	QM-44a	51.5
Polypaecilum insolitum	QM-7961	52.5
Scopulariopsis brevicaulis	QM-815	50
S. brevicaulis var. *glabra*	QM-6875	52.5
S. melanospora	QM-7884	53
S. repens	QM-399	51.5
Spadicoides xylogenum	QM-6817	54
Sporotrichum pruinosum	QM-168	58
S. pruinosum	QM-826	58
Trichoderma lignorum	QM-1275	51
T. viride	QM-1512	49.5
Trichothecium roseum	QM-102e	54
Verticillium niviostratum	QM-5187	35.5
Tuberculariaceae		
Epicoccum neglectum	QM-1070	51
Fusarium episphaeria	QM-7826	53
F. moniliforme	QM-527	51
F. moniliforme	QM-1224	50
F. moniliforme var. *minus*	QM-556	51
F. oxysporum	QM-21c	50.5
F. oxysporum	QM-47e	50.5
F. roseum	QM-38g	50
F. sambucum	QM-7162	51.5
F. scirpi	QM-660	51.5
F. solani	QM-21d	50.5
Myrothecium inundatum	QM-206	53
M. roridum	QM-188	50.5
Sphaeropsidales		
Chaetomella raphigera	QM-7359	53.5
Phoma pigmentivora	QM-502	49
P. terrestris	QM-120k	56

[a] Abbreviations: QM, Pioneering Research Center, U.S. Army Natick Laboratories, Natick, Mass. 01760; UT, University of Texas isolate, Austin 78712.

TABLE 13. *Statistical indices of the frequency distribution of GC content values for Deuteromycetes*[a]

Taxonomic group	N_s	N_i	$\bar{X}$	R	s^2	s	SE	PE
Deuteromycetes	163	220	52.1	35.5–64.5	10.67	3.27	0.25	2.20
Moniliales	159	216	52.1	35.5–64.5	10.68	3.27	0.25	2.20
Dematiaceae	27	35	52.4	40.5–64.5	23.55	4.85	0.91	3.27
Cladosporium	4	5	52.5	49.5–55				
Curvularia	6	8	53.3	52–56	1.80	1.34	0.54	0.90
Humicola	2	2	49	45.5–52				
Nigrospora	2	2	52	42–62				
Phialophora	3	3	47	40.5–51				
Stemphylium	2	2	55.5	53.5–57.5				
Moniliaceae	121	168	52.1	35.5–61	6.35	2.52	0.22	1.70
Aspergillus	36	56	53.5	48–61	5.85	2.42	0.40	1.63
Penicillium	66	90	51.5	47–60	3.85	1.96	0.24	1.32
Scopulariopsis	4	4	52	50–53				
Trichoderma	2	2	50	49.5–51				
Tuberculariaceae	11	13	51.2	50–53	1.01	1.00	0.31	0.67
Fusarium	8	10	51.1	50–53	0.78	0.88	0.31	0.59
Myrothecium	2	2	52	50–53				
Sphaeropsidales	3	3	52.8	49–56				
Phoma	2	2	52.5	49–56				

[a] Abbreviations are indicated in Tables 1 and 3.

analyzed was found to be 48.5 to 60%. It was disappointing to find that the species represented could not be grouped in any way but formed a continuous series with *C. murorum* (48.5% GC) at the bottom and *C. causiaeformis* (60% GC) at the top. Nor could any correlation be detected between GC content and various morphological characters such as shape of perithecium, asci, or ascospores or type of perithecial hairs, all of which are used to distinguish among species of this genus. Furthermore, in *C. globosum*, a notoriously variable species morphologically, the GC content of the four isolates available to us was remarkably constant!

With regard to the other orders, one might note that the values for *Gelasinospora* and *Sordaria* are the same as those described in the literature (*see* Table 2).

Deuteromycetes

A total of 220 isolates distributed among 163 species was surveyed. Individual values for the isolates are listed in Table 12. These values were grouped, and the indices were calculated and are indicated in Table 13.

With but four exceptions, the *Deuteromycetes* analyzed belong to the form-order *Moniliales*. The remarks below, therefore, pertain largely to that group.

Although small in comparison to the total, the number of deuteromycete species covered is large enough to be, perhaps, of some significance. In general, it may be said that nothing has been revealed to set these fungi apart from the other groups. The average GC content is close to that of the *Ascomycetes*, as might be expected, but it is equally close to that of the *Oömycetes* which would not be expected if such figures are to be regarded as indicators of relationship.

Dematiaceae and *Moniliaceae*, separated purely on color of conidiophore and conidia, are artificial groups. The *Tuberculariaceae* differ somewhat in the morphology of spore production from the others and might conceivably be regarded as a somewhat more natural group. The narrow range of GC content (50 to 53%) in this form-family (Table 13) might have been used as supporting evidence for that statement were it not for the fact that it is based almost entirely on the form-genus *Fusarium*.

In considering individual form-genera of the *Deuteromycetes*, we find them differing in homogeneity, but this again may be due in some instances to a difference in the number of species analyzed. *Cladosporium* (four species) shows a range of 49.5 to 55% GC, *Curvularia* (six species) 52 to 56%, and *Fusarium* (eight species) 50 to 53%. Again it is of interest to point out that *Fusarium*, considered as a very difficult form-genus taxonomically, because of the great variation exhibited within a species and even within a clone, has a relatively narrow range of GC content, contrary to expectations.

Form-genus Aspergillus. The GC content of the 41 species of *Aspergillus* analyzed (including five in the genera of Ascomycetes with *Asper-*

TABLE 14. *Distribution of GC content in groups within the form-genus Aspergillus*[a]

Group	R	N_s[b]	N_i
Cervinus	48–53	3	3
Flavus	49.5–52.5	5	10
Versicolor	52–54	3	4
Ochraceous	53.5	1	3
Clavatus	53–54	2	5
Glaucus	54.5–55	2[c]	2
Restrictus	52–61	2	2
Niger	52.5–55	7	13
Nidulans	54	1	2
Fumigatus	53–55	2	2
Candidus	53.5	1	1
Flavipes	54–58	3	3
Ustus	55	1	1
Terreus	55–57	3	5
Total	48–61	36	56

[a] Abbreviations are indicated in Tables 1 and 3.
[b] In determining the number of species, a variant was counted as a separate species.
[c] Includes one culture of *A. katsuobusi*, the identity of which is uncertain.

gillus conidial stages) varies from 48 to 61%. With one exception, different isolates of the same species agree within 2.5% in GC content. The exception is *Eurotium* (*Aspergillus*) *amstelodami* with a range of 4% (52 to 56%) in three isolates.

An attempt to correlate GC content with taxonomic groupings (40) of nonascosporic species based on morphology is illustrated in Table 14. This table shows that even though the GC ranges of many groups overlap, the groups may nevertheless be arranged in a series of more-or-less increasing GC content, with the *Cervinus* group, with a GC range of 48 to 53%, at one end of the scale and the Terreus group, with 55 to 57% GC, at the other. The main discordant note in the series is provided by *Aspergillus conicus* in the *Restrictus* group which has a GC content of 61%.

The *Ascomycetes* with *Aspergillus* type conidia all fall within the range of 52 to 55% GC, but many species of *Aspergillus* outside the cleistothecial group are also in this range so that no meaningful correlation can be established between sexuality and GC content. The same negative results are obtained when one attempts to correlate GC content with other morphological or physiological features such as uniseriate or biseriate sterigmata, color of conidial heads, etc.

It appears to us, nevertheless, that it may be worthwhile to investigate GC content of as many isolates as are available of all species that have been described. It is certainly possible that such an in-depth study of this ubiquitous form-genus may yield valuable results of taxonomic and phylogenetic significance.

Even less correlation exists in *Penicillium* between the morphological sections (41) and their GC content than in *Aspergillus*. If we list those sections of *Penicillium* from which more than one culture was included in this survey, we find little difference in the GC range of the groups: asymmetrica-divaricata, 47 to 61%; monoverticillata, 48.5 to 54%; biverticillata-symmetrica 48.5 to 55%; asymmetrica-fasciculata 49 to 54%; asymmetrica-velutina, 50 to 54.5%.

The picture does not change whether we include or exclude the eight species listed under the ascomycete genera *Eupenicillium* and *Tallaromyces* which have *Penicillium* conidial states. Thus, no evolutionary pattern is evident in *Penicillium* from this survey.

It will be noted that the *Deuteromycetes* investigated in the present research have been listed in accordance with the Saccardian system which places the *Deuteromycetes* into several form-families in four form-orders. The system is purely artificial, if convenient, and no one claims that its groupings reflect relationships. In recent years, Hughes, Tubaki, Subramanian, and others have stressed the manner in which conidia are produced rather than types, arrangement, and color of conidiophores and conidia, and a new system of groupings has emerged. The latest, most comprehensive treatise which classifies many deuteromycete genera in these new groupings, regardless of their position in the Saccardian system, is that of Barron (5).

TABLE 15. *Grouping of GC content of Deuteromycetes according to the series of Barron (5)*

Series	GC content range	
	A[a]	B[b]
Phialosporae	35.5–61	40.5–55.5
Aleurosporae	45.5–58	45.5–58
Porosporae	48.5–69.5	48.5–57.5
Annellosporae	50–56	50–56
Sympodulosporae	50.5–64.5	50.5–53
Arthrosporae[c]	54	54

[a] Gives the range of all the taxa that fall in the six series.
[b] Excludes a few taxa which show extreme %GC values.
[c] One culture of *Trichothecium roseum*, an anomalous fungus which may or may not belong to this series.

It appeared desirable, therefore, to see whether the GC range of the *Deuteromycetes* investigated by us in each of these groupings might support conclusions from developmental data. Table 15 arranges six of Barron's series, in which our taxa belong, in order of increasing lowest-GC value. Column A gives the range of all the taxa that fall in the six series; column B excludes a few taxa which show extreme GC values and which perhaps should be reinvestigated. As is evident from these groupings, there is a complete overlap which makes futile any attempt to support or contradict this system.

The number of determinations reported in the literature on *Deuteromycetes*, other than members of the *Cryptococcaceae*, is limited to 10 isolates representing 9 species. The GC values for these range from 46 to 52% with an average of 49.7% (Table 3). Clearly, *Deuteromycetes*, if one excepts the yeast forms to be discussed later, are characterized by an average GC content of about 50.0%.

Homobasidiomycetidae

As already indicated in the Introduction, experimental surveys other than the present one have been limited so far to yeasts and yeastlike fungi and to a few *Homobasidiomycetidae*. The %GC values are listed in Table 2 together with the few others that are also available in the literature. We will discuss first the case of the *Homobasidiomycetidae*.

The only survey of some scope is, as already indicated, that on some species belonging to the families *Hymenochaetaceae* and *Polyporaceae*. The *Polyporaceae* present a good case for the assessment of the taxonomic value of DNA base composition because modern systems of classification are based on cultural and physiological characters in addition to morphological ones. The major features of the survey on the polypores can be summarized as follows: (i) the GC contents of the DNA samples of 50 isolates averaged 55.6% and ranged from 50 to 59.5% GC. (ii) There were 16 species for which two or more isolates were analyzed. Of these, only four species had a range in %GC greater than 1.5. It would appear, therefore, that DNA GC content may possibly have some taxonomic significance at the species level, but many more species must be analyzed before conclusions can be reached. (iii) There were several instances in which newer classifications based on physiological characters produced a correlation between these and the GC contents. For example, brown polypores such as *Inonotus dryophilus* and *Phellinus gilvus* and *P. ferruginosus* are excluded from the family *Polyporaceae* and are included in the family *Hymenochaetaceae* along with the resupinate genus *Hymenochaete*. The four values of %GC for this group formed a discrete cluster not overlapping with the distribution of the other groups. (iv) The results confirm that the DNA is characterized by an average GC content significantly higher than 50% and that the compositional diversity for this class appears, as mentioned above, to be much smaller than that for classes considered to be less evolved such as *Zygomycetes* and *Ascomycetes* (Fig. 4).

Yeasts and Yeastlike Fungi

We have already indicated that caution should be exercised when comparing values obtained in various laboratories because different methods are often used for the determination of GC content and also because the origin of strains or isolates is often not indicated. Before making an attempt to evaluate the taxonomic and phylogenetic significance of GC content for this group of fungi, it is also very important to stress the fact that "the yeasts" do not necessarily represent a natural group. The morphological changes accompanying the life cycle (asexual or sexual) in the filamentous fungi are often missing among the yeasts and yeastlike fungi. Also, unlike the situation in many "filamentous" fungi, in "yeasts" the occurrence of heterocaryosis is almost completely absent so far as is known at present. With regard to phylogeny, some consider the true yeasts as having been derived from the mycelioid forms by reduction, thus as degenerate organisms, whereas others regard them as intermediate forms between *Zygomycetes* and *Ascomycetes*. The possible contribution of the analysis of DNA base composition to this controversy will be discussed in the last part of the present paper. Here, we wish to discuss the yeasts and yeastlike fungi themselves. All the %GC values listed in Table 2 were grouped, and average values characteristic for species were used for the calculations of statistical indices. Thus, when two or more strains or isolates of the same species were studied, their individual %GC values were averaged. The frequency distribution of %GC for 124 species distributed among *Ascoidiaceae*, *Endomycetaceae*, *Saccharomycetaceae*, *Cryptococcaceae*, and *Sporobolomycetaceae* is characterized by a range of 25 to 70% (Fig. 8). [The value of 70% corresponds to a single value, *Rhodotorula graminis* (Table 2), and is the highest ever reported for a fungus.] The overall shape of the histogram suggests the existence of a bimodality. It is interesting, therefore, to find out whether this situation is a re-

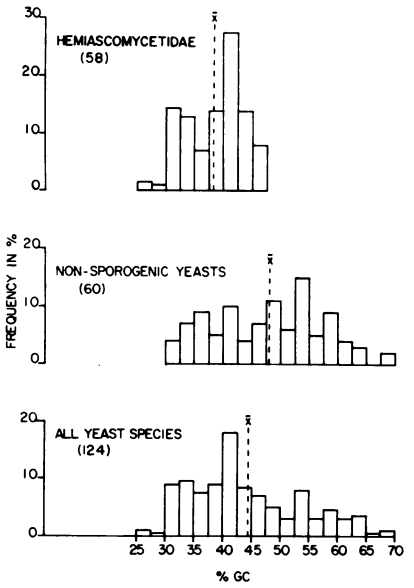

FIG. 8. *Frequency distribution (expressed in per cent) of the GC content values in Hemiascomycetidae, nonsporogenic yeasts, and in all the species of yeasts listed in Table 2. Numbers in parentheses indicate the number of species analyzed in each taxonomic group.* $\bar{X}$ = *average.*

flexion of taxonomic position or a sampling artifact. Using the same class interval values, the results of the survey on *Hemiascomycetidae* and on the anascosporogenous yeasts were grouped. As shown in Fig. 8, the frequency distributions of these two groups are quite different. (The same is true if, as shown in Fig. 9, the values are grouped as *Saccharomycetaceae* and *Cryptococcaceae*.) This leaves no doubt that the bimodality mentioned above is now resolved. The lower mode is that of the *Hemiascomycetidae* and the higher one that of the anascosporogenous yeasts. It should be noted in passing that the 124 species of yeast include 6 of *Sporobolomyces* which are not included in the histogram for anascosporogenous yeasts. The range of the *Hemiascomycetidae* is 25 to 47.5% GC, and it coincides (Fig. 5) with those (30 to 47.5) for the genera *Hansenula* and *Saccharomyces*. It might be remarked that only one species, *Pichia kluyveri* (*see* Table 2), had a GC content lower than 30%, and at the other end two species with values falling in the class 45 to 47.5% GC. Among the 59 species of anascosporogenous yeasts, there are 26 or 44% with GC content falling within 30 to 47.5% and the remaining 56% within 47.5 to 70%. The lower values are due primarily to some *Candida* and *Torulopsis* species (Fig. 5) and for which a total of 32 species out of a total of 59 for the nonsporogenous organisms was studied. Within the genus *Candida*, we found 65.4% of the species to have less than 47.5% GC. This 65.4% corresponds to 17 individual species of *Candida* which in turn correspond to 28.8% of the total number of anascosporogenous species analyzed.

Thus, among the anascosporogenous yeasts one finds only one genus, *Candida*, where more than 50% of the species analyzed have %GC values falling in the range characteristics for the ascosporogenous yeasts, suggesting that they are the corresponding imperfect forms as was suspected for a long time. The remainder of the genus *Candida*, together with the totality of the genera *Cryptococcus* and *Rhodotorula*, appear to have different relationships. The genus *Rhodotorula* has been suspected of being composed of *Sporobolomyces* species which have lost the ability to produce ballistospores. [It was recently suggested (49) that ballistospores are not basidiospores or meiospores because ballistospores of diploid and haploid colonies give, again, rise to the diplophase and haplophase, respectively.] Recently, Banno (4) obtained spore formation in mixed cultures of two strains of *Rhodotorula glutinis* which has a DNA with 64% GC (38) and established a new genus, *Rhodosporidium*, in the *Ustilaginaceae*. It is less evident but possible that this might also be the case for some *Cryptococcus* and *Candida* species.

Nakase and Komagata (38) surveyed several genera of anascosporogenous yeasts and found that organisms with strong urease activity had

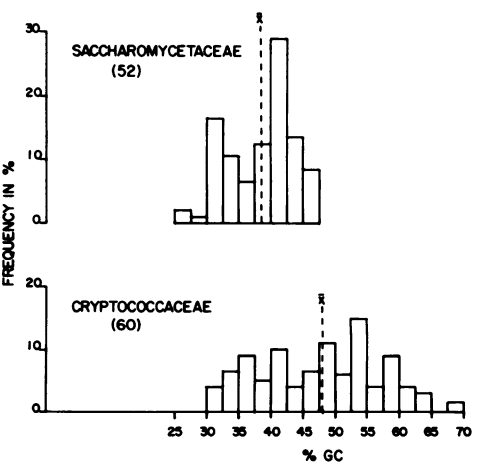

FIG. 9. *Frequency distribution (expressed in per cent) of the GC content values in Saccharomycetaceae and Cryptococcaceae. Numbers in parentheses indicate the number of species analyzed in each family.* $\bar{X}$ = *average.*

high GC contents without exception. This group included *Cryptococcus*, *Rhodotorula*, *Sporobolomyces*, *Torulopsis*, *Candida*, and *Trichosporon*, all genera which, according to the authors, "are supposed to be related to the heterobasidiomycetes."

As was indicated above, the frequency distribution of %GC values among all the yeasts analyzed was bimodal. Inspection of the histograms in Fig. 4 and 5 reveals that bimodality also prevails at the level of genera such as *Hansenula*, *Sporobolomyces*, *Rhodotorula*, and *Cryptococcus*. The first genus listed was analyzed in detail by Nakase and Komagata (39). They found that the GC content of DNA ranged from 28.5 to 46.3%. The first group, had GC content with maximal frequency in the range of 40 to 42%. The second possessed relatively low values with the maximal frequency in a range of 32 to 34%. They found that all the species which produce saturn-shaped ascospores belong to the first group. Species which produce hat-shaped ascospores were found in both groups. This intrageneric variation suggested to the authors heterogeneity of this genus. They concluded that GC content has a significance since "several small groups with similar DNA base ratio such as species arranged in each line of Wickerham's phylogenetic scheme or serological groups were discriminated within the genus *Hansenula*." They conclude: "since the taxon "Genus" should be comprised of closely related species, intrageneric variation of DNA base ratio should be within narrow range. Therefore, division of *Hansenula* into several small genera should be inevitable in the future."

The intrageneric variation in *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces* was analyzed by Storck et al. (46). These authors found that within each of these genera, the two groups of GC content were characterized by averages which on the basis of a "t" test appear to differ significantly from each other. Thus, as for the case of *Hansenula*, it is possible that the results of the DNA GC content analysis will suggest a reexamination of these genera and ultimately lead to a redistribution of the organisms into two or more genera. This might possibly be the case for other yeast genera such as *Saccharomyces* and *Candida* (Fig. 5). Significant intrageneric variation is not exclusive for yeasts and yeastlike fungi, and we have described it earlier for some *Mucorales* and *Oömycetes*. It remains, however, that by and large the greatest intrageneric diversity in GC content is found in this group of organisms. It is tempting to suggest that the cause for this diversity might be found in the fact that most yeasts exist as unicellular organisms and that this stage favors the selection of mutants in populations, thereby increasing the variation in the DNA composition within a taxon. Finally, in terminating the analysis of the base composition of yeast DNA one should emphasize that the GC content of *Dipodascus uninucleatus* and *D. albidus* are (Table 2) 43 and 33%, respectively. These values are near the range of overlap between *Zygomycetes* and *Ascomycetes*; thus, the suggestion that *Dipodascus* is a key intermediate (23) is still tenable. One might also point out that the same would apply to the *Endomycetales* (*see* Fig. 10).

In conclusion, one can state with some degree of confidence that the survey of yeasts and yeastlike fungi has been rewarding so far since the results point to the fact that when more analyses become available, regrouping of species might be necessary.

Phylogenetic Significance of the Diversity in GC Content of Fungal DNA

The question of the origin and evolution of the fungi has been debated by mycologists ever since the theory of evolution became accepted as the most logical explanation of the fundamental similarities exhibited by all living organisms. It is superfluous to trace here the various concepts concerning the origin of the fungi. Suffice it to say that two theories have dominated mycological thought for some years, one supported

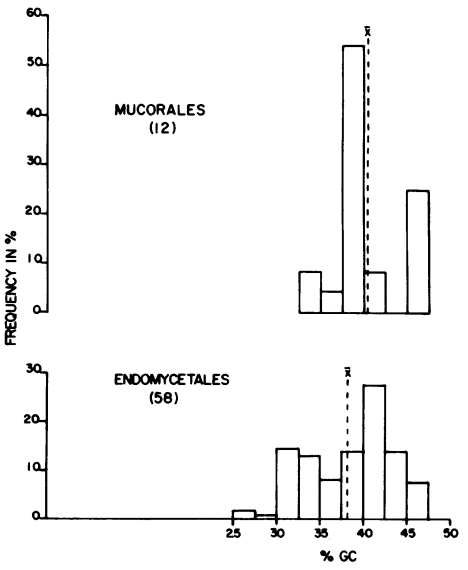

FIG. 10. *Frequency distribution (expressed in per cent) of the GC content values in Mucorales and Endomycetales as reported in Table 2. Numbers in parentheses indicate the number of species analyzed in each order.* $\bar{X}$ *= average.*

by Gäumann (23) in Europe, the other by Bessey (9, 10) in America. The former supposed that the fungi, with the exception of the *Oömycetes*, arose from an ancestral flagellate and developed monophyletically, whereas the *Oömycetes* originated independently from the heterosiphonaceous algae. The latter supported the view that the *Phycomycetes* originated from heterocont unicellular algae and evolved along three pathways, and that the *Ascomycetes* came from ancestral red algae and eventually gave rise to the *Basidiomycetes*. Both these theories are based on comparative morphology. In addition, several excursions of a general nature into other fields have been attempted. The first was by Mez (37) who based his phylogenetic system on serology. This was followed by Cantino (13) who attempted to determine whether physiological criteria could be utilized to determine phylogenetic relationships among aquatic *Phycomycetes*. Cantino's conclusions are quite interesting, but they relate only to one group of fungi in a special habitat. Although Mez's work was a pioneering effort, his results are not considered conclusive in the light of modern methods dealing with the pathway for lysine biosynthesis (52), the physicochemical characteristics of the enzymes involved in the biosynthesis of tryptophan (25), and the chemical composition of the cell wall (6).

With regard to the first type of study (52), 26 fungal species were surveyed in addition to others belonging to different taxonomic groups. Whereas the diaminopimelic acid pathway is present in bacteria, blue-green algae, green algae, vascular plants, and among fungi in the *Oömycete* orders, *Saprolegniales*, *Leptomitales*, *Lagenidales*, *Peronosporales*, and in the *Hyphochytriales*, it is the α-aminoadipic pathway which is found in *Chytridiales*, *Blastocladiales*, *Monoblepharidales*, *Mucorales*, *Endomycetales*, *Taphrinales*, *Plectascales*, *Pseudosphaeriales*, *Sphaeriales*, *Hypocreales*, *Pezizales*, and also among *Basidiomycetes* such as *Ustilaginales*, *Polyporales*, *Agaricales*, and *Lycoperdales*. Of significance is the fact that among those species in which the last type of pathway was found are *D. uninucleatus*, *Candida utilis*, and *Saccharomyces cerevisiae*. We should, as far as the present discussion is concerned, note that *Oömycetes* and *Zygomycetes* have a different pathway. This fact suggests an evolutionary independence of these two taxonomic groups. The presence of the same pathway in *Zygomycetes*, *Hemiascomycetidae*, *Euascomycetidae*, *Hetero-* and *Homobasidiomycetidae* and the *Deuteromycetes* argues for a monophyletic origin of these taxa.

The study of the enzymes involved in the biosynthesis of tryptophan (25) dealt with 22 different species of fungi. Among these, four different patterns of enzyme associations could be recognized. On this basis, biflagellated *Oömycetes* could be distinguished not only from the uniflagellated aquatic *Phycomycetes* but also from the *Zygomycetes* and the other classes of *Eumycotina*. Type I pattern was present in *Chytridiales* (*Rhizophlyctis*) and in *Aspergillales* (*Eurotiales*) (*Aspergillus*, *Byssochlamys*), type II in *Endomycetales* (*Dipodascus*, *Endomyces*, and *Saccharomyces*), and type III in *Zygomycetes* (*Mucor*, *Rhizopus*, *Phycomyces*). On the basis of these findings, the authors suggest to abandon *Zygomycetes* and *Endomycetales* as possible intermediates between the chytrids and the *Aspergillales* and to adopt a "more direct *Chytridiales* → *Aspergillales* relationship, with the *Zygomycetes* and *Endomycetales* being sidelines in evolution." This survey further revealed the same type of enzyme pattern in *Mucorales*, *Cryptococcus laurentii*, *Rhodotorula glutinis*, *S. salmonicolor*, *Tremella mesenterica*, and *Ustilago maydis* which was taken as an indication of a close relationship between "yeasts" and *Heterobasidiomycetidae*. Since the species of *Coprinus* and those of *Polyporus* have a different type of pattern, a polyphyletic origin of the *Basidiomycetes* was indicated.

The study of the chemical composition of the cell wall appears to show great sign of promise for the taxonomy not only of larger categories but also of smaller taxa such as genera. However, at the present time, generalizations based on the use of this criterion should be accepted with caution because, as in the case of the lysine biosynthetic pathway, and the pattern of the tryptophan biosynthetic enzymes, the number of species which has been analyzed is still too small. In his review (6) Bartnicki-Garcia establishes eight groups "based on dual combinations of those polysaccharides which appear to be the principal components of vegetative walls." The cell wall in the groups *Chytridiomycetes*, *Ascomycetes* (excluding *Saccharomycetaceae*), *Basidiomycetes* (excluding *Sporobolomycetaceae*), and *Deuteromycetes* (excluding *Cryptococcaceae* and *Rhodotorulaceae*) is composed primarily of chitin and glucan. *Oömycetes*, *Hyphochytridiomycetes*, and *Zygomycetes* have the following pairs, respectively: cellulose-glucan, cellulose-chitin, and chitosan-chitin. Thus, these results, in agreement with those for the tryptophan synthesis enzyme pattern, suggest a regrouping of the *Chytridiomycetes* with the *Ascomycetes* and the *Homobasidiomycetidae*. Of great interest also is the fact that on one hand *Saccharomycetaceae* and *Cryptococcaceae* fall into the group mannan-

glucan, and on the other hand the *Sporobolomycetaceae* and *Rhodotorulaceae* fall in the group mannan-chitin.

It is appropriate now to try to determine whether phylogenetic trend(s) can be developed on the basis of %GC determinations. If so, it becomes of interest to compare the results of such an analysis to those outlined above. Storck (45) showed earlier that there is a gradual and possibly significant rise in the average GC content from *Zygomycetes* to *Ascomycetes/Deuteromycetes* to *Basidiomycetes*. As shown in the present survey (*see* Tables 3 and 4), this trend is entirely confirmed. There is, of course, as might be expected, a considerable overlap in the %GC range between consecutive classes arranged in this order. The range for *Zygomycetes* is greater than that for the other classes analyzed. However, it must be kept in mind that whereas the figures for the *Zygomycetes* are fairly representative of the class, those for the *Ascomycetes* and *Deuteromycetes* are not.

The average and range for *Oömycetes* are almost exactly the same as those of *Ascomycetes*, which, if a %GC increase within the entire group of fungi is interpreted as a criterion of evolutionary advancement, would indicate either that the *Oömycetes* are more advanced than the *Zygomycetes* on a monophyletic scheme or that they have had a separate origin from all other fungi, as Gäumann postulates, and have evolved in a parallel manner. The latter hypothesis appears to be much more probable in view of the results of biochemical analysis outlined above and the probable gametangial meiosis resulting in a diploid thallus (12). It would be interesting now to have figures on the GC content of some *Chytridiomycetes* and *Hyphochytridiomycetes* to see how they would compare to those of the *Oömycetes* and *Zygomycetes*. The three published values (43) for *Blastocladiella*, *Rhizophlyctis*, and *Rhizophydium* set the %GC range for the *Chytridiomycetes* at 44 to 66%, which fits nowhere logically in this scheme.

The possible relationships of the yeasts in the phylogenetic system was discussed in the previous section. The reported GC range (26.5 to 47.5% GC) of the ascosporogenous yeasts (Table 3) falls within the range of the *Zygomycetes* (27.5 to 59% GC), reaching close to the lower limits (48.5 GC) of the relatively few *Ascomycetes* included in the present survey. Perhaps of greater significance is the fact that the average %GC for the *Zygomycetes* is closer to that of the *Hemiascomycetidae* than to that of the *Euascomycetidae*. This might be taken to indicate that the yeasts are primitive rather than reduced forms. In line with this phylogenetic scheme, the *Plectomycetes* (*Gymnoascaceae*, *Ophiostomataceae*, and *Eurotiaceae*) should have GC values intermediate between the yeasts and the *Pyrenomycetes/Loculoascomycetes*. There is a slight suggestion that this is the case as the following groupings indicate: *Plectomycetes: Gymnoascaceae*, 50 to 55.5 (average, 52.5); *Eurotiaceae*, 48.5 to 57 (average, 52.9); *Ophiostomataceae*, 53 (1 value); *Pyrenomycetes: Chaetomiaceae*, 48.5 to 60 (average, 55.7); *Sordariaceae*, 50.5 to 54 (average, 52.5); *Hypocreaceae*, 52 (1 value); *Loculoascomycetes: Pleosporaceae*, 56.5 (1 value); *Sporormiaceae*, 53.5 (1 value).

Taphrinales and *Discomycetes*, two phylogenetically interesting groups, are not included in this survey. Both Bessey and Gäumann (9, 23) consider the *Taphrinales* to be reduced *Discomycetes*, but the two theorists are at odds in their view of the phylogenetic position of the *Discomycetes*.

Following the same line of reasoning of considering high GC values as indicating advancement, we would have to concede that the yeasts with basidiomycetous affinities and high GC values are reduced *Basidiomycetes*. In view of what we have postulated for the ascomycetous yeasts, this appears somewhat anomalous but not impossible (*see* also results of cell wall analyses).

Thus, it is difficult from the data at hand to support or deny either of the well-known morphological theories on the phylogeny of the fungi. Some very general trends have been pointed out; but if DNA base composition is to be evaluated as a phylogenetic criterion, it is obvious that only when we have data for a very much greater number of fungi from all classes will this become possible.

In the previous paragraphs, it was suggested that the evolution of fungi could have been accompanied by a progressive increase in the GC content of DNA. It is perhaps appropriate at this point to discuss the possible theoretical and molecular basis for such a unidirectional change. Clearly, this discussion must be limited to fungi since no data are available for other groups of eucaryotic microbes and, furthermore, higher forms of life tend to have a narrow range of GC content values (18, 43). However, increment in %GC accompanying evolution is also found among bacteria for the *Actinomycetes*. These organisms have %GC ranging from about 63 to 75% (18, 24, 32) and are considered in view of the occurrence of the mycelial stage as the product of evolutionary convergence vis-a-vis the fungi. Some authors (22, 47) have formulated mathematical models accounting for slow changes in DNA base composition as a result

of single-base substitutions resulting from mutation. These studies permit an estimate of the time needed for significant changes in GC content, but there is nothing in their model that indicates or even suggests that there should be a unidirectional change toward higher GC content. Furthermore, these models apply to prokaryotic genomes and it is not certain that they could be used for eucaryotic organisms. Indeed, in higher forms there is a high redundancy in DNA (11), suggesting that in these forms the evolution of DNA is not merely the result of straightforward single-base substitutions. Again, for bacteria it was claimed that by mutation one could observe a substantial change in %GC. Although the results of such studies are controversial (30), they do not support our thesis since the observed GC content changes occur in both directions. In the case of *Escherichia coli*, a gene called "mutator" induced a high frequency of mutations in which AT pairs are replaced by GC ones (17). This mutation led to an increase in GC content of 0.3%. There is, to the best of our knowledge, no demonstration for the existence of such a type of gene in fungi. On an a priori basis, one could hold the view (55) that since AT base pairs are held together less strongly than GC base pairs, an increase in the GC content would increase the stability of DNA.

CONCLUSION

The taxonomic "resolving power" of the base composition of DNA varies with the group under consideration. In most cases, various taxonomic groups (species, genera, families, etc.) have a dispersion of %GC values which does not fluctuate by more than a certain value, irrespective of the specific denomination.

The phylogenetic "resolving power" of the base composition of DNA is limited for fungi and will remain so until a much larger number of species has been analyzed. There are practically no data on the amount of DNA per genome in fungi. There is indication from the study of other eucaryotic organisms that evolution has been accompanied by an increase in the amount of DNA per genome. Thus, final appraisal of the phylogenetic significance of GC content will require a knowledge of the extent of the variation of DNA per genome.

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ADDENDUM IN PROOF

We call to the reader's attention two articles now in press: one by S. A. Meyer and H. J. Phaff on the taxonomic significance of %GC in yeasts and another by C. E. Singer and B. N. Ames on ultraviolet light as a selective factor toward high %GC in bacteria.

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