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POLYPLOIDY IN DISCOMYCETES

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Polyploidy is quite a common phenomenon in discomycetes. The ploidy levels have been explored by means of microspectrophotometric determination of the relative nuclear DNA content. In rare cases all the examined species of a genus have the same relative DNA contents, i.e. are to be regarded as belonging to one definite ploidy level. Most genera, however, contain species with different ploidy levels. The lowest measured amount of DNA is presumed to represent the haploid state, which is not very common. The most frequent ploidy levels are diploid, triploid, and to some extent also tetraploids. Higher-ploids occur less frequently. Moreover a correlation between grade of ploidy and period of fructification, substrate type and altitude (or climate) has been found. Within the life cycle of a species the relative DNA content of the resting nucleus is constant. Fungal material of the same species but of different origin normally has same DNA values. Some exceptions are described as examples for cryptical speciation.

Polyploidy plays an important role in evolution and speciation. Rogers (1973) regarded it as 'a factor in the evolution' of many groups of fungi. However, in fungi very little is known about such phenomena, because the minute size of fungal chromosomes and their disinclination to individualize makes chromosome counting by light microscope almost impossible. In fact electron microscopical methods (e.g. counting of synaptonemal complexes) yield reliable chromosome numbers, but they are very time consuming. An alternative method for obtaining the grade of ploidy is by means of microspectrophotometry, e.g. with the fluorochrome DAPI (4', 6-diamidino-2-phenylindole), which binds quantitatively on doublestranded DNA. The fluorescence intensity of the DAPI-DNA-complex can be determined photometrically. Thus one can avoid complicated chromosome counting and still obtain reliable information about the range of ploidy. Recently, Wittmann-Meixner (1989) showed that polyploidy is quite common in higher Basidiomycetes (Boletales) also. Other authors too have been using microspectrophotometry, based on Feulgen staining for a long time, in order to determine ploidy levels, e.g. Therrien & al. (1989) in *Phytophthora.* Nowadays DAPI is often used as fluorochrome, e.g. Motta & al. (1986) in *Armillaria,* Bresinsky & al. (1987) in *Pleurotus,* Meixner & Bresinsky (1988) in species of Coniophoraceae, Talbot & al. (1988), Wittmann-Meixner & Bresinsky (1989) in Paxillaceae. Statements about chromosome numbers by means of this method, however, are in most cases not reliable because of possible intra- or interspecifically variation of chromosome sizes. Wittmann-Meixner & al. (1989) recently referred to this problem in their paper about different grades of correlation between relative nuclear DNA content and chromosome number.

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The purpose of this work was to investigate in connection with a doctoral thesis of the junior author whether polyploidy is also a common phenomenon in Ascomycetes. So far the DNA content of over 400 species, mainly of the orders Leotiales and Pezizales, have been examined.

MATERIALS AND METHODS

Origin of fungal material

Only the species cited in this paper are listed. Those which were used for the statistical work, however, will be mentioned with full data in the doctoral thesis in preparation. The material has been deposited mainly in the Herbarium of the University of Regensburg (REG), partially in that of H.O. Baral (Baral).

Calycellina lachnobrachya (Desm.) Baral – Switzerland, near Schaffhausen, on *Acer campestre* leaves, 11.XI.89, leg. *H. Baral & P. Blank*, det. H. Baral (REG); *Calycellina ulmariae* (Lasch) Korf – Tübingen, on *Filipendula ulmaria*, 27.IV.89, leg./det. *H. Baral* (Baral); *Cistella hymeniophila* (P.Karsten) Korf – Alpenpark Berchtesgaden, near Bucher-Brunnen, on *Antrodia serialis*, 18.X.85, leg./det. *H. Schmid-Heckel* (M); *C. rubescens* Raschle – CBS 602,77, *on Alnus viridis; Gyromitra esculenta* (Pers.) Fr. – NP Bayerischer Wald, 5.V.87, leg./det. *N. Luschka* (REG); *G. infula* (Schaeffer) Quélet – NP Bayerischer Wald, 8.X.86, leg. *N. Garrido*, det. A. Bresinsky (REG); *Helvella acetabulum* (L.) Quélet – near Regensburg, 22.VI.87, leg. *A. Rott*, det. E. Weber (REG); *H. alpestris* Boud. – Wettersteingebirge, 24.IX.89, leg. *A. Bresinsky*, det. E. Weber & A. Bresinsky (REG); *H. crispa* (Scop.) Fr. – Regensburg, 24.VI.87, leg. *N. Arnold*, det. A. Bresinsky & E. Weber (REG); *H. elastica* Bull. – near Regensburg, 9.X.86, leg./det. *H. Besl* (REG); *H. lacunosa* Afz. – Tenerife, near Aquamanza, 21.IV.89, leg./det. *E. Weber* (REG); *Hymenoscyphus caudatus* (P. Karsten) Dennis – NP Bayerischer Wald, on *Alnus* leaves, 23.IX.87, leg. *N. Luschka*, det. E. Weber (REG); *H. fructigenus* (Bull. ex Mérat) Gray – near Regensburg, on *Quercus* cupules, 2.IX.88, leg. *D. Rothe & M. Galuczka*, det. E. Weber (REG); *H. imberbis* (Bull.) Dennis – Hirschau/Opf., on wood, 21.X.89, leg./det. *E. Weber* (REG); *H. repandus* (Phill.) Dennis – NP Bayerischer Wald, on herbaceous stems, 29.V.89, leg. *N. Luschka*, det. E. Weber (REG); *H. rokebyensis* (Svrček) Matheis – near Tübingen, on *Fagus* cupules, 16.IX.89, leg. *H. Baral & E. Weber*, det. H. Baral (REG); *H. rumicis* (Velen.) Dennis – France, near Gérardmer, on *Rumex* fruits, 19.VI.90, leg./det. *E. Weber & H. Baral* (REG); *H. salicellus* (Fr.) Dennis – NP Bayerischer Wald, on *Salix*, 23.VIII.88, leg. *N. Luschka*, det. E. Weber (REG); *Incrupila viridipilosa* Graddon – near Tübingen, on *Acer*, 27.II.90, leg./det. *H. Baral* (Baral); *Incrupilella flexipila* Svrček – Luxemburg, near Reichelange, on *Salix*, 20.IV.90, leg. *H. Baral & G. Marson*, det. H. Baral (Baral); *Lachnellula calyciformis* (Batsch) Dharne – NP Bayerischer Wald, on *Picea abies*, 27.VII.89, leg./det. *E. Weber* (REG); *L. resinaria* (Cke. & Phill.) Rehm – NP Bayerischer Wald, on *Picea abies* resin, 17.XI.89, leg. *N. Luschka*, det. E. Weber & H. Baral (REG); *L. subtilissima* (Cooke) Dharne – NP Bayerischer Wald, on *Picea abies*, May 1988, leg. *N. Luschka*, det. E. Weber (REG); *L. suecica* (de Bary ex Fuckel) Nannf. – Switzerland, near Davos, on *Larix* twigs, 4.IX.90, leg./det. *E. Weber* (REG); *Lachnum impudicum* Baral (1) – near Gomadingen, on *Fagus sylvatica*, 24.IX.88, leg./det. *H. Baral* (Baral); (2) – Regensburg, on wood, 29.I.90, leg./det. *H. Baral* (REG); *L. subvirgineum* Baral nom. prov. 1985 – Switzerland, near Schaffhausen, on bark, 28.VII.88, leg./det. *H. Baral* (REG); *L. virgineum* (Batsch) P. Karsten – NP Bayerischer Wald, on wood, 5.V.89, leg. *N. Luschka*, det. E. Weber (REG); *Mollisa benesuada* (Tul.) Phill. – Luxemburg, near Walferdange, on *Alnus incana*, 18.IV.90, leg. *H. Baral & G. Martin*, det. H. Baral (Baral); *M. discolor* (Mont.) Phill. – near Tübingen, on *Prunus spinosa*, 2.XII.89, leg. *E. Weber & H. Baral*, det. H. Baral & E. Weber (Baral); *M.* spec. 401 – NP Bayerischer Wald, on *Fagus*, 28.VII.89, leg. *E. Weber & H. Baral*, det. H. Baral (Baral); *Morchella conica* Pers. – Tenerife, near Erjos, 15.IV.89, leg./det. *E. Weber* (REG); *Morchella esculenta* (L.) Pers. – near Burggriesbach, 1985, leg./det. *F. Fürnrohr* (REG); *Pezicula acericola* (Peck) Sacc. – Luxemburg, near Altrier, on *Acer*, 6.I.90, leg. *G. Marson*, det. H. Baral & G. Marson (Baral); *P. alba* Guthrie – near Tübingen, on *Malus*, 16.III.90, leg./det. *H. Baral* (Baral); *P. frangulae* (Fr.) Fuckel – France, near Gérardmer, on *Rhamnus frangula*, 18.VI.90, leg. *H. Baral & J. Deny*, det. H. Baral (Baral); *P. livida* (Berk.& Br.) Rehm – NP Bayerischer Wald, on *Abies*,

3.VII.88, leg./det. *N. Luschka* (REG); *P. sepium* (Desm.) Dennis – Luxemburg, near Syren, on *Crataegus*, 8.I.90, leg. *G. Marson*, det. G. Marson & H. Baral (Baral); *Proliferodiscus pulveraceus* (Alb.& Schw.) Baral – near Regensburg, on *Fraxinus*, 31.I.90, leg./det. *E. Weber & H. Baral* (REG); *Proliferodiscus* spec. 471 – NP Bayerischer Wald, on *Fagus*, 22.XI.89, leg. *N. Luschka*, det. H. Baral & E. Weber (REG); *Rhizina undulata* Fr. – NP Bayerischer Wald, 11.IX.89, leg./det. *N. Luschka* (REG); *Rutstroemia* cf. *firma* (Pers.) P. Karsten (1) – near Tübingen, on *Quercus*, 1.XII.89, leg./det. *H. Baral* (REG); (2) – NP Bayerischer Wald, on *Alnus*, 6.IX.89, leg. *N. Luschka*, det. E. Weber & H. Baral (REG); *Scutomollisia russea* Schmid-Heckel – NP Bayerischer Wald, on *Nardus stricta*, 28.VII.89, leg. *H. Baral & E. Weber*, det. H. Baral (Baral); *Underwoodia fuegiana* (Speg.) Gamundi – Argentinia, Parque Nacional Tierra del Fuego, 14.III.88, leg./det. *A. Bresinsky & N. Garrido* (REG); *Urceolella tetraspora* (Rehm) E.Müller – NP Bayerischer Wald, Rehberg, on ferns, 1.VI.89, leg. *N. Luschka*, det. E. Weber (REG).

The fruit-bodies were fixed in Carnoy (ethanol 96% : chloroform : acetic acid = 6 : 3 : 1) and stored at 5–10°C until needed. For nuclei staining the material was squeezed between slide and cover-slip and subjected to the DAPI-staining procedure already described in Wittmann-Meixner & Bresinsky (1989). Cultures were grown on microscope slides using van Uden's method (1951). For DNA measurements a Zeiss photomicroscope Universal with a microscope photometer 03 linked to a computer (Hewlett-Packard) were used. As internal standard served *Morchella esculenta* or *M. conica* (with 60 arbitrary units).

OBSERVATIONS

(a) Variations in nuclear DNA content within species

Generally, during the mitotic cell cycle fungal nuclei have to double their DNA content before division is possible. Especially in fast growing mycelium one can frequently find nuclei which have already started DNA replication and have therefore higher amounts (up to twice) of DNA compared to the basic value. For our investigations, however, it was necessary to use only nuclei which were in their resting phase and had so the basic DNA amount. Therefore we avoided measurements in regions with high mitotic activity, e.g. in hyphal tips. In order to ascertain that this basic amount of DNA is the same for the nuclei of a species we examined the nuclei of *Morchella esculenta* and *M. conica* (both have same DNA contents) at several stages of their life cycle. We proved that the measured relative DNA content was the same in young and old mycelia – beginning with just germinated spores – (cultured on different media), fruit-bodies, sclerotia or the conidial state (*Costantinella* as anamorph of *Morchella*).

Generally in the fruit-bodies of Leotiales and Pezizales observed the nuclei of sterile cells (such as paraphyses, excipulum cells etc., which in species of the Leotiales are normally monocaryotic, and in the Pezizales oligo- or polycaryotic) contained the same amount of DNA as the nuclei (normally two) in the ascogenous hyphae and as the nuclei in spores (at least initially). During ascus formation, however, the two nuclei in each cell of ascogenous hyphae double their DNA content (as before mitosis) but without a following separation, such that we can measure up to double the amount in each of the two nuclei in the young ascus. Then the two nuclei fuse forming a nucleus with four times the amount of DNA. Within the examined discomycetes this is the normal and most frequent case, which Rossen & Westergaard (1966) also found in *Neottiella rutilans*. The fusion nucleus then divides into two nuclei (first meiotic

division) each containing double the original amount of DNA, and subsequently into four nuclei (second meiotic division) each with the basic DNA value. After a further mitotic division there are eight nuclei and then normally eight spores.

Of the species examined *Urceolella tetraspora* was an exception to this pattern since no pre-meiotical replication of the two nuclei in the young ascus takes place, and therefore the fusion nucleus has only double the basic DNA content. Moreover only four spores are formed. This is certainly a secondary phenomenon arising from mutation because the other *Urceolella* species behave quite normally with respect to meiosis and spore formation. In additionally examined species from different families with four spores per ascus meiosis and spore formation take a normal course, however, four spores aborting prematurely.

More frequently, in species of Leotiales we found instead of dicaryotical ascogenous hyphae monocaryotical hyphae with double the amount of DNA in the nucleus. Beyond that, there were never croziers to be found in those cases. Yet, dicaryotical ascogenous hyphae have also been found in species without croziers.

It is also important to establish whether fruit-bodies of the same species but from different collections have the same DNA content. In more than 100 cases which have been studied in this respect up till now, the nuclei of fruit-bodies from one species were always found to contain the same amount of DNA. (Some apparent exceptions will be mentioned in the next chapter.)

(b) Different DNA contents as an indication for cryptical speciation

In some cases we found that different collections of apparently identical or very closely related species did not have the same relative DNA content. A selection of these species is listed in Table I.

Table I. Different relative DNA contents within morphologically identical (a) or little deviating species (b) of Leotiales.

Species	Relative DNA content (in a.u.) (± coeff. of variation)
(a) *Lachnum impudicum* (collected in September)	44 (± 10%)
Lachnum impudicum (collected in January)	66 (± 8%)
Cistella hymeniophila	44 (± 5%)
Cistella rubescens	64 (± 7%)
Rutstroemia cf. *firma (Quercus)*	92 (± 7%)
Rutstroemia cf. *firma (Alnus)*	129 (± 11%)
(b) *Lachnum subvirgineum* nom. prov. Baral 1985	41 (± 11%)
Lachnum virgineum	70 (± 6%)

Table II. Different ploidy levels in species of Leotiales and Pezizales.

Species	Relative DNA content (in a.u.) (± coeff. of variation)
***Proliferodiscus* and related species**	
Incrupilella flexipila	21 (± 8%)
Incrupila viridipilosa	21 (± 12%)
Proliferodiscus pulveraceus	41 (± 10%)
Proliferodiscus spec. 471	89 (± 9%)
Pezicula	
Pezicula alba	40 (± 8%)
Pezicula frangulae	63 (± 9%)
Pezicula livida	66 (± 9%)
Pezicula acericola	93 (± 7%)
Pezicula sepium	123 (± 7%)
Hymenoscyphus	
Hymenoscyphus rumicis	22 (± 11%)
Hymenoscyphus imberbis	43 (± 11%)
Hymenoscyphus repandus	62 (± 8%)
Hymenoscyphus fructigenus	67 (± 10%)
Hymenoscyphus salicellus	93 (± 7%)
Hymenoscyphus caudatus	97 (± 8%)
Hymenoscyphus rokebyensis	121 (± 6%)
Helvellaceae	
Rhizina undulata	19 (± 14%)
Gyromitra infula	39 (± 14%)
Gyromitra esculenta	40 (± 8%)
Helvella crispa	60 (± 8%)
Helvella lacunosa	61 (± 10%)
Helvella elastica	63 (± 12%)
Helvella acetabulum	122 (± 8%)
Helvella alpestris	130 (± 8%)
Underwoodia fuegiana	115 (± 7%)

Lachnum impudicum, for instance, when collected in the winter, contained one and a half time as much DNA as the material collected in early autumn, which might indicate that two different species are covered by one name, by analogy with *Lachnum virgineum* which contains one and a half time the DNA value of *L. subvirgineum*. The latter is a provisional species set up by Baral (in Baral & Krieglsteiner, 1985), distinguished from *L. virgineum* mainly because of the hairs with strongly swollen apices. A similar case is *Cistella hymeniophila* and *C. rubescens*. *C. hymeniophila* occurs on polypores; *C. rubescens* was recently described by Raschle (1978) on *Alnus viridis*. Both produce a red pigmentation in the substrate. Since there seem to be no other obvious differences, Baral (in Baral & Krieglsteiner, 1985) and others regard them as one species. However, the DNA content differed between the culture of

Raschle's type collection and the material collected on a polypore. Another example is *Rutstroemia firma*, obviously a collective species, which varies in its relative DNA content. One species is commonly found on *Quercus* twigs, the other occurs on *Alnus*.

(c) Different levels of ploidy within genera and families

Measurements of the relative nuclear DNA content of different species of one or more genera revealed clearly the predominance of certain ranges. Table II and III show some examples from different families of the Leotiales and Pezizales.

From the results of over 400 examined species it is evident that relative DNA contents of approximately 40–45 a.u. (= arbitrary units) and 60–70 a.u. occur most frequently. However, contents of 80–100 a.u., and even 120 a.u. are still fairly common and in a few cases even higher levels. Occasionally one finds values of approx. 20 a.u., being the lowest amount of DNA measured within the orders Leotiales and Pezizales, for example in *Incrupilella flexipila* and *Incrupila viridipilosa*, two very closely related species of Leotiales which might belong to the genus *Proliferodiscus*. In *Proliferodiscus pulveraceus* the amount of DNA is double, in *Proliferodiscus* spec. 471 four times that of the lowest amount (see Table II). A DNA content of approx. 20 a.u. is also found in other genera and families of the Leotiales, e.g. in *Poly-*

Table III

	LEOTIALES			PEZIZALES
x (20) (haploid)	*Incrupilella flexipila* *Incrupila viridipilosa*		*Hymenoscyphus rumicis*	*Rhizina undulata*
2x (40–45) (diploid)	*Proliferodiscus pulveraceus*	*Pezicula alba*	*Hymenoscyphus imberbis*	*Gyromitra infula esculenta*
3x (60–70) (triploid)	—	*Pezicula livida frangulae*	*Hymenoscyphus repandus fructigenus*	*Helvella crispa lacunosa elastica*
4x (80–100) (tetraploid)	*Proliferodiscus* spec. 471	*Pezicula acericola*	*Hymenoscyphus caudatus salicellus*	—
6x (120) (hexaploid)	—	*Pezicula sepium*	*Hymenoscyphus rokebyensis*	*Helvella acetabulum alpestris* *Underwoodia fuegiana*

Table IV. Correlation between substrate and relative DNA content (in Leotiales).

Type of substrate	On wood and bark		On stems and leaves of herbaceous plants		On leaves of trees		On fruits and flowers	
	a	b	c	d	a	b	a	d
Number of species examined	(31)	(127)	(58)	(62)	(12)	(25)	(14)	(25)
x (haploid)	6%	7%	3%	–	–	–	–	4%
2x-aneuploid	–	2%	2%	–	–	–	–	–
2x (diploid)	35%	44%	46%	44%	25%	4%	21%	16%
3x (triploid)	26%	22%	38%	32%	33%	36%	36%	44%
4x (tetraploid)	23%	17%	9%	14%	25%	44%	43%	12%
6x (hexaploid)	10%	6%	2%	5%	17%	12%	–	16%
8x (octaploid)	–	2%	–	5%	–	4%	–	–
10x								4%
14x								4%

a: conifers. b: deciduous trees and shrubs. c: monocotyledons. d: dicotyledons.

desmia pruinosa (Hyaloscyphaceae) or *Ionomidotis fulvotingens* and *Bulgaria inquinans* (Leotiaceae). This value is the highest common factor of most of the measured DNA contents. We therefore consider those species to be haploid. Species with doubled amounts (40–45 a.u.) would be diploid, those with values of 60–70 a.u. triploid, those between 80 and 100 a.u. tetraploid, those of approx. 120 a.u. hexaploid and so on. In many of the examined genera (e.g. *Pezicula, Calycellina, Mollisia, Ciboria*) a haploid species could not be found, but diploid, triploid, tetraploid, hexaploid species and to a certain extent higher ploidy levels with obviously comparable values were clearly present. In the Hyaloscyphaceae we can observe a clear predominance of diploid species (62%). In Dermateaceae and Leotiaceae diploid, triploid and tetraploid species have approximately equal distribution (tetraploids somewhat less).

(d) Correlation between ploidy grades and ecological conditions

In a few cases the relative DNA content of all examined species of a genus was the same, e.g. in *Scutellinia* or *Durella,* but in most of the genera different ploidy levels occur. A correlation between DNA content and substrate type, time of fructification or altitude could be established. (Only the order of Leotiales was taken into consideration.)

Table IV shows that fungi growing on wood or bark (of conifers or deciduous trees and shrubs) or on remains of herbaceous plants have the lowest DNA contents and ploidy levels. Nearly half of the examined species are haploid, diploid or '2x-aneuploid', with diploids clearly in the majority. '2x-aneuploid' means that the measured DNA value lies between x and

Table V. Correlation between period of fructification and relative DNA content (in Leotiales).

	All the year round	Mainly in winter	In spring and summer	Mainly in autumn
Number of species examined	(31)	(48)	(186)	(112)
x	10%	10%	3%	3%
2x-aneuploid	6%	2%	1%	1%
2x	**55%**	**48%**	**44%**	20%
3x	20%	19%	29%	**36%**
4x	6%	15%	16%	27%
6x	3%	5%	6%	8%
8x			1%	3%
10x				1%
14x				1%

2x and is interpreted in such a way that single chromosomes are added or lost. On the other hand species on leaves (of conifers or deciduous trees) have much higher DNA values and therefore ploidy levels (mainly triploid and tetraploid). The DNA values of species occurring on fruits or flowers have a very similar distribution as the latter ones.

Another possibility is to divide the examined species into different types according to fructification time, e.g. species fruiting all year round as opposed to those which fructify only in autumn and so on (see Table V). It is not always easy to classify species in this way because different authors often give different information – or none at all – about fructification times for a particular species. Moreover some species have only been found rarely, and therefore data are often lacking.

Fungi occurring all the year round or mainly in winter show approximately the same distribution of DNA contents and ploidy levels, and also the lowest values. In the group of fungi growing in spring and summer there is a slight shift to higher ploidy levels. (This could perhaps be explained by the presence of some montane species.) However, there is a remarkable distribution of ploidy levels in fungi occurring mainly in autumn. There are many more autumnal species in the triploid and tetraploid state than in species fruiting in the other seasons. A good example is the couple *Calycellina ulmariae* and *C. lachnobrachya*. The former, a diploid species, grows on *Filipendula ulmaria* in spring and summer; the latter which inhabits leaves of *Quercus* and *Acer*, however, is triploid and occurs in autumn. Moreover most of the species occurring on leaves (77% on the leaves of deciduous trees, 50% on needles) and many of those on fruits of deciduous trees grow in autumn, whereas fungi on herbaceous plants grow mainly in spring and summer (91%). Although the species on wood and bark show no clear preference of season, those which fruit in autumn have more higher-ploid species compared

Table VI. Correlation between altitude and relative DNA content in different species of Leotiales.

Species	Relative DNA content (in a.u.) (± coeff. of variation)
Lachnellula calyciformis	40 (±11%)
Lachnellula subtilissima	41 (±10%)
Lachnellula resinaria	65 (± 8%) montane
Lachnellula suecica	65 (± 7%) montane–subalpine
Mollisia benesuada	44 (±10%)
Mollisia discolor	61 (±13%)
Scutomollisia russea	82 (± 9%) montane
Mollisia spec. 401	131 (± 8%) montane

to the whole distribution. Furthermore, half the examined species of Leotiaceae fruits in autumn. On the other hand, half of the Hyaloscyphaceae species and three quarters of the Dermateaceae species fructify in spring and summer. This means that the distribution of the species of different families on different substrate types is not always equal, e.g. on herbaceous plants there are in comparison less members of Leotiaceae. For average distribution of ploidy levels within the mentioned families see under c.

As a further possibility we observed a correlation between DNA content and altitude (or climate). Montane and alpine (subalpine) species often show higher DNA values than related species growing at lower altitudes, a fact which has also been noted in higher plants in accordance with the 'Tischler-rule'. Examples within the family Helvellaceae are *Helvella alpestris* which occured at about 2.000 m above sea level and *Underwoodia fuegiana* which was found in the antarctic zone. Both have double the DNA content of most of the other *Helvella* species examined. Table VI shows some additional examples for this statement.

DISCUSSION

Our observations show clearly that polyploidy has to be regarded as quite a common phenomenon in higher Ascomycetes in accordance with studies in higher Basidiomycetes (Wittmann-Meixner, 1989). The relative nuclear DNA content assigned to the haploid state (20 a.u.) is the lowest level within the species of discomycetes investigated so far. All species with the whole multiple of this lowest amount of DNA are to be interpreted as diploid or higher-ploid. It is unlikely that a lower nuclear DNA content will be found within the taxonomic range of Leotiales and Pezizales. However, if relative DNA contents of 10 a.u. or less were found in some species, the grade of polyploidy in higher Ascomycetes would be even more prominent than shown by our investigation so far. Although it has not been proved conclusively, the haploid value for all genera of Leotiales and presumably of Pezizales is obviously the same. Indeed species with this haploid value have not been found in all genera so far. But

the measured DNA contents are quite comparable and consistent, and the genera, at least within the range of an order, are generally so closely related (although there are few exceptions) that it seems safe to assume this basic haploid value. In the Pezizales the DNA values and therefore ploidy levels are mostly the same as in the Leotiales. In the Pezizales the lowest relative DNA content corresponding to the haploid value has only been found once up till now, namely in *Rhizina undulata*. This species may be regarded as more primitive with respect to fruit-body morphology compared to the species of *Gyromitra* (diploid), *Helvella* (most species triploid), and *Underwoodia* (hexaploid in the investigated species) discussed here. Our data support the suggestion already raised for the higher Basidiomycetes that the species in discomycetes are most frequently on the diploid (and triploid level), rarely on the tetraploid and even more rarely on the haploid, hexaploid or higher ploidy levels. Haploid species can be regarded as being less derived than diploid and higher-ploid ones. Most of the higher-ploid species have probably developed more recently in comparison to the diploid ones (from diploid ancestors). These species seem to be well adapted to ecological niches in higher altitudes and to areas which have been influenced to a high degree by glaciation (in accordance with the Tischler-rule for vascular plants). The proportion of species showing higher ploidy levels than the diploid one is greater in autumnal species (nearly 80%) than in species fruiting all year round (nearly 30%), mainly in winter (40%) or in spring and summer (53%). Many autumnal species can probably be regarded as more derived because of higher ploidy grades. Fruiting in autumn perhaps requires a higher activity of metabolism and consequently higher amounts of DNA on account of greater fluctuations in temperature and a comparatively short period of fructification. Fungi fruiting in other periods have mostly more time available, especially those which fructify all the year round or in spring and summer (to early autumn). Species fruiting in winter start their fructification often already in the autumn and extend it to spring.

On the other hand leaves and fruits of trees are often inhabited by higher-ploid species fruiting preferably in autumn. This could be a hint that those substrates had been taken by higher evolved species.

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