Ribosomal DNA variation, recombination and inheritance in the basidiomycete *Trichaptum abietinum*: implications for reticulate evolution

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Two divergent nuclear ribosomal DNA (nrDNA) types, designated α and β , were found distributed in 11 North European populations of the basidiomycete *Trichaptum abietinum*. These types differed by a 220 bp indel in the internal transcribed spacer 1 (ITS1) sequence and a number of linked substitutions and small indel motives in the internal transcribed and intergenic spacers (ITS1, ITS2, IGS1 and IGS2). The α and β haplotypes co-occurred in heterozygous somatic individuals (dikaryons) and segregated in a Mendelian fashion in monokaryotic single spore progenies. This result suggests that the haplotypes are encoded in different nuclei of field-collected dikaryons and inherited as a single locus. No meiotic recombinants were observed among the sequenced monokaryons. Population genetic analyses by

PCR-RFLP revealed that a low frequency of evolutionary intermediate nrDNA types also existed in natural populations, presumably as a result of meiotic recombination of α and β nrDNA. The existence of divergent nrDNA types in *T. abietinum* could be a result of a former independent evolution followed by a hybridization event. Phylogenetic analyses of ITS sequences suggest that the sister taxon *T. fusco-violaceum* has been involved in the evolutionary history of *T. abietinum*. Sequence polymorphisms observed in the translation elongation factor 1 α (*efa*) and glyceraldehyde-3-phosphate dehydrogenase (*gpd*) genes, did not reveal two well-defined types of these genes. The results are discussed in the light of other evolutionary mechanisms as well.

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Introduction

The genetic composition and structure within species, populations and individuals reflect their evolutionary history and may indicate which evolutionary mechanisms have been in action. Divergent speciation, mainly caused by mutations, selection and genetic drift, results in differentiated gene lineages. Reticulate evolution, that is, speciation via hybridization, combines divergent DNA lineages and is well documented among plants. Hybrid speciation has recently also been postulated as a track to rapid evolution in a few mutualistic and plant pathogenic fungi (Tsai *et al*, 1994; Garbelotto *et al*, 1996; O'Donnell and Cigelnik, 1997; O'Donnell *et al*, 1998; Brasier *et al*, 1999; Newcombe *et al*, 2000; Nielsen and Yohalem, 2001). Several studies have pointed out the usefulness of nuclear ribosomal DNA (nrDNA) sequence data in tracking the history of a species, including reticulate evolution (Sang *et al*, 1995; Wendel *et al*, 1995; O'Donnell and Cigelnik, 1997; O'Donnell *et al*, 1998; Brasier *et al*, 1999; Hughes and Petersen, 2000; Newcombe *et al*, 2000). The nuclear ribosomal gene family consists of reiterated cistrons of the ribosomal genes (18S, 5.8S and 28S) interspersed by the internal transcribed and intergenic spacers (ITS and IGS). In plants, different

outcomes have been reported for the ribosomal repeat following hybridization: (a) both parental ITS spacer sequences were retained (eg Franzke and Mummenhoff, 1999), (b) the ribosomal repeat was homogenized to one parental type (eg Wendel *et al*, 1995), (c) the ribosomal repeat was homogenized but contained scattered elements of both parents (eg Sang *et al*, 1995).

Trichaptum abietinum (Dicks.:Fr.) Ryvarden is a widespread wood-decaying fungus in boreal coniferous forests of the northern hemisphere (Ryvarden and Gilbertson, 1994). It can give rise to hundreds of small poroid basidiocarps (fruit bodies) on coniferous logs. The life history of the fungus is that of a typical basidiomycete, with a predominant dikaryotic vegetative state (each cell including two nuclei) and a short-lived monokaryotic state following meiosis before dikaryon formation. In some basidiomycetes, different intersterile populations (intersterility groups), which are fully or partially interfertile, have been observed. Intersterility groups (ISGs) generally equate to intraspecific units, and some differ in adaptive characters, for example those occurring within the root rot fungus *Heterobasidion annosum* (Garbelotto *et al*, 1996). *T. abietinum* comprises at least two North American ISGs that are partially compatible with a third European ISG (Macrae, 1967; Magasi, 1976). Four other *Trichaptum* species co-occur with *T. abietinum* on the northern hemisphere; *T. fusco-violaceum*, which forms a toothed basidiocarp; *T. laricinum*, which forms a lamellate basidiocarp and *T. bifforme* and *T. subchartaceum*, which form poroid basidiocarps

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(Gilbertson and Ryvarden, 1987; Ryvarden and Gilbertson, 1994). Phylogenetic analyses of ITS2 nrDNA and mtSSU sequences in *Trichaptum* have shown that the strains of each species formed monophyletic groups (Ko and Jung, 2002). However, ITS1 nrDNA sequence data subdivided the *T. abietinum* strains into three subgroups with *T. fusco-violaceum* included in one of the groups (Ko and Jung, 2002). It has been suggested that *T. abietinum* may be in the process of differentiation into new physiological or geographical taxa (Ko *et al.*, 1997).

In this study, we report two divergent types of nuclear ribosomal repeat units in *T. abietinum*, designated α and β , as revealed by sequence and RFLP analyses of the nrDNA ITS and IGS regions in monokaryons and dikaryons from 11 populations of *T. abietinum* in Fennoscandia. To assess whether the observed nrDNA patterns were a result of reticulate evolution or other evolutionary mechanisms, we also examined partial sequences from the translation elongation factor 1 α (*efa*) and glyceraldehyde-3-phosphate dehydrogenase (*gpd*) genes and the mode of nrDNA inheritance in spore progenies. The results are discussed.

Materials and methods

Sample

In all, 249 basidiocarps from 11 populations of *T. abietinum* in coniferous forests of Fennoscandia were sampled in order to examine the geographic range of genetic variation at population level. Somatic cultures (dikaryons) were obtained from all basidiocarps. For sequencing, 10 single spore cultures (monokaryons) were obtained from germinating basidiospores of nine different fruit bodies from four populations. The isolates were grown on 2% PDA at 25°C in darkness. To determine

whether the nrDNA polymorphisms are inherited in simple Mendelian ratios, we sampled 56 (2 × 28) single spore cultures (monokaryons), from two additional fruit bodies (TaGu1 and TaGu4), and performed a segregation experiment. The existence of somatic and single spore culture isolates relied on microscopic observation of respectively clamp connections on hyphae (dikaryons) or their absence (monokaryons).

Molecular methods

DNA was extracted by the CTAB miniprep method described by Murray and Thompson (1980) with some minor modifications: DNA was resuspended in 100 µl sterile milliQ H₂O at the final step of extraction, and DNA templates were diluted 50–100-fold before PCR amplification. Target sequences were amplified using the thermal profiles and primers given in Table 1. The primer Ta300, positioned ca. 350 bp upstream from the nuclear small subunit (18S) rRNA gene, was designed based on comparison with a partial sequence of the IGS2 in the material, which we derived from amplification with the primers 5SA' (Anderson and Stasovski, 1992) and CNS1 (White *et al.*, 1990). The primer pairs EF595F/EF1160R (Kauserud and Schumacher, 2001), and EF1160F/EF1750R (this study), were designed based on comparison with an accessioned *efa* sequence of *Schizophyllum commune* Fr. in GenBank (EMBL accession number X94913) (Table 1). PCR was performed in 40 µl reactions containing 23.5 µl 50 × diluted template DNA and 16.5 µl reaction mix (final concentrations: 4 × 250 µM dNTPs, 0.625 µM of each primer, 2 mM MgCl₂ and 1 unit Dynazyme™ II DNA polymerase (Finnzymes Oy, Espoo, Finland) on a Genius Operator (Techné). Monokaryon amplicons were sequenced manually using the PCR primers, employing the ThermoSequenase radiolabelled terminator cycle sequencing kit (Amersham Pharmacia

Table 1 Primers and thermal profiles employed for PCR amplification of target sequences

Target sequence	Primer pairs	References	Thermal profile		
			Denat.	Repeated part	Exten.
ITS	ITS5	White <i>et al.</i> (1990)	94°C	94°C–54°C–72°C (36 rep)	72°C
	ITS4	White <i>et al.</i> (1990)	4 min	30 s–35 s–40 s	10 min
ITS2	ITS3	White <i>et al.</i> (1990)	94°C	94°C–52°C–72°C (36 rep)	72°C
	ITS4	White <i>et al.</i> (1990)	4 min	30 s–35 s–40 s	10 min
IGS1	CNL12	White <i>et al.</i> (1990)	94°C	94°C–55°C–72°C (36 rep)	72°C
	5SA	Anderson and Stasovski (1992)	4 min	30 s–35 s–40 s	10 min
IGS2	5SA'	Anderson and Stasovski (1992)	94°C	94°C–58°C–72°C (37 rep)	72°C
	CNS1	White <i>et al.</i> (1990)	4 min	30 s–35 s–40 s	10 min
IGS2-segment	Ta300 ^a	This study	94°C	94°C–55°C–72°C (37 rep)	72°C
	CNS1	White <i>et al.</i> (1990)	4 min	30 s–35 s–40 s	10 min
<i>gpd</i> segment	CTK-052	Kreuzinger <i>et al.</i> (1996)	94°C	94°C–55°C–72°C (37 rep)	72°C
	CTK-032	Kreuzinger <i>et al.</i> (1996)	4 min	30 s–35 s–40 s	10 min
<i>efa</i> part 1	EF595F ^b	Kauserud and Schumacher (2001)	94°C	94°C–55°C–72°C (37 rep)	72°C
	EF1160R ^c	Kauserud and Schumacher (2001)	4 min	30 s–35 s–40 s	10 min
<i>efa</i> part 2	EF1160F ^d	This study	94°C	94°C–55°C–72°C (37 rep)	72°C
	EF1750R ^e	This study	4 min	30 s–35 s–40 s	10 min

^aPrimer sequence (5'–3'): AGCCGGTTGTTGGTAGTCCTC.

^bPrimer sequence (5'–3'): CGTGACTTCATCAAGAACATG.

^cPrimer sequence (5'–3'): CCGATCTTGACGTCCTG.

^dPrimer sequence (5'–3'): CAGGACGTCTACAAGATCGG.

^ePrimer sequence (5'–3'): GTACTCGTTGTAGGACTCAA.

Biotech Inc., OH, USA) and α -³³P-ddNTPs. EMBL/GenBank/DDBJ accession numbers of sequences are: AJ309808–AJ309815 for the divergent ITS1, IGS1, IGS2 and ITS2 haplotypes, AJ309882–AJ309891 for the five *efa* haplotypes (two parts) and AJ309892–AJ309901 for the five *gpd* haplotypes (two parts). Identical nrDNA spacer sequences were obtained on complementary strands and in isolates from different populations, ruling out the possibility of PCR or sequencing artifacts. For restriction analyses, 10 µl of ITS2 and IGS2 PCR products were digested in 25 µl volumes containing 12 µl H₂O, 2.5 µl buffer and 0.5 µl *Rsa*I and *Dde*I, respectively, following the manufacturer's instructions (Promega, Southampton). Restriction products were separated on a 2% agarose gel stained with ethidium bromide, using 0.5 TBE as the running buffer, and photographed over UV light.

Sequence alignments and phylogenetic analyses

Available ITS nrDNA sequences from *Trichaptum* spp. were retrieved from GenBank (Table 2) and aligned in BioEdit (Hall, 1999) together with sequences obtained in this study. One large indel region of the ITS1 alignment (characters 38–332) was excluded prior to analyses. Maximum parsimony analyses of the aligned ITS1 and ITS2 sequences were performed separately in PAUP* v. 4.02b (Swofford, 1999). Parsimony analyses were carried out using the branch-and-bound option with the following parameter settings: gaps treated as missing, equal character weights, MultTrees option in effect, maxtrees setting to 50 000 and branches having minimum length zero allowed to collapse yielding polytomies. To examine the support for inferred relations, bootstrap analyses (1000 replicates) were performed (Felsenstein, 1985) using default options.

Results

Sequence variation in the nrDNA spacers

PCR amplification of the ITS nrDNA region (ITS1–5.8S–ITS2) in dikaryons of *T. abietinum* gave two amplicons of

different lengths, designated α (596 bp) and β (818 bp). These co-occurred in the same (heterozygotes) or in different (homozygotes) dikaryotic isolates of the geographic populations (Figure 1a).

Seven single spore isolates (monokaryons) from four different populations yielded amplicons of either the α or β type, each isolate possessing a single ITS sequence of α or β type. Four monokaryons derived from the populations of Skotjernfjell and Kuhmo possessed the ITS β type, and three monokaryons from the populations of Lierne, Umeå and Skotjernfjell possessed the ITS α type (Figure 2a). A 220 bp indel, positioned 29 bp downstream in the aligned ITS1, and seven minor indels and substitutions consistently distinguished the α and β ITS types into two main ITS1 types, designated ITS1 α (239 bp) and ITS1 β (461 bp) (Figure 2a). Substitutions in positions 113 (A/G) and 189 (GC/AT) of the ITS2 (202 bp) distinguished two ITS2 types, designated ITS2 α and ITS2 β (Figure 2a).

PCR amplification of the partial IGS1 in *T. abietinum* dikaryons gave two amplicons of different lengths, recognized as IGS1 α (407 bp) and IGS1 β (410 bp) in the subsequently sequenced monokaryons. Indels at positions 358 and 374, recognized as single motif microsatellites (a G following six G's, and TT following 11 T's), distinguished the α and β IGS1 sequences (Figure 2a). The single-banded partial IGS2 amplicons of *T. abietinum* dikaryons were resolved as two IGS2 haplotypes of the same length (352 bp), distinguished as IGS2 α and IGS2 β based on indels at positions 26 (–/C) and 37 (G/–) upstream from the 18S gene (Figure 2a). A sequence linkage was observed between the ITS/IGS α spacer versions, and between the ITS/IGS β spacer versions. Thus, two distinct nrDNA types (linkage groups) were observed among the sequenced monokaryons (Figure 2a).

Sequence variation in *efa* and *gpd*

Sequence analyses of two amplified portions (505 and 480 bp) of the *efa* gene from seven monokaryons representing three populations, (Skotjernfjell, Kuhmo and Umeå), revealed 10 polymorphisms, which consti-

Table 2 GenBank sequences used in this study, published by Ko and Jung (2002)

Taxon	Strain number	Locality	GenBank accession nos.	
			ITS1	ITS2
<i>T. abietinum</i>	CBS347.68	Canada	AF266676	AF267651
<i>T. abietinum</i>	CCFC008387	Canada	AF266677	AF267652
<i>T. abietinum</i>	CBS357.68	Canada	AF267648	AF266681
<i>T. abietinum</i>	CCFC009931	Canada	AF267646	AF267653
<i>T. abietinum</i>	CCFC010156	Canada	AF267647	AF267654
<i>T. abietinum</i>	SFC950815-17	Korea	AF266680	AF267655
<i>T. abietinum</i>	SFC960608-11	Korea	AF267644	AF267656
<i>T. abietinum</i>	SFC961028-11	Korea	AF267645	AF267657
<i>T. biforme</i>	FP-86522	USA		U63476
<i>T. biforme</i>	HHB-7316	USA		U63473
<i>T. fusco-violaceum</i>	FP-133997	USA		U63472
<i>T. fusco-violaceum</i>	HHB-4016	USA		U63478
<i>T. subchartaceum</i>	CCFC003928	Canada	AF267650	AF267659
<i>T. subchartaceum</i>	CCFC003932	USA	AF266678	AF266679
<i>T. laricinum</i>	RLG-4665	USA		U63471
<i>T. laricinum</i>	RLG-6936	USA		U63477

Figure 1 (a) Agarose gel showing ITS amplicons of 21 dikaryotic isolates from the Lierne population (Norway). Two distinct ITS products were found: a short version (596 bp) and a long version (818 bp), separated by a 220 bp indel in ITS1. The two ITS products reflect the distribution of ITS1 α and β versions. In heterozygous isolates including both versions (labelled $\alpha\beta$), the short version was favoured in the PCR reaction, giving a more prominent ITS1 α and a weaker ITS1 β . Artificial heteroduplex bands are also visible in heterozygous isolates. (b) The ITS2 region digested by *Rsa*I. The different restriction patterns reflect the ITS2 α and β types. (c) The partial IGS2 region digested by *Dde*I. The different restriction patterns reflect the IGS2 α and β types. Five isolates (2, 4, 7, 14 and 21) show recombinant nrDNA genotypes (marked *) that possess recombined α and β nrDNA spacer combinations. m = size markers (X-174 DNA digested with *Hae*III).

Figure 2 Distribution of sequence polymorphisms in target sequences: (a) the ITS1 (239/461 bp), ITS2 (202 bp), IGS1 (407/410 bp) and the partial IGS2 nrDNA sequences (352 bp), (b) two amplified portions (505+480 bp) of the *efa* gene and (c) sequenced 218+309 bp portions of the *gpd* gene. Isolate codes are shown to the left in each matrix. The two first letters of isolate codes indicate taxon name (Ta = *T. abietinum*); the third and fourth letters indicate population (eg Um = Umeå), and the numbers refer to isolates. The position of the polymorphic sites in the aligned sequence matrices is written vertically above columns. The dotted line and grey shadings show the positions of sequence polymorphisms. In one position of *efa* (173) and in four positions of *gpd*, three different character states occurred among the haplotypes (i = 220 bp indel in ITS1, * = position upstream from the 18S gene, W = A/T, N = A/C/G/T).

tuted five distinct *efa* haplotypes (Figure 2b). The two most divergent *efa* haplotypes came from the same population (Skotjernfjell) and did not share any of the 10 polymorphisms. Two sequences from Skotjernfjell (TaSf38.3 and TaSf1-18.2) yielded the same *efa* haplotype, while a third sequence (TaSf13.1), constituting another *efa* haplotype, was identical to one of the sequences derived from the Kuhmo population (TaKu6.2). The three remaining *efa* sequences from Kuhmo, Umeå and Skotjernfjell yielded unique haplotypes. In one position (173, part 2) three character states (T, C or gap) occurred (Figure 2b).

A ca. 1 kbp portion of the *gpd* gene was amplified from six monokaryotic single spore isolates representing two populations (Skotjernfjell and Kuhmo), and sequenced from each end. A total of 38 substitutions and minor indel motives appeared in the 549 (218+309)bp long partial sequences of the *gpd* gene, making up five distinct *gpd* haplotypes (Figure 2c). Two sequences from Skotjernfjell (TaSf38.3 and TaSf4-2.3) yielded identical *gpd* haplotypes. Two highly divergent *gpd* haplotypes (TaSf38.3 and TaSf1-18.2), derived from one of the populations, (Skotjernfjell) shared only two out of 38 polymorphic sites. The three remaining *gpd* haplotypes, derived from both populations, were evolutionary intermediate in sequence composition (see Figure 2c).

ITS phylogenies

Phylogenetic analyses of the 268-character ITS1 and 236-character ITS2 data matrices (of which 57 and 65 characters were parsimony informative) gave three and four MPTs, 95 and 90 steps long (Figure 3). The ITS1 and ITS2 MPTs all recognized four groups of *Trichaptum* strains: one *T. abietinum*/*T. fusco-violaceum* clade, one *T.*

biforme, one *T. subchartaceum* and one *T. laricinum* clade. In the ITS1 tree, there was support for various subgroups within the *T. abietinum*/*fusco-violaceum* clade and the *T. fusco-violaceum* strains were included as parts of one of the *T. abietinum* subclades (87% bootstrap support). On the contrary, the *T. fusco-violaceum* strains were placed at the base within the *T. abietinum*/*T. fusco-violaceum* clade in the ITS2 tree (Figure 3).

An alignment of the variable characters of the *T. abietinum* and *T. fusco-violaceum* ITS1 sequences, clearly demonstrated the existence of three ITS1 length types with sparse sequence variation in the worldwide sample of *T. abietinum* (Figure 4). The ITS1 α type of the North European *T. abietinum* strains is also present in three Canadian strains, and the β type in three Korean strains. A third type, of intermediate length (425–431 bp), also occurred in *T. abietinum* and in two *T. fusco-violaceum* strains. The ITS1 α and β types of the North European specimens shared five minor indels not observed in the other strains (Figure 4).

nrDNA recombination

Amplification of the ITS1 from 249 dikaryotic isolates, constituting all geographic populations (11), gave varying frequencies of the α and β ITS1 nrDNA versions across populations (Table 3, Figure 5a). We used Webcutter 2.0 (Heiman, 1997; <http://www.firstmarket.com/cutter/cut2.html>), and found that the endonucleases *RsaI* and *DdeI* were able to digest and discriminate between the versions of the ITS2 and IGS2 sequences obtained, based on sequence polymorphisms in positions 113 (ITS2) and 37 (IGS2) (cf. Figure 2a). No suitable endonucleases were found for IGS1 restriction. In order to investigate whether the distribution of ITS2/

Figure 3 (a) ITS1 and (b) ITS2 phylogenies of *Trichaptum* specimens, obtained by use of the branch-and-bound search option in PAUP* v. 4.02b (Swofford, 1999). Sequences are labelled with taxon names, geographical origin of specimens (in clamps) and collection names. (a) One out of three most parsimonious ITS1 phylograms, 95 steps long (RI = 0.954, HI = 0.074). (b) One out of four most parsimonious ITS2 phylograms, 90 steps long (RI = 0.911, HI = 0.089). Stippled lines collapsed in the strict consensus trees. Bootstrap support values (> 50%) are shown below nodes.

Figure 4 ITS1 sequence alignment of *T. abietinum* and *T. fusco-violaceum* (the latter indicated by *) including only variable characters. Three main ITS1 length types can be recognized. Dark grey shading indicates regions shared by all the three ITS1 length types, medium grey indicates regions shared by the two length types, and light grey indicates regions included in only one length type. The region between the arrows was deleted prior to phylogenetic analysis.

IGS2 α coalesced with the distribution of ITS1 α , and ITS2/IGS2 β with ITS1 β , we performed *RsaI* and *DdeI* restriction digest analyses of ITS2 and IGS2 amplicons of all dikaryotic isolates. Of the 249 dikaryons 85.5% (213) had combinations of solely ITS1/ITS2/IGS2 α or ITS1/ITS2/IGS2 β nrDNA versions, referred to here as nonrecombinant α or β nrDNA; and 14.5% (36) showed intralocus recombinant nrDNA genotypes, possessing combinations of α and β nrDNA versions (cf. Figure 1, Table 3). The intralocus recombination ratios were 5.0% between ITS1 and ITS2, 10.1% between ITS2 and IGS2 and 15.1% between ITS1 and IGS2. The proportion of nrDNA recombinants varied in the different populations.

No recombinants were observed in the Lammi population (Finland), whereas 12 (52.2%) were observed in the Voss population (Norway) (cf. Table 3, Figure 5b). The proportion of α and β spacer types varied overall from 58.8% α in ITS1 to 61.6% α in ITS2 to 65.1% α in IGS2, with a proportional decline in the ITS1, ITS2 and IGS2 β spacer types. A similar pattern was observed in 10 out of the 11 populations studied.

Inheritance of nrDNA spacers

Analysis of ITS1 α and β distribution in a total of 2×28 single spore progenies (monokaryons), representing two

Table 3 Distribution of ITS1 α /ITS1 β , ITS2 α /ITS2 β and IGS2 α /IGS2 β in 11 populations of *Trichaptum abietinum*. Recombination frequencies between spacer versions are calculated

Population	n	Haplotype distribution (%)			Recombination (%)		
		ITS1 α/β	ITS2 α/β	IGS2 α/β	ITS1-ITS2	ITS2-IGS2	ITS1-IGS2
Skotjernfjell	26	48.1/51.9	50.0/50.0	51.9/48.1	3.8	3.8	7.7
Sigdal	33	61.4/38.6	63.6/36.4	68.2/31.8	4.5	18.2	22.7
Lierne	21	71.4/28.6	73.8/26.2	73.8/26.2	4.8	19.0	23.8
Uppsala	21	61.9/38.1	61.9/38.1	66.7/33.3	0.0	9.5	9.5
Lammi	21	73.8/26.2	73.8/26.2	73.8/26.2	0.0	0.0	0.0
Grimstad	19	57.9/42.1	63.2/36.8	65.8/34.2	5.3	5.3	10.5
Voss	23	41.3/58.7	50.0/50.0	69.6/30.4	17.4	34.8	52.2
Umeå	20	45.0/55.0	45.0/55.0	45.0/55.0	0.0	10.0	10.0
Rovaniemi	16	50.0/50.0	59.4/40.6	62.5/37.5	17.6	5.9	23.5
Kuhmo	23	78.3/21.7	78.3/21.7	80.4/19.6	0.0	4.3	4.3
Evenstad	26	57.7/42.3	59.6/40.4	59.6/40.4	3.8	0.0	3.8
Total	249	58.8/41.2	61.6/38.4	65.1/34.9	5.0	10.1	15.1

Figure 5 (a) The distribution of α (black section) and β (grey section) ITS1 haplotypes in 11 geographic *T. abietinum* populations. (b) The distribution of nonrecombinant α and β nrDNA haplotypes (grey section) and recombinant nrDNA haplotypes (black section) in the populations, including both α and β spacer versions.

fruit bodies (TaGu1 and TaGu4) known to possess both ITS1 versions (dikaryons), gave a 1:1 (TaGu1) and 4:3 (TaGu4) segregation of the ITS1 α and ITS1 β versions and were consistent with a 1:1 ratio (χ^2 test, $P < 0.05$). In order to investigate whether meiotic recombination between α and β nrDNA versions had occurred, restriction analyses of ITS2 and the partial IGS2 amplicons from the two spore families, employing the *Rsa*I and *Dde*I endonucleases, were performed. The ITS2/IGS2-RFLP analyses gave segregation patterns concordant with the ITS1 sequence data, demonstrating cosegregation and a tight linkage between the ITS1/ITS2/IGS2 α and the ITS1/

ITS2/IGS2 β spacer types, respectively (data not shown). No meiotic recombinants or progenies that included both α and β nrDNA were detected among the 56 monokaryons studied.

Discussion

This study has shown that there are two fairly divergent types of ribosomal repeat units present in North European populations of *T. abietinum* and that these behave like standard alleles at the population level and in their segregation. Recombination is suppressed within

them, but does occur within a population-level sample. A third ITS type, recently published by Ko and Jung (2002), has so far not been observed in the North European material.

The Mendelian segregation pattern of the nrDNA variants suggests that the observed intraspecific and sometimes within-individual ITS/IGS nrDNA heterogeneity is caused by divergent nrDNA versions encoded in different nuclei in *T. abietinum*. Several alternative explanations may account for the observed pattern of multiple nrDNA types. The most plausible explanation may be that the nrDNA types evolved independently and diverged some time in the past and were later united in a hybridization event. During divergent speciation, it is predicted that sibling species undergo ITS nrDNA sequence homogenization (Arnheim *et al.*, 1980; Zimmer *et al.*, 1980) and achieve a more or less homogenous ITS sequence distinct from other species. In contrast, speciation via hybridization may lead to a species that harbours divergent, but homologous sequences from the parental lineages.

The fact that the nrDNA spacer sequences (ITS1, ITS2, IGS1 and the partial IGS2) from monokaryons derived from four North European geographic populations all yielded either the α or β nrDNA version is suggestive of a past independent evolutionary history of the two European nrDNA lineages. In a recombining lineage with a common evolutionary history, it is to be expected that mutations distribute more or less at random and that particular linkage groups, such as observed in the nrDNA of *T. abietinum*, are non-existent. The somewhat incongruent topologies of the ITS1 and ITS2 trees suggest that recombination has occurred between different nrDNA lineages. The existence of at least three main ITS1 length types in *T. abietinum*, where one of the types is shared by the sister taxon *T. fusco-violaceum*, suggests that *T. fusco-violaceum* may have been involved in the evolutionary history of *T. abietinum*. The ITS1 α type observed in this study was similar in length to an ITS1 type beforehand observed in both North America and Korea, while the β type was similar to a unique Korean type (Ko and Jung, 2002). The fact that the North European α and β types shared a number of unique polymorphisms not observed in non-European *T. abietinum*/*T. fusco-violaceum* specimens indicate that they have recombined and shared a common evolutionary history for some time.

Recently, there have been some reports of ongoing hybridization events in basidiomycetes. Newcombe *et al.* (2000) reported a range of hybrid *Melampsora* rust genotypes between *M. medusae* and *M. occidentalis*, having ITS versions different from the parental species in both homozygous (one version) and heterozygous (both versions) combinations. A similar pattern has been detected in *Flammulina*, where a rare natural hybrid contained ITS nrDNA elements from the parental species *F. velutipes* and *F. rossica* (Hughes and Petersen, 2000). In Californian populations of *H. annosum* (Fr.) Bref., two ISGs as well as a hybrid group between the two were detected (Garbelotto *et al.*, 1996). The genetic pattern observed in the geographic *T. abietinum* populations of Fennoscandia may have a similar explanation, representing a combination of ancient evolutionary divergence in geoisolation as well as more recent

genetic recombination of previously isolated populations.

The likelihood of hybridization probably increases when previously geographically isolated populations or taxa that lack strong reproductive barriers come into contact and share the same niche (Brasier, 2000). Three ISGs have been documented in *T. abietinum*; two in North America which are intersterile, and a third European group which is partially interfertile with the North American groups (Macrae, 1967; Magasi, 1976). Unpublished data suggest that the individuals of the present North European populations all belong to the European ISG. Our data indicate that the European populations may represent a mixture of other lineages that could be intersterile but interfertile with the European populations. Additional mating experiments are needed to explore this topic in more detail.

As an alternative hypothesis, it has been suggested that the extensive ITS1 length variation observed in *T. abietinum* could be a result of gene duplication (Ko and Jung, 2002). In the study of Ko and Jung (2002), only heterokaryotic specimens were examined; thus, whether different nrDNA types were restricted to different nuclei and consequently may represent mere allelic variants, was not evidenced. The Mendelian segregation pattern of ITS types observed in the present study appears to rule out the possibility of paralogous α or β nrDNA loci in *T. abietinum*, at least in the North European populations. It has to be mentioned, though, that paralogous nrDNA have been observed in some fungi (O'Donnell and Cigelnik, 1997; O'Donnell *et al.*, 1998; Kim and Breuil, 2001), a phenomenon frequently observed in polyploid plant (hybrid) species (eg Sang *et al.*, 1995; Wendel *et al.*, 1995).

The PCR-RFLP analyses of field samples (dikaryons) gave absence or low frequencies of evolutionary intermediate nrDNA types of mixed α and β sequences (except for the Voss population). However, recombinant nrDNA types were not observed among the sequenced single spore isolates, probably because of the low number of analysed isolates. The proportion of recombinant nrDNA among populations varied on a spatial scale, which may be attributed to stochastic genetic drift. The Voss population, isolated from the other populations by high mountains, had higher proportion of nrDNA recombinants, which may be attributed to a past founder event. Admittedly, our assignment of α and β nrDNA types in dikaryotic field samples was based on digest profiles of the spacer regions, which cannot fully determine whether the nrDNA type is of α , β or a recombinant form. Thus, the possibility that some nrDNA variants have been overlooked cannot be ruled out.

The sequence variation observed in the *efa* and *gpd* genes, may also well reflect former divergent evolutionary lineages, like the α and β nrDNA versions, but these data are less convincing. In isolation, the observed sequence patterns in *efa* and *gpd* could be explained by accumulation of mutations over time in a recombining taxon. However, recombination will gradually lead to a breakdown of associations between linked polymorphisms. Hybrids or introgressants are unlikely to be detected when recombination (and homogenization) has occurred over a long period of time, which may be the case regarding *efa* and *gpd*. Another possibility that cannot be fully dismissed is that genes from one lineage

have been eliminated after a hybridization event (Wendel *et al*, 1995).

In the segregation analyses of basidiospore progenies, the α or β nrDNA spacer versions cosegregated, and no meiotic recombinants including novel associations of the α and β nrDNA were found. This demonstrates that nrDNA is inherited as a single locus in Mendelian fashion, as has also been observed in other basidiomycetes, for example, *Laccaria bicolor* (Maire) P.D. Orton (Selosse *et al*, 1996) and *Pleurotus cornucopiae* (Paulet) Rolland (Iracabal and Labarere, 1994). A low frequency of IGS2 recombinants was observed in *L. bicolor* (Selosse *et al*, 1996), while no recombinants was observed in *Coprinus cinereus* (Schaeff:Fr.) S.F. Gray (Cassidy *et al*, 1984). The absence of recombinants in the progenies of *T. abietinum* suggests that meiotic recombination within the nrDNA gene family may be suppressed, as observed previously in yeast (Petes and Botstein, 1977). Another possible explanation is that very few linked nrDNA repeats exist. However, nothing is known at present about size and organization of the nrDNA clusters in *T. abietinum*. A higher sample size may possibly have detected meiotic recombinants also in *T. abietinum*.

As a concluding remark, it must be emphasized that a refined phylogenetic study of worldwide *Trichaptum* spp. specimens, including nrDNA and other unlinked gene sequences, is needed in order to understand the natural history of *T. abietinum* and its allies more fully.

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