Taxonomy and population structure of E-strain mycorrhizal fungi inferred from ribosomal and mitochondrial DNA polymorphisms

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E-strain fungi form ecto- and ectendomycorrhizal associations with many trees, and are important mycorrhizal symbionts in disturbed forest habitats and conifer nurseries. Unlike the majority of mycorrhizal fungi, which are basidiomycetes, E-strain fungi are ascomycetes belonging to the genus *Wilcoxina* (Pezizales). We analysed variation in the nuclear and mitochondrial ribosomal RNA genes to elucidate species concepts among E-strain fungi and to examine their population structure. We found that most E-strain isolates can be assigned to two taxa, *W. mikolae* and *W. rehmii*, that have different habitat preferences. *Wilcoxina mikolae* is the predominant taxon in disturbed forest habitats on soils that are often low in organic matter, such as burned sites, while *W. rehmii* prefers peaty soils. Analysis of mitochondrial DNA also revealed that within each species, isolates could be differentiated based upon host preference. This is the first report of population subdivision based upon host in E-strain fungi.

E-strain mycorrhizae were first described from conifer nurseries in Finland by Laiho & Mikola (1964). They have the morphological characteristics of ectomycorrhizae, except the mantle is very thin or sometimes lacking, and the hyphae of the Hartig-net may penetrate root cortical cells on some hosts (Mikola, 1965; Wilcox, Ganmore-Newmann & Wang, 1974; Wilcox, Yang & Lo-Buglio, 1983; Piché, Ackerley & Peterson, 1986). E-strain mycorrhizae are widely distributed on coniferous and deciduous hosts in nurseries and burned or disturbed forest sites in Finland (Mikola, 1965), the United States (Laiho, 1965; Wilcox *et al.*, 1974, 1983; Yang & Wilcox, 1984) and Canada (Danielson, 1982; Danielson, Visser & Parkinson, 1983; Danielson, Zak & Parkinson, 1984).

The taxonomic position of the fungi that form E-strain mycorrhizae was unclear to the original researchers, since sexual fruiting bodies were not found (see Mikola, 1965). Later, an analysis of cultural characteristics led Danielson (1982) to postulate that the fungi involved were ascomycetes belonging to the order Pezizales. This was confirmed by Yang & Wilcox (1984), who were the first to discover and identify the sexual stage of the fungus. Later Yang & Korf (1985) delineated a new genus, *Wilcoxina*, for E-strain fungi.

In a previous paper (Egger & Fortin, 1990) we showed that the majority of E-strain fungi could be referred to taxa in the genus *Wilcoxina* based upon cultural characters and DNA restriction fragment polymorphisms in the nuclear ribosomal RNA genes. In this paper we analyse the restriction fragment variation in both nuclear and mitochondrial ribosomal RNA genes in order to elucidate the taxonomy and population structure of E-strain fungi.

MATERIALS AND METHODS

Cultures of six fungal taxa, *Geopora* sp., *Trichophaea hemisphaerioides* (Mont.) Graddon, *Tricharina praecox* (Karst.) Dennis var. *intermedia* Egger, Yang & Korf, *Wilcoxina mikolae* (Yang & Wilcox) Yang & Korf var. *mikolae*, *W. mikolae* (Yang & Wilcox) Yang & Korf var. *tetraspora* Wilcox, Yang & Korf, and *W. rehmii* Yang & Korf were obtained by germination of ascospores from fresh ascocarps or dried herbarium specimens. E-strain isolates were obtained by germination of chlamydospores sieved from soil or by isolation from surface-sterilized mycorrhizal root tips. Distribution of voucher specimens and original cultures is documented in Yang & Korf (1985). Subcultures of isolates are deposited in the University of Alberta Microfungus Collection (UAMH), Edmonton, Canada and the Department of Agriculture Culture Collection (DAOM), Ottawa, Canada.

E-strain isolates from Alberta represent four population samples from three geographical regions. The first sample was isolated from jack pine seedlings (*Pinus banksiana* Lamb.) planted in a shallow mineral overburden collected near Bitumont (57° 25′ N, 111° 45′ W). The second sample was isolated from jack pine seedlings planted in peat, or oil sand tailings amended with mineral soil and peat, collected near Ft. McMurray (57° 5′ N, 111° 45′ W). Since these two populations were geographically close to one another and from similar lowland boreal forest habitats (altitude approx. 370 m)

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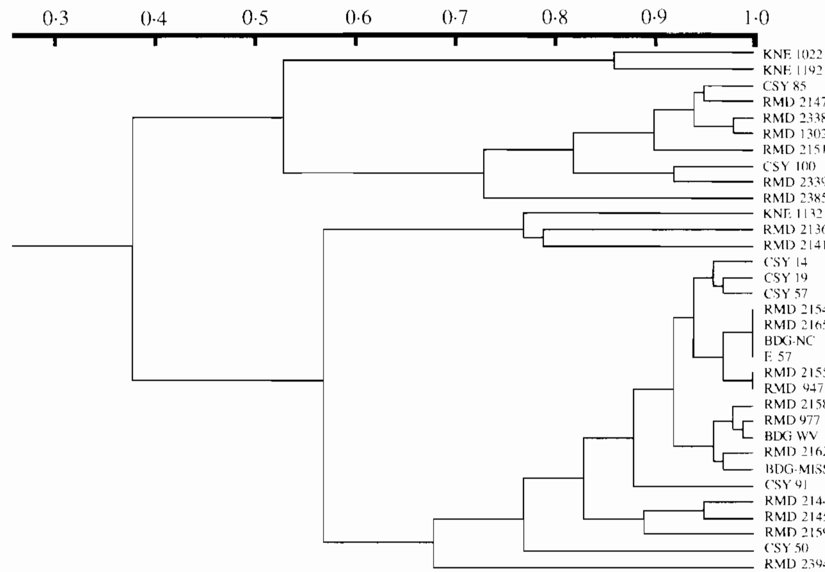

Fig. 2. Dendrogram of E-strain isolates based upon UPGMA cluster analysis of Jaccard's similarity matrix generated from restriction fragment polymorphisms in nuclear ribosomal RNA genes.

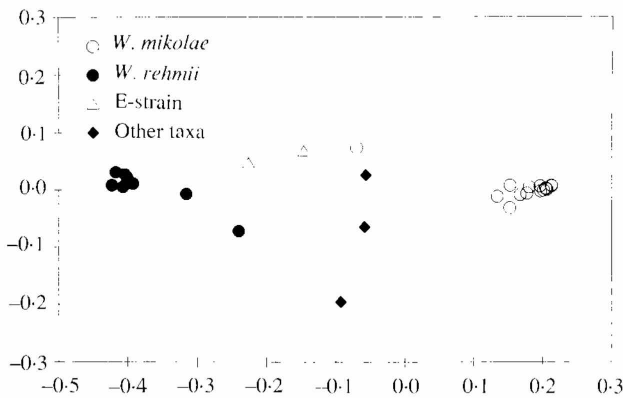

Fig. 3. Ordination of E-strain isolates by correspondence analysis (CA) based upon restriction fragment polymorphisms in nuclear ribosomal RNA genes. The first factor of the CA (*x* axis) is plotted against the second factor (*y* axis).

subcluster contained the other outgroup taxon, *Tricharina praecox*, as well as two unusual E-strain isolates (RMD 2136, 2141) that showed an affinity to the '*W. mikolae*' group, but that differed in several important polymorphisms. The last sub-cluster contained the known taxa of *W. mikolae* and E-strain isolates placed in the '*W. mikolae*' group by Egger & Fortin (1990). Two isolates occupied a peripheral position in this last cluster, RMD 2394 and CSY 50. RMD 2394 was placed in a peripheral position primarily because of it had a much larger spacer fragment than other isolates, otherwise it was similar to other members of the '*W. mikolae*' group. CSY 50 is an isolate of *W. mikolae* var. *tetraspora* which contains many polymorphisms present in both *W. mikolae* and *W. rehmii*. The co-occurrence of polymorphisms suggests that CSY 50 could be a hybrid between these taxa, which would explain its intermediate position; we are currently evaluating this hypothesis.

At the 2·5 and 5% homology test levels (analyses not shown) higher level clusters were not as well formulated. The cophenetic correlation coefficient indicated that the 7·5%

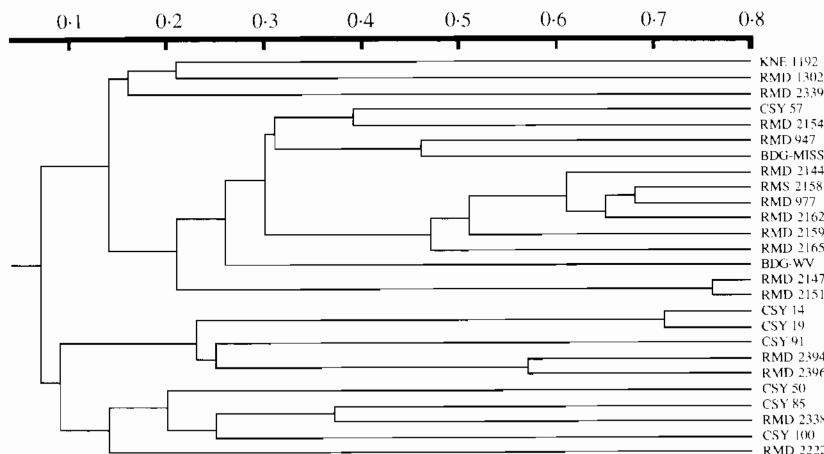

Fig. 4. Dendrogram of E-strain isolates based upon UPGMA cluster analysis of Jaccard's similarity matrix generated from restriction fragment polymorphisms in mitochondrial ribosomal RNA genes.

Table 1. Fungal isolates examined, including host, substrate, and geographical information

Number*	Name†	Host	Substrate	Origin	Ref.‡
KNE 1022	*Trichophaea hemisphaerioides*	Unknown	Burnt Soil	Victoria, B.C.	
KNE 1132	*Tricharina praecox*	Unknown	Burnt Soil	Nelson, B.C.	1
KNE 1192	*Geopora* sp.	Unknown	Soil	Victoria, B.C.	
CSY 14	*Wilcoxina mikolae* var. *mikolae*	*Pinus resinosa*	Nursery Soil	Oregon, U.S.A.	1
CSY 19	*W. mikolae* var. *mikolae*	*Pinus resinosa*	Nursery Soil	Oregon, U.S.A.	1
CSY 50	*W. mikolae* var. *tetraspora*	*Pinus resinosa*	Nursery Soil	New York, U.S.A.	1
CSY 57	*W. mikolae* var. *tetraspora*	*Pinus resinosa*	Nursery Soil	New York, U.S.A.	1
CSY 85	*Wilcoxina rehmii*	Unknown	Soil	Norway	1
CSY 91	*W. mikolae* var. *mikolae*	Unknown	Burnt Soil	Holland	1
CSY 100	*Wilcoxina rehmii*	?*Pinus* sp.	Soil	Spain	1
RMD 947	'*Wilcoxina mikolae*'	*Picea glauca*	Mine Spoil	Luscar, Alb.	2
RMD 977	'*Wilcoxina mikolae*'	*Picea glauca*	Mine Spoil	Luscar, Alb.	2
RMD 1302	'*Wilcoxina rehmii*'	*Pinus banksiana*	Tailings/Peat	Canmore, Alb.	2
RMD 2136	E-strain	*Pinus banksiana*	Tailings/Peat	Canmore, Alb.	2
RMD 2141	E-strain	*Pinus banksiana*	Tailings	?Canmore, Alb.	2
RMD 2144	'*Wilcoxina mikolae*'	*Picea glauca*	Mine Spoil	Luscar, Alb.	2
RMD 2145	'*Wilcoxina mikolae*'	*Picea glauca*	Mine Spoil	Luscar, Alb.	2
RMD 2147	'*Wilcoxina rehmii*'	*Picea glauca*	Spoil/Peat	Luscar/Canmore	2
RMD 2151	'*Wilcoxina rehmii*'	*Picea glauca*	Spoil/Peat	Luscar/Canmore	2
RMD 2154	'*Wilcoxina mikolae*'	*Picea glauca*	Mine Spoil	Luscar, Alb.	2
RMD 2155	'*Wilcoxina mikolae*'	*Picea glauca*	Mine Spoil	Luscar, Alb.	2
RMD 2158	'*Wilcoxina mikolae*'	*Picea glauca*	Mine Spoil	Luscar, Alb.	2
RMD 2159	'*Wilcoxina mikolae*'	*Picea glauca*	Mine Spoil	Luscar, Alb.	2
RMD 2162	'*Wilcoxina mikolae*'	*Picea glauca*	Mine Spoil	Luscar, Alb.	2
RMD 2165	'*Wilcoxina mikolae*'	*Picea glauca*	Mine Spoil	Luscar, Alb.	2
RMD 2222	'*Wilcoxina rehmii*'	*Pinus banksiana*	Peat	Canmore, Alb.	3
RMD 2338	'*Wilcoxina rehmii*'	*Pinus banksiana*	Mineral Soil	Bitumont, Alb.	4
RMD 2339	'*Wilcoxina rehmii*'	*Pinus banksiana*	Mineral Soil	Bitumont, Alb.	4
RMD 2385	'*Wilcoxina rehmii*'	*Pinus banksiana*	Peat	Ft. McMurray, Alb.	5
RMD 2394	'*Wilcoxina mikolae*'	*Pinus banksiana*	Soil/Peat	Ft. McMurray, Alb.	6
RMD 2396	'*Wilcoxina mikolae*'	*Pinus banksiana*	Soil/Peat	Ft. McMurray, Alb.	6
BDG-MISS	'*Wilcoxina mikolae*'	?*Pinus resinosa*	Nursery Soil	MS, U.S.A.	7
BDG-NC	'*Wilcoxina mikolae*'	*Pinus resinosa*	Nursery Soil	NC, U.S.A.	7
BDG-WV	'*Wilcoxina mikolae*'	?*Pinus resinosa*	Nursery Soil	WV, U.S.A.	7

* Cultures isolated by: KNE, K. N. Egger; CSY, C. S. Yang; RMD, R. M. Danielson; BDG, H. E. Wilcox.

† Names in quotes are hypothesized E-strain taxa from Egger & Fortin (1990).

‡ 1, Yang & Korf (1985); 2, Danielson (1991); 3, Danielson *et al.* (1984); 4, Danielson *et al.* (1983); 5, Danielson & Visser (1988); 6, Danielson & Visser (1989); 7, Wilcox *et al.* (1983).

we will refer to them as the Ft. McMurray population. The third sample was isolated from white spruce seedlings (*Picea glauca* (Moenche) Voss) planted in soil collected from a mine spoil in a subalpine habitat (altitude 1680 m) near Luscar (53° N, 117° 24′ W). The fourth sample was isolated from white spruce and jack pine seedlings planted in peat originating from a subalpine site (altitude 1320 m) near Canmore (51° 07′ N, 115° 20′ W) or planted in mine spoil or oil sand tailings amended with the Canmore peat. Since the oil sand tailings did not contain mycorrhizal inoculum, the mycorrhizal fungi must have originated from the peat; the seedlings planted in mine spoil could have obtained inoculum from the spoil or the peat. Since the Luscar and Canmore sites were situated in similar subalpine habitats, they are contrasted with the two lowland boreal forest sites near Ft. McMurray. Sources of isolates, taxonomic placement, and associated collection information are summarized in Table 1.

DNA extraction and analysis

DNA extraction, digestion, transfer to membranes, and hybridization to detect polymorphisms in ribosomal DNA is as described in Egger & Fortin (1990). To detect polymorphisms in mitochondrial DNA (mtDNA), total DNAs digested with the restriction endonucleases *Bam*H I, *Eco*R I, and *Hind* III were transferred to nylon membranes and hybridized with the plasmids pHP2 and pHPR4, which contain inserts that code for the mitochondrial ribosomal RNA genes from *Neurospora crassa* (Heckman & RajBhandary, 1979). The plasmid pHP2 contains an insert of approximately 2·6 kilobases (kb) which codes for part of the upstream spacer, the 17 S small subunit gene, and a downstream tRNA gene; the plasmid pHPR4 contains an insert of approximately 3·1 kb which codes for part of the upstream spacer, most of the 24 S large subunit gene, and part of the downstream intron.

Plasmid DNA was isolated by the alkali-lysis method of Maniatis, Fritsch & Sambrook (1982) and purified on a caesium chloride-ethidium bromide gradient. For each hybridization approximately 1 μg of plasmid DNA was labelled with $dCT^{32}P$ by nick translation. Membranes were hybridized at moderate stringency (60 °C, 1 M-NaCl) and washed as described in Chomezynski & Qasba (1984). Autoradiography used Kodak X-Omat AR film with intensifying screens at −80° for 12–72 h.

Data analysis

Size of restriction fragments was estimated by comparison with a DNA standard (lambda phage DNA digested with *Hind* III) and entered into a computer database. Fragments were then sorted according to size for each enzyme/probe combination and homology inferred based upon the degree of size overlap between fragments. Separate data matrices were generated for the nuclear and mitochondrial data sets using three homology test levels: 7·5, 5 and 2·5%. At each level, fragments differing by less than the prescribed value were considered homologous. The data sets were then analysed using the numerical taxonomy package NTSYS-pc with Supplementary programs (Rohlf, 1987). A total of 132 polymorphic restriction fragments distributed among 33 individuals were coded for nuclear ribosomal RNA genes, and 218 fragments distributed among 26 individuals for mitochondrial ribosomal RNA genes.

For cluster analysis, a similarity matrix was generated from each data matrix using the program SIMQUAL and Jaccard's similarity coefficient, which scores the number of common bands divided by the total number of bands present in the two samples being compared. Cluster analysis was performed using the program SAHN by the unweighted pair-group method using arithmetic averages (UPGMA). A cophenetic correlation coefficient was also calculated as a measure of the goodness of fit for the cluster analysis (Rohlf, 1987).

Ordination was preformed directly on the data matrix using correspondence analysis. According to Lebart, Morineau & Warwick (1984) this is an appropriate method of analysis for presence/absence data, and results were very similar to Principal Components Analysis (results not shown). First, row and column factors (eigenvectors) were extracted by the program CORRESP, then objects were projected onto three axes corresponding to the three largest row factors using the program PROJ.

RESULTS

Hybridizations of the *Bam*H I digests yielded few polymorphisms and were not further analysed. Hybridizations of *Eco*R I digested DNAs with pHPR4 and *Hind* III digested DNAs with pHP2 exhibited moderate levels of polymorphism (Table 2), while *Hind* III digested DNAs hybridized with pHPR4 yielded the most complex restriction fragment patterns (Fig. 1). Not all isolates yielded restriction fragment patterns for both nuclear and mitochondrial data sets.

Data for the nuclear ribosomal RNA gene polymorphisms was taken from Egger & Fortin (1990). Dendrogram topology and level of branching depended upon the homology test level. At the 7·5% level the dendrogram clearly defined two main clusters with each of these divided into two subclusters (Fig. 2). The first subcluster contained two outgroup taxa (*T. hemisphaerioides* and *Geopora* sp.). The second contained the known taxa of *W. rehmii*, as well as E-strain isolates placed in the '*W. rehmii*' group in Egger & Fortin (1990). The third

Table 2. Restriction fragment size estimates (kilobases) of mitochondrial DNA polymorphisms with two enzyme/probe combinations

Isolate	*Hind* III/pHP2	*Eco*R I/pHPR4
KNE 1192	12·4, 9·3	3·4
CSY 14	3·5, 2·1	9·4, 4·0, 1·3
CSY 19	3·5, 2·8	9·3, 4·1, 1·3
CSY 50	3·6, 3·1, 2·9, 2·2, 1·8	6·2, 4·0, 1·6
CSY 57	3·5	12·2, 5·4
CSY 85	3·7	13·0, 4·5
CSY 91	3·2, 2·9	8·9, 4·2, 1·3
CSY 100	3·7, 2·8	7·6, 3·2
RMD 947	3·5	12·8
RMD 977	3·5	15·4
RMD 1302	3·7, 2·4, 2·2	3·4
RMD 2144	3·5	12·2
RMD 2147	3·6, 2·4, 2·3	3·8
RMD 2151	3·6, 2·4, 2·3	3·7
RMD 2154	3·5	17·4
RMD 2158	3·5	11·6
RMD 2159	3·6	11·6
RMD 2162	3·5	11·6
RMD 2165	3·6	10·4
RMD 2222	3·4, 2·8	7·6, 3·6, 1·9, 1·5
RMD 2338	3·7, 2·7, 0·3	5·8, 4·6
RMD 2339	3·3, 1·3, 0·3	5·2, 4·7, 3·8
RMD 2394	2·9, 1·7	6·6, 4·5, 4·1, 1·5
RMD 2396	2·9, 2·0	6·5, 4·5, 4·0, 1·5
BDG-MISS	3·5	5·5
BDG WV	3·5, 2·9, 1·8	7·7, 4·4, 1·6

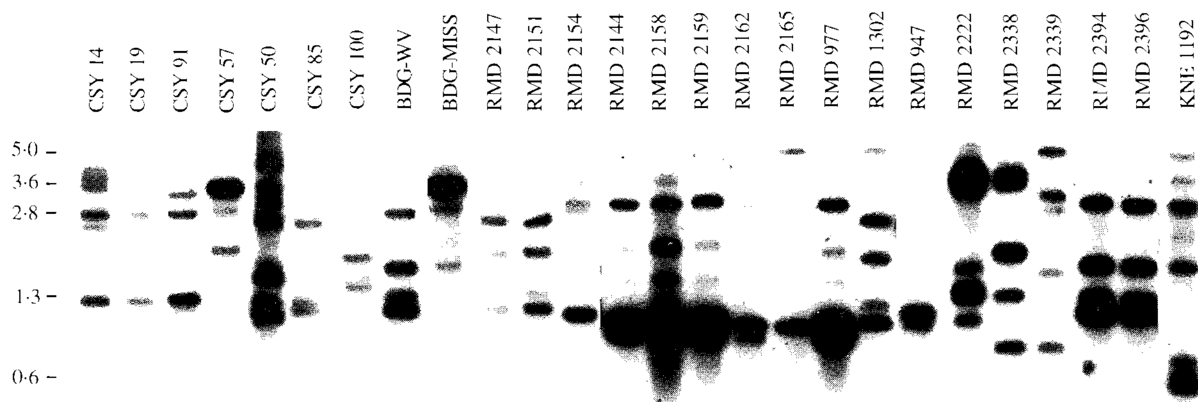

Fig. 1. Autoradiograph of total cellular DNAs from E-strain isolates digested with *Hind* III and hybridized with the mitochondrial probe pHPR4. Isolate numbers are listed along the top, with fragment size on the left.

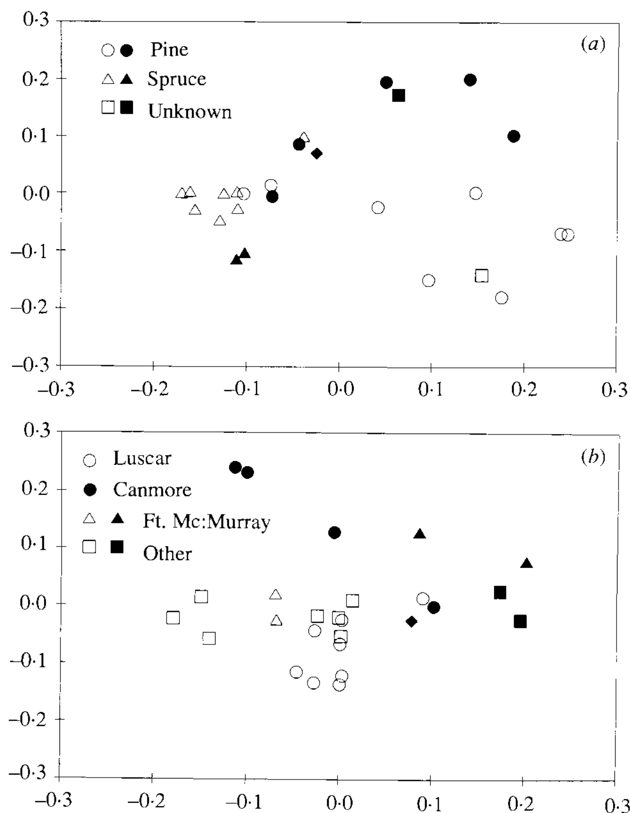

Fig. 5 (*a, b*). Ordination of E-strain isolates by correspondence analysis (CA) based upon restriction fragment polymorphisms in mitochondrial ribosomal RNA genes. In (*a*) the first factor of the CA (x axis) is plotted against the second factor (y axis). In (*b*) the second factor (x axis) is plotted against the third factor (y axis). Open symbols represent taxa of *Wilcoxina mikolae* and closed symbols taxa of *Wilcoxina rehmii*, except for the closed diamond which designates the outgroup taxon *Geopora* sp.

homology test level gave the best fit to the data of the levels tested, with the highest value at the 7·5% level (0·965 38) and declining from the 5% (0·958 54) to the 2·5% (0·831 75) test levels. The 7·5% homology data set was used for further analysis.

The first factor of the correspondence analysis of the nuclear data matrix clearly differentiated the two main taxa of *Wilcoxina* identified in Egger & Fortin (1990), with the *W. rehmii* group on the left and the *W. mikolae* group on the right (Fig. 3). The three outgroup taxa (K1022, K1132, and K1192) and the putative hybrid isolate (CSY 50) occupied intermediate positions between the two *Wilcoxina* groups, as did the two unusual E-strain isolates (RMD 2136, 2141) that grouped with *T. praecox* (K1132) in the cluster analysis.

Analysis of mtDNA polymorphisms yielded a different pattern of relationships among isolates. In contrast to the nuclear DNA analysis, relationships among isolates were best represented at the 2·5% homology test level (Fig. 4). Although there was considerable variation in higher level clusters, lower level clusters tended to group isolates of the same fungal taxon or on the same host. At the 7·5 and 5% homology test levels (analyses not shown) higher level clusters were not as well formed. The cophenetic correlation indicated that the 2·5% homology test level provided the best fit to the data, with the highest correlation coefficient occurring at 2·5% (0·888 67), and declining from the 5% (0·862 60) to the 7·5% (0·798 78) test levels. The 2·5% homology data matrix was used for further analysis.

Correspondence analysis of the mitochondrial data matrix showed the relationships among individual isolates more clearly than the cluster analysis. The first factor (Fig. 5*a*) separated isolates primary upon the basis of host, with isolates from pine predominating in the right quadrant and isolates from spruce concentrated in the left quadrant. The second and third factors (Fig. 5*b*) tended to separate the isolates into taxa, with *W. rehmii* isolates occupying the upper right and *W. mikolae* isolates occupying the lower left quadrants. Within each taxon there was also a tendency for isolates to cluster into groups that reflected geographical origin, with the lowland Ft. McMurray populations separating from the alpine Luscar and Canmore populations.

A combined data matrix was generated from the nuclear and mitochondrial data sets. The UPGMA cluster analysis of the combined data set (Fig. 6) yielded two major subclusters corresponding to the *W. mikolae* and *W. rehmii* groups. Within these clusters isolates were grouped primarily according to host and to some extent geographical origin of the isolates. The cophenetic correlation coefficient for this analysis was 0·968 85.

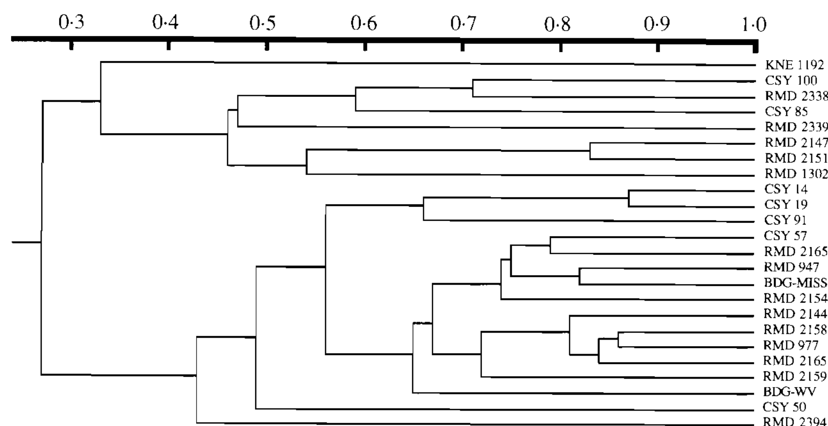

Fig. 6. Dendrogram of E-strain isolates based upon UPGMA cluster analysis of Jaccard's similarity matrix generated from restriction fragment polymorphisms in both nuclear and mitochondrial ribosomal RNA genes.

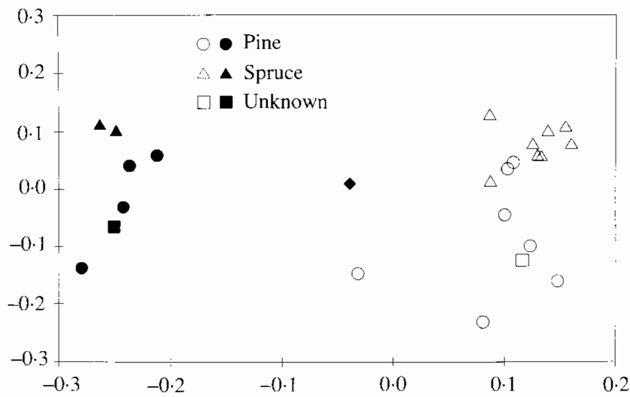

Fig. 7. Ordination of E-strain isolates by correspondence analysis (CA) based upon restriction fragment polymorphisms in both nuclear and mitochondrial ribosomal RNA genes. The first factor of the CA (x axis) is plotted against the second factor (y axis). Open symbols represent taxa of *Wilcoxina mikolae* and closed symbols taxa of *Wilcoxina rehmii*, except for the closed diamond which designates the outgroup taxon *Geopora* sp.

Correspondence analysis of the combined polymorphism data matrix clearly differentiated the two species of *Wilcoxina* on the first axis (Fig. 7), with the outgroup (K1192) and the possible hybrid (CSY 50) occupying intermediate positions. The second axis primarily distinguished isolates based upon host preference.

DISCUSSION

Upon initial examination it was not clear why the 7·5% homology test level was best for nuclear rDNA polymorphisms while the 2·5% level was best for mtDNA polymorphisms. However, subsequent restriction mapping studies (data not shown) suggest that the 7·5% homology test level performed better because most polymorphisms were due to variation in the size of fragments coding for portions of the intergenic spacer (IGS). The size of the IGS is known to vary within species, usually due to variation in number of internal repeats (Rogers & Bendich, 1987). At the 7·5% homology test level these variable fragments were judged to be homologous, which is appropriate if most variation is due to numbers of internal repeats rather than sequence variation.

The 2·5% test level performed best for the mitochondrial data because there was more variation in restriction fragment size and fragments that were similar in size were less likely to code for homologous sequences. By using the lowest homology test level the probability of errors caused by judging non-homologous fragments of similar size as homologous was minimized.

Restriction fragment polymorphisms in nuclear versus mitochondrial DNAs yielded different patterns of similarity among isolates. Polymorphisms in nuclear ribosomal RNA genes were correlated with taxonomic species. Similar results have been shown for other fungi (Anderson, Petsche & Smith, 1987; Kohn *et al.*, 1988), although some fungi show higher levels of variation (Vilgalys & Gonzalez, 1990). Mitochondrial polymorphisms were more variable and grouped isolates based primarily upon host and fungal taxon. This observation is not unexpected since mtDNA generally evolves more rapidly than nuclear DNA, exhibits low levels of recombination, and has more limited dispersal because it is typically maternally inherited (Taylor, 1986). There were also suggestions that mtDNA polymorphisms grouped isolates based upon geographical origin, although there were too few isolates to establish any clear trends.

This is the first time that population subdivision by host has been observed in E-strain fungi. At this point it is not clear whether population subdivision is due to differences in host compatibility or if it is a hierarchical structure imposed by differences in host distribution. However, there is evidence of morphological differentiation between host populations, with isolates from spruce lacking the characteristic chlamydospores seen in isolates from pine (Egger & Fortin, 1990), suggesting that populations are specialized to infect certain hosts. In addition, if a shift from ectomycorrhizal to ectendomycorrhizal infection is a manifestation of incompatibility between host and fungus, then incompatibility appears to be a common phenomenon when E-strain isolates are inoculated onto different hosts in axenic culture (Wilcox *et al.*, 1983). More work needs to be done to evaluate host compatibility. However, this study supports the statement of Wilcox *et al.* (1983) that 'specificity in the field appears to be more complex than originally perceived'.

We are confident in assigning most E-strain isolates to two taxa, *W. mikolae* and *W. rehmii*, but several isolates were atypical. We suspect that one atypical isolate, *W. mikolae* var. *tetraspora* (CSY 50), is a hybrid between *W. mikolae* and *W. rehmii*. *W. mikolae* var. *tetraspora* was differentiated from var. *mikolae* by the presence of four-spored asci (Yang & Korf, 1985). In addition, there were two atypical E-strain isolates (RMD 2136, 2141) that were morphologically similar to *W. mikolae* but contained several polymorphisms that indicated affinities to *W. rehmii* and *T. praecox*. It is possible that these isolates represent another taxon of *Wilcoxina*. We hope that further work on restriction mapping of the ribosomal DNA of representative isolates will help resolve the relationships of these isolates to other *Wilcoxina* taxa.

Finally, we can draw conclusions about habitat preference of E-strain taxa. It has been hypothesized that E-strain isolates from peat represent a nonchlamydosporic E-strain taxon (Zak, Danielson & Parkinson, 1982; Danielson *et al.*, 1984). Our study supports this hypothesis and identifies this taxon as *W. rehmii*, which occurs in peaty soils and does not produce chlamydospores (Egger & Fortin, 1990). *Wilcoxina mikolae* was not associated with peat, being more common in disturbed soils that are often low in organic content, such as burned soil or coal mine spoil. Habitat differences also provide evidence that the two atypical E-strains (RMD 2136 and 2144) are a different taxon since they originated from peat, an unusual substrate for *W. mikolae*.

Presence of two or more E-strain taxa may also explain differences in successional patterns with regard to replacement of *Thelephora terrestris* by E-strain (and vice versa) in nursery conditions and on disturbed soils, as suggested by Danielson (1990). Thus it may be critical to recognize E-strain fungi at the species level rather than at higher taxonomic levels in order to document successional or competitive interactions, and to predict effectiveness of inoculation programs.

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